To the Graduate Council:

I am submitting herewith a thesis written by John M. Bogert entitled "Cellular Response of Two Turfgrass Cultivars to Selected Herbicides." I have examined the final copy of this thesis for form and content and 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.

Lloyd M. Callahan

Lloyd M. Callahan, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council

Vice Provost
and Dean of The Graduate School

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master's degree at The University of Tennessee, Knoxville, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of the source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in his absence, by the Head of Interlibrary Services when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without by written permission.

Signature John M. Bogert

Date August 19, 1988

CELLULAR RESPONSE OF TWO TURFGRASS CULTIVARS TO SELECTED HERBICIDES

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

John M. Bogert

December 1988

ACKNOWLEDGMENTS

The author takes this opportunity to extend special thanks and appreciation to Dr. Lloyd M. Callahan, Professor in Ornamental Horticulture and Landscape Design, for serving as major professor and committee chairman, giving insight and guidance on the research project, and editing the manuscript.

Appreciation is expressed to Dr. Effin T. Graham, Professor in Ornamental Horticulture and Landscape Design, for his patience and instruction involving histological procedures and review of the thesis.

He also wishes to thank Dr. G. Neil Rhodes, Jr., Assistant Professor in Plant and Soil Science, for serving on the committee and reviewing the manuscript.

Appreciation is extended to Dr. G. Douglas Crater, Head of the Department of Ornamental Horticulture and Landscape Design, for review of the thesis.

The author also expresses appreciation to Dr. Ernest C. Bernard, Professor in Entomology and Plant Pathology for the use of laboratory equipment.

Special appreciation and gratitude is extended to all faculty, staff, and fellow students, especially Sandy, Joe, and Dennis, for their support, encouragement, and friendship.

He is especially grateful to his parents, grandparents, sister, and family for their unlimited encouragement, love, and understanding during his graduate studies.

Finally he wishes to thank Linda for her unending love, sacrifice, and frienship throughout his studies.

ABSTRACT

Root meristem cell, foliage, and root growth responses of Penncross creeping bentgrass (Agrostis palustris Huds.) and Tifgreen bermudagrass [Cynodon dactylon (L.) Pers. x Cynodon transvaalensis Burtt-Davy] to postemergence herbicides in solution culture were investigated with visual ratings, root and shoot ash weights, paraffin sections and photomicroscopy. The capacity of these cultivars to recover from herbicide injury was evaluated. Bentazon [3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4-(3H)-one 2,2-dioxide] and bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) treatments resulted in reduced shoot and root weights of both cultivars. Cellular manifestations of herbicide injury were not easily detectable. Severe reductions of shoot and root weights of bentgrass and bermudagrass were noted for treatments of asulam [methyl[(4-aminophenyl)sulfonyl] carbamate] and sethoxydim [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one]. Prolific lateral root initiation abnormally close to root tips of bentgrass was induced by asulam. Bentgrass plants were killed following treatments of asulam and sethoxydim. Pronamide [3,5-dichloro(N-1,1-dimethyl-2-propynl)benzamide] exposure killed test plants of both cultivars.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

FIGURE PAGE

FIGURE PAGE

FIGURE PAGE

FIGURE PAGE

CHAPTER 1

INTRODUCTION

Turfgrass management has evolved into a highly sophisticated and demanding science. Herbicides continue to be an important tool for weed control in management programs. An understanding of the effects of herbicides on turfgrasses is important for their proper use in weed control programs.

Most research with herbicides focuses on control of weeds and visual foliar response of the turfgrass. Some research has shown that selected herbicides result in extensive injury to turfgrass roots or root meristems (8, 9, 10, 12). Cellular abnormalities in roots in response to herbicide treatments has been documented. Enlarged cortical cells, reduced root cap size, enlarged epidermal cells, and proliferation of the pericycle are examples of cell abnormalities observed (10, 21, 46). Greater knowledge and understanding of herbicide influence on root meristems is needed for continued acceptance and use of these chemicals in the future.

In response to this need, this study was undertaken to determine the anatomical and morphological responses of roots of 'Penncross' creeping bentgrass (Agrostis palustris Huds.) and 'Tifgreen' bermudagrass [Cynodon dactylon (L.) Pers. x Cynodon transvaalensis Burtt-Davy] to selected

herbicides. The capacity of the herbicide-treated turfgrasses to recover following a period of root exposure to these chemicals was also tested.

CHAPTER II

LITERATURE REVIEW

Most research with postemergence herbicides deals with weed control and foliar response of turfgrass following treatment. Few researchers have reported on turfgrass root responses to the herbicides used in this study.

The following review of literature will discuss each herbicide separately.

A. ASULAM

Asulam [methyl[(4-aminophenyl)sulfonyl]carbamate] is labeled for control of crabgrass (Digitaria spp.), goosegrass [Eleusine indica (L.) Gaetrn.], and sandbur (Cenchrus pauciflorus Benth.) in established stands of St. Augustinegrass [Stenotaphrum secundatum (Walt.) Kuntze] and 'Tifway' bermudagrass. Like many other carbamate herbicides, asulam kills susceptible plants by interfering with cell division in plant meristems (2).

Neel and Burt (44) observed the tolerance of five turfgrasses to a single application of asulam at 1.1, 2.2, or 4.5 kg/ha. Five St. Augustinegrass cultivars and 'Emerald' Zoysia (Zoysia japonica x Zoysia tenuifolia Willd ex Trin.) showed excellent tolerance to these rates. Centipedegrass [Eremochloa ophiuroides (Munro.) Hack.] was very sensitive to the 2.2 and 4.5 Kg/ha rates. Four

cultivars of bermudagrass differed in their tolerance to asulam. Injury was severe to the cultivars 'Tifgreen' and 'Tifdwarf' but less severe to 'Tifway' and 'Ormond' bermudagrass. Similar findings were reported by Murdoch and Iked (43) in an earlier study using a single application of asulam at 2.2 kg/ha to the same bermudagrass cultivars.

Burt and Carlyle (6) noted little or no injury to St. Augustinegrass from a single application of asulam at 2.2 kg/ha. Furthermore, they found that shoot growth of bahiagrass (Paspalum notatum Flugge.) and common bermudagrass [Cynodon dactylon (L.) Pers.] were significantly suppressed by the same treatment. A report by Bingham (4) noted that asulam applied soon after bermudagrass spring green-up was much more injurious than applications made in late summer.

Arnold and Aitken (1) observed the control of perennial grasses with asulam in pecan (Carya spp.) orchards. A single 8.8 kg/ha application gave excellent control of bahiagrass for at least 15 weeks.

In field studies, Blevins and Rieck (5) reported good control of johnsongrass [Sorghum halepense (L.) Pers.] in a tall fescue (Festuca arundinacea Schreb.) stand with asulam at rates of 3.36 kg/ha or greater. None of the chemical rates up to 13.45 kg/ha, caused visual injury to the tall fescue. However, greenhouse studies with asulam applied to

tall fescue seedlings resulted in much greater injury to the seedlings than to mature tall fescue plants.

B. BENTAZON

Bentazon [3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4-(3H)-one 2,2-dioxide] is labeled for yellow nutsedge (Cyperus esculentus L.) control in established bluegrass (Poa spp.), fescue (Festuca spp.), bentgrass (Agrostis spp.), bermudagrass, bahiagrass, centipedegrass, Zoysia, ryegrass (Lolium spp.), and St. Augustinegrass. Bentazon controls weeds by inhibiting photosynthetic carbon dioxide fixation and photosynthetic electron transport (15).

Hawf (23) reported slight injury to 'Astoria' colonial bentgrass (Agrostis tenuis Sibth.) following applications in late spring of 1.65 and 3.3 kg/ha of bentazon to a mixture of 'Astoria', Kentucky bluegrass (Poa pratensis L.) and 'Kentucky 31' tall fescue. However, the bentgrass resumed normal growth following the initial injury. Tall fescue showed slight leaf tip chlorosis with the 1.65 kg/ha rate and leaf tip chlorosis plus 10 percent thinning with the 3.3 kg/ha rate. Hall and Parochetti (20) found that 0.83 and 1.65 kg/ha of bentazon significantly reduced stand density of tall fescue. Johnson (36) reported severe injury to field grown tall fescue two weeks after treatment with 2.2 and 4.4 kg/ha of bentazon. Eight weeks after treatment the tall fescue appeared to have fully recovered. However, a

double application of bentazon resulted in lower stand densities than a single application.

Several researchers testing the effects of bentazon for postemergence control of nutsedge in Kentucky bluegrass reported excellent tolerance of the turfgrass to rates of 1.1 and 2.2 kg/ha applied during late June and July (28, 29, 30, 31, 39).

Johnson (36), in both field and greenhouse studies, reported that 'Emerald' Zoysia, centipedegrass, St. Augustinegrass, and 'Tifway' bermudagrass generally appeared tolerant to bentazon. None of the field grown turfgrasses appeared permanently injured following treatments with 2.2 and 4.4 kg/ha. In greenhouse pot studies, 'Emerald' Zoysia, centipedegrass, and St. Augustinegrass were temporarily injured. However, topgrowth of these turfgrasses appeared unaffected by bentazon 16 weeks after treatment. Only 'Emerald' Zoysia showed a reduction in root growth. 'Tifway' bermudagrass exhibited no root or shoot injury.

C. BROMOXYNIL

Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) is labeled for the control of selected broadleaf weeds in established Kentucky bluegrass, fescue, ryegrass, bermudagrass, St. Augustinegrass, bentgrass and Zoysia, excluding bentgrass putting greens. Bromoxynil has a

complex mode of action which includes uncoupling of oxidative phosphorylation and inhibition of the photosynthetic electron transport in chloroplasts (14).

Troutman, et al. (47) noted poor winter weed control in dormant common bermudagrass with bromoxynil at 0.55, 1.1, and 1.65 kg/ha applied in late February. They found that none of the treatments delayed spring green-up or resulted in reductions in subsequent growth.

Ebdon and Jagschitz (17) reported excellent tolerance of a 'Kingstown' velvet bentgrass ((Agrostis canina L.) green to 1.1 and 2.2 kg/ha of bromoxynil in a test for prostrate spurge (Euphorbia supina Raf. ex Boiss.) control. They also reported that an 'Exeter' colonial bentgrass and a common Kentucky bluegrass lawn sustained very little or no injury from similar bromoxynil treatments. Jagschitz (32) further reported that lawns of 'Touchdown' Kentucky bluegrass, 'Jamestown' red fescue (Festuca rubra L.), 'Exeter' colonial bentgrass and putting greens of 'Kingstown' velvet bentgrass, 'Emerald' creeping bentgrass, and 'Penncross' creeping bentgrass exhibited tolerance to bromoxynil applications of 4.4 kg/ha.

Dernoeden and Fry (16) found that bromoxynil applied at 0.55 kg/ha resulted in poor dandelion (Taraxacum officinale Weber) control in test plots containing an unknown blend of Kentucky bluegrass cultivars. The Kentucky bluegrasses showed no visual symptoms of injury to the chemical.

Jagschitz (27) studied the effects of bromoxynil on mature and seedling turfgrass stands. A single application at 2.2 kg/ha to a mature stand mixture of Kentucky bluegrass, red fescue, and colonial bentgrass resulted in only slight injury to all of the species in the mixture. Applications of bromoxynil at 0.55 and 1.1 kg/ha to Kentucky bluegrass and red fescue seedlings in the one-leaf and in the three- to six-leaf stages resulted in only slight reductions in stand densities.

Morrow and Canode (42) reported that a single application of bromoxynil at 1.68 kg/ha increased the first seed crop yield of 'Pennlawn' red fescue over an application rate of 0.56 kg/ha. The increase in seed yield appeared to be due to reduction of competitive broadleaf weeds with the higher rate. No injury to the red fescue was apparent.

D. PRONAMIDE

Pronamide [3,5-dichloro(N-1,1-dimethyl-2-propynyl)benzamide] is labeled for preemergence and post-emergence control of annual bluegrass (Poa annua L.) in bermudagrass turf. The label cautions against the use of pronamide in stands of dichondra (Dichondra micrantha Urb.), bluegrass, annual ryegrass (Lolium multiflorum Lam.), perennial ryegrass (Lolium perenne L.), fescue, and bentgrass. Pronamide acts on the root meristems of susceptible plants by inhibiting cell division and

subsequently causing unorganized differentiation (3).

Johnson (35) found in field studies that two applications of pronamide at 0.83 and 1.65 kg/ha resulted in significantly better growth of newly planted 'Tifway' bermudagrass than single applications. The 1.65 kg/ha treatments slightly retarded growth five weeks after planting as compared to treatments at 0.83 kg/ha. The bermudagrass fully recovered by nine weeks. In another study, Johnson (37) reported that pronamide applied at 0.8, 1.7, and 3.4 kg/ha to 'Tifdwarf' bermudagrass overseeded with perennial ryegrass did not affect the spring green-up or general health of the bermudagrass. All treatments of pronamide increased early spring coverage of the bermudagrass by greatly reducing the competitive stand of perennial ryegrass in the plots.

According to Johnson (35), pronamide applied at 0.83 and 1.65 kg/ha did not reduce root growth of centipedegrass plugs grown in pots in the greenhouse. Centipedegrass sprigged into an established common bermudagrass stand in the field exhibited no visual injury following three repeat applications of pronamide at 0.83 kg/ha (38). In an earlier study by Johnson (34), centipedegrass sprig survival rate was shown to increase when pronamide was applied three weeks after sprigging as compared to applications immediately following sprigging. A single application of 1.68 kg/ha immediately after sprigging resulted in only a 59 percent

survival rate. However, the same application made three weeks after sprigging gave an 83 percent survival rate.

Hartwig (22) found that 'Kentucky 31' tall fescue was somewhat tolerant to pronamide applied at 0.55 kg/ha. Applications of 1.1 and 1.65 kg/ha showed severe injury to the plants and resulted in large reductions of dry matter yield.

