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I am submitting herewith a thesis written by Eddie Dean Seagle entitled "Cellular Responses in Roots of Bentgrass and Bermudagrass to Selected Herbicides." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ornamental Horticulture and Landscape Design.

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CELLULAR RESPONSES IN ROOTS OF BENTGRASS AND BERMUDAGRASS TO SELECTED HERBICIDES

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Eddie Dean Seagle

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ABSTRACT

Cellular responses in roots of Penncross creeping bentgrass (*Agrostis palustris* Huds.) and Tifgreen bermudagrass (*Cynodon dactylon* (L.) Pers. x *Cynodon transvaalensis* Burtt-Davy) to selected herbicides were determined by a histological study. The most severe tissue abnormalities in Penncross bentgrass occurred following treatments of benefin (N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine) and terbutol (2,6-di-tert-butyl-p-tolyl methylcarbamate). Root cell damage in bentgrass was less severe with bandane (polychlorodicyclopentadiene isomers), bensulide [O,O-diisopropyl phosphorodithioate S-ester with N-(2-mercaptoethyl)benzenesulfonamide], DCPA (dimethyl tetrachloroterephthalate) and siduron [1-(2-methylcyclohexyl)-3-phenylurea]. The most severe tissue malformations in Tifgreen bermudagrass resulted from DCPA, siduron and terbutol. Cellular damage in roots of bermudagrass was less severe from bandane and bensulide, and slight with benefin.

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CHAPTER I

INTRODUCTION

Weeds are a problem nearly everywhere turfgrasses are grown. A desire for an attractive weed-free turf in recent years has led to the increased use of herbicides. Herbicides are an important management tool in good turfgrass culture but they can create serious problems even when used properly (5). When an herbicide comes in contact with a plant, changes in the morphology and anatomy may occur and various physiological and biochemical processes can be affected (30). In recent years, herbicides which were thought to be safe to turfgrasses have been shown to cause extensive cellular damage (9). Information on the nature of herbicide injury to turfgrasses can influence the continued acceptance of these chemicals in management programs.

To understand better how herbicides may affect turfgrasses, a study was conducted to investigate the potential phytotoxic effects of selected herbicides to turfgrass roots. The specific objective of this study was to determine the anatomical and morphological responses of cells in the roots of Penncross creeping bentgrass (*Agrostis palustris* Huds.) and Tifgreen bermudagrass

(*Cynodon dactylon* (L.) Pers. x *Cynodon transvaalensis* Burtt-Davy) to selected herbicides.

CHAPTER II

REVIEW OF LITERATURE

Use of herbicides in turfgrass management programs is increasing and often faster than research can verify their level of safety to turfgrasses. A desirable herbicide is one that gives excellent control of a target weed or weeds with minimal turfgrass injury. Effects of herbicides in the cells of turfgrasses must be better understood in order to establish their general level of safety.

Each of the herbicides investigated in this study will be discussed separately in the following literature review.

I. BANDANE

Bandane (polychlorodicyclopentadiene isomers) is a chlorinated hydrocarbon compound labeled for use in turf as a preemergence herbicide to control crabgrass (*Digitaria* spp.). Burt and Simmons (4) reported that bandane applied at 44 kg/ha (40 lb/A) three days before seeding of Kentucky bluegrass, Chewings fescue, and tall fescue significantly reduced turfgrass emergence. When the turfgrasses were seeded 17 days after treatment, little or no reduction in emergence occurred. The time period between the date of herbicide application and planting

appeared critical to the safety and survival of all turfgrass species.

In work with common Kentucky bluegrass sod plugs, Smith and Callahan (39) reported no significant reduction in root length with 44 kg/ha (40 lb/A) of bandane, but a significant reduction occurred with an 88 kg/ha (80 lb/A) rate. Top growth of the Kentucky bluegrass was reduced significantly at both rates.

Turgeon et al. (41, 42) reported marked reduction in turf quality and rooting with an increased proneness to stripe smut disease in Kenblue and Merion Kentucky bluegrass, and Pennlawn red fescue when bandane was applied at 44 kg/ha (40 lb/A). Their investigations further showed that bandane at 39 kg/ha (35 lb/A) applied in four consecutive years could be associated with increased occurrence of leaf spot disease, higher wilting tendency, reduced shoot and root growth, and substantial thatch development in Kenblue Kentucky bluegrass (43). Hall et al. (23) stated that continuous applications of bandane at the rate of 39 kg/ha (35 lb/A) in a mixture of Kentucky bluegrass and red fescue was deleterious to the turf quality. Furthermore, continuous herbicide treatments significantly reduced the number of roots at the 7.6 cm (3 in) depth and reduced the germination potential of the overseeded Kentucky bluegrass.

Callahan (6) reported that bandane treatments at 44, 66 and 88 kg/ha (40, 60 and 80 lb/A) the first year and one-half these rates the second and third years in a Penncross bentgrass green resulted in slight injury the first year, slight sod loss the second year, and severe sod loss the third year. The bandane treated plots were more susceptible to pathogenic diseases and wilting injury.

Bandane was reported to cause little or no observed injury in grasses such as Pensacola bahiagrass, Emerald zoysia, Bitter Blue St. Augustinegrass, and Ormond and Tifgreen bermudagrass (3, 13). However, Callahan (7) investigated bandane treatments at 44 and 88 kg/ha (40 and 80 lb/A) the first year, and at one-half these rates the second and third years in a Tifgreen bermudagrass green. Results showed no foliage injury or sod loss the first year. Moderate injury occurred the second year with foliage symptoms resembling wilt suggesting herbicide induced physiological stress from root injury. The third year the turf showed severe injury. Furthermore, bandane continued to cause phytotoxicity injury to the Tifgreen bermudagrass from residues up to three years after the last annual herbicide treatment.

II. BENEFIN

Benefin (N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine) is a dinitro analine compound labeled for use as a selective, preemergence herbicide for the control of crabgrass, goosegrass and other annual weedy grasses in turf.

Benefin has been reported to consistently result in significantly reducing the density of Italian ryegrass and rough bluegrass (12). Callahan (5, 6) emphasized that herbicides, including benefin, often weaken root systems to the extent that they become incapable of functioning properly under adverse environmental conditions. He found in a three-year study using benefin at 2.2, 3.3 and 4.4 kg/ha (2, 3 and 4 lb/A) in a Penncross bentgrass green, moderate injury and increased disease and wilting the first year, moderate injury the second year, and severe sod loss the third year. During the fourth year, which was an observation year, sod loss persisted through late spring before recovery with a brief sod loss later in the season following a high incidence of Rhizoctonia brown patch in the benefin treated plots. The intensity of disease activity was very severe and very difficult to halt when compared to untreated plots.

Benefin has been reported to cause a marked reduction in overseeded Pennfine perennial ryegrass without causing

observable injury to the bermudagrass (32, 33). Other investigators have reported that benefin did not significantly reduce root growth of several bermudagrass hybrids (27, 38). However, studies by Coats and Ward (10) showed that benefin slightly inhibited rooting of No Mow, Tifdwarf, Tifgreen, Tifway and common bermudagrass. Treatments with benefin in February delayed the growth of common bermudagrass in a golf putting green for two or three weeks (16). Benefin applications later in the spring for three consecutive years in a Tifgreen bermudagrass green resulted in only slight injury and no sod loss the first year, moderate injury and high percentage sod loss the second year, and severe injury and sod loss the third year (7). Observations during the fourth year indicated slight injury or foliage wilt from residue, with full recovery later into the season.

III. BENSULIDE

Bensulide [O,O-diisopropyl phosphorodithioate S-ester with N-(2-mercaptoethyl)benzenesulfonamide] is a sulfonamide compound labeled for use as a selective, preemergence herbicide for the control of annual bluegrass, crabgrass, goosegrass and several annual broadleaf weeds in turf.

Smith and Callahan (39) reported that bensulide reduced root growth of common Kentucky bluegrass growing

in pot cultures. Furthermore, Hall et al. (23) concluded that repeated applications of bensulide at 14 kg/ha (12.5 lb/A) to turf considerably reduces the probability of achieving success with Kentucky bluegrass overseedings. Although, Johnson (28) reported that bensulide at 11 kg/ha (10 lb/A) resulted in no observed injury to a mature stand of Kentucky bluegrass.

Other researchers have reported that bensulide affects established annual bluegrass by causing leaf discoloration and root length reduction without causing any observable injury to creeping bentgrass (35). Bensulide also caused no observable injury to creeping or velvet bentgrass putting green turf (37). However, phytotoxic symptoms have been observed in annual ryegrass, red fescue and rough bluegrass (44). Furthermore, bensulide applied in three consecutive annual applications to a Penncross bentgrass green resulted in no observable injury the first year, no sod loss the second year, but severe sod loss the third year (6). Fall reseeding in the third year was unsuccessful and the sod loss continued into the following season with Rhizoctonia brown patch disease becoming very severe.

Several researchers working with Tifgreen bermudagrass have found bensulide to retard the development of stolons, rhizomes and roots (11, 14, 17). Callahan (7) showed that bensulide in a Tifgreen bermudagrass green

caused a slight foliage browning and no sod loss the first and second years. Slight to moderate injury and slight sod loss occurred the third year. Foliage wilt and slight foliage browning continued to persist during the season following testing.

IV. DCPA

Another chemical labeled for use in turf as a selective, preemergence herbicide for the control of annual bluegrass, crabgrass, goosegrass and certain annual broadleaf weeds is DCPA (dimethyl tetrachloroterephthalate), a phthalic acid compound.

DCPA has been found to inhibit root regrowth of Kentucky bluegrass sod plugs grown in culture containers under greenhouse conditions (15, 39). Although some researchers have reported no observed injury with DCPA in mature Kentucky bluegrass sod (4, 20), Goss (21, 22) observed stunted, yellow annual bluegrass seedlings removed from a DCPA treated plot.

Several investigators have reported that DCPA injured red fescue and colonial bentgrass (20, 21, 22, 34). DCPA reduced turf uniformity and bud counts in Toronto bentgrass (36). DCPA applied for three consecutive years in a Penncross bentgrass green showed slight injury the first year and moderate sod loss the second and third years (6). During the season following testing, slight sod loss and

increased occurrence of Rhizoctonia brown patch was observed.

Some researchers have reported that DCPA caused little or no observable injury to Tifgreen, Ormond and Texturf bermudagrass (3, 14). However, Fullerton et al. (18, 19) found that DCPA caused a decrease in verdure, and stolon and root weights in Tifgreen bermudagrass. The results further indicated that DCPA was implicated in increased winter kill. Studies by Bingham (1, 2) showed that DCPA reduced initiation of roots and stolons in Tifgreen bermudagrass. Histological studies of root tips of the bermudagrass further showed that cell division had ceased while cell enlargement continued for some time with cells becoming excessively large and irregularly shaped. Huffine (25) reported that DCPA significantly inhibited rooting in U-3 bermudagrass.

Coats et al. (12) reported that DCPA applied in February delayed the breaking of dormancy in Tifdwarf bermudagrass. Coats et al. (10) later determined that the February treatments with DCPA resulted in slow growth and increased incidence of Sclerotinia dollar spot in Tifgreen bermudagrass. In another investigation, DCPA applied in a Tifgreen bermudagrass green for three consecutive years showed moderate injury the first and second years, and severe injury and sod loss the third year (7). During the season following testing, foliage wilt and sod loss persisted.

V. SIDURON

Siduron [1-(2-methylcyclohexyl)-3-phenylurea] is a substituted urea compound labeled for use in turf as a selective, preemergence herbicide for the control of crabgrass and a few other annual weedy grasses and broadleaf weeds. Smith and Callahan (39) reported that siduron in sod plugs of Kentucky bluegrass under greenhouse conditions caused little significant reduction in root growth. However, Turgeon et al. (42) found that siduron reduced root growth of Kenblue Kentucky bluegrass and clipping yields in red fescue in field plots.

Splittstoesser and Hopen (40) reported that siduron caused no visible injury to the top growth of Cohansey creeping bentgrass, and that only an extremely high rate of 110 kg/ha (100 lb/A) reduced root growth. However, much lower rates of 0.8 and 1.7 kg/ha (0.75 and 1.5 lb/A) injured both top and root growth of IaGreen creeping bentgrass in a greenhouse study (40). Juska et al. (29) found that Washington creeping bentgrass was severely injured by siduron but Penncross creeping bentgrass showed no visible injury. However, studies with siduron applied for three consecutive years in a Penncross creeping bentgrass green resulted in no observable injury the first and second years, but moderate sod loss in the third year (6).

Investigations with common bermudagrass showed that siduron reduced root initiation on stolons (1). The root

tips appeared enlarged. Microscopic observations revealed that cell division had ceased. Another investigator used siduron for three consecutive years in a Tifgreen bermudagrass green and reported severe foliage injury and sod loss in all three years of the study (7). Observations during the season following the test showed that foliage injury or sod loss did not persist.

VI. TERBUTOL

Terbutol (2,6-di-tert-butyl-p-tolyl methylcarbamate) is a carbamate compound labeled for use as a selective, preemergence herbicide for the control of crabgrass in turf. Engel and Callahan (15) reported that terbutol soil residue samples from field treatments caused serious inhibition of root development of Merion Kentucky bluegrass sod plugs in greenhouse culture studies. A few years later Smith and Callahan (39) confirmed these findings.

Turgeon et al. (41, 42) reported that terbutol reduced clipping yield of Pennlawn red fescue. Also, they observed visual injury in Kenblue Kentucky bluegrass with a significant reduction in root growth and increased thatch accumulation (41, 42).

Callahan (5, 6) found that three consecutive annual treatments with terbutol in a Penncross creeping bentgrass green resulted in moderate injury the first and second years, and severe injury the third year. Severe injury

persisting from residues of terbutol was observed during the fourth year. Furthermore, incidence of Pythium blight was very high in terbutol treated plots when compared to the untreated plots.

