THE ISOLATION OF A TOXIN FROM SPRING DEAD SPOT AREAS IN BERMUDAGRASS (*CYNODON* L.C. RICH.) TURF

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PREFACE

The experiments cited in this dissertation are reported in chronological order. Considerable time during this study was devoted to the development of the methodology required for representative bioassays. This necessitated constant adjustments in procedures. It is possible that the results of the earlier bioassays would be drastically different if they were repeated, using later techniques.

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CHAPTER I

INTRODUCTION

Bermudagrass (Cynodon dactylon (L.) Pers.) is perhaps the best adapted and most versatile turfgrass for use in warm humid regions of the United States. It provides a dense, low growing turf as a result of its creeping growth habit. The stolons, and rhizomes, which are characteristic of bermudagrass growth, greatly contribute to the abundance of meristematic tissue in bermudagrasses. Recuperative potential, therefore, is excellent.

Not only is the potential great, but the rate of growth is probably the highest of all warm season turfgrasses. (Busey and Myers, 1979). Both factors are the main contributors to the rapid recovery of bermudagrass from injury. Establishment of this grass is also quick.

Busey and Myers (1979) have measured the daily growth rate of single common bermudagrass plants as an increase of 9.15 percent of their fresh weight. They calculated that if a one-square meter common bermudagrass turf could continue to grow at the 9.15 percent rate for one year, it would cover an area equal to 50 percent of the land area of the world. Due to self-inhibition, however, this rate has never been observed in turf.

This incredible growth rate is one of the most important factors that characterizes the excellent adaptation of bermudagrass as a turf type grass. Unfortunately, it is also one of the leading contributors to a major problem in bermuda turfs. Restriction of bermudagrass to a limited area is usually a seasonlong problem. With both stolons and rhizomes rapidly spreading laterally the turf eventually grows beyond its borders.

One look at a sidewalk, adjacent to a bermudagrass turf, will convince anyone that even concrete does little to halt this advancement! Barriers both above and below ground, however, are used extensively with some degree of success. Cultivation is perhaps the most effective control, but it is usually short lived. At present, only non-selective herbicides are available for bermudagrass control. The application of one of these chemicals severely limits the use of the treated area.

For unlimited use of these areas, therefore, a chemical control would have to be selective for only bermudagrass. Seasonlong soil activity would also help minimize the time and cost involved in application.

Spring dead spot (SDS) is a disease of bermudagrass which can be characterized by the appearance of small circular areas of dead grass noticeable first as it breaks dormancy in the spring. These necrotic areas remain devoid of bermudagrass throughout the next year or two. Weeds, how-

ever, readily invade the dead areas and persist with seemingly no ill effects. An area infected with SDS is illustrated in Figure 1.

This selectivity of the SDS environment might be the key to selective biological control of bermudagrass. Since the spots normally do not increase in size through the period of active bermudagrass growth, the restriction of bermudagrass development by an active pathogen is questionable. However, the presence of a fungus produced toxin is very probable.

If this toxin is produced only during active pathogen growth, then it would appear to be very resistant to degradation. Possibly, persisting for the entire season! Leaching of the toxin is minimal. This is supported by the lack of pronounced down hill movement of SDS on sloped turfs.

Other toxin mechanisms are also possible. An extemely slow growing fungus which continuously excretes a toxin could also produce the observed symptoms. A degradation by-product of a pathogen metabolite is also a possibility.

This research was initiated to investigate the possibility of a SDS related toxin, regardless of the mechanism of production. The objectives of this research were:

1. To isolate the toxic agent(s) of spring dead spot.
2. To characterize any isolated toxin.
3. To determine the ability of these toxins to eradicate bermudagrass.

Figure 1. Spring Dead Spot in Bermudagrass Turf on the Apron of the Seventeenth Green at Ponca City Country Club

CHAPTER II

REVIEW OF LITERATURE

Etiology of Spring Dead Spot

Spring dead spot (SDS) is a disease of bermudagrass which is observed in early spring as well defined, circular areas, that seem to remain dormant after the surrounding turf has greened-up. With close examination, however, Wadsworth and Young, (1960) reported the roots and rhizomes in the dead spots were black and rotted.

They reported that spring dead spot was first observed in Stillwater, Oklahoma during the spring of 1954. Mr. Bob Dunning, a Tulsa area golf course superintendant, related to Wadsworth and Young that he observed spring dead spot as early as 1936. Although the disease may have occurred earlier, it did not become a serious problem until the late 50's. This was probably due to the greatly increased use of bermudagrass as a fine turf according to Wadsworth and Young(1960). They also observed that SDS was found only in high maintenance turf.

The greatest incidence of SDS is found in the transition zone of bermudagrass adaptation. Frederiksen (1964) described the area as being just south of the line roughly

from Tulsa to Kansas City, to St. Louis, to Indianapolis, to Philadelphia to central New Jersey. McCoy (1967) has stated that SDS only occurs where it is cold enough in winter to induce dormancy in bermudagrass. He feels there is a correlation between disease severity and the length of the dormant season.

In most cases, all the grass in a SDS area is dead. There are exceptions, however, according to Wadsworth and Young (1960), in a few spots, tufts of grass may survive in the center. Regrowth of the bermudagrass in these areas is usually delayed for 3 or 4 years. When regrowth does begin it is from the outside in. This can occur when stolons from the perimeter of the area bridge the spot and become rooted on the far side, according to Wadsworth and Young (1960). They also stated that roots arising from nodes in contact with the dead spot area soon become blackened and rot away.

According to Wadsworth and Young (1960), SDS has only been observed on bermudagrass. They stated that the disease occurs on all varieties with the most extensive damage occurring on U-3.

Kozelnicky (1974) reports that SDS is not associated with any one soil type, or topography. He also has only observed SDS in bermudagrass under high management. In an attempt to reproduce SDS, Kozelnicky (1979), inoculated healthy, mature bermudagrass (Tifway and Tifgreen) with Helminthosporium rostratum, H. spiciferum, Fusarium roseum,

and Curvularia spp. singly and in all combinations. No SDS disease symptoms were detected.

In surveying roots of SDS affected grass, Kozelnicky (1974), found at least five genera of nematodes. They were found only in low numbers, however, and he assumed they had no role in the disease incidence. It was also determined that mycoplasma were not causal agents.

SDS Research in Arkansas

Research conducted in Arkansas by Dale and Diaz (1963), indicated that soil in SDS and healthy turf areas did not differ in pH, fertility level, or organic matter content. Examinations of roots and counts of nematode populations in the soil showed little evidence that nematodes were a factor in the disease. They also reported that in most instances SDS areas filled in with crabgrass or bluegrass and they did not appear to be affected with the disease.

Dale and McCoy (1964) observed the presence of a scale insect Odonaspis ruthae in SDS areas on a lawn in Little Rock, Arkansas. Rhizomes in the center of these infected spots were dead and the ones on the margins were declining. Adjacent lawns were not affected. Except for this one isolated instance, harmful soil insects were not found in any other SDS areas and were discounted as a causal agent.

In 1979, Dale found that when SDS areas were thoroughly tilled, and resprigged with bermudagrass, the spots filled in normally. He also stated that the symptoms of SDS in

Arkansas were the same as those observed in other areas, but the amount of spotting was not as prevalent as in some other areas.

Although, there is no expression of symptoms in the above ground portions of SDS areas during the dormant season, roots appear to rapidly blacken and die before "green-up" of the shoots. Kozelnicky (1979) and Lucas (1979) have observed this root necrosis repeatedly in January and February. Kozelnicky also stated that "the amount of grass that dies is dependent on the severity of the winter."

Factors Leading to Disease development

Madison (1970) has proposed that "close mowing, high fertility, thatch, and high traffic are factors that favor SDS development." He stated that "low, wet-spots that receive traffic have the densest soil and the highest disease incidence." Madison feels these areas are also subject to warming trends, followed by sharp drops in temperature. He concluded that SDS is a management disease, in the sense that it appears on well-fertilized, closely, and often mowed turf.

Organisms Associated With SDS Areas

In Georgia, Kozelnicky et al.(1967), grew five roots and stolons of bermudagrass from SDS areas on water agar,

and hempseed agar. The fungi most frequently isolated were species of Fusarium, Helminthosporium, Curvularia, Rhizoctonia, Pythium, Gliocladium, and Helicocephalum. Spiral, dagger, stubby root, and ring nematodes were found in root lesions. Roots and stolons sampled from the center of SDS infections had lower populations of microorganisms than roots and stolons from the outside perimeter. No single fungus was found to predominate the population during the year.

Helminthosporium spiciferum- a Possible Causal Agent

Dale (1979) isolated various fungi from seedlings grown in SDS soil and from roots of older plants growing at the margins of SDS areas. One Fungus, Helminthosporium spiciferum, was predominant in most isolations. In addition to H. spiciferum, Dale also observed that Polymyxa, Olpidium, and Pythium sometimes occur in SDS areas. Although these fungi might weaken, predispose, or kill bermudagrass plants, he was not able to determine their relationship to SDS in Arkansas.

In examining plants at the margins of SDS areas, Dale often noticed that the lower leaves had numerous lesions and chlorotic spots due to H. spiciferum. He considers H. spiciferum a weak parasite, but said it is pathogenic to young grass seedlings. He also stated that "during certain periods of dormancy it might be pathogenic to mature plants."

In 1970, Freeman isolated H. spiciferum from seed of two lots of Arizona produced, common bermudagrass seed. About 25 percent of the seed was contaminated and caused necrosis to the germinated seedlings. Surface sterilizing with a 15 percent clorox solution provided an effective control.

In an attempt to provide a clue to the pathway of infection of H. spiciferum, Gudanskas (1962) tried to establish a disease on Coastal bermudagrass by three methods. He added H. spiciferum to the soil, sprayed on the foliage, and directly injected into crown and stem tissue. Infection only occurred in the directly injected plants. Roots of these diseased plants were rotted and brown in color.

Wadsworth and Young (1960) isolated H. spiciferum from soil in SDS areas in Oklahoma more frequently than any other fungus. However, Wadsworth (1966), when working with SDS area soils from California, rarely isolated H. spiciferum. He found a species of Ophiobolus in 67 percent of the soil samples. In an investigation in 1966, Wadsworth used five isolates of H. spiciferum (Bainier) Nicot., one isolate of H. cynodontis Marignoni, and three isolates of Ophiobolus sp. to determine their pathogenicity toward common and U-3 bermudagrass. All the isolates produced some root-rotting of bermudagrass at low temperatures (6 degrees C). The rotting was not severe enough however, for the expression of any above-ground symptoms. Since SDS is not generally observed in bermudagrass turf until the third spring after

establishment, Wadsworth suggested that high levels of inoculum were needed for disease expression. Therefore, greenhouse soil-inoculations, such as those tested, would probably have too low of levels of inoculum.

Other Possible Causal Organisms

Reportedly H. spiciferum and phytototoxins are not the only agents that have been related to the the incident of SDS. Wilcoxen (1976) stated that possibly a complex of Helminthosporium, Fusarium, and Rhizoctonia species produce the collective effect known as SDS.

Lucas (1979) has associated some unidentified basidiomycetes species with SDS areas. Cultures of these fungi reduced the root systems of bermudagrass grown in a greenhouse.

In 1931, Broadfoot and Cormack (1941) isolated a low-temperature basidiomycete from turf grass damaged by snow mold. The fungus was found to have a wide host range and grew on most media at 0-18 C. No fruiting bodies were ever produced, therefore, the fungus was never identified.

A disease similar to SDS was first described by Smith (1971) on Couch grass (bermudagrass) in New South Wales. Smith also named this disease spring dead spot. He observed the symptoms of SDS from autumn to mid-summer, but most often in autumn after wet, cold weather. Smith found that the spots did not increase in size during dormancy.

In summer the spots healed slowly by growing in from the edges, but the larger spots completely healed only when they were resprigged. By mid-summer symptoms of the disease have usually disappeared. SDS occurred yearly in the same spots, with increased size, however, seasonal conditions may interrupt the pattern.

Roots and stolons of infected grass are severely rotted and a septate, dark brown mycelium which forms runner hyphae is associated with the diseased parts acording to Smith. Numerous dark, brown, flattened sclerotia occur on the stolons and sometimes in the infected roots. The sclerotia range from 40 to 400 micrometers(um) in diameter. Thick-walled, carbonacious ascocarps with well-developed necks are occasionally present on infected stolons. Smith consistently isolated (Leptosphaeria narmari J. Walker and A.M. Smith) from roots, sclerotia and ascospores from infected plants.

Single ascospore isolates of L. narmari from couch grass were maintained at 25 C on potato dextrose agar (PDA) and were used as inoculum. One half centimeter(cm) squares of L. narmari on PDA were buried two cm from couch seeds in three soil mixes an unsterilized sandy loam, a pasteurized sandy loam, and a pasteurized mix of half sandy loam and half sand in 14 cm clay pots. This experiment was also repeated using inoculated roots. Turf established from seed for five months was also inoculated with L. narmari placed

in 6 cm holes. Disease symptoms were found in all inoculated pots and the uninoculated pots remained healthy. L. narmari, sclerotia, and ascocarps were then re-isolated from the diseased grass. In the established turf, which grew well until winter, disease symptoms also developed. L. narmari and sclerotia were again isolated from infected roots.

The temperature range for optimum growth of L. narmari in sandy loam soil was found by Smith to be between 10 and 20 degrees C. He was successful in controlling SDS with either Thiram (80%W/W) at 4.3 ounces(oz.) per 1000 square feet or Nabam (30%W/W) at 17 fluid (fl oz.) per 1000 square feet. These were applied every four weeks from fall to early spring. All fungicides were drenched in with 60 gallons (gal.) water per 1000 square feet.

Spring Dead Spot Toxin

Bermudagrass rarely re-established in SDS infected areas, therefore Wadsworth, (1966) concluded that it seemed possible that a phytotoxin might be involved with the disease development. He felt that if a toxin were produced, it would likely be insoluble in water, because the dead spots persist for several years and usually remain circular on hills or slopes. It was, therefore, decided to test culture filtrates of the isolates rather than SDS soil extracts. The results of all tests appeared to be negative. Wadsworth (1966) concluded that leachates from diseased spots would

ultimately have to be tested to determine the presence of a toxin.

Diaz (1964) also consistently isolated H. spiciferum from bermudagrass seedlings grown in SDS infested soil. Using these isolates to infest sterilized soil, Diaz found a 40 percent reduction in bermudagrass seedling emergence from those grown in non-infested soil The seedling survival rate was reduced by 80 percent. With similar methods, he obtained a 27 percent decrease in Kentucky bluegrass (Poa pratensis) germination and 269 percent increase in crabgrass (Digitaria sp.) germination when grown in H. spiciferum infested soil. Mature bermudagrass plants however, were not affected.

Wadsworth et al. (1968) compared the pH of soil from SDS infected areas in four species of bermudagrass with that found in healthy turf. Even though the average pH of the diseased turf was less than healthy turf, the difference in pH was thought to be insignificant in disease development.

Diaz (1964) also observed that filtrates from H. spiciferum cultures inhibited germination and growth of bermudagrass seed and young seedlings, and caused chlorosis and wilting of older plants. The three week old culture filtrates were found to contain 40 parts per million (ppm) of nitrites. Diaz proposed that this high level of nitrites might occur in dead-spots due to increased nitrogen fertilization (nitrates) which H. spiciferum converted to nitrites.

In 1967, McCoy making isolates from SDS infected soil in Oklahoma, also found H. spiciferum in the greatest numbers of samples. Using these isolates and an isolate of Ophiobolus sp. that Wadsworth had obtained from SDS infected soils in California, McCoy attempted to quantify their growth reguirements. He found that H. spiciferum had the greatest radial growth of mycelium at 30C. Sudden temperature changes did not significantly affect the growth rate.

He found the two fungi, H. spiciferum and Ophiobolus sp both grew well over a wide range of pH, the optimum being 5.0 to 8.0, and 5.0 to 7.0, respectively. Both fungi grew well on any of three sources of nitrogen, L-asparagine, KNO3, and NH4NO3. McCoy observed that ethanol extracts of culture filtrates of H. spiciferum were not toxic to bermudagrass. The filtrate of decomposing bermudagrass thatch, however, caused severe inhibition of the growth of bermudagrass roots in the laboratory and greenhouse.

H. spiciferum did infect bermudagrass cuttings, but at warm temperatures (25C) damage to the host was less severe.

Kozelnicky (1974,1979) also searched in vain to isolate a toxin from SDS. Leachates from SDS-infected sod were collected and subsequently passed through "healthy" sod. A slight reduction in topgrowth, as evidenced in clipping weights, was observed. Ryegrass was then used to bioassay SDS filtrate for toxic effects. It seemed that the leachate substantially reduced the respiration rate of the ryegrass, but the results after three trials were inconclusive.

Seeds of common bermudagrass, Pennfine perennial ryegrass, Penncross bentgrass, Golden Cross Bantam sweet corn, Rogers barley, Bragg soybeans, Top Crop beans and Yellow Straightneck squash also were planted in SDS infected soil as an assay of toxin. All species exhibited stimulated growth for the first two weeks after seeding, however, the trend then reversed and all plants then declined. Kozelnicky noted that Poa annua was an exception to this pattern and shows continued extraordinary growth in SDS soil.

The possibility of a mycotoxin, or a toxic intermediate, or derivative from decomposing plant residues, is also supported by work done on other crops or organisms. Meehan and Murphy (1947) observed blighted leaves in oats, while Helminthosporium victoriae was isolated only from basal portions of the plant. They found the presence of a toxin which was relatively stable. It withstood antoclaving at 15 psi. for 20 minutes.

Luke and Wheeler (1955), working with the same toxin, found it to be quite stable at a pH of 4, but it was rapidly destroyed by heat when neutral or alkaline. It was also found to be highly specific for susceptible varieties of oats whose root growth was reduced even in dilutions of one ppm. Dilutions of 10 ppm, however, had no effect on resistant oat varieties, or other grasses, or vegetables.

A host specific toxin has also been associated with Helminthosporium carbonum. Scheffer and Ullstrup (1965)

used a susceptible corn line in a bioassay to confirm the presence of a toxin. A resistant corn line was unaffected. Victoxinine, a host-specific toxin, was isolated from H. carbonum and confirmed by paper chromatography.

Toxins From Decomposing Plants

Patrick et al.(1963) observed injury to roots of lettuce and spinach seedlings in fields with fresh crop residue. They found that the injury was mainly confined to those parts that were in direct contact with decomposing plant parts. Organisms that were isolated from root lesions were mostly non-pathogenic. Although some toxic soil extracts were obtained, there was great variability in their duration and distribution in the field.

In 1963, Toussoun and Patrick worked with the following bean root-rot organisms, Fusarium solani F. phaseoli, Thielaviopsis basicola, and Rhizoctonia solani. All these organisms showed an increase in pathogenicity on beans that were first exposed to toxic bean decomposition products.

Cultural Controls

The evaluation of possible cultural controls of SDS were conducted by Kozelnicky (1974) for four years. Complete rejuvenation, aerifying, vertigrooving, and aerifying/vertigrooving of dead spots was performed. These treatments were superimposed with applications of lime, K20, sewage sludge, singly, and in all combinations. Only com-

plete rejuvenation by rototilling to a depth of 12 inches reduced the amount of spots permanently. All other treatments were inconsistent from one year to the next.

In a greenhouse experiment, Kozelnicky (1974) incorporated gypsum (CaSO4) into a clay soil and a sandy loam soil. Using healthy U-3 and Tifway bermudagrass, he showed that the pH of soil and availability of calcium and magnesium increased with the higher concentrations of gypsum, but phosphorus and potasssium levels decreased as the gypsum concentrations increased. No correlation between this mechanism and the occurance of SDS was proposed, however.

Chemical Control of SDS

Control of SDS in the United States has been rather inconsistent. Early work by Waldsworth (1961) indicated that Dieldrin, an insecticide, controlled SDS satisfactory.

A SDS control study involving eight chemical treatments was started in two common bermudagrass lawns at Stillwater in the fall of 1965 (Wadsworth et al. (1967). Again the results showed Dieldrin to be the most effective treatment, however, no definite conclusions were drawn. In another study slight control was obtained with Du-ter, Spring Bak, Polycide, and Dieldrin. This study was repeated for a second year and again the results were inconclusive.

Wadsworth et al (1967) also began control trials on a golf course fairway at Quail Creek Country Club in Oklahoma

City, Oklahoma in 1965. The U-3 bermudagrass fairways were mostly on heavy clay soils. Nineteen chemical treatments were applied to 10x100 feet plots. Half of each plot also was treated with Vineland Chemical Company's wetting agent, #958. None of the treatments gave significant control of SDS, although, the four insecticides, Dieldrin, Aldrin, Kepone, and Chlordane 75 showed slight control.

McCoy (1967) added 15 chemicals (fungicides and insecticides) at three concentrations to liquid cultures of H. spediferum and Ophiobolus sp. Dieldrin was found to be fungistatic with less growth occurring with increasing concentrations. Phenmad, Cu 8-hydroxyquinolinate, Panogen, and BOS allowed no growth of either fungus. The other chemicals tested gave varying degrees of fungal inhibition. In a field experiment, eight chemicals were used as drenches for SDS control in two infected lawns. Only Dieldrin gave any significant degree of control.

As reported by Wilcoxen (1976), a test was conducted in 1973 at the Cherokee Town and Country Club, Dunwoody, Georgia. He used Actidione-Thiram and Daconil 2787 at three oz. of fungicide per 1000 square feet. One area was sprayed in spring, fall, and winter, while another received only a fall and winter treatment. Both areas showed a great reduction in the number of diseased spots. The area that was treated in the spring also healed much quicker than the untreated area.

Collins (1976) evaluated two fungicides in combination with aerification for their effect on SDS. Aerification showed little effect on the action of either nabam or sodium azide. Nabam, when applied monthly from October through March, had the highest percent of recovery in SDS areas of 46.5 percent. The overall average recovery for sodium azide, however, was 36.8 percent.

In a five year study, Kozelnicky (1974-1979) found no consistent control from any of the following fungicides: Benomyl, Dac 2787, Demosary Dyrene, Fore Manzate D, MF324, Panogen, Panterra, PCNB, Spring-bak and Super-x. In an experiment on a Tifgreen bermudagrass golf green, however, five fungicides were found to reduce the number of dead spots over a two year period with no reappearance of spots in treated plots the third year. On the basis of all his research, Kozelnicky has made the following proposals to reduce the incidence of SDS:

1. Regulate the nutrient supply, especially nitrogen, which should be kept to a minimum.
2. Control thatch.
3. Prevent or relieve soil compaction.
4. Regulate water supply carefully.
5. Use preventive schedule of fungicides for the control of all turf diseases.
6. The best time of application is early spring into summer.

CHAPTER III

METHODS AND MATERIALS

Collection of SDS Soil

The initial phase of this investigation was to collect soil from SDS areas. This soil was used in all the subsequent soil extractions. All collections were taken between April 26, 1979, and May 21, 1979.

Despite the severe winter of 1978-1979, the reported incidence of SDS was minimal. Therefore, very few sites were available for collection of SDS samples. In all, samples were obtained from eight sites, located on three golf courses and two homelawns. The site, location, soil texture, and herbicides applied to the site within two months of the sampling date are listed in Table I.

A golf course green hole cutter was used to extract all soil core samples. Three cores were lifted from each dead spot and adjacent healthy turf. The cores were taken in an approximate triangular pattern. After removal from the hole cutter, each core was divided into four parts. First, all thatch material was removed from the top of the core. The remaining soil was divided as follows; the first three centimeters (cm), 3 to 6 cm, and 6 to 9 cm. Any remaining soil

was discarded. In general, three spring dead spots and one healthy area were sampled for each site.

TABLE I

SITE, LOCATION, SOIL TEXTURE AND PREEMERGENCE HERBICIDE APPLIED TO SDS SOIL SAMPLES

Location	Site	Soil Texture	Preemergence Herbicide
Southern Hills Country Club	Eighteenth Fairway	Loam	Benefin
Southern Hills Country Club	Twelfth Rough	Loam	None
Southern Hills Country Club	Sixteenth Fairway	Loam	Benefin
Ponca City Country Club	Tenth Fairway	Silt Loam	None
Ponca City Country Club	Seventeenth Green Apron	Sandy Loam	Benefin
Cushing	Homelawn	----	Benefin
Cushing Country Club	Fourteenth Fairway	Loam	None
Stillwater	Homelawn	Loam	Benefin

Preliminary Tests

A screening for toxin activity was initiated in a greenhouse on June 18, 1979. Seven sites were selected as a

representative group of the sampled population. A split-plot design with four replications was used. The main plots were a factorial arrangement of site and SDS or check (healthy soil sampled from an adjacent area). Subplots were the four depth fractions of each sample: thatch, soil surface to three cm, 3 to 6 cm, and 6 to 9 cm.

The soil for each subplot was placed in 5cm square peat pot. Twenty-five hulled seeds of an experimental bermudagrass hybrid, Guymon X 10978b were placed in the top five millimeters (mm) of soil.

Guymon X 10978b is a F1 progeny of an Oklahoma common strain referred to as "Guymon", and an introduced strain from israel identified as accession 10978. Guymon X 10978b was selected for use in the experiment because it exhibited excellent seedling vigor in previous work (Fermanian 1978).

Each replication was bordered on all sides by a single row of untreated pots. The pots were placed on a mist-table to provide uniform periodic wetting. The mist cycle was for two seconds, every 12 minutes. This sequence was repeated for eights hours each day. Seedling emergence counts were made at 10, 35, and 70 days after seeding.

Helminthosporium spiciferum

Extract Bioassay

Helminthosporium spiciferum is the fungus most often isolated from SDS soil. McCoy (1967) and Wadsworth (1966)

each attempted to extract with water or ethanol, H. spiciferum produced toxins from the filtrate of their liquid culture medium. These experiments showed variable results.

Since the nature of the toxin(s) and the medium requirements needed to enhance its production are unknown. An investigation of the effect that growth medium has on toxin production was needed. An experiment was initiated, August 27, 1979, to extract H. spiciferum produced toxins from the filtrate of several culture media. The following three media were used:

1. nutrient broth
   a. nutrient broth 8 gm
   b. yeast extract 5 gm
2. Richard's Solution
   a. potassium nitrate 10 gm
   b. potassium phosphate, monobasic 5 gm
   c. magnesium sulfate 2.5 gm
   d. ferric chloride .02 gm
   e. sucrose 50 gm
   f. V-8 juice 100 ml
   g. yeast extract 5 gm
3. V-8 broth
   a. V-8 juice 200 ml
   b. calcium carbonate 5 gm
   c. yeast extract 5 gm

All media were made up to a volume of 1000 ml. Approximately 500 ml of each medium was not inoculated, to serve as a check. The cultures and their checks were placed on a shaker and allowed to grow for 17 days at 25C.

The filtrate of the cultures was attained by vacuum. All solutions were vacuum drawn through several layers of "Whatman" No. 4, filter paper. Sterilization was achieved through a 0.2 micrometer (um) millipore filter. The filtrate solutions were then refrigerated for later use in the bioassay.

The bioassay used to detect the presence of H. spiciferum produced toxins was 1) to test the presence of germination inhibitors utilizing Guymon X 10978b bermudagrass seed as the assay crop and 2) three node lengths of bermudagrass stolons were grown in the culture filtrates to detect the inhibition of meristematic tissue.

In the germination test, 25 seeds were placed on two layers of absorbent tissue substrate moistened with five ml of culture filtrate, in a 7 X 7 X 2.5cm box. In both seed and stolon assays the filtrates were arranged in a split-plot design with multiple observations. Contamination of the germination boxes and test tubes was encountered in a preliminary test, therefore, multiple observations and four replications were used to provide a measure of the variability in the preparation technique. The three media were mainplots while the inoculated and uninoculated portions of each medium were the subplots.

The stolon bioassay was evaluated at 7, 14, and 21 days. The total number of nodes that were initiating new shoots or roots were recorded. Percent seed germination was counted after seven day intervals for three weeks.

Plant Clippings Extract Bioassay

Previous studies by McCoy (1967) showed a reduction in root growth of bermudagrass when grown in an environment of decomposing bermudagrass plant parts (thatch). In this study clippings of common bermudagrass were used to simulate thatch. In addition clippings of knotweed (Polygonum aviculare L.)were also tested. Knotweed is often found in bermudagrass turf and sometimes displays antagonistic symptoms. The clippings of both plants were mixed with water in a 1 to 18 ratio. The mixtures were then antocalved at 120C for 1 hr. at 1.03 bars. After cooling, the mixtures were filtered and a bioassay of the extract was prepared by the methods previously described in the H. spiciferum filtrate study. The method of evaluation of both bermudagrass stolons and seed was identical to the methods used in the assay of the culture filtrates. The extracts were arranged in a randomized block design with multiple observations and four replications.

