# A STUDY OF MUGWORT: I. GROWTH HABITS AND CONTROL II. EFFECTS OF 2,3,6-TRICHLOROPHENYLACETIC ACID ON CERTAIN RESPIRATORY ENZYMES

by

ASA BENJAMIN ROGERSON

Thesis submitted to the Graduate Faculty of the

Virginia Polytechnic Institute

in candidacy for the degree of

MASTER OF SCIENCE

in

Plant Physiology

September, 1964

Blacksburg, Virginia

TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF FIGURES

ACKNOWLEDGEMENTS

The author wishes to express sincere appreciation to all persons who helped during this study. He would like to express special thanks to Dr. W. E. Chappell for the opportunity to do graduate work and to Dr. S. W. Bingham for his help and guidance throughout the study and preparation of this thesis.

The author is grateful to H. L. Witt who helped with this study and who was always ready to help in any way.

Thanks is also due for the materials furnished by Amchem Products, Inc., Diamond Alkali Company, Dow Chemical Company, and Velsicol Chemical Corporation.

# INTRODUCTION

At various times, certain plants become very troublesome in that their presence is a nuisance in the area in which they are located. This situation eventually leads to programs for evaluating control measures. Before a plant species can be attacked from the control standpoint, something must be known of its distribution and general growth characteristics.

Artemisia vulgaris L. (mugwort) is a perennial plant which has become a problem along the eastern seacoast, especially among woody ornamental nursery crops. Mugwort has a very dense rhizome system that is concentrated near the surface of untilled soil and becomes a competitor with other plants in these areas.

Early work on chemical control of mugwort was done by Pridham (1954). A control program involving mugwort was begun at VPI in 1961, and this study was a part of that program.

The first objective was to determine the optimum sized mugwort rhizome section that would produce uniform plants for later studies. Also sections were cut from different positions along a rhizome to determine if there was any difference in viability of the younger and older axillary buds.

The second objective was to observe mugwort throughout a growing season, noting growth patterns, amount of spread and total production in a certain area.

A third objective involved chemical methods of control of mugwort as several herbicides were applied at different dates to an infested area near Fredericksburg, Virginia. The herbicide used in laboratory and greenhouse studies was 2,3,6-trichlorophenylacetic acid (fenac) at various concentrations. Rhizomes were planted in soil-fenac mixtures to determine rates that would keep the rhizome buds from producing new plants. Fenac-nutrient solution mixtures were used to determine the level of fenac that was necessary to keep the rhizome buds from initiating new shoots.

The fourth objective was to determine the effects of two rates of fenac on respiration and some respiratory enzymes. Various enzyme inhibitors were used in the manometric studies to help determine which respiratory enzymes were functional in mugwort plants.

# LITERATURE REVIEW

## Control of mugwort

Pridham (1954) did some of the first chemical control research on mugwort in which he evaluated 4,6-dinitro-o-sec-butylphenol (DNBP), isopropyl N-(3-chlorophenyl)carbamate (CIPC), 3,6-endoxohexahydrophthalic acid (endothal), and 3-(4-chlorophenyl)-1,1-dimethylurea (monuron). Results showed a decline of mugwort rhizomes, but it was suggested that a series of application would be needed for control.

When nursery stock remains on one location for a period of years, the area may become infested with mugwort. Pridham (1957) used 3-amino-1,2,4-triazole (amitrole) alone or in mixtures with a residual herbicide in the summer and fall to reduce the population of mugwort rhizomes. It was observed that much of the shoot regrowth was dwarfed and white or pink in color.

Bing and Pridham (1963) stated that some nurseries kept mugwort under control by frequent cultivation and hand weeding in dry weather, although most nurseries find it impossible to control by mechanical means. These authors recommend rototilling ethyl N,N-di-n-propylthiolcarbamate (EPTC) into the soil or spraying with 2(2,4,5-trichlorophenoxy)propionic acid (silvex). Granular 2,3,6-trichlorophenylacetic acid (fenac) or spray applications of fenac, that were applied in May, caused severe distortion of foliage but no apparent control at 4 and 8 lb/A.

Further work with EPTC by Bing and Pridham (1964), showed that increased incorporation of the chemical into the soil increased the control of mugwort. They suggest that after treating an area with EPTC at 15 lb/A, at least a two week waiting period is needed before planting another crop.

Bingham (1963) conducted a series of control tests on mugwort. Fenac and 2-methoxy-3,6-dichlorobenzoic acid (dicamba) were most effective when applied in the early stages of vegetative growth. Later in the season, fenac gave better control than dicamba. N,oleoyl-1,3-propylene diamine salt of 2,4-dichlorophenoxy-acetic acid (2,4-D, OPDS) and butoxyethanol ester of 2,4-dichlorophenoxy-acetic acid (2,4-D ester) plus butoxyethanol ester of 2,4,5-trichloro-phenoxyacetic acid (2,4,5-T) gave good initial control of shoots but rhizomes were not controlled. After two months, shoot growth was again vigorous. When mugwort was mowed and dicamba and 2,4-D were applied alone, only fair control was observed. Fenac was more effective when applied to new growth following mowing than when applied to large mugwort plants.

Rabbit and Cook (1964) investigated the control of solid mugwort stands and found the most promising treatment to be silvex in combination with amitrole. EPTC resulted in control provided the chemical was completely incorporated into the soil. Incorportation is almost impossible in solid stands of mugwort, however.

## Rhizome Section Studies

Few references were found in the literature on growth studies of mugwort rhizomes, but several workers have studied Johnsongrass and quackgrass rhizomes.

Vengris (1962) observed the effect of rhizome length and depth of planting on the mechanical and chemical control of quackgrass. It was found that the percentage of buds producing shoots was much greater with one-bud sections than with five-bud sections when planted at a depth of one inch, although plants from the one-bud sections were weaker. By cutting the rhizomes into shorter pieces, buds were encouraged to develop. This was possibly caused by the destruction of apical dominance. When the planting depth of the one-bud sections was increased from one to three inches, the number of new plants was greatly reduced. The author suggests that if quackgrass rhizomes could be mechanically cut into small sections, chemicals would probably result in better control of the weaker plants.

Development of Johnsongrass from rhizomes was observed by McWhorter (1961). Growth from rhizomes originated at the axillary buds. When the new plant had grown for about three weeks, a rhizome spur originated at its base, and new shoots were initiated. Nineteen to twenty-four days after emergence, the old rhizomes had generally disintegrated. Little difference was noted in Johnsongrass development from seeds or rhizomes.

Anderson et al (1960) observed Johnsongrass development and found that seedlings initiated a rhizome system four to five weeks after emergence through the matured seed stage of growth. Rhizomes, which developed during one growing season, overwintered and gave rise to new plants the following year. Old rhizomes did not function further after the new plants arose.

Johnson and Buchholtz (1962) worked with quackgrass rhizome sections to determine the periods of dormancy in the rhizomes. They found that activity of the buds decreased from April through May and during June the buds were quite dormant. They observed that quackgrass rhizomes followed a cycle of decomposition from year to year. Rhizomes that produced the first plants in spring were decomposed by the following year.

Pridham (1963) used mugwort rhizomes sections of two to four inches to produce plants for herbicide screening work. He also used stem cuttings four to six inches in length that would develop roots and rhizomes when placed in a rooting media. Seeds were collected and planted at various times but this method was not satisfactory because of the low germination of mugwort seed.

Rogerson and Bingham (1964) observed new rhizome initiation from mugwort plants about four weeks after transplanting. Between the fourth and sixth weeks of growth, there was rapid increase in rhizomes. Branching of rhizomes began after nine weeks and new plants were rapidly arising between 9 and 15 weeks.

## Effect of Fenac on Plants

Zimmerman and Hitchcock (1937) observed phenylacetic acid to have growth regulating properties. Various forms of phenylacetic acid were worked with through the years, and Pybus et al (1958) reported that fenac had as much effect on peas and wheat as 2,3,6-trichlorobenzoic acid (TBA) or 2,4-dichlorophenoxyacetic acid (2,4-D).

Fenac has gained usage on troublesome annual and perennial broadleaf weeds on highways, ditchbanks, and fencerows. Also this herbicide

is used in specific crops such as sugar cane to control Johnsongrass. Millhollon (1963) states that the carboxyl group appears to be a necessary part of the fenac molecule since similiar compounds without the carboxyl group do not control Johnsongrass in sugar cane.

Wiese and Rea (1961) used fenac in an eradication program of Convolvulus arvensis (field bindweed), Franseria tomentosa (woolyleaf franseria), Hoffmanseggia densiflora (hogpotato), and Sida hederacea (alkali sida), and they found 20 lb/A was as effective or more effective than 800 lb/A sodium chlorate or 60 lb/A fenuron. Fenac appeared to sterilize the soil for a longer period than the substituted benzoic acids.

Dowler et al (1963) observed the effect of soil type on soil incorporated herbicides for witchweed control. Fenac was applied at several rates and incorporated into six soil types which were heavily infested with witchweed. At 2 and 4 lb/A, fenac approached 100 per cent control and did not cause significant crop injury. Although the 8 lb/A gave excellent control, severe injury to corn was observed.

Fenac was applied as soil-incorporated treatments at various times during the fall preceding corn planting and also at planting time in the spring by Sand et al (1964). Excellent control of witchweed resulted from fenac at 8 lb/A, but as noted by Dowler et al (1963), corn injury was severe.

## Terminal Oxidases, Catalase and Peroxidase

Plant oxidases contain a metal group essential to their functioning and are completely specific for their oxidant, which is molecular

oxygen. Four plant enzymes included as terminal oxidases of respiration in higher plants are polyphenol oxidase, cytochrome oxidase, ascorbic acid oxidase, and glycolic acid oxidase. All are widespread in higher plants.

The term "phenol oxidase" is used to include all terminology describing the enzyme which catalyzes the oxidation of mono- and ortho-diphenolic substances. Tyrosinase, polyphenol oxidase, "DOPA oxidase", potato oxidase and catechol oxidase are used generally depending on the substrate supplied or the one giving the greatest oxygen uptake. Little is known of the physical properties of the enzyme except that the highly purified protein has a molecular weight of approximately 100,000. This weight corresponds to a copper content of four atoms of copper per mole (Bonner, 1957).

Lardy (1949) states that phenol oxidase catalyzes the oxidation of orthodihydric phenols to quinones, and subsequent oxidations give rise to melanin. Phenol oxidase substrates can function as electron carriers.

Phenol oxidase was the most active terminal oxidase in corn (Funderburk and Porter, 1961); potatoes (Boswell and Whiting, 1938); and spinach (Bonner and Wildman, 1946).

Szent-Gyorgyi (1930), working with cabbage leaves, reported the oxidation of ascorbic acid by a specific enzyme that he named "hexoxidase." This name was later changed to ascorbic acid oxidase.

In a review by Mapson (1958), he states that enzymes are essential for any reaction between ascorbic acid and oxygen, because within the physiological range of pH, ascorbic acid is not autooxidisable. Enzyme

systems are found in plants that are capable of catalyzing the oxidation of ascorbic acid. These systems are divided into two categories: (a) ones in which the oxidation of ascorbate is secondary to the oxidation of the substrate of the enzyme and, (b) ones in which there is a direct link between ascorbic acid, enzyme, and oxygen. Phenol oxidase, cytochrome oxidase, and peroxidase are included in the first category. In the second category only ascorbic acid oxidase is known, and this enzyme is very substrate specific.

There is conflict in the literature as to the main function of ascorbic acid oxidase. James (1953b) gave evidence that this oxidase is the terminal oxidase in barley plants. He observed a replacement of a cytochrome oxidase system with an ascorbic acid oxidase as roots matured. His work was based on the decline in sensitivity of respiration to carbon monoxide. This indicated a change from an iron catalyzed to a copper catalyzed system. Bendall and Hill (1956) state that a fall in carbon monoxide sensitivity may just indicate a change from a carbon monoxide sensitive system to an insensitive one. Eichenberger and Thimann (1957) found an active ascorbic acid oxidase in pea stem internodes which was unaffected by carbon monoxide. Oxygen uptake occurred at a rate only one-fortieth as great as that for which ascorbic acid oxidase could account. Eighty per cent of oxygen uptake was inhibited by carbon monoxide and was light reversible, so they concluded that cytochrome oxidase was the terminal oxidase, and ascorbic acid oxidase was probably non-functional.

Kiraly and Farkas (1957) support cytochrome oxidase as the terminal oxidase in healthy wheat plants. When wheat leaves became

infected with stem rust, increased respiration was noted along with an increase in ascorbic acid oxidase activity. They suggested that ascorbic acid oxidase may be present in an inactive state in healthy plants and only becomes operative in infected plants. This oxidase could possibly act as the terminal oxidase of the parasitically stimulated respiration.

Mapson (1958) states that it is uncertain whether ascorbic acid oxidase ever functions as a terminal oxidase, but at some time an environmental or pathological condition could result in respiration via ascorbic acid oxidase. Ascorbic acid oxidase was reported as the most active terminal oxidase in cotton (Bingham and Porter, 1961) and barley (James, 1953b).

In recent years, cytochrome oxidase has been considered by various workers to be the most important terminal oxidase in higher plants. Keilin (1925) was one of the first workers with the cytochromes. Cytochrome oxidase is almost universal in higher plant tissues, and the probable importance of this enzyme system is in acting as a link which connects the cytochromes to other respiratory intermediates (Beevers, 1961). Webster (1952) worked with 54 species representing 23 families of dicotyledonous plants, and over 90 per cent of the species contained a cytochrome oxidase system.

Various inhibitors have been used to distinguish between the terminal oxidase systems. An important method of distinguishing a metal-containing oxidase is cyanide and azide inhibition (Beevers, 1961). This system has met opposition by the fact that when respiration is inhibited in young plant tissue, older tissue may not be inhibited

and actually stimulated in some cases. In a review of inhibitors, James (1953a) emphasizes the importance of using the correct inhibitor concentrations in order not to inhibit a complete system. Carbon monoxide has been used to distinguish between the terminal oxidases containing iron and copper. At a carbon monoxide to oxygen ratio of 19 to 1, respiratory activity is inhibited in the dark. When light is applied, the iron carbonyls are decomposed and copper carbonyls are light-stable. Therefore, in a cytochrome system, oxygen uptake would begin when light is applied, but the phenol oxidase system would not be affected. Sodium diethyldithiocarbamate chelates copper enzymes, but hardly affects cytochrome oxidase at 0.2 mM (Albert and Gledhill, 1949).

Selective inhibitors are important tools for elucidating the mechanism of respiration, but it is very important not to accept a limited series of results at face value (James, 1953a).

Catalase, a metalloprotein enzyme with hematin as its prosthetic group, catalyzes the decomposition of hydrogen peroxide to water and molecular oxygen (Lardy, 1949). Sumner and Dounce, (1937) were the first workers to crystallize catalase. All catalases thus far isolated have been found to contain four hematin groups in a protein with a molecular weight of approximately 240,000 (Nicholls and Schonbaum, 1963).