Smith and Ayers (38) stated that terbutol did not affect root growth of Tifdwarf, Tifgreen and Tifway bermudagrass. However, Coats and Ward (11) found that terbutol inhibited rooting of No Mow, Tifdwarf, Tifgreen, Tifway and common bermudagrass. Callahan (7) found that terbutol caused slight injury to a Tifgreen bermudagrass green the first year of application, moderate injury the second year, and severe injury the third year. Also, slight injury from residues continued to occur throughout the fourth season following the test period.

CHAPTER III

MATERIALS AND PROCEDURES

This study was developed to determine the response of cells in the root tips of Penncross creeping bentgrass and Tifgreen bermudagrass to several preemergence type herbicides.

The herbicide treatment portion of the study was conducted under greenhouse conditions. The histological determinations were conducted in the laboratory. Greenhouse and laboratory procedures will be discussed separately.

Investigations were conducted in the greenhouse and laboratory facilities of the Department of Ornamental Horticulture and Landscape Design of the University of Tennessee, Knoxville, from March 1976 to November 1977.

I. GREENHOUSE PROCEDURES

Plant Preparation

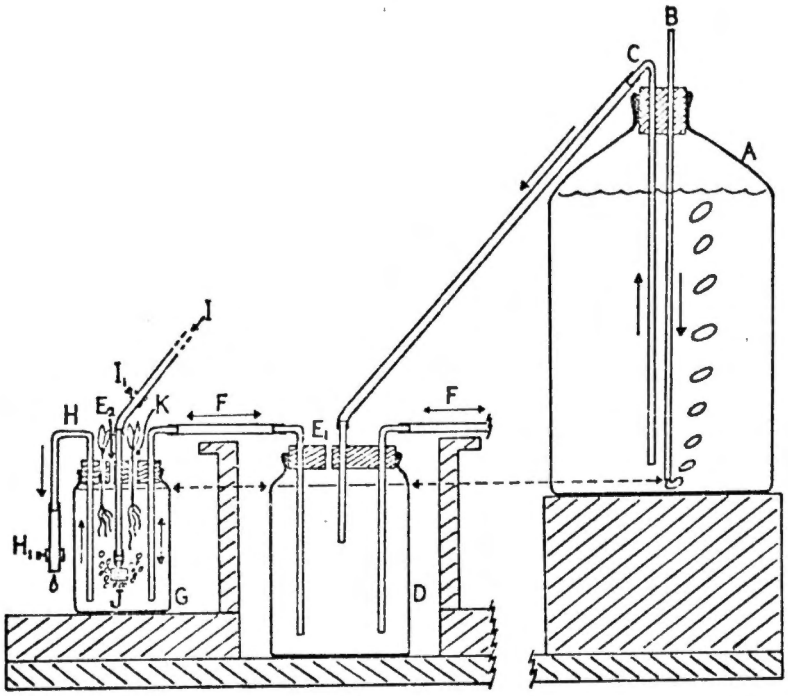
This study utilized healthy plants of Penncross bentgrass and Tifgreen bermudagrass grown in pure medium sand in a greenhouse environment similar to the actual test conditions. Bentgrass was seeded and bermudagrass sprigged in the flats of sand. Nutrient treatments of a modified Hoagland solution provided the necessary macro- and micro-elements.

When the bentgrass and bermudagrass plants were approximately 12 weeks of age, healthy individual plants of uniform size and appearance were selected and transferred to culture jars. As each seedling was selected, the sand was carefully washed from the root system and the roots excised 5 cm below the crown.

Apparatus and Test Procedures

The turfgrass plants were transferred to the culture jars containing modified Hoagland nutrient solution in a specially designed continuous flow and constant-level solution culture renewal system (8). The principle of continuous flow of renewed solution for growing plants in nutrient culture is a widely adopted concept (24).

The operation of the continuous flow and constant-level solution renewal system is shown in Figures 1 and 2 as a side and top view diagram of its three basic parts. An 18 L reservoir jar was used (Figure 1, A). The operation of the system is unique in that it functions as a "closed" system between the reservoir jar (Figure 1, A) and the distributor jar (Figure 1, D). The level of the solution in the distributing jar was altered by raising or lowering of the air inlet tube (Figure 1, B) in the air-tight reservoir jar. The actual solution level was determined by the location of the bottom end of the air inlet tube in the reservoir jar which operated on the principle of a Mariotte's bottle. Silicone grease was used to maintain an air-tight



A = Continuous flow nutrient solution reservoir. A rubber stopper in the top forms an air-tight seal.

B = Air inlet tube controls the head of solution flow into the distributor jar (D).

C = Siphon delivery tube from reservoir (A) to distributor jar (D).

D = Distributor jar for supporting several smaller plant culture jars (G).

E_1, E_2 = Open air holes in distributor jar (D) and plant culture jars (G).

F = Siphon connector tube between distributor jar (D) and plant culture jars (G).

G = Plant culture jars.

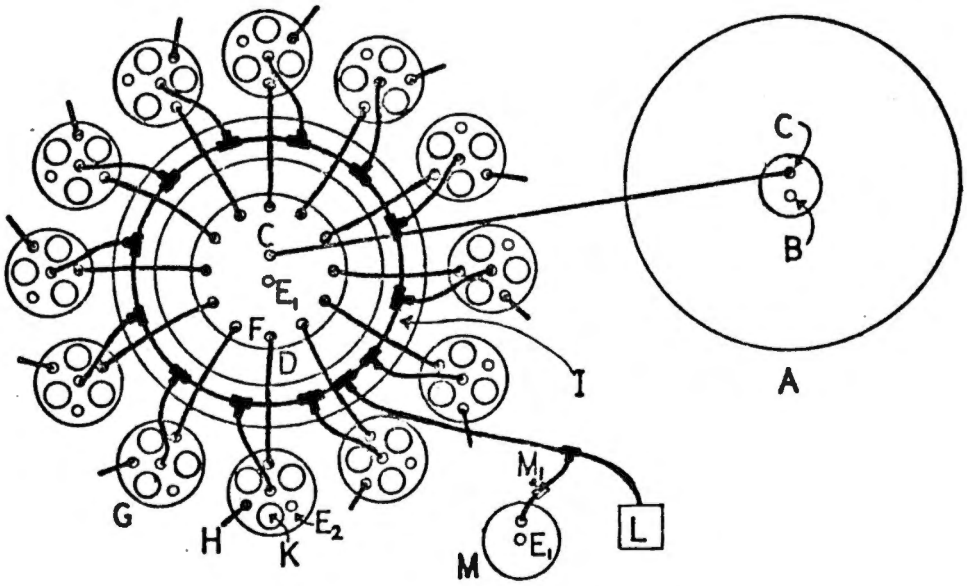
H = Siphon drain tube, and regulating screw clamp (H_1).

I = Air induction system for forced aeration, and regulating screw clamp (I_1).

J = Aquarium-type airstone for aerating the solution.

K = Three 15 mm dia. holes in the rubber stopper of the plant culture jars (G) for mounting plants.

Figure 1. Side view of a single continuous flow nutrient solution renewal system showing only one of the 12 plant culture jars (drawing not to scale).



L = Electric air-pump for forced air induction.

M = Bleeder jar for drain-off of excess air pressure; regulated by a screw clamp (M_1).

Figure 2. Top view of a complete continuous flow nutrient solution renewal system (drawing not to scale).

seal of the rubber stopper in the top of the reservoir jar. The continuous flow of the solution from the reservoir jar to the distributor jar was at a rate of solution replacement needed in the distributor jar to maintain the prescribed solution level for at least 24 hours. The solution from the reservoir jar to the distributor jar moved through a flexible polyvinylchloride (PVC) siphon delivery tube (Figure 1, C).

The distributor jar (Figure 1, D) was of 3.8 L (1 gal) capacity so that it could easily support solution needs of several plant culture jars (Figure 1, G). The system developed here had 12 smaller plant culture jars attached to the distributor jar (only one is shown in Figure 1). The operation of the system between the distributor jar and the culture jars functioned as an "open" system. The distributor jar and culture jars have open air holes in their lids (Figure 1, E_1 and E_2) for maintaining an automatic adjusting constant solution level between jar D (Figure 1) and jars G (Figure 1) with the siphon connector tube (Figure 1, F). The connecting length, which was PVC tubing (Figure 1, C and F) between the two glass tubes, was kept taut to prevent air bubble accumulation which could interrupt the normal flow of the solution.

The capacity of the plant culture jars was 1.9 L (2 qt). Rubber stopper lids with three holes (Figure 1, K),

approximately 15 mm diameter, were used for mounting test plants in the culture jars. Each culture jar contained nine plants of the same cultivar, three plants for each of the three holes in the rubber stopper lid, wrapped in non-absorbant cotton. The plant roots were suspended below the rubber stopper so that the crowns either came in contact with, or were positioned just above, the solution level in the culture jars. Rubber stopper lids were selected over screw-type lids for the convenience of frequent handling to observe roots and for collecting root tissue. The rubber stoppers were not forced into the jar for a tight seal but were simply set into the jar opening with a gentle firmness since this was an "open" system and exact positioning was not critical.

The siphon drain tube (Figure 1, H), which was supported through a hole in the rubber stopper into the plant culture jars, was used to maintain a very slow drip-type drainage. Extraction of water and nutrients from the solution by the plants, and steady drainage, resulted in a lowering of the solution level in the culture jars. This lowering in turn automatically drew fresh nutrient solution from the distributor jar. As solution in the distributor jar was lowered, it automatically maintained itself by drawing fresh solution from the reservoir jar. The siphon drain tube also helped remove potential impurities or nutrient salt sediment which occassionally

may build up in the bottom of the plant culture jars. The rate of drainage flow was regulated with a screw clamp (Figure 1, H_1), located near the bottom of the PVC tube extension attached to the glass siphon drain tube (Figure 1, H).

A forced air induction system (Figure 1, I), supplied by an electric air pump (Figure 2, L) individually aerated each culture jar. A screw clamp (Figure 1, I_1) was attached to the PVC air tube close to the culture jar in order to regulate the air flow. An aquarium-type airstone (Figure 1, J) was positioned approximately 5 cm above the bottom of the siphon tubes (Figure 1, F and H) in the culture jars. This prevented the entrance of air bubbles into these tubes which would prevent the transfer of solution from the distributor jar to the culture jars, and the siphon drain tube. The airstone also slightly agitated the solution in the culture jars thus preventing sedimentation. Agitation of solution in the distribution jar was not necessary since rate of solution flow through this stage of the system to the culture jars was steady enough to maintain sufficient stirring action.

The central arrangement of the 12 plant culture jars around the distributor jar provided a very compact system, permitting three systems to be used on the greenhouse bench (Figure 2). An arrangement in this manner aided also in efficiency of the aeration system since less total tube

length was needed. The air pump was more efficient since it could easily produce more air pressure than was needed to aerate the system. Excess pressure was bled-off with a bleeder jar (Figure 2, M). The bleeder jar had a glass tube submerged in water and connected to the main PVC air line with a glass "T" connector. A screw clamp (Figure 2, M_1) attached to the bleeder line served to regulate air flow to the bleeder jar. The circular arrangement of air line branching off to the culture jars was achieved with the use of glass "T" connectors. The aeration tube system (Figure 1, I, page 16), was supported on top of a circular vertical frame rest and conveniently above plant culture jars (Figures 1 and 2).

Calibration of the system was necessary to determine the rate of solution volume interchange in a culture jar due to drainage of effluent from the jars, and transpiration of the plants growing in the nutrient solution. This information helped determine the frequency at which the reservoir jar needed to be replaced.

All of the jars and some of the glass and PVC tubing were painted black as simple prevention of algae formation. An unpainted window was left in each jar with aluminum foil taped over the window so the solution level could be monitored. Remaining glass and PVC tubing were wrapped with aluminum foil.

Straight glass tubing comprised a portion of the length indicated as PVC tubing as an aid to maintaining rigidity for more reliable solution flow. The glass tubing used in the system described was 8 mm outside diameter and 6 mm inside diameter.

The greenhouse temperature ranged from 17 to 26 ^{o}C (62 to 78 ^{o}F) during the day and 19 to 22 ^{o}C (67 to 72 ^{o}F) during the night. Temperature and humidity were measured by a Belfort hygrothermograph operating on a one-week interval chart. The relative humidity ranged from 41 to 67 percent during the day and 51 to 67 percent at night during the period of the study. Light was provided by a flourescent light panel with eight tubes regulated by a 24-hour time clock permitting 12 hours of light and 12 hours of dark. A Ll-COR-185 photometer was used to take light readings occasionally to assure adequate illumination of the plants for optimum growth. The temperature of the nutrient solution averaged 21 ^{o}C (70 ^{o}F) with a pH of 6.8. This entire system was contained inside a growth chamber-type frame house structure mounted on a greenhouse bench.

Herbicide Treatments

The herbicides used, their formulation, and respective rate of application in kilograms of active ingredient per hectare, and corresponding pounds per acre in parenthesis were: bandane, granular at 39 kg/ha (35 lb/A); benefin, granular at 3.3 kg/ha (3 lb/A);

bensulide, granular at 17 kg/ha (15 lb/A); DCPA, wettable powder at 17 kg/ha (15 lb/A); siduron, wettable powder at 13 kg/ha (12 lb/A); and terbutol, granular at 11 kg/ha (10 lb/A). These herbicides were weighed in the laboratory using a Mettler analytical balance. Each herbicide was repeated in three culture jars, or three replications, plus check jars without any herbicide, for each of the two test species. The necessity of three replications of each treatment was actually a precaution to assure a sufficient supply of root tissue since some herbicides might permit very little root regrowth. From a statistical standpoint the replications protected against treatment error.

Herbicide exposure intervals consisted of two test phases. When the excised root seedlings were mounted in the culture jars of nutrient solution the test phase began. The first test phase called for the herbicide treatments to be delayed for 14 days so the plants could begin growing a new root system. Herbicide treatments were made on the fifteenth day and at 72-hour intervals thereafter for the next two weeks, giving a total of one herbicide treatment and four renewal treatments. Each herbicide renewal treatment was the same as the original rate. Turfgrass root tissue was collected on the fourteenth day, again on the twentieth day, and on the twenty-eighth day, the last day of test phase one.