Ethanol and Water Extract Bioassays

Water and ethanol were used as solvents in an attempt

to extract any SDS toxins from the soil samples. The four depth fractions, previously described, from a SDS and a healthy soil sample obtained from a Stillwater homelawn were split-in-half. One-half was used in the ethanol extract, while the other was extracted with water. The procedure used for the extraction, filtration, and bioassay of the extract was as follows:

1. 100 gm of soil was weighed into a 1000 ml flask.
2. 500 ml of either ethanol or water was added
3. The flasks were plugged and shook on a wrist-action shaker for 1.5 hr.
4. All solutions were vacuum-filtered through No. 2 filter paper.
5. The aqueous solutions required the use of a high pressure filtration apparatus for the first filtration.
6. All extracts were then filtered through a 0.2 micrometer, millipore filter.
7. Five ml of each ethanol solution was added to 100 X 15 mm sterile glass, petri dishes, containing one piece of No. 2 filter paper.
8. The lids of the dishes were propped up slightly to allow the ethanol to evaporate.
9. After allowing the ethanol dishes to evaporate for two days, 5 ml of sterile distilled water was added.
10. Five ml of the water extracts were transferred to a sterile, petri dish.
11. Bermudagrass (Guymon X 10978b) and lettuce (Mesa 659) were used in the bioassay.
12. 25 seeds of either bermudagrass or lettuce were added to a petri dish after surface sterilization.

13. Surface sterilization was accomplished by:

    a. A ten second submersion in a 0.5 percent sodium hypochlorite solution,

    b. followed by three, 10 second rinses in sterile water

Identical designs, except for randomization, were arranged for both the lettuce and the bermudagrass seed. A randomized block design with three replications was used. Both experiments were placed in the germinator on November 11, 1979. Eight days later the total number of seeds that germinated in each dish were counted.

Ether Extract Bioassay

In the initial soil bioassay there were indications that the SDS soil sampled from the apron of the seventeenth green at the Ponca City Country Club might contain a toxin. Therefore, on December 6, 1979 an experiment was designed to extract this toxin, with diethyl ether.

The following procedure was used for the extraction and subsequent bioassay:

1. 668 gm of soil, either SDS or healthy soil, with an equal volume (w/v) of water.

2. After 72 hr. the solutions were centrifuged at 5,000 rpm for one hour.

3. The supernate was mixed with 10 ml of diethyl ether and then allowed to separate.

4. The ether fraction was removed and allowed to evaporate.

5. 50 ml of distilled water was added to the residue.

6. A dilution series was prepared as follows:

   a. 25 ml of the aqueous solution was added to 25 ml of distilled water.

   b. Finally, 25 ml from step (a) was brought to 50 ml volume with distilled water.

   c. Six milliliters of each dilution, and 6 ml of distilled water to serve as a check were added to 7 X 7 X 2.5cm germination boxes with two layers of substrate.

7. 50 lettuce (Mesa 659) seeds were then added to each box.

The boxes were arranged in a randomized block designed, with four replications. They were placed in the germinator set for an alternating cycle of 16 hrs of dark at 20C and 8 hrs of light at 30C on December 7, 1979, and seven days later the total number of germinated seeds were counted.

Methanol Extract Bioassays

First Extract

Another experiment was started on January 15, 1980, to extract toxins from a SDS soil sample, using an 80 percent methanol solution as the extractant. The 3 cm fractions of SDS and healthy soil obtained from a Stillwater homelawn were used. The soil extraction procedure and the bioassay of the extract was as follows:

1. An 80 percent methanol solution was prepared.

2. The soil samples, approximately 400 gm each were mixed with an equal volume of the methanol solution (w/v).

3. After shaking for approximately one minute, the mixtures were allowed to stand for 24 hr.

4. The mixtures were filtered several times by vacuum through "Whatman" No. 31 filter paper.

5. The extract was flash-evaporated at 60 degrees centigrade to remove the methanol.

6. After evaporation of all the methanol, the remaining aqueous solution was cooled, and refrigerated until used in the bioassay.

7. The SDS extract was diluted as outlined for the ether extract bioassay.

8. The undiluted, healthy soil extract, distilled water, 0.5 percent methanol solution, and a one percent methanol solution were used as controls.

9. Six ml of each methanol extract dilution, healthy soil extract, and control solution were placed in a 7 X 7 X 2.5cm germination box with two layers of substrate.

10. 50 seeds of either bermudagrass (Guymon X 10978b), or lettuce (Mesa 659), were then added to each box.

All germination boxes were arranged in a single randomized block design with four replications. The boxes were placed in the germinator on January 21, 1980. The germinated lettuce seeds were counted three days later. After seven days, the boxes containing bermudagrass seedlings were evaluated for percent germination, shoot length, and root length.

Second Extract

A second methanol extraction was begun on January 29, 1980. Soil sampled from a Stillwater homelawn was used again. A 6 cm fraction of SDS soil was also used. The extraction of the soil samples and the preparation of bioassay treatments were the same as outlined for the first study.

The experimental design, seed used, and length of time in the germinator was identical to that used in the first study. Evaluation of the growth of both the lettuce, and bermudagrass, was also the same as that for the first extract, however, the root length of the lettuce seedlings was also recorded.

Third Extract

A third and more detailed methanol extract experiment was initiated on February 18, 1980. The preparation and extraction of the soil samples was the same as the previous two experiments.

A major change in the design was made, however. Only bermudagrass seed (Guymon X 10978b) was used in the assay. The germination boxes were arranged in a split-unit design with four replications. The main-units consisted of a factorial arrangement of extracts from SDS and healthy soil samples, from four locations. The extracts from the four depth fractions of each sample were the sub-units.

All boxes were placed in the germinator on February 29, 1980. Seven days later each box was evaluated for percent germination, shoot length, and root length.

Characterization of Methanol Extracted Toxins

Paper and Thin Layer Chromatography

Both paper chromatography (PC) and thin layer chromatography (TLC) were utilized in the attempted characterization of SDS toxin. In the case of PC, however, a technique for the effective separation of any of the extracts was not found. Therefore, experiments involving PC will not be discussed.

The parameters that were common to all TLC runs are listed in Table II. Many solvent systems were tried as in PC. The chloroform:methanol:water system listed on Table II was, however, the most efficient in separating compounds. It was, therefore, the standard solvent used for single, or first dimension runs.

In two dimensional chromatography, a less polar solvent was used to effect a separation of any non-polar compounds. The only solvent that was effective, was a 1:1 ratio of benzene and ethyl acetate. The detecion of all compounds was achieved by viewing the TLC plates under ultraviolet (UV) light. Verification that all compounds on the plates fluoresced under UV illumination was achieved by placing the plate in an iodine chamber.

TABLE II

STANDARD CONDITIONS FOR SDS THIN LAYER CHROMATOGRAPHY RUNS

Conditions	Standards*
Plates	pre-poured silica gel G
Dimension of Layer	200 X 200 mm
Layer Thickness	250 micrometers
Starting Point	30 mms from lower edge 30 mms from left edge samples were 10 mm apart
Length of Run	approximately 150 mm
Sample Size	40-60 microliters
Solvent	chloroform : methanol : water 80 : 20 : 1

*As modified from Stahl (1967)

Column Chromatography

Although TLC was fairly efficient in separating the soil extracts, the quantities of separated compounds were too small to be bioassayed. A preparative technique was needed therefore, to provide larger fractions for assay.

A procedure for column chromatography (CC) as modified from Marvel and Rands (1950) was employed. A 3 cm diameter glass column was used (Figure 2). It was wet packed with a silicic acid

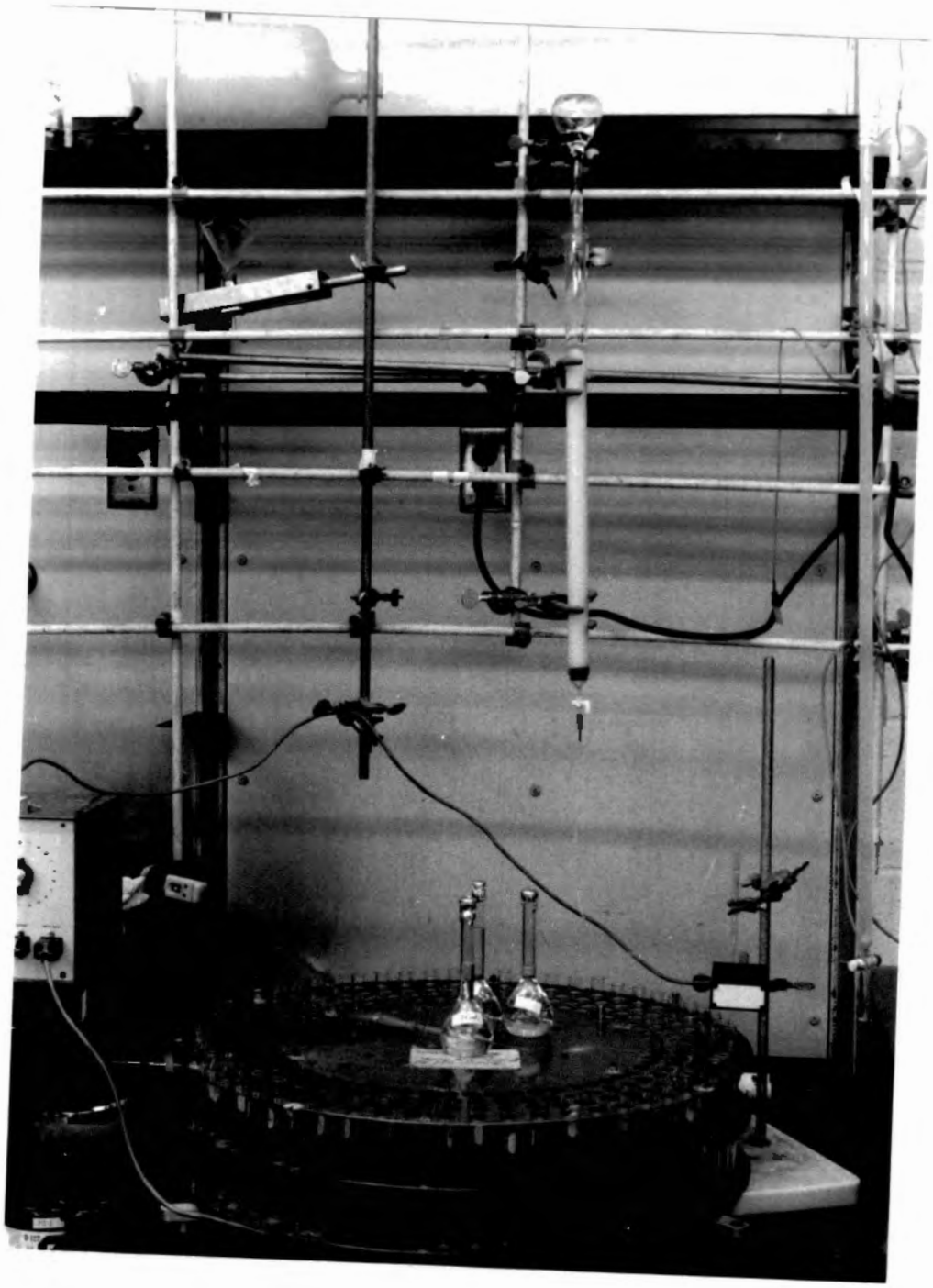


Figure 2. Column and Fraction Collector Used in Column Chromatography

slurry in a 50:50 chloroform-hexane mixture. Water was substituted for 0.5N H_2SO_4, as the stationary phase absorbant. All the SDS soil extracts, from either the Stillwater or Cushing homelawns, were combined into single samples. The two composite samples were lyophilized and redissolved in approximately 2 ml of methanol. After the column was packed, a 1 ml sample was carefully placed on the top of the packings.

The column was developed with an active phase solvent of increasing polarity. A chloroform:methanol:water solvent was added 100 ml at a time. The initial 100 ml of solvent, was composed of 89 ml of chloroform, 10 ml methanol, and 1 ml of water. In each subsequent 100 ml aliquot, the methanol portion was increased by five percent, and the chloroform portion reduced by an equal amount. After the methanol portion reached 35 percent, a final 100 ml aliquot of the solvent, with 35 percent methanol, was added. In the final aliquot, however, 1 ml of acetic acid was substituted for the water. This stripped the column of any remaining sample.

The effluent was collected in test tubes on a fraction collector, at the rate of approximately 2-3 ml per minute. The tubes were changed after they had collected approximately 10 ml of effluent. All tubes were placed in a 50C water bath to promote the evaporation of all liquids.

CC Effluent Bioassay

After drying, the tubes were prepared for the bioassay as follows:

1. 1 ml of methanol was added to each tube.
2. 0.5 ml of the methanol solution was placed on two layers of 3 cm filter paper, in 35 X 10 X 1.5mm petri dishes.
3. One fraction consisted of three concurrent tubes, each tube serving as a replication.
4. After the 0.5 ml of methanol had evaporated, 0.8 ml of distilled water was added to each dish.
5. 10 lettuce seeds (Mesa 659) were placed in each dish.
6. On April 18, 1980, all dishes, after being arranged in a randomized block design, were placed in a germinator.

After three days, the dishes were removed and evaluated for percent germination, and total seedling length (shoot + root).

CHAPTER IV

RESULTS AND DISCUSSION

Preliminary Soil Tests

Evaluations of seed germination and growth of bermudagrass in theses preliminary bioassay were conducted on three different dates. Each date was analyzed separately as a split-plot experiment. The analysis of variance for each date is listed in Appendix Table VIII.

All sources of variation, except for depth of sample and depth of sample X type of sample, exhibited a significant F-value when tested at the five percent level of probability. This was true for all three dates. The implications of these results are misleading, however.

As shown in Figures 3, 4, and 5, at each site, with the exception of the Stillwater homelawn and the apron of the seventeenth green at Ponca City C.C., the mean germination and growth of bermudagrass was greater for the SDS soil. Unlike Kozelnicky's (1974) previous findings, this stimulated growth in SDS soil persisted for up to 70 days.

When the mean germination and growth of bermudagrass in the check soil exceeded that in the SDS soil, the differences were found insignificant when tested by the least sig-

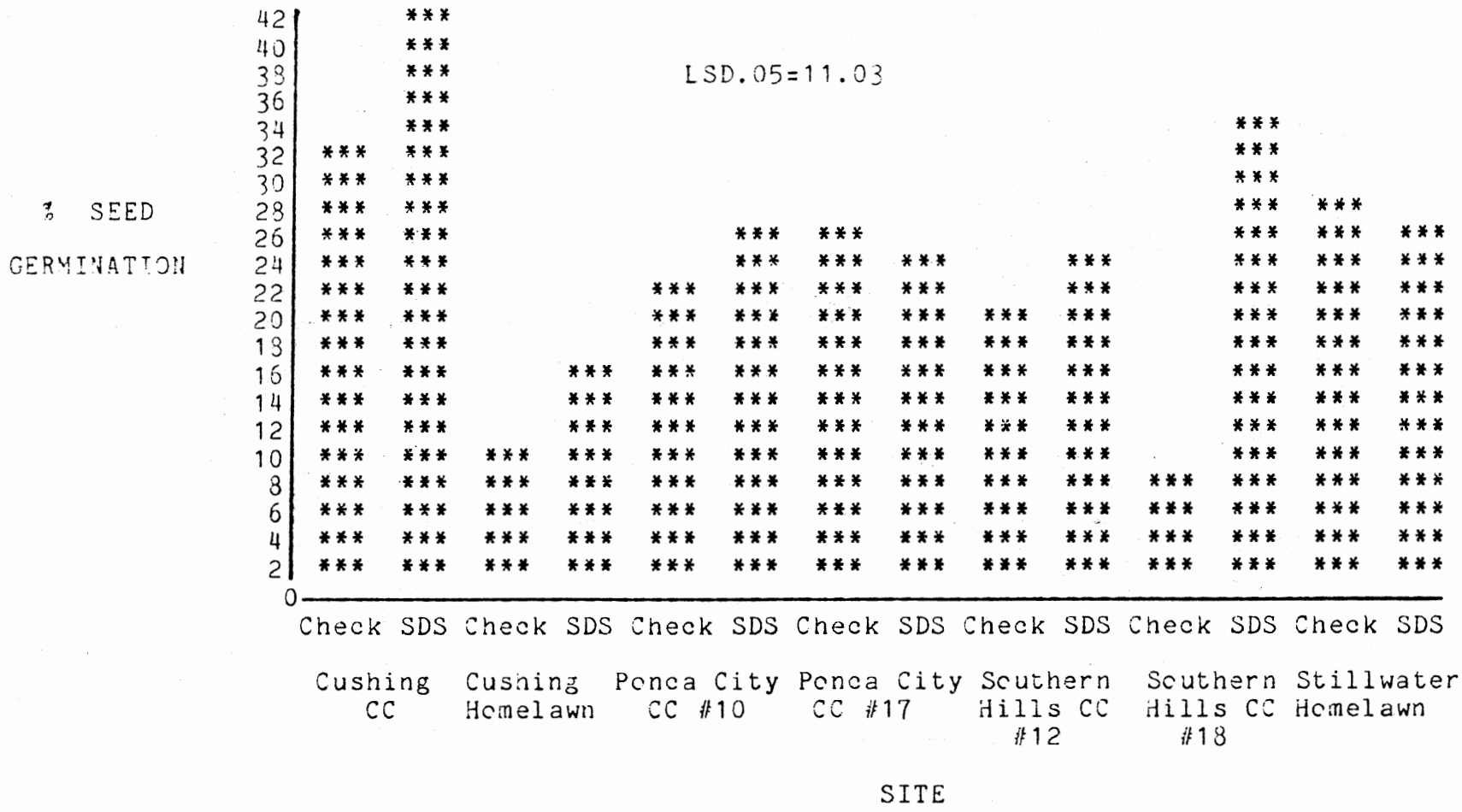


Figure 3. Germination and Growth of Bermudagrass Seedlings Grown Ten Days in SDS and Healthy Soil From Seven Sites

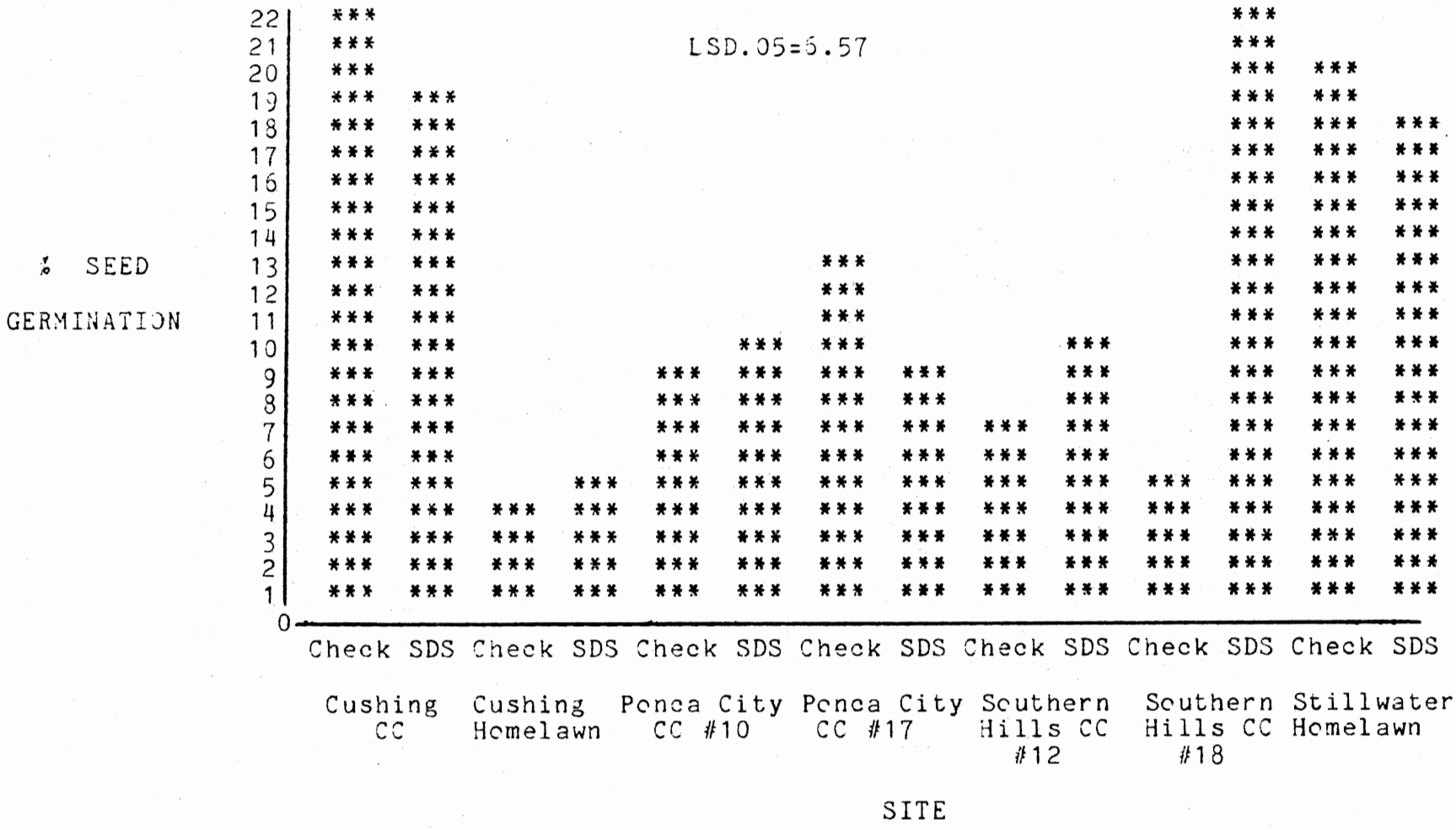


Figure 4. Germination and Growth of Bermudagrass Seedlings Grown Thirty-Five Days in SDS and Healthy Soil From Seven Sites

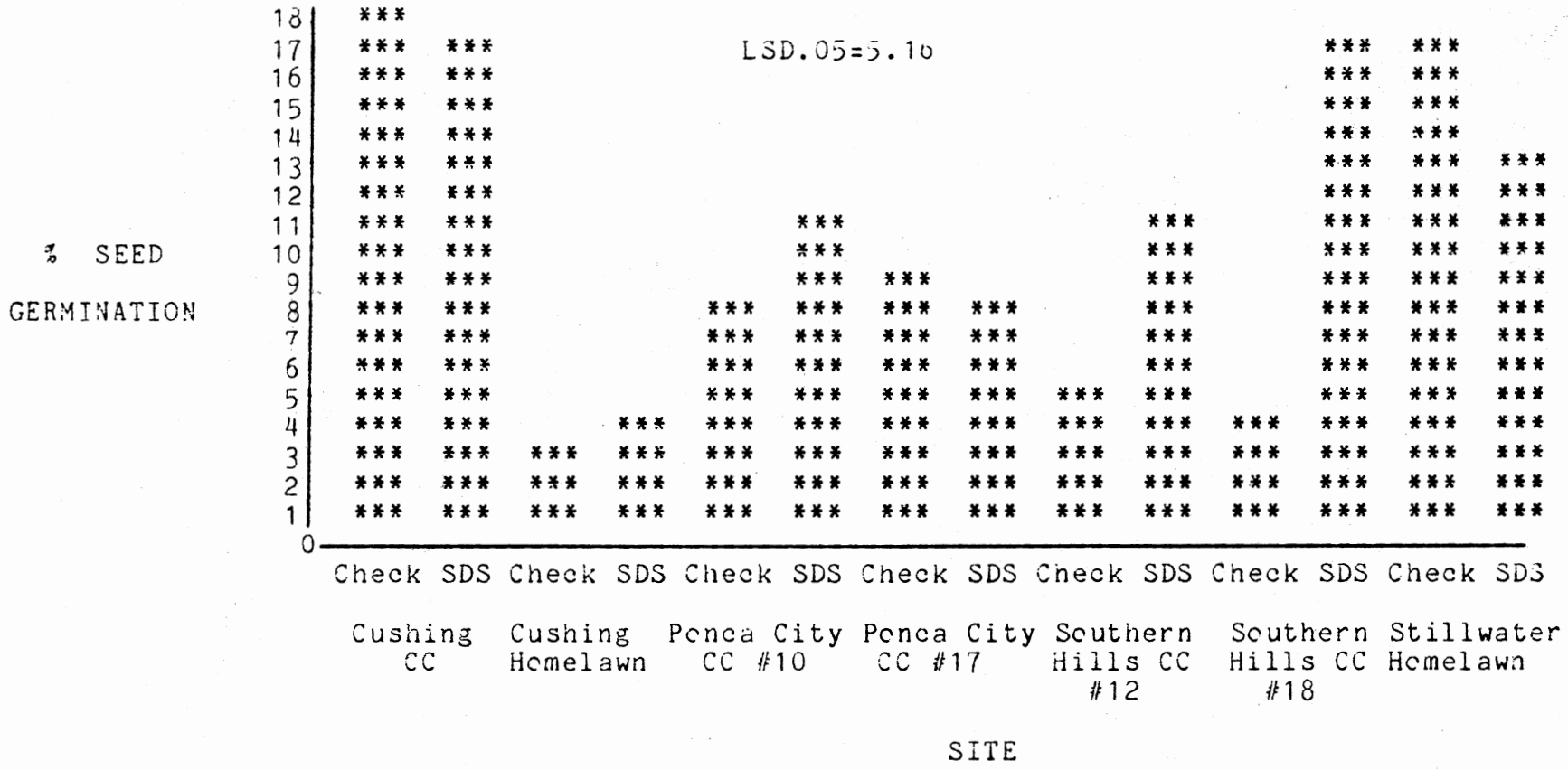


Figure 5. Germination and Growth of Bermudagrass Seedlings Grown Seventy Days in SDS and Healthy Soil From Seven Sites

nificant difference (LSD). Any differences in germination and growth of bermudagrass at different soil sample depths or their interactions are meaningless without the establishment of SDS toxic effects first.

Helminthosporium spiciferum

Extract Bioassay

The evaluation of bermudagrass seed germination was analyzed as a split-plot design. The analysis of variance of germination is listed in Appendix Table IX. Although there were highly significant differences in germination between different media, there were no significant differences in germination of inoculated, or sterile cultures, of the same medium.

All media exhibited reduced germination of bermudagrass when compared to distilled water (54 percent). Both nutrient broth (two percent) and Richard's solution (zero germination) were so drastically reduced in bermudagrass seed germination that the probability that a toxin originating from the media and not the fungus is high.

In the experiment where stolons were used, both the initiation of new shoots and roots were analyzed separately for each week of evaluation. The analysis of variance for shoot counts is shown in Appendix Table X, while the root count analysis of variance is shown in Appendix Table XI.

The only source of variation that showed significant differences among mean shoot counts was for treatment (inoculated or sterile) on one date. Although there were no significant differences, as in the germination experiment, the nutrient broth and Richard's solution showed greater inhibition of shoot initiation than the V-8 broth treatments.

Growth inhibition in nutrient broth, and Richard's solution, was evident in root initiation. A F-test of media mean squares for root initiation was significant in the analysis of both of the first two dates. Differences in root initiation between treatments were not significant.

Plant Clippings Extract Bioassay

As in the H. spiciferum extract bioassay, the assay of plant clipping extracts were analyzed as a split-plot design. Seed germination of bermudagrass was not effected by bermudagrass extract, knotweed extract, or distilled water. The analysis of variance for bermudagrass germination in plant clipping extracts is shown in Appendix Table XII.

Although no significant differences in bermudagrass seed germination were found among extracts or water, the root length of bermudagrass seedlings were reduced in the bermudagrass clipping broth. This effect can be seen in Figure 6. Bermudagrass seedlings germinated on knotweed extract had normal roots.

