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A study of factors effecting the herbicidal control of yellow nutgrass (Cyperus esculentus L.)

Alvin D. Rutledge

To the Graduate Council:

I am submitting herewith a dissertation written by Alvin D. Rutledge entitled "A study of factors effecting the herbicidal control of yellow nutgrass (Cyperus esculentus L.)." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

H. D. Swingle, Major Professor

We have read this dissertation and recommend its acceptance:

D. L. Coffey, B. S. Pickett, Gordon E. Hunt

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Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

March 30, 1970

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Homer D. Sprague
Major Professor

We have read this dissertation
and recommend its acceptance:

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Vice Chancellor for
Graduate Studies and Research

A STUDY OF FACTORS EFFECTING THE HERBICIDAL CONTROL OF YELLOW NUTGRASS (*CYPERUS ESCULENTUS L.*)

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Alvin D. Rutledge

June 1970

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ABSTRACT

The objective of this research was to determine if tuber scarification and potassium gibberellate treatment improved the effectiveness of four herbicides in killing yellow nutgrass (Cyperus esculentus L.). Scarification was studied as a method for improving herbicidal penetration into the nutgrass tuber. Potassium gibberellate was evaluated for its effectivensss in promoting starch hydrolysis in the mother tuber. It was applied as a soak to the germinating tubers alone and in combination with soil applications of s-ethyl dipropylthiocarbamate (EPTC) or 2'chloro-2,6-diethyl-n-methoxymethyl acetanilide (alachlor). Potassium gibberellate was also applied alone as a foliar spray and in combination with a foliar application of 3-amino-s-triazole(amitrole) or 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea(linuron).

Plant height, fresh and dry weight, total air dry root weight, and free glucose and starch content of the mother tubers were determined at one, five, and nine weeks after herbicide treatment.

Tuber scarification did not improve herbicidal effectiveness in killing yellow nutgrass. The tuber soak of potassium gibberellate did not influence plant growth or form of tuber carbohydrate content. Foliar applications of potassium gibberellate increased plant height, but the increase was not associated with a change in type of carbohydrate of the mother tuber, and did not improve the herbicidal effectiveness in killing nutgrass.

EPTC greatly inhibited nutgrass plant and root growth. There was a delay in the availability of free glucose in EPTC treated tubers.

Starch utilization in the mother tubers was greatly inhibited, but hydrolysis occurred over the nine-week period.

Alachlor inhibited nutgrass foliage and root growth for four weeks after treatment. Starch utilization was inhibited by alachlor, but hydrolysis occurred over the nine-week period.

Foliar applications of amitrole and linuron were effective in reducing nutgrass plant growth. Free glucose decreased in tubers treated with these chemicals. There was no significant difference in the initial and final starch content of the amitrole, or linuron treated tubers. However, starch content in amitrole and linuron treated tubers was significantly lower than that of the non-treated control at the termination of the experiment. Neither the amitrole nor the linuron treated tubers appeared viable at the termination of the experiment.

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INTRODUCTION

Although there is considerable usage of herbicides in the southeast, the growth of nutgrass species continues to pose a problem. This is partially attributed to a decrease in cultivation resulting from increased herbicide utilization. None of the herbicides now used on vegetables are sufficiently selective to control nutgrass for long periods of time. Therefore, nutgrass benefits from reduced competition from other weeds.

For a chemical to modify the growth of any plant, it must enter the plant. A variety of environmental forces act to change structurally, to destroy, or to remove externally applied herbicides rendering them internally unavailable. These include leaching, volatilization, photodecomposition, microbial degradation, chemical conversion, and herbicidal adsorption to soil colloids. The morphological, biochemical, and physiological factors operate to control herbicidal entry and distribution within the nutgrass plant. Internally, physiological activity such as adsorption to inactive sites and complex formations with other compounds may reduce the effectiveness of an herbicide.

Research with selective soil applied herbicides which provide short time nutgrass control indicates that nutgrass tubers can germinate and grow normally once the herbicide has been broken down either in the soil or in the plant. Perhaps this recovered germination (sprouting) is partially due to insufficient penetration of the herbicide into the

tuber. As a result, the chemical never completely upsets the metabolic pathways essential for nutgrass growth. In fact, radioautography has shown that EPTC (s-ethyl dipropylthiocarbamate) accumulates within the hard, brownish outer coat of the nutgrass tuber and never fully enters the physiologically active part of the plant (5). Thus, the effectiveness of EPTC in killing the nutgrass tuber becomes limited.

The effectiveness of a given herbicide might be improved by combining materials to enhance herbicidal penetration with growth regulator treatments which affect certain growth processes. When the pathway for improved penetration has been provided and the growth regulator has been applied, a followup application of the herbicide to active tissue should increase the probability of killing the nutgrass tuber.

This study was initiated to investigate the effects of tuber scarification, gibberellic acid, and four herbicides upon the growth and development of nutgrass (Cyperus esculentus L.) plants and the subsequent sugar and starch content of the mother tuber.

To facilitate the discussion of herbicides in this text, the common or designated and chemical names of all compounds referred to are summarized in Table I.

TABLE I

COMMON AND CHEMICAL NAMES OF HERBICIDES
REFERRED TO IN THE TEXT

	Common Name or Designation	Chemical Name
1.	AMA	Amine methylarsonate
2.	Amiben	3-amino-2, 5-dichlorobenzoic acid
3.	Amitrole	3-amino-s-triazole
4.	Alachlor	2' chloro-2, 6-diethyl-n-methoxymethyl acetanilide
5.	Atrazine	2-chloro-4-(ethylamino)-6-(isopropylamino-s-triazine)
6.	Barban	4-chloro-2-butynyl-m-chlorocarbanilate
7.	Bromacil	5-bromo-3-sec-butyl-6-methyluracil
8.	Butylate	S-ethyl diisobutylthiocarbamate
9.	CP-44939	2- tert-2-chloro-n-methoxymethyl-6 methylacetanilide
10.	CP-52223	2-chloro-n-(isobutoxymethyl)-2', 6'-acetoxylidide
11.	Dalapon	2, 2-dichloropropionic acid
12.	Dicamba	3, 6-dichloro-o-anisic acid
13.	Dichlobenil	2, 6-dichlorobenzonitrile
14.	Dinoseb	2-sec-butyl-4, 6-dinitrophenol
15.	Diphenamid	N, N-dimethyl-2, 2-diphenylacetamide
16.	EPTC	S-ethyl dipropylthiocarbamate
17.	Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea
18.	.....	N-hydroxymethyl-2, 6-dichlorothiobenzamide

TABLE I (continued)

Common Name or Designation	Chemical Name
19. Pebulate	S-propyl butylethylthiocarbamate
20. Propachlor	2-chloro-n-isopropylacetanilide
21. Simazine	2-chloro-4, 6-bis(ethylamino)-s-triazine
22. Terbacil	3-tert-butyl-5-chloro-6-methyluracil
23. 2, 4-D	(2, 4-dichlorophenoxy) acetic acid

CHAPTER II

REVIEW OF LITERATURE

I. BOTANICAL DESCRIPTION OF NUTGRASS

Nutgrass is very difficult to control because of its mode of reproduction. It can reproduce both sexually and asexually. It is a perennial sedge which forms weak, filiform stolons (11) and differs from annuals because it does not die after seed production. Instead, it survives the winter as a storage tuber (12).

Yellow nutgrass (Cyperus esculentus L.) is described (5) as having stout three-sided culms between 15 and 30 inches tall, and three-sided with yellowish, green stems. The leaves are about one-fourth to one-half inches wide, with a heavy mid-vein and slightly roughened edges. The involucre has three to six leaf like bracts extending beyond the umbel rays, which are often compound. The spikes are straw colored or pale yellow-brown. The whole plant is conspicuous due to its light coloring which is plainly visible among grasses. The scales of the spikelets are oblong-ovate, oppressed at the base but loose at the tip, and three to five nerved with narrow, scarious margins. The achenes are small, oblong, ovoid, three-sided, and light, yellowish-brown (5).

Tubers are usually oval and may attain a size of one-half to one inch. They are covered with a light to dark brown coat with many root-like projections over the surface. The interior is a hard, white, starchy material. Tubers may analyze 0.7 percent protein, 6.6 percent

fat, and 10.5 percent carbohydrates, on a dry weight basis (5). Taylorson (45) reports that reducing sugars of sprouted tubers ranges from about 1 to 2 percent while total sugar ranges from about 5 to 14 percent.

II. NUTGRASS REPRODUCTION

Yellow nutgrass reproduces chiefly by tubers and rhizomes (46). One tuber produces from zero to seven shoots, but a given shoot produces many rhizomes which end in new shoots or tubers. Tumbleson and Kommedale (46) have shown that one tuber can produce 1900 plants and 6900 tubers in one year.

When a tuber germinates, one or more slender rhizomes originate from the buds at the apical end. As a rhizome nears the soil surface, a new plant with a crown, top growth, and roots develops. The new plant then produces many slender rhizomes which terminate in vegetative growth during a long photoperiod (5).

The crown at the base of the nutgrass is an important zone for regeneration of new growth. Regrowth occurs from this area when nutgrass is killed back by contact herbicides (5). A small percentage of parent tubers will also resprout when the top growth is killed by herbicides or tillage. When the tubers are killed, the chief means of nutgrass dissemination is destroyed (5). Therefore, it is important to kill the tuber to reduce the spread of nutgrass.

Hauser (16) studied nutgrass reproduction over a two-year period. During the first year, he found that many tubers sprouted within seven to ten days after planting. Within three to four weeks, many new shoots

had emerged which extended outward from the planting site. Within five weeks, plants from adjacent three foot spacings were almost overlapping, which indicated a rapid rhizome growth and a high degree of non-dormancy in the underground structures (16).

In comparing nutgrass growth during the first and second years, Hauser (16) found that one tillage at the beginning of the second season did not materially affect the number of tubers formed. Reductions were greatest in thinly planted areas.

III. FACTORS AFFECTING NUTGRASS REPRODUCTION

Tuber germination is greatly influenced by oxygen concentration according to Negi and Normand (31) and Palmer and Porter (35). The exclusion of all oxygen or a pretreatment with 100 percent oxygen resulted in a greater percentage germination than did exposure to normal atmospheric oxygen. Pretreatment with 100 percent oxygen resulted in a stimulation only when the duration was less than 48 hours (31, 35).

Bundy et al. (7) reported that overall plant growth and development of vegetative shoots of northern nutgrass is promoted by increasing the photoperiod, light intensity, soil temperature, and soil moisture. Bell et al. (5) agree with Bundy et al. (7) that tuber development, on the other hand, is promoted by decreasing the photoperiod under reduced light intensity.

Tubers buried in the soil for 12 months do not germinate according to Bundy et al. (7). Tubers placed on top of the soil in the field in October for five months germinated 12 percent in April even though temperatures dropped to as low as three degrees Fahrenheit. Tubers

placed at constant temperatures of zero, five, and twenty degrees Fahrenheit, remained viable for 13 days or less.

Garg et al. (12) indicated that tuber differentiation on rhizomes is more pronounced under twelve and one-half hour photoperiods than under fourteen hour photoperiods. These investigators also found that 1000 parts per million (ppm) of gibberellic acid promoted tuberization under fifteen and one-half hour photoperiods. However, zero, and ten ppm of gibberellic acid under short photoperiods promoted more tubers than did long photoperiods (12).