E. SETHOXYDIM

Sethoxydim [2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one] is labeled for annual and perennial grass control in soybeans (Glycine max (L.) Merr.), cotton (Gossypium spp.), sugar beets (Beta spp.), and certain nursery and ornamental crops. It is also currently labeled for use in established centipedegrass for large crabgrass (Digitaria sanguinalis (L.) Scop.) control and for use in tall fescue for seedhead suppression along roadsides, rights-of-way, and nonfood crop alleyways. Sethoxydim affects the root and shoot meristem of susceptible species. It inhibits acetyl-coenzyme A carboxylase (7).

Hicks and Jordan (25) reported that single foliar applications at 0.28, 0.56, and 0.84 kg/ha of sethoxydim gave 53, 77, and 98 percent control respectively, of common bermudagrass After four weeks, all of these treated plants exhibited no regrowth. In another portion of this study,

fresh weights of bermudagrass clippings were collected at three hour intervals. After 6 hours, sethoxydim-treated bermudagrass showed significant reductions in clipping fresh weights.

Higgins et al. (26) reported unacceptable color and stand density ratings of 'Penncross' creeping bentgrass and 'Tifgreen II' bermudagrass at 14 and 28 days following sethoxydim at 0.1, 0.2, and 0.3 kg/ha.

Cisar and Jagschitz (13) showed that two 0.138 kg/ha treatments with sethoxydim in July severely thinned perennial ryegrass in a perennial ryegrass-red fescue lawn. Red fescue exhibited acceptable tolerance to this application schedule.

Yahres and Jagschitz (48) reported variable crabgrass control using sethoxydim in tests in Kentucky bluegrass turf. In both tests, a single and double application of sethoxydim was applied in July at 0.22 and 0.44 kg/ha and both resulted in severe injury to the Kentucky bluegrass.

McCarty, et al. (41) applied sethoxydim postemergence to centipedegrass at 0.1, 0.2, and 0.3 kg/ha in two split treatments 28 days apart. Centipedegrass showed no change in stand density or color following treatments.

The use of sethoxydim for suppression or control of bahiagrass in centipedegrass roadside turf was shown by Lewis and DiPaola (40). Sethoxydim suppressed or completely controlled bahiagrass and did not significantly discolor or

reduce stand density of centipedegrass when applied at rates ranging from 0.224 to 0.672 kg/ha.

CHAPTER III

MATERIALS AND METHODS

These investigations were conducted to determine anatomical and morphological responses of cells in the apical region of the roots of 'Penncross' creeping bentgrass and 'Tifgreen' bermudagrass to selected postemergence herbicides. Solution culture in the greenhouse was chosen as the media for herbicide treatments of the two turfgrasses. Histological procedures and ash weight analyses were conducted in the laboratory. Greenhouse procedures will be discussed separately from laboratory procedures.

These studies were conducted in the facilities of the Department of Ornamental Horticulture and Landscape Design at the University of Tennessee, Knoxville, from June 1986 to June 1988.

A. GREENHOUSE PROCEDURES

Test Plants

'Penncross' creeping bentgrass seed was sown in flats of pure sand and placed under mist in the greenhouse for two weeks to aid good germination and uniform seedling emergence. 'Tifgreen' bermudagrass plants were sprigged in flats of pure sand and placed under mist for one week to aid

good establishment. Flats of both bentgrass and bermudagrass were removed from mist after establishment and grown under environmental conditions similar to actual test conditions. A modified Hoagland (24) solution was applied at four day intervals to provide necessary mineral nutrition.

Approximately 10 weeks after establishment, healthy plants of uniform size were removed from the pure sand. The roots of each plant were gently washed, excised 2.5 cm below the crown, and mounted in 1.9 L culture jars.

Apparatus and Test Procedures

The apparatus used to conduct these investigations was a continuous flow, constant-level solution culture renewal system (11) containing a modified Hoagland solution (24). Three complete systems were used. Each system contained an 18 L nutrient solution stock jar, a 4 L central distributor jar, and twelve 1.9 L culture jars. Culture jars were fitted with a rubber stopper lid with three holes approximately 15 mm in diameter. Nine plants were contained in each culture jar, three plants in each hole. There were a total of six culture jars for each chemical treatment with each culture jar serving as a replication. Each system contained two of the culture jars for each treatment randomly arranged. Plants were wrapped in non-absorbent cotton and mounted so that the roots were suspended in the solution, and the

crowns suspended at or above the level of solution in the jar.

The solution culture system was set on a greenhouse bench for both turfgrass studies. Four 350 watt bulbs were suspended 72 cm above the plants to supplement natural light and were controlled by a time clock providing 14 hours of light and 10 hours of darkness each 24-hour period.

Greenhouse temperature and humidity readings were collected with the use of a Belfort hygrothermograph stationed on the bench with the solution culture system. The greenhouse temperature averages from September 10 to November 5, 1986 for the bermudagrass experiments were 34 C (94 F) high and 20 C (68 F) low, with average percent relative humidities of 15 percent day and 80 percent night. Temperature averages from March 24 to May 19, 1987 for the bentgrass tests were 35 C (95 F) high and 17 C (62 F) low with average percent relative humidities of 16 percent day and 92 percent night.

Herbicide Treatments

Herbicides, their formulations, and rate of application used in each solution culture jar are shown in Table 1. Formulations included four emulsifiable concentrates (EC) and one wettable powder (W). Rates are expressed in kilograms of active ingredient per hectare (kg/ha) and the corresponding pounds of active ingredient per acre (lb/A).

Table 1. Herbicides used, their formulations, respective rates of application, amounts added to each solution culture jar per treatment, and corresponding molarity.

Herbicide Common name	Formulation	Rate kg/ha	Rate lb/A	Amount of herbicide per solution culture jar in ml or mg / 123 cm2	Corresponding herbicide Molarity
Asulam	3.34 EC	2.2	2.0	0.5 ml	6.30 µM
Bentazon	4 EC	1.1	1.0	0.5 ml	2.95 µM
Bromoxynil	2 EC	0.4125	0.375	0.5 ml	1.01 µM
Pronamide	50 W	1.1	1.0	2.8 mg	5.70 µM
Sethoxydim (+ Prime oil)	1.5 EC	0.4	0.4	0.5 ml	0.67 µM

All treatments were made using mean or minimum rates within the label recommendations. Amount of herbicide used was reduced from a surface area of kg/ha to ml or mg/123 cm^2, which was the surface area of solution in the culture jars. Rates were then converted to Molarity of active ingredient and expressed as micromoles (μM) based on rate per volume of solution in the culture jar.

Herbicides were measured or weighed in the laboratory just prior to application. Designated amounts of each of the emulsifiable concentrate herbicides were diluted in distilled water with the total volume equaling one liter. The contents were then stirred thoroughly to create a uniform suspension. From each suspension, 0.5 ml was pipetted into 5 ml microbeakers, brought up to 5 ml volume with distilled water, and then sealed. The remainder of the bulk suspension was sealed and stored in a dark cabinet until the next treatment four days hence. After two treatment withdrawals from the bulk suspension, it was discarded and a new suspension was prepared. The wettable powder herbicide was weighed on an analytical balance, placed in 5 ml microbeakers, and sealed.

Initial treatments were made on the same day the plants were mounted. Supplemental treatments were applied at four day intervals over a period of 28 days, giving a total of seven treatments. Calculations of solution drip rate from the culture jars showed a complete solution change-over

approximately every three days, thus eliminating or greatly reducing herbicide concentration in the solution. Since mean or minimum herbicide rates were being used, on a surface area basis, it was estimated that fortification treatments at four day intervals for the 28 day period would not result in significantly elevating herbicide concentration in the solution culture jars above the high label rate.

At the end of the 28 day period, root tips were collected for histological processing. Following the herbicide treatment period, plants were transferred into herbicide-free nutrient solution for a 28 day recovery period. Root tips were again collected at the conclusion of the recovery period.

Shoot and Root Growth Response Measurement

Visual shoot and root color ratings were made at weekly intervals throughout the herbicide exposure and non-herbicide periods. Shoot color rating scale used was as follows: M=medium, L=light, Y=yellow, and B=brown. Scale used for rating root color was W=white and B=brown. Ratings were made subjectively without comparison to untreated check plants.

Root Tip Collection

Root tips were collected by lifting the rubber stopper lids from the jars and excising tissue with a small pair of

scissors. Approximately 20 root tips were collected at random from each treatment where possible. Due to the phytotoxic effects of some herbicides, fewer than 20 root tips were collected from some treatments. Root tips were then placed in Craf III killing and fixing solution (45). For ease of handling, a root tip portion longer than necessary (approx. 10 mm) was removed. The apical 2 mm and the sub-apical 2 mm root tip regions were excised from the 10 mm portion and separated in the laboratory with the aid of a dissecting microscope immediately prior to further histological processing.

Ashing Weights

After root tissue collection, turfgrass plants were removed from the culture jar lids. Plants in each jar were excised just above and below the crown, leaving only the roots and the shoots and discarding crowns. This is a practice commonly followed with early stage turfgrass seedlings due to the potential for error since crowns varied considerably in mass and weight. The nine root systems in each culture jar were combined and ash weights determined. The nine shoot systems in each culture jar were also combined and ashed in the same manner as for roots. This procedure was followed for each replication per herbicide treatment and grass species. Root weights by the ashing method were used due to possible errors resulting from

residual sand adhering to the 2.5 cm of roots retained when seedlings were mounted in the culture jars. Possible weight errors could also result from nutrient salt residues on roots. Ashing removes all organic matter leaving only inert residues reflecting accurate weights. Salt residues on shoots posed a potential for weight errors, thus the ashing method was again used to maintain consistency with methods used.

Plant tissue was placed in a drying oven for two days at 105 C. Roots and shoots were weighed in their respective crucibles and pre-ashed. The pre-ashing method consisted of pre-heating the ashing oven to 300 C, placing the crucibles with their tissue inside and heating at 300 C for 10 minutes after the oven began to cycle, and then raising the temperature of the oven to 400 C for four hours. Plant tissue was removed from the oven, allowed to cool, and weighed again to determine ash weights. Ash weights were subjected to analysis of variance and mean separations with the Waller-Duncan K-Ratio T test.

B. LABORATORY PROCEDURES

Histological Procedures

Root tips were removed from the fixative, placed in small vials, and processed through an alcohol dehydration series starting at 5 percent ethyl alcohol (ETOH) and progressing through a 30 percent concentration. The

dehydration series continued with 50 percent ETOH/tertiary butyl alcohol (TBA) and 50 percent water, 70 percent ETOH/TBA and 30 percent water, 85 percent ETOH/TBA and 15 percent water, 95 percent ETOH/TBA and 5 percent water, and then 100 percent ETOH/TBA mixture. This TBA dehydration series was a modification of Johansen's (33) series. Erythosin B was added to the 100 percent ETOH/TBA to impart color. The root tips were then placed in three changes of 90 percent TBA and 10 percent isopropyl alcohol. Each of the steps in this dehydration series lasted approximately 15 minutes. Following dehydration, the vials were placed in an oven at 60 C and paraffin pellets added periodically to replace the evaporating alcohol and infiltrate the root tissue. Vials remained in the oven until all the alcohol had been evaporated. Root tips were embedded in paraffin using stainless steel molds and plastic block rings. Paraffin blocks were sectioned at 10 μm using a rotary microtome and a steel knife.

Paraffin was removed from the sections by passing the slides through four changes of AmeriClear histological clearing solvent (Stephens Scientific Division, Cornwell Corporation, Denville, NJ). The sectioned tissue was then rehydrated by passing it through a series of decreasing alcohol concentrations starting with two changes of 100 percent ETOH and ending with 30 percent ETOH. Slides were placed in a water rinse before staining. Tissue was stained

with Gill's hematoxylin (19) for six minutes, rinsed in two changes of water, and dehydrated with an alcohol series beginning with 30 percent ETOH and finishing with three changes of 100 percent of ETOH. Following dehydration, slides were passed through four changes of AmeriClear histological clearing solvent. Cover glasses were placed over the tissue with Pro-Texx Mounting Medium (Lerner Laboratories, Pittsburg, PA).

Photomicrography and Root Drawings

Photomicrographs were made with 35 mm Technical Pan film. Photomicrographs at 35, 70 and 175 magnification(X) are included to illustrate selected cellular detail. Drawings of root tissue were made with a drawing tube at 80X. Apical drawings show meristematic zone, root cap, cortex, epidermis, pericycle, and vascular or stelar elements. Photomicrographs are of selected locations within the root meristem. Drawings illustrate principle cellular growth and cellular interrelationships all along the respective 2000 μm root length with greater cell clarity.

CHAPTER IV

RESULTS AND DISCUSSION

Shoot and root growth and root meristem cellular responses to each herbicide for both turfgrass test species will be discussed separately. All plant responses to each herbicide will be compared also to untreated check plants.

A. SHOOT AND ROOT GROWTH RESPONSE

Bermudagrass Shoot Response

Leaf color. Rapidly growing untreated bermudagrass check plants exhibited healthy, medium to light green leaves during the eight week test period (Table 2). Asulam, bentazon, and bromoxynil treated plants also exhibited medium to light green leaves during the same test period. During the 28 day recovery period, plants which had been exposed to bentazon eventually developed medium green leaves. Pronamide treatments resulted in brownish-green leaves halfway through the 28 day herbicide exposure period and completely died halfway through the non-herbicide recovery period. The phytotoxic effects of pronamide to roots were not surprising due to the report by Audus (2) that this chemical acts on the root meristem by inhibiting cell division and inducing unorganized differentiation of root cells.

Sethoxydim treatments resulted in brownish-green leaves

Table 2. Leaf color ratings for 'Tifgreen' bermudagrass during the herbicide exposure and non-herbicide recovery period from September 10 to November 5, 1986.[1]

Chemical	Leaf color rating dates[2]						
	Herbicide exposure				Recovery period		
	Sept. 18	Sept. 25	Oct. 2	Oct. 9	Oct. 15	Oct. 22	Oct. 30
Untreated Check	MG	MG	LG	LG	MG	LG	LG
Asulam	MG	MG	MG	LG	LG	LG	LG
Bentazon	MG	MG	LG	LG	LG	MG	MG
Bromoxynil	MG	LG	LG	LG	LG	MG	LG
Pronamide	MG	MG	BG	BG	BG	BG	B
Sethoxydim	MG	MG	LG	BG	BG	BG	BG

[1]Initial herbicide treatment was made on September 10, 1986.