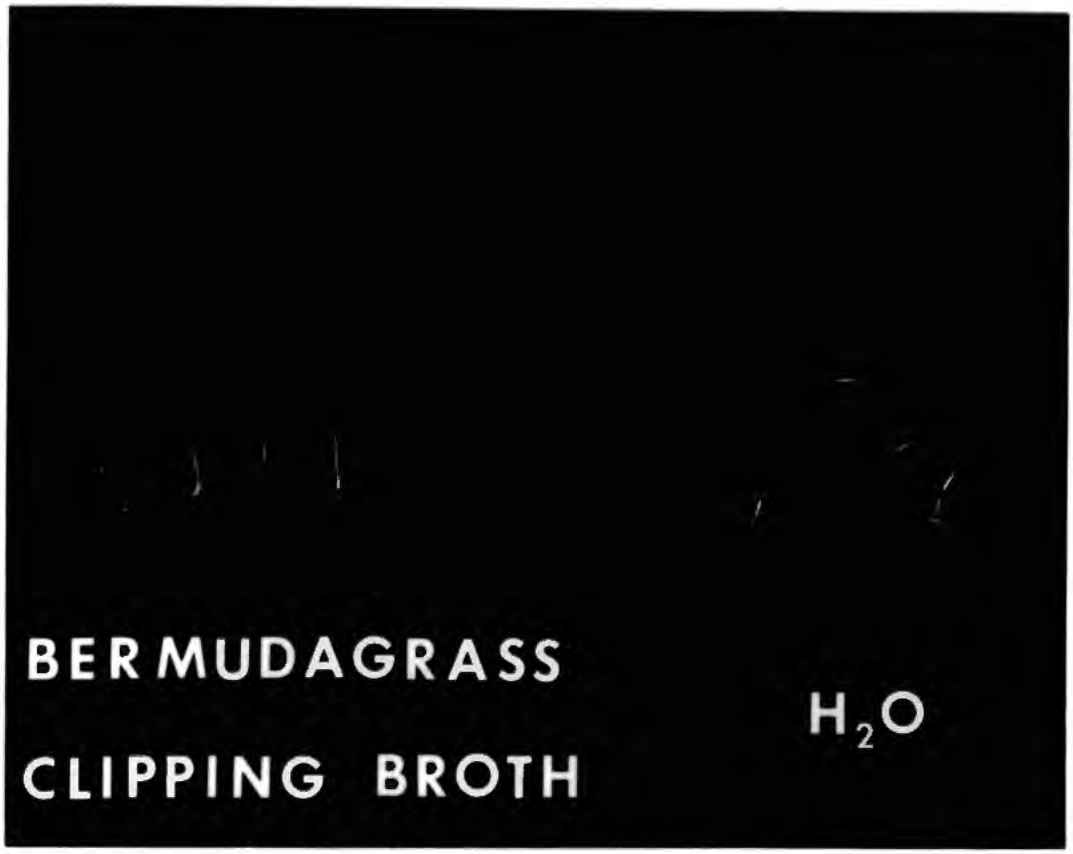


Figure 6. Bermudagrass Seedlings Grown in Bermudagrass Clipping Extract and Water

Evaluation of shoot and root initiation at nodes of bermudagrass stolons indicated there were no significant differences between stolons grown in bermudagrass extract, knotweed extract, or distilled water. The analyses of variance for stolon shoot and root initiation in plant extracts are listed in Appendix Tables XIII and XIV, respectively.

In both the H. spiciferum and plant clipping extract bioassays, the variation among similar treatments on different tray (replications) levels was not significantly different than the variation of similar treatments on the same tray. This was true for the studies on bermudgrass seed germination, and shoot and root initiation, from bermudagrass stolons.

Ethanol and Water Extract Bioassays

The evaluation of both lettuce and bermudagrass seed germination was analyzed as a split-plot design. The analysis of variance for each is listed in Appendix Table XV. No significant differences in germination of either species tested was found in any group of means.

Germination was generally very good. The overall mean germination for bermudagrass and lettuce was 63.4 and 97.4 percent, respectively. The germination of bermudagrass and lettuce seed on substrate moistened with distilled water was 66.7 and 100 percent, respectivily.

Ether Extract Bioassay

The bioassay of the ether extracts was analyzed as a randomized block experiment. Like the water and ethanol extract assay results the difference in germination of lettuce seeds among treatments was small, but in general germination was high. Significant differences in germination were not found among any dilution of the SDS extract or the control extract.

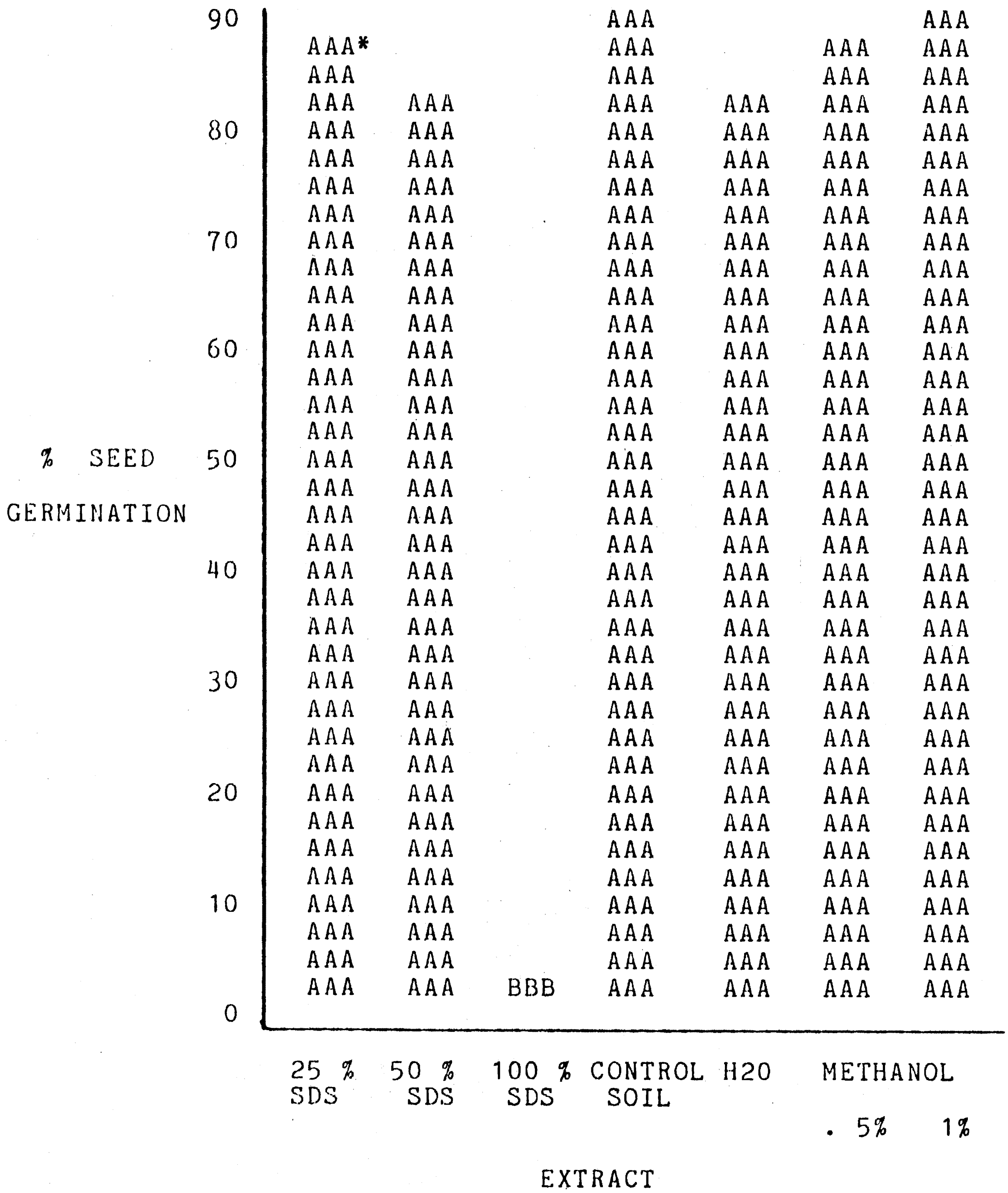
The analysis of variance for lettuce seed germination on substrate impregnated with the ether soluble fraction of SDS soil extract are shown in Appendix Table XVI.

Methanol Extract Bioassay

First Extract

The inital methanol extract bioassay was analyzed as a randomized block experiment. The analysis of variance for lettuce and bermudagrass seed germination and seedling shoot and root length are presented in Appendix Tables XVII and XVIII, respectively.

Significant differences in extract treatment means were found in all areas evaluated. Lettuce germination and both shoot and root length of bermudagrass seedlings had highly significant differences in their means. The mean germination of lettuce seed in the different extracts is illustrated in Figure 7. Out of a possible 200 seeds only two germinated.



* Bars containing a common letter are not significantly different at the 5% level of probability based on Duncan's multiple range test.

Figure 7. Mean Seed Germination of Lettuce Germinated on Substrate Moistened With the Methanol Extracts of Spring Dead Spot and Healthy Soil From a Stillwater Homelawn

Figure 8 illustrates the lettuce seed condition at the time of evaluation.

Bermudagrass seed germination was also significantly reduced in the undiluted SDS extract, as compared to the healthy soil extract. A photograph of the bermudagrass seedlings at the time of evaluation is shown in Figure 9. Figure 10 compares the mean germination of bermudagrass in seven extracts.

The mean shoot lengths of bermudagrass seedlings were evaluated by Duncan's Multiple Range Test (DMRT) after finding a significant F-value for extracts in a preliminary analysis of variance. (Appendix Table XVIII) The length of seedling shoots was significantly shorter than in any other group. It should also be noted that the seedling shoot length in both methanol solutions was significantly longer than any other group, Figure 11.

Evaluation of the bermudagrass seedling root lengths by DMRT showed differences similar to those for shoot length. Seedlings grown in the undiluted SDS extract had significantly shorter roots than any other group. The seedlings grown in either methanol solution again had the longest length which suggests methanol residues were not a factor in the test results. DMRT for root length means is illustrated in Figure 12.

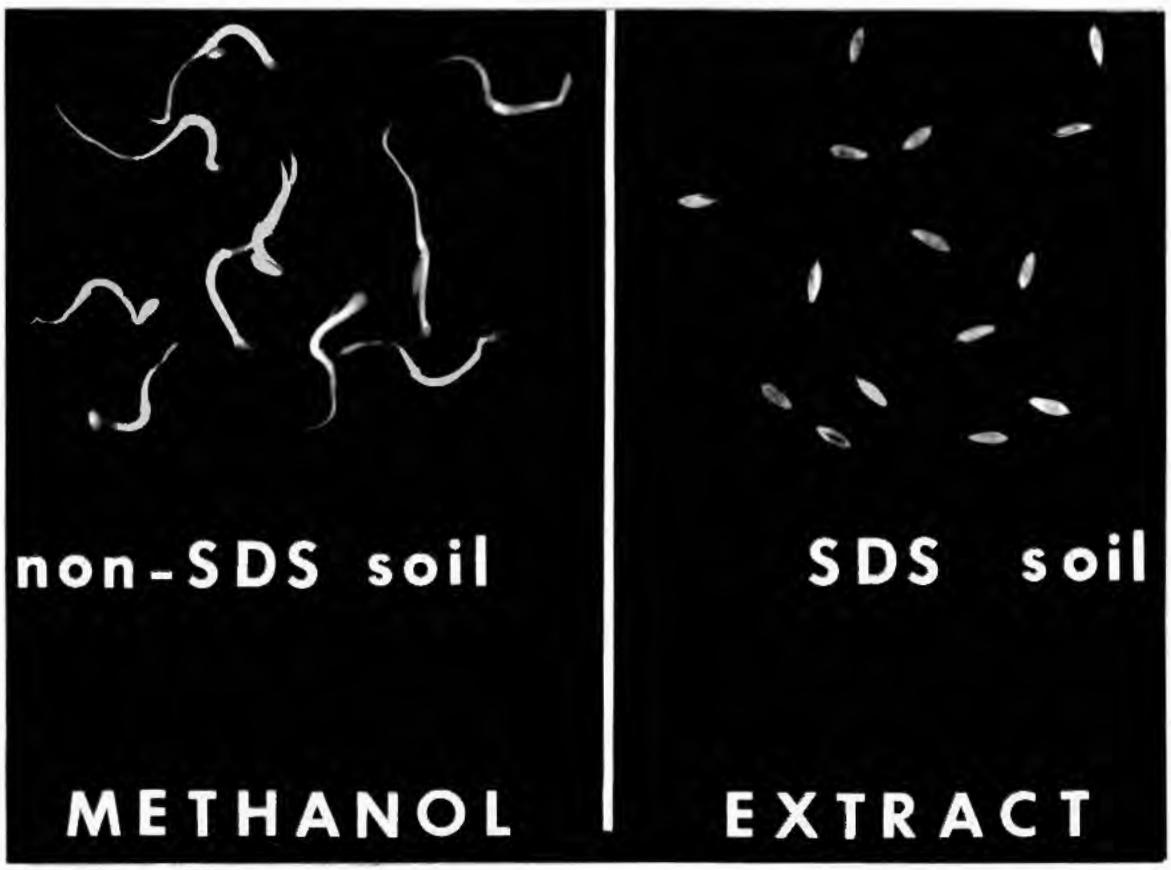


Figure 8. Lettuce Seedlings Germinated on Substrate Moistened With the Methanol Extracts of SDS and Healthy Soil From a Stillwater Homelawn

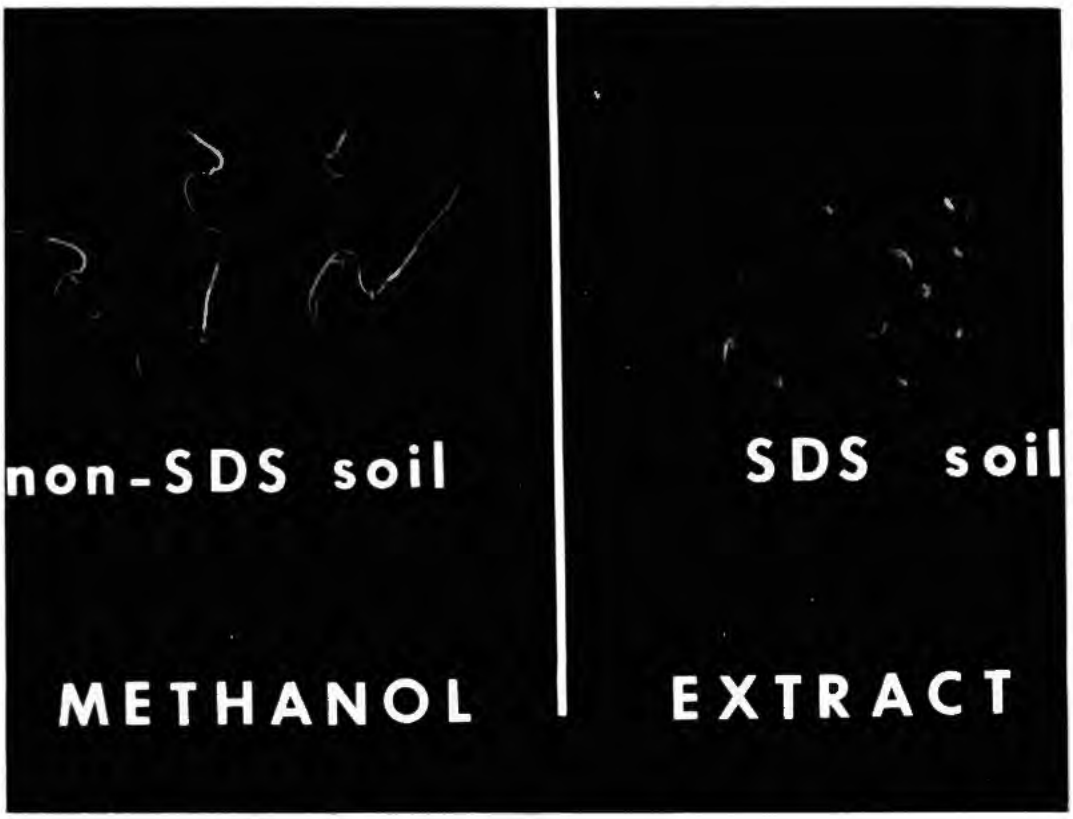
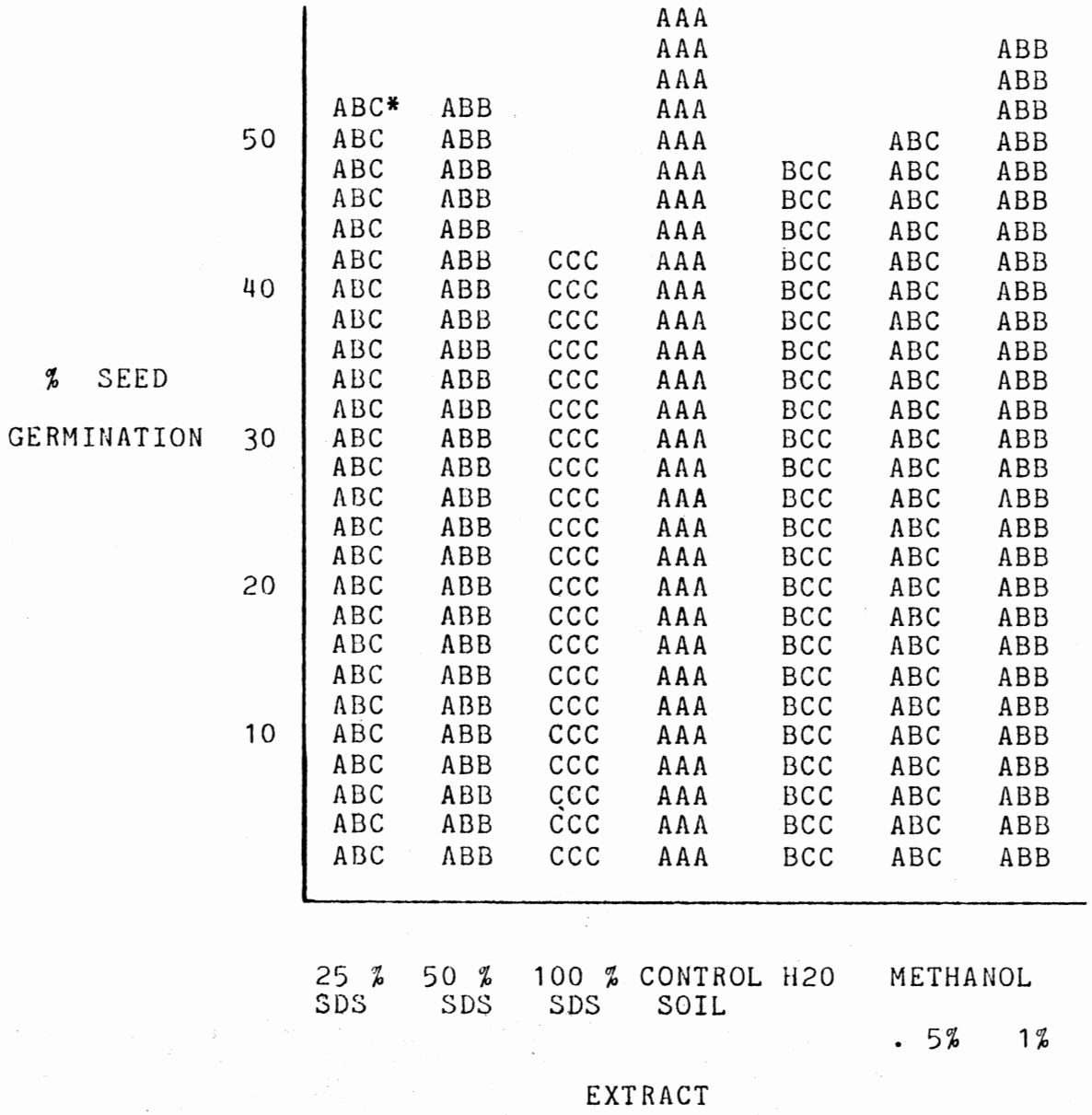
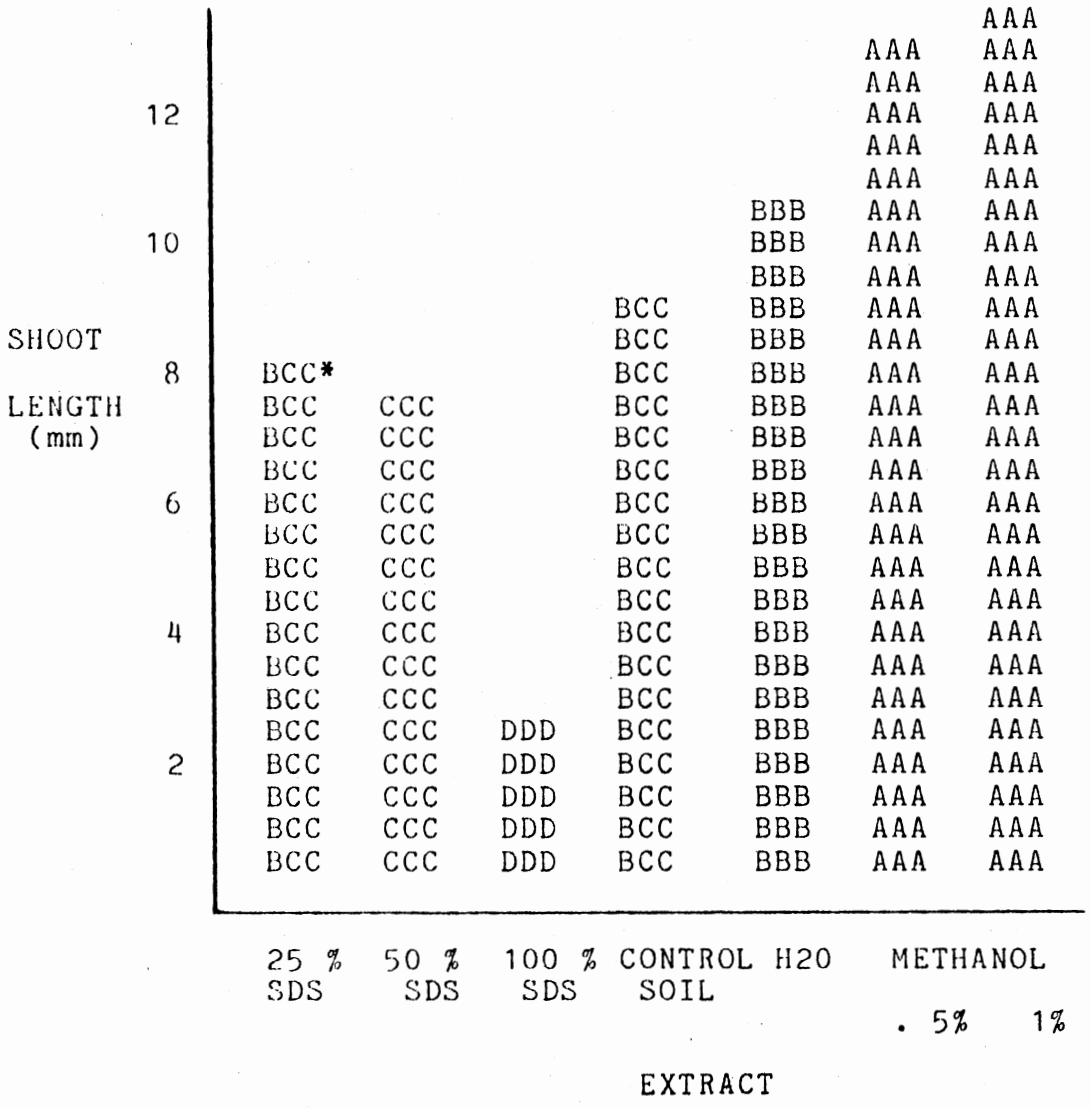


Figure 9. Bermudagrass Seedlings Germinated on Substrate Moistened With the Methanol Extracts of SDS and Healthy Soil From a Stillwater Homelawn



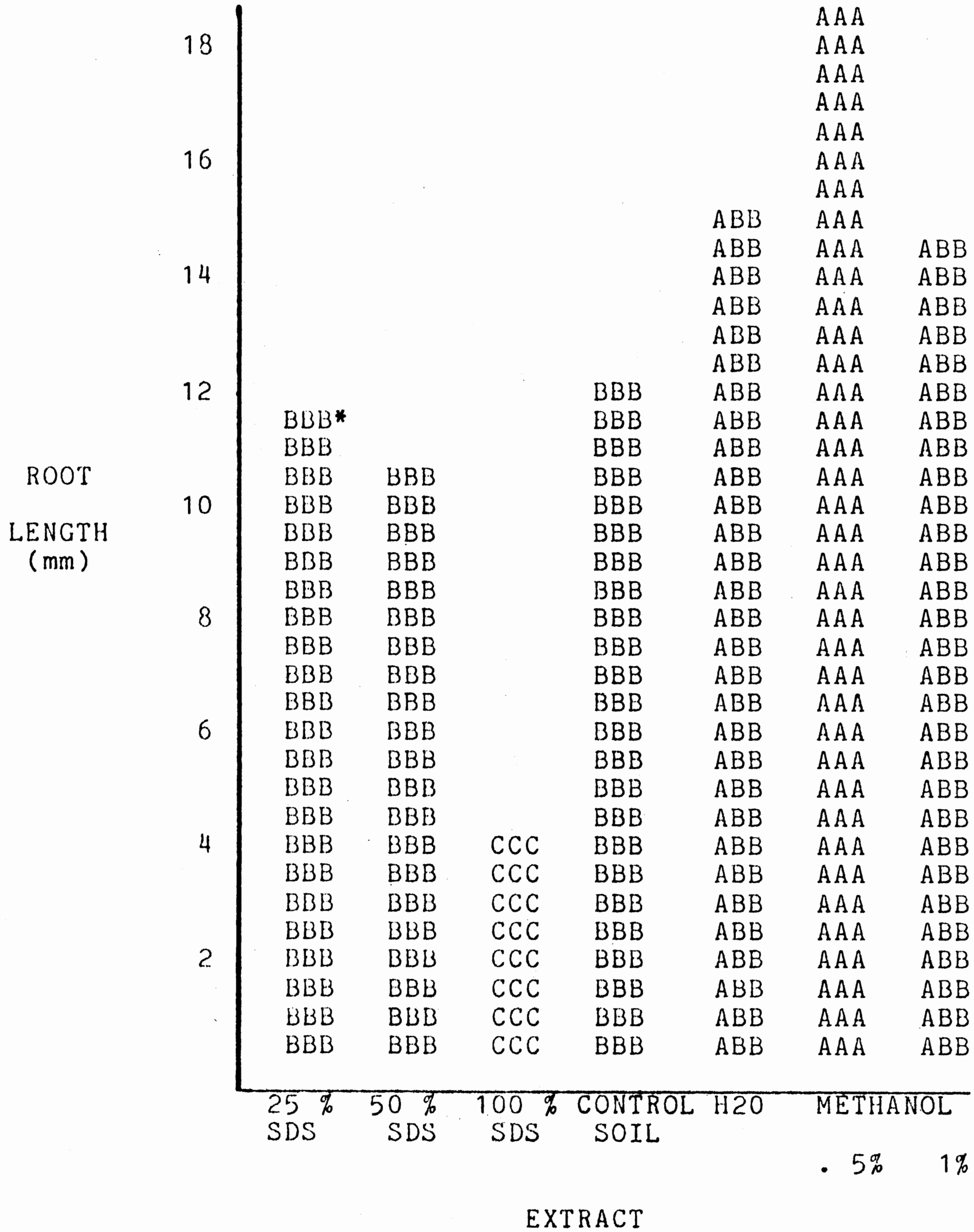
* Bars containing a common letter are not significantly different at the 5% level of probability based on Duncan's multiple range test.

Figure 10. Mean Seed Germination of Bermudagrass Germinated on Substrate Moistened With the Methanol Extracts of SDS and Healthy Soil From a Stillwater Homelawn



* Bars containing a common letter are not significantly different at the 5% level of probability based on Duncan's multiple range test.

Figure 11. Mean Shoot Length of Bermudagrass Seedlings Germinated on Substrate Moistened With the Methanol Extracts of SDS and Healthy Soil From a Stillwater Homelawn



* Bars containing a common letter are not significantly different at the 5% level of probability based on Duncan's multiple range test.

Figure 12. Mean Root Length of Bermudagrass Seedlings Germinated on Substrate Moistened With the Methanol Extracts of SDS and Healthy Soil From a Stillwater Homelawn

Second Extract

Germination studies utilizing the second methanol soil extracts were analyzed as a randomized block experiment. Analysis of variance tables computed for lettuce germination and lettuce seedling root length are presented in Appendix Table XIX. The analyses of variance for bermudagrass germination, seedling shoot, and root length is presented in Appendix Table XX.

Unlike the first extract bioassay, lettuce seed germination in the control soil extract was not significantly different than the germination in the undiluted SDS extract. There were, however, significant differences in lettuce seed germination among the control soil extract, undiluted SDS extract, the 50 percent SDS extract, and all the other extracts. The lettuce seed germination means were tested by DMRT and are shown in Table III.

When tested by DMRT, the lettuce root length means showed similar groupings as the germination means. Again, there was no significant difference in root length between lettuce grown in the undiluted SDS extract at either depth or the control soil extract. The lettuce root means are shown in Table IV. It should be noted that the lettuce seedling roots in water were significantly longer than the roots of any other group.

The greatest support of evidence of the presence of SDS toxin in the second methanol extract, came from the DMRT of

bermudagrass seed germination means. (Figure 13) The undiluted SDS extract for both depths significantly lowered the germiation percentages in comparision to the control.