The specificity of the catalase reaction depends upon the protein moiety and how this is attached to the hematin groups (Lardy, 1949). Keilin and Hartree (1945) suggested that hydrogen peroxide decomposition by catalase involved a change in valence of the catalase iron and an oxidation of the ferrous catalase by molecular oxygen.

In earlier work by Sumner (1941), he postulated no change in valence of iron.

Catalase activity has been studied in recent herbicide work by several workers. The effect of atrazine on catalase in resistant and susceptible lines of corn was studied by Eastin et al (1964). It was found that catalase activity was lower as effects of atrazine increased in the line of corn susceptible to atrazine. They postulated that the Hill reaction of photosynthesis was inhibited thereby causing a reduction in catalase activity. Palmer and Porter (1959), while studying effects of amitrol on nut grass tubers, found that catalase activity in germinated tubers was 18.5 times higher than tubers that were inhibited from germinating by amitrol.

Bingham and Porter (1961) observed catalase activity of cotton cotyledons following application of DCMA. It was found that catalase activity was held at the original level by DCMA for an eight-day testing period, while activity in control tissue increased two-fold.

In the presence of hydrogen peroxide, peroxidase catalyzes the oxidation of many phenols and amines. Much of the early work on peroxidases was done on a qualitative basis and the results were confusing (Lardy, 1949).

Kenten and Mann (1953) demonstrated the oxidation of a variety of substances in the presence of manganese and a phenol without any addition of hydrogen peroxide. Peroxidases are sensitive to cyanide and azide inhibition but are resistant to carbon monoxide inhibition (Beevers, 1961).

Eastin et al (1964) found that atrazine apparently inhibits some peroxidase mechanism in resistant corn lines and stimulates it in corn

that was susceptible to atrazine. Bingham and Porter (1961) found peroxidase activity in cotton leaves, that had been treated with DCMA, was one-fourth that of untreated leaves after six days. Peroxidase activity of nutgrass was lower in amitrol treated tubers than in untreated tubers (Palmer and Porter, 1959).

# MATERIAL AND METHODS

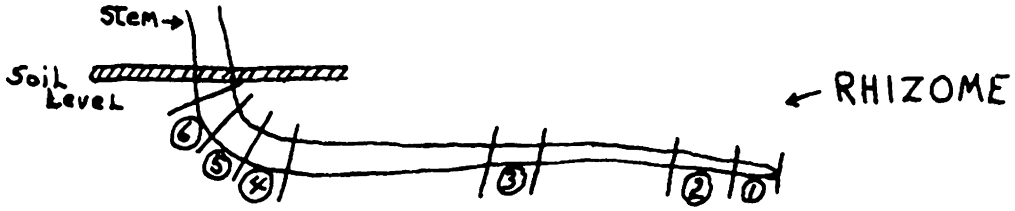
## I. Growth Studies

### A. Rhizome Section Experiment (Length)

An experiment was performed to determine the length of rhizome section that would produce uniform mugwort plants. Rhizomes were cut into one, two, four, and eight inch sections, and these sections were placed an inch deep in metal flats that contained a fertilized silt loam soil. There were 36-one inch, 24-two inch, 16-four inch, and 5-eight inch sections per respective flat. Three flats of each length were planted and watered daily. Plant counts were made three and five weeks after planting.

### B. Rhizome Section Experiment (Position)

During the growth of mugwort rhizomes, the older part of the rhizomes take on a brownish color. To determine if the older axillary buds of the mugwort rhizomes were functional, two-inch rhizome sections from six position, shown in the following diagram, were taken from 15 week old rhizomes and compared for viability.




The six-2 inch sections were randomly placed in a metal flat that contained a silt loam soil and the test was replicated six times.

Counts of plants were made at two and four weeks.

C. Field Growth Study

An experimental area 20' x 75' was located near the VPI campus. The Groseclose silt loam soil was disked, harrowed and fertilized with 5-10-10 fertilizer at the rate of 500 lb/A on April 15, 1963. Methyl bromide was applied at the rate of 1 lb per 100 sq. ft. to fumigate the area immediately after the fertilizer was applied.

This area was divided into five blocks, each containing twenty-four 3' x 3' plots. Into each plot of 3' x 3', a small mugwort plant, which had grown from a two-inch rhizome section, was transplanted on April 22, 1963. These plants averaged two inches tall and had no rhizome system.

Irrigation was applied several times during April and May. Harvest dates were June 17, July 2, July 23, and September 11, -7, 9, 12, and 18 weeks after transplanting, respectively. Second year data were taken in May, 1964. At each harvest date, four plots were harvested at random from each of the five blocks.

Entire plants were removed from the 3' x 3' plots on the harvest dates, and the soil was removed from the rhizome-root system. The plant was separated into shoots, which included all growth above the soil line, and the subterranean parts--the rhizomes and roots.

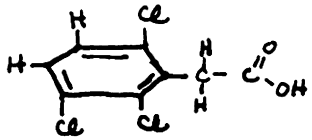
At harvest the following items were recorded in each plot: shoot weight, rhizome-root weight, number of new rhizomes, rhizome length and shoot height. Plant averages are expressed as the means of 20 plants.

## II. Herbicide Studies

### A. The Herbicide

Fenac is the common name for 2,3,6-trichlorophenylacetic acid. In this study, liquid Fenac Industrial (1.5 lb/gal.), which was water soluble, was used.

Fenac has the following formula:




This herbicide exhibits its effect after being absorbed through the roots. No evidence has been shown where fenac appreciably penetrates shoots.

### B. Soil-herbicide Mixture

In the first soil-herbicide study, fenac was applied at the rates of 0, 1, 2, 4, 8, 12, 16, and 32 ppm. A stock solution of fenac was prepared and the desired amount of herbicide was hand mixed thoroughly with a known volume of soil on a plastic sheet. The soil mixture was put into one-quart plastic containers and a two-inch rhizome section was planted in each container. Each treatment was replicated five times. One hundred ml of water was added to each container twice a week.

In this test, rates of 2 ppm and above completely destroyed the rhizome sections. Another experiment was run by the procedure described above. In the second experiment, rates of fenac were 0, ¼, ½, 1, and 2 ppm.

After eight weeks the plants were harvested and oven dried for 24 hours at 80° C. Dry weights were recorded and subjected to an analysis of variance.

C. Nutrient solution-herbicide mixture

Fenac was added to nutrient solution at 0, 1/8, 1/4, 1/2, 1, 2, 5, and 10 ppm. Ten ml of each concentration was measured into petri dishes which contained a piece of blotter paper that covered the bottom of the dish. A two-inch rhizome section was placed in each dish. Each rate was replicated five times.

This test was conducted in the laboratory and light from 40 watt cool white fluorescent lamps produced a 16 hour light and 8 hour dark period. After 25 days each rhizome was checked for growth. Growth was expressed as total mm of shoots per rhizome, and the treatments were subjected to an analysis of variance.

II. Respiration and Respiratory Enzyme Studies

A. Growth of Plants

1. Enzyme Studies

Two-inch rhizome sections were planted about an inch deep in pots which contained a silt loam soil. Pots remained in the greenhouse and nutrient solution was applied at intervals of about two weeks After about 12 weeks the plants were sprayed with fenac at 0, 5, or 10 lb/A in 60 gallons of water similating field spraying. Plants were kept in the greenhouse until used at 0, 3, 7, and 15 days after treatment.

2. Inhibitor Studies

Two 12 week old mugwort plants were washed free of soil and transferred to trays that contained nutrient solution. Fenac was added to one tray to make a mixture of 5 ppm.

After 15 days, the plants were taken from the solutions, and rhizome sections were prepared for infiltration of the various inhibitors of respiration.

B. Preparation of Rhizome Sections

1. Rhizome Sections--Rhizomes from one plant of each treatment were washed free of soil and then blotted with paper towels. One inch sections were sliced, weighed and transferred to Warburg flasks which contained sucrose-phosphate buffer at 0.02 M and 0.05 M with respect to sucrose and potassium phosphate. When respiratory inhibitors were used, the inhibitor was mixed with the buffer.

2. Vacuum Infiltration--Rhizome sections were placed in beakers that contained an inhibitor solution that was to be vacuum infiltrated. Beakers were placed in a vacuum dessicator which was attached to a water aspirator and a vacuum was applied to the dessicator for 10 minutes. The vacuum was slowly released and applied for 10 minutes more. After releasing the vacuum, the sections were removed from the solution, blotted and placed in Warburg flasks that contained the appropriate inhibitor solution.

D. Preparation of Homogenates and Supernatants

Four grams of rhizomes were cut and placed in an omni mixer which contained 50 ml of 0.05 M potassium phosphate buffer of pH 6.0. Unless otherwise stated, this buffer was used for all enzyme studies. The mixer was connected to a powerstat and operated at 90 volts for 5 minutes. An ice bath surrounded the mixing vessel to keep the temperature below $5^{\circ}$ C.

Homogenates were strained through a double layer of cheesecloth and used for the studies involving polyphenol oxidase, ascorbic acid oxidase, catalase, and peroxidase. In studies involving catalase and peroxidase activity, the homogenate was centrifuged at 2,000 x g for 15 minutes.

E. Determination of Oxygen Uptake and Carbon Dioxide Production

Respiratory activity of rhizome sections and homogenates was determined by using a Warburg respirometer and conventional methods described by Umbreit et al (1964). In flasks where oxygen uptake alone was determined, a piece of folded filter paper and 0.15 ml of 20 per cent KOH was placed in the center well. Flasks which contained rhizome sections also received 2.0 ml of sucrose-phosphate buffer. Duplicate flasks were run for all treatments.

Manometer readings were recorded at 15 minute intervals for one hour except in the catalase and peroxidase studies. All studies were run in a constant 25° C water bath. Oxygen uptake was expressed as $Q_{O_2}(N)$, which means microliters of oxygen uptake per milligram of nitrogen per hour.

F. Estimation of Enzyme Activity

1. Polyphenol oxidase--A 3.0 ml aliquot of homogenate was placed in the main compartment of Warburg flasks and 0.5 ml of 0.12 M catechol was added to the side arm. The catechol was adjusted to pH 6.0 just before addition to the flask. After 15 minutes equilibration, the manometers were closed and the catechol was tipped into the homogenate. Readings were taken at 15-minute intervals.

Some homogenate was boiled for three minutes and checked for activity using the above procedure.

2. Ascorbic acid oxidase--A 3.0 ml aliquot of homogenate was added to the main compartment of a Warburg flask and 0.5 ml of 0.12 M ascorbic acid was added to the side arm. The ascorbic acid was prepared in 0.05 M phosphate buffer and adjusted to pH 6.0. After equilibration, the substrate was tipped into the main compartment, the manometers were closed and then read at intervals of 15 minutes.

3. Catalase--A modified procedure of Appleman (1951) was used. The main compartment of the Warburg flask received 2 ml of 10 mM $H_2O_2$. One-half ml of supernatant was added to the side arms. After equilibration the enzyme source was tipped into the main compartment and oxygen production was recorded at two minute intervals for 10 minutes.

4. Peroxidase--A manometric procedure given by Ettori (1949) was used. The main compartment received the following: 0.2 ml of 5 per cent pyrogallol, 1.5 ml of $H_2O$ and 0.2 ml of one per cent $H_2O_2$. A 0.5 ml of supernatant was added to the side arms. After equilibration the enzyme source was tipped into the main compartment and readings recorded at two minute intervals for 10 minutes. A second set of duplicate flask were included in which 0.15 ml of 20 per cent KOH and a piece of filter paper were placed in center wells. These flasks gave an estimation of the oxygen produced by catalase activity which had to be accounted for in the total gas production in the other flasks. Peroxidase activity is expressed in terms of carbon dioxide production in the conversion of pyrogallol to purpurogallin.

G. Nitrogen Determination

Protein nitrogen in homogenates and supernatants was determined by a modified procedure of Kabat and Mayer (1961).

Three ml of the nitrogen source was added to five ml of digestion mixture (2g $CuSO_4$ + 2g $H_2SeO_3$ + 100 g $Na_2SO_4$ + 500 ml distilled water + 500 ml of concentrated $H_2SO_4$) in a 125 ml Kjeldahl flask. The sample was digested until the mixture became greenish-blue and then continued for 15 minutes. After cooling, 20 ml of distilled water was added to each flask. Duplicate flasks were run for each determination.

Ten ml of 2 per cent boric acid was placed in 100 ml beakers and then 3 drops of boric acid indicator (80 ml 0.1 per cent brom cresol green + 10 ml of 0.1 per cent methyl red) was added to each beaker.

Distillation of the samples consisted of adding 15 ml of 40 per cent NaOH to the distillation flask and bubbling steam into the flask. Twenty ml of distillate was trapped in the boric acid.

The sample was then titrated back to the original color with dilute $H_2SO_4$ (acid factor equal to 0.124). The ml of acid required times 0.124 equals the mg of nitrogen in the sample.

IV. Artemisia Control Tests at Fredericksburg, Virginia

During 1963, four experiments were initiated on heavily infested mugwort area near the Rappahannock river at Fredericksburg, Virginia. This control test was a followup of the 1962 experiments of Bingham (1963) and further evaluated some of the promising herbicides for mugwort control. A list of the chemicals used in the experiments is shown in table 1.

Table 1. A list of the herbicides used with formulations and designated names.

| Chemical name | Formulation | Designated name |
|---|---|---|
| 2,4-dichlorophenoxyacetic acid | | |
| Butoxy ethanol ester | Emulsifiable, 4 lbs. acid equiv./gal. | 2,4-D ester |
| Oleyl 1,3 propylene diamine salt | Emulsifiable, 4 lbs. acid equiv/gal. | 2,4-D, OPDS |
| 2,3,6-trichlorophenylacetic acid | | |
| Sodium salt | Water soluble, 1½ lbs. acid equiv./gal. | Fenac |
| 2-methoxy-3,6-dichlorobenzoic acid | | |
| Dimethylamine salt | Water soluble, 4 lbs. acid equiv./gal. | Dicamba |
| 4-amino-3,5,6-trichloropicolinic acid | Water soluble, 2 lbs. acid equiv./gal. | Tordon |

The chemicals in experiment 1 were applied March 5, 1963 when the mugwort had just begun to appear. Experiments 2, 3, and 4 were applied April 4, May 21, and July 15, respectively. Plots were 10' x 10' or larger. Knapsack sprayers were used in applying the herbicides at 60 gpa total spray volume.

A randomized block design with four replications was used in each experiment. Experiments 1, 2, and 3 were evaluated October, 1963. During evaluation, random one foot square areas were sampled in each plot and data were recorded for: number of shoots, height of shoots, weight of shoots, and weight of the rhizome-root system to a depth of four inches. Visual ratings were made at this harvest and also in March, 1964. Experiment 4 was visually rated July, 1964.