[2]Leaf color codes: B=Brown, G=Green, L=Light, M=Medium.

by the end of the herbicide exposure period. Although sethoxydim treated plants were still alive during the non-herbicide recovery period, plants were rapidly advancing in senescence.

Shoot weights. Although bermudagrass plants treated with bentazon and bromoxynil exhibited significantly lower shoot weights than untreated checks, shoot growth was still very good (Figures 1-6; Table 3). These effects were generally supported by Troutman et al. (47) who reported no reductions in growth of common bermudagrass treated with bromoxynil.

Significant reductions in bermudagrass shoot weights also resulted with treatments of asulam and sethoxydim (Figures 7-10; Table 3). In both cases, shoot mass was sparse and growth rate was slow. Neel and Burt (44) reported severe injury to 'Tifgreen' bermudagrass following treatments with asulam. Pronamide treatments resulted in shoot masses of approximately one-tenth the weight of untreated checks before plants died (Figures 11 and 12; Table 3).

Bermudagrass Root Response

Root color. Roots of untreated bermudagrass check plants exhibited a characteristic white to brownish-white color throughout the eight week test period (Table 4). Bentazon and bromoxynil treatments resulted in root color

Figure 1. Photograph of untreated check of a 'Tifgreen' bermudagrass plant after four weeks in nutrient culture.

Figure 2. Photograph of untreated check of a 'Tifgreen' bermudagrass plant after eight weeks in nutrient culture.

Figure 3. Photograph of a bentazon treated 'Tifgreen' bermudagrass plant after four weeks in nutrient culture.

Figure 4. Photograph of a bentazon treated 'Tifgreen' bermudagrass plant after eight weeks in nutrient culture.

Figure 5. Photograph of a bromoxynil treated 'Tifgreen' bermudagrass plant after four weeks in nutrient culture.

Figure 6. Photograph of a bromoxynil treated 'Tifgreen' bermudagrass plant after eight weeks in nutrient culture.

Table 3. Shoot and root ash weights for 'Tifgreen' bermudagrass at the end of the eight week test period on November 5, 1986.

Chemical	Mean ash weights (mg)[1]	
	shoot	root
Untreated Check	2277 a	391 a
Bromoxynil	2014 b	264 b
Bentazon	1973 b	278 b
Sethoxydim	922 c	135 c
Asulam	851 c	127 c
Pronamide	241 d	24 d

[1]Means within columns followed by the same letter are not significantly different according to the Waller-Duncan K-Ratio T Test.

Figure 7. Photograph of an asulam treated 'Tifgreen' bermudagrass plant after four weeks in nutrient culture.

Figure 8. Photograph of an asulam treated 'Tifgreen' bermudagrass plant after eight weeks in nutrient culture.

Figure 9. Photograph of a sethoxydim treated 'Tifgreen' bermudagrass plant after four weeks in nutrient culture.

Figure 10. Photograph of a sethoxydim treated 'Tifgreen' bermudagrass plant after eight weeks in nutrient culture.

Figure 11. Photograph of a pronamide treated 'Tifgreen' bermudagrass plant after four weeks in nutrient culture.

Figure 12. Photograph of a pronamide treated 'Tifgreen' bermudagrass plant after eight weeks in nutrient culture.

Table 4. Root color ratings for 'Tifgreen' bermudagrass during the herbicide exposure and non-herbicide recovery period from September 10 to November 5, 1986.[1]

Chemical	Root color rating dates[2]						
	Herbicide exposure				Recovery period		
	Sept. 18	Sept. 25	Oct. 2	Oct. 9	Oct. 15	Oct. 22	Oct. 30
Untreated Check	W	W	BW	BW	BW	W	BW
Asulam	W	BW	B	B	BW	BW	BW
Bentazon	W	W	BW	BW	BW	BW	BW
Bromoxynil	W	W	BW	BW	BW	BW	BW
Pronamide	B	B	B	B	B	B	B
Sethoxydim	W	BW	BW	B	BW	BW	BW

[1]Initial herbicide treatment was made on September 10, 1986.

[2]Root color codes: B=Brown, W=White.

responses similar to check plants. Roots of asulam and sethoxydim treatments developed a brown color by the end of the herbicide exposure period but generally recovered during the four week non-herbicide treatment period. Pronamide exposed roots quickly browned and died, and decayed rapidly.

Root weights. Root systems of bentazon and bromoxynil treated plants showed significant reductions in weight, but they still exhibited good root mass and weight compared to untreated plants (Table 3). Asulam and sethoxydim treatments resulted in greatly reduced root growth and weights of approximately 65 percent below that of check plants (Table 3). The very small mean root weight following pronamide treatments reflect the high degree of phytotoxicity of this chemical to bermudagrass (Table 3).

Bentgrass Shoot Response

Leaf color. In general, the herbicides tested appeared more phytotoxic to bentgrass than to bermudagrass (Table 5). Untreated bentgrass check plants developed light to medium green leaves throughout the eight week test period. Leaves of bentazon and bromoxynil treated plants exhibited an initial yellow-green but eventually developed a light green coloration during the eight week test period. Asulam and sethoxydim resulted in brownish-green leaves, and pronamide brown leaves by the end of the four week herbicide exposure period. Higgins et al. (26) reported unacceptable color and

Table 5. Leaf color ratings for 'Penncross' creeping bentgrass during the herbicide exposure and non-herbicide recovery period from March 24 to May 19, 1987.[1]

Chemical	Leaf color rating dates[2]							
	Herbicide exposure				Recovery period			
	Mar. 31	Apr. 7	Apr. 14	Apr. 21	Apr. 28	May 5	May 12	May 19
Untreated Check	LG	LG	LG	LG	MG	LG	MG	MG
Asulam	LG	LG	BG	BG	B	B	B	B
Bentazon	YG	LG	LG	LG	LG	LG	LG	LG
Bromoxynil	YG	LG	LG	MG	MG	MG	LG	LG
Pronamide	MG	MG	BG	B	B	B	B	B
Sethoxydim	MG	BG	BG	BG	B	B	B	B

[1]Initial herbicide treatment was made on March 24, 1987.

[2]Leaf color codes: B=Brown, G=Green, L=Light, M=Medium, Y=Yellow.

stand density for 'Penncross' bentgrass following sethoxydim treatments. The entire shoot systems of all three herbicide treatments were brown going into the recovery period. Shoot systems of these three herbicide treatments were completely dead within a few days into the four week recovery period.

Shoot weights. Although bentazon and bromoxynil treatments resulted in significantly reduced shoot growth weights as compared to check plants, shoot growth still appeared good (Figures 13-18; Table 6). Hawf (23) reported that 'Astoria' colonial bentgrass exhibited slight initial shoot injury from bentazon but subsequently resumed normal growth. Shoot growth following treatment of asulam, pronamide, and sethoxydim was only sparse as reflected by the very low ash weights (Figures 19-24; Table 6).

Bentgrass Root Response

Root color. Untreated plants exhibited healthy, white roots throughout the entire test period (Table 7). Bentazon and bromoxynil treatments exhibited white roots throughout the herbicide exposure period. However, roots turned brownish-white about halfway through the non-herbicide recovery period. Brown senescing roots developed halfway through the herbicide exposure period with pronamide and sethoxydim, and by the end of this period with asulam. These three herbicides resulted in roots that were dead or soon died going into the non-herbicide recovery period.

Figure 13. Photograph of an untreated check of a 'Penncross' bentgrass plant after four weeks in nutrient culture.

Figure 14. Photograph of an untreated check of a 'Penncross' bentgrass plant after eight weeks in nutrient culture.

Figure 15. Photograph of a bentazon treated 'Penncross' bentgrass plant after four weeks in nutrient culture.

Figure 16. Photograph of a bentazon treated 'Penncross' bentgrass plant after eight weeks in nutrient culture.

Figure 17. Photograph of a bromoxynil treated 'Penncross' bentgrass plant after four weeks in nutrient culture.

Figure 18. Photograph of a bromoxynil treated 'Penncross' bentgrass plant after eight weeks in nutrient culture.

Table 6. Shoot and root ash weights for 'Penncross' creeping bentgrass at the end of the eight week test period on May 19, 1987.

Chemical	Mean ash weights (mg)[1]	
	shoot	root
Untreated Check	8718 a	706 a
Bentazon	7088 b	473 b
Bromoxynil	7081 b	477 b
Asulam	284 c	17 c
Pronamide	63 d	9 c
Sethoxydim	66 d	8 c

[1] Means within columns followed by the same letter are not significantly different according to the Waller-Duncan K-Ratio T Test.

Figure 19. Photograph of an asulam treated 'Penncross' bentgrass plant after four weeks in nutrient culture.

Figure 20. Photograph of an asulam treated 'Penncross' bentgrass plant after eight weeks in nutrient culture.

Figure 21. Photograph of a pronamide treated 'Penncross' bentgrass plant after four weeks in nutrient culture.

Figure 22. Photograph of a pronamide treated 'Penncross' bentgrass plant after eight weeks in nutrient culture.

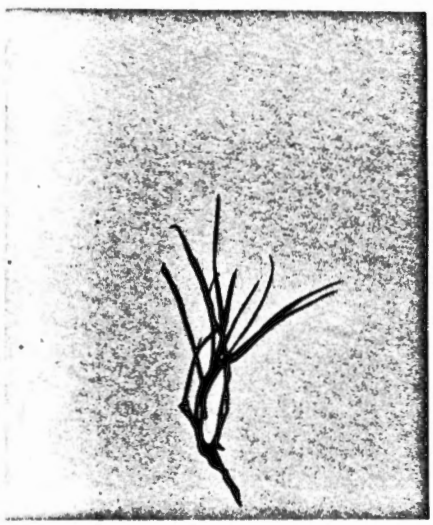

Figure 23. Photograph of a sethoxydim treated 'Penncross' bentgrass plant after four weeks in nutrient culture.

Figure 24. Photograph of a sethoxydim treated 'Penncross' bentgrass plant after eight weeks in nutrient culture.

Table 7. Root color ratings for 'Penncross' creeping bentgrass during the herbicide exposure and non-herbicide recovery period from March 24 to May 19, 1987.[1]

Chemical	Root color rating dates[2]							
	Herbicide exposure				Recovery period			
	Mar. 31	Apr. 7	Apr. 14	Apr. 21	Apr. 28	May 5	May 12	May 19
Untreated Check	W	W	W	W	W	W	W	W
Asulam	BW	BW	BW	B	B	B	B	B
Bentazon	W	W	W	W	W	W	BW	BW
Bromoxynil	W	W	W	W	W	W	W	BW
Pronamide	BW	BW	B	B	B	B	B	B
Sethoxydim	BW	B	B	B	B	B	B	B

[1]Initial herbicide treatment was made on March 24, 1987.

[2]Root color codes: B=Brown, W=White.

Root weights. Root weights following bentazon and bromoxynil treatments were a little more than one-half that of untreated checks (Table 6). The dead roots following asulam, pronamide, and sethoxydim treatments are reflected in the extremely low values exhibited for these three chemicals (Table 6).

B. ROOT MERISTEM CELLULAR RESPONSES

Bermudagrass

Untreated check. Cellular organization of the apical and subapical 2000 µm of 'Tifgreen' bermudagrass after four weeks in nutrient culture are shown in Figures 25 and 26. Root caps were normally developed and approximately 250 µm in length. Meristematic cells were round in shape. Cortical cells appeared characteristically arranged in columns back from the tip. These cells were square-shaped close to the tip and became rectangular moving back from the tip. Epidermal cells were compressed near the root tip. Stelar elements were well defined throughout this region. The subapical 2000 µm region was characterized by columnar rows of rectangular shaped cortical cells. Epidermal cells were evident along the entire length of this region. Stelar definition was excellent. Lateral roots were not commonly observed in the subapical secton. Harper (21) reported that the presence of lateral root initials in this region for bermudagrass was normal.

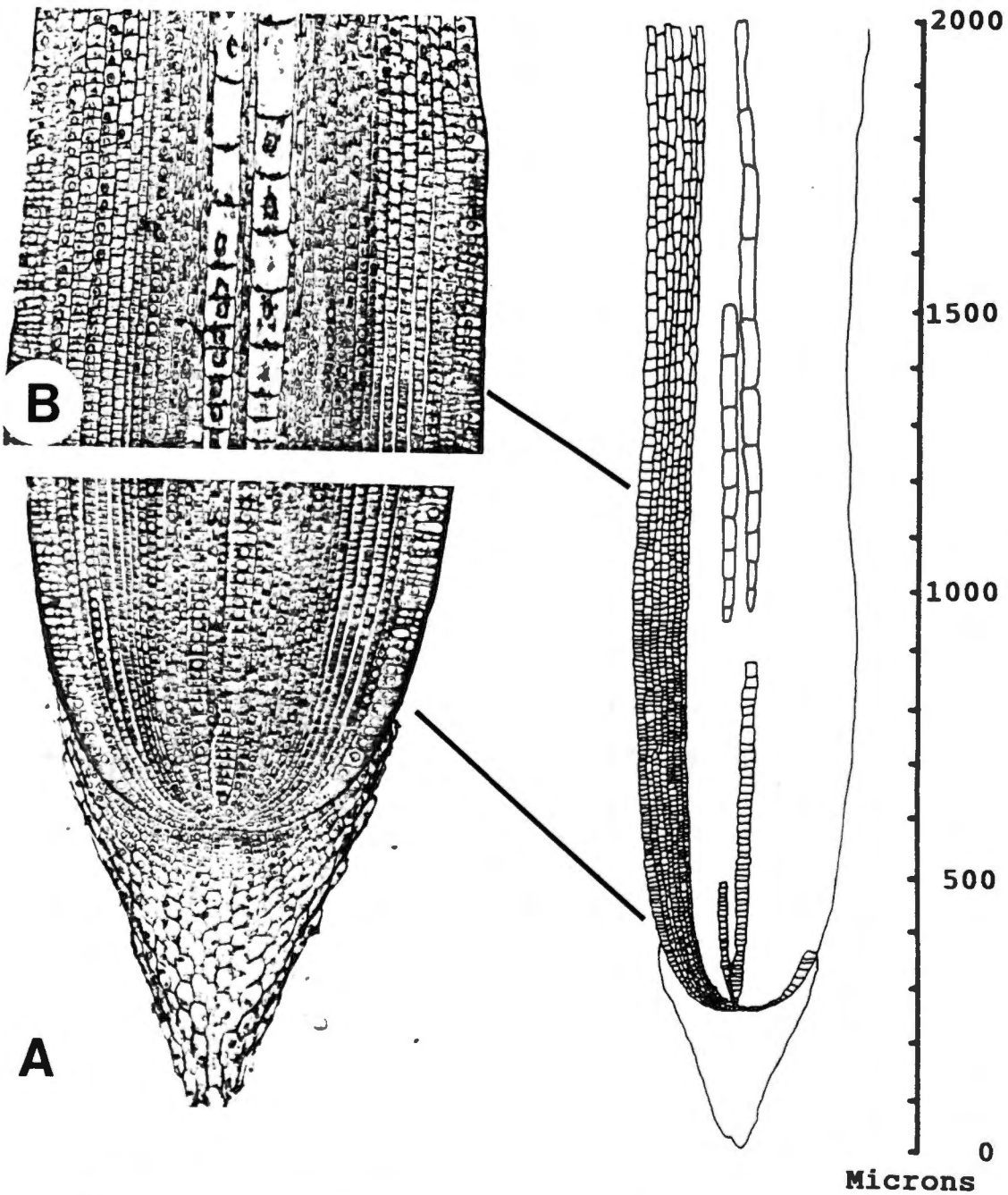

Figure 25. Diagram of a median longitudinal section of the apical 2000 µm of an untreated check of a 'Tifgreen' bermudagrass root after four weeks in nutrient culture. Drawing at 80X, photomicrographs A and B at 175X.