For the second test phase the roots of the test cultivars were excised to 5 cm below the crown, seedlings mounted in the culture jars of nutrient solution, and herbicides added immediately. Herbicide treatments were made on the first day and renewed at 72-hour intervals thereafter for the next four weeks, or for a total of nine herbicide applications. Each herbicide renewal application was for the same rate as the first treatment. The first tissue samples were taken on the third day, again on the fifteenth day, and on the twenty-seventh day, the last day of the test phase.

Specimen Collections

When collecting root tissue, the rubber stopper lid on the culture jars was moved slightly to one side to allow passage for sampling. The roots suspended in nutrient solution were individually excised and immediately placed in a vial of water for holding to prevent damage. After several roots were removed from the culture jars the rubber stopper lid was replaced. Root tip segments of 2000 microns (2 mm) length were then excised from the root by rapidly placing the root across the dampened index finger and cutting with a razorblade. The root tip was immediately placed in a small vial of Karnovsky's fixative (3 percent paraformaldehyde and 3 percent glutaraldehyde). The collection of root tip segments combined all three replications of a

respective treatment into one collection vial. Each herbicide, untreated check, and the three sampling dates for each test phase were kept separate.

II. LABORATORY PROCEDURES

Histological Procedures

Tissue remained overnight in the fixing solution. The following morning a vacuum pump was connected to each vial for 10 minutes to insure removal of all air from the samples. A dehydration series was begun using ethyl alcohol from the 10 percent through the 40 percent concentration. At the 50 percent concentration stage, the dehydration series was continued using increasing amounts of tertiary butyl alcohol (TBA), and decreasing amounts of ethyl alcohol terminating in three changes of pure TBA. During dehydration the samples were temporarily stained with erythrosin B for ease of identification when it came time to embed the samples in paraffin.

Samples were rinsed three times in pure TBA, which had to be kept warm to prevent the formation of crystals at room temperature. Paraffin pellets were added to the sample vials and allowed to combine with a small quantity of TBA as the temperature was raised to 60 ^{o}C in the paraffin oven. The vials were then allowed to remain uncovered in the oven overnight at 60 ^{o}C to permit the TBA to evaporate from the vials. Samples were then embedded

in molten paraffin using stainless steel embedding molds and plastic block rings. Paraffin blocks were allowed to chill a few hours in the refrigerator after which the molds were released. Paraffin specimens were then placed on a microtome for ribbon sectioning at a thickness of six microns. These ribbons were placed on prewashed glass slides that had been cleaned in warm water and detergent. Slides were then kept on a warming plate for at least 24 hours before staining to assure strong adhesive bonding between the tissue sections and the glass.

Slides were then passed through xylene rinses to remove the paraffin from around the samples, and then through a series of ethyl alcohol hydrations from 100 to 50 percent concentration. Slide sections were stained using a new staining technique developed by Dr. E. T. Graham.[1] Sections were stained one minute in 0.1 percent w/v safranin 0 and rinsed very rapidly in water. Then the sections were stained 10 minutes in aniline blue-orange G in citrate buffer at pH 3.5, rinsed very rapidly and plunged into TBA to stop further extraction of the stain by the water rinse. Aniline blue-orange G has been previously used as a phospholipid stain in histochemistry (26, 31), but was

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[1]Dr. Effin T. Graham, Associate Professor, Department of Ornamental Horticulture and Landscape Design, The University of Tennessee, Knoxville.

used as a general histological stain in sequence with safranin for the purpose of this investigation.

The slides were then passed through a series of five rinses composed of 95 percent TBA and 5 percent isopropyl alcohol, then through a series of four xylene rinses, and cover glasses set over the specimens with Lipshaw mounting resin. The purpose of the isopropyl alcohol in the TBA was to prevent crystallization at room temperature.

Photomicrography

Slides were observed and studied under a Wild Heerbrugg M20EB light microscope using Wild fluotar objectives. Photomicrographs were made using a Nikon M-35S camera attached to the microscope. Kodak Panatomic-X film was used. Camera lucida drawings were made using a Bausch and Lomb Tri-Simplex Micro-Projector.

CHAPTER IV

RESULTS AND DISCUSSION

Discussion of root cellular responses are presented according to the specific herbicide treatment as compared to the untreated check for Penncross bentgrass and Tifgreen bermudagrass. Camera lucida drawings at 97.5 magnification and photomicrographs at 113, 281 and 347 magnifications are used to show cellular responses in roots. Each drawing identifies the root cap, meristematic region, cell elongation zone, epidermis, cortex, pericycle and stelar elements of the root tip.

I. UNTREATED CHECK

Bentgrass

The anatomical and morphological arrangement of the cells in the apical (2 mm) and subapical (2 mm) regions of a healthy Penncross bentgrass root are shown in Figures 3 and 4, respectively. The meristematic zone shows normal cell differentiation into column-type rows of epidermal, cortical and stelar cells (Figure 3). The meristematic cells were characteristically rounded then differentiated into typical square-shaped cells with progressive growth of the root tip. Further back from the tip, approximately 600 μ, cell elongation became more

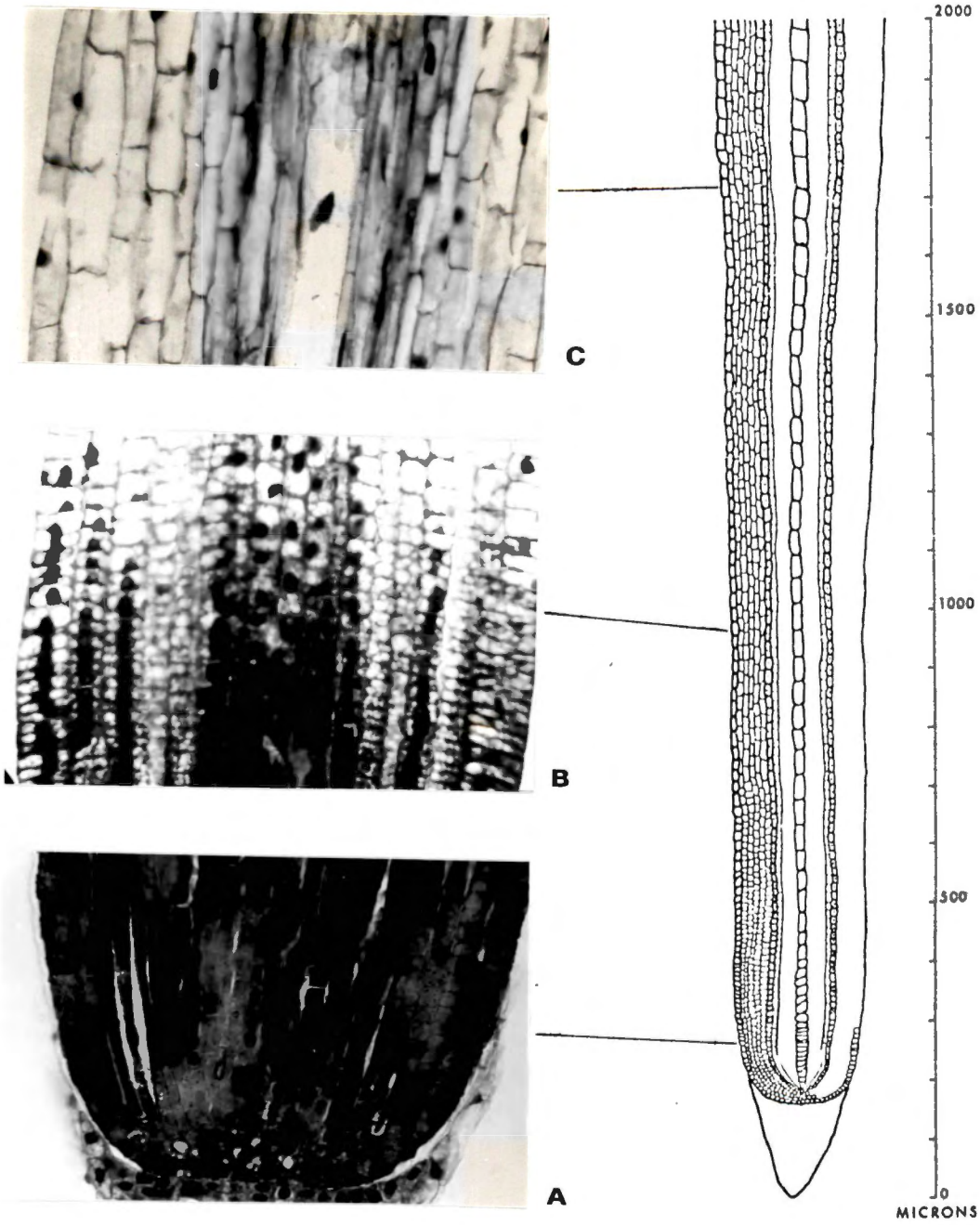

Figure 3. Diagram of a median longitudinal section of the apical 2000 microns of a normal, healthy Penncross bentgrass root. Camera lucida drawing at 97.5X and photomicrographs A, B and C at 281X.

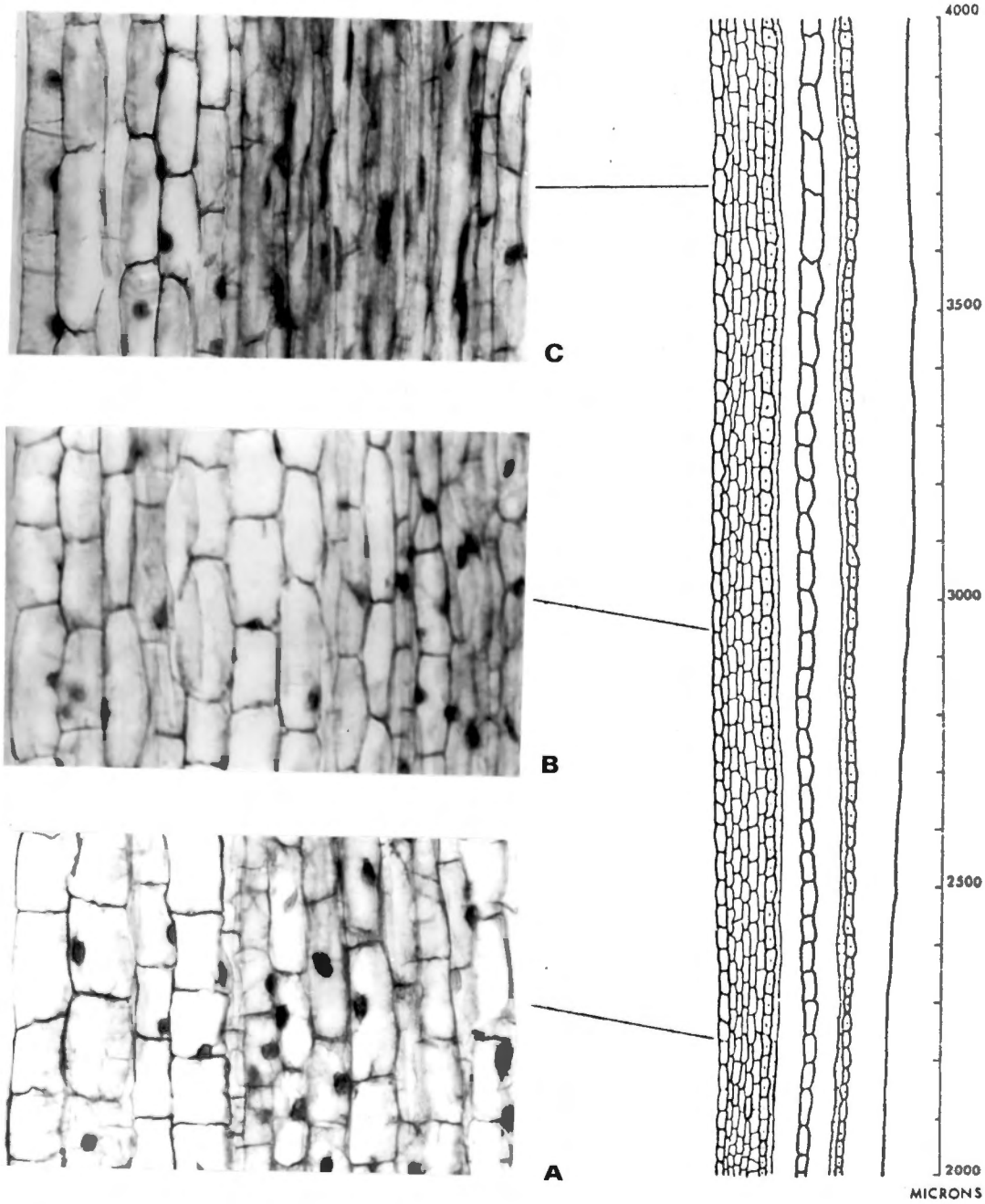

Figure 4. Diagram of a median longitudinal section of the subapical 2000 microns of a normal, healthy Penncross bentgrass root. Camera lucida drawing at 97.5X and photomicrographs A, B and C at 347X.

rectangular in shape and maintained a columnar arrangement. Various stelar elements such as the pericycle, xylem and phloem, were also well-defined in this region. The general absence of lateral roots and root hairs appeared typical in this region. The root cap was fully developed and found to be approximately 175 μ in length. A large number of new roots of bentgrass were produced in the solution culture jars during the 28-day period of the greenhouse portion of the experiment.

The degree of stainability of the healthy cells was very intense. Massive amounts of the aniline blue-orange G stain was absorbed by the cellular components of the root tip. This was a result of material in the root tip which was preserved using Karnovsky's fixative. Other fixatives may have chemically eliminated this intense staining material.

In the subapical region (2 mm), the cells appeared more elongated and rectangular in shape (Figure 4). The columnar appearance and organized arrangement of the cells were maintained with stelar elements well-defined. This region revealed a general absence of lateral roots and root hairs. The degree of stainability was lighter than the apical 2 mm section of the root. Smaller quantities of the aniline blue-orange G stain were absorbed by cellular components.