Bell et al. (4,5) concluded that germination of nutgrass seed is favored by alternating daily temperatures of 85 to 96°F. for eight hours and 70°F. for sixteen hours. Alternating freezing and thawing or wetting and drying reduced the percentage germination but did not kill the seed. Light or absence of light had little effect on germination when temperature conditions were optimum (4,5).

IV. NUTGRASS TUBER RESPIRATION

Palmer and Porter (36) found that carbon dioxide evolution in germinating tubers is initially greater than oxygen consumption. Dormant tubers have a respiratory quotient of unity that persists for five and one-half days after germination. This would suggest, according to Palmer and Porter (36) that organic acids are the substrates being respired. However, Beevers (3) points out that the frequency with which values close to unity have been observed is related to the part played by sugars as the respiratory substrate. In recent years, appreciable changes from unity have been found to occur as a result of incomplete sugar oxidation,

oxidations and decarboxylations which are unrelated to respiration, and fat utilization in the glyoxylate cycle (3). Thus, many factors may contribute to the respiratory quotient observed in nutgrass tubers.

Hardcastle and Wilkinson (13) found that concentration of dichlobenil, length of exposure to the herbicide, or interaction of these two factors did not appear to affect the respiratory activity of dormant tubers. Ashton (2) reported that EPTC stimulated tuber respiration on a fresh weight basis.

V. NUTGRASS TUBER ENZYME ACTIVITY

Catalase activity is 18.5 times higher in untreated, germinated tubers than in tubers treated with amitrole according to Palmer and Porter (37). Since catalase catalyzes the following reaction:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

it is possible that amitrole inhibits catalase to such an extent that toxic concentrations of hydrogen peroxide accumulates within the cell (39, 48).

Peroxidase activity is two and one-half times higher in the germinated tuber than in the dormant tuber (36). Germinating purple nutgrass tubers which had been exposed to the higher rates of amitrole exhibited approximately the same perioxidase activity as did dormant tubers. Consequently, amitrole treatment was effective in reducing peroxidase activity.

Amylases catalyze starch hydrolysis. They are known as alpha (α) and beta (β) amylase. Alpha amylase splits the 1,4 glucosidic bonds,

except for maltose, in apparently random fashion. Beta amylase, on the other hand, attacks polysaccharide chains effecting successful removal of maltose units from the non-reducing ends. Neither type of enzyme acts on α-1,6 or β-1, 4 bonds (39, 48).

Penner (38) reported that amylase from germinating barley is inhibited by the herbicides amiben, diphenamid, 2,4-D, and dicamba. Of these herbicides, 2,4-D slightly, and amiben severely, reduced amylase activity during barley germination.

Gibberellic acid stimulated both germination and amylase activity in barley according to Paleg (33). Amylytic activity can be induced by GA_3 in isolated endosperm which results in the formation and release of reducing sugars, particularly of maltose and glucose. This effect is believed to be due to amylase activity.

Yung and Mann (49) showed that amylase activity was severely inhibited when barban was added to embryo-free barley seeds within four or five hours after gibberellin treatment. Addition of barban seven or more hours after gibberellin treatment was almost without effect.

VI. CHEMICAL CONTROL OF NUTGRASS

Leyden (28) applied bromacil and terbacil at 4 and 2 pounds per acre as a foliar spray or as an incorporated treatment in non-cropland. Some sprouting of new nutgrass tubers occurred in treated areas, but these plants typically yellowed and died. Dry weight of tubers recovered from bromacil, terbacil, and non-treated plots was 8, 17, and 85 grams, respectively. Almost all of the tubers from untreated plots appeared viable while those from treated plots did not.

Terbacil and bromacil at 10 pounds per acre are equally effective on purple nutgrass, but maximum control does not occur until one year after treatment (47). With these chemicals, tubers sprout and grow normally until the third to fourth leaf stage when the shoots slowly become chlorotic and die. After each cultivation, new tubers sprout and die in the same manner.

Dichlobenil incorporation gave nearly perfect nutgrass control at the 20 pound rate, and only slightly less at the 5 and 10 pound rates (41). Surface applications at the same rates were ineffective. In an experiment conducted by Taylorson (45) dichlobenil resulted in excellent nutgrass control for about 18 months after which reinfestation occurred from a few surviving plants.

Atrazine appears to be an effective nutgrass control as an incorporated post-emergence application according to Durfee et al. (10). Hargan et al. (14) indicated that disking after atrazine application greatly enhances nutgrass control when applied to sprouting tubers, but plowing down decreased its effectiveness. Post-emergence applications are reported to be slightly more effective (10,14).

Vernolate applied as a subsurface application at the 2 to 4 inch depths generally controls nutgrass much better than does preplanted, incorporated treatments (20).

EPTC is one of the most specific chemicals for nutgrass when used at 6 to 10 pounds per acre (25). Holt et al. (23) found that nutgrass tubers germinated readily in soil treated with EPTC. However, growth was suppressed and emergence delayed. The delay was dependent on the rate of application. After shoots emerged through the tuber epidermis,

the herbicide appeared to stop further cell division and subsequent elongation of the shoot (23).

A granular formulation of EPTC is effective longer than an emulsifiable formulation incorporated in the same way according to Holt et al. (23). In samples taken 14 months after herbicide application, tubers that did not germinate after three months were dissected and examined. In general, such tubers were nearly decomposed and necrotic throughout.

Hauser (18,20) reported that it required twice as much incorporated EPTC to give nutgrass control equivalent to a subsurface application. A surface application of 8 pounds per acre resulted in poor control. A subsurface application of 2 pounds per acre at three-fourths inch was also ineffective (19).

A single application of EPTC one or two weeks after nutgrass emergence prevented foliage growth during the summer (17). However, single shoot growth resumed in some plots five months after application. These weakened shoots were winter killed and no new growth occurred during the second year. EPTC applications at later stages provided good control (17).

Hargan et al. (14) found that preplowing and surface application of EPTC gave inadequate nutgrass control. The rate of EPTC is not as important as the timing and disking-in of the application. Disking immediately following treatment gave particularly good results when EPTC was applied post-emergence to nutgrass (14).

Amitrole appears to hinder or inhibit chlorophyll formation, which probably accounts for the failure to form chloroplasts. When this

happens, plants lose their photosynthetic capabilities and are unable to survive (25).

Hill et al. (21) found that amitrole greatly reduced nutgrass tuber viability while Hauser (17) indicated that tuber control decreased with delayed amitrole applications after emergence.

Linuron is ineffective in controlling nutgrass when used pre-emergence. Post-emergence applications which are disked-in resulted in good initial control of nutgrass as illustrated by Hargan et al. (14). These investigators also reported that only temporary control resulted from spraying plants four to six inches tall while spraying 20 to 24 inch plants resulted in full season kill of the tops. Linuron effectiveness is enhanced by irrigation immediately after spraying (14).

VII. HERBICIDE TRANSLOCATION IN NUTGRASS

Bell et al. (5) and Hill et al. (21) indicate that radioactive amitrole is translocated into the tubers and seeds of nutgrass. Tubers which contain amitrole rarely grow and seed germination is decreased.

Working with soil-applied C^{14}-labeled dalapon, Saidak (40) found very little accumulation of the herbicide within nutgrass roots and tubers. This same type of relationship was found to exist when leaves were dipped in radioactive dalapon.

According to Magalhaes et al. (29), labeled dicamba is slowly translocated and exhibits both symplastic and apoplastic movement after application to a fully expanded leaf. Labeled dicamba accumulated principally in the actively growing plant parts, but radioactivity was detected throughout the aerial organs. Detection of radioactive dicamba

in "daughter" plants indicated a translocation of the chemical through the rhizomes and tubers. However, very little radioactivity was detected within the rhizomes and tubers (29).

When AMA was applied to one shoot of a tuber which had two shoots, the non-treated shoot also showed toxicity symptoms (24). AMA toxicity symptoms were also observed in shoots arising from tubers connected by rhizomes to the tuber from which the original treated shoot had arisen. Symptoms were also present in shoots arising from tubers which were separated along the same rhizome by as many as two, three, or four tubers from the treated shoot.

Arsenate moves into tubers from shoots treated with AMA. All tubers along chains in which the terminal tuber shoot had been treated contained more arsenate than untreated tubers. The amount of arsenate accumulating in tubers in a chain subtending a treated shoot decreased as the distance (in tuber numbers) from the treated shoot increased. AMA movement appeared to be enhanced if there was a shoot on each end of the chain (24).

Crafts (18) reported that S^{35} labeled EPTC is readily translocated through nutgrass plants. Bell et al. (5) also concluded that radio-labeled EPTC moved easily through nutgrass plants but did not accumulate within the tuber. Instead, it accumulated in the external coat of the tuber and apparently never really upset tuber metabolism.

CHAPTER III

MATERIALS AND METHODS

I. ACCUMULATION OF RESEARCH MATERIAL

In September and October of 1968, nutgrass plants and tubers were collected from The University of Tennessee Plant Sciences Farm and planted in sand in the greenhouse. Plants were watered one or two times per week with a complete Hoagland's (22) nutrient solution at half strength. Plants were grown under long day growing conditions to enhance the development of new tubers. Incandescent light bulbs were spaced three feet apart four feet above the plants to supplement natural light from 6 a.m. to 8 p.m. The temperature was maintained at 23 to 30°C.

During the first week of July, 1969, newly developed, mature tubers were removed from the sand and washed over one-eighth inch wire mesh for separation. The new tubers were stored at 5°C. until ready for treatment. The tubers were removed from storage as needed and soaked for one hour in a 1 percent ethylene chlorohydrin solution to promote germination. The soak and cold treatment appeared to be quite sufficient since approximately 80 percent of the tubers germinated in all of the cases tested.

The tubers grown in this phase were utilized for study in the following experiments.

II. PRELIMINARY SCREENING EXPERIMENTS

Sixteen herbicides, eight having greater effectiveness when applied to the soil and eight having greater effectiveness as a foliar application, were included in this phase of the study. The objective at this point was an evaluation of the relative effectiveness of several herbicides in controlling nutgrass emergence and foliage kill.

From this screening, four foliar-applied and four soil-applied herbicides were selected for further evaluation based on their nutgrass activity and possible selectivity to vegetable crops.

Gibberellic acid enhances the synthesis and release of α-amylase in germinating barley seeds. Alpha amylase is one of the enzymes responsible for the starch to glucose conversion. Barley seeds and nutgrass tubers are high in starch. A rapid starch depletion of the nutgrass tuber induced by gibberellic acid treatment may be conducive to a more effective herbicidal control of nutgrass.

A two-phase study utilizing potassium gibberellate and herbicide treatments was initiated to obtain preliminary information on the effectiveness of these chemicals in controlling nutgrass.

Foliar Application of Potassium Gibberellate and Herbicides to Nutgrass

On August 11, 1969, 10 tubers per flat were planted into a soil mixture containing one part sand, one part peat, and two parts of soil. The experimental design was a split plot containing four replications of one flat each. Whole plots consisted of three potassium gibberellate concentrations and the sub-plots consisted of five herbicide treatments (Table II).