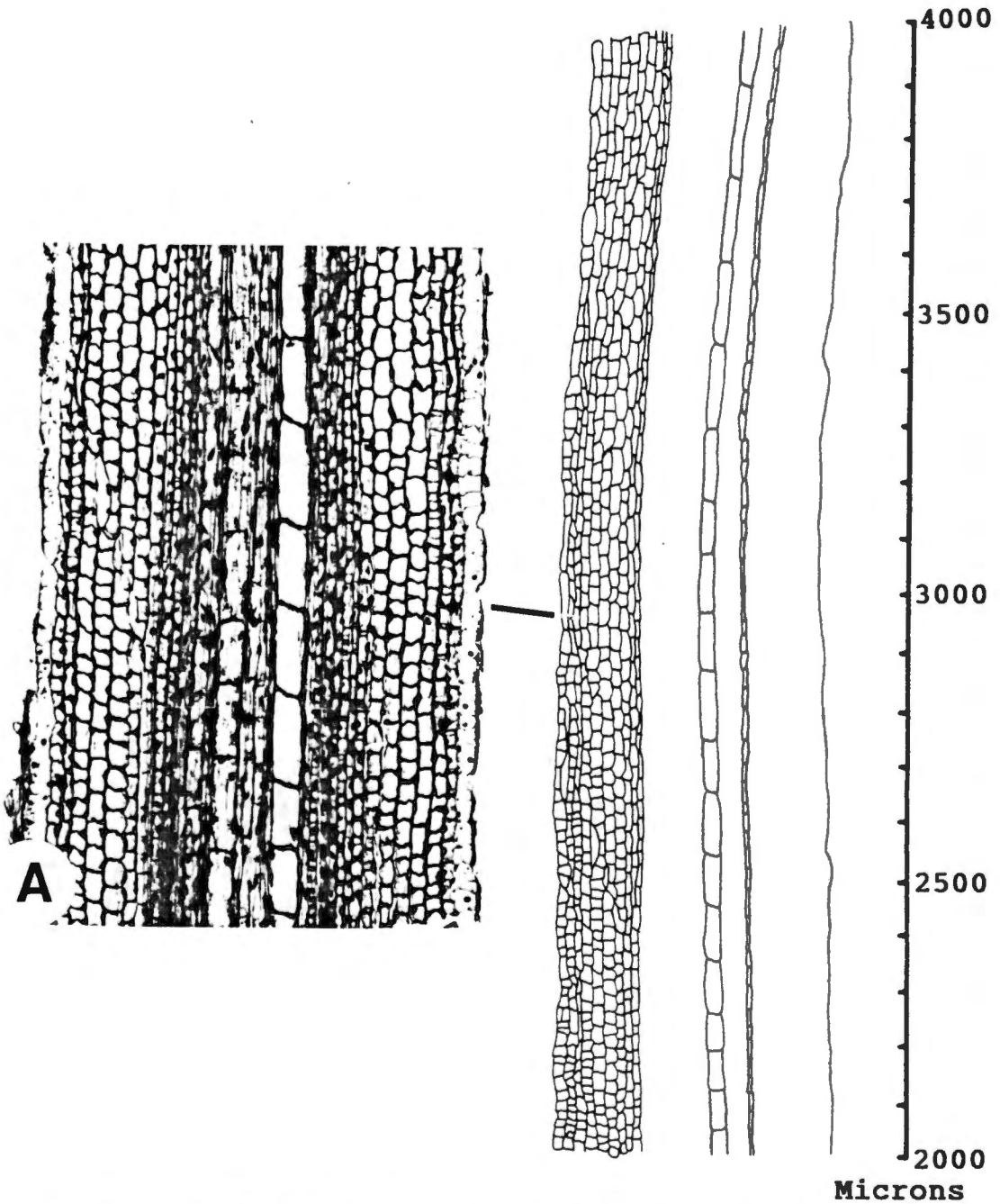

Figure 26. Diagram of a median longitudinal section of the subapical 2000 μm of an untreated check of a 'Tifgreen' bermudagrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

Anatomical and morphological cellular arrangements of 'Tifgreen' bermudagrass root tips at eight weeks were indistinguishable from root tips following four weeks in nutrient culture (Figures 27 and 28).

Asulam. Apical 2000 µm root sections of 'Tifgreen' bermudagrass treated with asulam showed extensive cellular abnormalities after four weeks of herbicide exposure (Figure 29). Root caps were greatly reduced in size. Cortical cells were enlarged near the root tip and began losing columnar arrangement. Lateral root initials were beginning to appear abnormally close to the root tip (200 µm). Cells were generally in a fragile weakened state, and some shattering occurred during histological processing. Columnar arrangement of cortical root cells was disrupted in the subapical 2000 µm after four weeks (Figure 30). Cortical cells were also enlarged compared to untreated check roots. Stelar elements were well defined. Subapical tissue was also weakened as evidenced by sloughing epidermal cells and shattered cortical cells.

After four weeks in herbicide free nutrient culture, 'Tifgreen' bermudagrass root tips previously exposed to asulam showed no manifestations of herbicide-induced injury (Figure 31). Root caps were well developed. Meristem cells appeared characteristically rounded. Cortical cells were square near the root tip and assumed normal rectangular organization back from the tip. Stelar cells were well

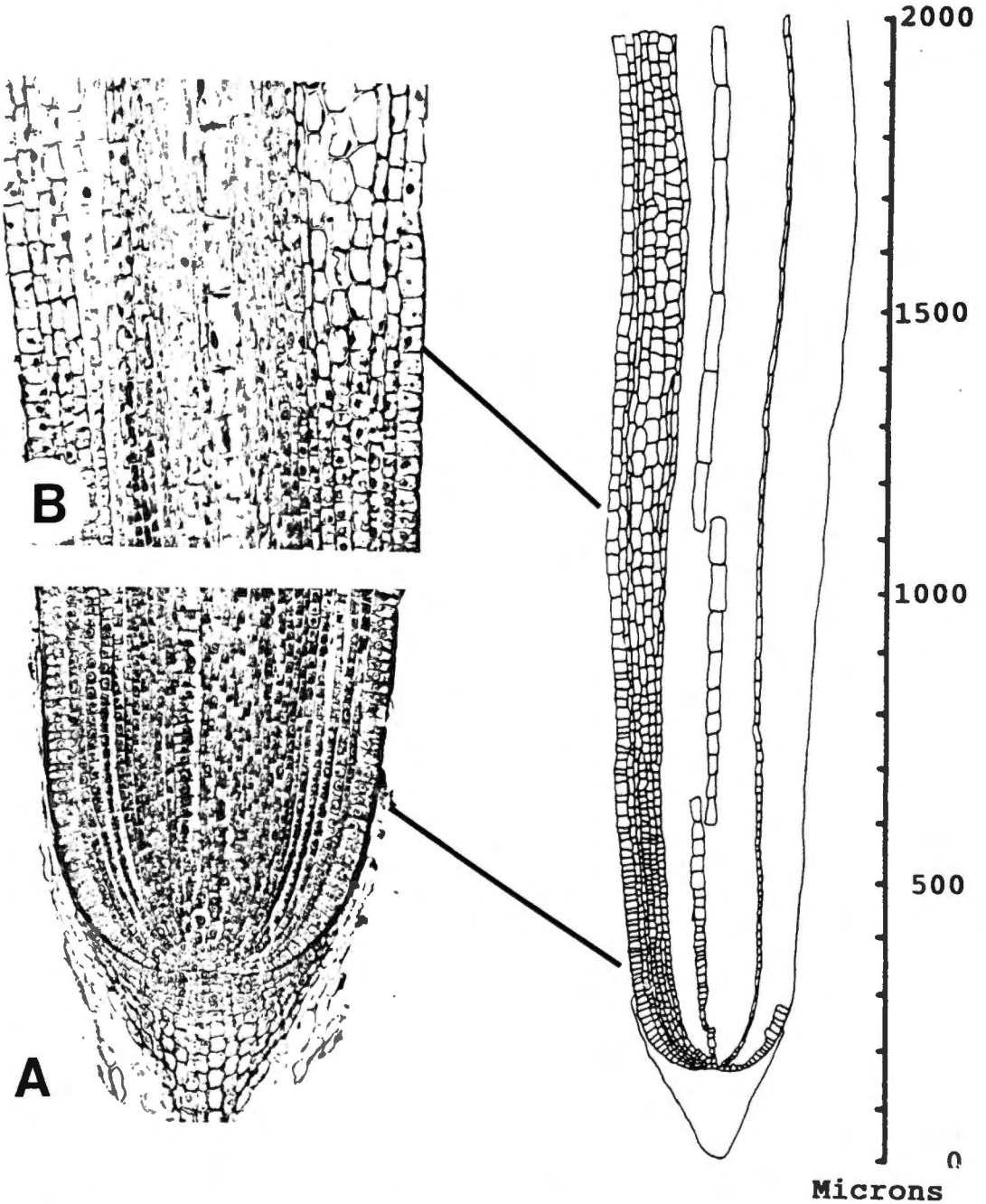

Figure 27. Diagram of a median longitudinal section of the apical 2000 μm of an untreated check of a 'Tifgreen' bermudagrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrographs A and B at 175X.

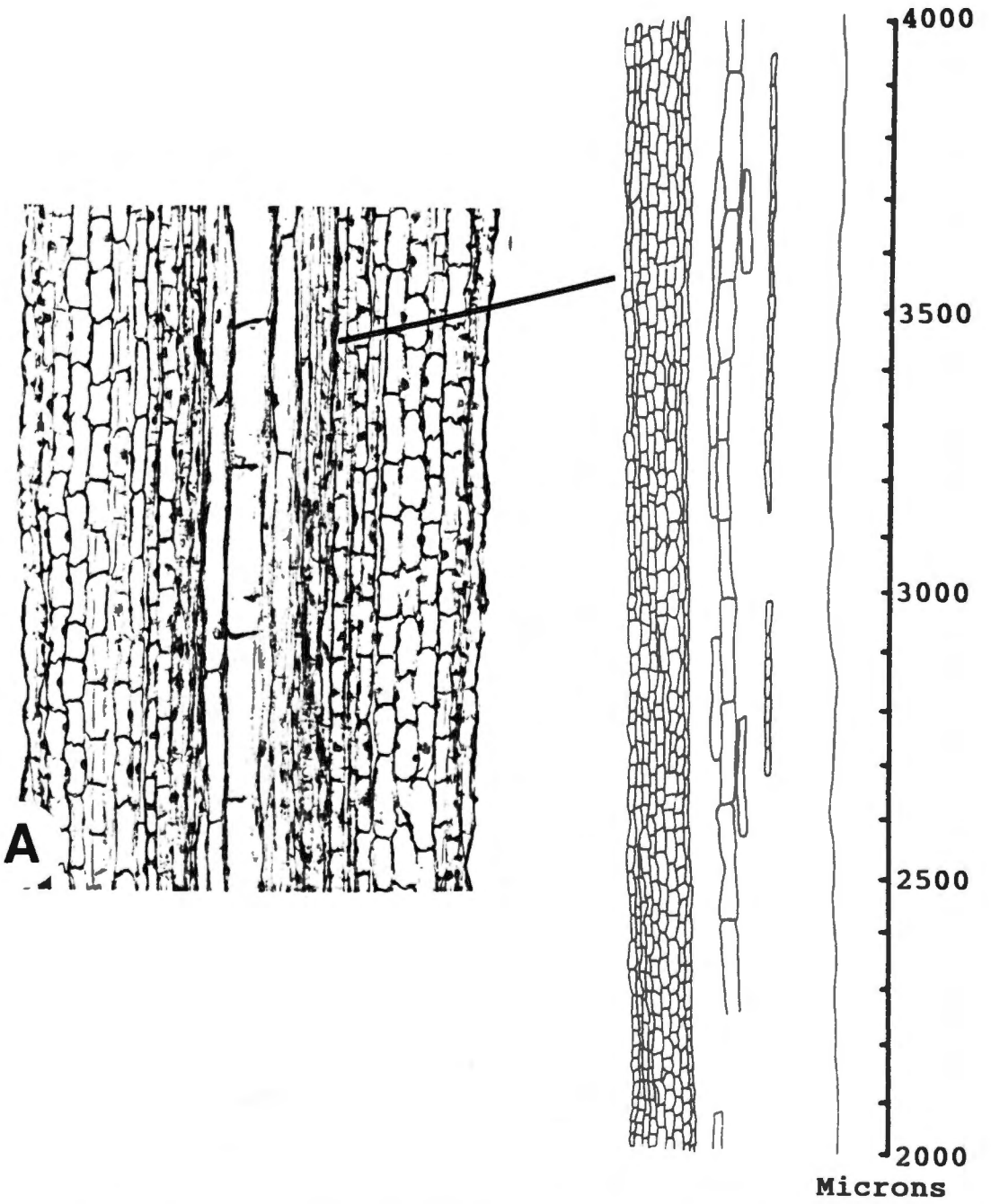

Figure 28. Diagram of a median longitudinal section of the subapical 2000 μm of an untreated check of a 'Tifgreen' bermudagrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