Bermudagrass

The shape and form of cells in the apical (2 mm) and subapical (2 mm) regions of a healthy Tifgreen bermudagrass root are shown in Figures 5 and 6, respectively. Like normal, healthy cells in bentgrass root tips, meristematic cells in the root tips of Tifgreen bermudagrass differentiated into columnar rows of epidermal and cortical cells (Figure 5). The meristematic region showed rounded cells differentiating into square-type cells. At approximately 700 μ from the root tip, the cells became more rectangular in shape and maintained uniform columns. Stelar elements were well-defined in this zone. The general absence of lateral roots and root hairs appeared characteristic for this region. The thickness of bermudagrass root tips were almost twice that of healthy bentgrass tips. The healthy plants of bermudagrass produced a large number of roots in solution culture jars during the greenhouse experiment.

Stainability of this region was very intense. Cellular components in the root tips absorbed large amounts of the aniline blue-orange G stain. The zone at about 1800 μ back from the tip absorbed less stain than those components closer to the tip.

In the subapical region (2 mm), the structure and arrangement of the differentiated cells observed in the

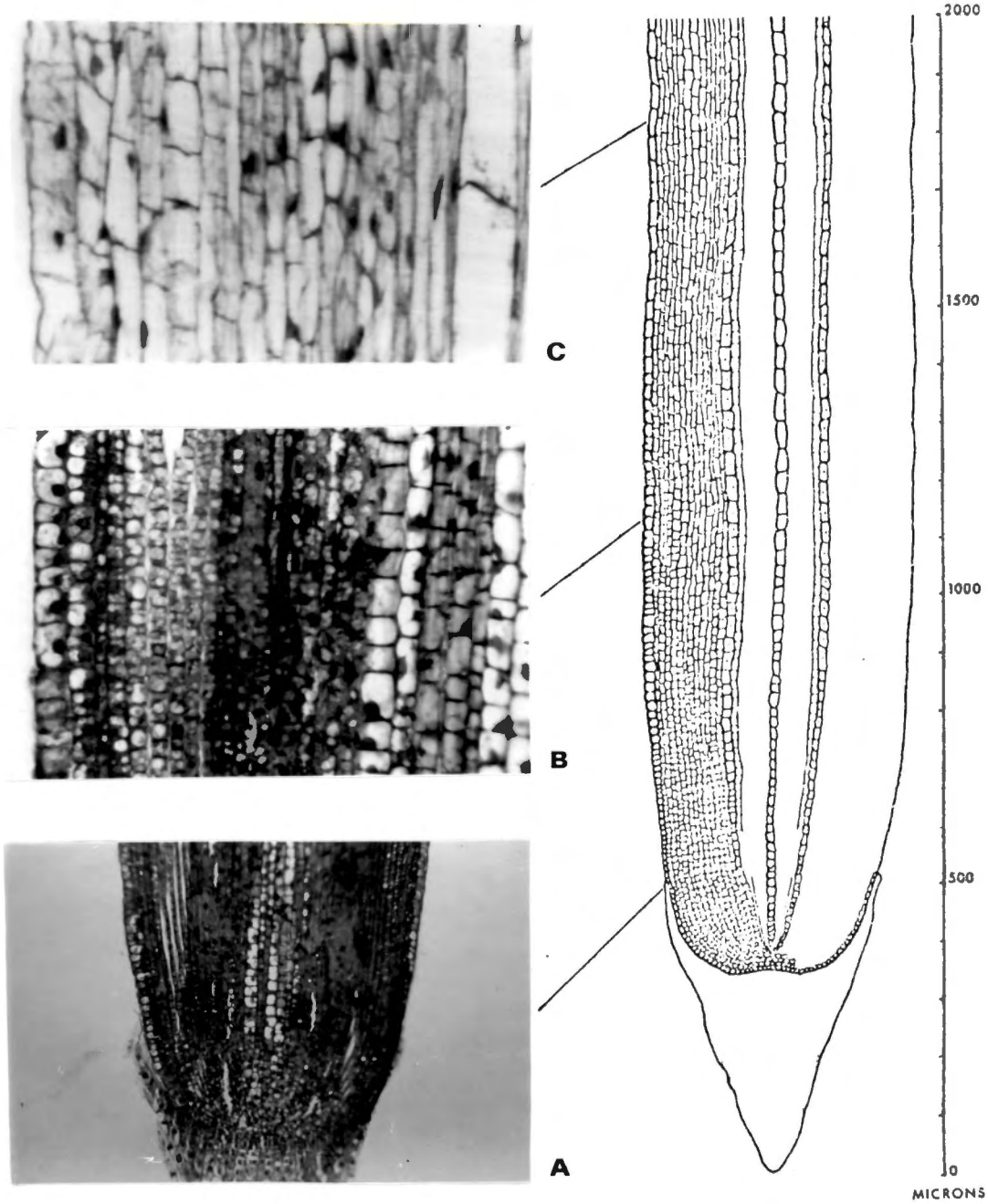

Figure 5. Diagram of a median longitudinal section of the apical 2000 microns of a normal, healthy Tifgreen bermudagrass root. Camera lucida drawing at 97.5X and photomicrographs A at 113X, B at 281X, and C at 347X.

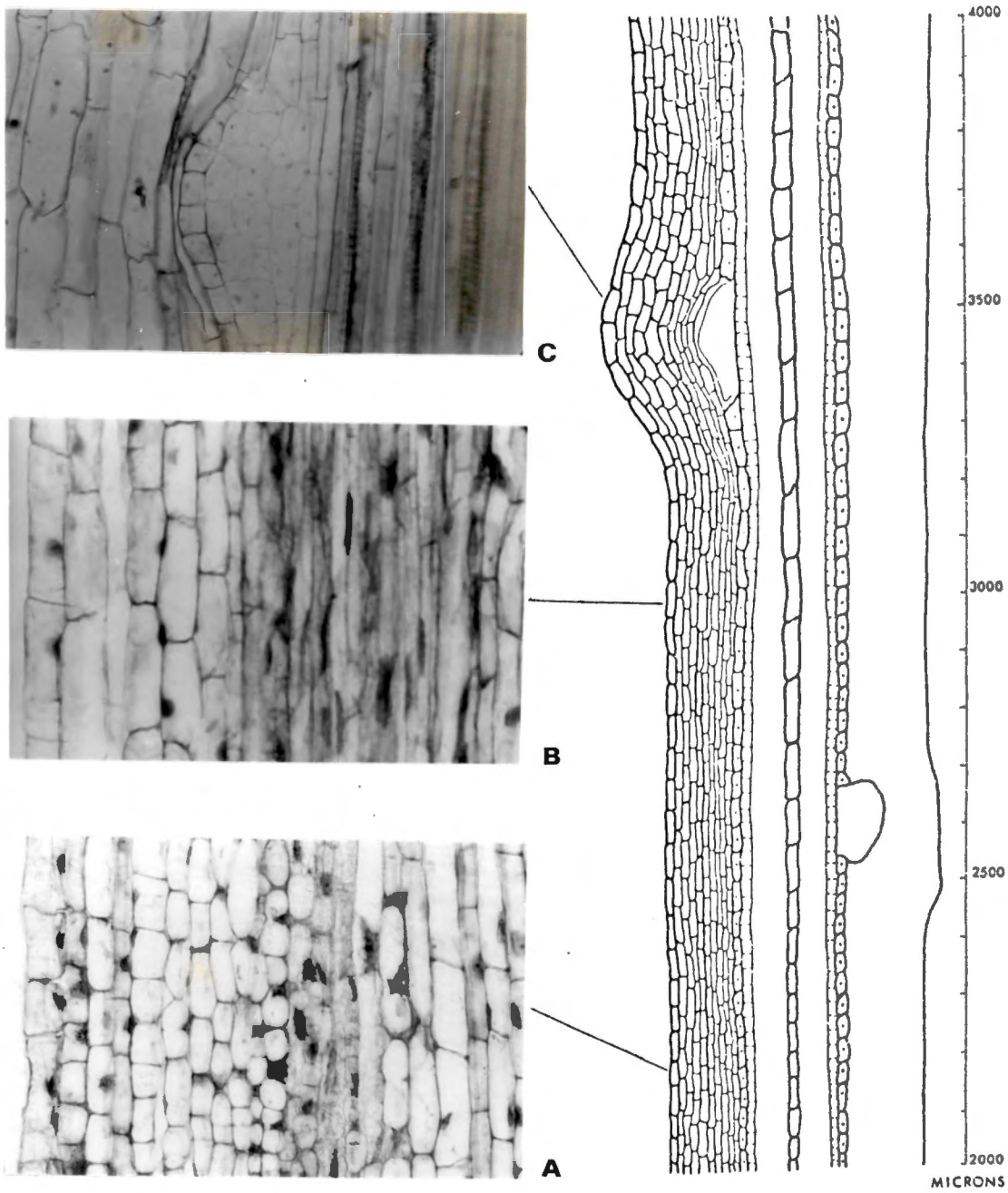

Figure 6. Diagram of a median longitudinal section of the subapical 2000 microns of a normal, healthy Tifgreen bermudagrass root. Camera lucida drawing at 97.5X and photomicrographs A and B at 347X, and C at 113X.

apical tip section were maintained (Figure 6). However, the cells became more elongated and maintained their columnar arrangement. Cells in the subapical zone appeared more mature than those in the apical 2 mm tip region. Lateral root formation appeared to be a common characteristic of the subapical region, and were observed as close as 2600 μ from the root tip. The absence of root hairs also appeared normal for this region. Stelar elements were very prominent. The degree of stainability in this region was less than the apical 2 mm sections.

II. BANDANE

Bentgrass

Bandane caused some tissue abnormalities in the root tip of Penncross bentgrass, although not as pronounced as some of the other herbicides tested (Figure 7). Epidermal cells were compressed within 900 μ of the tip. The columnar organization of the cortical cells was not as precise as the check. Lateral roots and root hairs were not found in this region. Investigations by another researcher have indicated bandane to be slow in showing injury to Penncross bentgrass (5, 6). Under the short-term conditions of this study, bandane exposure to new root development may not have been long enough to cause severe cellular malformations. Fewer numbers of roots were observed in the solution culture jars in the greenhouse experiment as compared to the check.

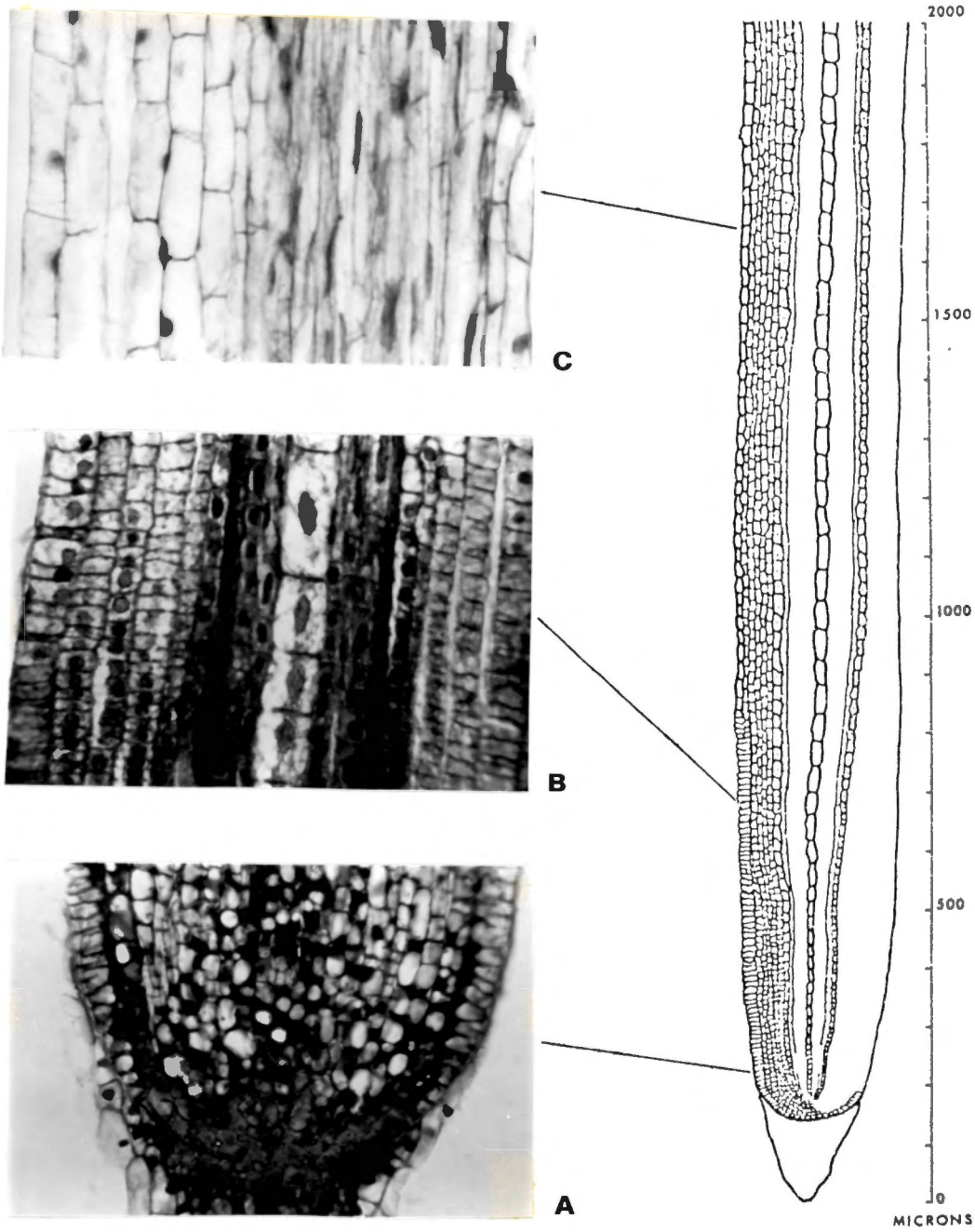

Figure 7. Diagram of a median longitudinal section of the apical 2000 microns of a bandane-affected Penncross bentgrass root. Camera lucida drawing at 97.5X and photomicrographs A, B and C at 281X.