Each of the items was subjected to an analysis of variance and treatment means were compared using Duncan's multiple range test (1955).

# RESULTS AND DISCUSSION

## I. Rhizome Section Experiment

### A. Length of Sections

The number of shoots initiating from the axillary buds of rhizome sections three and five weeks after planting are presented in Table 2. After three weeks, 50 per cent of the two-inch sections had initiated shoots while all of the eight-inch sections had one or more shoots.

At five weeks, each of the two-inch sections and 60 per cent of the one-inch sections had produced one shoot per plant. There was about one shoot per two inches of rhizome regardless of length.

It was concluded that there probably was not enough food reserve in all of the one-inch sections to get shoots started. Because one bud from each of the two-inch sections sent up a shoot, this length was decided best for producing uniform plants.

Original rhizome sections remained firm throughout the growing period and stayed attached to the plant. This indicates that mugwort rhizomes remain longer than Johnsongrass because McWhorter (1961) observed disintegration of Johnsongrass rhizomes after about 24 days.

### B. Position Experiment for Viability

Two-inch rhizome sections were taken from six positions along a 12 to 18 inch rhizome to determine if there was any difference in viability of axillary buds.

The number of rhizome sections that produced shoots from six

Table 2. Number of shoots produced by mugwort rhizome sections of various lengths planted at a depth of one inch.

| No. of rhizomes per flat | Length of rhizome sections | No. of shoots 3 wks. | 5wks. | Per cent shoots from rhizomes 3 wks. | 5 wks. | Average no. of shoots per inch |
|---|---|---|---|---|---|---|
| 36 | 1" | 8 | 22 | 22 | 61 | 0.6 |
| 24 | 2" | 12 | 24 | 50 | 100 | 0.5 |
| 16 | 4" | 9 | 21 | 56 | 100[a] | 0.3 |
| 5 | 8" | 9 | 20 | 100[a] | 100[a] | 0.5 |

[a]More than one shoot from some of the longer rhizome sections.

positions along a rhizome after two, four, and five weeks are shown in Table 3.

Observations showed that 100 per cent of the sections from position six produced shoots within a week after planting. This region represents the area of new rhizome development at the base of the main plant. At two weeks only seventeen per cent of sections from positions four and five produced shoots.

After five weeks, each of the sections from the six positions had initiated shoots.

By cutting the rhizomes, apical dominance was probably destroyed, and a bud from each position produced a shoot. There did not appear to be any difference in viability of buds in the older or younger areas of the rhizomes, although positions three and six produced plants faster.

## II. Growth Experiment Near the VPI Campus

Mugwort development was slow during the first four weeks after it was transplanted, although the area was irrigated several times. It was observed that the first rhizome spurs were initiated after about four weeks.

At the first harvest, seven weeks after transplanting, there had been only a few small showers. Plants in several of the plots were still small, although most plants were beginning rapid growth. Entire plant systems were dug up and soil was removed as seen

Table 3. Number of sections producing shoots from different positions on mugwort rhizomes two, four, and five weeks after planting.

| Rhizome position* | No. of rhizome sections producing shoots | | | Per cent of rhizome sections producing shoots | | |
|---|---|---|---|---|---|---|
| | Weeks after planting | | | Weeks after planting | | |
| | 2 | 4 | 5 | 2 | 4 | 5 |
| 1 | 4 | 6 | 6 | 66 | 100 | 100 |
| 2 | 2 | 5 | 6 | 33 | 83 | 100 |
| 3 | 5 | 6 | 6 | 83 | 100 | 100 |
| 4 | 1 | 5 | 6 | 17 | 83 | 100 |
| 5 | 1 | 5 | 6 | 17 | 83 | 100 |
| 6 | 6 | 6 | 6 | 100 | 100 | 100 |

*Rhizome positions are indicated in a diagram (page 20).

in Figure 1A. Plants had grown from the height of about three inches to an average of ten inches. Shoots averaged 18 g and rhizomes 27 g on a green weight basis. No rhizomes were found lower than two inches below the soil, and they spread almost entirely in a horizontal movement. There was an average of 12 rhizomes per plant which gave a total rhizome length of 103 inches. During this harvest only two plots had secondary plants. Secondary plants are plants that had arisen from axillary buds of the new rhizomes.

The second harvest came after nine weeks, and rhizomes were increasing and secondary plants were appearing (Figure 1B). Shoots averaged 17 inches tall, and shoot weight had increased 62 per cent. There was an average of 14 rhizomes per plant that gave a total length of 158 inches. At this time, secondary plants were about two inches high, and some of the rhizomes were branching (Figure 2).

There was an increase of 138 per cent in shoot weight between the ninth and twelfth week. Rhizomes were becoming highly branched, and more secondary plants were observed. Total rhizome length had increased to 277 inches per plant with a 135 per cent increase in weight.

One of the larger mugwort plants at this harvest is shown in Figure 3. Some of the secondary plants were arising from as far as 18 inches from the base of the main plant. Rhizomes were observed to come out of the ground and penetrate again (Figure 4). The section of the rhizome exposed to light took on a purple pigmentation, and the epidermis seemed tougher, while the underground portion was white and fleshy.

A.

B.

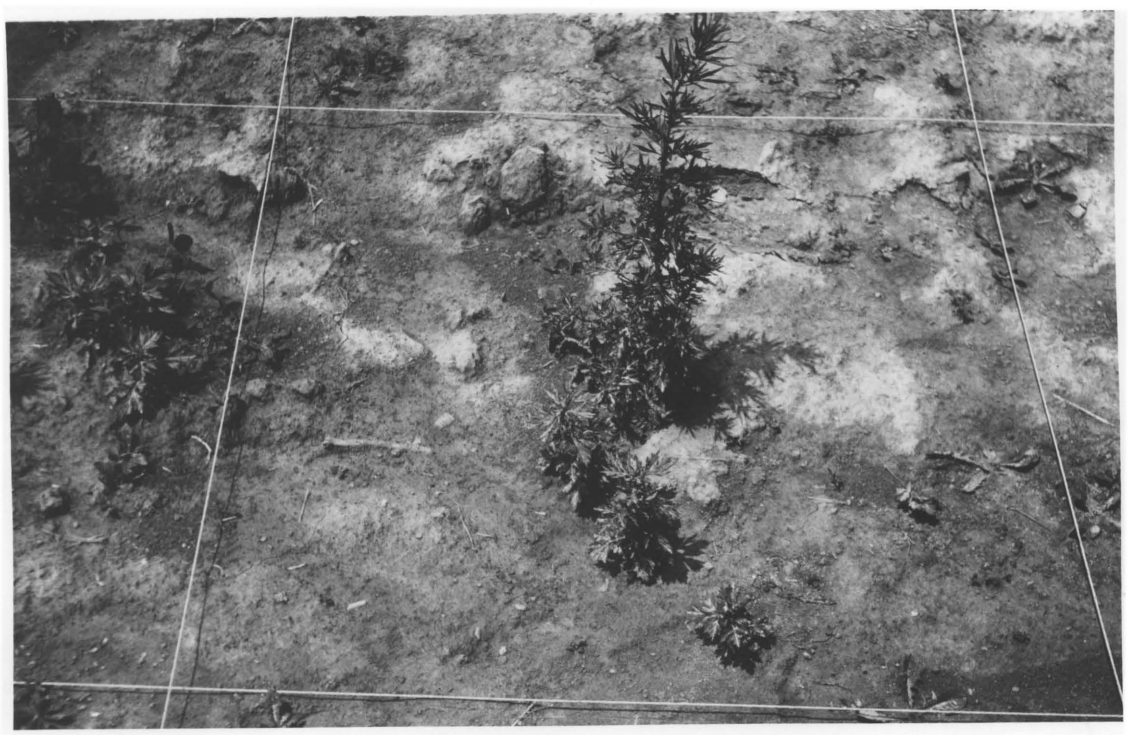


**Figure 1.** A mugwort plant 9 weeks after transplanting. (A) After harvest. (B) Before harvest.

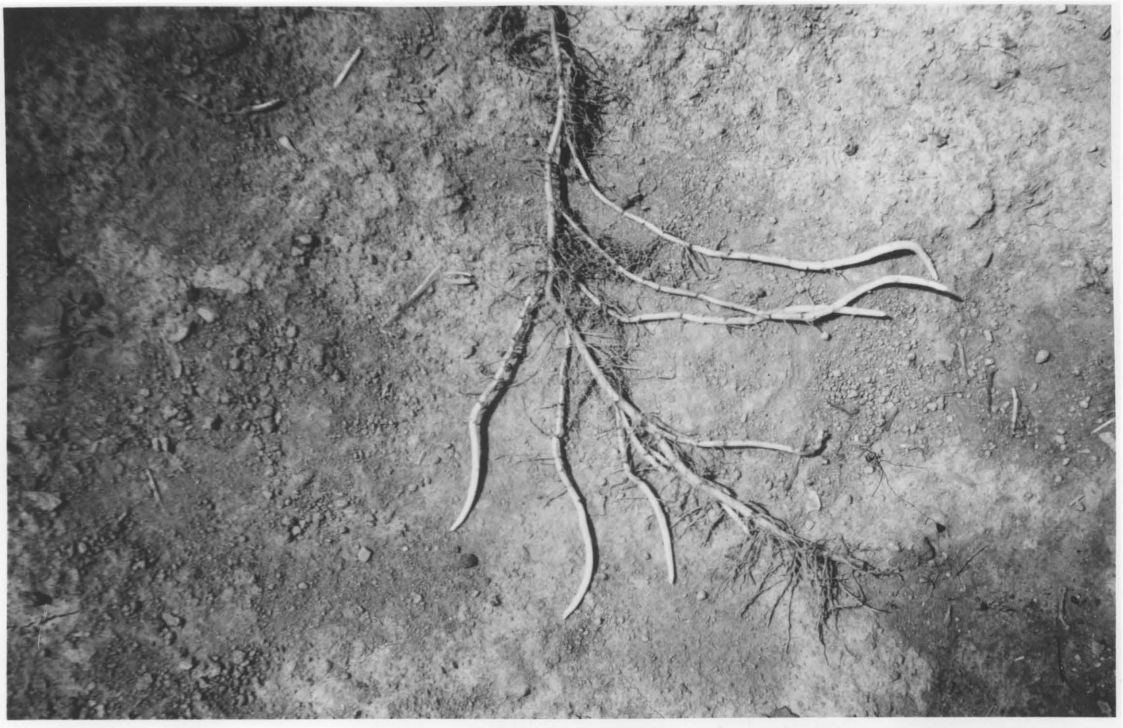


Figure 2. A rhizome that had become highly branched after 9 weeks.

Figure 3. Mugwort plant 12 weeks after transplanting.

Figure 4. Portion of an older rhizome where younger branched rhizomes came out of the soil and penetrated again.

Several rhizomes were observed to be completely covered by a matted network of roots where no branching of rhizomes occurred (Figure 5). Dry weather might have caused this thickening of the roots at certain regions.

Flowering was first observed about August 15, and nearly all plants had produced flowers by September 10. The small, yellow flowers were arranged in clusters along the top branches.

Mature plants were harvested 18 weeks after transplanting (Figure 6). During the six weeks since the third harvest, there was a four-fold increase in shoot weight. The plots were almost covered with an average of 15 secondary plants per plot, and these secondary plants ranged from two to 35 inches, while the main plants averaged 36 inches.

Rhizome weight increased over 300 per cent with a total rhizome length of 77 feet per plant. During this harvest, it was very difficult to separate and measure the matted rhizome system. Seasonal increases are summarized in Figures 7 and 8.

The area was left through the winter, and mugwort was observed appearing in early March, 1964. Scattered plants could be seen later in March with rapid regrowth from rhizomes in April. Three plots were harvested May, 1964 and new plants completely covered what had been the 3 x 3 ft plots. The weight of the plant system was five times higher than when sampled at the end of the 1963 growing period.

From this test, it was seen that mugwort will infest an area very fast. Several workers have observed that when mugwort is

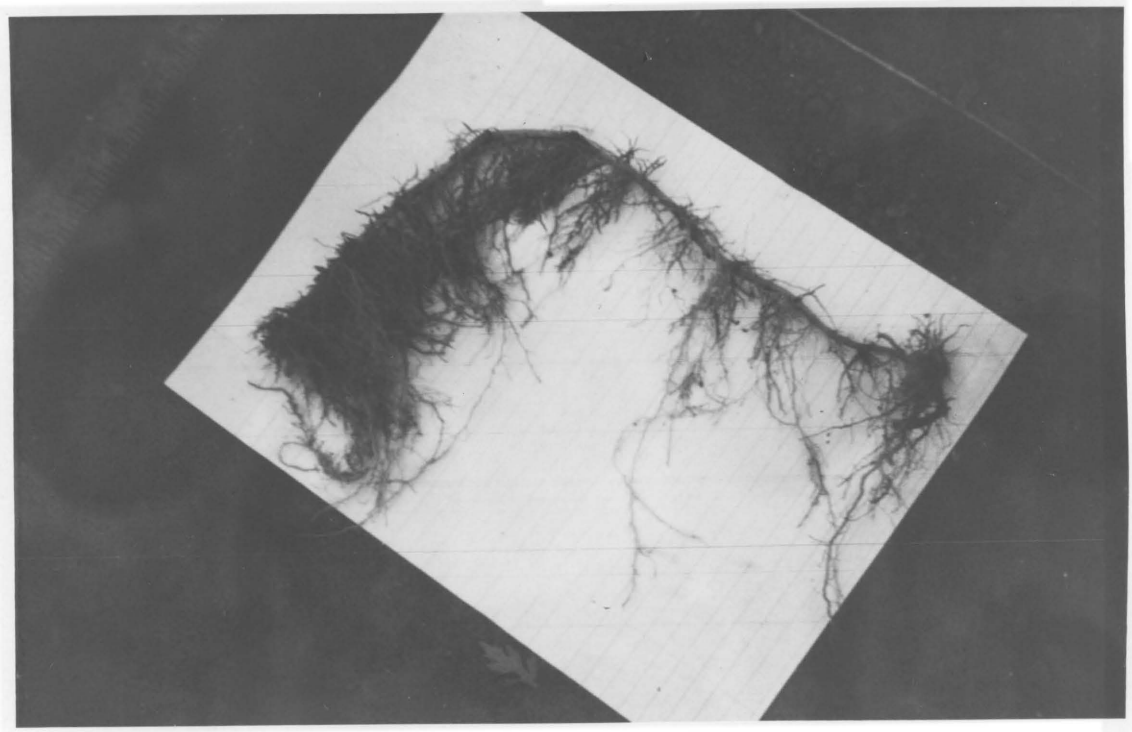


Figure 5. Roots became thick and completely covered the rhizomes in certain regions.