TABLE III

DUNCAN'S MULTIPLE RANGE TEST OF LETTUCE SEED GERMINATION MEANS IN THE SECOND METHANOL EXTRACT OF SDS AND HEALTHY SOIL FROM A STILLWATER HOMELAWN

Extract	Mean
	% Germination
12.5% 3cm SDS	93.50 a*
25% 3cm SDS	88.50 a
50% 3cm SDS	4.50 c
100% 3cm SDS	0 c
12.5% 6cm SDS	90.00 a
25% 6cm SDS	78.50 b
50% 6cm SDS	0 c
100% 6cm SDS	0 c
100% Control Soil	0 c
Water	91.50 a

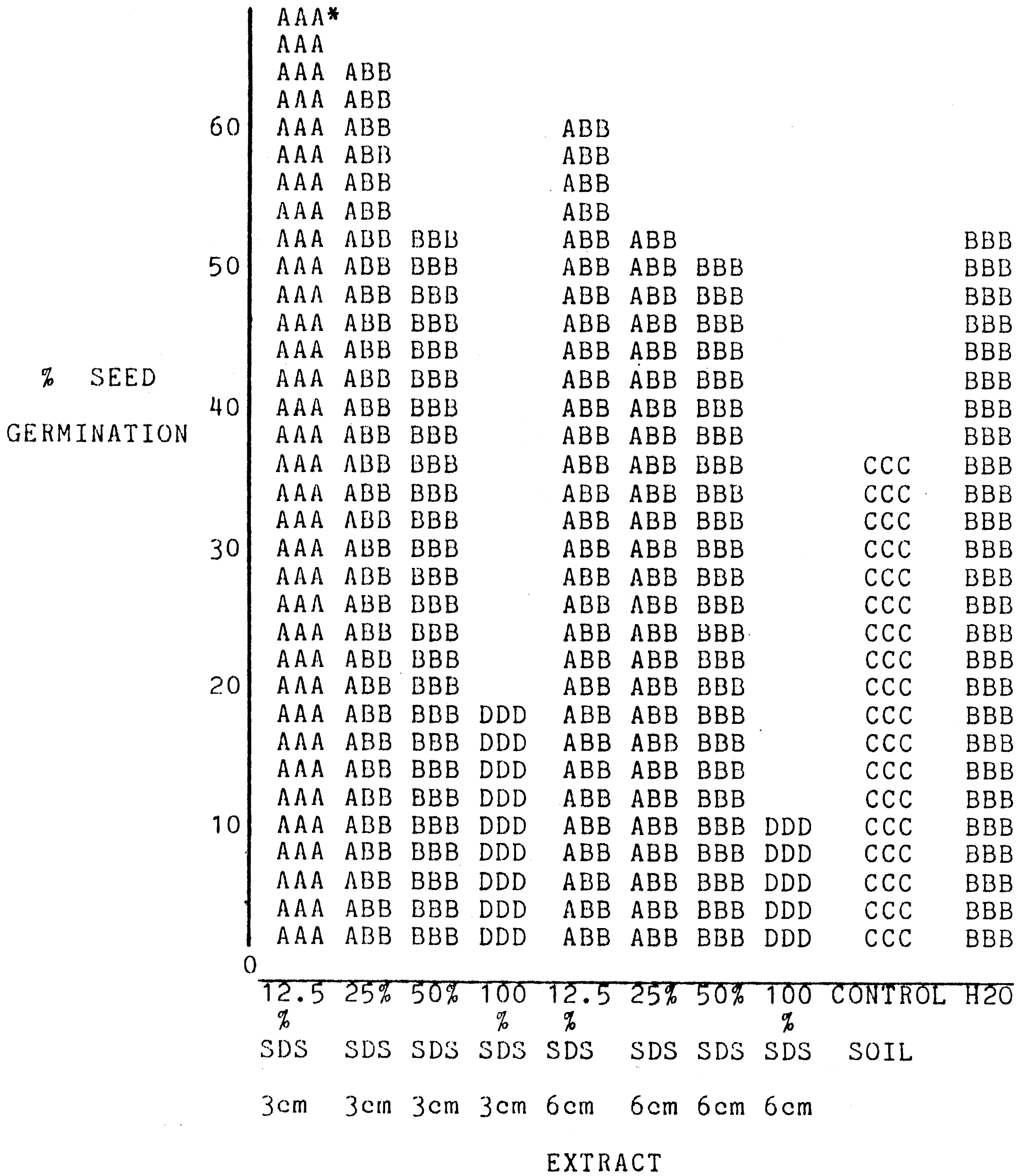
* Means followed by a common letter are not significantly different at the 5% level of probability.

TABLE IV

DUNCAN'S MULTIPLE RANGE TEST OF LETTUCE SEEDLING ROOT LENGTH MEANS IN THE SECOND METHANOL EXTRACT OF SDS AND HEALTHY SOIL FROM A STILLWATER HOMELAWN

Extract	Root Length
	mm
12.5% 3cm SDS	8.25 b
25% 3cm SDS	5.50 c
50% 3cm SDS	0.25 d
100% 3cm SDS	0 d
12.5% 6cm SDS	9.50 b
25% 6cm SDS	5.50 c
50% 6cm SDS	0 d
100% 6cm SDS	0 d
100% Control Soil	0 d
Water	13.00 a

* Means followed by a common letter are not significantly different at the 5% level of probability.



* Bars containing a common letter are not significantly different at the 5% level of probability based on Duncan's multiple range test.

Figure 13. Mean Seed Germination of Bermudagrass Germinated on Substrate Moistened With the Second Methanol Extracts of SDS and Healthy Soil From a Stillwater Homelawn

Significant differences in both bermudagrass shoot and root lengths were found between the soil extracts. For both the shoot and root length means however, there were no significant differences between either undiluted SDS extract or the control soil. The bermudagrass seedlings in water had the greatest shoot and root lengths, therefore, all extracts showed some degree of reduction in shoot and root length. The bermudagrass seedling shoot and root length means are presented in Tables V and VI, respectively.

Third Extract

The third methanol extract bioassay was analyzed in the two parts, in the following order:

1. The total experiment was analyzed as a split-plot design. The main plots were a factorial arrangement of type of extract, and location of samples. (Appendix Table XXI)

2. The data for each location were analyzed separately as a split-plot design. The main plots were type of extract. (SDS or Healthy Soil)

Before the entire experiment analysis could be computed, a regression equation had to be formulated for the subplot values. Approximate values for germination, shoot length, and root length for both the Cushing homelawn healthy soil, thatch sample and the Cushing C.C. SDS thatch sample were selected from a regression line for each replication. These values were used in all analyses of

variance to provide a balanced design. Eight degrees of freedom were subtracted from the subplot error term. This procedure was necessary because thatch was not present at the time of sampling at these two sites.

TABLE V

DUNCAN'S MULTIPLE RANGE TEST OF BERMUDAGRASS SEEDLING SHOOT LENGTH MEANS IN THE SECOND METHANOL EXTRACTS OF SDS AND HEALTHY SOIL FROM A STILLWATER HOMELAWN

Extract	Shoot Length
	mm
12.5% 3cm SDS	8.00 ab*
25% 3cm SDS	7.75 abc
50% 3cm SDS	5.00 def
100% 3cm SDS	2.75 efg
12.5% 6cm SDS	5.75 bcd
25% 6cm SDS	7.75 abcd
50% 6cm SDS	5.25 cde
100% 6cm SDS	1.75 g
100% Control Soil	2.50 g
Water	

* Means followed by a common letter are not significantly different at the 5% level of probability.

TABLE VI

DUNCAN'S MULTIPLE RANGE TEST OF BERMUDAGRASS SEEDLING ROOT LENGTH MEANS IN THE SECOND METHANOL EXTRACTS OF SDS AND HEALTHY SOIL FROM A STILLWATER HOMELAWN

Extract	Root Length
	mm
12.5% 3cm SDS	7.75 ab*
25% 3cm SDS	7.25 ab
50% 3cm SDS	3.00 c
100% 3cm SDS	2.75 c
12.5% 6cm SDS	5.00 bc
25% 6cm SDS	7.75 ab
50% 6cm SDS	4.50 bc
100% 6cm SDS	1.50 c
100% Control Soil	2.25 c
Water	10.00 a

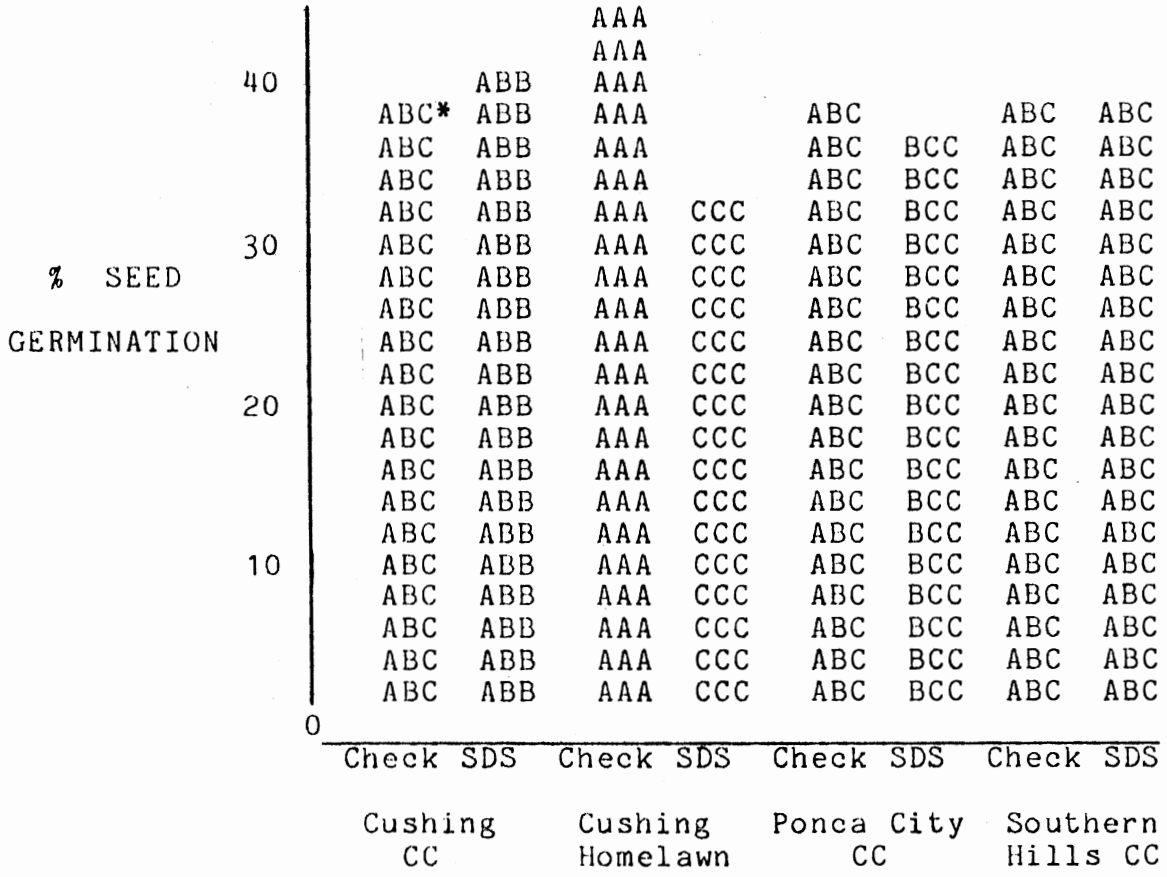
* Means followed by a common letter are not significantly different at the 5% level of probability.

A significant F-value was found for the type X location interation. These interaction means were tested by DMRT and are shown in Figure 14. Although much overlapping of means occurred, one clear separation should be noted. The bermudagrass in SDS extract from the homelawn in Cushing had significantly lower germination than the bermudagrass in healthy soil extract from the same site.

No significant differences in seedling shoot lengths were found between SDS and healthy soil extracts from the same location, except from Southern Hills C.C. which promoted greater seedling shoot length in the SDS soil. Significant differences in shoot lengths between different extracts between locations, however, did occur. The shoot length means for the location X type interaction is presented in Table VII.

The analysis of variance for root lengths of bermudagrass seedlings showed a significant F-value for the location X type interaction. Significant differences in root length means were also found between SDS and healthy soil in the same locations for Cushing C.C., Cushing Homelawn, and the Ponca City C.C.

Ponca City C.C. and Cushing C.C., however, showed greater seedling root length in the SDS extract. The location X type interaction means for root length are shown in Figure 15.



* Bars containing a common letter are not significantly different at the 5% level of probability based on Duncan's multiple range test.

Figure 14. Mean Seed Germination of Bermudagrass Germinated on Substrate Moistened With the Third Methanol Extracts of SDS and Healthy Soil From Four Locations

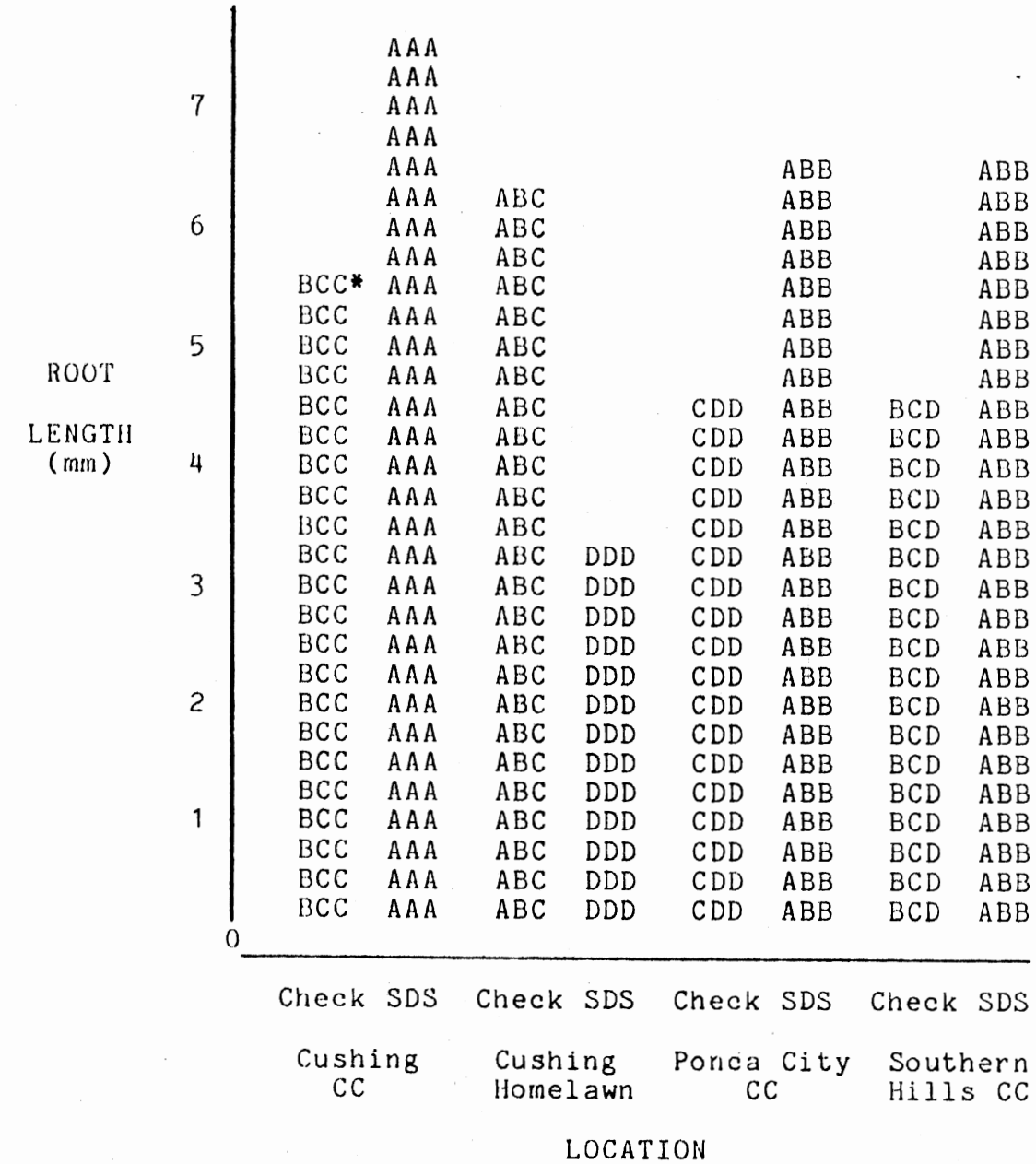
TABLE VII

DUNCAN'S MULTIPLE RANGE TEST OF BERMUDAGRASS SEEDLING SHOOT LENGTH MEANS IN THE METHANOL EXTRACTS OF SDS AND HEALTHY SOIL FROM FOUR LOCATIONS

Location X Type	Shoot Length
	mm
Cushing CC, Check	8.81 ab*
Cushing CC, SDS	8.31 ab
Cushing Homelawn, Check	8.12 abc
Cushing Homelawn, SDS	6.00 c
Ponca City CC, Check	8.56 ab
Ponca City CC, SDS	9.81 a
Southern Hills CC, Check	7.19 c
Southern Hills CC, SDS	8.87 ab

* Means followed by a common letter are not significantly different at the 5% level of probability.

The analyses of variance for bermudagrass seed germination, shoot length, and root length in the third methanol extracts, showed significant F-values for depth, depth X type, depth X location, and depth X location X type interactions ,therefore, no interpretations will be made.



* Bars containing a common letter are not significantly different at the 5% level of probability based on Duncan's multiple range test.

Figure 15. Mean Root Length of Bermudagrass Seedlings Germinated on Substrate Moistened With the Third Methanol Extracts of SDS and Healthy Soil From Four Locations

A more valid interpretation can be made by examining the anaysis of variance by location. Because no significant differences in germination, shoot length, and root length means among location X type interactions were found for the Cushing C.C. and Southern Hills C.C. locations, an analysis of variance for either will not be presented. The analyses of variance for bermudagrass germination, shoot length, and root length in the methanol extract at the Cushing homelawn and Ponca City C.C. locations are listed in Appendix Tables XXII and XXIII, respectively.

The analyses of variance of the Ponca City C.C. soil extract showed with few exceptions no significant F-values for all groups of means. The exceptions, however, had greater values for the SDS extract than the control soil extract.

No significant differences in bermudagrass seed germination for depth X type interaction means from the Cushing homelawn location were observed. For both seedling shoot length and root length, the 3 and 6 cm depth extract of SDS soil had significantly lower values than the extracts from corresponding depths of healthy soil. Figure 16 and 17 show the depth X type interaction means for shoot length, and root length, at the Cushing homelawn location, respectively.

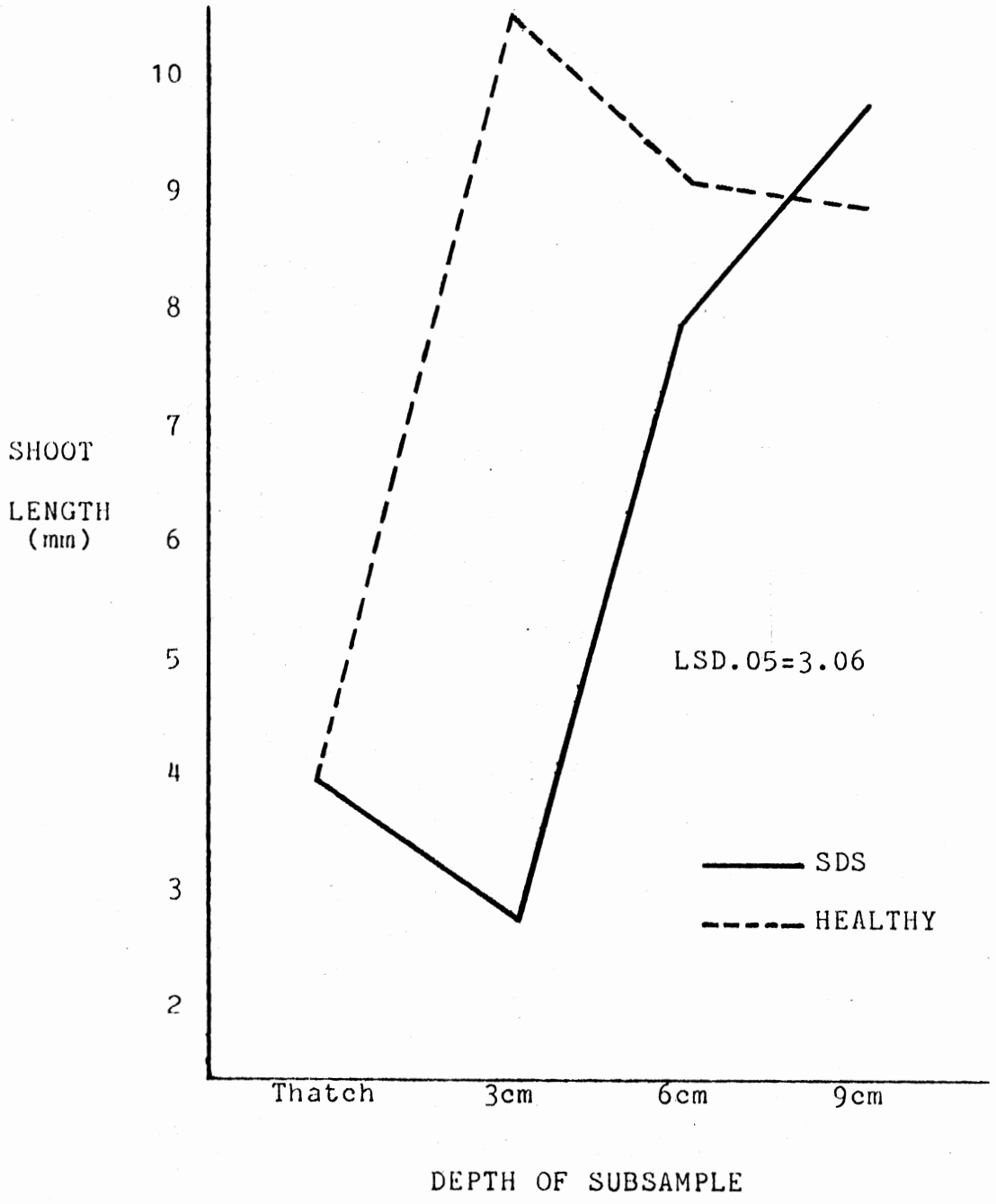


Figure 16. Mean Shoot Length of Bermudagrass Seedlings Germinated on Substrate Moistened With the Third Methanol Extracts of Four Depths of SDS and Healthy Soil From a Cushing Homelawn

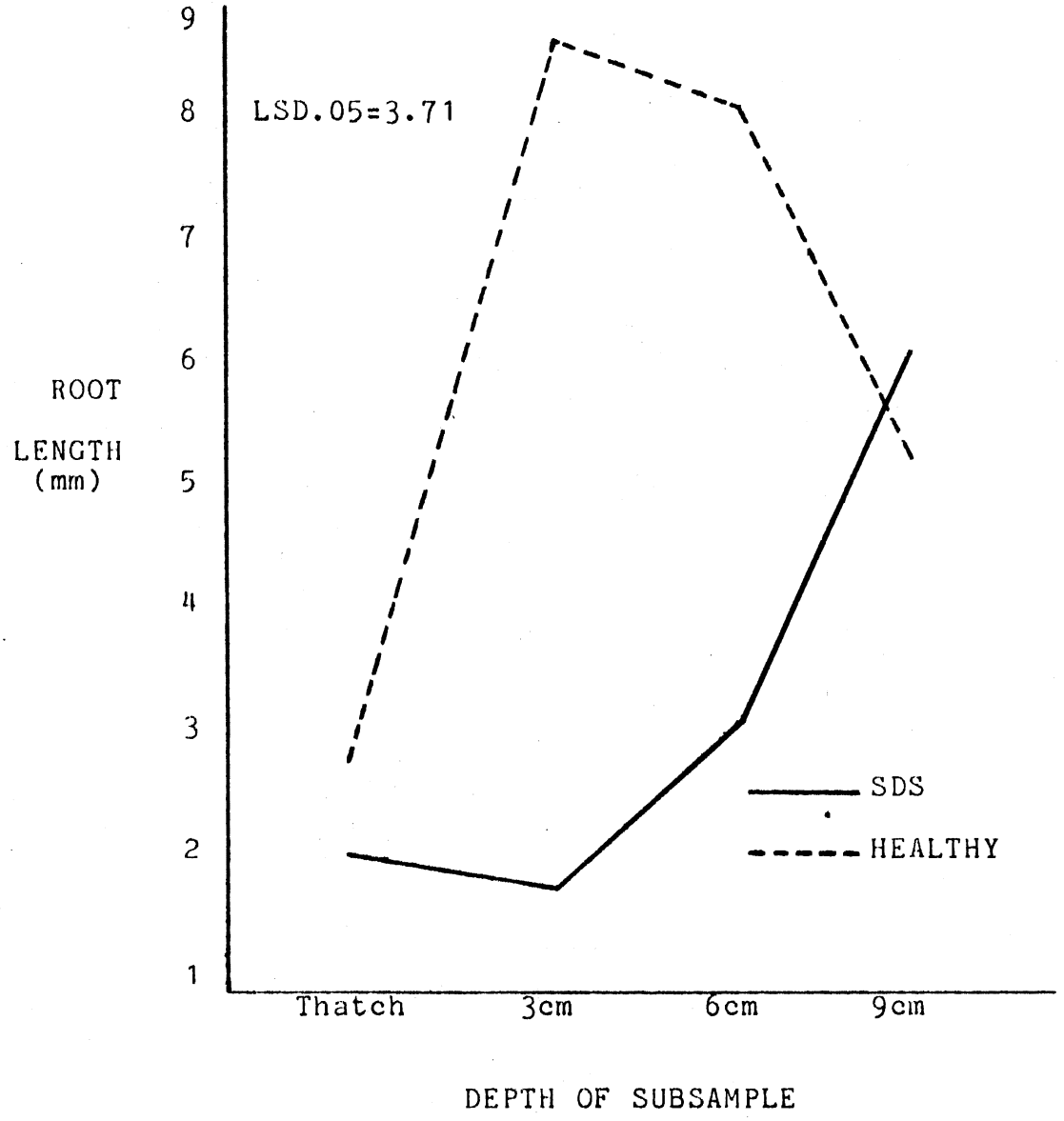


Figure 17. Mean Root Length of Bermudagrass Seedlings Germinated on Substrate Moistened With the Third Methanol Extracts of Four Depths of SDS and Healthy Soil From a Cushing Homelawn

Characterization of Methanol Extracted Toxins

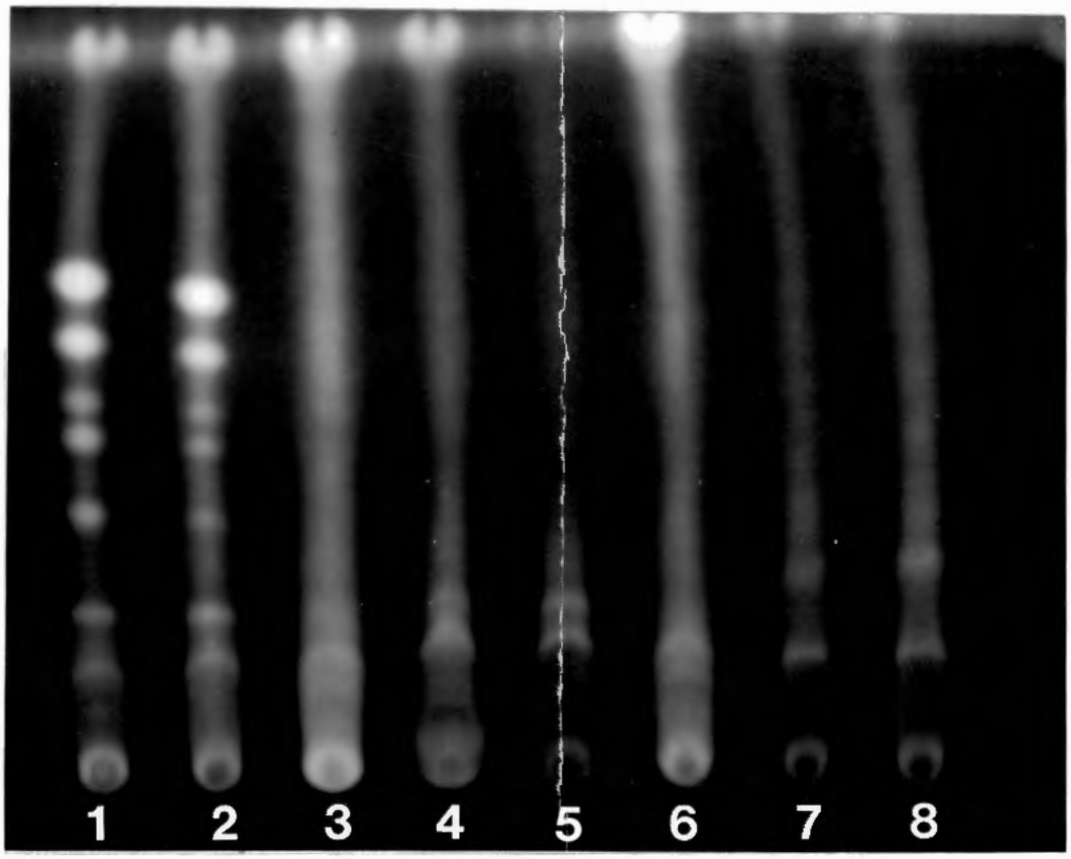
Thin Layer Chromatography

The TLC plate shown in Figure 18 is a composite of several preliminary runs. The source of each sample, Rf value, and color under UV illumination are listed in Figure 19. The solvent for this one dimension plate was an 80:20:1 ratio of chloroform-methanol-water. The sample load size was 40 microliters.