The stainability of the bandane-affected bentgrass roots was very intense, with the meristematic zone of the tips absorbing the greater amount of the aniline blue-orange G stain.

Bermudagrass

The bermudagrass root tips, like bentgrass, showed less severity of tissue abnormalities with exposure to bandane than found with several of the other herbicides (Figure 8). However, bandane caused compressed epidermal and stelar cells in the meristematic zone. Columnar organization of cortical cells showed slight disruption. Neither lateral roots nor root hairs were found in the apical region. Fewer numbers of roots were observed in the solution culture jars in the greenhouse experiment as compared to the check. Results found support reports that bandane tends to manifest injury slowly in Tifgreen bermudagrass (7).

The meristematic zone absorbed large quantities of the aniline blue-orange G stain.

III. BENEFIN

Bentgrass

Benefin caused extensive and severe cellular disruptions and malformations in the root tips of Penn-cross bentgrass (Figure 9). Epidermal cells showed

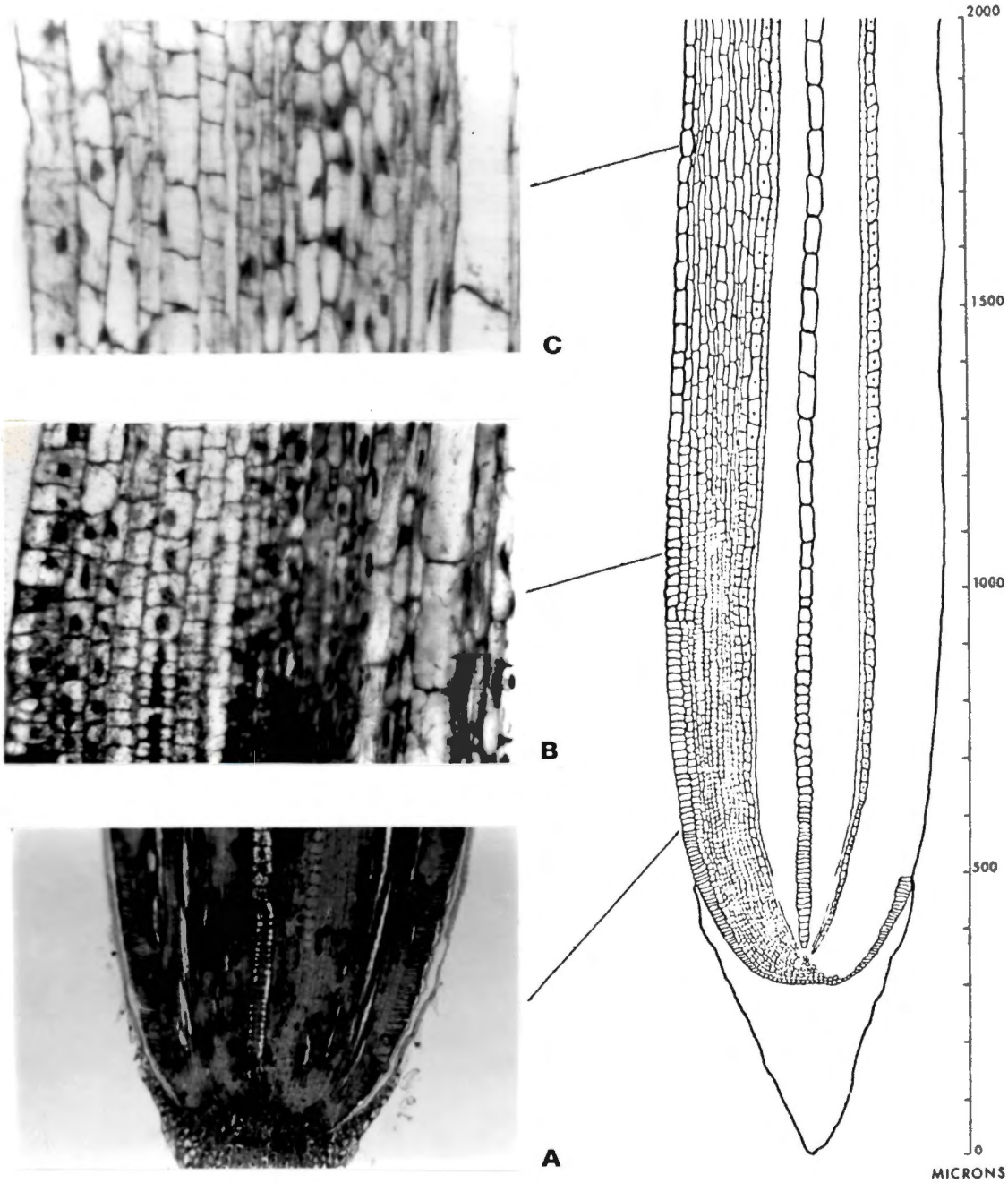

Figure 8. Diagram of a median longitudinal section of the apical 2000 microns of a bandane-affected Tifgreen bermudagrass root. Camera lucida drawing at 97.5X and photomicrographs A at 113X, B at 281X, and C at 347X.

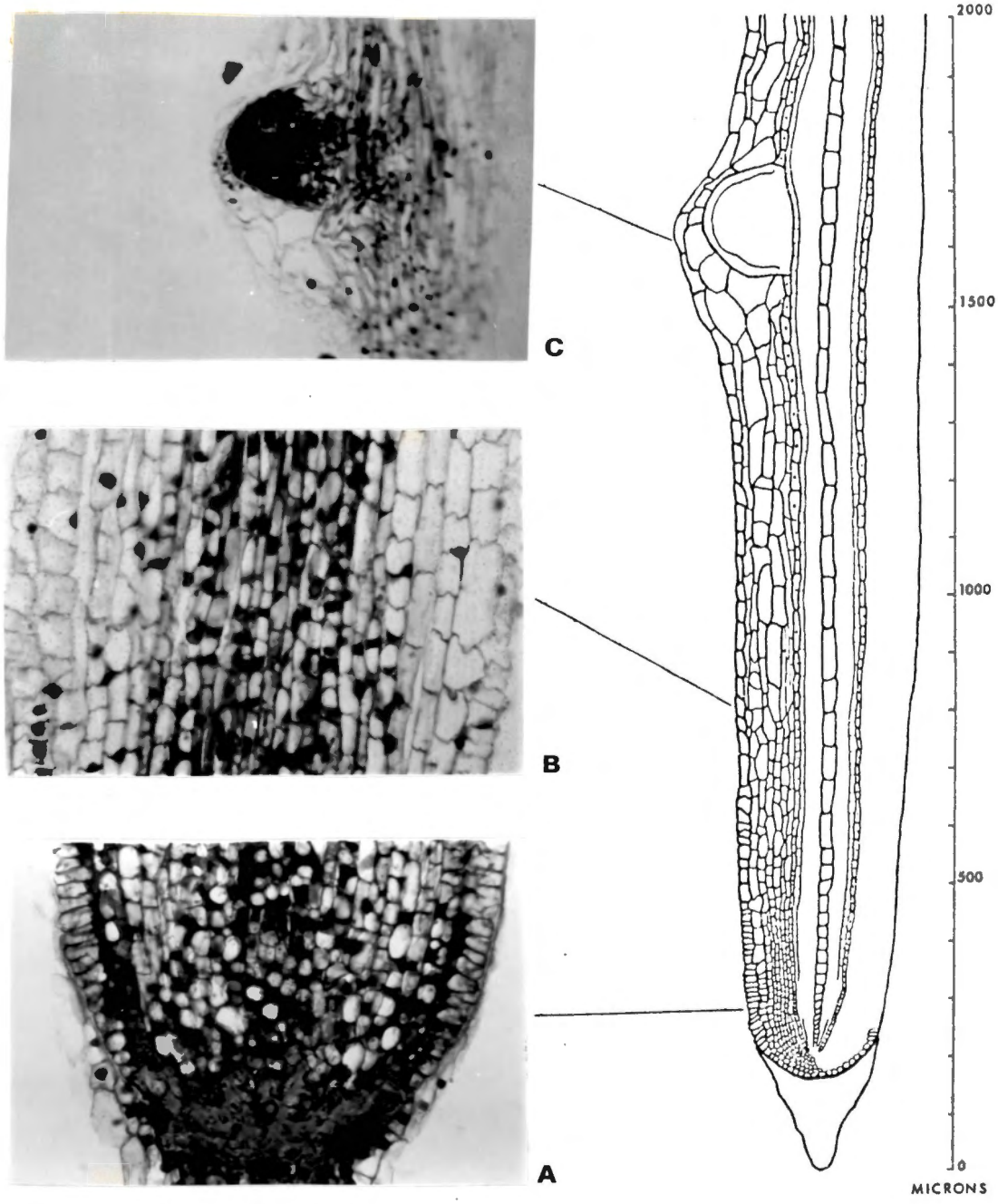

Figure 9. Diagram of a median longitudinal section of the apical 2000 microns of a benefin-affected Penncross bentgrass root. Camera lucida drawing at 97.5X and photomicrographs A and B at 281X, and C at 113X.

compression within 500 μ of the root tip and extensive irregularity in size and shape of cells. Cortical cells showed columnar disruption with cells greatly enlarged. Lateral roots occurred abnormally close (1600 μ) to the root tip with enlarged cortical cells which easily ruptured. Root hairs were not observed in this region. Fewer numbers of roots were observed in the solution culture jars in the greenhouse experiment as compared to the check. Results supported findings by other investigations that benefin readily injured bentgrass (5, 6).

The stainability of the meristematic zone was intensive, while less aniline blue-orange G stain was absorbed farther from the root tips. The lateral root formations absorbed much more stain than surrounding cortical tissue.

Bermudagrass

Tissue abnormalities were only slight in the benefin affected Tifgreen bermudagrass root tips (Figure 10). Cellular maturation and elongation was only in slight difference compared to the check. Neither lateral roots nor root hairs were observed in this zone. Root numbers were only slightly fewer in the benefin-treated solution culture jars in the greenhouse experiment when compared to the check. Benefin has been reported to cause low intensity injury to Tifgreen bermudagrass (7, 10, 16). These results

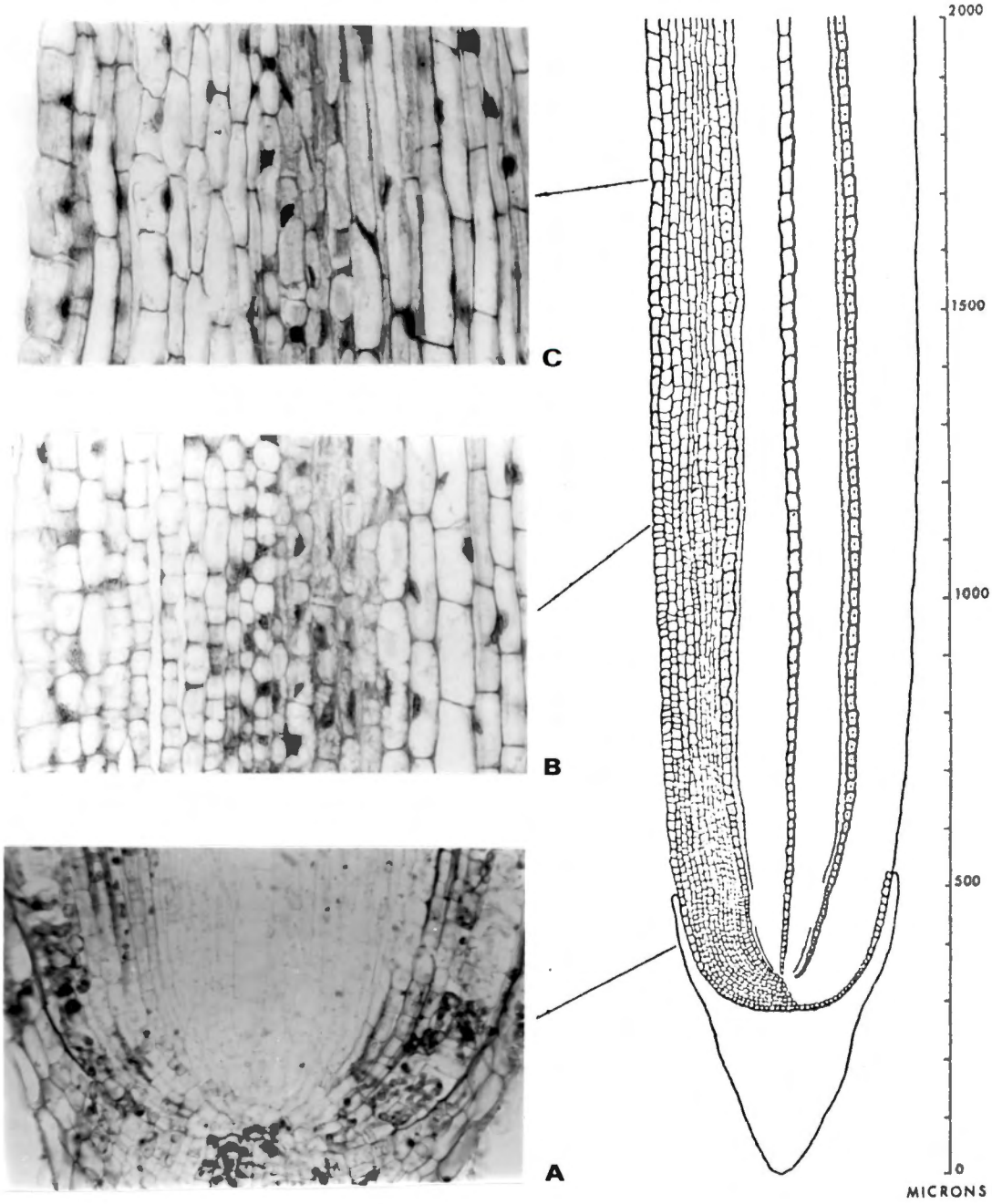

Figure 10. Diagram of a median longitudinal section of the apical 2000 microns of a benefin-affected Tifgreen bermudagrass root. Camera lucida drawing at 97.5X and photomicrographs A at 281X, and B and C at 347X.

indicated a similar response. The intensity of stain absorbed in the benefin affected root tips was very slight.