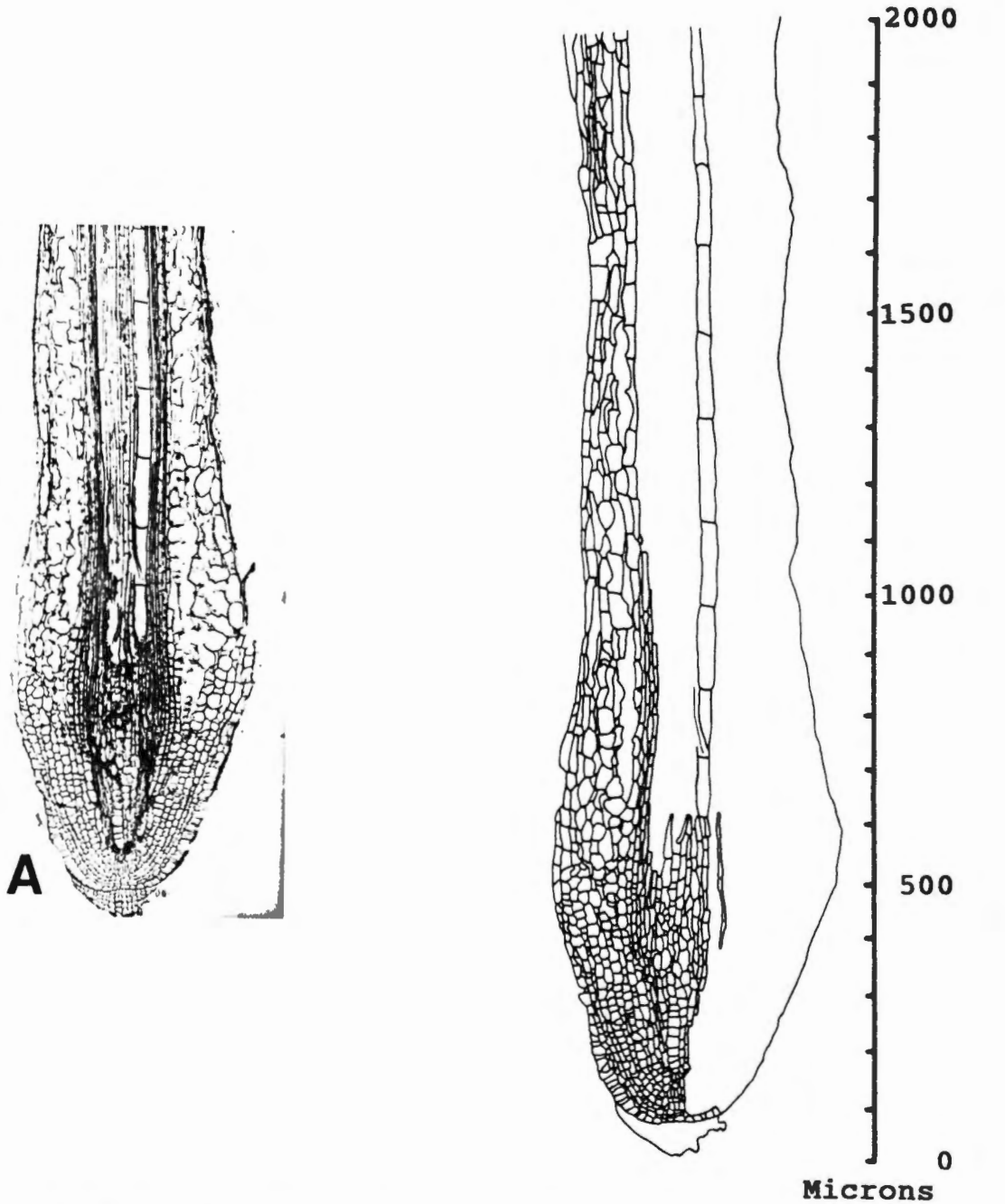

Figure 29. Diagram of a median longitudinal section of the apical 2000 μm of an asulam treated 'Tifgreen' bermudagrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 70X.

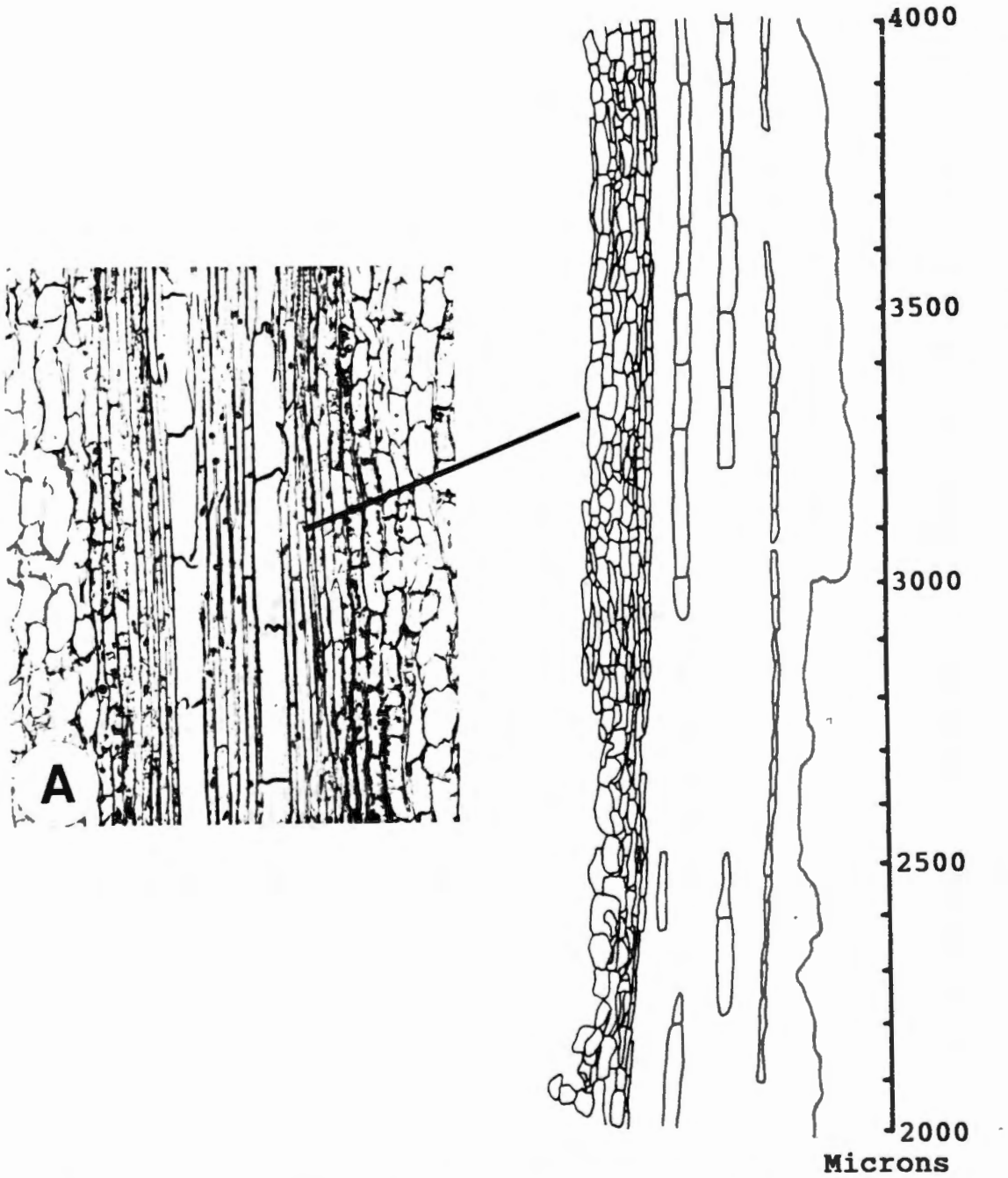

Figure 30. Diagram of an intermittent median longitudinal section of the subapical 2000 μm of an asulam treated 'Tifgreen' bermudagrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

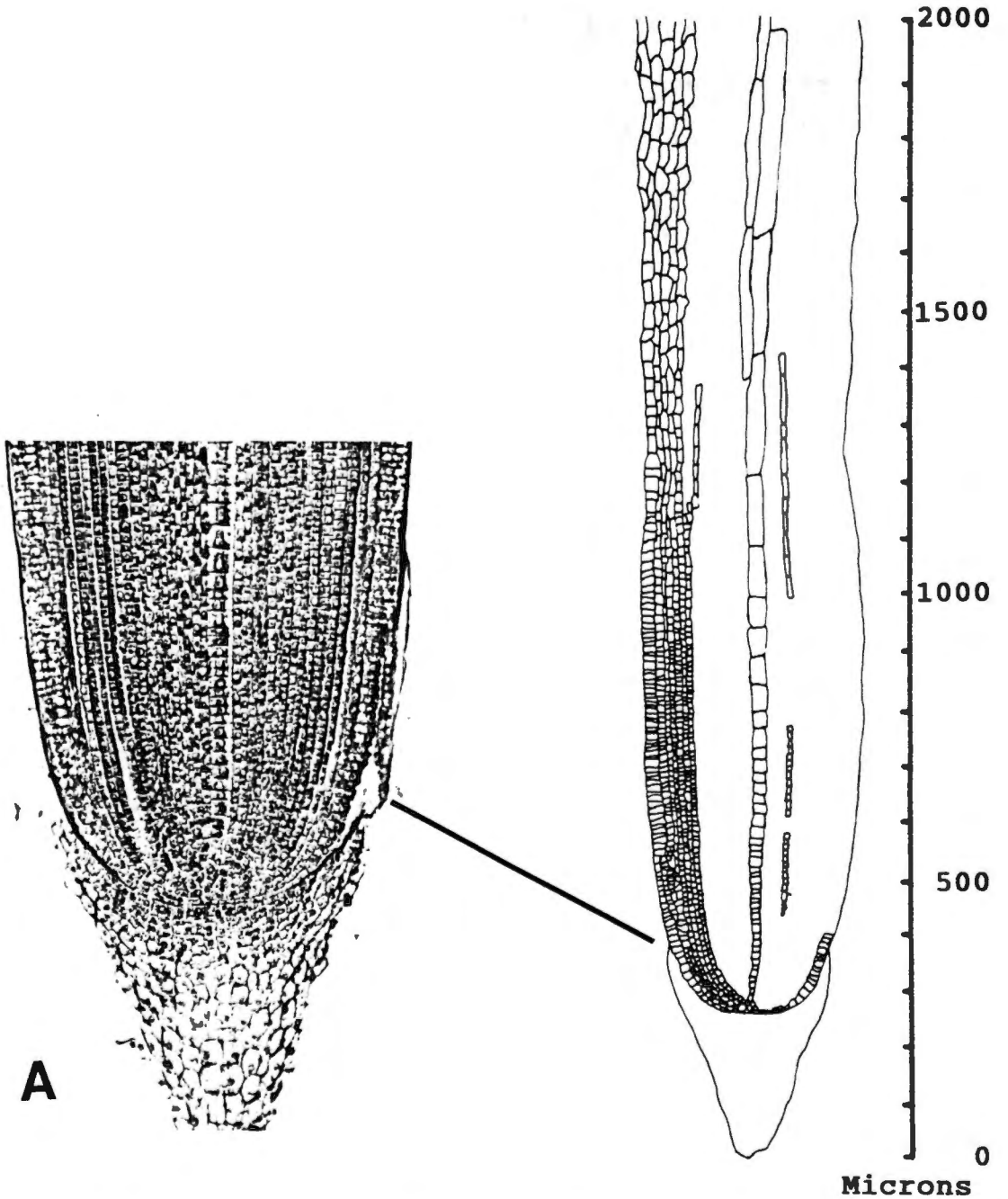

Figure 31. Diagram of a median longitudinal section of the apical 2000 μm of an asulam treated 'Tifgreen' bermudagrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

defined. Subapical root sections exhibited atypically elongated cortical cells (Figure 32). Stelar cells were well defined but were somewhat inconsistent, indicating slight root curvature.

Bentazon. The apical 2000 μm of 'Tifgreen' bermudagrass root tips exhibited only slight evidence of herbicide injury after four weeks exposure to bentazon (Figure 33). Root caps were well developed and cortical cell shape and size appeared normal, but columnar organization was somewhat inconsistent. Epidermal and cortical cells exhibited good strength throughout this region. Root tips displayed some curvature resulting in intermittent sectioning of stelar elements. After four weeks of herbicide exposure, the bermudagrass subapical root region continued to exhibit curvature with cortical cells appearing enlarged and lacking good columnar arrangement (Figure 34). Massive lateral roots exhibited abnormal rupturing through the cortex and epidermis.