The extract obtained from the Stillwater soil was red to pinkish in color while the extract from the Cushing soil was light yellow. On the TLC plate, no visible spots were evident for any of the Cushing or Ponca City soil extracts. The two Stillwater extracts, however, showed spots having Rf values of .68, .66, .59, and .58 had a visible reddish-pink color. It should also be noted that extracts 1, 2, 4, and 5 showed significant toxic activity in at least one of the bioassays, while extracts 3, 6, 7 and 8 showed no activity.

When the TLC plate was sprayed with ninhydrin reagent, no color developed. There was also a negative reaction to an aniline phtholate reagent. Therefore, the presence of an amino acid, amine, aminosugar, or a reducing sugar on the plate was considered unlikely.

Two additional TLC plates were prepared, similar to the first plate. The same eight extracts used on the first



1) Stillwater homelawn, 3cm SDS 2) Stillwater homelawn, control soil 3) Ponca City C.C., thatch SDS 4) Cushing homelawn, 3cm SDS 5) Cushing homelawn, 6cm SDS 6) Ponca City C.C., thatch control 7) Cushing homelawn, 3cm control 8) Cushing homelawn 6cm control

Figure 18. Thin Layer Chromatograph of Methanol Extracts of SDS and Healthy Soil

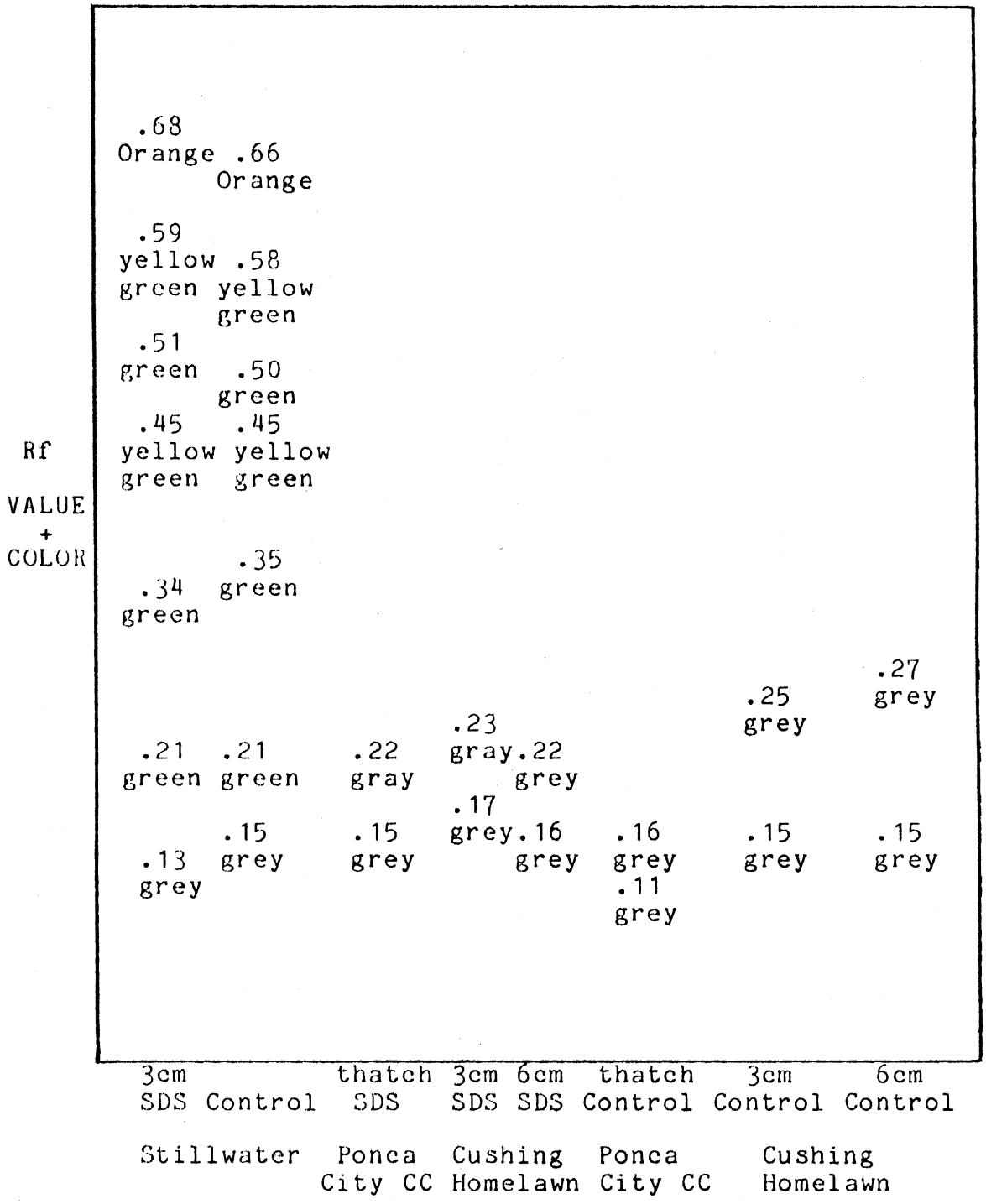


Figure 19. Rf-Values and Flourescent Color Under UV Illumination for Methanol Extracts of SDS and Healthy Soil on a Thin Layer Chromatograph

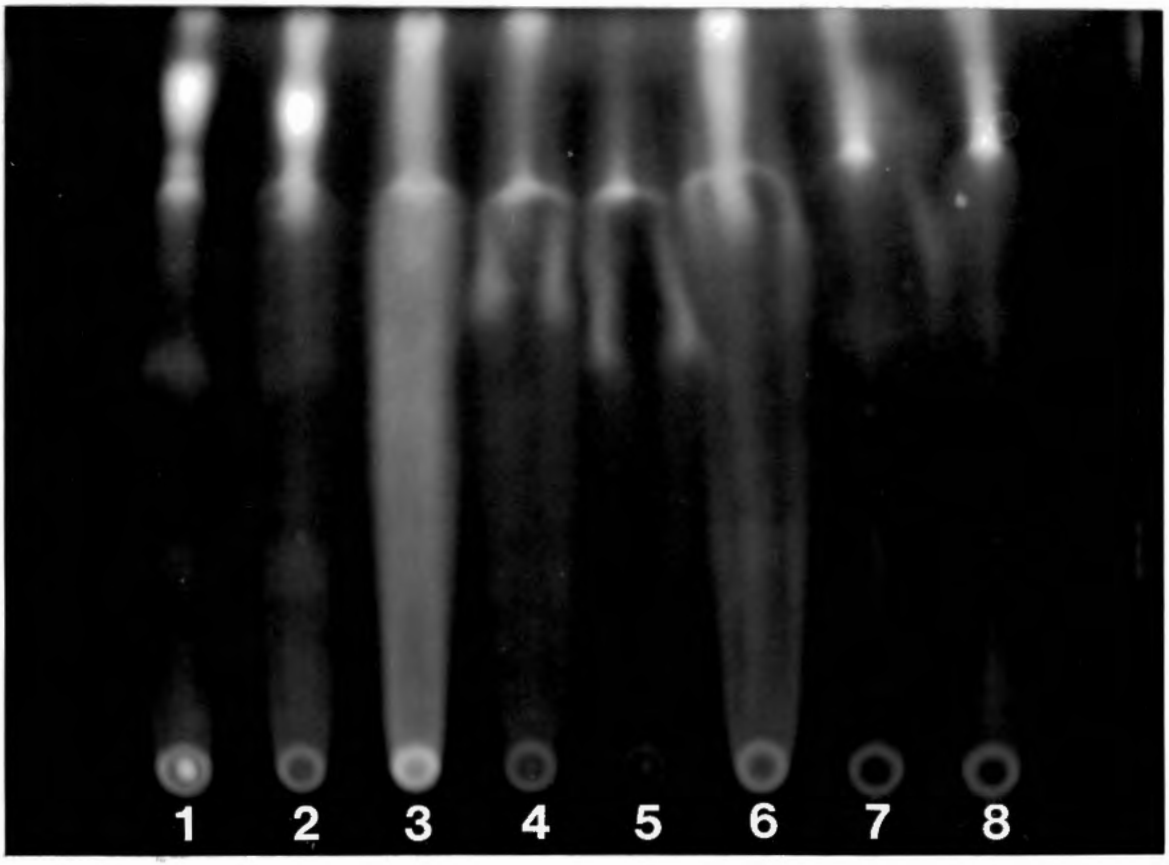
plate were spotted on each plate in the same order. The sample size, however, was increased to 60 microliters of undiluted extract.

Each plate was developed in a different solvent. To find the pH effect on sample migration, one plate was partitioned with a 65:35:1 ratio (V/V/V) of chloroform-methanol-acetic acid, and for another, NH_4OH was substituted for the acid in the solvent. Photographs of the acidic and basic TLC plates are shown in Figure 20 and 21, respectively.

Although plate resolution was very poor, one important point should be noted. In the basic solvent, part of each sample remained at the origin. This was also true for the plate that was developed with a neutral solvent (water). However, on the plate developed in acidic solvent, no sample remained at the origin. This phenomenon was the most intense for the Stillwater and Cushing SDS extracts.

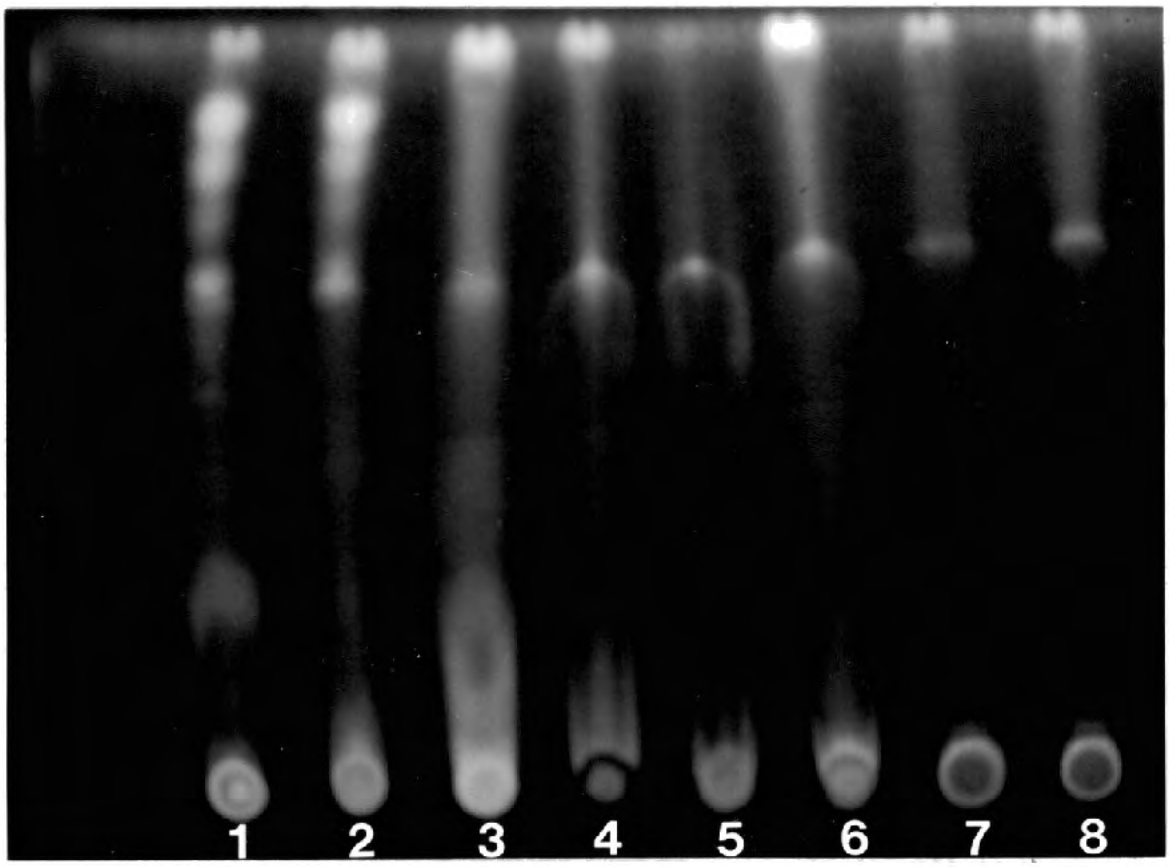
CC Effluent Bioassay

The effluents from each CC run were analyzed separately. The analysis of variance for lettuce seed germination, and seedling length (shoot + root) from the Stillwater extract are shown in Appendix Table XXIV. The analysis of variance for the Cushing extract CC run is shown in Appendix Table XXV. Examination of the means for either germination percentages or total length, in the Stillwater extract, did



1) Stillwater homelawn, 3cm SDS 2) Stillwater homelawn, control soil 3) Ponca City C.C., thatch SDS 4) Cushing homelawn, 3cm SDS 5) Cushing homelawn, 6cm SDS 6) Ponca City C.C., thatch control 7) Cushing homelawn, 3cm control 8) Cushing homelawn 6cm control

Figure 20. Thin Layer Chromatograph of Methanol Extracts of SDS and Healthy Soil Developed in an Acidic Solvent



1) Stillwater homelawn, 3cm SDS 2) Stillwater homelawn, control soil 3) Ponca City C.C., thatch SDS 4) Cushing homelawn, 3cm SDS 5) Cushing homelawn, 6cm SDS 6) Ponca City C.C., thatch control 7) Cushing homelawn, 3cm control 8) Cushing homelawn 6cm control

Figure 21. Thin Layer Chromatograph of Methanol Extracts of SDS and Healthy Soil Developed in an Basic Solvent

not reveal any clear cut differences. The problem seems to center around the arbitrary selection of treatment samples. For several selected treatments, the effluent from one tube, or replication, caused very poor germination, while another tube in the same treatmment had very high germination. These extremes were, therefore, not shown in the treatment mean.

Four scatter diagrams, two for each location, were constructed using the data from one tube for each point. Lettuce seed germination and seedling length for the Stillwater extract CC run are shown in Figures 22 and 23, respectively. Lettuce seed germination and seedling length for the Cushing extract CC run are shown in Figures 24 and 25 , respectively.

In the Bioassay of the Stillwater effluents, germination of lettuce was most reduced in tubes 40, 53, 57, and 85. Tubes 53 and 57 also had reduced seedling lengths. For the Cushing run, tubes 30, 39, and 79 reduced the germination. The greatest reduction in germination for the Cushing effluents, however, was found in tubes 117 to 126. These fractions were all collected after the last aliquot of solvent, containing acetic acid, was added to the column.

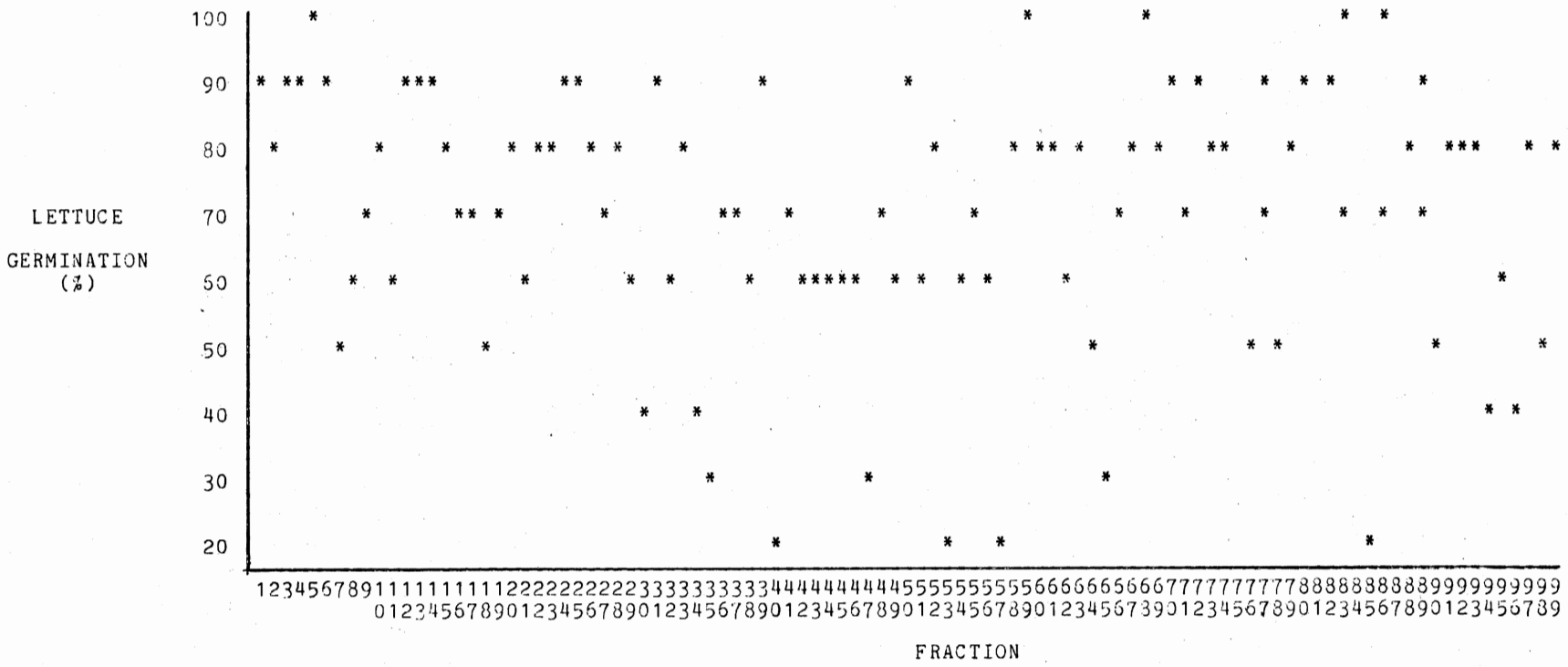


Figure 22. Seed Germination of Lettuce on the CC Effluent of the Methanol Extract of SDS Soil From a Stillwater Homelawn

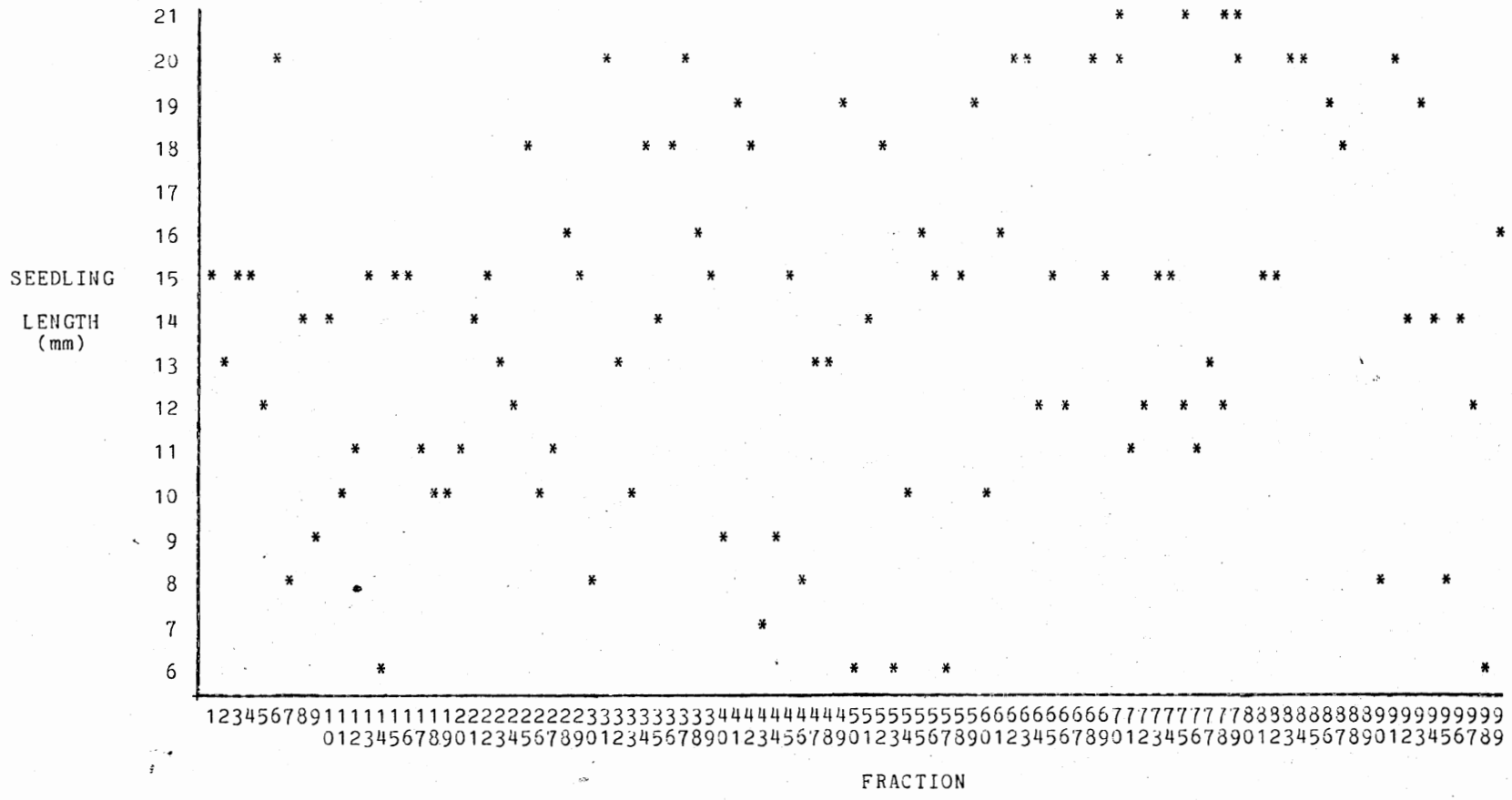


Figure 23. Seedling Length of Lettuce on the CC Effluent of the Methanol Extract of SDS Soil From a Stillwater Homelawn

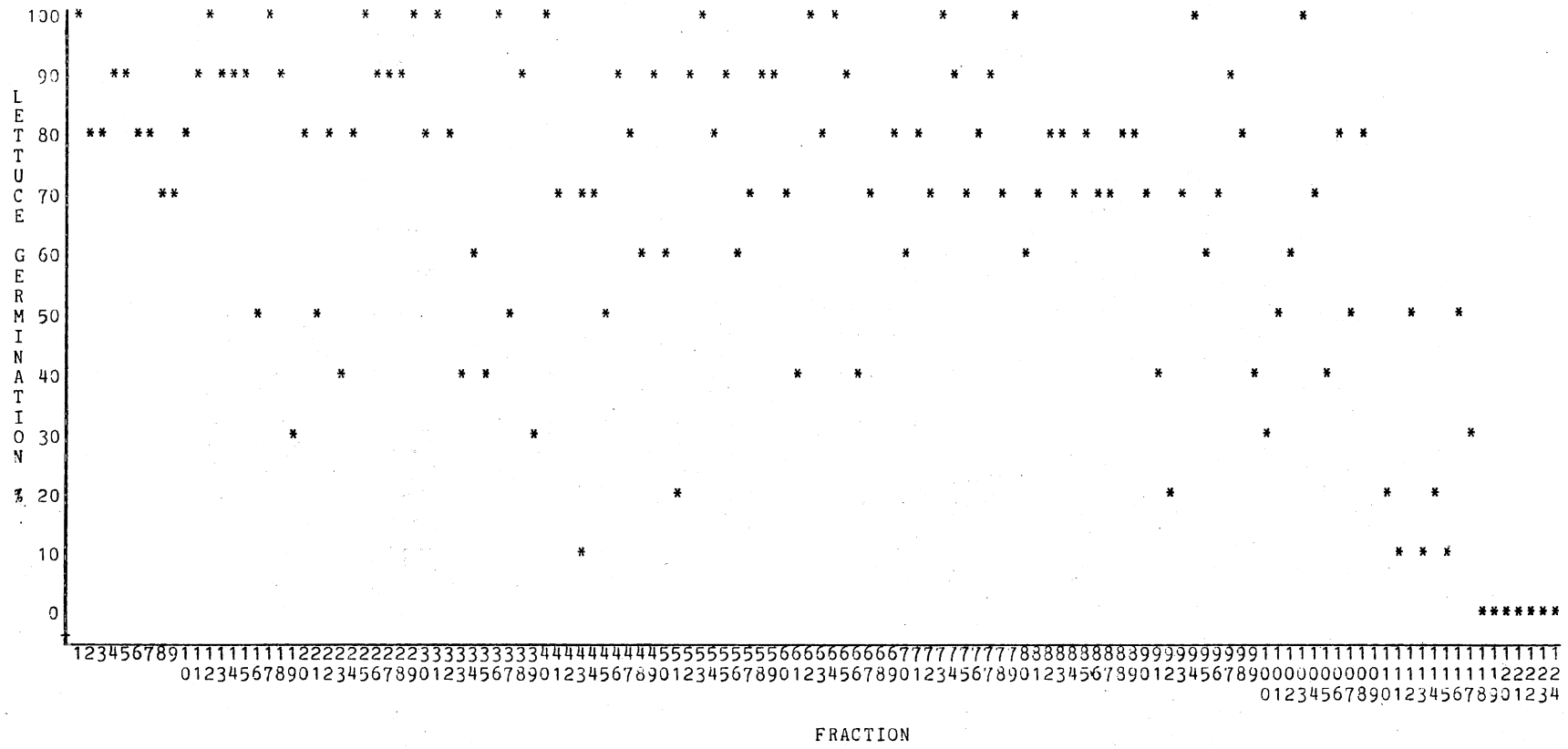


Figure 24. Seed Germination of Lettuce on the CC Effluent of the Methanol Extract of SDS Soil From a Cushing Homelawn

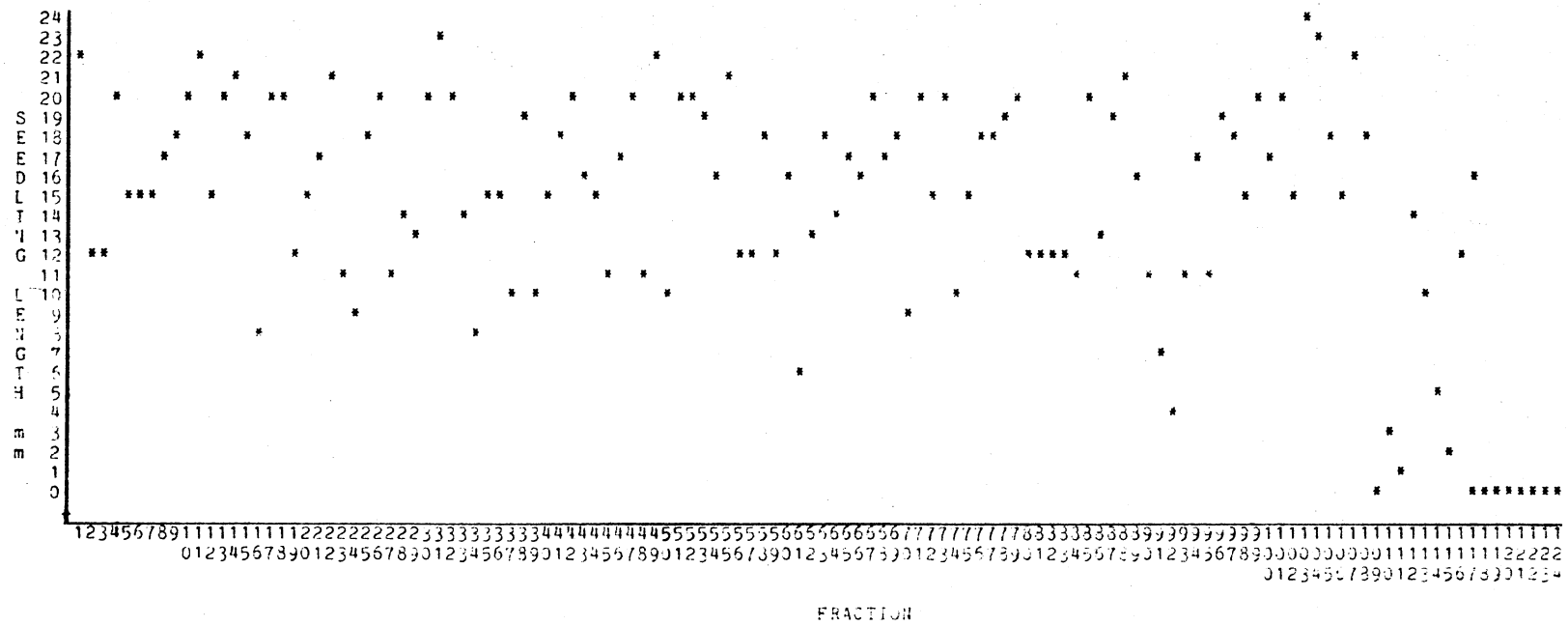


Figure 25. Seedling Length of Lettuce Grown on the CC Effluent of the Methanol Extract of SDS Soil From a Cushing Homelawn

This phenomenon is similar to the effect shown on the acidic TLC plate. It is probable that the SDS toxin is tightly held to the stationary phase under neutral to basic pH, but released at low pH. This toxic fraction was not seen for the Stillwater run, because enough fractions were not collected.