Figure 6. Mugwort plant 18 weeks after transplanting.

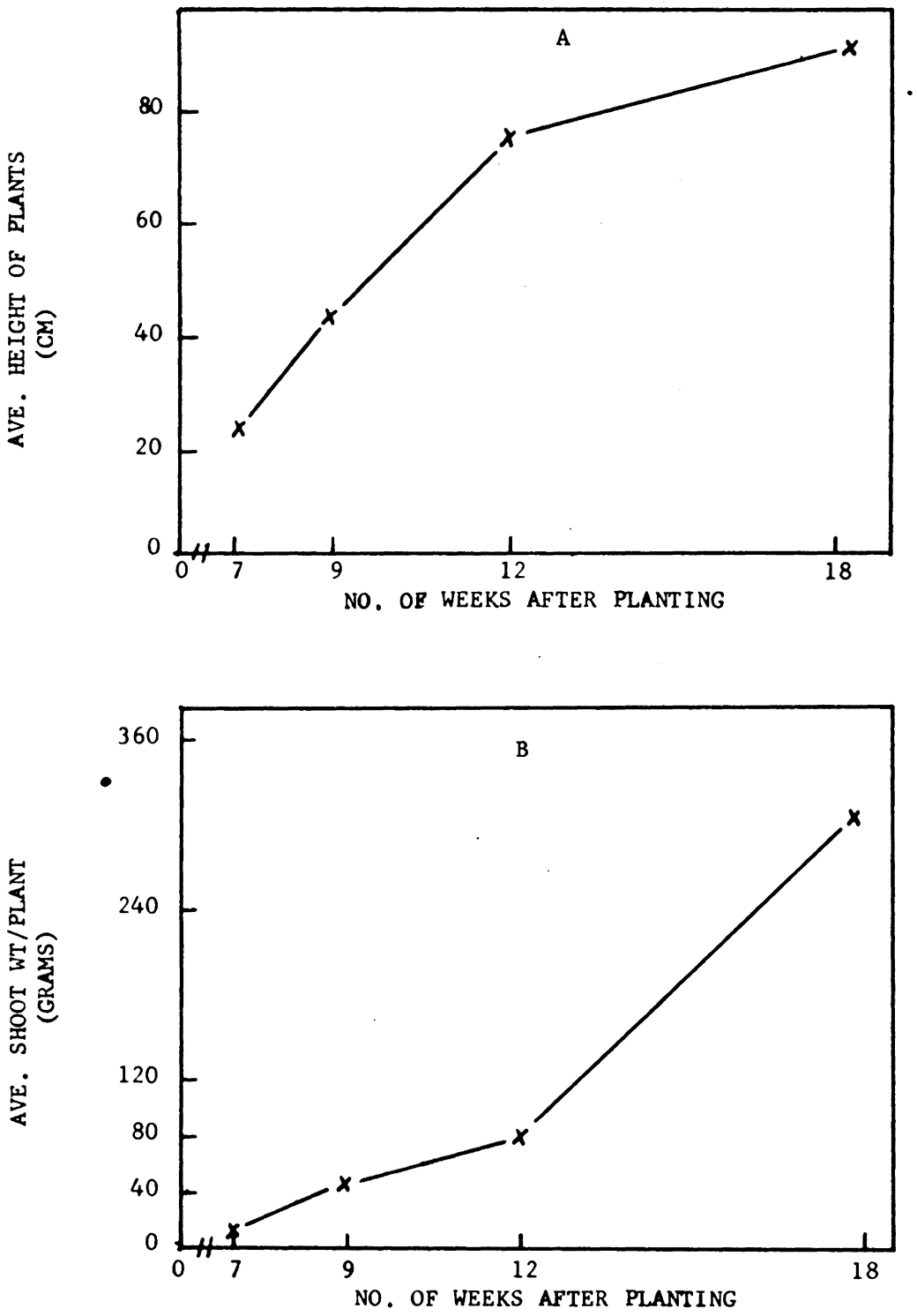



Figure 7. The increase in (A) height of shoots and (B) weight of shoots during the 1963 season.

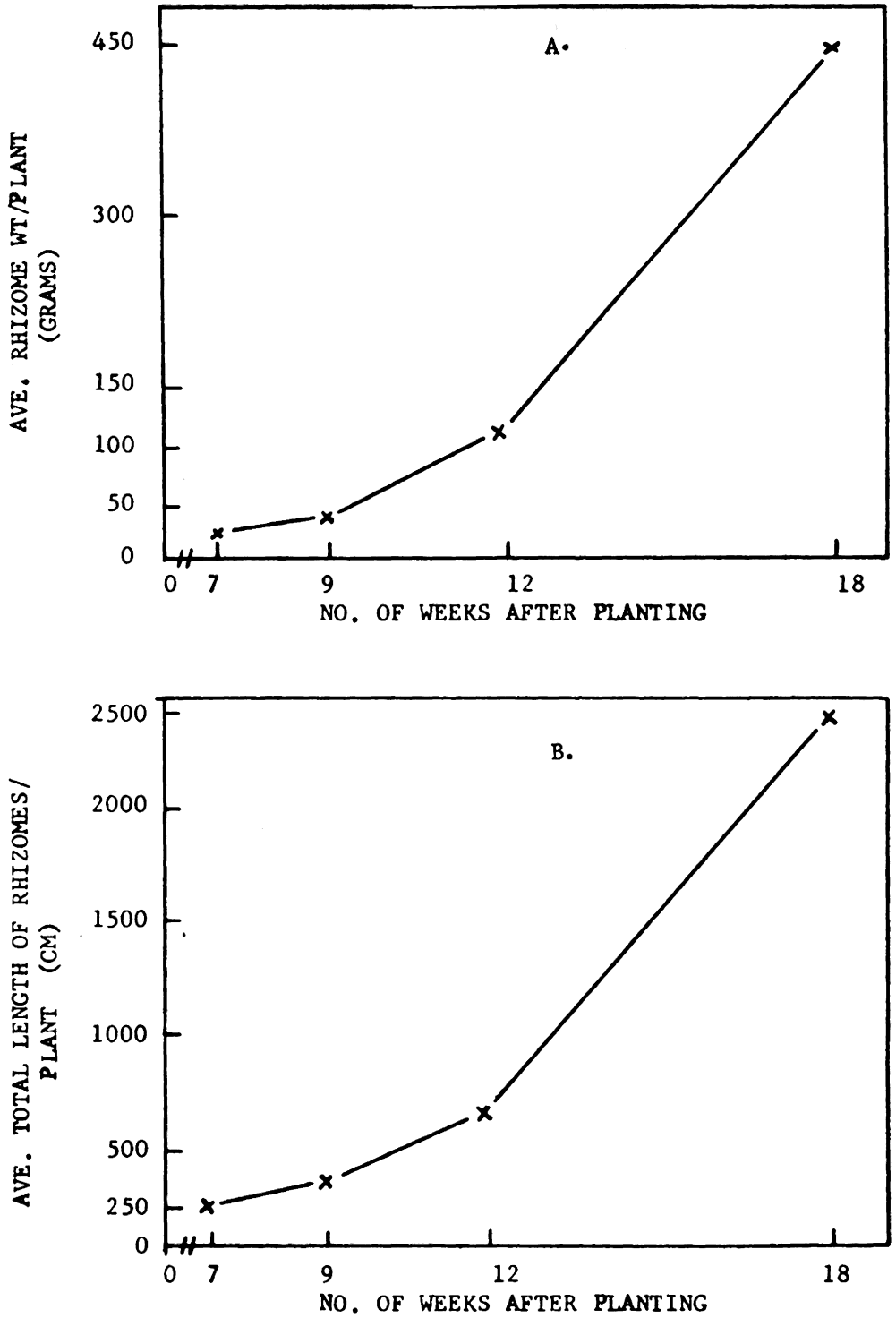



Figure 8. The increase in (A) rhizome weight per plant and (B) length of rhizomes per plant during the 1963 season.

growing in an area, very few other plants are found. Because of the early growth of this plant, it gets well established and probably keeps other plants out mainly through competition.

## III. Rhizome-Herbicide Experiments

### A. Soil-fenac experiment

Several of the fenac rates caused disintegration of the rhizome sections after six weeks.

The only sympton from the one-quarter ppm treatment was shoots appeared about a week later than the shoots from the control treatment. The one-half ppm treatment caused the shoots to have a stunted look with twisting of the leaves. Two of the five replications in the one ppm treatment produced shoots, but they were very chlorotic. Observation of the other three replications indicated that the axillary buds started growth, but were stopped before the buds penetrated the soil surface. Effects of the various fenac mixtures on shoot development are shown in Table 4.

### B. Nutrient solution-fenac experiment

Shoot growth was observed from all sections except the five and ten ppm treatments. Sections from the one-fourth and one-eighth ppm treatments produced more total growth than the control sections. There was possibly some growth stimulation by these two rates. Shoots were observed in the one and two ppm treatments, but injury was apparent. At five ppm fenac or above, mugwort rhizomes did not initiate shoots.

Table 4. Effect of various amounts of soil applied fenac on development of shoots from mugwort rhizomes.

| Fenac (ppm) | 2.0 | 1.5 | 1.0 | 0.5 | 0.25 | 0 |
|---|---|---|---|---|---|---|
| Shoots (Grams/section)* | 0 | 0 | 0.02 | 0.07 | 0.39 | 0.94 |

*Any two means not underscored by the same line are significantly different at the 1% level.

After 25 days, rhizome sections in the ten ppm treatment were turning brown and becoming soft, a sympton that had been observed through field spraying of mugwort. Effects of the various nutrient solution-fenac mixtures on total shoot length of mugwort sections are shown in Table 5.

Table 5. Effect of various nutrient solution-fenac mixtures on the development of shoots from mugwort rhizomes.

| Fenac, ppm | 10 | 5 | 2 | 1 | 0 | 1/2 | 1/8 | 1/4 |
|---|---|---|---|---|---|---|---|---|
| Shoot length (mm/section)* | 0 | 0 | 13 | 28 | 40 | 50 | 58 | 78 |

*Any two means not underscored by the same line are significantly different at the 5% level.

## IV. Mugwort Control Experiments At Fredericksburg, Va.

Shoot and rhizome weight per sq. ft. at the end of a growing period plus visual control ratings at two dates were used to determine the degree of mugwort control by various herbicide treatments.

Data from March treatments are shown in Figure 9. When these treatments were applied, mugwort had just begun to appear. Nearly all treatments gave initial control for about three weeks, at which time plants began to reappear in the plots treated with 2,4-D at 5 lb/A, and in the plots treated with dicamba at 5 lb/A. Fenac at 2.5 lb/A did not immediately kill back the small mugwort but started showing more effect after about five weeks. Higher rates of fenac and several mixtures of herbicides gave good season long control.

An interesting observation was noted in the 5 lb/A dicamba plots. When dicamba was first applied to the small mugwort, it seemed to kill all the shoots, but in five weeks the mugwort came back very thick. At the end of the season, the rhizome weight was higher in the dicamba plots than in the control plots. There seemed to be a stimulation of new plants. Results were almost the same for 2,4-D. Fenac at 10 lb/A gave perfect control of mugwort.

Because of the potential infestation from a small number of rhizomes, all of the rhizomes need to be destroyed, so that no new plants will be initiated. Therefore, visual control ratings were made at the end of the 1963 growing period, and also in the spring of 1964 to help evaluate the effectiveness of various herbicide treatments. Control ratings for the March treatments are shown in Table 6.

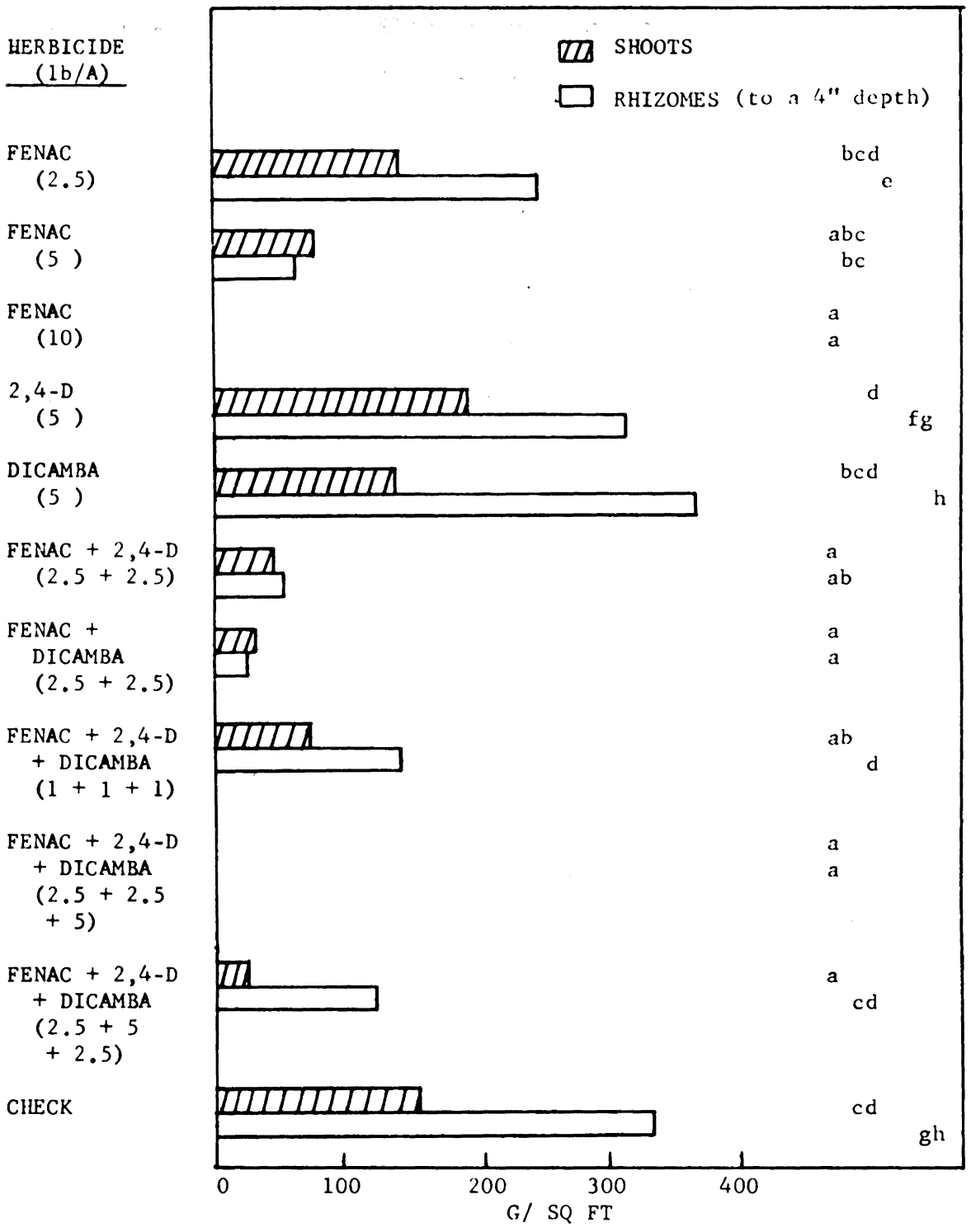



Figure 9. Weights of shoots and rhizomes in October, 1963, after various herbicides were applied in March, 1963.