TABLE II

POTASSIUM GIBBERELLATE AND HERBICIDES USED AS A FOLIAR APPLICATION TO NUTGRASS

Potassium Gibberellate (ppm)	HERBICIDES		
	Name	lb./A	Formulation
1. 25	1. None	--	--
2. 50	2. Amitrole	2	2 lb./gal. emulsion concentrate
3. 100	3. Linuron	6	50% wettable powder
	4. Simazine	3	80% wettable powder
	5. Terbacil	2	80% wettable powder

The potassium gibberellate solutions were applied by spraying until runoff to one and one-half week old nutgrass foliage. Herbicides were applied to the foliage 24 hours after potassium gibberellate application. The delay in herbicide application was to allow time for the potassium gibberellate to enter the plant.

Height and fresh weight of the shoots of the treated plants was determined two weeks after herbicide application. Tubers were collected, placed in cold storage for four weeks, and regerminated to evaluate the killing effectiveness of these chemicals. The data are recorded as the percentage of regermination.

Potassium Gibberellate Soak of Germinating Tubers and Soil Herbicide Treatment

A second lot of germinated tubers was divided into three groups, placed in 25, 50, and 100 ppm of potassium gibberellate solutions derived from a 75 percent material. They were then shaken on an automatic shaker for six hours and planted in soils treated with the herbicides shown in Table III. The herbicides were applied at the desired rate in 900 gallons of water per acre and thoroughly mixed by tumbling of the soil. The experimental design was identical to that described in the preceding experiment.

The percentage emergence of the treated tubers was determined at three and five days after planting. The original tubers were collected after four weeks growth, placed in cold storage for four weeks, and regerminated. The effect of the combination of potassium gibberellate and herbicides was observed by determining the percentage of tuber regermination.

TABLE III

POTASSIUM GIBBERELLATE TREATMENTS USED TO SOAK GERMINATING TUBERS AND SOIL-APPLIED HERBICIDES APPLIED TO NUTGRASS

Potassium Gibberellate (ppm)	HERBICIDES		
	Name	lb./A	Formulation
1. 25	1. None	--	--
2. 50	2. EPTC	8	6 lb./gal. emulsion concentrate
3. 100	3. Vernolate	6	6 lb./gal. emulsion concentrate
	4. Dichlobenil	10	50% wettable powder
	5. Alachlor	4	4 lb./gal. emulsion concentrate

III. PRIMARY EXPERIMENT

Scarification, Potassium Gibberellate, and Herbicide Treatment

From the preceding experiments, two soil-applied and two foliar-applied herbicides were selected for further study in combination with a tuber scarification treatment and a 100 ppm of potassium gibberellate treatment. The herbicides studied were EPTC, alachlor, amitrole, and linuron.

Since EPTC does not penetrate into the starchy portion of the tuber (5), a study of tuber scarification combined with potassium gibberellate and herbicide treatment was made to evaluate the effects on nutgrass growth and mother tuber carbohydrate content. Theoretically, improved herbicide penetration by scarification and enhanced starch breakdown by potassium gibberellate should result in greater effectiveness in the herbicidal control of nutgrass.

On September 17, tubers which had received a cold treatment were scarified by rubbing against a medium grade of sand paper until very slight visual damage could be detected on the outer coat of the tuber.

The experiment was a split plot design with three replications of one flat each. The whole plots were scarified or non-scarified tubers, and the sub-plots were the potassium gibberellate and herbicide treatments. The respective treatments are summarized in Table IV.

Tubers designated to receive the soil-applied herbicides were germinated and soaked in 100 ppm of potassium gibberellate prior to planting. The tubers were planted in sterilized media of one part sand, one part peat, and one part soil and containing either EPTC or alachlor.

TABLE IV

TREATMENTS USED TO EVALUATE SCARIFICATION, POTASSIUM GIBBERELLATE, AND HERBICIDE EFFECTIVENESS ON NUTGRASS FOLIAGE GROWTH AND CARBOHYDRATE CONTENT OF TUBERS

	SUB-PLOTS			
Whole Plots	Potassium Gibberellate	Herbicide	lb./A	Formulation
1. Scarified tubers	1. None	None	--	--
2. Non-scarified tubers	2. 100 ppm soak on germinating tubers	None	--	--
	3. 100 ppm soak on germinating tubers	EPTC	8	6 lb./gal. emulsion concentrate
	4. 100 ppm soak on germinating tubers	Alachlor	4	4 lb./gal. emulsion concentrate
	5. 100 ppm spray on foliage	None	--	--
	6. 100 ppm spray on foliage	Amitrole	2	2 lb./gal. emulsion concentrate
	7. 100 ppm spray on foliage	Linuron	6	50% wettable powder

They were grown in the greenhouse at temperatures of 21 to 27°C. under long day conditions.

Tubers designated to receive the foliar applications of potassium gibberellate, amitrole, and linuron were planted in the soil mixture described above at the same time and grown for one and one-half weeks. Potassium gibberellate was then applied and the herbicide applications were made 24 hours later. The entire experiment was conducted in duplicate.

Foliage height, fresh and dry weight, total air dry root weight, and carbohydrate content of the mother tuber were measured at one, five, and nine weeks from herbicide treatment. The total air dry root weight in this text includes all the underground growing parts except that of the mother tuber. The author realizes that a tuber or rhizome is not a root, but it is discussed in this manner in the text. Furthermore, two random samples were selected from each treatment and the percentage of total air dry, underground plant weight due to new tubers was determined.

Glucose and starch determinations were made by modifications of the procedures described by the AOAC (1), McRary and Slattery (30), Nelson (32), and Somogyi (44). To determine these compounds, two fractions were made of the mother tuber. Fraction one consisted of washing a known fresh weight of tubers, homogenizing in 40 mls. of 80 percent ethanol for one minute, centrifuging at 1.4×10^4 g, and filtering to remove the residue. The residue was dried and kept for starch analysis. The filtrate was made to 250 mls. with distilled water and the content of alcohol soluable reducing substance was determined and reported as glucose. This fraction was expressed as the percentage of

glucose in the "free state." All determinations are expressed on a percentage-of-dry-matter basis.

For starch determinations, 10 mls. of distilled water were added to a 250 mg. sample of dried tissue from fraction one. The residue was refluxed in a boiling water bath for 30 minutes with a 25 ml. inverted Erlenmeyer flask acting as a condenser. The sample was cooled in a water bath, and then incubated for 44 $\pm$ 0.5 hours at 37°C. The incubation medium consisted of a solution containing 10 mls. of acetic acid-sodium acetate buffer of pH 4.2, and 10 mls. of a "Clarase 900"* form of takadiastase containing five gms. of clarase per liter of solution. One gram of powdered thymol per liter of solution was added to the enzyme and buffer solutions to serve as an antibacterial agent.

After incubation, the residue was filtered through Whatman No. 42 filter paper, washed with five mls. of 0.7N HCL and refluxed in a boiling water bath as described previously to complete hydrolysis. After refluxing, the sample was made to 250 mls. with distilled water. For determinations, it was necessary to further dilute this fraction at a ratio of 1 ml. of sample per 3 mls. of water.

To determine glucose reduction, 2 mls. of copper reagent were added to 2 mls. of the final diluted sample in a 50 ml. test tube. The samples were then heated in boiling water for 15 minutes using 25 mm. funnels as reflux condensers. After heating, the samples were cooled to room temperature and 2 mls. of arsenomolybdate and 25 mls. of distilled

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*"Clarase 900" form of takadiastase was obtained from Miles Laboratories, Inc., Elkhart, Indiana.

water added. A 3 ml. aliquot of this sample was added to clean cuvettes and the absorbance read on a Beckman DU spectrophotometer at 510 mu.

The absorbance of four replications of standard solutions containing 0, 0.05, 0.10, 0.15, 0.20, and 0.25 mg. of α-D-glucose per ml. of solution was determined. The various amounts of standard glucose were divided by their corresponding absorbance and the resulting factors averaged to give the necessary conversion factor. The absorbance of the unknown sample was multiplied by this factor to give the milligrams per milliliter of glucose present in the sample. The percentage of glucose was then determined by the following formula:

$$\text{percent glucose} = \frac{\text{mg. glucose/ml. in sample}}{\text{mg. dry matter/ml. in sample}} \times 100$$

The percentage of starch was then calculated by the following formula:

$$\text{percent starch} = \frac{\text{percent glucose}}{111.1} \times 100$$

The second determination is possible since 100 parts of starch should theoretically yield 111.1 parts of glucose (39).

IV. SECONDARY EXPERIMENTS

The following experiments were initiated as a result of some findings on tuber scarification and starch hydrolysis in the primary experiment.

Effect of a Water Soak on Scarified Tuber Germination

This study was initiated to determine the effects of a water soak on the germination of scarified tubers. Tubers were scarified as described earlier, weighed, and allowed to soak in water for 0, 2, 4, 8, and 24 hours. At the termination of the soaking period, the tubers were wiped dry, reweighed, and 10 tubers were replicated four times in sand-filled petri plates to which was added 40 mls. of water. Each plate was covered and sealed with masking tape to avoid moisture loss. The tubers were germinated in the dark at 33°C. for 72 hours and the percentage of germination determined.

Herbicidal Effect on Starch Hydrolysis

This study was initiated to investigate the effects of EPTC and alachlor on the "Clarase 900" enzyme utilized to convert starch to glucose in the nutgrass tissue. This study would be expected to provide preliminary information on the effectiveness of EPTC and alachlor in inhibiting the amylase system responsible for the starch to glucose conversion.

Dry nutgrass tissue which had sugars in the "free state" removed was incubated as described previously except that 10 mls. of 0, 2, 4, 6, 8, and 10 ppm of EPTC and alachlor were added separately to the incubation medium. Each treatment was replicated four times.

After incubation, the percentage of glucose was determined as described previously.

The data collected in these experiments were subjected to the "Analysis of Variance" as described by LeClerg et al. (27) and Snedecor (42) and analyzed on the 7040 computer at The University of Tennessee Computer Center.

CHAPTER IV

RESULTS AND DISCUSSION

I. PRELIMINARY SCREENING EXPERIMENTS

A summary of the nutgrass growth as affected by the first 16 herbicides screened in this study is shown in Table V. These observations were taken one month after treatment. In general, all of the soil-applied herbicides showed activity against early growth of yellow nutgrass. EPTC and dichlobenil appeared to inhibit nutgrass growth for longer periods than did any of the other soil-applied herbicides. EPTC and alachlor were selected for further study because they have been reported to be more selective in vegetable crops than any of the other soil-applied herbicides used in this phase of research.

Of the foliar-applied herbicides used in this study, amitrole and linuron showed the greatest effectiveness in killing the nutgrass foliage. These two herbicides are not widely used for weed control in vegetable crops, but their apparent activity in nutgrass control and their selectivity in certain economic crops indicate a need for further study.

Foliar Application of Potassium Gibberellate and Herbicides to Nutgrass

The effect of foliar applications of potassium gibberellate on plant height, fresh weight, and the percentage regermination of mother tubers is summarized in Table VI. Height was the only factor affected by potassium gibberellate during this phase of research. Plant height

TABLE V

EFFECT OF SIXTEEN HERBICIDES ON NUTGRASS GROWTH

Herbicide	Area Applied	Rate lb./A	Observation
1. Alachlor	soil, inc.	4	Shoots emerged but were killed. Tubers remained viable.
2. Amitrole	foliage	2	Foliage became very chlorotic and eventually died.
3. Atrazine	foliage	6	Shoots grew more rapidly than the control. Shoots were dark green.
4. Bromacil	foliage	2	Shoot growth reduced with considerable chlorosis.
5. Butylate	soil, inc.	6	Shoots emerged and grew for four days. Tubers were viable.
6. Control	-----	--	Tuber growth had darkened. Foliage showed vigorous growth.
7. CP-44939	soil, inc.	4	Shoots had some stunted growth but died later.
8. CP-52223	soil, inc.	4	Effects similar to CP-44939.
9. Dichlobenil	soil, inc.	10	Tubers germinated but growth was inhibited. Tubers remained viable.
10. Dinoseb	foliage	6	No observable effect.
11. EPTC	soil, inc.	8	Effects very similar to dichlobenil.
12. Linuron	foliage	6	Foliage became severely chlorotic and died.
13. Pebulate	soil, inc.	6	Shoots emerged but were killed. Tubers were viable.
14. Propachlor	soil, inc.	4	Toxicity occurred after one and one-half weeks.
15. Simazine	foliage	3	Shoot growth was reduced and leaves became chlorotic.
16. Terbacil	foliage	2	Effects similar to bromacil.
17. Vernolate	soil, inc.	6	Growth similar to EPTC.