IV. BENSULIDE

Bentgrass

Epidermal cells following bensulide exposure were greatly compressed for over 1300μ of the Penncross bentgrass root tip (Figure 11). Cell columnar arrangement showed slight irregular development. Lateral roots and root hairs were not observed in this region. Numbers of roots produced in the solution culture jars were slightly reduced as compared to check containers. Other investigations have shown bensulide to be relatively slow in its effect on Penncross bentgrass under field conditions (5, 6).

The meristematic zone absorbed a large amount of the aniline blue-orange G stain. Farther from the root tips, less stain was absorbed.

Bermudagrass

The effects of bensulide caused compressed epidermal cells close to the root tips but only slight cortical cell columnar disruption in Tifgreen bermudagrass (Figure 12). However, cortical cells appeared abnormally enlarged progressing back from the tips. Lateral roots and root hairs

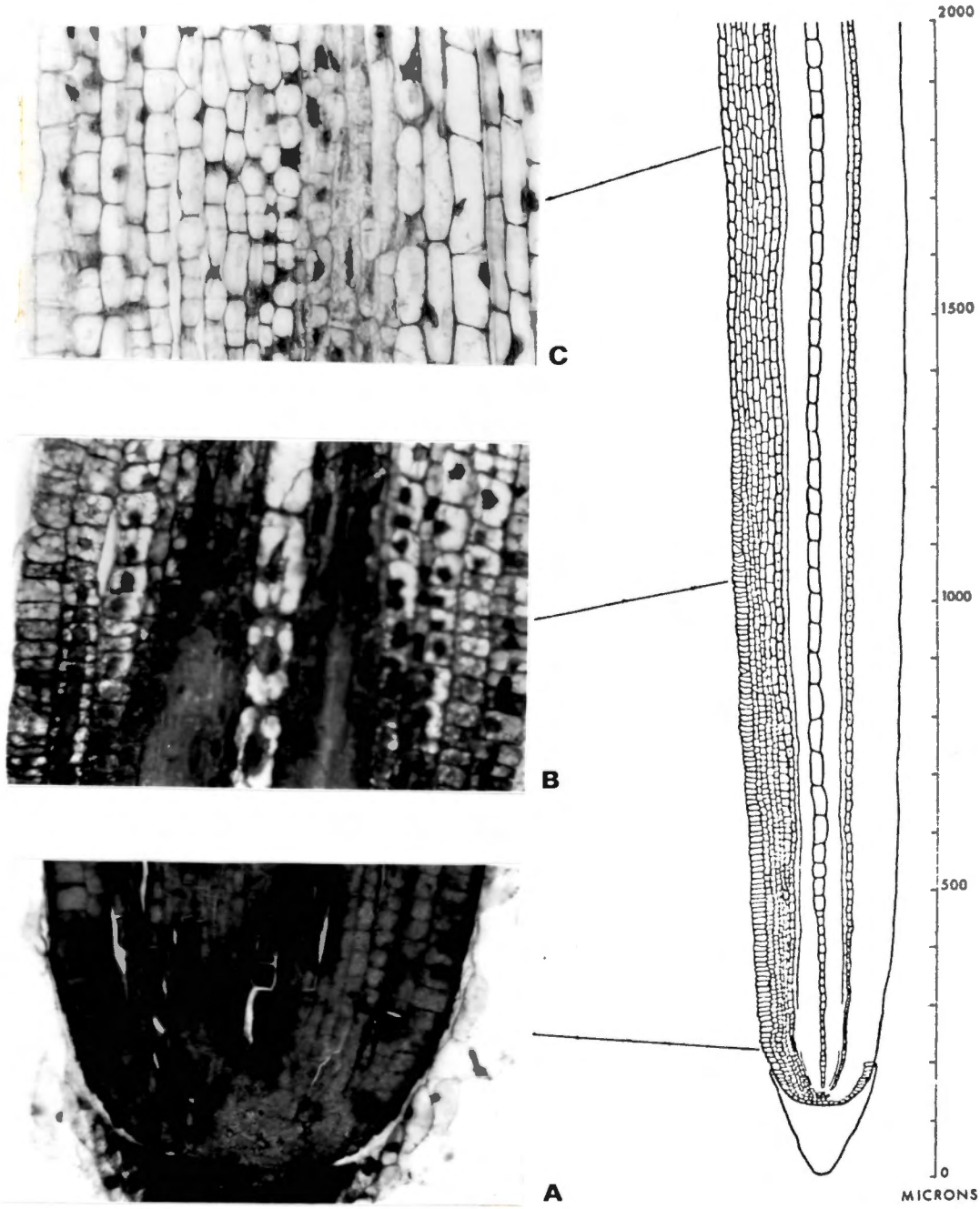

Figure 11. Diagram of a median longitudinal section of the apical 2000 microns of a bensulide-affected Penncross bentgrass root. Camera lucida drawing at 97.5X and photomicrographs A and B at 281X, and C at 347X.

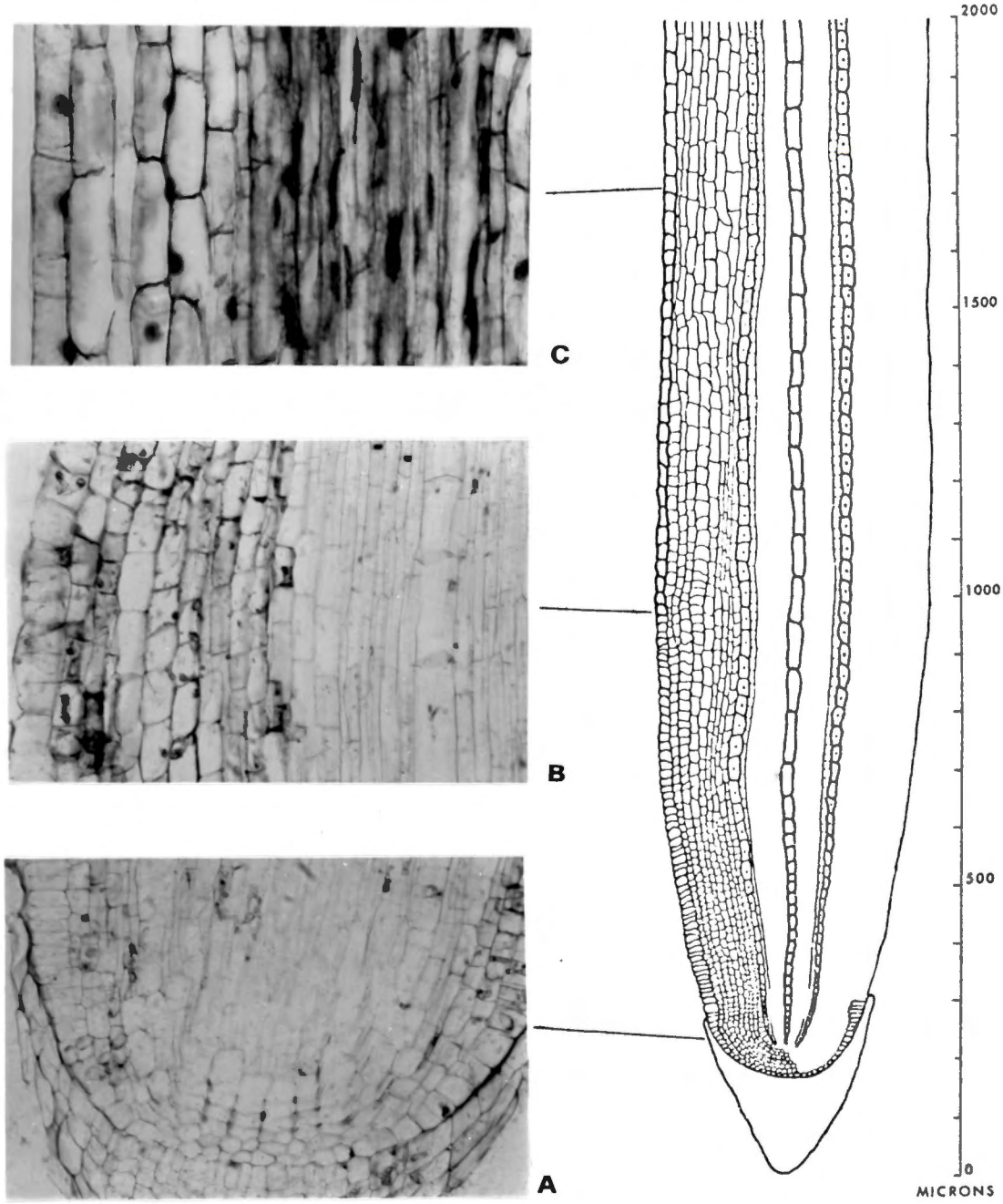

Figure 12. Diagram of a median longitudinal section of the apical 2000 microns of a bensulide-affected Tifgreen bermudagrass root. Camera lucida drawing at 97.5X and photomicrographs A and B at 281X, and C at 347X.

were not found in this region. The number of roots observed in the solution culture jars was fewer in number as compared to the check. The effects of bensulide in Tifgreen bermudagrass appeared slow in developing, a characteristic reported by several other investigators (7, 11, 14, 17). The degree of stainability in the meristematic region was very light.

V. DCPA

Bentgrass

The effects of DCPA in Penncross bentgrass were manifested as compressed epidermal and cortical cells within 700 μ of the tip, cortical cells abnormally enlarged progressing back from the tip, but only slight disruption of cell columnar arrangement (Figure 13). However, DCPA caused abnormally enlarged roots of over one and one-half times thicker than healthy bentgrass roots. Lateral roots and root hairs were absent in this region. Fewer roots developed in the solution culture jars with exposure to DCPA as compared to the checks. Results supported other findings that DCPA caused slight to moderate injury to bentgrass (5, 6, 20, 21, 22, 26, 35). The aniline blue-orange G stain was absorbed very intensively in the meristematic zone.

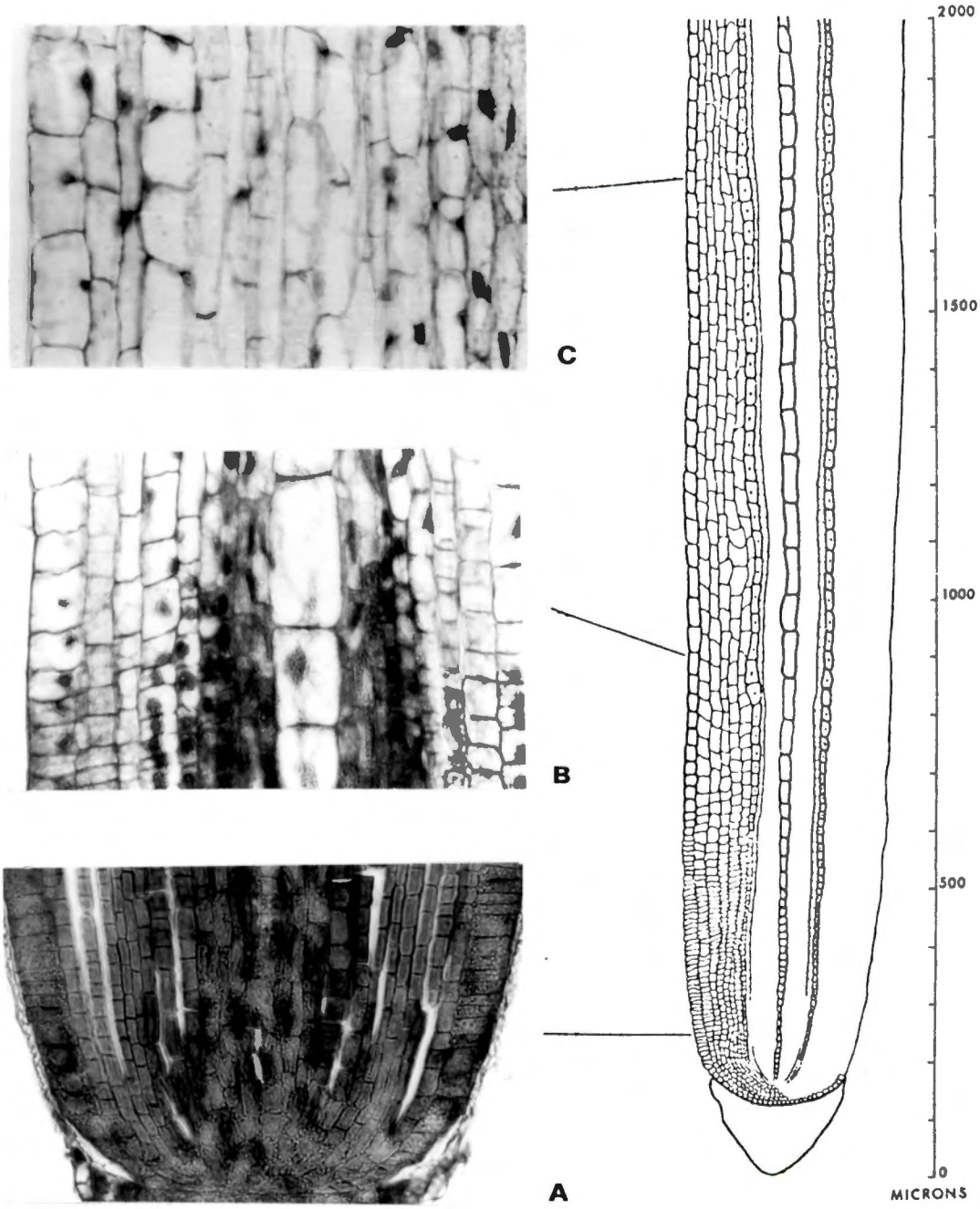

Figure 13. Diagram of a median longitudinal section of the apical 2000 microns of a DCPA-affected Penncross bentgrass root. Camera lucida drawing at 97.5X and photomicrographs A, B and C at 347X.