Table 6. Effect of March, 1963 application of herbicides for the control of mugwort.

| Herbicide | Rate (lb/A) | Weed Control* October, 1963 | | March, 1964 | |
|---|---|---|---|---|---|
| Fenac | 2.5 | 1.0 | c | 0.0 | e |
| Fenac | 5.0 | 6.3 | b | 5.3 | cd |
| Fenac | 10.0 | 10.0 | a | 10.0 | a |
| Dicamba | 5.0 | 1.8 | c | 0.0 | e |
| 2,4-D | 5.0 | 2.0 | c | 0.0 | e |
| Fenac + dicamba | 2.5 + 2.5 | 8.2 | ab | 5.0 | d |
| Fenac + 2,4-D | 2.5 + 2.5 | 9.0 | ab | 7.3 | b |
| Fenac + dicamba + 2,4-D | 1 + 1 + 1 | 9.3 | ab | 6.7 | bc |
| Fenac + dicamba + 2,4-D | 2.5 + 5.0 + 2.5 | 10.0 | a | 9.9 | a |
| Fenac + dicamba + 2,4-D | 2.5 + 2.5 + 5.0 | 9.3 | ab | 9.3 | a |
| Check | --- | 0.0 | c | 0.0 | e |

*Weed control ratings are based on 0 = no control and 10 = complete control.

Mugwort was about six inches high when the April treatments were applied, and data from these plots are shown in Figure 10. A mixture of dicamba at 2.5 lb/A plus 2,4-D at 2.5 lb/A resulted in almost 100 per cent control at the end of the season, which was very surprising because when either was used alone in the March application, no appreciable control was observed. The liquid formulation of fenac tended to give better rhizome control than a granular formulation when both were applied at equal rates. Fenac at 10 lb/A gave 100 per cent control, and this rate seemed to sterilize the soil during the summer because no other plants appeared. Control ratings for the April test are shown in Table 7.

When the May treatments were applied, mugwort was about 18 inches tall. The data (Figure 11) would indicate that the May treatment of dicamba at 5 lb/A, and a fenac, 2,4-D, dicamba mixture of 1, 1, and 1 lb/A respectively, gave better control than the March treatments. There was more time for recovery from the March treatments, and this probably accounted for the increased weight. Dicamba at 5 lb/A and 2,4-D, OPDS at 5 lb/A resulted in poor control. Tordon at 0.7 and 3.5 lb/A, respectively, gave 100 per cent control. Control ratings for the May test are shown in Table 8.

Because the July experiment had only been applied for seven weeks, it was not checked when the other experiments were evaluated. A visual rating made in July, 1964 indicated that only Tordon at 0.7 lb/A was completely free of mugwort. Tordon plus 2,4-D at 0.35 and 1 lb/A respectively gave about 90 per cent control. Fenac treatments at 2.5 and 5 lb/A, and Tordon treatments at 0.18 and 0.35 lb/A appeared to give good control at the end of the 1963 season, but no control was observed July, 1964.

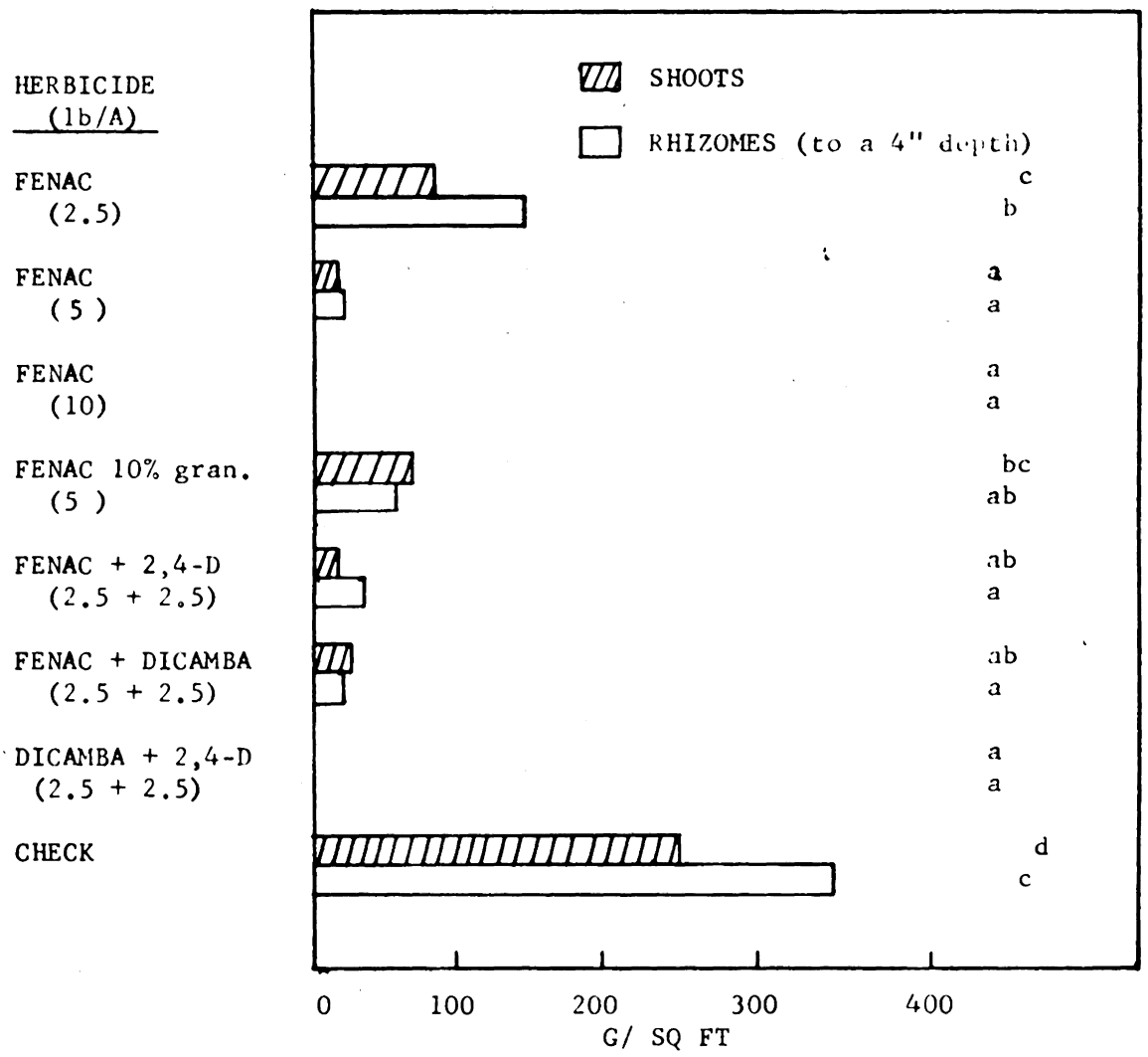



Figure 10. Weights of shoots and rhizomes in October, 1963, after various herbicides were applied in April, 1963.

Table 7. Effect of April, 1963 application of herbicides for the control of mugwort.

| Herbicide | Rate (lbs/A) | Weed Control* October, 1963 | | Weed Control* March, 1964 | |
|---|---|---|---|---|---|
| Fenac | 2.5 | 3.3 | c | 0.5 | c |
| Fenac | 5.0 | 9.7 | a | 9.4 | a |
| Fenac | 10.0 | 10.0 | a | 10.0 | a |
| Fenac 10% gran. | 5.0 | 4.8 | b | 0.5 | c |
| Fenac + 2,4-D | 2.5 + 2.5 | 8.8 | a | 7.0 | b |
| Fenac + dicamba | 2.5 + 2.5 | 9.5 | a | 6.3 | b |
| Dicamba + 2,4-D | 2.5 + 2.5 | 9.7 | a | 9.9 | a |
| Check | --- | 0.0 | d | 0.0 | c |

*Weed control ratings are based on 0= no control and 10 = complete control.

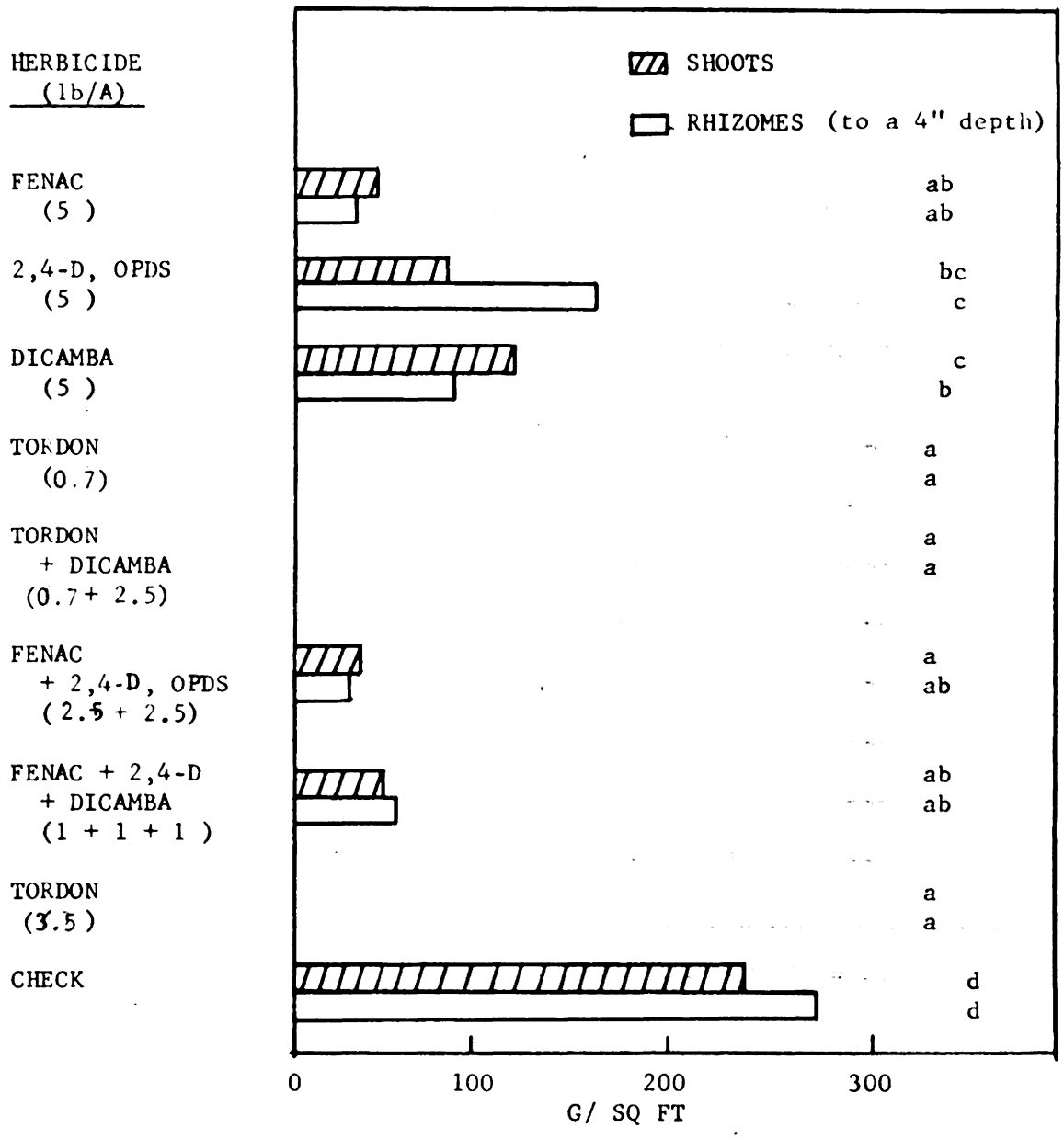



Figure 11. Weights of shoots and rhizomes in October after various herbicides were applied in May, 1963.

Table 8. Effect of May, 1963 application of herbicides for the control of mugwort.

| Herbicide | Rate (lbs/A) | Weed Control* October, 1963 | | Weed Control* March, 1964 | |
|---|---|---|---|---|---|
| Fenac | 5.0 | 9.2 | ab | 8.0 | b |
| 2,4-D, OPDS | 5.0 | 3.5 | d | 0.0 | e |
| Dicamba | 5.0 | 4.5 | c | 0.0 | e |
| Tordon | 0.7 | 10.0 | a | 10.0 | a |
| Tordon + dicamba | 0.7 + 2.5 | 10.0 | a | 9.9 | a |
| Fenac + 2,4-D, OPDS | 2.5 + 2.5 | 9.2 | ab | 6.5 | c |
| Fenac + 2,4-D + dicamba | 1 + 1 + 1 | 8.3 | b | 5.0 | d |
| Tordon | 3.5 | 10.0 | a | 10.0 | a |
| Check | --- | 0.0 | e | 0.0 | e |

*Weed control ratings are based on 0 = no control and 10 = complete control.

## V. Effect of Fenac on Respiration and Some Respiratory Enzymes of Mugwort Plants

Respiration of mugwort rhizomes was studied at intervals during a 15 day period following fenac treatment (Figure 12). A slight decrease in oxygen uptake was noted in both the treated and untreated rhizomes after 15 days, although respiration tended to be higher in fenac treated plants.

The effect of two respiratory inhibitors on untreated and fenac treated rhizomes was studied.

There was 24 and 40 per cent inhibition of oxygen uptake in untreated and treated rhizomes, respectively (Figure 13A) by iodoacetic acid (1 mM). According to James (1953a), iodoacetic acid probably inhibits triosephosphate dehydrogenase.

Sodium fluoride (10 mM) resulted in 54 and 40 per cent inhibition of oxygen uptake in untreated and treated rhizomes, respectively (Figure 13B). James (1953a) stated that sodium flouride inhibits the enzyme responsible for the formation of phosphopyruvic acid from phosphoglyceric acid.

Because of the decreased oxygen uptake from rhizomes after these inhibitors were added, this indicates that some step in the respiratory process was being inhibited.

Polyphenol oxidase, ascorbic acid oxidase, catalase, and peroxidase activities in rhizomes were measured at various intervals during a 15 day period after plants were treated with fenac at 0, 5, or 10 lb/A.