TABLE VI

EFFECT OF POTASSIUM GIBBERELLATE ON PLANT HEIGHT, FRESH WEIGHT, AND PERCENTAGE REGERMINATION OF MOTHER TUBERS

Potassium Gibberellate (ppm)	FOLIAGE*		Tuber Regermination* (%)
	Height** (inches)	Fresh Weight** (gms./flat)	
1. 25	4.9b	1.5a	22.5a
2. 50	5.8a	1.6a	29.5a
3. 100	5.7a	1.5a	23.0a

*Values followed by the same letter in the same vertical column do not differ at the .05 level (Duncan's Multiple Range).

**Taken two weeks after herbicide treatment.

did not differ between plants treated with 50 and 100 ppm of potassium gibberellate, but height was increased in these treatments above the 25 ppm treatment. The effect of foliar-applied herbicides on nutgrass plant height, fresh weight, and percentage of tuber regermination is summarized in Table VII. All of the herbicides tested reduced plant height, fresh weight of once-planted tubers, and the percentage of regermination of the same tubers after four weeks of storage at 5°C. However, differences were observed among the herbicides in each of the above characters. Linuron was the most effective herbicide in reducing plant height and fresh weight. Amitrole significantly reduced tuber regermination, but it did not completely inhibit regermination as did linuron, simazine, and terbacil. These data indicate that the foliar-applied herbicides are highly effective in killing the mother tuber.

Potassium Gibberellate Soak of Germinating Tubers and Soil Herbicide Treatment

Emergence of nutgrass tubers appeared to be increased by a 100 ppm potassium gibberellate soak of the germinating tubers (Table VIII). However, it did not influence nutgrass regermination of the same tubers after storage for four weeks at 5°C.

The soil-applied herbicides effectively reduced the emergence of tubers, but they did not affect the percentage regermination of the same tubers after storage at 5°C. (Table IX). These data indicate that the soil-applied herbicides are highly effective in preventing shoot growth, but they are not effective in killing the mother tuber.

TABLE VII

EFFECT OF FOLIAR APPLICATION OF HERBICIDES ON PLANT HEIGHT, FRESH WEIGHT, AND TUBER REGERMINATION

Herbicide	Rate lb./A	FOLIAGE* Height** (inches)	FOLIAGE* Fresh Weight** (gms./flat)	Tuber Regermination* (%)
1. None	--	8.0a	3.6a	58.3a
2. Amitrole	2	6.4b	1.9b	15.3b
3. Linuron	6	3.3c	0.3c	0.0c
4. Simazine	3	5.5b	1.6b	0.0c
5. Terbacil	2	4.1c	1.4b	0.0c

*Values followed by the same letter in the same vertical column do not differ at the .05 level (Duncan's Multiple Range).

**Taken two weeks after herbicide treatment.

TABLE VIII

EFFECT OF POTASSIUM GIBBERELLATE SOAK ON THE PERCENTAGE EMERGENCE AND REGERMINATION OF MOTHER TUBERS

Potassium Gibberellate (ppm)	Emergence* (%)	Regermination* (%)
1. 25	58.8b	83.5a
2. 50	63.8b	78.0a
3. 100	75.8a	84.0a

*Values followed by the same letter in the same vertical column do not differ at the .05 level (Duncan's Multiple Range).

TABLE IX

EFFECT OF SOIL-APPLIED HERBICIDES ON THE PERCENTAGE EMERGENCE AND REGERMINATION OF MOTHER TUBERS

Herbicide	Rate lb./A	Emergence* (%)	Regermination* (%)
1. None	--	96.3a	85.8a
2. EPTC	8	75.0b	80.8a
3. Vernolate	6	83.8b	77.0a
4. Dichlobenil	10	38.8d	75.0a
5. Alachlor	4	36.7d	87.5a

*Values followed by the same letter in the same vertical column do not differ at the .05 level (Duncan's Multiple Range).

II. PRIMARY EXPERIMENT

Scarification, Potassium Gibberellate, and Herbicide Treatment

There was a delay in the germination of scarified tubers (Figure 1). This appeared to be related to injury of the growing points because microsections showed evidence of dead cells in the very young shoots. No evidence of dead tissue in the non-scarified shoots was found.

Secondary experiments indicated that germination of scarified tubers was delayed when allowed to soak in water from 8 to 24 hours. The delay in germination did not appear to be related to water inhibition since the weight of the tubers did not change during the soaking period.

Although there was a delay in germination of the scarified tubers, the growth of shoots appeared to overcome this effect rapidly because none of the growth characteristics (Table X) and carbohydrate content of the mother tuber (Table XI) were not affected by scarification. Therefore, it appears that scarification did not improve herbicidal effectiveness in controlling nutgrass.

In general, there was an increase in total nutgrass growth with the sampling period (Table XII). However, only the percentage of free glucose decreased with sampling date (Table XIII). This would indicate either a rapid utilization of free glucose or a rapid conversion to storage carbohydrates.

Table XIV summarizes the effect of potassium gibberellate and herbicide treatment on plant height, fresh and dry weight, and total air dry root weight of nutgrass. Plants from tubers which had received

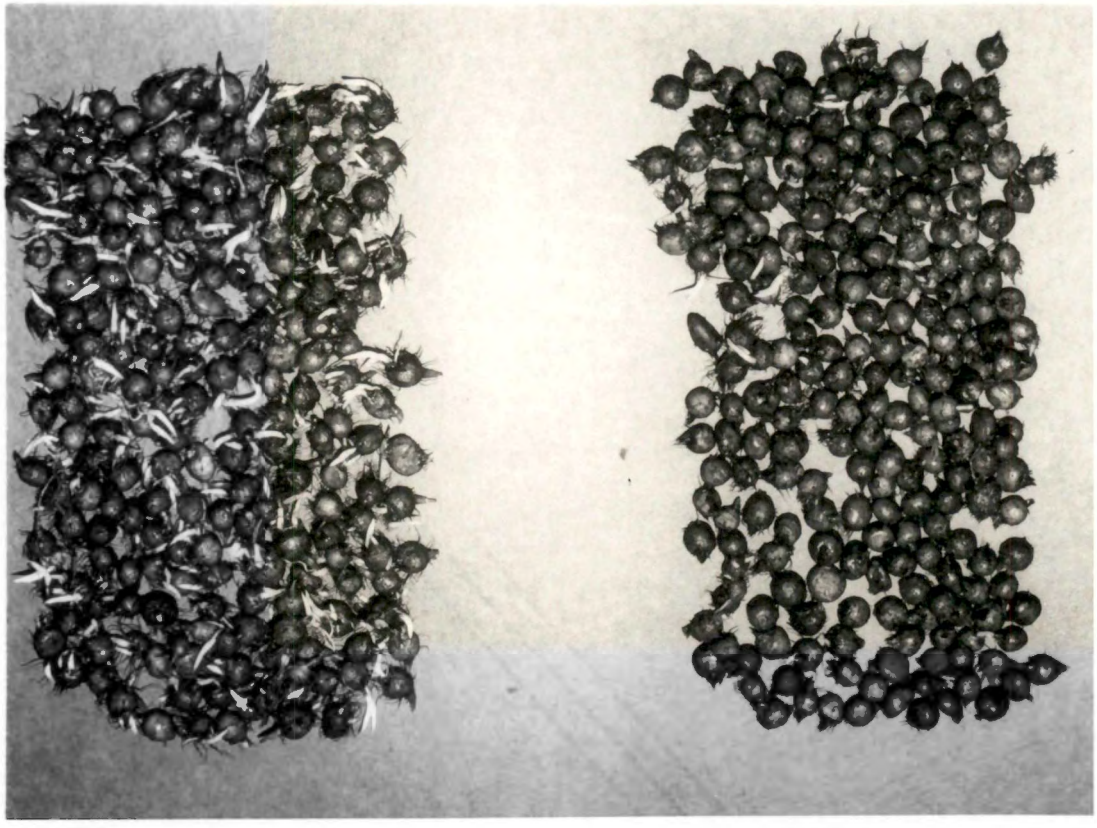


Figure 1. Germination of scarified and non-scarified tubers three days after scarification; non-scarified tubers on left, scarified tubers on right.

TABLE X

EFFECT OF SCARIFICATION ON PLANT HEIGHT, FRESH AND DRY WEIGHT, AND AIR DRY ROOT WEIGHT OF NUTGRASS*

Treatment	FOLIAGE CHARACTERISTICS						Air dry root wt.(mg.) produced per mother tuber	
	Height (inches)		Fresh wt.(mg.) produced per mother tuber		Dry wt.(mg.) produced per mother tuber			
	Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2
1. Scarification	7.1a	7.3a	585a	451a	126a	105a	213a	216a
2. Non-scarification	7.1a	7.1a	580a	514b	130a	128b	232a	255b

*Values followed by the same letter in the same vertical column do not differ at the .05 level (Duncan's Multiple Range).

TABLE XI

EFFECT OF SCARIFICATION ON CARBOHYDRATE CONTENT OF NUTGRASS TUBERS*

Treatment	Free Glucose (%)		Starch (%)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
1. Scarification	2.5a	3.1a	21.4a	22.8a
2. Non-scarification	3.2a	2.7a	22.9a	25.0a

*Values followed by the same letter in the same vertical column do not differ at the .05 level (Duncan's Multiple Range).

TABLE XII

CHANGE IN PLANT HEIGHT, FRESH AND DRY WEIGHT, AND AIR DRY ROOT WEIGHT WITH SAMPLING DATE*

Sampling Date	FOLIAGE CHARACTERISTICS						Air dry root wt.(mg.) produced per mother tuber	
	Height (inches)		Fresh wt.(mg.) produced per mother tuber		Dry wt.(mg.) produced per mother tuber			
	Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2
1. 1 week post-treatment	5.7b	5.5b	525b	417b	74b	66b	171c	145c
2. 5 weeks post-treatment	7.9a	7.7a	636a	614a	146a	140a	201b	239b
3. 9 weeks post-treatment	7.7a	8.5a	436c	417b	148a	143a	327a	353a

*Values followed by the same letter in the same vertical column do not differ at the .05 level (Duncan's Multiple Range).

TABLE XIII

CHANGE IN CARBOHYDRATE CONTENT OF NUTGRASS TUBERS ASSOCIATED WITH SAMPLING DATE*

Sampling Date	Free Glucose (%)		Starch (%)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
1. 1 week post-treatment	3.4a	3.2a	22.9a	22.0a
2. 5 weeks post-treatment	3.7a	3.6a	21.6a	24.6a
3. 9 weeks post-treatment	1.4b	1.9b	21.9a	25.2a

*Values followed by the same letter in the same vertical column do not differ at the .05 level (Duncan's Multiple Range).