Bermudagrass

Some of the most extensive root abnormalities observed in this study occurred with DCPA treatment of Tifgreen bermudagrass (Figure 14). The root tip showed abnormal narrowing with epidermal cells greatly compressed within 600 μ of the tip. Cell columns were seriously disrupted with epidermal and cortical cells greatly enlarged. Lateral roots proliferated abnormally close (1500 μ) to the tip accompanied by hypertrophic cortical cells. Many enlarged cells ruptured easily. Root hairs were not observed in this region. The number of roots observed in the solution culture jars in the greenhouse experiment was considerably fewer than in the check. These results strengthened findings by other researchers that DCPA caused severe injury to Tifgreen bermudagrass (1, 2, 7, 18, 19). The stainability of cells in the meristematic zone was very light.

VI. SIDURON

Bentgrass

The effects of siduron were only slight in the root tips of Penncross bentgrass (Figure 15). Tissue abnormalities appeared as compressed epidermal cells within 1000 μ of the root tip and slight cortical cell columnar disruption. No lateral roots or root hairs occurred in this region. Penncross bentgrass produced a large number of roots in the

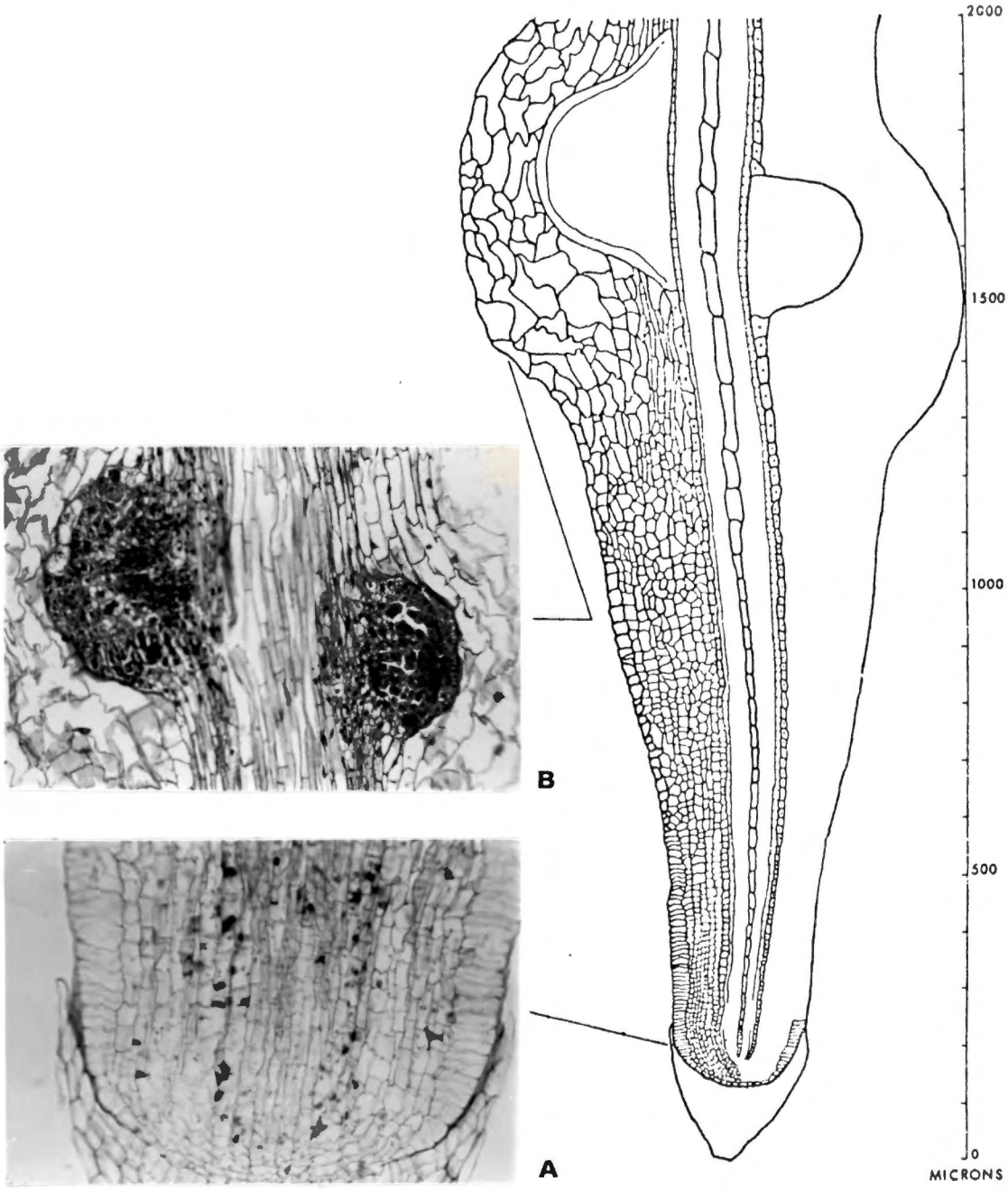

Figure 14. Diagram of a median longitudinal section of the apical 2000 microns of a DCPA-affected Tifgreen bermudagrass root. Camera lucida drawing at 97.5X and photomicrographs A at 281X and B at 113X.

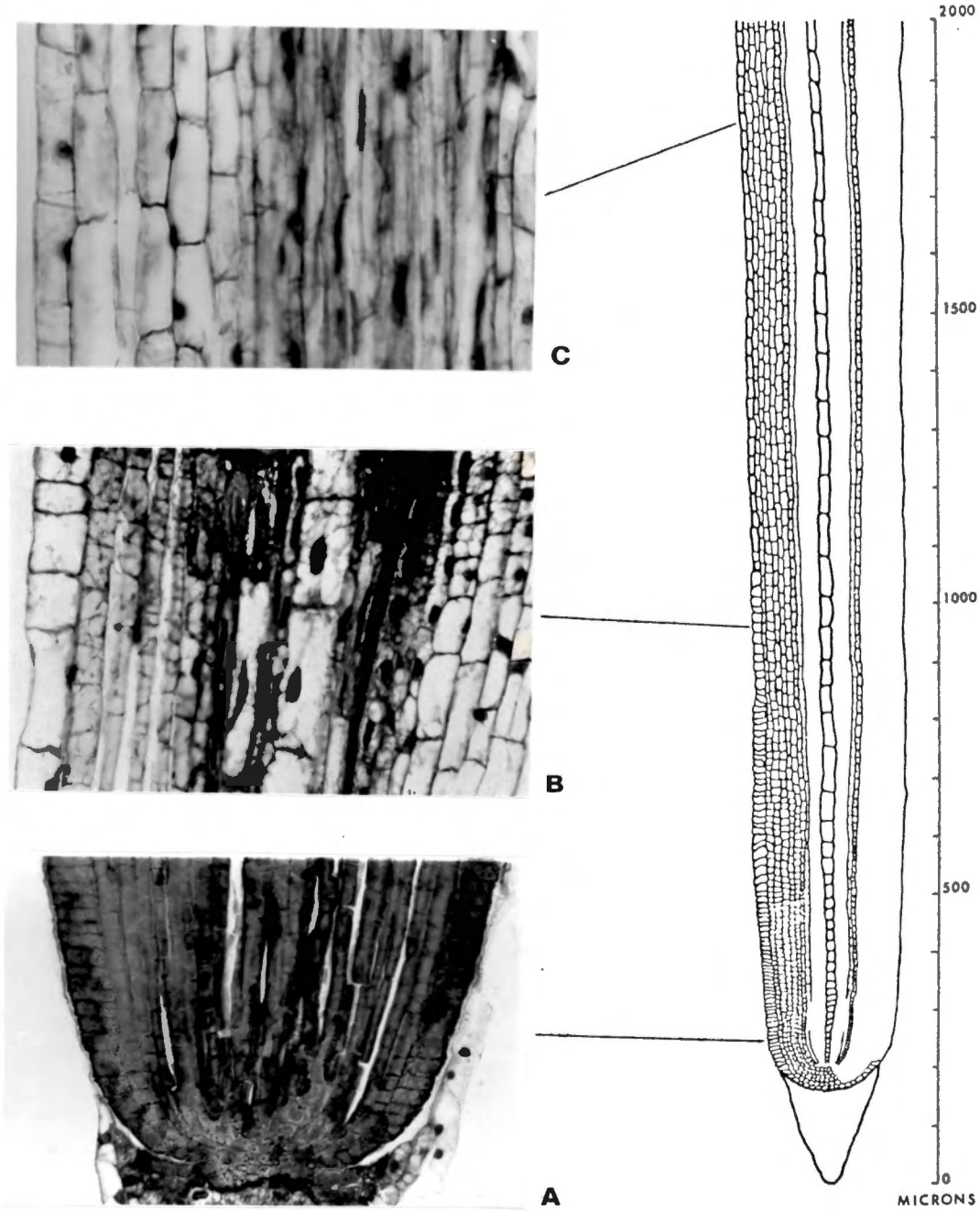

Figure 15. Diagram of a median longitudinal section of the apical 2000 microns of a siduron-affected Penncross bentgrass root. Camera lucida drawings at 97.5X and photomicrographs A and B at 281X, and C at 347X.

siduron-treated culture jars in the greenhouse experiment when compared to the checks. Penncross bentgrass appeared to show some resistance to siduron, a characteristic indicated by other investigators (6, 24). The meristematic cells of the tips absorbed a large amount of the aniline blue-orange G stain.

Bermudagrass

Extensive tissue abnormalities were found in roots of Tifgreen bermudagrass following siduron treatments (Figure 16). The affected roots showed epidermal cells moderately compressed close to the tips, and cortical cell differentiation showed disruption and columnar disorganization. Lateral root proliferations occurred abnormally close (1500 μ) to the tip accompanied by hypertrophic cortical cells, although no root hairs were found. Many of the cortical cells ruptured easily. Fewer numbers of roots were observed in the siduron-treated solution culture jars in the greenhouse experiment as compared to the checks. Other investigators have reported siduron severely injures or kills Tifgreen bermudagrass (1, 7).

The meristematic zone absorbed very little of the aniline blue-orange G stain. Lateral root formations absorbed more of this stain.

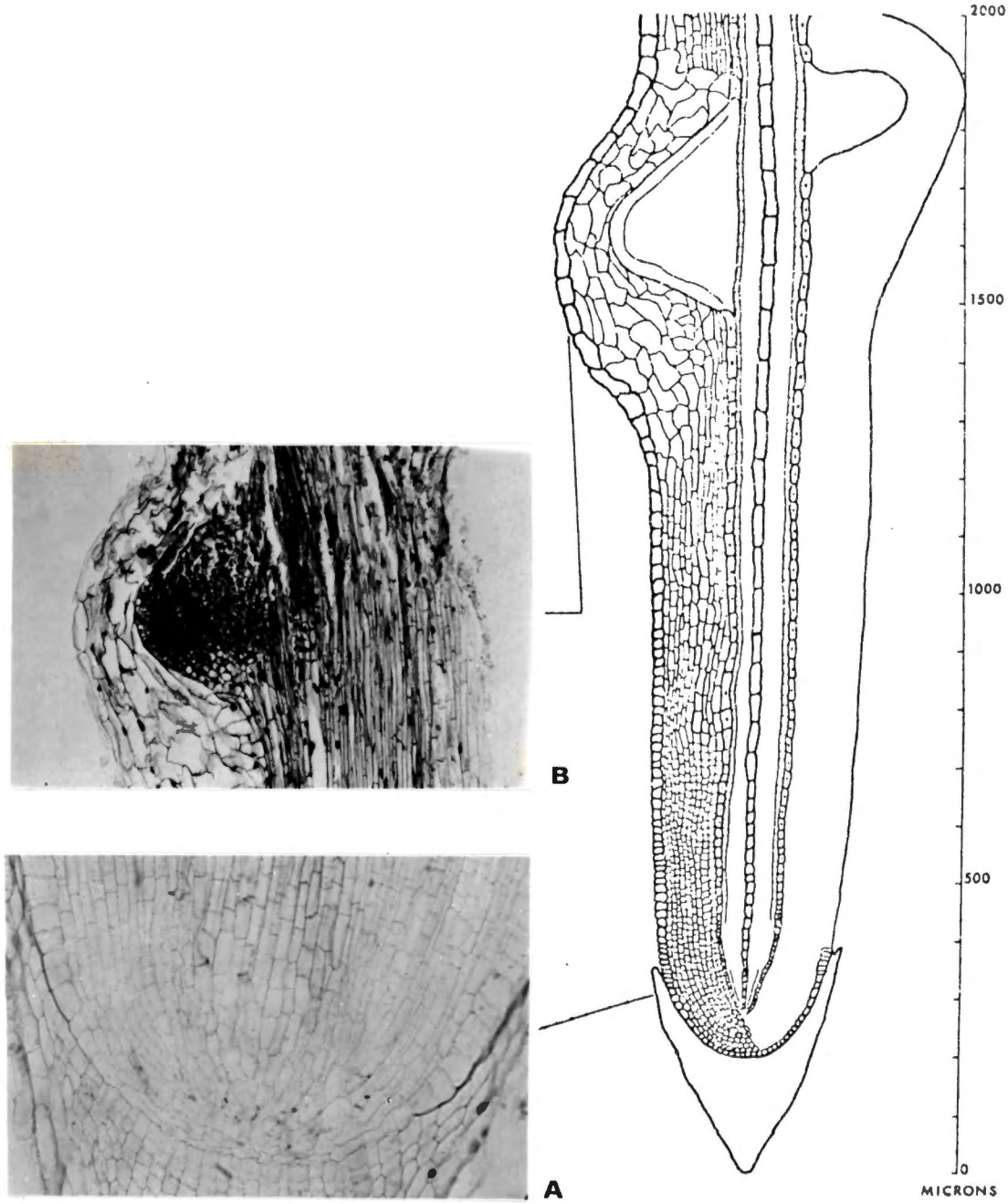

Figure 16. Diagram of a median longitudinal section of the apical 2000 microns of a siduron-affected Tifgreen bermudagrass root. Camera lucida drawing at 97.5X and photomicrographs A at 281X and B at 113X.