Following four weeks growth in herbicide free nutrient solution, root tips rapidly developed extensive lateral root abnormalities not observed during the four week bentazon exposure period (Figure 35). Root curvature developed resulting in intermittent sectioning. Massive, localized lateral roots occurred abnormally close (550 μm) to the root tip. Hypertrophic and disorganized cortical cells developed adjacent to the lateral root initials. The

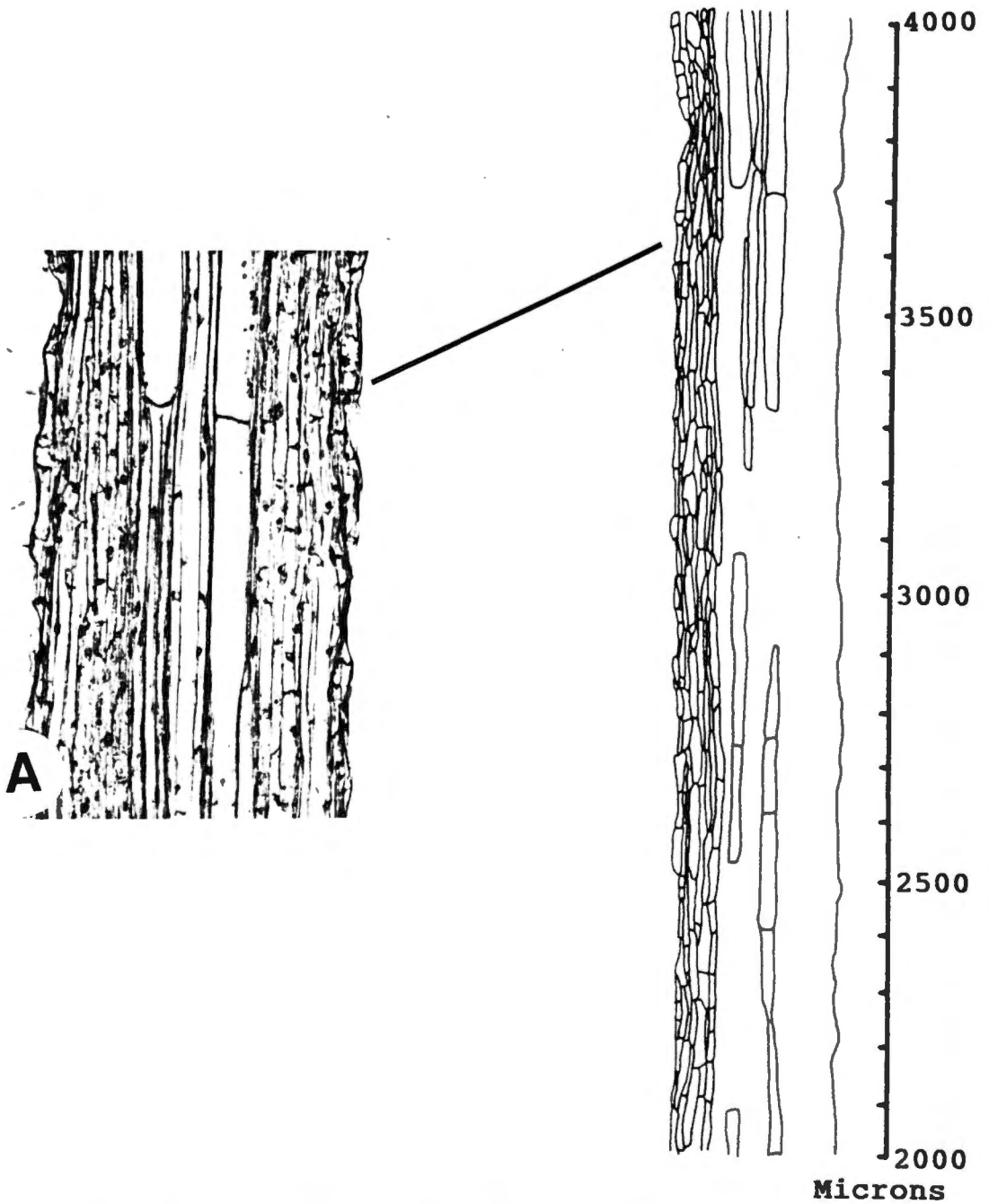

Figure 32. Diagram of an intermittent median longitudinal section of the subapical 2000 μm of an asulam treated 'Tifgreen' bermudagrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

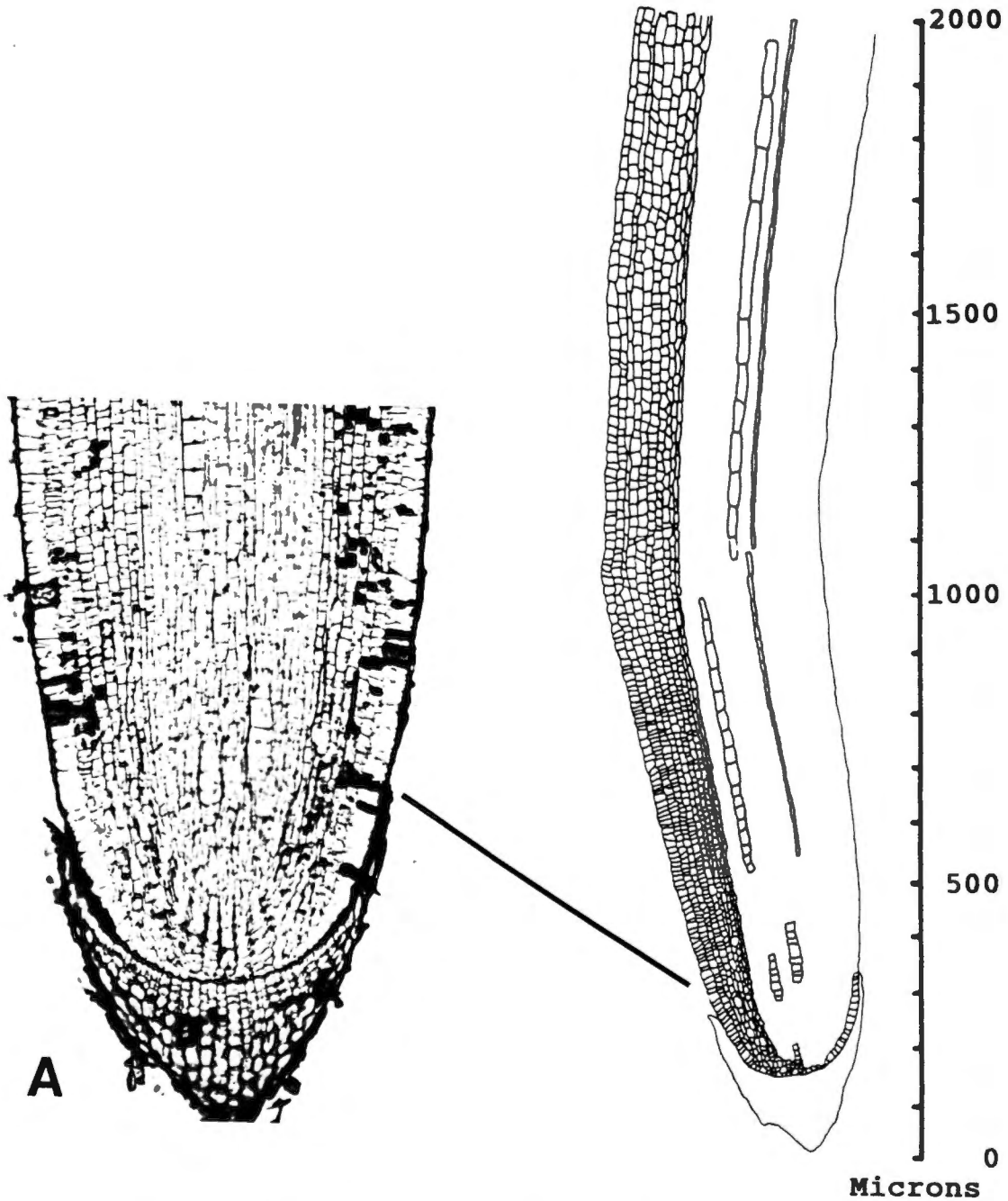

Figure 33. Diagram of a median longitudinal section of the apical 2000 μm of a bentazon treated 'Tifgreen' bermudagrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

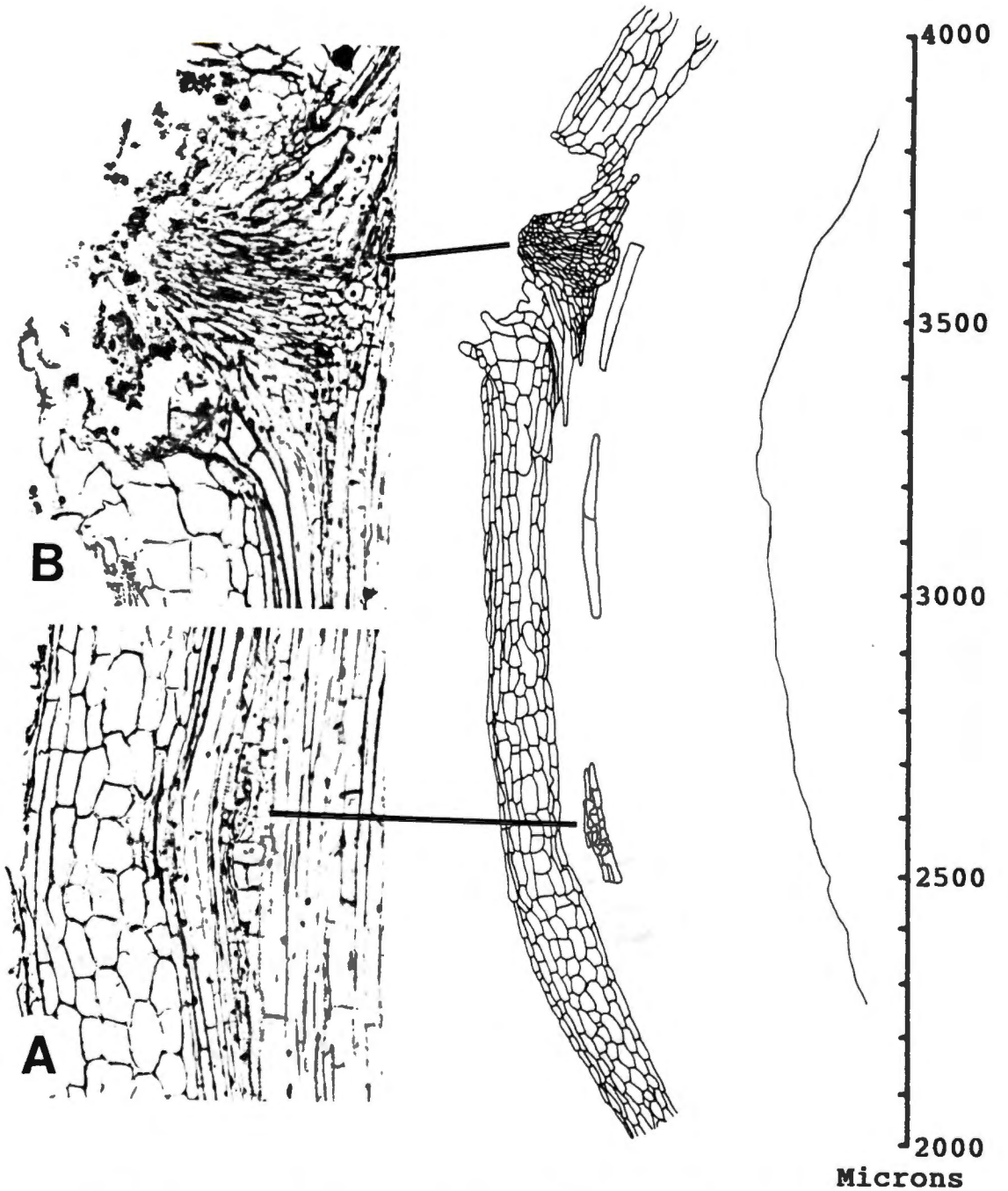

Figure 34. Diagram of an intermittent median longitudinal section of the subapical 2000 µm of a bentazon treated 'Tifgreen' bermudagrass root after four weeks in nutrient culture. Drawing at 80X, photomicrographs A and B at 175X.

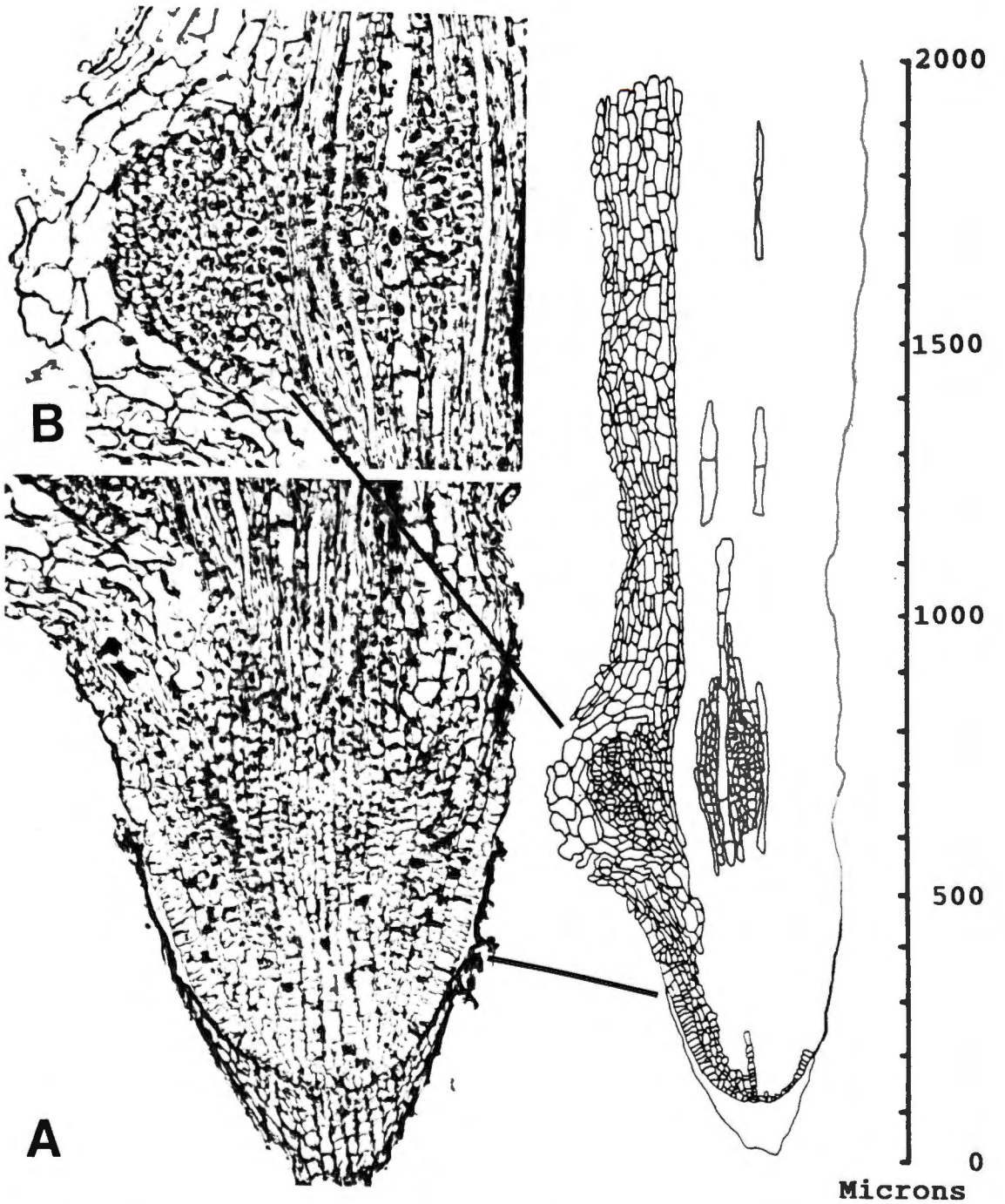

Figure 35. Diagram of an intermittent median longitudinal section of the apical 2000 μm of a bentazon treated 'Tifgreen' bermudagrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrographs A and B at 175X.

subapical 2000 μm root sections exhibited massive proliferations of lateral root initials during the non-herbicide recovery period (Figure 36). Hypertrophic cortical cells also accompanied lateral root initials. Stelar definition was inconsistent indicating curvature.