TABLE XIV

EFFECT OF POTASSIUM GIBBERELLATE AND HERBICIDE TREATMENT ON PLANT HEIGHT, FRESH AND DRY WEIGHT, AND AIR DRY ROOT WEIGHT OF NUTGRASS*,**

TREATMENTS		Rate	FOLIAGE CHARACTERISTICS						Air dry root wt.(mg.) produced per mother tuber	
			Height (inches)		Fresh wt.(mg.) produced per mother tuber		Dry wt.(mg.) produced per mother tuber			
Potassium Gibberellate	Herbicides	(lb./A)	Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2
1. None	None	--	6.9bc	7.4b	744ab	671b	154b	158ab	373a	454a
2. 100 ppm tuber soak	None	--	7.3bc	7.4bc	627ab	624bc	146b	156ab	425a	358a
3. 100 ppm tuber soak	EPTC, pre-emergence	8	0.2d	0.7d	8c	15e	6e	4d	35c	39b
4. 100 ppm tuber soak	Alachlor, pre-emergence	4	5.4c	5.2c	346bc	245d	73c	61cd	100bc	121b
5. 100 ppm foliage spray	None	--	10.7a	11.3a	960a	936a	218a	206a	400a	504a
6. 100 ppm foliage spray	Amitrole, foliage spray	2	8.9ab	9.4a	588ab	519bcd	148b	124bc	190b	145b
7. 100 ppm foliage spray	Linuron, foliage spray	6	10.0a	9.2ab	457b	374cd	118bc	108bc	107bc	100b

*Values followed by the same letter in the same vertical column do not differ at the .05 level (Duncan's Multiple Range).

**Values represent an average of all sampling dates.

potassium gibberellate as a 100 ppm soak of the germinating tubers plus soil applications of EPTC were significantly reduced in plant height, fresh and dry weight, and total air dry root weight in both experiments when compared to the non-treated control. Potassium gibberellate as a 100 ppm soak of the germinating tuber plus alachlor treatment showed variability in reducing plant height and fresh weight in each experiment, but these two chemicals significantly reduced plant dry weight and total air dry root weight in both experiments (Table XIV). However, the tuber soak of potassium gibberellate did not result in significant changes in nutgrass growth (Table XIV) or carbohydrate content of the mother tuber (Table XV).

Very little nutgrass growth occurred throughout the experiment in plots which had received EPTC applications (Figure 2). Nutgrass growth did not occur in the alachlor treated plots for about four weeks after treatment. After this time, alachlor appeared to lose its effectiveness and growth occurred rapidly. In fact, the growth occurring four weeks after treatment and continuing throughout the experiment accounted for the major growth evaluations summarized in Table XIV for the alachlor treated tubers.

Foliar applications of potassium gibberellate alone significantly increased foliage dry weight and total air dry root weight above the combined potassium gibberellate and amitrole or linuron treatments (Table XIV). Consistent reductions in total air dry root weight occurred in the combined potassium gibberellate and amitrole or linuron treatments when compared to the non-treated control. However, these effects were not associated with accelerated glucose utilization or starch hydrolysis by

TABLE XV

EFFECT OF POTASSIUM GIBBERELLATE AND HERBICIDE TREATMENT ON CARBOHYDRATE CONTENT OF NUTGRASS TUBERS*

Potassium Gibberellate	Herbicides	Rate (lb./A)	Free glucose (%)		Starch (%)	
			Exp.1	Exp.2	Exp.1	Exp.2
1. None	None	--	3.1ab	3.4ab	20.8bc	23.6ab
2. 100 ppm tuber soak	None	--	3.7a	3.6a	24.7b	27.1ab
3. 100 ppm tuber soak	EPTC, pre-emergence	8	3.2ab	2.3ab	35.7a	30.2ab
4. 100 ppm tuber soak	Alachlor, pre-emergence	4	2.4ab	2.7ab	29.1a	31.3a
5. 100 ppm foliage spray	None	--	3.2ab	2.8ab	20.5bcd	24.5ab
6. 100 ppm foliage spray	Amitrole, foliage spray	2	2.2b	3.2ab	15.1cd	16.3bc
7. 100 ppm foliage	Linuron, foliage	6	2.4ab	2.2b	12.4d	14.4c

*Values followed by the same letter in the same vertical column do not differ at the .05 level (Duncan's Multiple Range).

Figure 2. Nutgrass growth in potassium gibberellate and herbicide treated plots ten weeks from treatment.

foliar applications of potassium gibberellate because they were not reduced by potassium gibberellate treatment (Table XV).

Nutgrass receiving foliar applications of potassium gibberellate, amitrole, or linuron was in a very vigorous state of growth at the time of application. About four days after application, the amitrole treated plants began to show chlorosis at the base of the leaf. The chlorosis continued to move toward the leaf tip until the leaf was killed. Linuron treated plants also showed considerable chlorosis or death at the termination of the experiment. In fact, linuron appeared to be more rapid in its phytotoxic effects than did amitrole. However, amitrole and linuron did not differ in their total effects on nutgrass growth (Table XIV, page 40).

New tubers had not developed in any of the treatments at one-week from application, but were present in all plots except the EPTC treated plots at five-weeks from application. They did not develop in the EPTC treated plots during the entire nine-week period. However, mother tubers in these plots appeared very viable at the termination of the experiment. Although EPTC apparently inhibits new tuber development, it does not appear to kill the mother tuber in nine weeks of direct soil contact.

As foliage growth occurred in the alachlor treated plots, the percentage of root growth measured as new tuber development increased rapidly (Table XVI). Although alachlor prevented foliage growth for about four weeks, it did not appear to upset the photosynthetic capability of new leaves once growth occurred. This conclusion is based on the fact that new tuber development (Table XVI) increased rapidly after foliage growth occurred.

TABLE XVI

EFFECT OF POTASSIUM GIBBERELLATE AND HERBICIDE TREATMENT ON THE PERCENTAGE OF UNDERGROUND GROWTH DUE TO NEW TUBERS

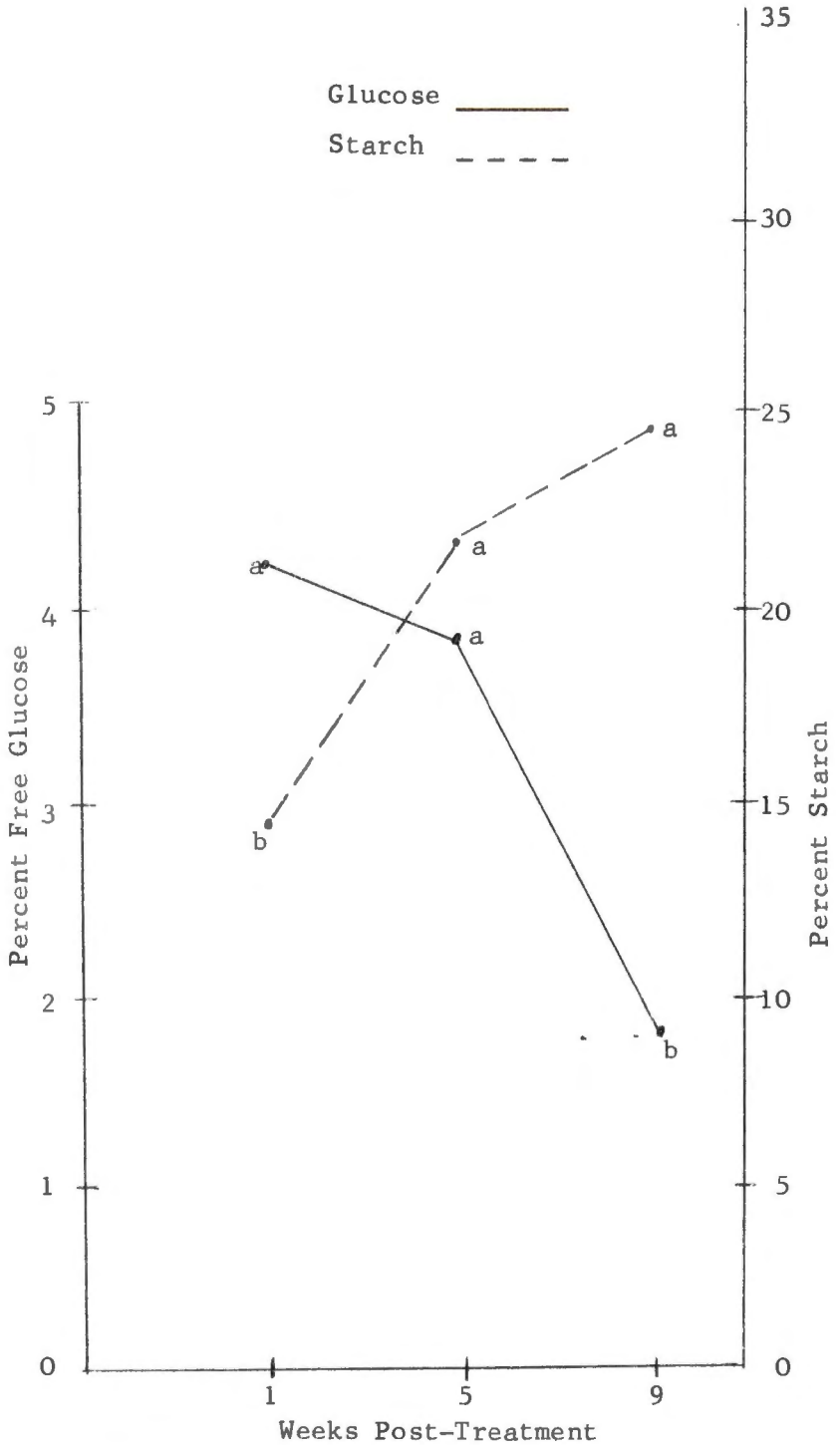
Potassium Gibberellate	Herbicides	Rate (lb./A)	PERCENTAGE OF NEW TUBERS* Weeks post-treatment					
			1		5		9	
			Exp.1	Exp.2	Exp.1	Exp.2	Exp.1	Exp.2
1. None	None	--	0.0	0.0	72.4	77.1	77.9	89.3
2. 100 ppm tuber soak	None	--	0.0	0.0	72.1	75.8	82.1	81.7
3. 100 ppm tuber soak	EPTC, pre-emergence	8	0.0	0.0	0.0	0.0	0.0	0.0
4. 100 ppm tuber soak	Alachlor, pre-emergence	4	0.0	0.0	35.1	31.8	41.7	59.2
5. 100 ppm foliage spray	None	--	0.0	0.0	76.9	75.8	82.4	71.8
6. 100 ppm foliage spray	Amitrole, foliage spray	2	0.0	0.0	73.9	49.8	74.5	62.2
7. 100 ppm foliage spray	Linuron, foliage spray	6	0.0	0.0	58.7	49.0	76.6	78.9

*Values represent an average of two samples per treatment.

Foliar applications of amitrole or linuron may reduce the total root growth of one and one-half week old nutgrass (Table XIV, page 40), but the percentage of growth as measured by new tubers is not reduced (Table XVI). This is probably due to the early capability of nutgrass to convert sugar to storage carbohydrate if the plants can undergo photosynthesis for one and one-half weeks before herbicide treatment. However, the new tubers did not appear to be at the same stage of maturity at the end of nine weeks as did the non-treated tubers. As long as there is incomplete kill of nutgrass foliage after herbicide treatment, the potential for new tubers to reach reproductive potential remains high. Therefore, it appears necessary to make one or two repeated applications of amitrole or linuron to nutgrass foliage to prevent the chances of nutgrass reinfestation.