Preliminary work, using catechol as the substrate, indicated that polyphenol oxidase was quite active in mugwort rhizomes. When rhizomes

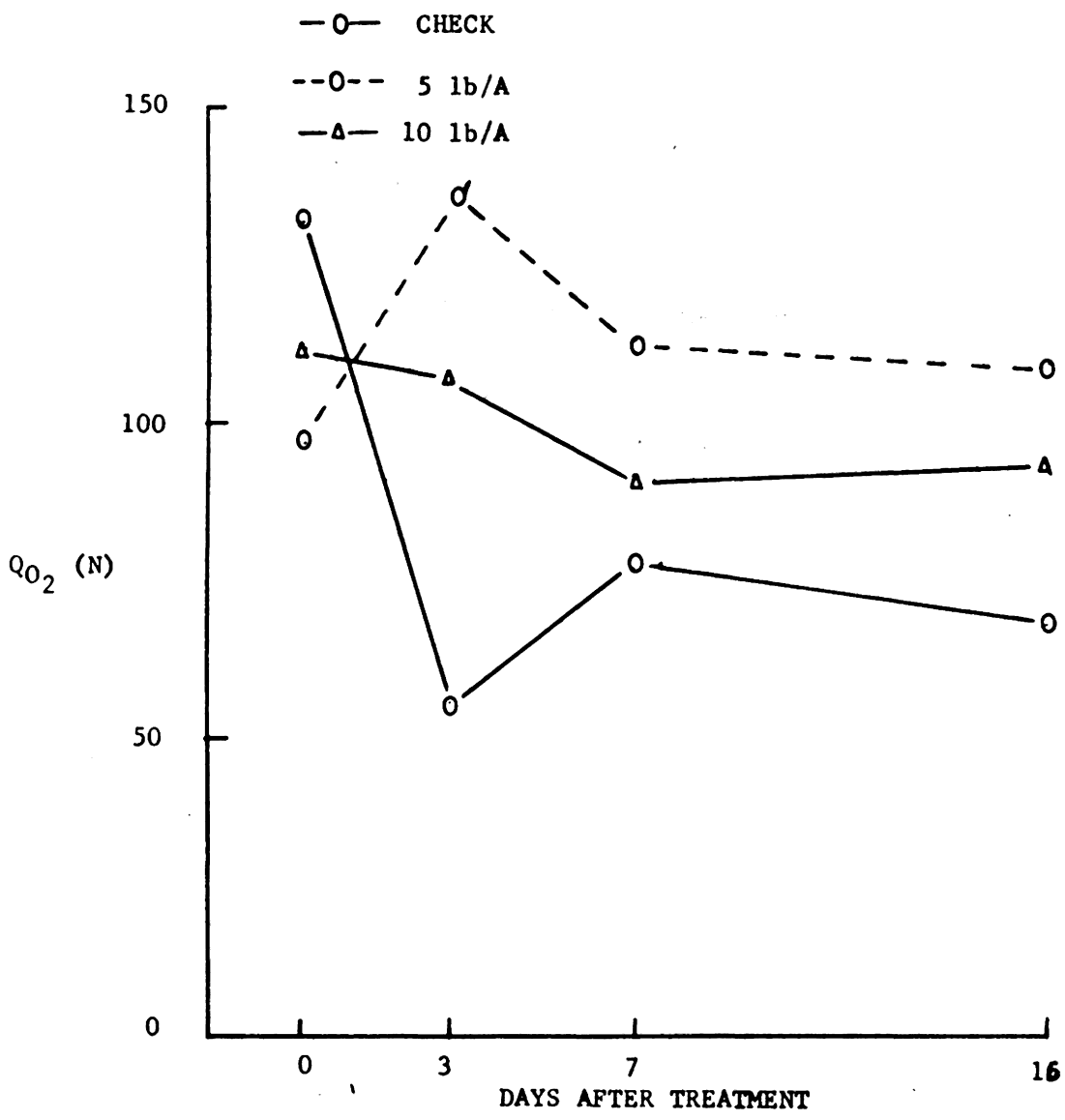



Figure 12. Effect of 0, 5, and 10 lb/A fenac on mugwort rhizome respiration.

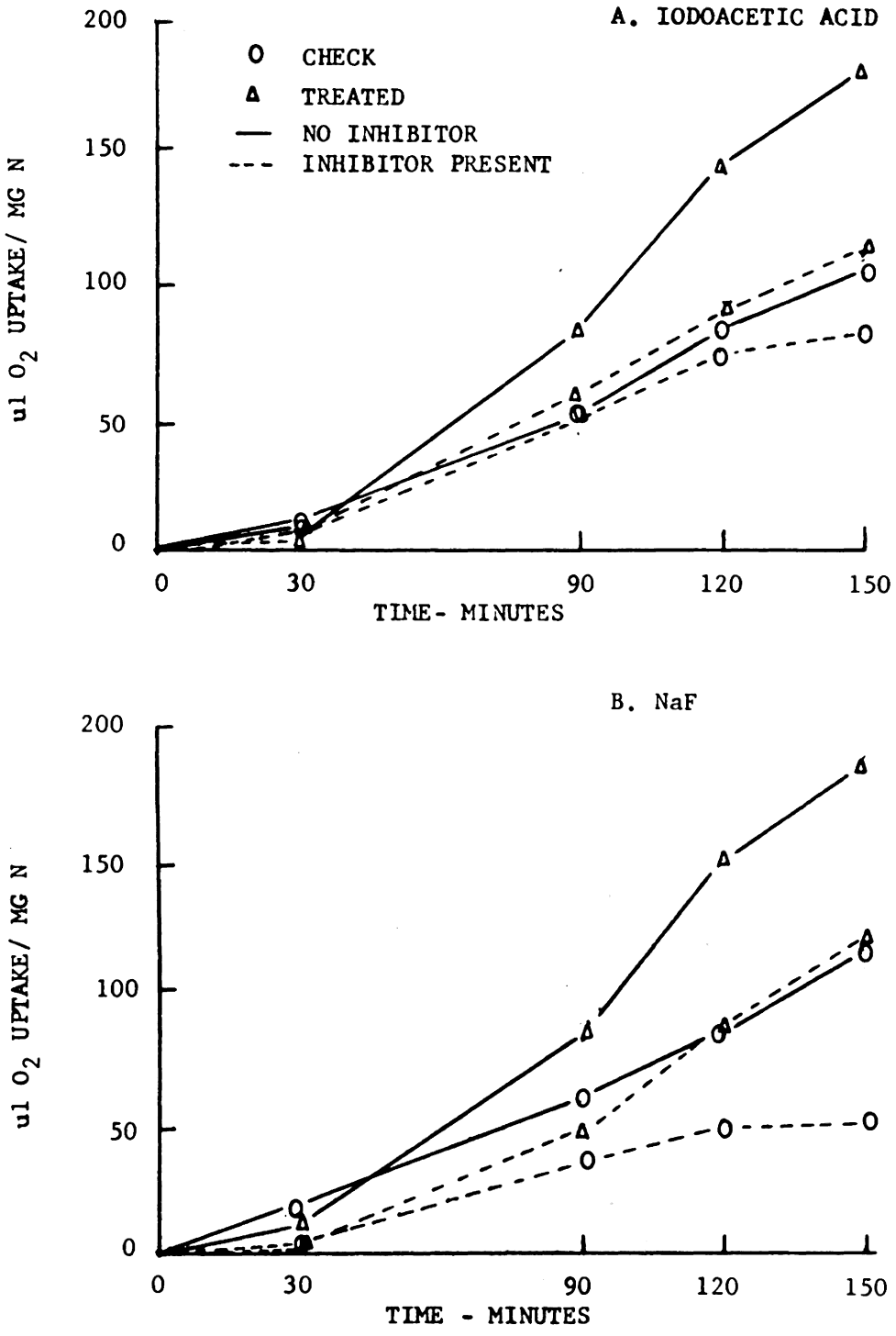



Figure 13. The effect of (A) Iodoacetic acid and (B) Sodium fluoride on the oxygen uptake of untreated and fenac treated mugwort rhizomes (15 days after treatment).

were injured or exposed to air, they became brown indicating a probable polyphenol oxidase system.

Three days after treatment, there was little change in oxygen uptake of treated and untreated plants. At seven days, there was a 41 and 27 per cent increase in oxygen uptake in the 5 and 10 lb/A treated plants, respectively (Figure 14). Rhizomes from plants that were treated at 10 lb/A were showing very serious damage by the end of the 15 day testing period. Fenac tended to increase polyphenol oxidase activity as evaluated using catechol as the substrate.

To gain more evidence that polyphenol oxidase was functioning in the rhizomes, some of the homogenate was boiled. There was no oxidation of catechol by the boiled homogenate indicating the enzyme was destroyed during boiling.

Two inhibitors of copper enzymes were used for additional evidence. Dieca (0.2 mM) resulted in 28 and 15 per cent inhibition of oxygen uptake for untreated and treated rhizomes, respectively (Figure 15A). Thiourea (20 mM) inhibition was 27 and 13 per cent for untreated and treated rhizomes, respectively (Figure 15B).

Ascorbic acid oxidase activity was evaluated by using ascorbic acid as a substrate. It was found that ascorbic acid oxidase activity was about one-third as great as polyphenol oxidase activity (Figure 16). As the oxygen uptake was higher in the treated plants after 15 days, it was concluded that the fenac damage to the rhizomes possibly caused a slight stimulation of ascorbic acid oxidase.

To determine if ascorbic acid was reducing quinone back to catechol in the polyphenol oxidase system, catechol and ascorbic acid were combined,

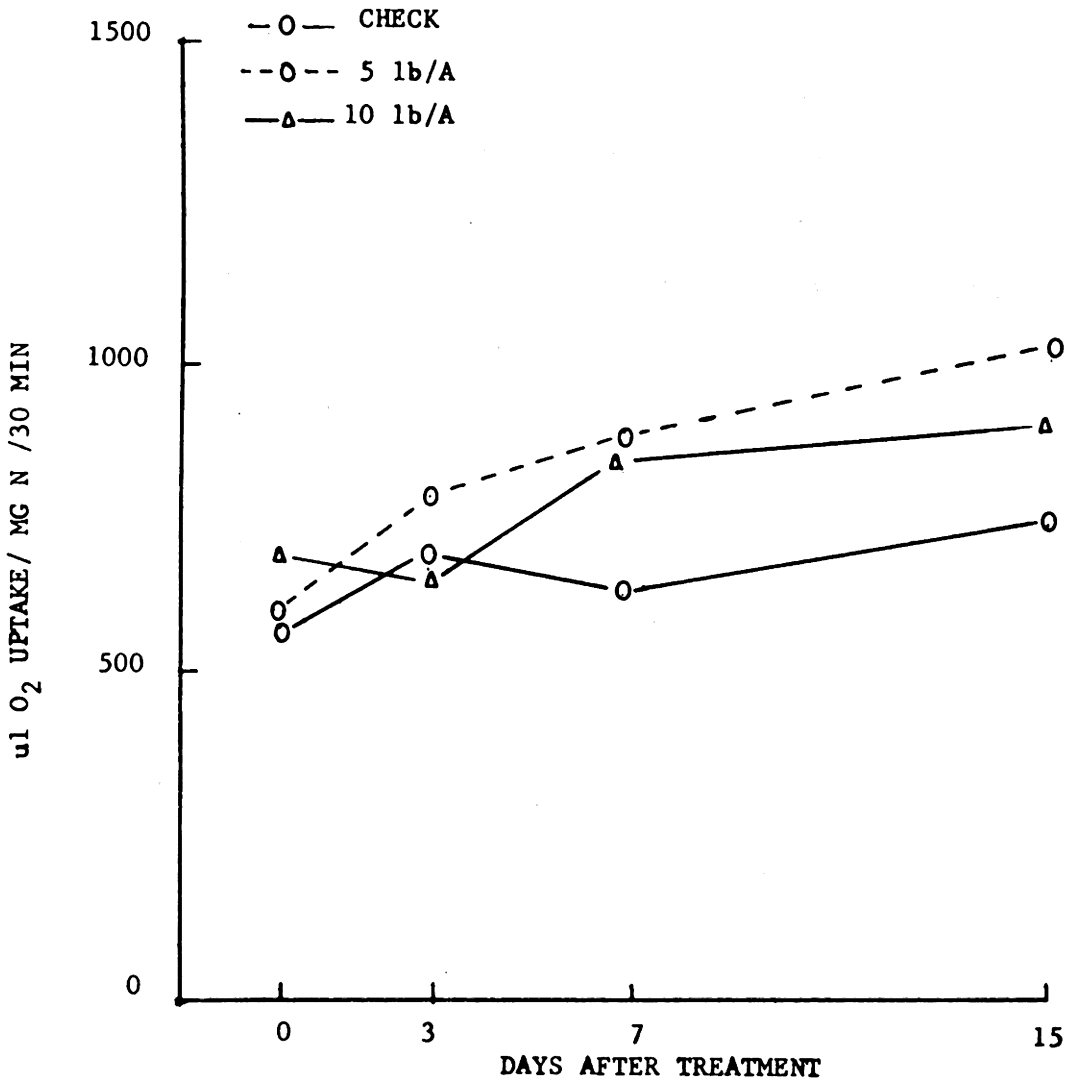



Figure 14. The effect of 0, 5, and 10 lb/A fenac on the oxidation of catechol.