The strong polarity of the toxin would explain its persistance, and lack of mobility in the soil environment. It is probably absorbed to the soil cation exchange complex, and slowly released.

The purity of these toxins is unknown, however. Additional separation procedures will have to be performed before the identification of any compounds can be made.

CHAPTER V

SUMMARY AND CONCLUSIONS

No conclusive evidence for toxin activity was found in the soil, H. speciferum, plant extract, ethanol and water extract, or ether extract bioassays. In the soil bioassay, a significant increase in germination and growth occurred in the SDS soil from several locations. Although the bermudagrass clipping extract did not effect bermudagrass germination, the seedlings were stunted and had reduced roots.

Significant differences in either lettuce or bermudagrass germination between healthy and SDS soil extracts were observed in all three methanol extract bioassays. This activity was generally lost when the SDS extract was diluted. Bermudagrass seedlings that did germinate in SDS extract, often had greatly reduced shoot and roots. No significant differences in germination were observed among depth subsamples at any location. The toxic activity however, was greatest in the 3 and 6 cm fractions. Since either the SDS or healthy soil thatch subsample was not available at either the Stillwater or Cushing homelawn locations, information on toxin activity for this fraction is lacking.

The lyophilized Stillwater SDS extract can be described as reddish-pink crystals, in small aggregates, while the Cushing SDS extract contained yellow crystals in a large, foam-like mass. Both extracts were soluble in methanol, water, and acetic acid, but insoluble in ethanol, chloroform, or heptane.

Using TlC, the Stillwater SDS extracts were separated into eight fractions. All fractions were fluoresced under UV illumination. Compounds remaining at the origin under neutral to basic development were released when the solvent was acidic. The Cushing SDS extracts were only separated into two fractions.

Significant toxin activity was only observed in the last ten fractions from the Cushing SDS extract CC run. This activity probably would also have been observed in the Stillwater SDS extract run if more fractions had been collected. It is very likely that the compounds left at the origin in the neutral TLC run are the same compound as in the last ten fractions of the Cushing extract CC run. Further analyses, however, are needed to confirm this, and to identify any compounds.

It can be concluded from these investigations that at least some SDS soil contain toxins whose origin is unknown. These toxins are most likely tightly bound to the soil exchange complex and only slowly released.

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APPENDIX A

PRELIMINARY BIOASSAYS

TABLE VIII

ANALYSIS OF VARIANCE OF BERMUDAGRASS SEED GERMINATION IN THE BIOASSAY OF SDS AND HEALTHY SOIL FROM SEVEN SITES

Source	Degrees of Freedom	28 June 1979		23 July 1979		20 August 1979	
		Mean Square	F	Mean Square	F	Mean Square	F
Replication	3	430.55		105.71		79.31	
Soil Type	1	2828.64	11.92**	292.57	3.47	380.64	7.32**
Site	6	1704.62	7.18**	1046.50	12.42**	594.98	11.44**
Soil Type X Site	6	767.48	3.23*	387.07	4.60**	277.64	5.34**
Error A	39	237.26		84.23		52.03	
Depth	3	89.90	0.76	319.43	6.26**	105.98	3.45*
Depth X soil Type	3	90.93	0.78	35.62	0.70	24.64	0.80
Depth X Site	18	252.02	2.15**	139.71	2.74**	105.42	3.45**
Depth X Site X Soil Type	18	363.32	3.10**	129.67	2.54**	88.98	2.89**
Error B	126	117.29		51.03		30.74	
Corrected Total	223	244.85		111.39		70.17	

*,** Exceeds 5% ands 1% Level of Significance, Respectively

TABLE IX

ANALYSIS OF VARIANCE OF BERMUDAGRASS SEED GERMINATION FOR THE BIOASSAYS OF H. SPICIFERUM CULTURE FILTRATES

Source	Degrees of Freedom	Mean Square	F
Replication	3	19.00	
Medium	2	4941.00	128.90**
Error A	6	38.33	
Treatment	1	176.33	2.10
Medium X Treatment	2	64.33	0.77
Error B	9	83.89	0.66
Sampling Error	24	127.67	
Corrected Total	47	304.11	

**Exceeds 1% Level of Significance

MEAN GERMINATION OF BERMUDAGRASS GROWN
IN THREE CULTURE FILTRATES
OF HELMINTHOSPORIUM SPECIFERUM

BAR CHART OF MEANS

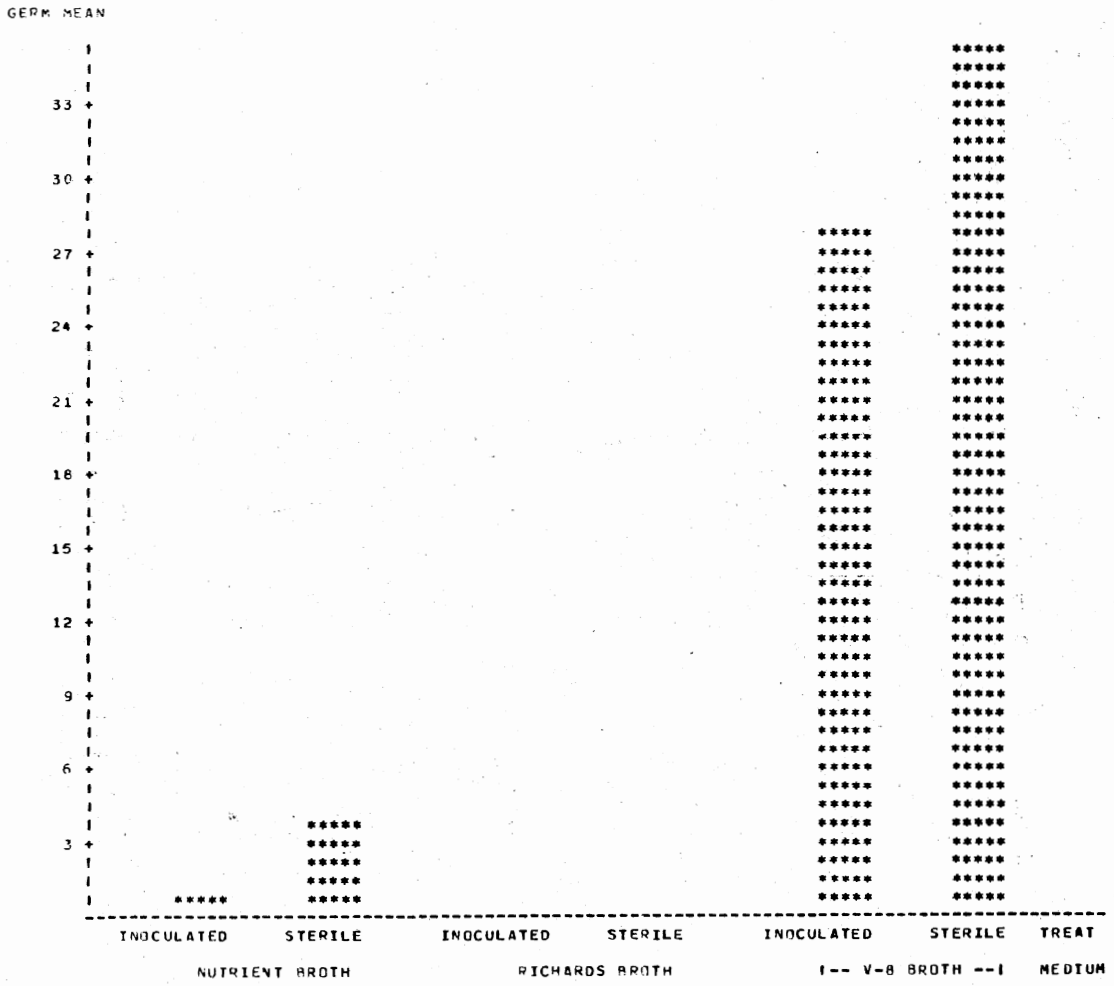


TABLE X

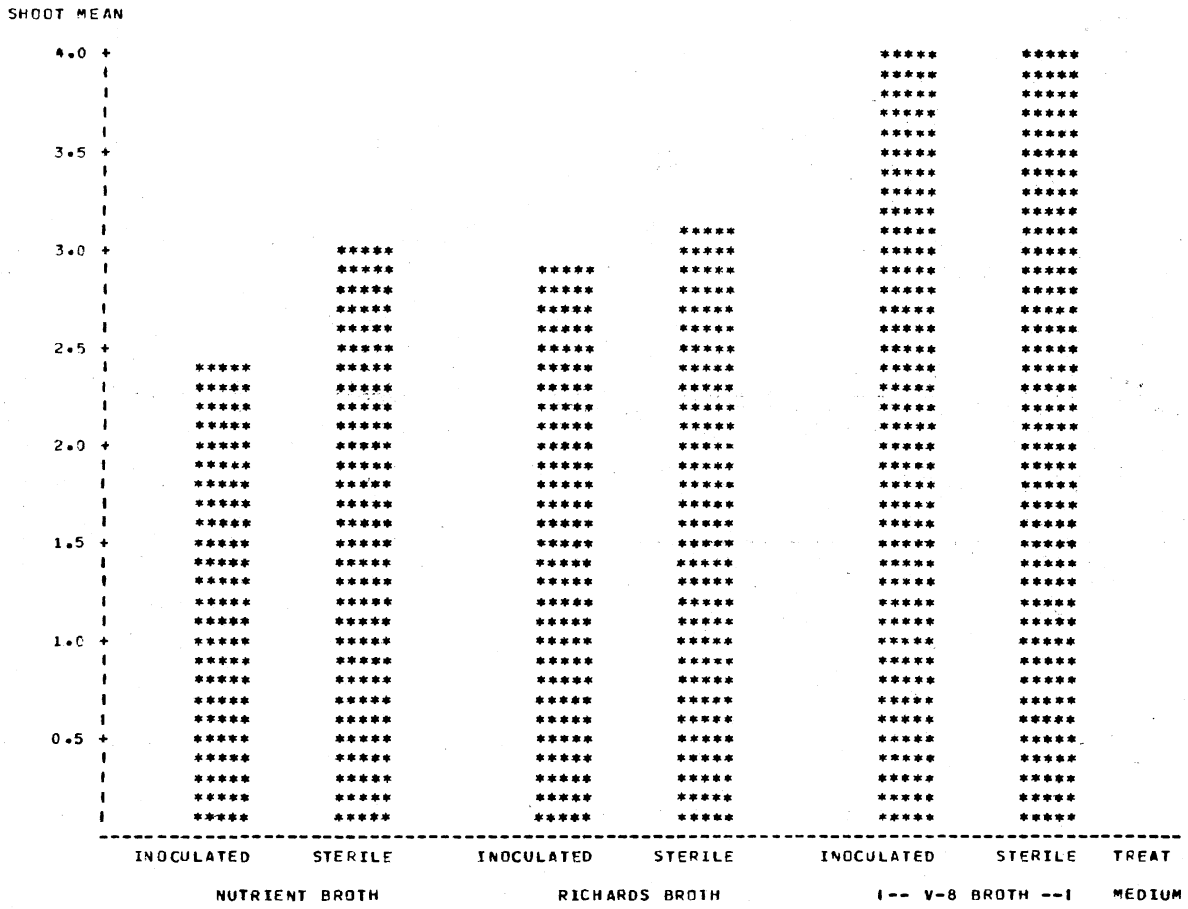
ANALYSIS OF VARIANCE OF BERMUDAGRASS STOLON SHOOT INITIATION FOR THE BIOASSAY OF *H. SPECIFERUM* CULTURE FILTRATES

Source	Degrees of Freedom	01 October 1979		08 October 1979		15 October 1979	
		Mean Square	F	Mean Square	F	Mean Square	F
Replication	3	2.58		3.02		2.69	
Media	2	7.52	2.06	4.75	4.32	6.06	4.10
Error A	6	3.66		1.08		1.48	
Treatment	1	1.02	2.07	1.02	1.21	6.02	6.42*
Treatment X Media	2	0.40	0.80	0.08	0.10	0.14	0.16
Error B	9	0.49	0.25	0.84	0.44	0.94	0.53
Sampling Error	24	1.98		1.90		1.77	
Corrected Total	47	2.10		1.69		1.84	

*Exceeds 5% Level of Significance

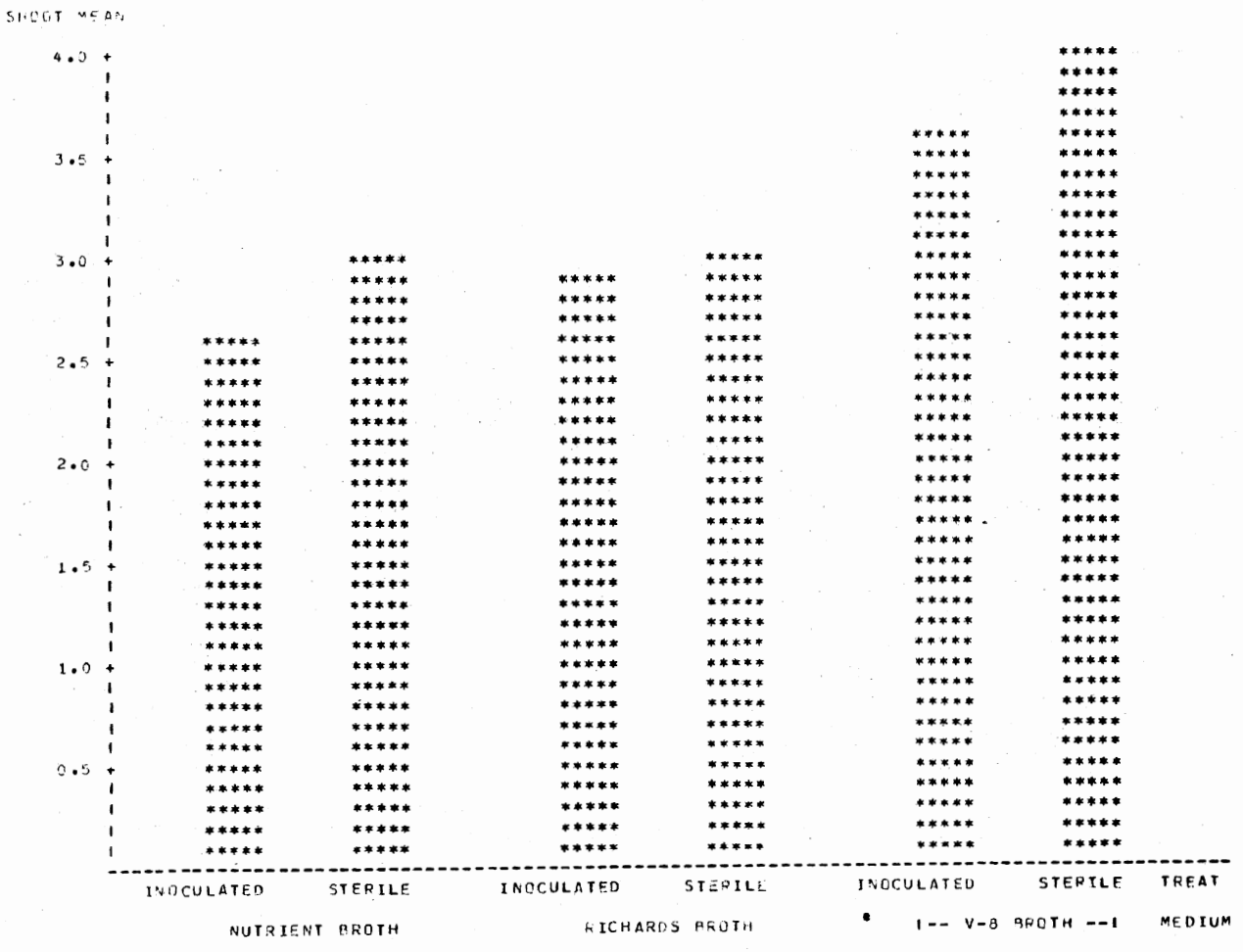
MEAN SHOOT INIATIATION OF BERMUDAGRASS STOLONS
IN THREE CULTURE FILTRATES
OF HELMINTHOSPORIUM SPECIFERUM
DATE=OCTR01

BAR CHART OF MEANS



MEAN SHOOT INIATIATION OF BERMUDAGRASS STOLONS
IN THREE CULTURE FILTRATES
OF HELMINTHOSPORIUM SPECIFERUM
DATE=OCTR09

BAR CHART OF MEANS



MEAN SHOOT INIATIATION OF BERMUDAGRASS STOLONS
IN THREE CULTURE FILTRATES
OF HELMINTHOSPORIUM SPECIFERUM
DATE=OCTR15

BAR CHART OF MEANS

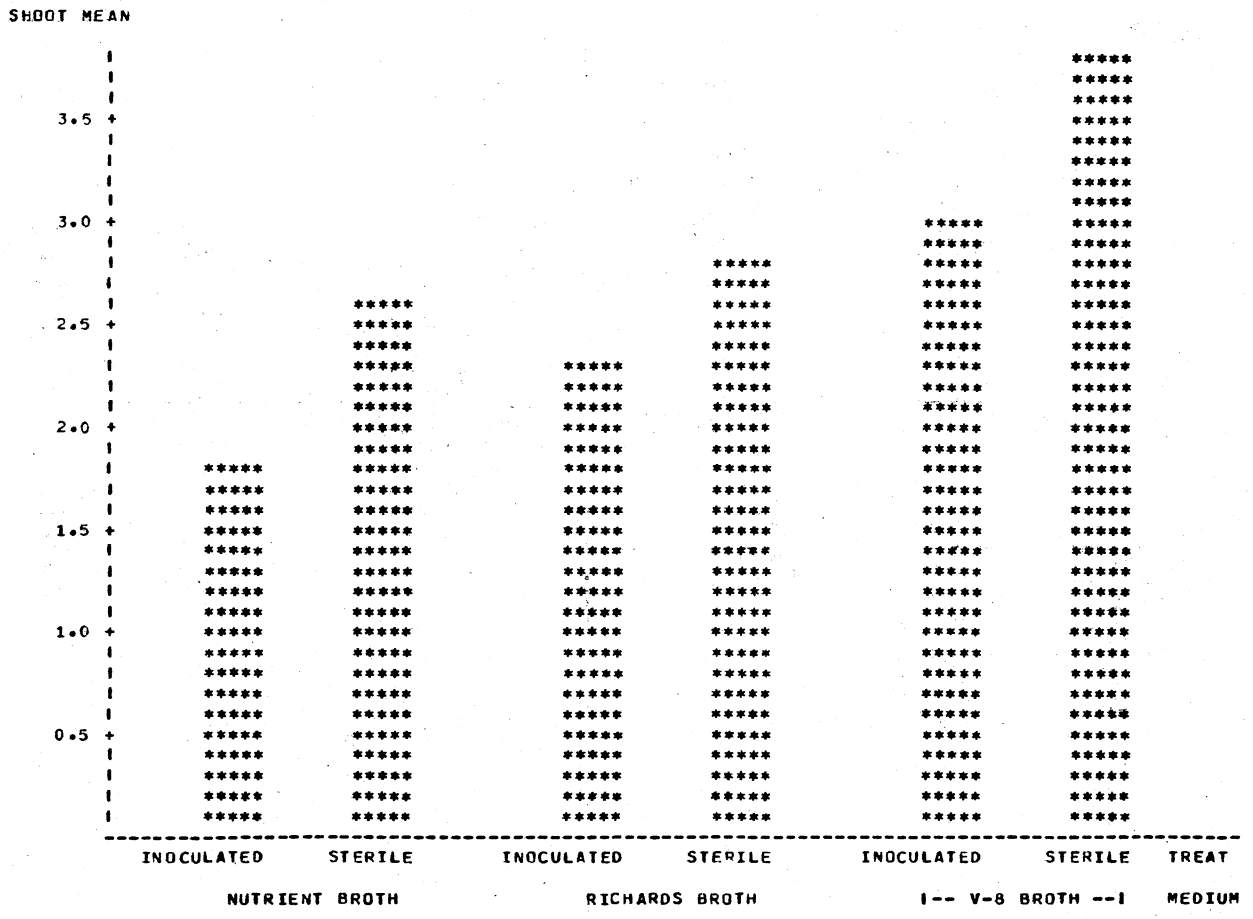


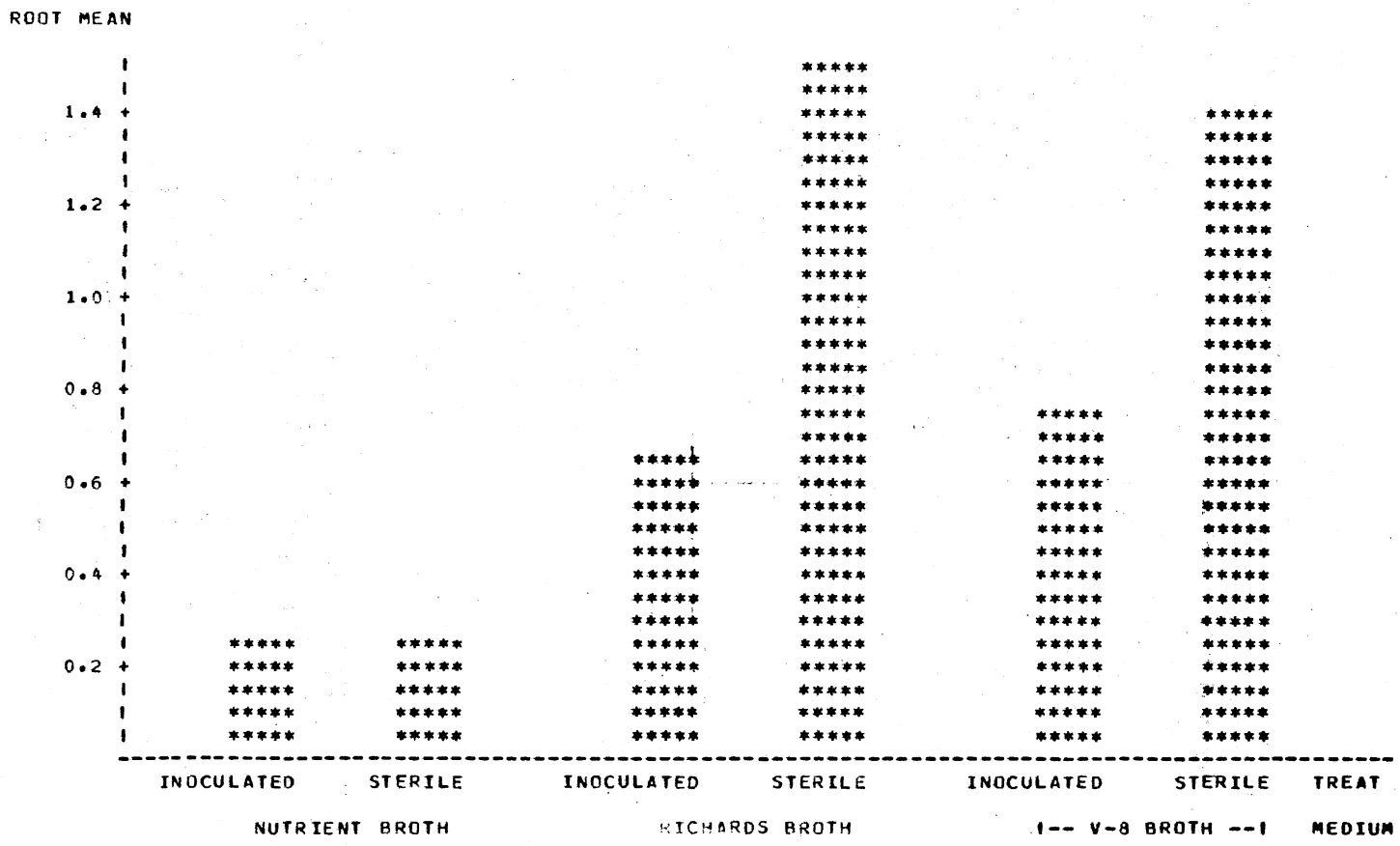
TABLE XI

ANALYSIS OF VARIANCE OF BERMUDAGRASS STOLON ROOT INITIATION FOR THE BIOASSAY OF *H. SPECIFERUM* CULTURE FILTRATES

Source	Degrees of Freedom	01 October 1979		08 October 1979		15 October 1979	
		Mean Square	F	Mean Square	F	Mean Square	F
Replication	3	0.08		0.35		0.25	
Media	2	3.52	6.76	2.33	7.00	1.56	5.00
Error A	6	0.52		0.33		0.31	
Treatment	1	3.00	4.60	0.19	0.66	0.08	0.32
Treatment X Media	2	0.81	1.24	0.25	0.88	0.02	0.08
Error B	9	0.65	0.58	0.28	0.41	0.26	0.58
Sampling Error	24	1.12		0.69		0.46	
Corrected Total	47	1.02		0.58		0.41	

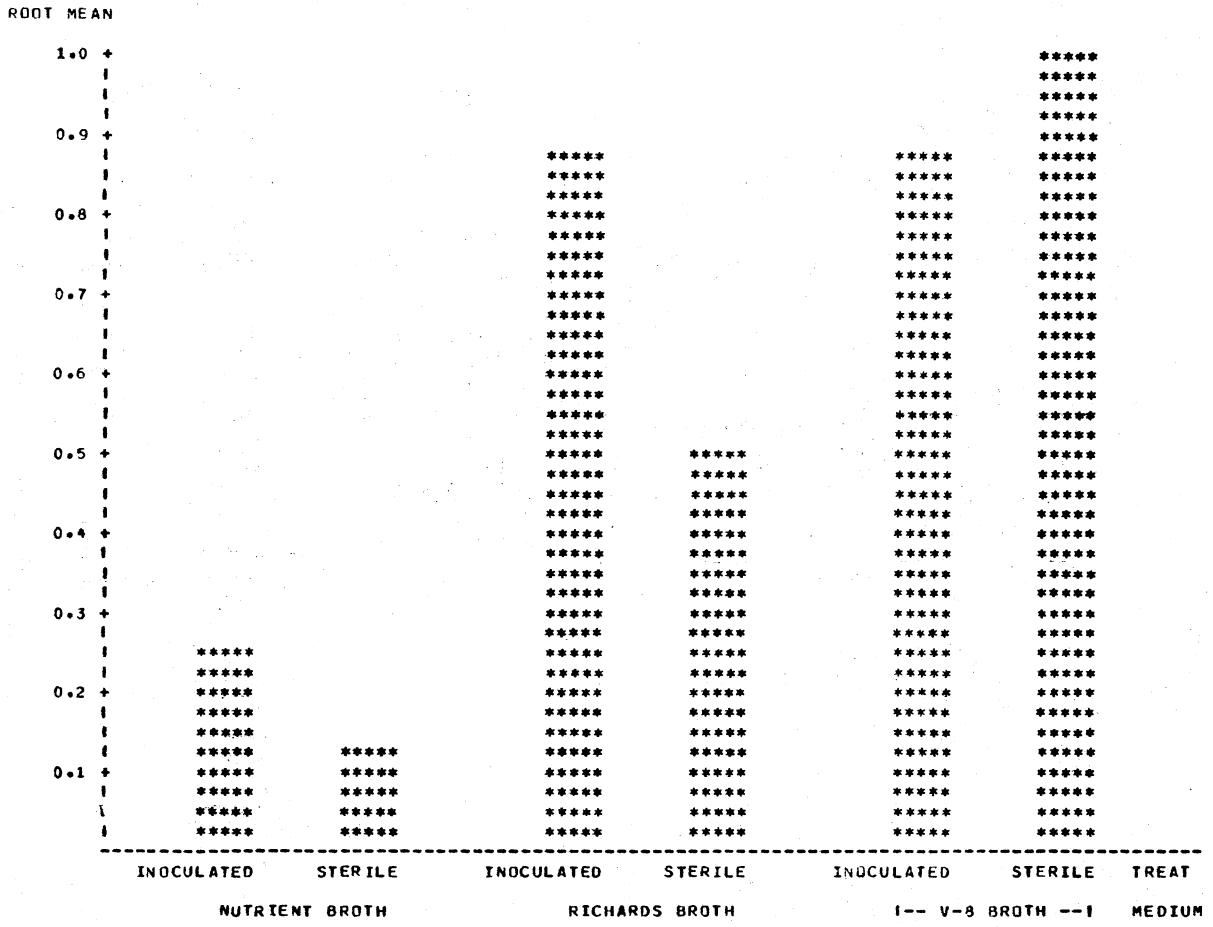
MEAN ROOT INIATIATION OF BERMUDAGRASS STOLONS
IN THREE CULTURE FILTRATES
OF HELMINTHOSPORIUM SPECIFERUM
DATE=OCTR01