The results with amitrole are in agreement with those of Hill (21). If the idea that an incomplete foliage kill of nutgrass allows the potential for new tuber development to remain high is correct, it may offer some explanation as to why Hauser (17) did not obtain good nutgrass control with post-emergence applications of amitrole.

In non-treated tubers, a high percentage of free glucose was available for utilization in the early stages of growth (Figure 3). Free glucose in mother tubers decreased over the entire experiment, but it was associated with a corresponding increase in starch content (Figure 3). One week after herbicide treatment, an average of 4.1 percent of free glucose was present in the non-treated tubers while an average of 1.75 percent was present at the termination of the experiment.



NOTE: Values followed by the same letter on the same line do not differ at the .05 level (Duncan's Multiple Range). Values are an average of the two experiments.

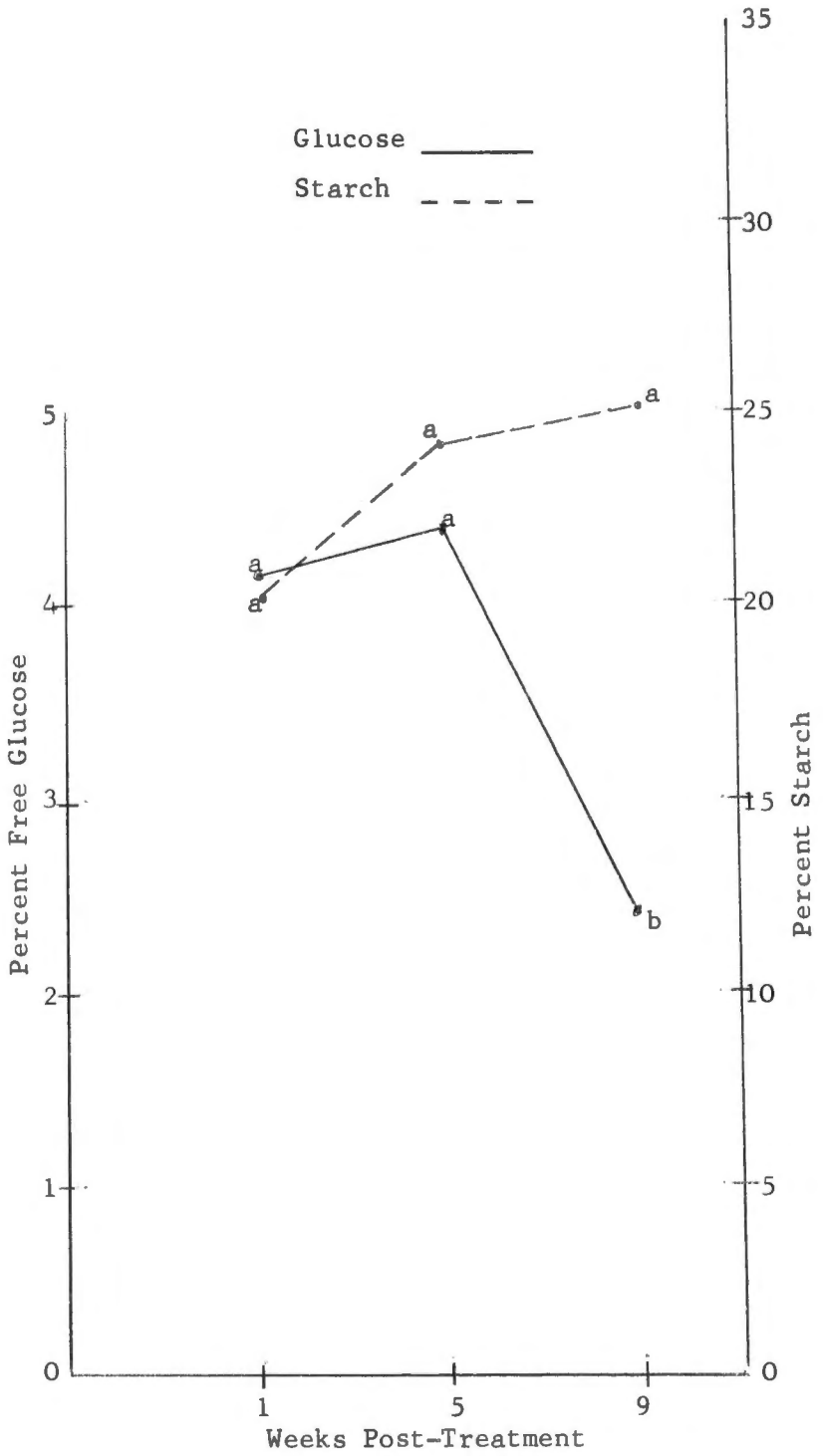
Figure 3. Free glucose and starch changes in non-treated mother tubers.

Starch content in the mother tubers was in close agreement with those of Duple and Holt (9) who report a range of 10 to 28 percent in purple nutgrass tubers. In this study, starch content in the non-treated tubers ranged from 14 percent one week after treatment to 24.5 percent at the termination of the experiment (Figure 3).

These data suggest that healthy nutgrass can convert photosynthetic products to storage carbohydrate rapidly. The rapid conversion into starch probably offers some explanation for the very rapid growth of new tubers (Table XVI, page 45) and for the difficulty in controlling nutgrass.

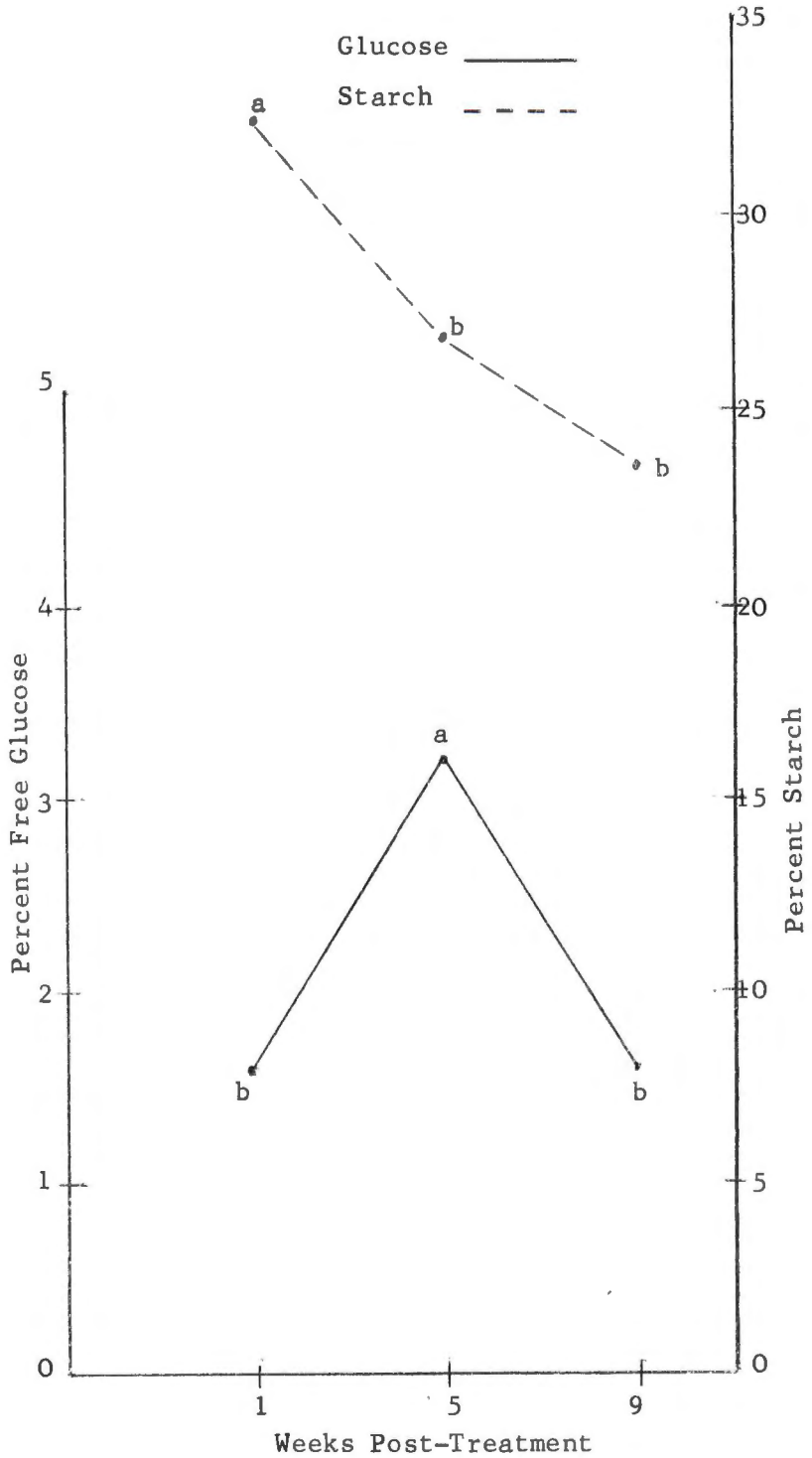
The potassium gibberellate soak of germinating nutgrass tubers shows a response in free glucose and starch content (Figure 4) like the non-treated tubers (Figure 3). The two treatments did not differ from each other in effecting glucose content at any time throughout the experiment. Although the starch content in the tubers soaked with 100 ppm of potassium gibberellate appeared higher at one week from treatment than in the non-treated tubers (Figures 3 and 4), it was not significant with sampling date (Table XIII, page 39). Therefore, potassium gibberellate applied as a tuber soak does not cause a rapid utilization of glucose or a rapid starch hydrolysis in the nutgrass tuber. Thus, potassium gibberellate did not appear to contribute to the effectiveness of soil-applied herbicides.