VII. TERBUTOL

Bentgrass

Penncross bentgrass roots appeared very sensitive to terbutol (Figure 17). Roots showed abnormal thickening with both epidermal and cortical cells compressed close to the tips. Cell columnar formation showed disruption with epidermal and cortical cells abnormally enlarged. Lateral roots occurred abnormally close (1400 μ) to the root tip accompanied by hypertrophic cortical cells, although no root hairs were found. The number of roots observed in the culture jars in the greenhouse experiment was fewer than in the checks. Terbutol has been reported to cause severe injury to Penncross bentgrass under field conditions (5, 6). The amount of stain absorbed by cells in the meristematic zone in the root tips was very heavy.

Bermudagrass

Terbutol caused some of the most severe and extensive tissue abnormalities in Tifgreen bermudagrass of the herbicides studied (Figure 18). Abnormalities observed in this study included a narrowed root tip as compared to healthy roots, cell column disruption, irregularly shaped and enlarged cortical and epidermal cells, and lateral roots occurring abnormally close (1300 μ) to the tip with accompanying hypertrophic cortical cells. Root hairs were absent from this region. The number of roots observed in

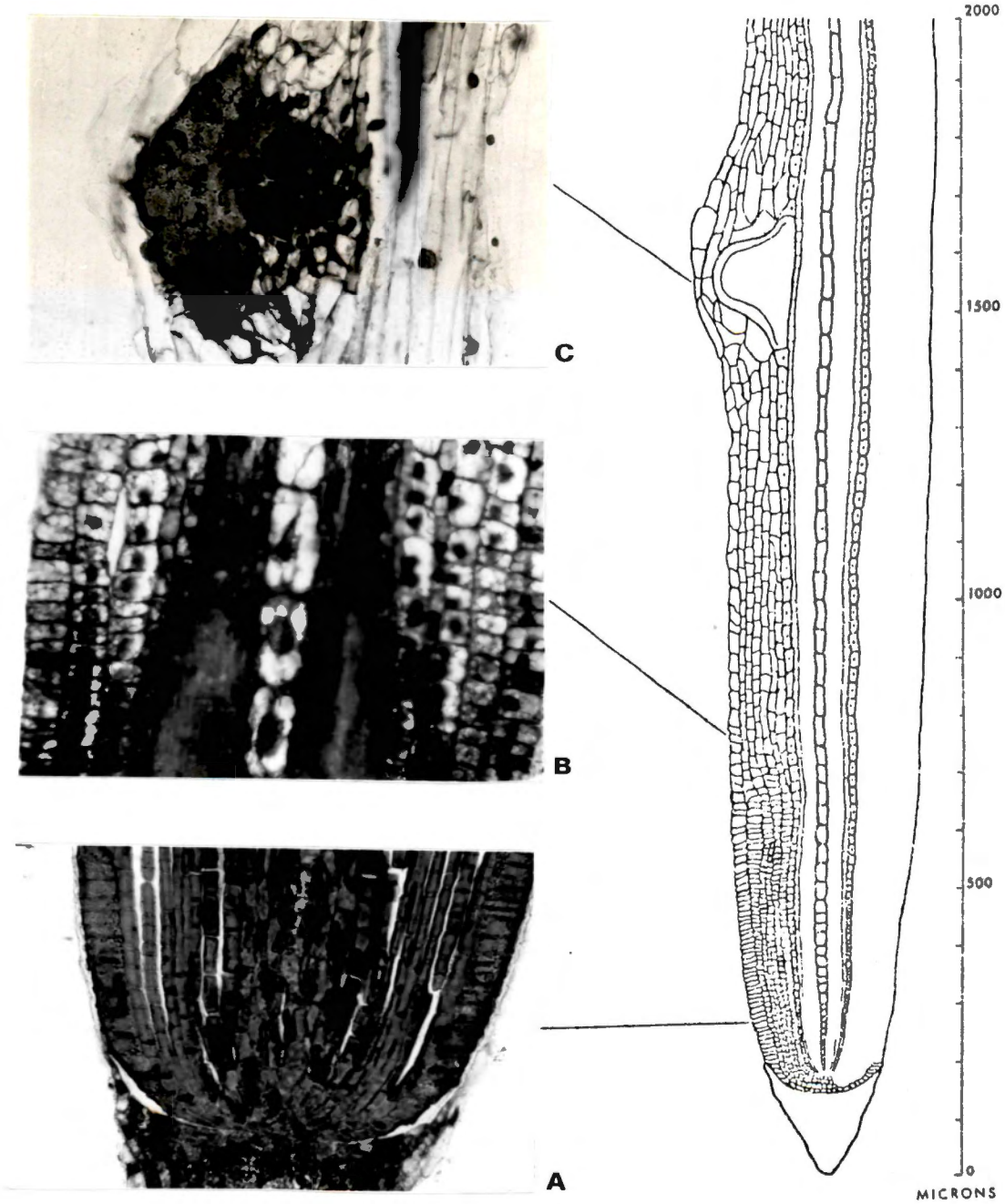

Figure 17. Diagram of a median longitudinal section of the apical 2000 microns of a terbutol-affected Penncross bentgrass root. Camera lucida drawing at 97.5X and photomicrographs A and B at 281X, and C at 113X.

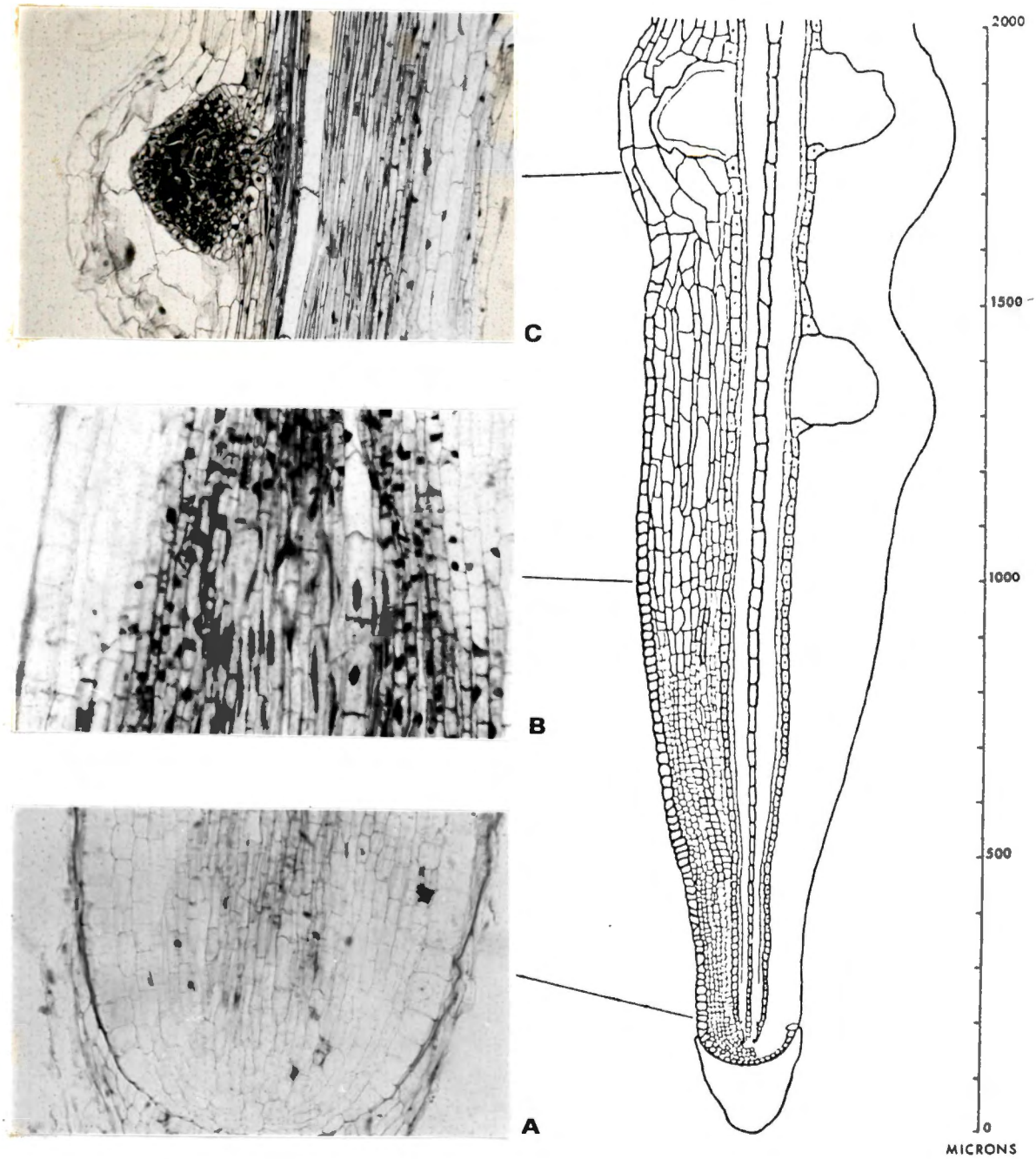

Figure 18. Diagram of a median longitudinal section of the apical 2000 microns of a terbutol-affected Tifgreen bermudagrass root. Camera lucida drawing at 97.5X and photomicrographs A and B at 281X, and C at 113X.

the culture jars in the greenhouse was much fewer than the checks. Terbutol injury has been reported to be severe in Tifgreen bermudagrass under field conditions (7, 11).

Small amounts of the aniline blue-orange G stain were absorbed in the meristematic zone of the root tips. The lateral root initials absorbed more of the stain than the surrounding tissue.

VIII. SAMPLING DATES

Results of the greenhouse solution culture tissue collection dates showed some variation between sampling dates for the two test phases. Herbicide induced cell malformations were recognizable in the bentgrass and bermudagrass root tips by the second date of the three sample collection dates in both test phases. The first test phase began October 14, 1976 and samples collected October 27, November 2, and November 10, 1976. The second test phase began January 8, 1977, with tissue samplings on January 10, January 22, and February 3, 1977. Results of the tissue abnormalities in the final samplings from the second test phase were more pronounced than from the first test phase.

Figures 9 (page 39), 10 (page 41), 12 (page 44), 15 (page 49), and 17 were selected from the last sampling date of the first test phase. Figures 3 (page 29),

4 (page 30), 5 (page 33), 6 (Page 34), 7 (page 36), 8 (page 38), 11 (page 43), 13 (page 46), 14 (page 47), 16 and 18 were chosen from the last sampling date of the second test phase.

Plants of bentgrass began showing damage at 20 days in the first test phase from treatments of benefin, DCPA and terbutol; and at 28 days from bandane, bensulide and siduron. Damage in bentgrass roots was observed at 15 days in the second test phase with benefin, DCPA and terbutol; and at 27 days with bandane, bensulide and siduron.

Damage in bermudagrass roots was observed at 20 days in the first test phase using DCPA, siduron and terbutol; and at 28 days from bandane, benefin and bensulide. Plants of bermudagrass began showing damage at 15 days in the second test phase using DCPA, siduron and terbutol; and at 27 days from bandane, benefin and bensulide.

CHAPTER V

SUMMARY

A histological study of the effects of a single rate with repeated applications of several selective, pre-emergence-type herbicides on cellular organization of the root tips in Penncross creeping bentgrass and Tifgreen bermudagrass was conducted from March 1976 to November 1977. The primary objective was to determine the anatomical and morphological responses in the apical 2 mm region of the roots in both species.

I. BENTGRASS

Bandane, benefin, bensulide, DCPA, siduron and terbutol caused compressed epidermal cells close to the root tips in Penncross bentgrass. Compressed cortical cells close to the tips resulted from benefin, DCPA and terbutol. Both cortical cells and the roots showed abnormal enlargement from DCPA and terbutol. Columnar disruption of cortical cells occurred using all six herbicides. Benefin and terbutol caused lateral root formations abnormally close to the root tips with accompanied hypertrophic cortical cells.

II. BERMUDAGRASS

Compressed epidermal cells close to the root tips in Tifgreen bermudagrass occurred when bandane, bensulide, DCPA and siduron were applied. The stelar cells close to the tips showed compression from bandane. Slight cortical cell compression close to the tip resulted from terbutol, and enlarged cortical cells occurred using bensulide, DCPA, siduron and terbutol. An abnormal narrowing of the root tips occurred from DCPA and terbutol with cortical cell columnar disruption resulting from treatments of benefin, bensulide, DCPA, siduron and terbutol. Treatments of DCPA, siduron and terbutol caused massed lateral root formations abnormally close to the root tips with associated hypertrophic cortical cells.

III. STAINABILITY

The meristematic zone in the root tips of the Penncross bentgrass and Tifgreen bermudagrass absorbed varying amounts of the aniline blue-orange G stain. The bentgrass roots absorbed large amounts of the stain for the herbicide treatments and the untreated check. The bermudagrass roots treated with benefin, bensulide, DCPA, siduron and terbutol absorbed very little of the stain; whereas, the bandane treated roots and the check absorbed large amounts of the stain. The differences in staining

levels appeared to correlate with the amounts of cytoplasmic material present in each root tip.

CHAPTER VI

CONCLUSIONS

Results of this study show that of the herbicides used, benefin and terbutol caused the most severe cellular damage in Penncross bentgrass roots. Moderate root cellular damage occurred from bandane, bensulide, DCPA and siduron.

DCPA, siduron and terbutol caused the most severe tissue abnormalities in the root tips of Tifgreen bermudagrass. Bandane and bensulide were moderate, and benefin slight in their effects on Tifgreen bermudagrass.

Additional research is needed to determine cellular responses in bentgrass from treatments with bandane, bensulide, DCPA and siduron; and in bermudagrass from treatments of bandane, benefin and bensulide under the conditions of a longer testing period.

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