BAR CHART OF MEANS



MEAN ROOT INIATIATION OF BERMUDAGRASS STOLONS
IN THREE CULTURE FILTRATES
OF HELMINTHOSPORIUM SPECIFERUM
DATE=OCTR08

BAR CHART OF MEANS



MEAN ROOT INIATIATION OF BERMUDAGRASS STOLONS
IN THREE CULTURE FILTRATES
OF HELMINTHOSPORIUM SPECIFERUM
DATE=OCTR15

BAR CHART OF MEANS

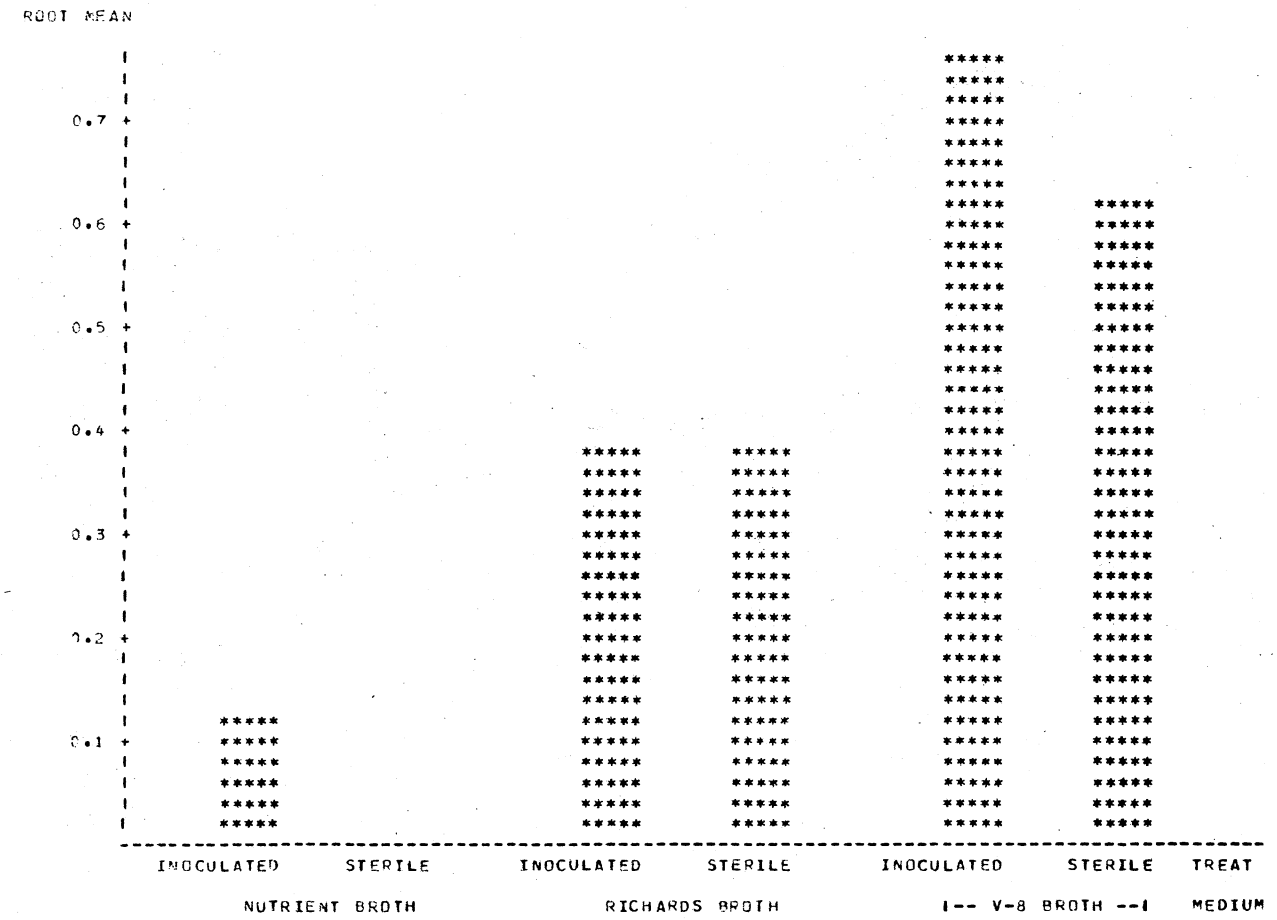


TABLE XII

ANALYSIS OF VARIANCE OF BERMUDAGRASS SEED GERMINATION FOR THE BIOASSAY OF PLANT CLIPPING EXTRACTS

Source	Degrees of Freedom	Mean Square	F
Replication	3	232.89	
Species	2	69.78	0.47
Error	6	148.00	1.24
Sampling Error	24	119.56	
Corrected Total	35	131.30	

MEAN GERMINATION OF BERMUDAGRASS GROWN
IN THE FILTRATE OF AUTOCLAVED
BERMUDAGRASS OR KNOTWEED CLIPPINGS

BAR CHART OF MEANS

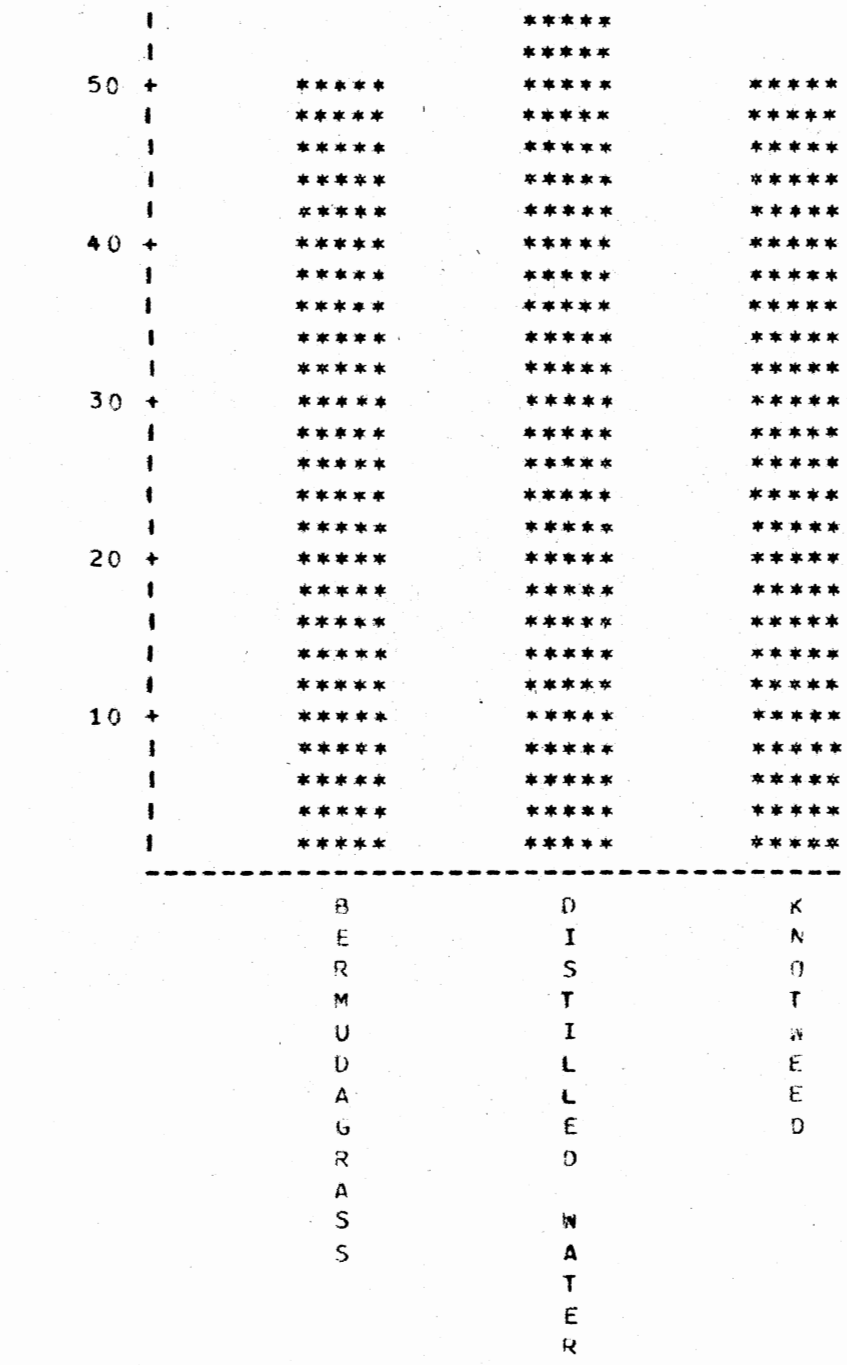


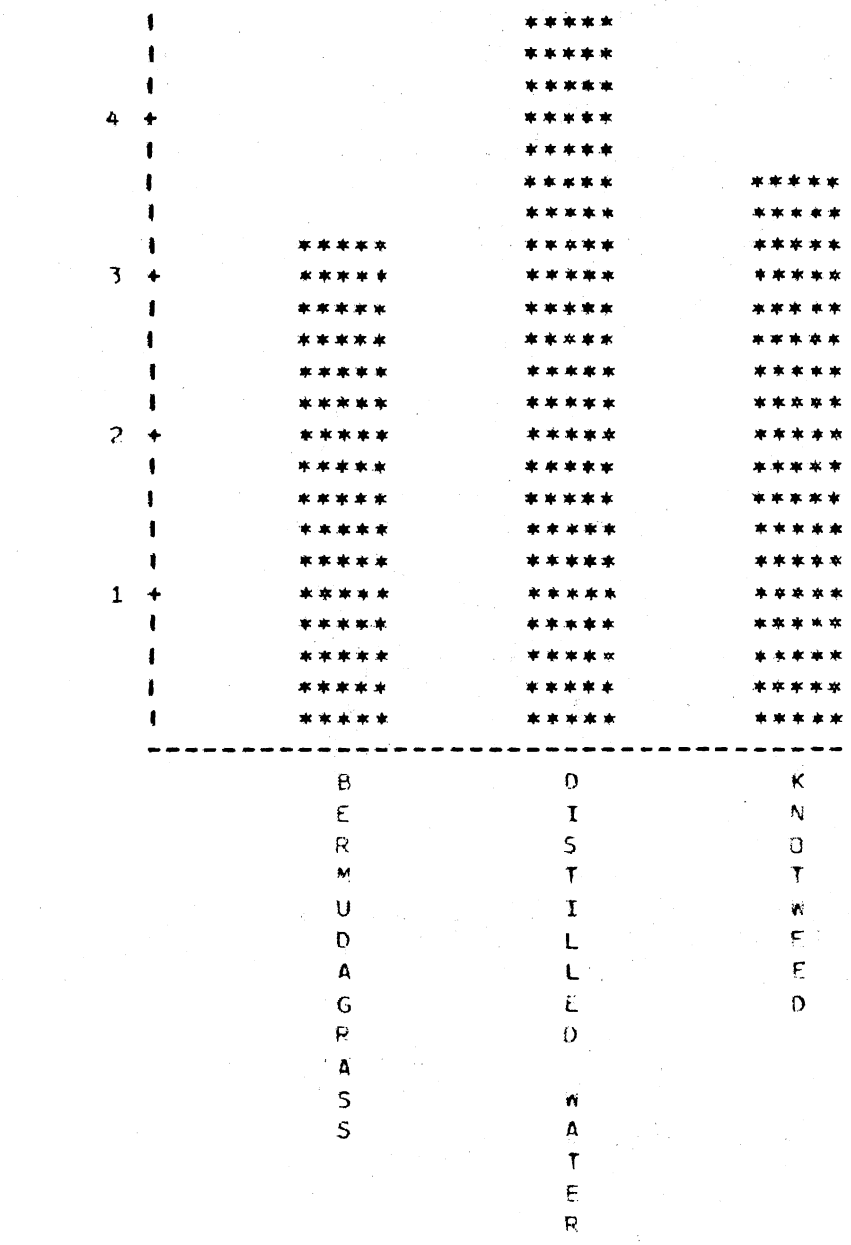
TABLE XIII

ANALYSIS OF VARIANCE OF BERMUDAGRASS STOLON SHOOT INITIATION FOR THE BIOASSAY OF PLANT CLIPPING EXTRACTS

Source	Degrees of Freedom	01 October 1979		08 October 1979		15 October 1979	
		Mean Square	F	Mean Square	F	Mean Square	F
Replication	3	0.32		3.21		0.96	
Media	2	5.25	2.37	3.25	2.45	7.75	4.00
Error	6	2.21	2.04	1.32	0.92	1.94	1.10
Sampling Error	24	1.08		1.44		1.75	
Corrected Total	35	1.45		1.68		2.06	

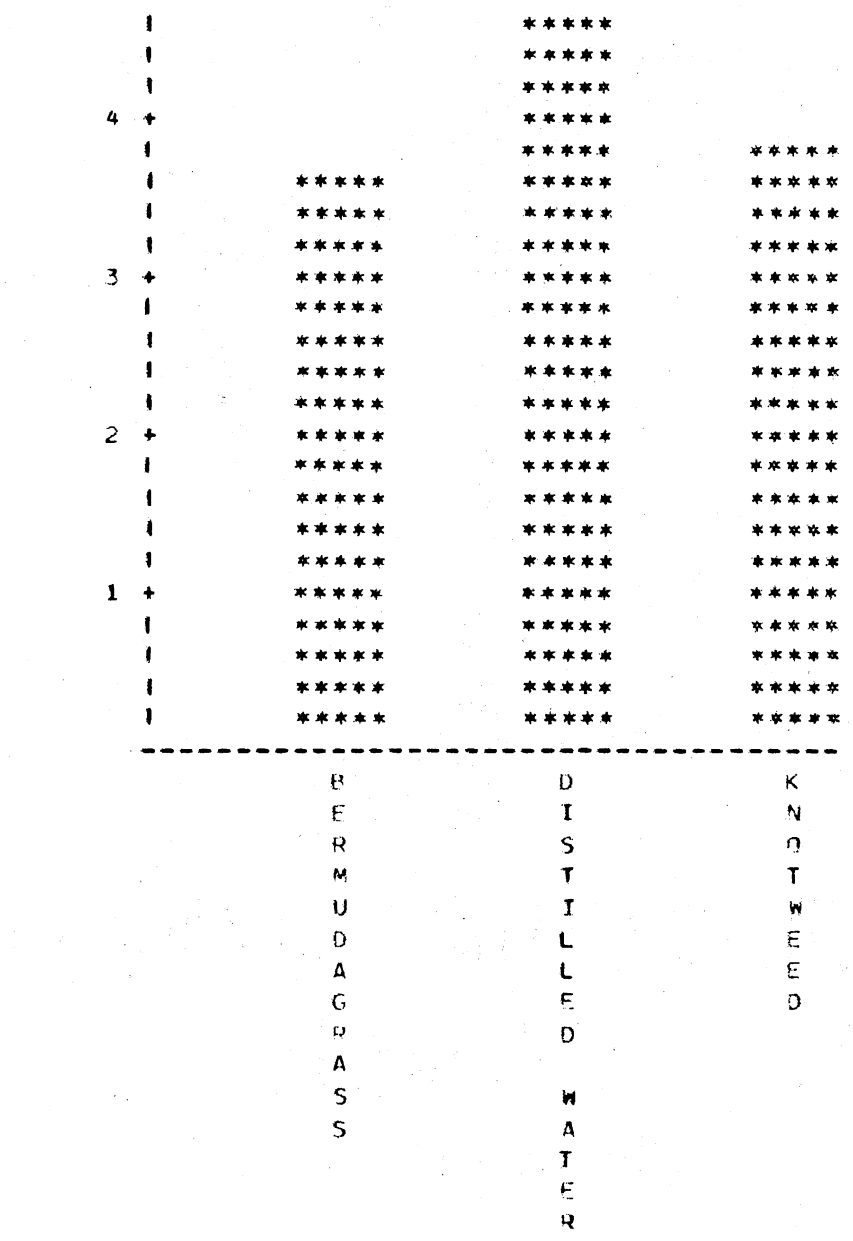
MEAN SHOOT INIATIATION OF BERMUDAGRASS STOLONS
IN THE FILTRATE OF AUTOCLAVED
BERMUDAGRASS OR KNOTWEED CLIPPINGS
DATE=OCTR01

BAR CHART OF MEANS



MEAN SHOOT INIATIATION OF BERMUDAGRASS STOLONS
IN THE FILTRATE OF AUTOCLAVED
BERMUDAGRASS OR KNOTWEED CLIPPINGS
DATE=OCTR08

BAR CHART OF MEANS



MEAN SHOOT INIATIATION OF BERMUDAGRASS STOLONS
IN THE FILTRATE OF AUTOCLAVED
BERMUDAGRASS OR KNOTWEED CLIPPINGS
DATE=OCTR15

BAR CHART OF MEANS

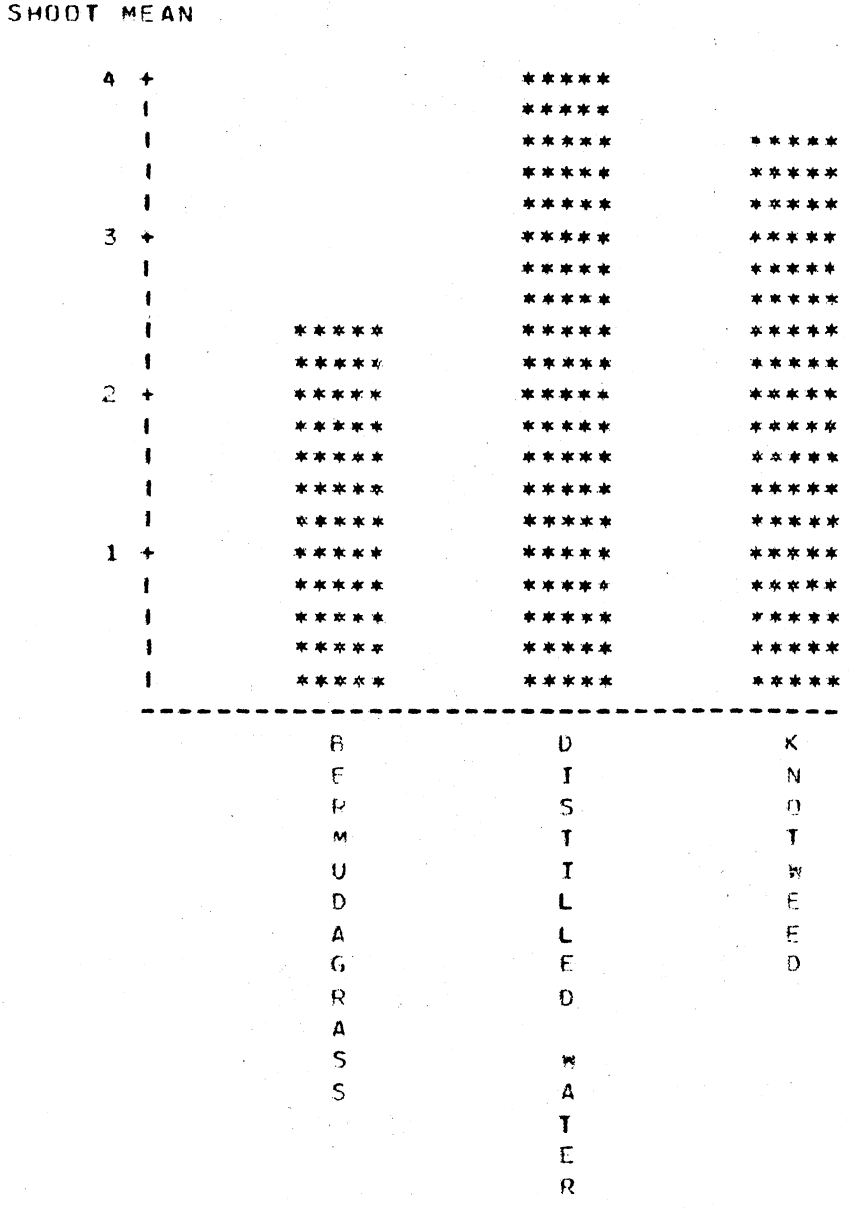


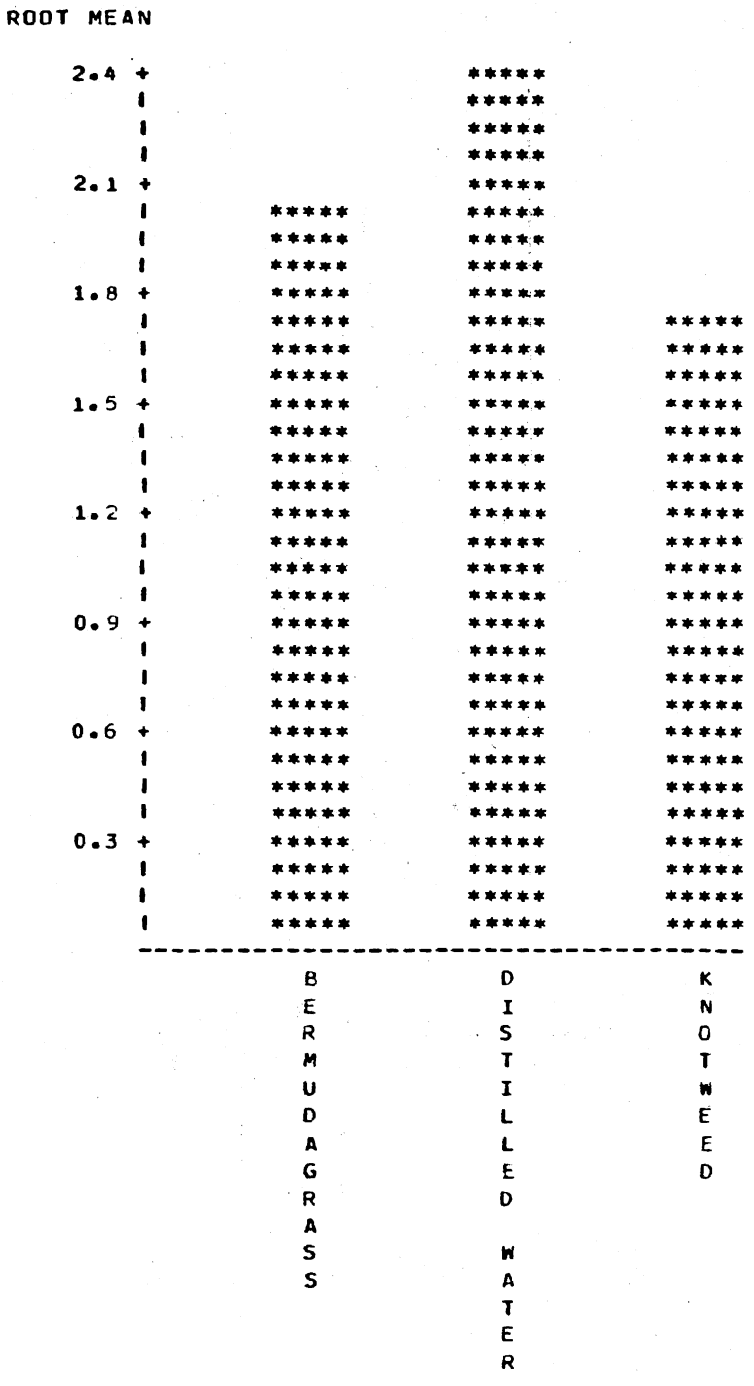
TABLE XIV

ANALYSIS OF VARIANCE OF BERMUDAGRASS STOLON ROOT INITIATION FOR THE BIOASSAY OF PLANT CLIPPING EXTRACTS

Source	Degrees of Freedom	01 October 1979		08 October 1979		15 October 1979	
		Mean Square	F	Mean Square	F	Mean Square	F
Replication	3	3.30		2.11		1.14	
Media	2	1.36	0.95	0.44	0.44	0.19	0.04
Error	6	1.44	0.52	1.00	0.60	4.86	7.00
Sampling Error	24	0.94		1.67		0.69	
Corrected Total	35	1.25		1.52		1.42	

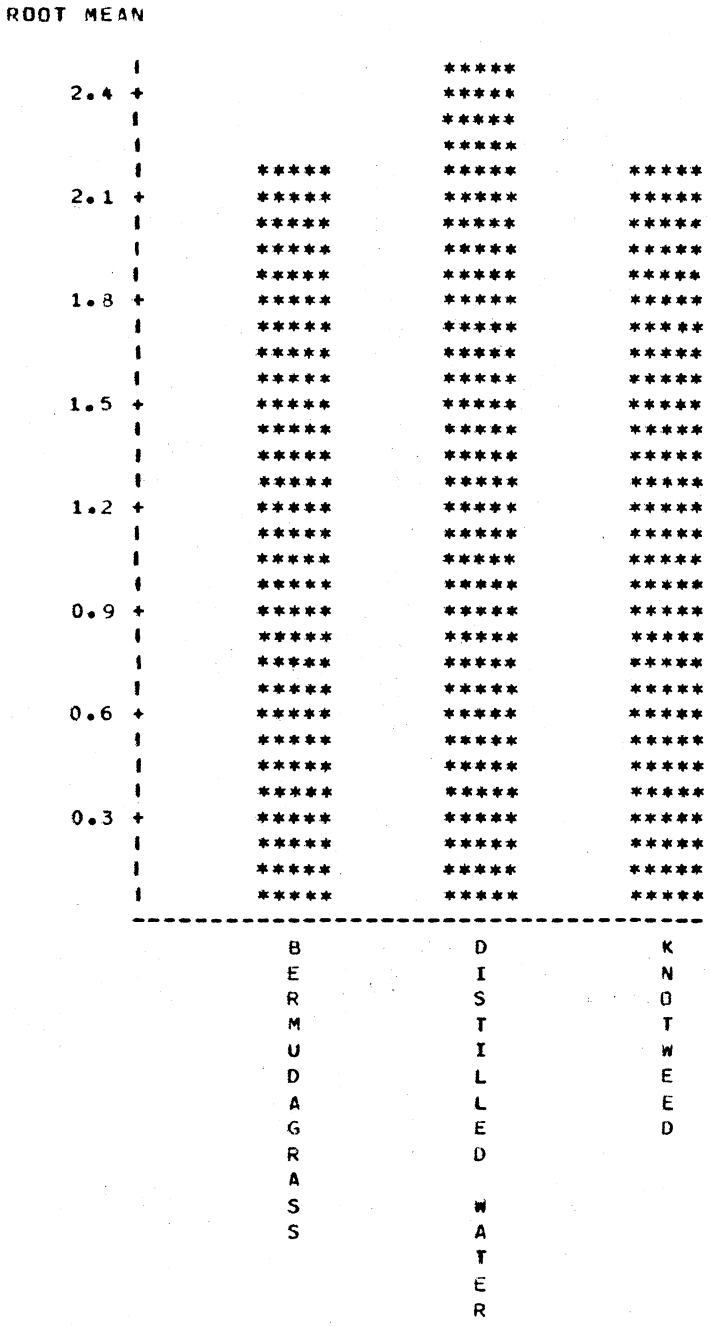
MEAN ROOT INIATIATION OF BERMUDAGRASS STOLONS
IN THE FILTRATE OF AUTOCLAVED
BERMUDAGRASS OR KNOTWEED CLIPPINGS
DATE=OCTR01

BAR CHART OF MEANS



MEAN ROOT INIATIATION OF BERMUDAGRASS STOLONS
IN THE FILTRATE OF AUTOCLAVED
BERMUDAGRASS OR KNOTWEED CLIPPINGS
DATE=OCTR08

BAR CHART OF MEANS



MEAN ROOT INIATIATION OF BERMUDAGRASS STOLONS
IN THE FILTRATE OF AUTOCLAVED
BERMUDAGRASS OR KNOTWEED CLIPPINGS
DATE=OCTR15