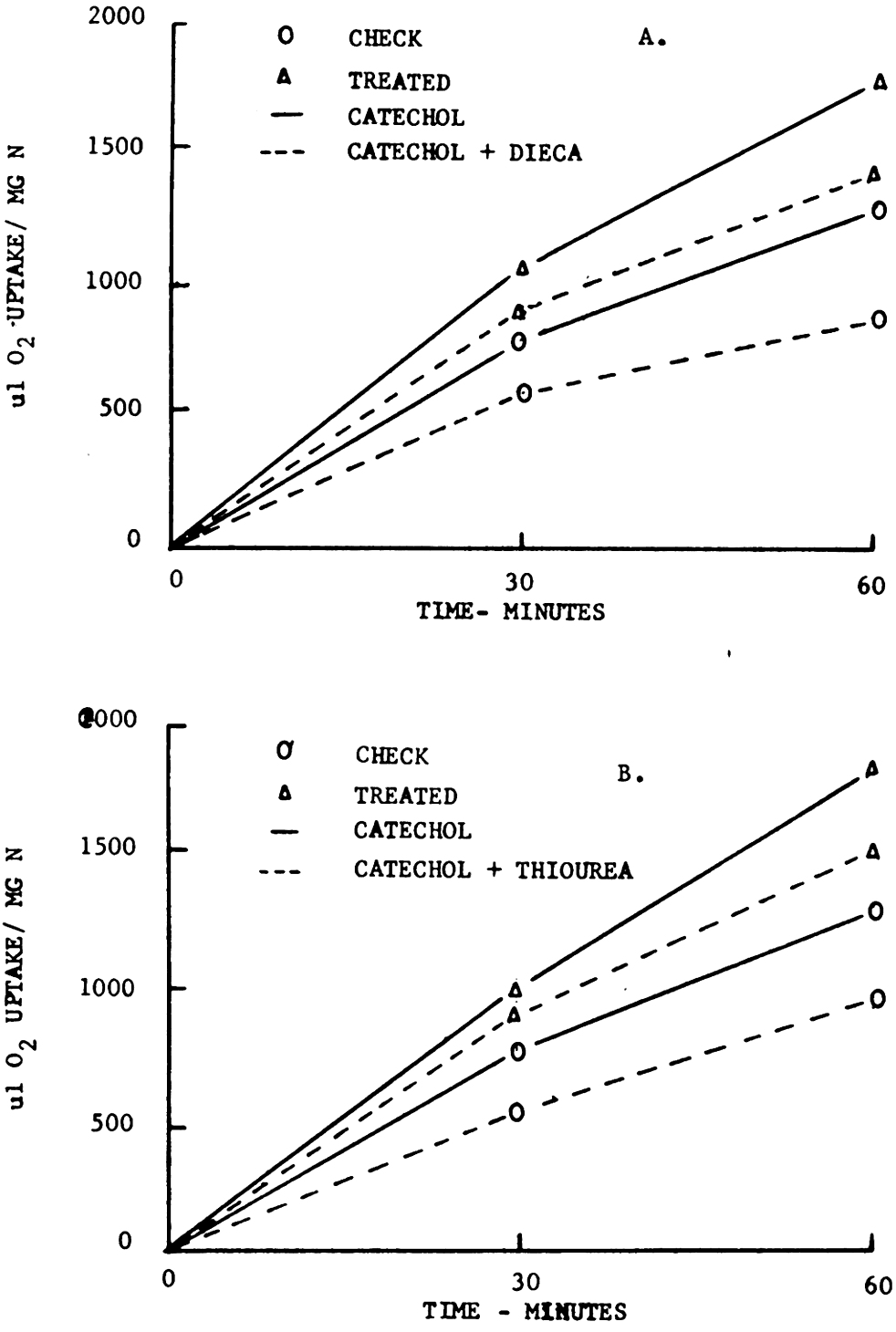



Figure 15. The effect of (A) dieca and (B) thiourea on the oxidation of catechol in homogenates of treated and untreated mugwort rhizomes.

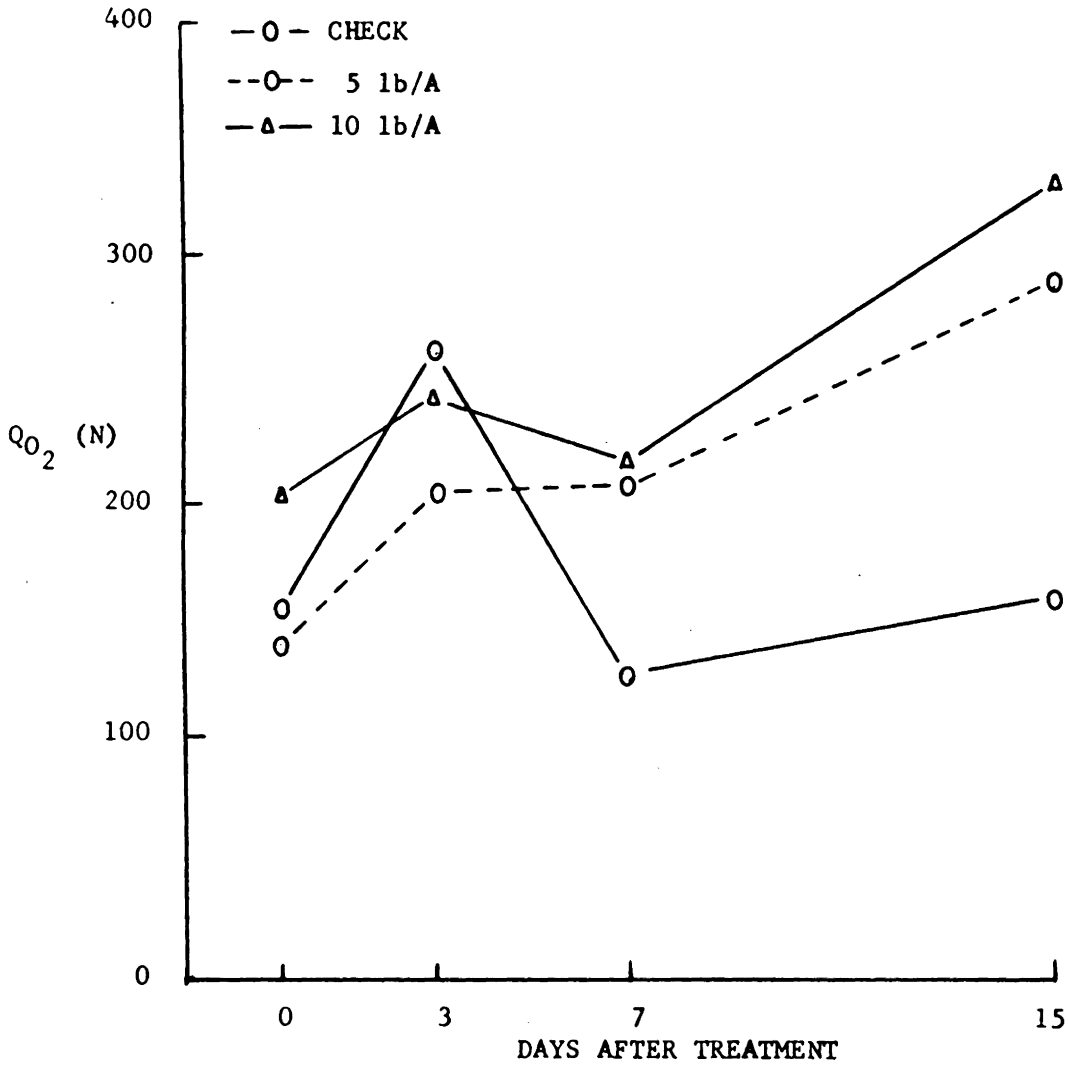



Figure 16. The oxidation of ascorbic acid by mugwort rhizome homogenates treated with 0, 5, and 10 lb/A fenac.

and the oxygen uptake evaluated. An additive effect was observed as shown in Figure 17, and this gave an indication that the small increase in the combined substrate activity was coming from the ascorbic acid oxidase.

Catalase activity was studied at intervals during a 15 day period after fenac treatment. Treated and untreated rhizomes showed about the same activity for seven days. There was about twice as much activity in the treated plants after 15 days (Figure 18A).

Potassium cyanide inhibits metalloprotein enzymes which contain hematin as their prosthetic group. The use of potassium cyanide (1 mM) resulted in about 50 per cent inhibition of catalase activity in treated and untreated rhizomes (Figure 18B).

A very active peroxidase system was found in the rhizomes. Fenac caused quite a stimulation in the activity seven days after treatment (Figure 19). There was about three times more activity in the treated plants than in the untreated plants at the end of the testing period.

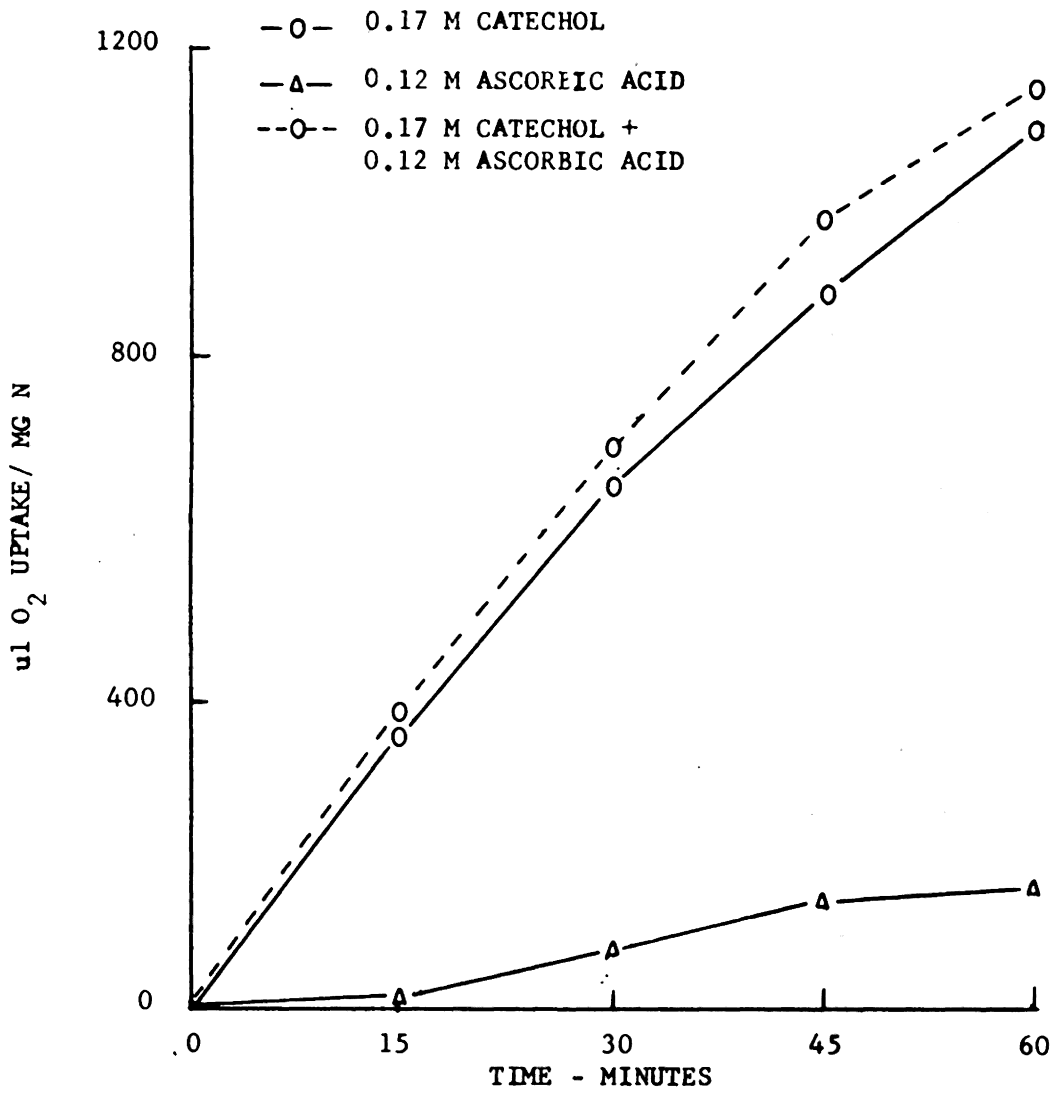



Figure 17. The oxidation of combined and separate substrates, catechol and ascorbic acid, in homogenates of untreated mugwort rhizomes.

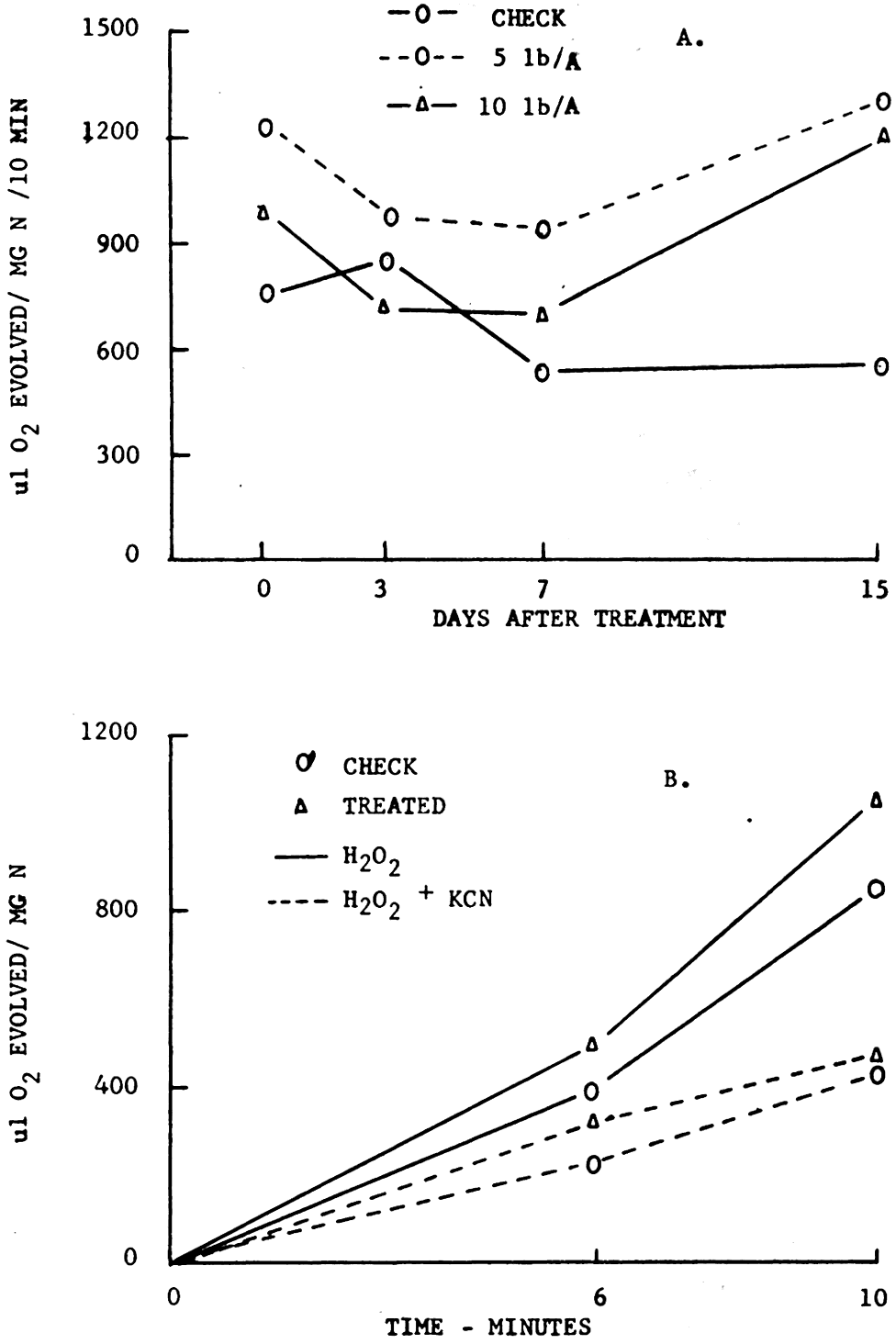



Figure 18. A. Catalase activity of mugwort rhizome homogenates after treatment with fenac. B. The inhibition of catalase activity by 1 mM KCN in untreated and fenac treated rhizomes.