Bromoxynil. Apical 2000 μm sections of bermudagrass roots following four weeks of exposure to bromoxynil showed cellular organization similar to that of untreated check roots (Figure 37). Slight root curvature occurred but a consistent median longitudinal section could still be made. The corresponding subapical root tissue did not survive the histological processing, thus, no results could be shown.

Following the four week non-herbicide recovery period, root apex cells continued to appear normal (Figure 38). Subapical tissue collected for the same period exhibited more lateral root initials than normal (21) (Figure 39). Several lateral root initials occurred grouped in the 3100 μm region. Stelar definition was inconsistent in this region. Cortical cells were somewhat columnar but enlarged.

Pronamide. Pronamide-treated root apices exhibited advanced stages of senescence by the end of the four week treatment period and shattered easily during histological processing (Figure 40). Root caps were suppressed. Lateral root proliferations occurred abnormally close to the root tip. Hypertrophic cortical cells evolved with no distinct columnar arrangement. Stelar elements were poorly defined,

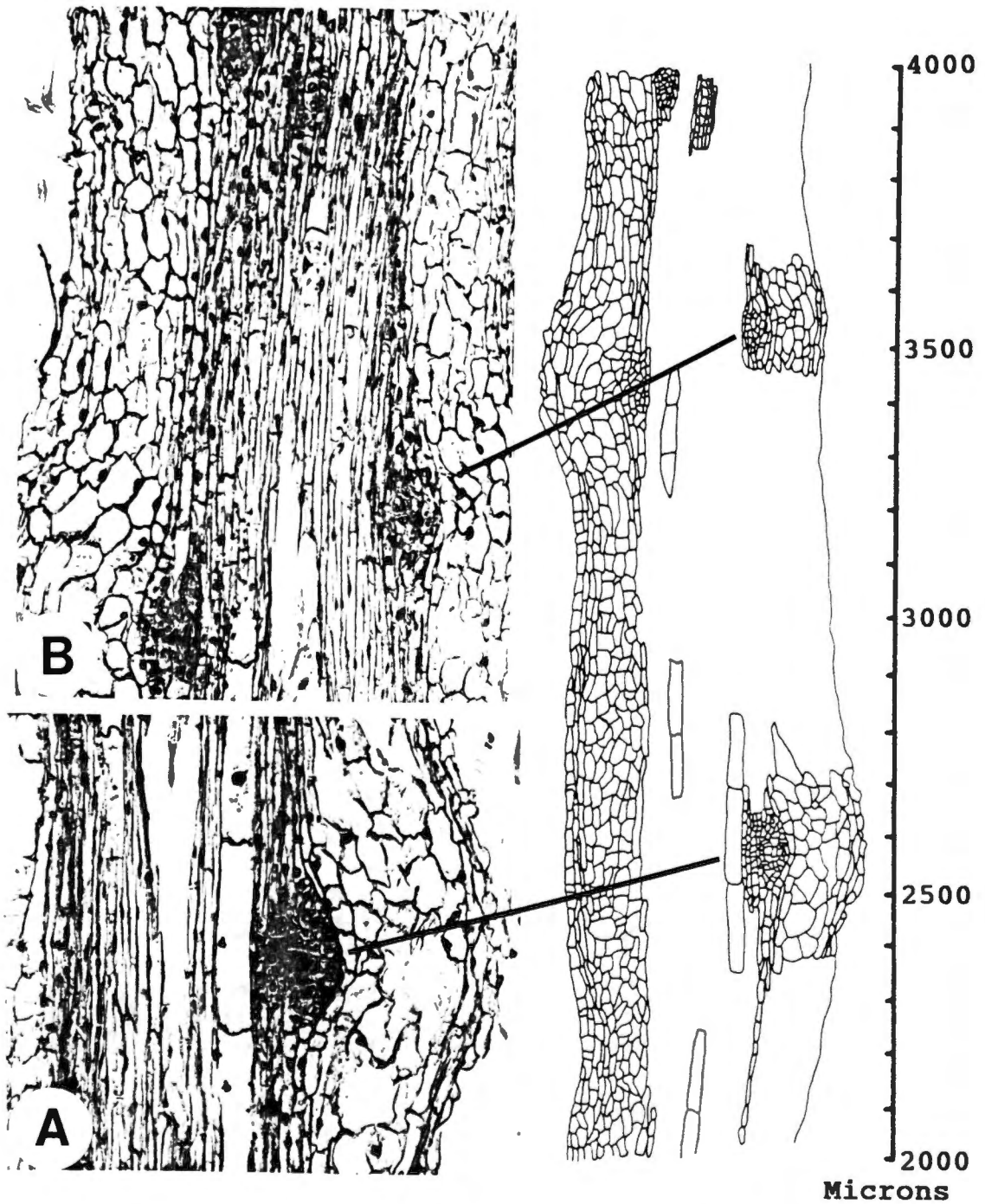

Figure 36. Diagram of an intermittent median longitudinal section of the subapical 2000 µm of a bentazon treated 'Tifgreen' bermudagrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrographs A and B at 175X.

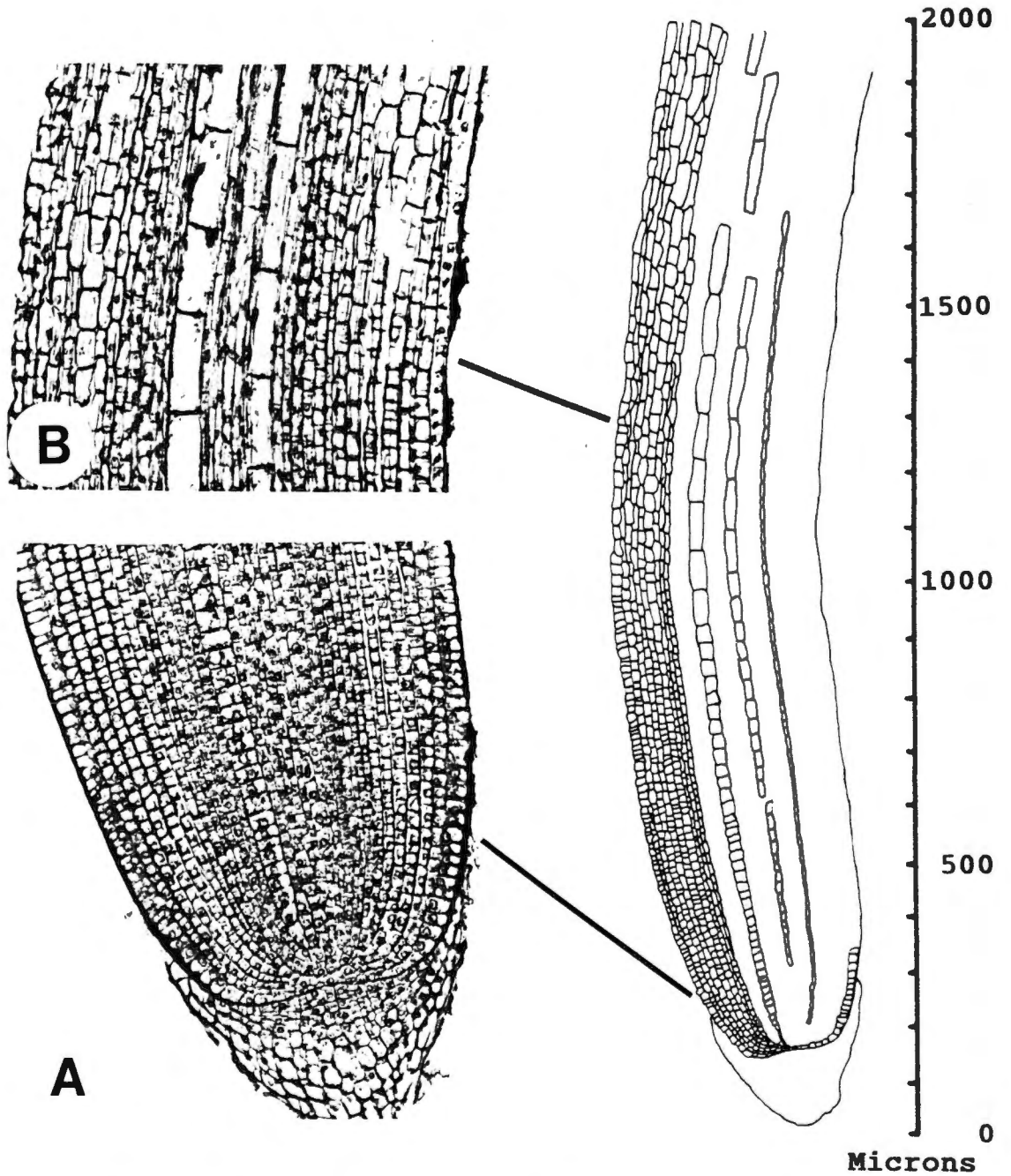

Figure 37. Diagram of a median longitudinal section of the apical 2000 μm of a bromoxynil treated 'Tifgreen' bermudagrass after four weeks in nutrient culture. Drawing at 80X, photomicrographs A and B at 175X.

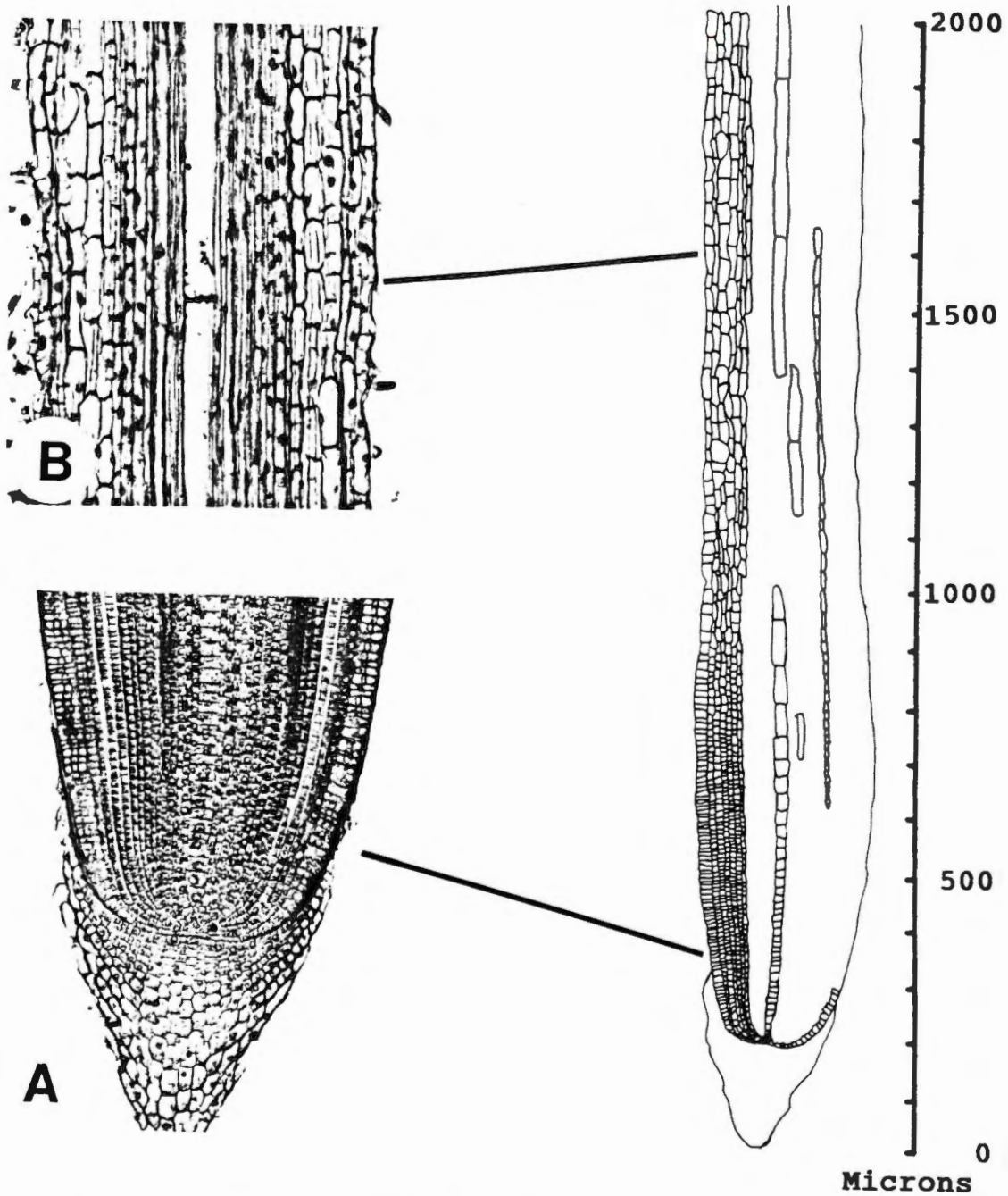

Figure 38. Diagram of a median longitudinal section of the apical 2000 μm of a bromoxynil treated 'Tifgreen' bermudagrass after eight weeks in nutrient culture. Drawing at 80X, photomicrographs A and B at 175X.