BAR CHART OF MEANS

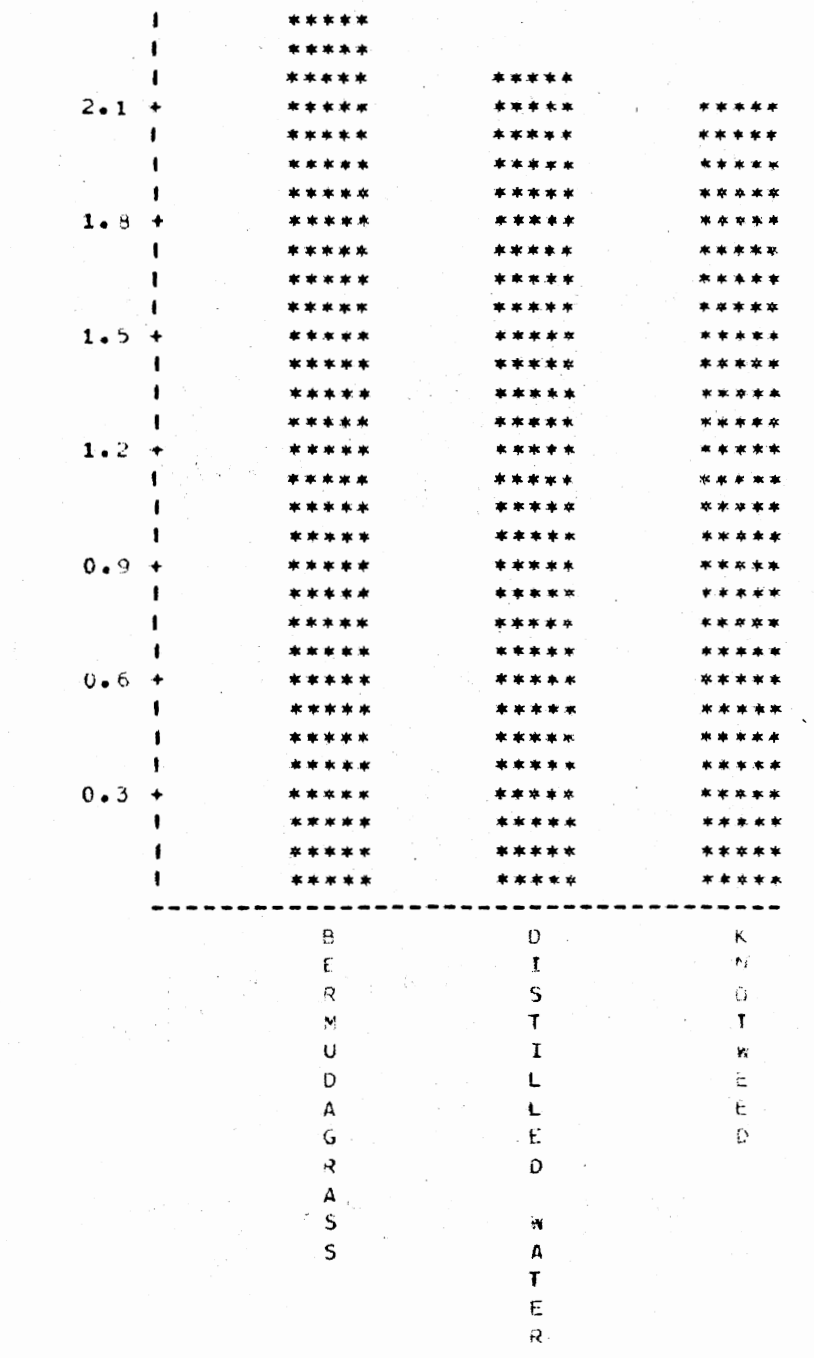


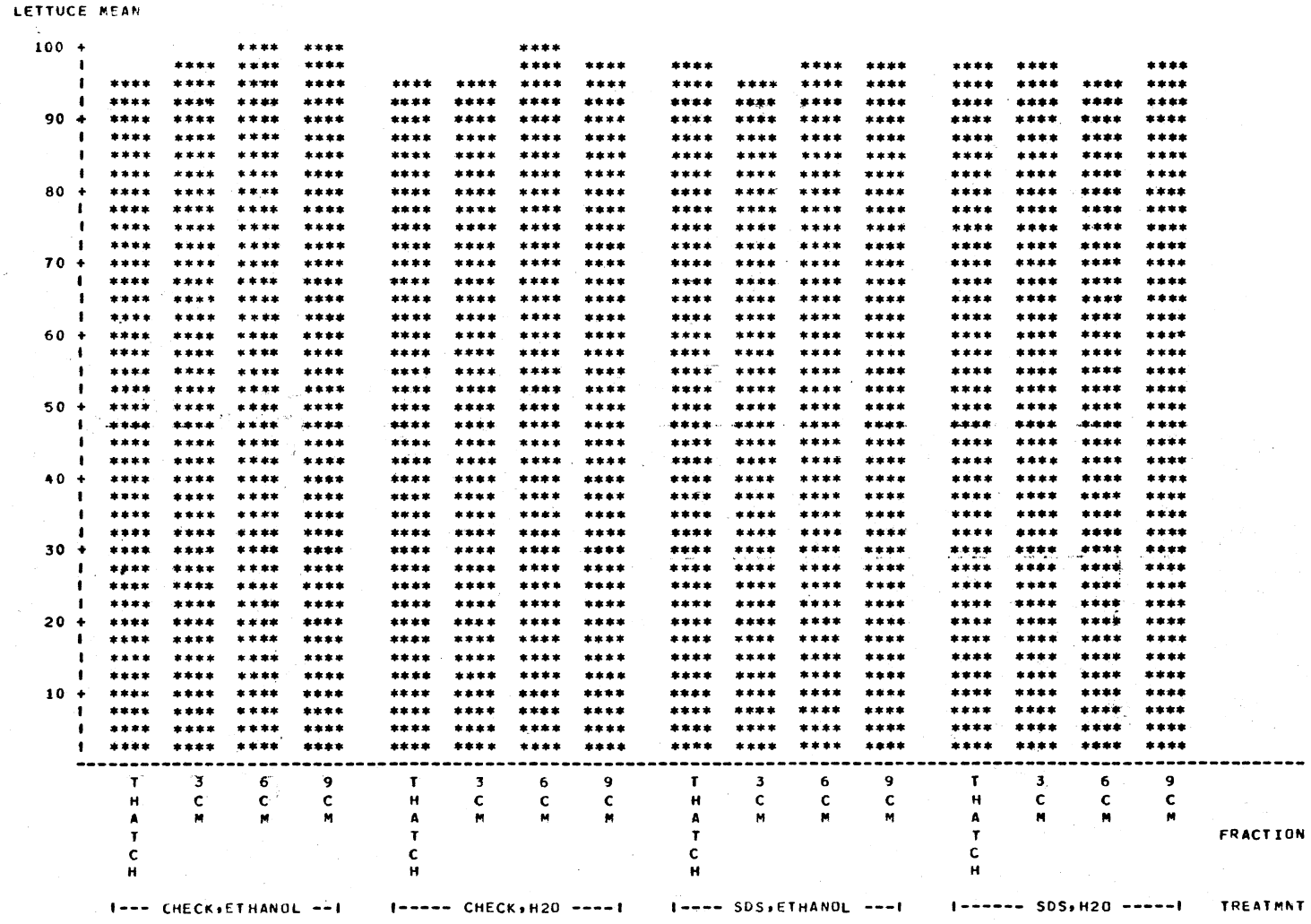
TABLE XV

ANALYSIS OF VARIANCE OF BERMUDAGRASS AND LETTUCE SEED GERMINATION FOR THE BIOASSAY OF ETHANOL AND WATER EXTRACTS OF SDS SOIL

Source	Degrees of Freedom	Bermudagrass Mean Square	F	Lettuce Mean Square	F
Replication	2	233.14		10.19	
Extract	3	16.67	0.22	5.41	0.60
Error A	6	76.56		9.08	
Depth	3	156.28	1.94	18.30	1.66
Extract X Depth	9	180.09	2.23	11.04	1.00
Error B	24	80.68		11.02	
Corrected Total	47	106.42		10.85	

MEAN LETTUCE GERMINATION IN WATER AND ETHANOL EXTRACTS
OF SPRING DEAD SPOT AND HEALTHY SOIL AT FOUR DEPTHS

BAR CHART OF MEANS



MEAN BERMUDAGRASS GERMINATION IN WATER AND ETHANOL EXTRACTS
OF SPRING DEAD SPOT AND HEALTHY SOIL AT FOUR DEPTHS

BAR CHART OF MEANS

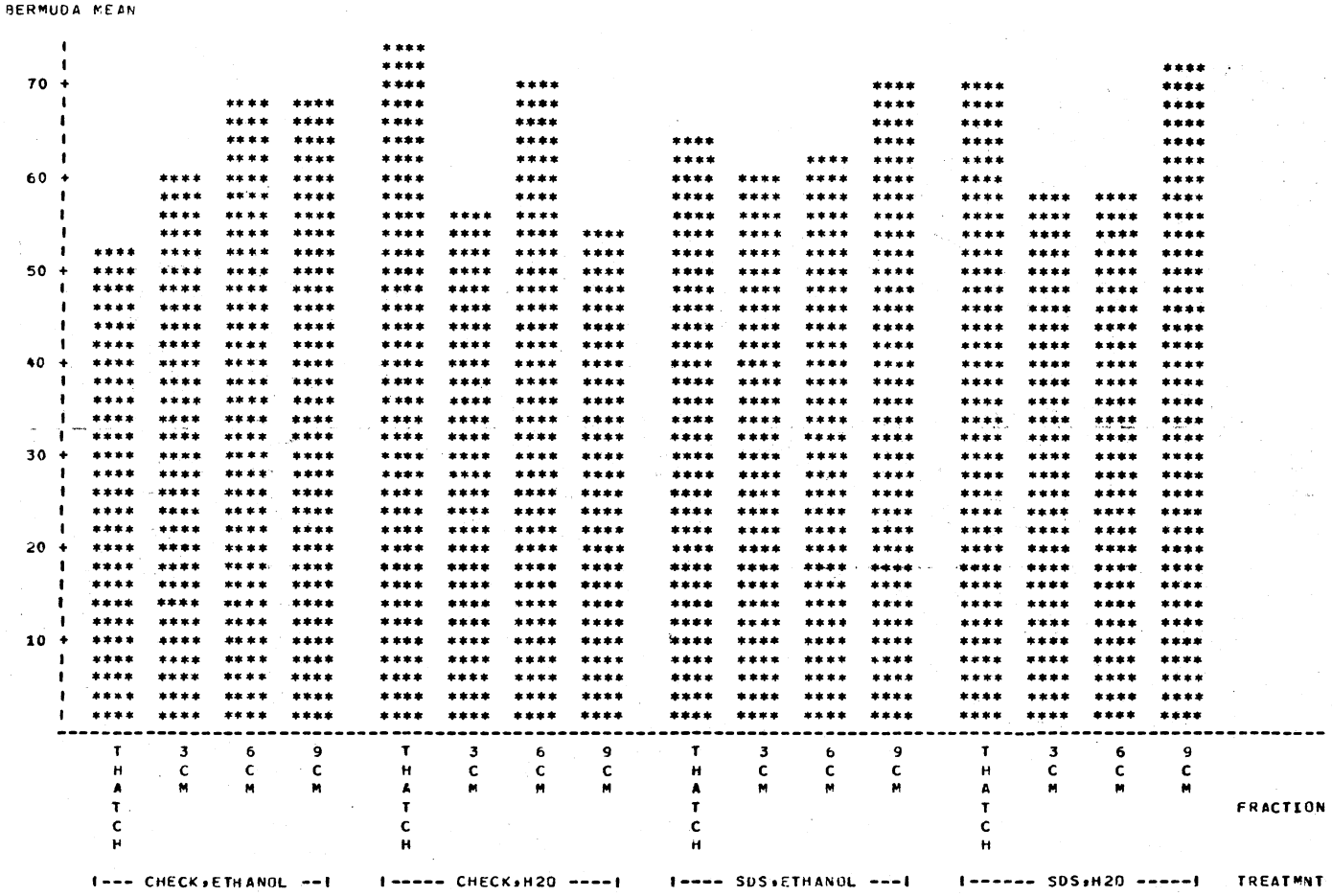


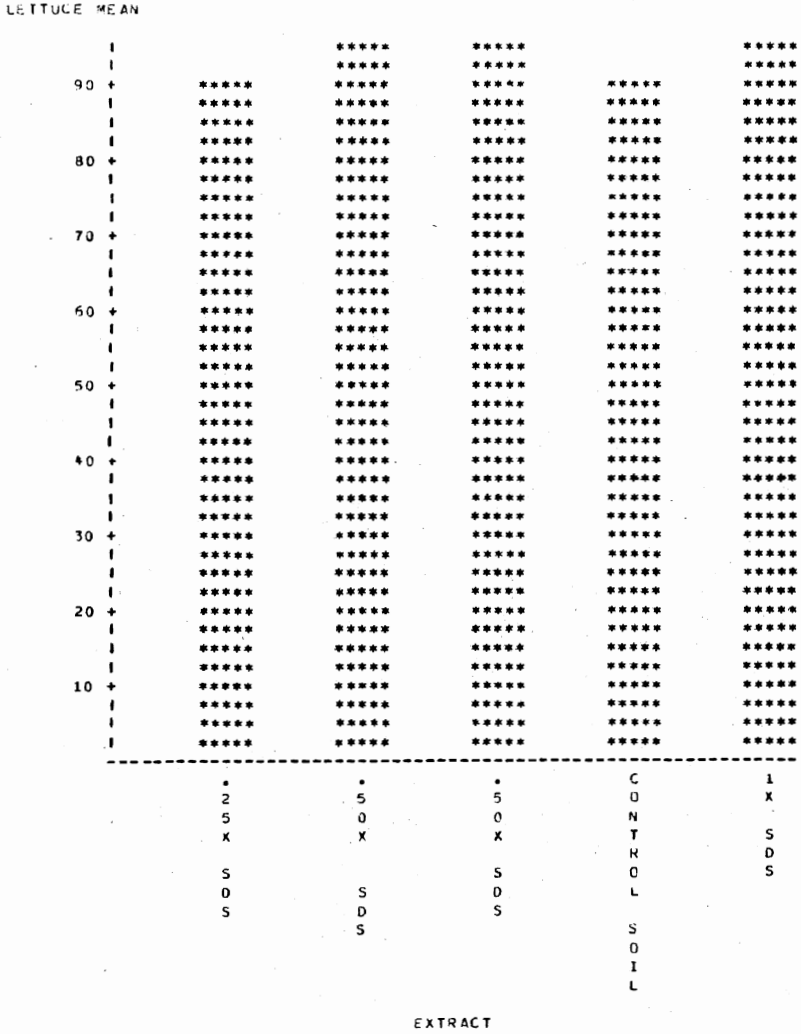
TABLE XVI

ANALYSIS OF VARIANCE OF LETTUCE SEED GERMINATION FOR THE BIOASSAY OF ETHER EXTRACT OF SDS SOIL

Source	Degrees of Freedom	Mean Square	F
Replication	3	2.23	
Concentration	3	6.39	1.85
Error	9	3.45	
Corrected Total	15	3.80	

MEAN GERMINATION OF LETTUCE IN AN ETHER EXTRACT
OF THE WATER FILTRATE OF SPRING DEAD SPOT SOIL

BAR CHART OF MEANS



APPENDIX B

METHANOL EXTRACT BIOASSAYS

TABLE XVII

ANALYSIS OF VARIANCE OF BERMUDAGRASS AND LETTUCE SEED GERMINATION FOR THE BIOASSAY OF THE FIRST METHANOL EXTRACT OF SDS AND HEALTHY SOIL

Source	Degrees of Freedom	Bermudagrass Mean Square	F	Lettuce Mean Square	F
Replication	3	121.29		70.29	
Extract	9	83.33	3.20*	4352.67	115.70**
Error	27	26.06		37.62	
Corrected Total	39	49.37		1000.15	

*Exceeds 5% Level of Significane
**Exceeds 1% Level of Significance

TABLE XVIII

ANALYSIS OF VARIANCE OF BERMUDAGRASS SHOOT LENGTH AND ROOT LENGTH FOR THE ASSAY OF THE FIRST METHANOL EXTRACT OF SDS AND HEALTHY SOIL

Source	Degrees of Freedom	Shoot Length Mean Square	Shoot Length F	Root Length Mean Square	Root Length F
Replication	3	4.51		4.24	
Extract	9	55.54	26.16**	83.04	8.31**
Error	27	2.12		9.99	
Corrected Total	39	14.26		25.58	

**Exceeds 1% Level of Significance

TABLE XIX

ANALYSIS OF VARIANCE OF LETTUCE SEED GERMINATION AND ROOT LENGTH FOR THE SECOND METHANOL EXTRACT ASSAY OF SDS AND HEALTHY SOIL

Source	Degrees of Freedom	Germination		Root Length	
		Mean Square	F	Mean Square	F
Replication	3	34.50		0.67	
Extract	9	8574.68	526.77**	93.99	93.99**
Error	27	16.28		1.00	
Corrected Total	39	1992.69		22.57	

**Exceeds 1% Level of Significance

TABLE XX

ANALYSIS OF VARIANCE OF BERMUDAGRASS SEED GERMINATION, SHOOT, AND ROOT LENGTH FOR THE SECOND METHANOL EXTRACT ASSAY OF SDS AND HEATHLY SOIL

Source	Degrees of Freedom	Germination		Shoot Length		Root Length	
		Mean Square	F	Mean Square	F	Mean Square	F
Replication	3	81.97		4.90		7.69	
Extract	9	1432.46	16.22**	24.16	8.60**	32.89	5.82**
Error	27	88.37		2.81		5.65	
Corrected Total	39	398.03		7.89		12.10	

**Exceeds 1% Level of Significance

TABLE XXI

ANALYSIS OF VARIANCE OF BERMUDAGRASS SEED GERMINATION, SHOOT, AND ROOT LENGTH FOR THE THIRD METHANOL EXTRACT ASSAY OF SDS AND HEATHLY SOIL FROM FOUR LOCATIONS

Source	Degrees of Freedom	Germination		Shoot Length		Root Length	
		Mean Square	F	Mean Square	F	Mean Square	F
Replication	3	208.68		10.63		29.18	
Soil Type	1	392.00	4.13	0.20	0.02	19.53	3.45
Site	3	31.78	0.33	25.90	3.34*	16.14	2.85
Soil Type X Site	3	303.42	3.20*	24.40	3.14*	49.43	8.74*
Error A	21	94.96		7.76		5.65	
Depth	3	652.93	8.14**	113.49	24.31**	61.43	9.88*
Depth X soil Type	3	67.94	0.85	12.90	2.76*	0.72	0.12
Depth X Site	9	239.48	2.99**	21.14	4.53*	10.28	1.65
Depth X Site X Soil Type	9	84.58	1.05	7.22	1.55	15.20	2.44**
Error B	64	80.18		4.67		6.21	
Corrected Total	119	119.56		10.75		10.39	

*,** Exceeds 5% ands 1% Level of Significance, Respectively

TABLE XXII

ANALYSIS OF VARIANCE OF BERMUDAGRASS SEED GERMINATION, SHOOT, AND ROOT LENGTH FOR THE THIRD METHANOL EXTRACT ASSAY OF SDS AND HEATHLY SOIL FROM A CUSHING HOMELAWN

Source	Degrees of Freedom	Germination		Shoot Length		Root Length	
		Mean Square	F	Mean Square	F	Mean Square	F
Replication	3	16.08		3.12		8.36	
Soil Type	1	1225.12	8.66	36.12	4.84	69.03	7.12
Error A	3	141.38		7.46		9.70	
Depth	3	195.75	1.51	42.38	18.98**	19.78	3.02*
Depth X soil Type	3	110.88	0.86	29.88	13.38**	25.44	3.89**
Error B	14	129.58		2.23		6.54	
Corrected Total	27	164.13		11.70		12.98	

*,** Exceeds 5% ands 1% Level of Significance, Respectively

TABLE XXIII

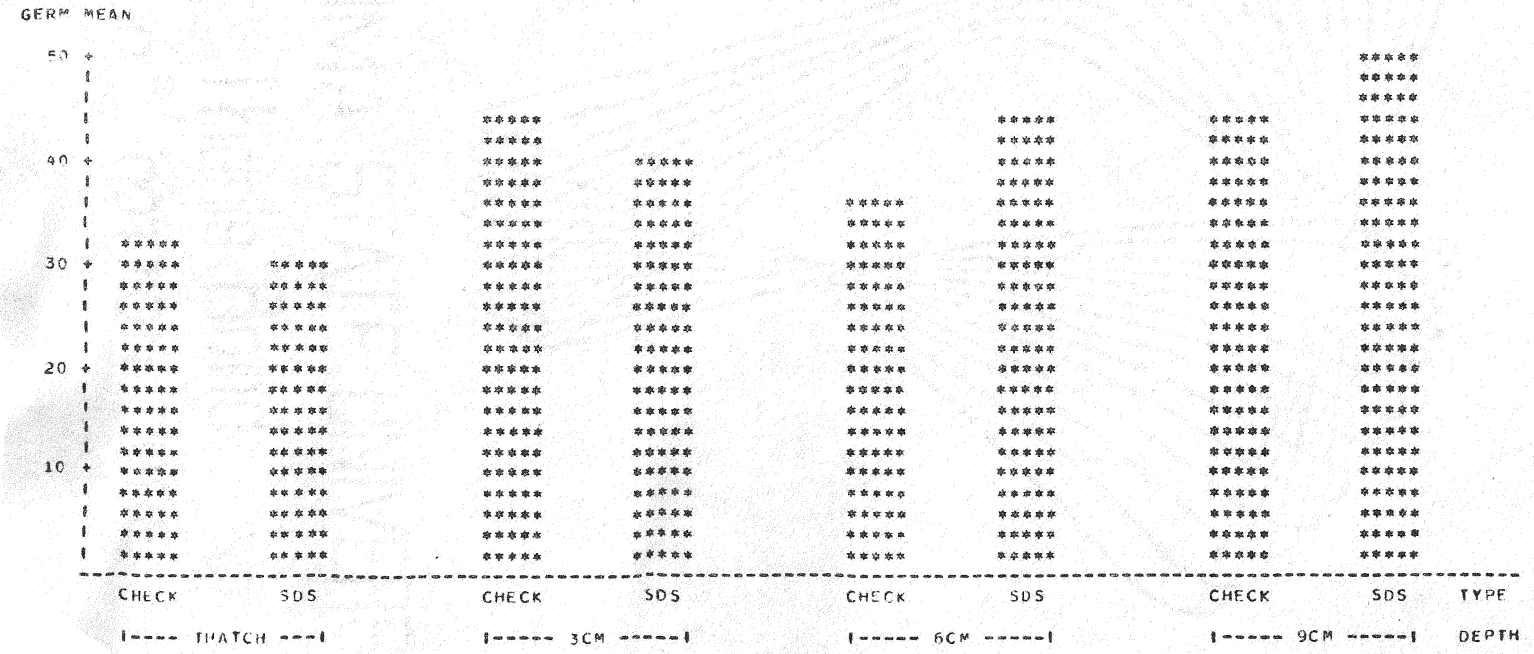
ANALYSIS OF VARIANCE OF BERMUDAGRASS SEED GERMINATION, SHOOT, AND ROOT LENGTH FOR THE THIRD METHANOL EXTRACT ASSAY OF SDS AND HEATHLY SOIL FROM THE PONCA CITY C.C.

Source	Degrees of Freedom	Germination		Shoot Length		Root Length	
		Mean Square	F	Mean Square	F	Mean Square	F
Replication	3	302.33		23.88		14.42	
Soil Type	1	24.50	0.23	12.50	1.28	32.00	12.39*
Error A	3	106.17		9.75		2.58	
Depth	3	752.83	8.09**	11.46	1.72	25.08	5.47**
Depth X soil Type	3	103.50	1.11	0.33	0.05	5.42	1.20
Error B	18	93.06		6.67		4.50	
Corrected Total	31	177.27		8.67		8.24	

*,** Exceeds 5% ands 1% Level of Significance, Respectively

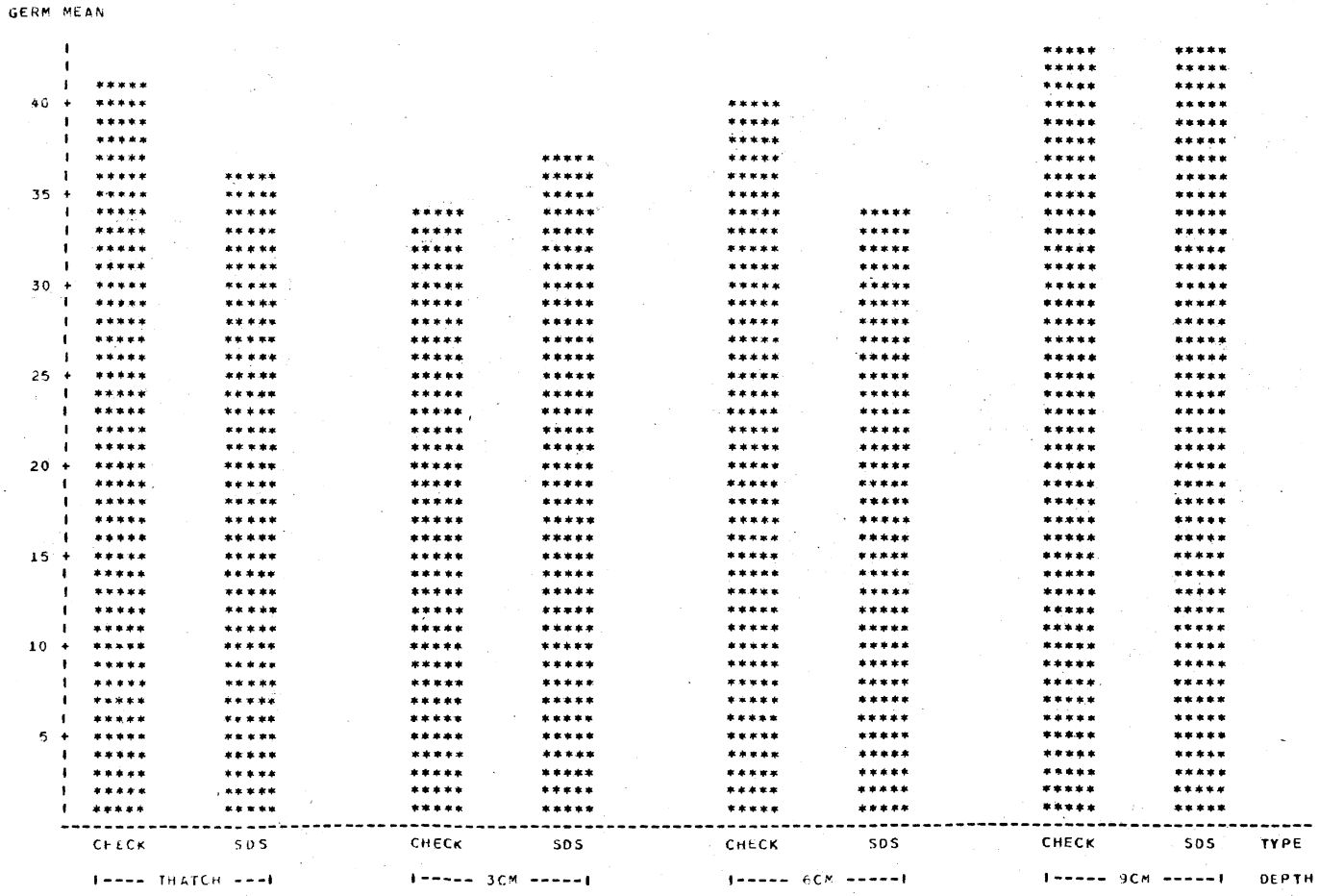
MEAN GERMINATION OF BERMUDAGRASS IN THE METHANOL EXTRACT
OF SPRING DEAD SPOT AND HEALTHY SOIL
AT FOUR DEPTHS AND FOUR LOCATIONS
LOCATION=CUSHING CC

BAR CHART OF MEANS



MEAN GERMINATION OF BERMUDAGRASS IN THE METHANOL EXTRACT
OF SPRING DEAD SPOT AND HEALTHY SOIL
AT FOUR DEPTHS AND FOUR LOCATIONS
LOCATION=SOUTHERN HILL CC

BAR CHART OF MEANS



APPENDIX C

COLUMN CHROMATOGRAPHY EFFLUENT BIOASSAYS

TABLE XXIV

ANALYSIS OF VARIANCE OF LETTUCE SEED GERMINATION AND SEEDLING LENGTH FOR THE COLUMN CHROMATOGRAPHY EFFLUENT ASSAY OF SDS SOIL FROM A STILLWATER HOMELAWN

Source	Degrees of Freedom	Germination Mean Square	Germination F	Seedling Length Mean Square	Seedling Length F
Replication	2	28.28		39.46	
Fraction	32	565.59	1.91*	22.27	1.50
Error	64	295.99		14.81	
Corrected Total	98	378.56		17.75	

*Exceeds 5% Level of Significance

TABLE XXV

ANALYSIS OF VARIANCE OF LETTUCE SEED GERMINATION AND SEEDLING LENGTH FOR THE COLUMN CHROMATOGRAPHY EFFLUENT ASSAY OF SDS SOIL FROM A CUSHING HOMELAWN

Source	Degrees of Freedom	Germination Mean Square	Germination F	Seedling Length Mean Square	Seedling Length F
Replication	2	1393.65		7.72	
Fraction	41	1922.08	5.12**	81.82	4.66**
Error	82	375.05		17.54	
Corrected Total	125	898.77		38.47	

**Exceeds 1% Level of Significance

VITA

Thomas Walter Fermanian

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE ISOLATION OF A TOXIN FROM SPRING DEAD SPOT AREAS IN BERMUDAGRASS (CYNODON L.C. RICH.) TURF

Major Field: Crop Science

Biographical:

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Education: Graduated from Solomon Juneau High School, Milwaukee, Wisconsin, in June, 1968; received Bachelor of Science degree from the University of Wisconsin-Whitewater in 1972 with a major in Biology; received Master of Science degree in Agronomy from Oklahoma State University in 1978; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in July, 1980.

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