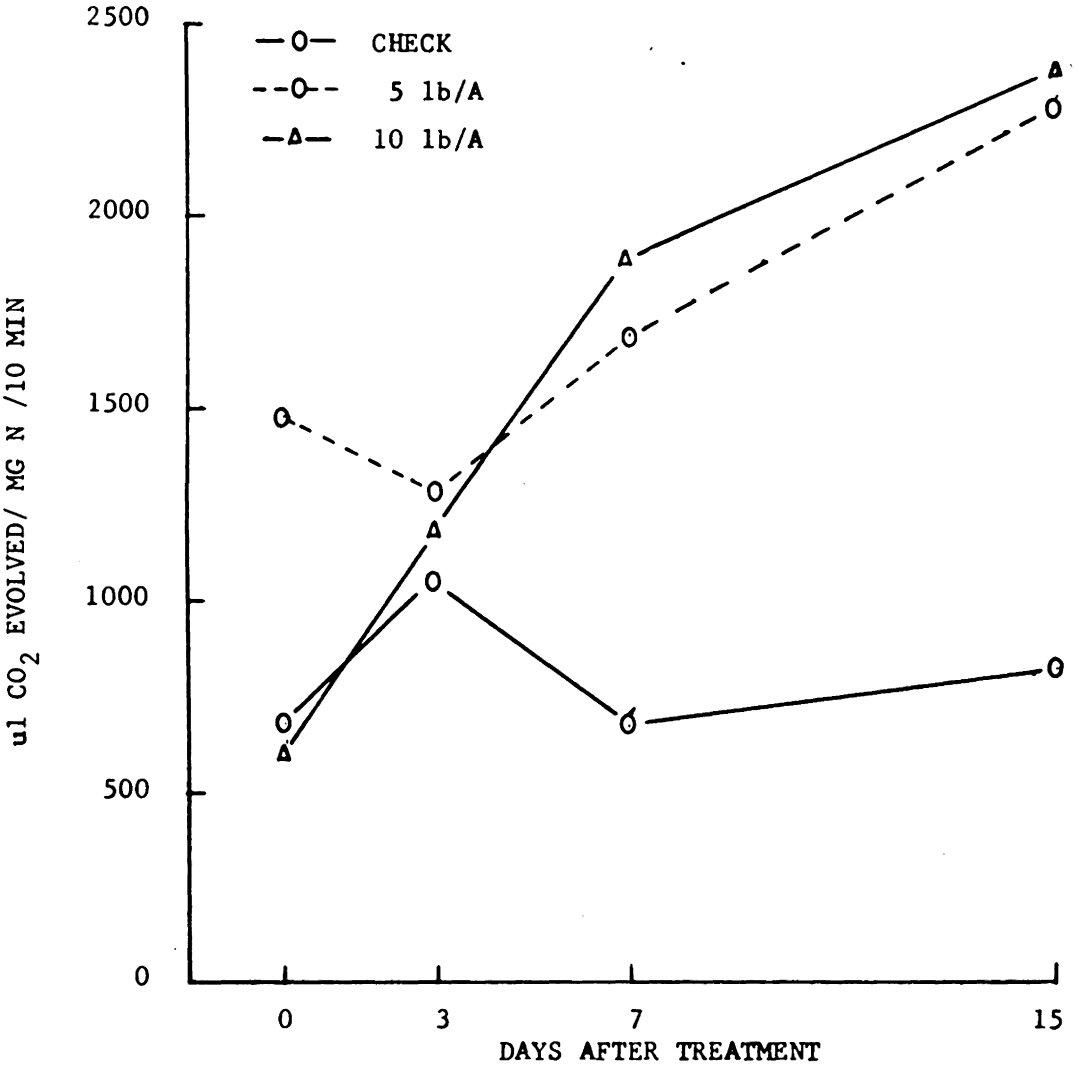



Figure 19. The effect of fenac at 0, 5, and 10 lb/A on peroxidase activity.

## SUMMARY

Studies on growth, control measures, and effects of fenac on respiration and respiratory enzymes of mugwort were conducted.

In a rhizome section study, each of the two-inch sections initiated one shoot, while there was more than one shoot from four and eight-inch sections. Only 61 per cent of the one-inch sections initiated shoots.

After five weeks, all positions along 12 to 18 inch mugwort rhizomes produced shoots, so there was no difference in viability of older or younger areas of rhizomes.

In the field growth study, rhizome spurs were initiated on the transplanted mugwort plants after about four weeks. From the seventh through the twelfth week, there was rapid growth with a large increase in rhizome length and weight. Secondary plants were initiated from new rhizomes about seven weeks after transplanting. Throughout this study, rhizomes were not found below two or three inches in the soil. Flowering was first observed about August 15. When the mugwort plants had completely flowered, there was an average of about 80 feet of rhizomes per plant.

Rhizomes overwintered and began to produce new plants in March, 1964, the next year after the experiment was started.

In a greenhouse study, fenac was mixed with soil and rhizome sections were planted. One ppm fenac was almost enough to prevent sections from initiating shoots. Shoot growth was not observed at two ppm fenac.

In a laboratory study, fenac was mixed with nutrient solution at various rates and shoot growth was measured. Growth was observed at rates of two ppm and below. There was a significant stimulation of growth from the one-fourth ppm treatment when compared with the control growth.

Field control studies involved mainly the use of fenac at various rates alone or in combination with other herbicides. Fenac at 2.5 lb/A did not result in any appreciable control of mugwort. Dicamba and 2,4-D, when each was used alone at 5 lb/A in March, gave no control and actually tended to stimulate growth. When these herbicides were used in combination at 2.5 and 2.5 lb/A, respectively, during April, more than 95 per cent control was observed. A mixture of fenac, 2,4-D, and dicamba at 2.5, 2.5, and 5 lb/A, respectively, applied in March, resulted in about 99 per cent control a year later. Tordon applied at 0.7 lb/A in May gave 100 per cent control of mugwort.

In the respiration studies, fenac caused a small increase in oxygen uptake of mugwort rhizomes. Iodoacetic acid and sodium flouride inhibited oxygen uptake of both fenac treated and untreated rhizomes.

Of the terminal oxidases studied, polyphenol oxidase was the most active. Fenac tended to cause a small increase in the polyphenol oxidase activity as evaluated using catechol as the substrate. Ascorbic acid oxidase activity appeared very low in mugwort rhizome homogenates.

Fenac caused an increase in catalase activity after seven days.

The most significant effect from fenac was observed in the peroxidase study. Between the third and fifteenth day after treatment, there was three

times more activity in the fenac treated plants than in untreated plants.

During the respiratory enzyme studies, no significant differences were noted between the 5 and 10 lb/A fenac treatments.

# BIBLIOGRAPHY

Albert, A. and W. S. Gledhill. 1947. The choice of a chelating agent for inactivating trace metals. Biochem. J. 41: 529-533.

Appleman, D. 1951. Manometric determination of catalase activity: Apparatus and methods. Anal. Chem. 23: 1727-1732.

Anderson, L. E., A. P. Appleby, and J. W. Weseloh. 1960. Characteristics of Johnsongrass rhizomes. Weeds 8: 402-406.

Beevers, H. 1961. Respiratory Metabolism in Plants. Row, Peterson and Co., White Plains, N. Y. pp. 65-118.

Bendall, D. S. and R. Hill. 1956. Cytochrome components in the spadix of Acum macullatum. New Phytologist 55: 206-212.

Bing, A. and A. M. S. Pridham. 1963. Field trials for control of Artemisia vulgaris. Proc. NEWCC. 17: 202-205.

Bing, A. and A. M. S. Pridham. 1964. The use of EPTC for control of Artemisia vulgaris L. Proc. NEWCC 18: 242-244.

Bingham, S. W. 1963. Chemical control of Artemisia vulgaris L. Proc. SWC. 16: 170-171.

Bingham, S. W. and W. K. Porter, Jr. 1961. Glycolic acid oxidase, catalase, and peroxidase of cotton tissue. Weeds 9: 299-306.

Bonner, J. and S. G. Wildman. 1946. Enzymatic mechanisms in the respiration of spinach leaves. Arch. Biochem. 10: 497-518.

Bonner, W. D. 1957. Soluble oxidases and their function. Ann. Rev. Plant Physiol. 8: 427-452.

Boswell, J. G. and G. C. Whiting. 1938. A study of the polyphenol oxidase system in potato tubers. Annals of Botany 2: 847-863.

Dowler, C. C., P. F. Sand, and E. L. Robinson, 1963. The effect of soil types on preplanting soil-incorporated herbicides for witchweed control. Weeds 11: 276-279.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

Eastin, E. F., R. D. Palmer and C. O. Grogan. 1964. Effect of atrazine on catalase and peroxidase in resistant and susceptible lines of corn. Weeds 12: 64-65.

Eichenberger, E. and K. V. Thiamn. 1957. Terminal oxidases and growth in plant tissues. IV. On the terminal oxidases of etiolated pea internodes. Arch Biochem. Biophys. 67: 466-478.

Ettori. J. 1949. The estimation of peroxidase activity. Biochem. J. 44: 35-38.

Funderburk, H. H., Jr., and W. K. Porter. 1961. Effects of N-(3,4-dichlorophenyl) methacrylamide on growth and certain respiratory enzymes of corn. Weeds 9: 538-544.

James, W. O. 1953a. The use of respiratory inhibitors. Ann. Rev. Plant Physiol. 4: 59-90.

James, W. O. 1953b. The terminal oxidases in the respiration of the embryos and young roots of barley. Proc. Roy. Soc. (B) 141: 289-299.

Johnson, B. G. and K. P. Buchholtz. 1961. An in vitro method of evaluating the activity of buds on the rhizomes of quackgrass. Weeds 9: 600-606.

Kabat, E. A. and M. N. Mayer. 1961. Experimental Imnochemistry. 2nd ed. Charles C. Thomas Publisher. pp. 476-483.

Keilin, D. 1925. On cytochrome c, a respiratory pigment common to animals, yeast, and higher plants. Proc. Roy. Soc. (B) 141: 312-339.

Keilin, D. and E. F. Hartree. 1945. Properties of azide-catalase. Biochem. J. 39: 148-157.

Kenton, R. H. and P. J. Mann. 1953. The oxidation of certain dicarboxylic acids by peroxidase systems in presence of manganese. Biochem. J. 53: 498-505.

Kiraly, Z. and G. L. Farkas. 1957. On the role of ascorbic acid oxidase in the parasitically increased respiration of wheat. Arch. Biochem. Biophys. 66: 474-485.

Lardy, H. A. 1949. Respiratory Enzymes. Burgess Publishing Co., Minneapolis, Minn. pp. 159-178.

McWhorter, C. G. 1961. Morphology and development of Johnson grass plants from seeds and rhizomes. Weeds 9: 558-562.

Mapson, L. W. 1958. Metabolism of ascorbic acid plants: Part I: Function. Ann. Rev. Plant Physiol. 9: 119-151.

Millhollon, R. W. 1963. An evaluation of fenac and related compounds for the control of seedling Johnsongrass and other weeds in sugarcane. Proc. SWC. 16: 91-93.

Nicholls, P. and G. R. Schonbaum. 1963. In 'The Enzymes'. 2nd ed., Boyer, P. D., H. Lardy and K. Myrback. Academic Press, New York, p. 158.

Palmer, R. D. and W. K. Porter. 1959. The metabolism of nut grass (Cyperus rotundus). IV. The activities of certain enzymes from tubers treated with amitrol. Weeds 7: 511-517.

Rridham, A. M. S. 1954. Artemisia vulgaris-control in nursery plantings. Proc. NEWCC. 8: 233-234.

Pridham, A. M. S. 1957. Response of Artemisia vulgaris to amino triazole in amounts up to 16 lb/A. Proc. NEWCC. 11: 80-82.

Pridham, A. M. S. 1963. Propagation of Artemisia vulgaris from stem cuttings. Proc. NEWCC. 17: 332.

Pybus, M. B., R. L. Wain, and F. Wightman. 1958. New plant growth substances with selective herbicidal activity. Nature 182: 1094-1095.

Rabbitt, A. E. and R. N. Cook. 1964. Control of Artemisia vulgaris around established shrubs. Proc. NEWCC. 18: 239-241.

Rogerson, A. B. and S. W. Bingham. 1964. A growth study and seasonal characteristics of Artemisia vulgaris. Proc. SWC. 17: 360-363.

Sand, P. F., E. L. Robinson, and C. Dowler. 1964. The effect of several herbicides on the control of witchweed in corn. Weeds 12: 7-10.

Sumner, J. B. 1941. The chemical nature of catalase. Adv. in Enz. 1: 163-176.

Sumner, J. B. and A. L. Dounce. 1937. Crystalline catalase. Science 85: 366-367.

Szent-Gyorgyi, A. 1930. On the mechanism of biological oxidation and function of the suprarenal gland. Science 72: 125-126.

Umbriet, W. W., R. H. Burris, and F. F. Stauffer. 1964. Manometric Techniques. 4th ed., Burgess Pub. Co., Minneapolis, Minn. pp. 1-18.

Vengris, J. 1962. The effect of rhizome length and depth of planting on the mechanical and chemical control of quackgrass. Weeds 10: 71-74.

Webster, G. C. 1952. The occurrence of a cytochrome oxidase in the tissues of higher plants. Am. J. Botany 39: 739-745.

Wiese, A. F., and H. E. Rea. 1961. Control of field bindweed and other perennial weeds with benzoic and phenylacetic acids. Weeds 9: 423-428.

Zimmerman, P. W. and A. E. Hitchcock. 1937. Comparative effectiveness of acids, esters and salts as growth substances and methods of evaluating them. Cont. Boyce Thompson Inst. 8: 337-350.

The vita has been removed from the scanned document

ABSTRACT

Several studies were conducted with Artemisia vulgaris (mugwort). The first study involved the observation of shoot initiation from various length rhizome sections and positions. A second study was conducted to observe growth habits of mugwort and to determine the amount of infestation and production in a growing season. The third study was conducted to determine fenac rates that would prevent shoot growth from mugwort rhizomes. Also, various herbicides were evaluated in field experiments for the control of mugwort. The last study involved the effects of fenac on respiration and some respiratory enzymes.

The most uniform plants were observed from two-inch rhizome sections. There was no difference in bud viability of older or younger areas of rhizomes.

New rhizome production became rapid about seven weeks after small mugwort plants were transplanted to the field. There was a continuous increase in shoots and rhizomes throughout the growing period. New plants were initiated in the spring of the next year from rhizomes that had overwintered in the soil.

Fenac, when mixed with soil at two ppm or above, prevented shoot initiation from rhizome buds. When fenac was present in nutrient solution at rates above two ppm, no shoot growth was observed. In the field control experiments, fenac, dicamba, and Tordon tended to give the best control of mugwort.

There tended to be an increase in the enzymatic activity of mugwort after the plants were sprayed with fenac.