Tubers receiving soil applications of either EPTC or alachlor showed very similar responses in free glucose and starch content (Figures 5 and 6). These treatments appeared to delay the availability of free glucose (Figures 3, 5, and 6). However, free glucose content of tubers



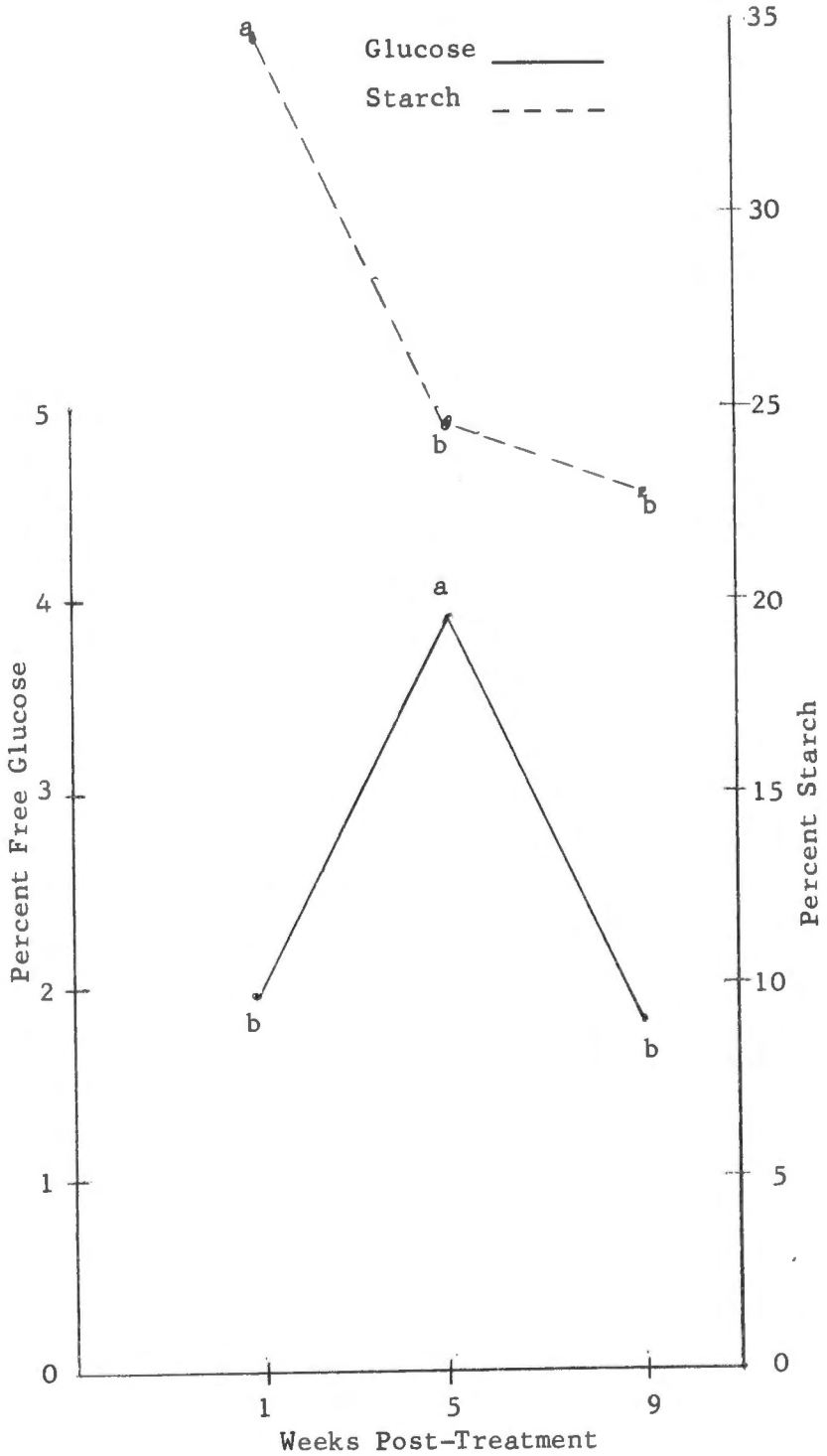
NOTE: Values followed by the same letter on the same line do not differ at the .05 level (Duncan's Multiple Range). Values are an average of the two experiments.

Figure 4. Free glucose and starch changes in potassium gibberellate treated mother tubers.



NOTE: Values followed by the same letter on the same line do not differ at the .05 level (Duncan's Multiple Range). Values are an average of the two experiments.

Figure 5. Effect of potassium gibberellate and EPTC on free glucose and starch changes of mother tubers.



NOTE: Values followed by the same letter on the same line do not differ at the .05 level (Duncan's Multiple Range). Values are an average of the two experiments.

Figure 6. Effect of potassium gibberellate and alachlor on free glucose and starch changes of mother tubers.

in these treatments differed from that of the non-treated control only at the one-week post-treatment period. No attempt is made to explain this response because the two herbicides are not chemically related, their mode of action is different, and tuber respiration would probably be different because plant growth in these two treatments differed over the nine-week period.

There appeared to be a delay in free glucose availability in the tubers treated with EPTC or alachlor (Figures 3, 5, and 6, pages 47, 50, and 51, respectively). However, the delay does not appear to be related to an inhibition of the amylase enzyme system because the presence of separate additions of each herbicide to the incubation medium did not reduce the conversion of starch to glucose in the secondary experiments (Table XVII). Bonner and Varner (6) point out that maltase is also active in the enzyme system responsible for the starch to glucose conversion. Therefore, it is possible that these herbicides are more active on the maltase system than on the amylase system.

The starch content in the EPTC and alachlor treated tubers was significantly greater than that of the non-treated tubers at the one-week post-treatment period (Figures 3, 5, and 6). Iodine stained micro-sections also revealed a high starch content in the EPTC tubers at about three weeks after treatment. There was a continuous decline in starch content of the EPTC and alachlor treated tubers (Figures 5 and 6) as opposed to a continual increase in the non-treated tubers (Figure 3). This would suggest that hydrolytic enzymes may have still been active in the herbicide treated tubers even though very little new growth resulted.

TABLE XVII

EFFECT OF EPTC AND ALACHLOR ON CLARASE 900 ACTIVITY IN NUTGRASS TUBER TISSUE

Herbicide (ppm)	Percent Glucose*	
	EPTC	Alachlor
0	38.4a	35.8a
2	30.0a	38.0a
4	29.6a	34.6a
6	32.1a	32.1a
8	31.1a	37.9a
10	29.2a	33.1a

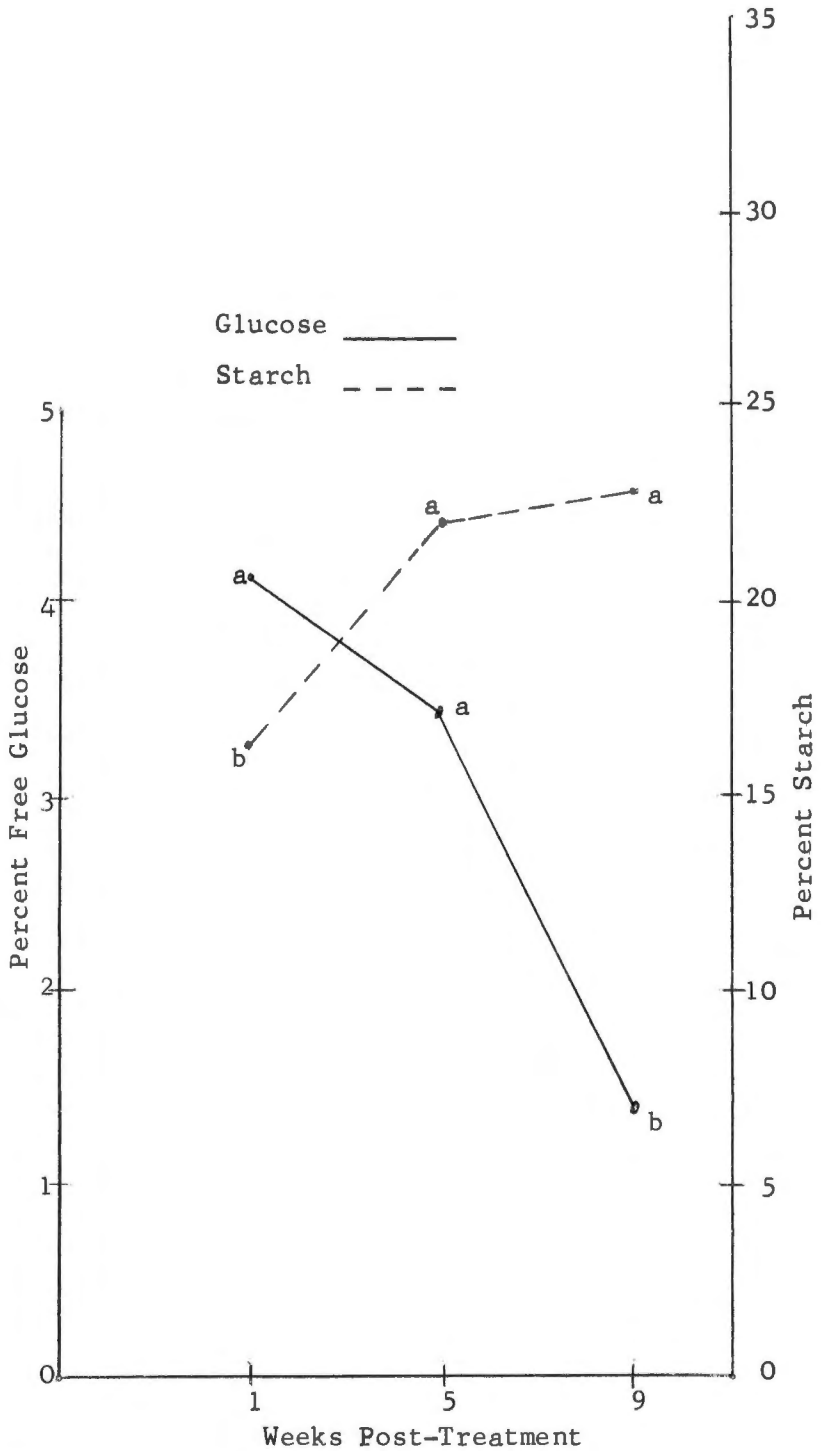
*Values followed by the same letter in the same vertical column do not differ at the .05 level (Duncan's Multiple Range).

Hastings and Kust (15) report that carbohydrate content in yellow rocket (Barbarea vulgaris) roots increase in the fall and decrease in the spring and summer. Perhaps further study with EPTC and alachlor is needed to determine if repeated applications would eventually deplete the nutgrass tuber of storage material to the point that it would not regerminate.

The apparent slowdown in starch hydrolysis in alachlor treated tubers (Figure 6, page 51) at five-weeks post-treatment was probably associated with the foliage growth occurring at that time. New foliage growth would probably initiate a sugar to starch reaction resulting in a change of starch content. However, these tubers appeared to have lost their capability to readily initiate a buildup of starch in mother tubers because starch had not shown a complete reversal at the termination of the experiment. Although the mother tubers had not begun to show an accumulation of starch at the termination of the experiment (Figure 6), this effect does not appear to alter the capability of new tuber formation (Table XVI, page 45).

At the termination of the experiment, there was no difference in starch content between the EPTC or alachlor treated tubers and the non-treated control (Figures 3, 5, and 6, pages 47, 50, and 51, respectively). Therefore, sufficient starch appeared to be available in the chemically treated tubers after nine weeks to furnish any glucose needed for growth when placed under optimum growing conditions.

The effect of foliar applications of 100 ppm potassium gibberellate on free glucose and starch content of mother tubers is illustrated in Figure 7. In general, both components followed the same trend as the



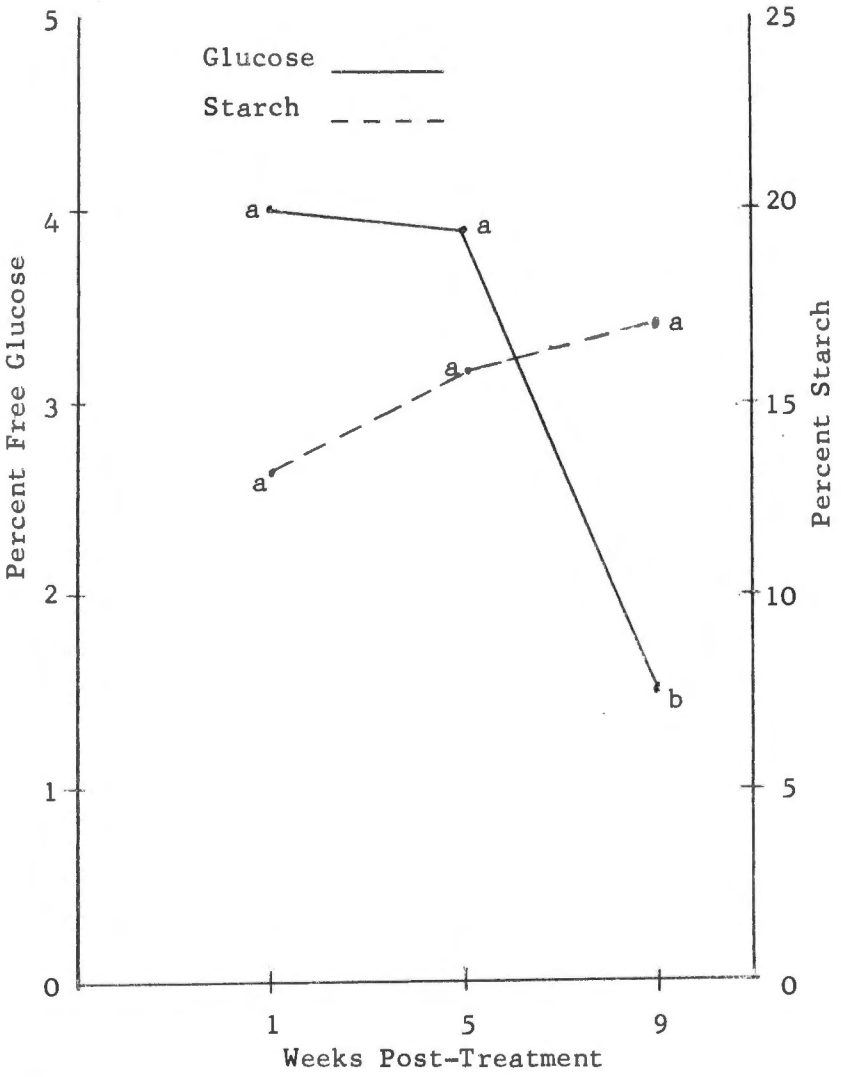
NOTE: Values followed by the same letter on the same line do not differ at the .05 level (Duncan's Multiple Range). Values are an average of the two experiments.

Figure 7. Effect of foliar-applied potassium gibberellate on free glucose and starch changes in mother tubers.

non-treated control (Figure 3, page 47). Neither free glucose nor starch content differed from that in the control at any of the sampling dates. The rate of starch increase declined after five-weeks post-treatment to the extent that it did not differ at the nine-week post-treatment period. Foliage browning began to occur at about four weeks post-treatment. This is probably related to the high amount of root growth occurring in these plots at four weeks from treatment. Therefore, the competition for nutrients was great and this led to foliage necrosis.

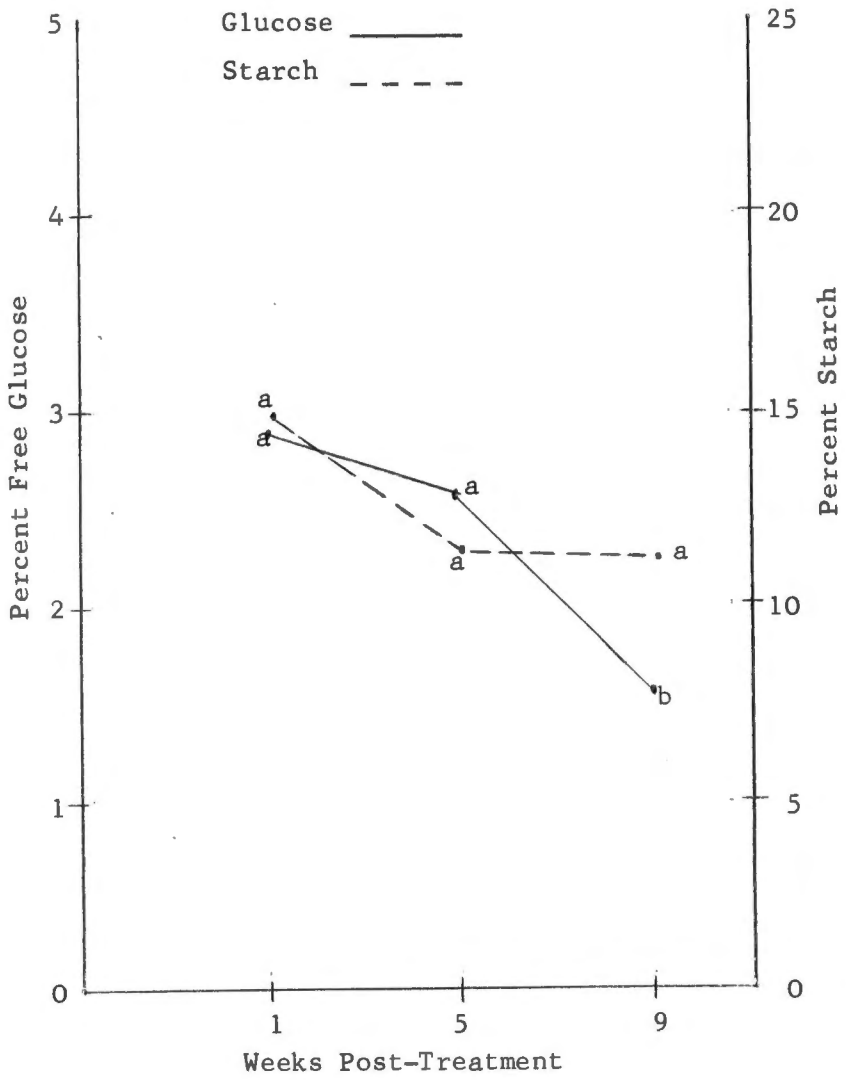
The effect of potassium gibberellate and amitrole treatment on free glucose and starch changes in mother tubers is illustrated in Figure 8. In general, there was a decrease in free glucose throughout the experiment. However, the initial and final starch content in the amitrole treated tubers did not differ significantly. The final starch content in these tubers was significantly less than that of the non-treated control (Figure 3, page 47, and 8).

The amounts of free glucose and starch as effected by foliar applications of potassium gibberellate and linuron in nutgrass tubers is illustrated in Figure 9. A decrease in free glucose with sampling date is observable. Consistent differences did not occur between the initial and final starch content in these tubers. The final starch content in the linuron treated tubers was significantly lower than in the non-treated tubers (Figures 3 and 9).



NOTE: Values followed by the same letter on the same line do not differ at the .05 level (Duncan's Multiple Range). Values are an average of the two experiments.

Figure 8. Effect of foliar applications of potassium gibberellate and amitrole on free glucose and starch changes of mother tubers.



NOTE: Values followed by the same letter on the same line do not differ at the .05 level (Duncan's Multiple Range). Values are an average of the two experiments.

Figure 9. Effect of foliar applications of potassium gibberellate and linuron on free glucose and starch changes in mother tubers.

CHAPTER V

SUMMARY

This study was initiated in July, 1969, to determine if tuber scarification or potassium gibberellate treatment improved the herbicidal effectiveness in killing nutgrass.

The study was conducted in three phases. It included a preliminary screening phase, a primary experiment, and two secondary experiments. In the preliminary screening phase, 16 soil- and foliar-applied herbicides were evaluated for their effects on killing nutgrass. From this study, the chemical treatments were selected for evaluation in the primary experiment.

The secondary experiments were initiated in an attempt to explain why scarification delayed germination and why there was a delay of free glucose availability in EPTC and alachlor treated tubers in the primary experiment. The results of the secondary experiments are summarized in conjunction with the results of the primary experiment.

The experimental design of the primary experiment was a split plot with three replicates of one flat each. The whole units included either scarified or non-scarified tubers. The subunits were as follows.

1. Non-treated tubers.
2. One hundred ppm potassium gibberellate soak of germinating tubers.
3. One hundred ppm potassium gibberellate soak of germinating tubers subjected to an 8 lb./A. application of EPTC.

4. One hundred ppm potassium gibberellate soak of germinating tubers subjected to a 4 lb./A. soil application of alachlor.
5. One hundred ppm foliar application of potassium gibberellate.
6. One hundred ppm foliar application of potassium gibberellate followed by 2 lb./A. of amitrole.
7. One hundred ppm foliar application of potassium gibberellate followed by 6 lb./A. of linuron.

The entire experiment was conducted in duplicate in the horticulture research greenhouse at The University of Tennessee in Knoxville during the summer and fall of 1969.

Plant height, fresh and dry weight, total air dry root weight, and free glucose and starch content of the mother tuber were determined at one, five, and nine weeks after herbicide treatment.

Tuber scarification delayed germination, but this appeared to be partially related to physical injury of the growing point of the tuber and to length of exposure in water. Water inhibition did not appear to play a part in this effect. Scarification did not appear to improve the effectiveness of either a soil- or foliar-applied herbicide in killing nutgrass.

A 100 ppm potassium gibberellate soak of germinating tubers did not alter nutgrass growth or mother tuber carbohydrate content after nine-weeks of growth when compared to the non-treated control. The 100 ppm foliar spray of potassium gibberellate increased plant height, but this effect was not associated with a corresponding change in carbohydrate content of the mother tuber. Therefore, neither a tuber soak nor a foliar application of 100 ppm potassium gibberellate would seem to

improve the herbicidal effectiveness in killing nutgrass since it did not contribute to a rapid hydrolysis of reserve carbohydrate in the mother tuber.

The soil application of 8 lb./A. of EPTC completely inhibited foliage and root growth of nutgrass for nine-weeks post-treatment. There was a delay in the availability of free glucose in EPTC treated tubers. However, this delay did not appear to be due to an inhibition of the amylase enzyme system responsible for the starch to glucose conversion. Starch content of the mother tubers from the EPTC treatments remained at a very high content even though the results indicate a decrease in the reserve starch over the nine-week period. The interior of the tuber tissue was very solid and white, indicating that the tuber had not been killed by a nine-week soil exposure to EPTC.

The 4 lb./A. soil application of alachlor inhibited nutgrass foliage and root growth for about four weeks after treatment. After that time, the chemical seemed to lose its effectiveness and nutgrass growth occurred rapidly. Free glucose and starch content of the mother tubers followed a similar pattern to that of the EPTC treated tubers. A delay in glucose availability in the alachlor treated tubers did not appear to be due to an inhibition of the amylase enzyme system by the herbicide.

One foliar application of 2 lb./A. of amitrole or 6 lb./A. of linuron was effective in reducing nutgrass shoot growth, total air dry root weight, and greatly reduced or completely inhibited starch formation in the mother tuber. Linuron appeared to be more rapid in killing the

foliage than did amitrole. However, one application of these compounds to one and one-half week-old foliage did not result in complete kill of the foliage within nine weeks. Therefore, one or two repeat applications of amitrole or linuron appear necessary to obtain complete foliage kill and reduce the possibility of new tuber maturation and nutgrass reinfestation.

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VITA

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Upon graduation from the University, he taught vocational agriculture for one year at Marshall, Virginia, and for one year at Sweetwater, Tennessee. In 1964, he entered graduate school at The University of Tennessee and received the Master of Science degree with a major in Horticulture in 1967. In 1970, he was granted the Doctor of Philosophy degree from The University of Tennessee with a major in Agriculture Plant and Soil Science. During both of the advanced degrees, he was employed by The University of Tennessee Agriculture Experiment Station as a Research Assistant in Horticulture.

He is married to the former Doris Lynn Dickey of Sweetwater, Tennessee.