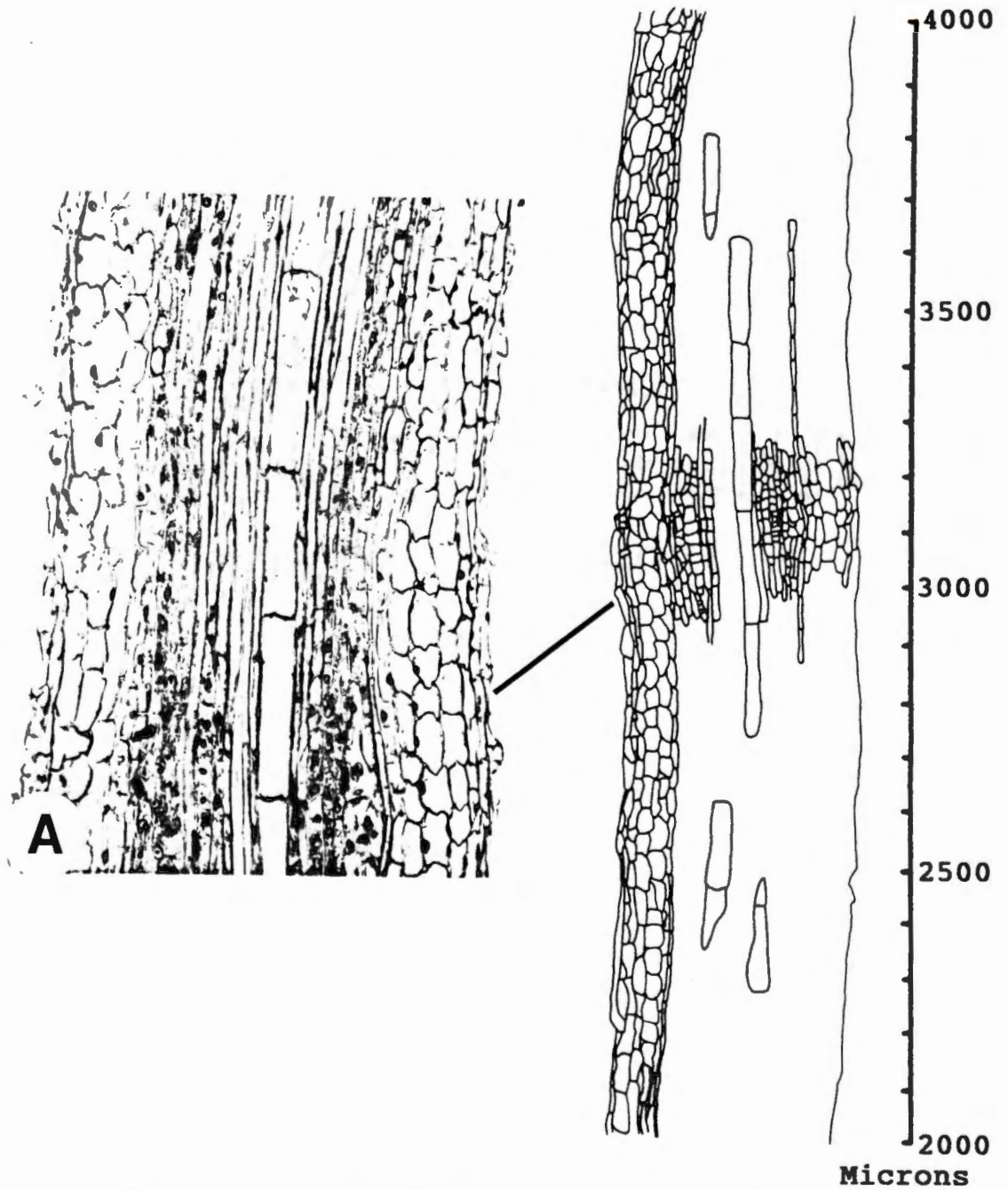

Figure 39. Diagram of an intermittent median longitudinal section of the subapical 2000 μm of a bromoxynil treated 'Tifgreen' bermudagrass after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

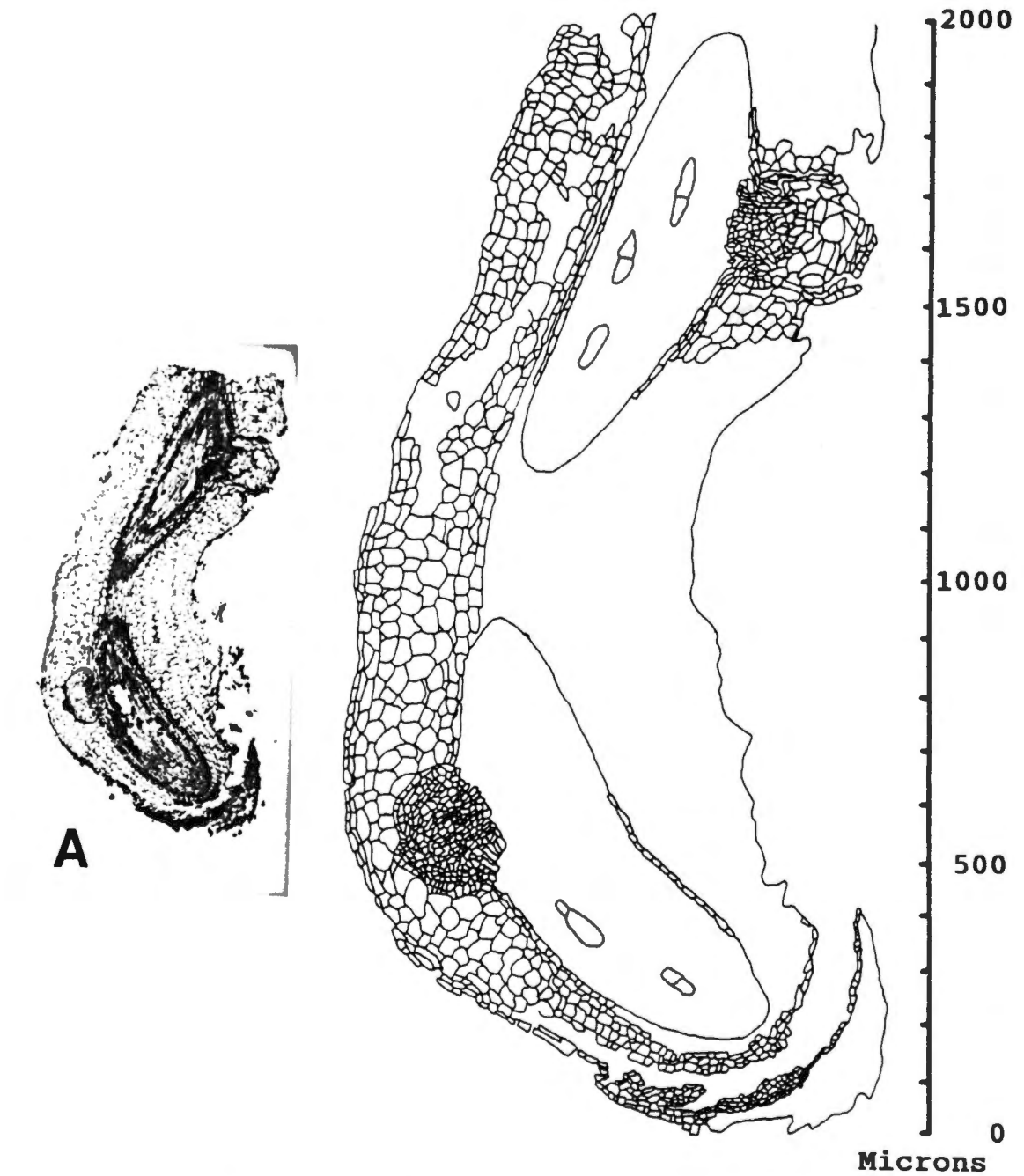

Figure 40. Diagram of an intermittent median longitudinal section of the apical 2000 μm of a pronamide treated 'Tifgreen' bermudagrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 35X.

and severe root curvature made it impossible to cut median longitudinal sections. Roots were abnormally enlarged as compared to untreated check plants. The subapical region continued to show abnormal cell development (Figure 41). Lateral root initiations were prevalent. Cortical cells were enlarged, fragile, and easily shattered during histological processing. Stelar definition improved slightly in this region.

'Tifgreen' bermudagrass plants exposed to four weeks of pronamide died during the four week recovery period and root tissue was too decomposed for histological processing.

Sethoxydim. Sethoxydim-treated roots of bermudagrass exhibited only slight cellular disorders in the apical 2000 μm (Figure 42). Although root cap development appeared normal, early stages of deterioration were evident. Cortical cell columnar arrangement was generally good, but root curvature prevented continual median longitudinal sectioning. An occasional abnormal lateral root initial occurred in this region. The subapical 2000 μm region exhibited weak cell walls and sloughing epidermal cells (Figure 43). Stelar elements were inconsistent and influenced in part by root curvature. Lateral root initials were more prevalent than considered normal (21).

After four weeks of growth in non-herbicide nutrient culture, newly forming cells in root tips showed good recovery from sethoxydim injury (Figure 44). Root caps

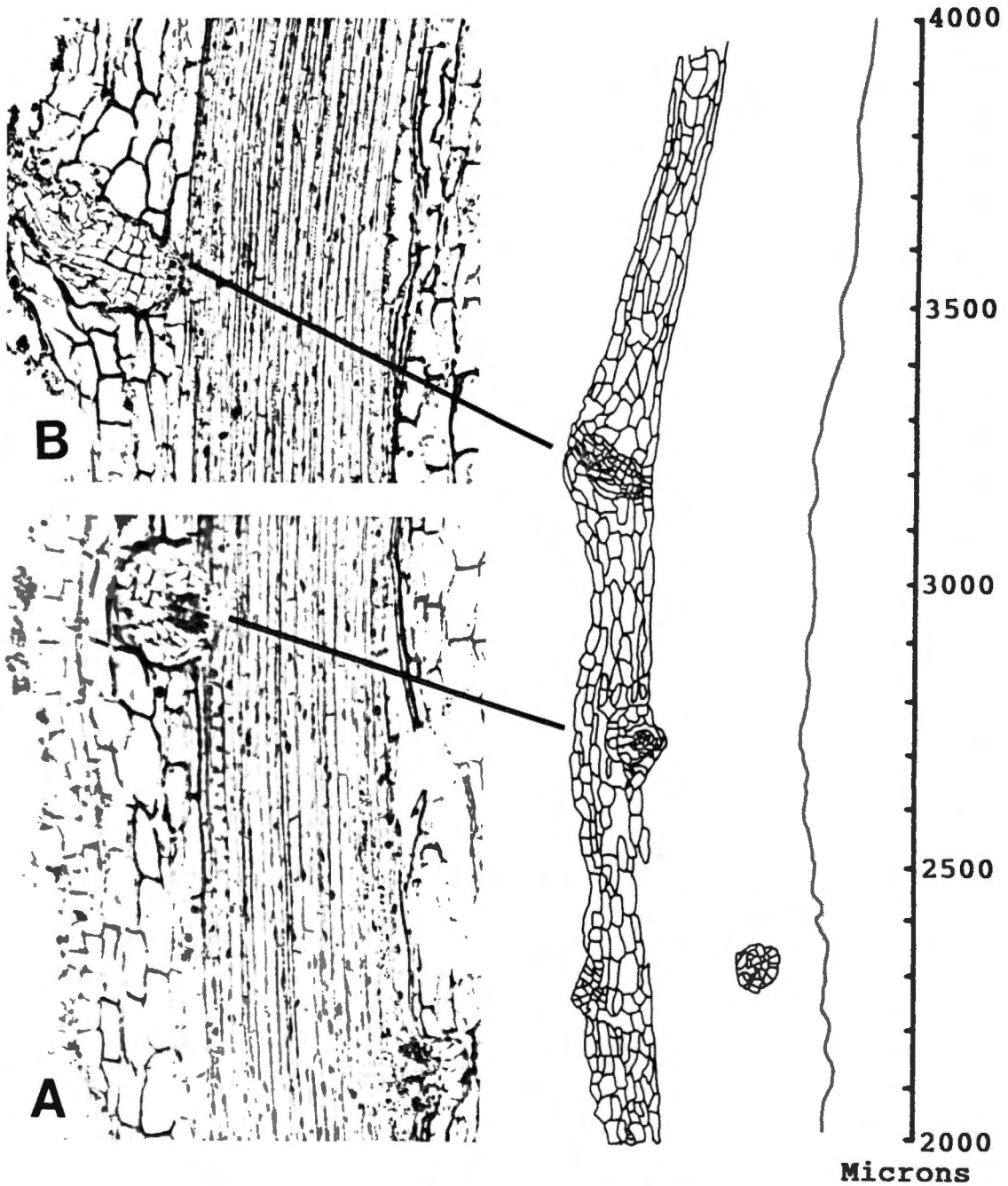

Figure 41. Diagram of a median longitudinal section of the subapical 2000 μm of a pronamide treated 'Tifgreen' bermudagrass root after four weeks in nutrient culture. Drawing at 80X, photomicrographs A and B at 175X.

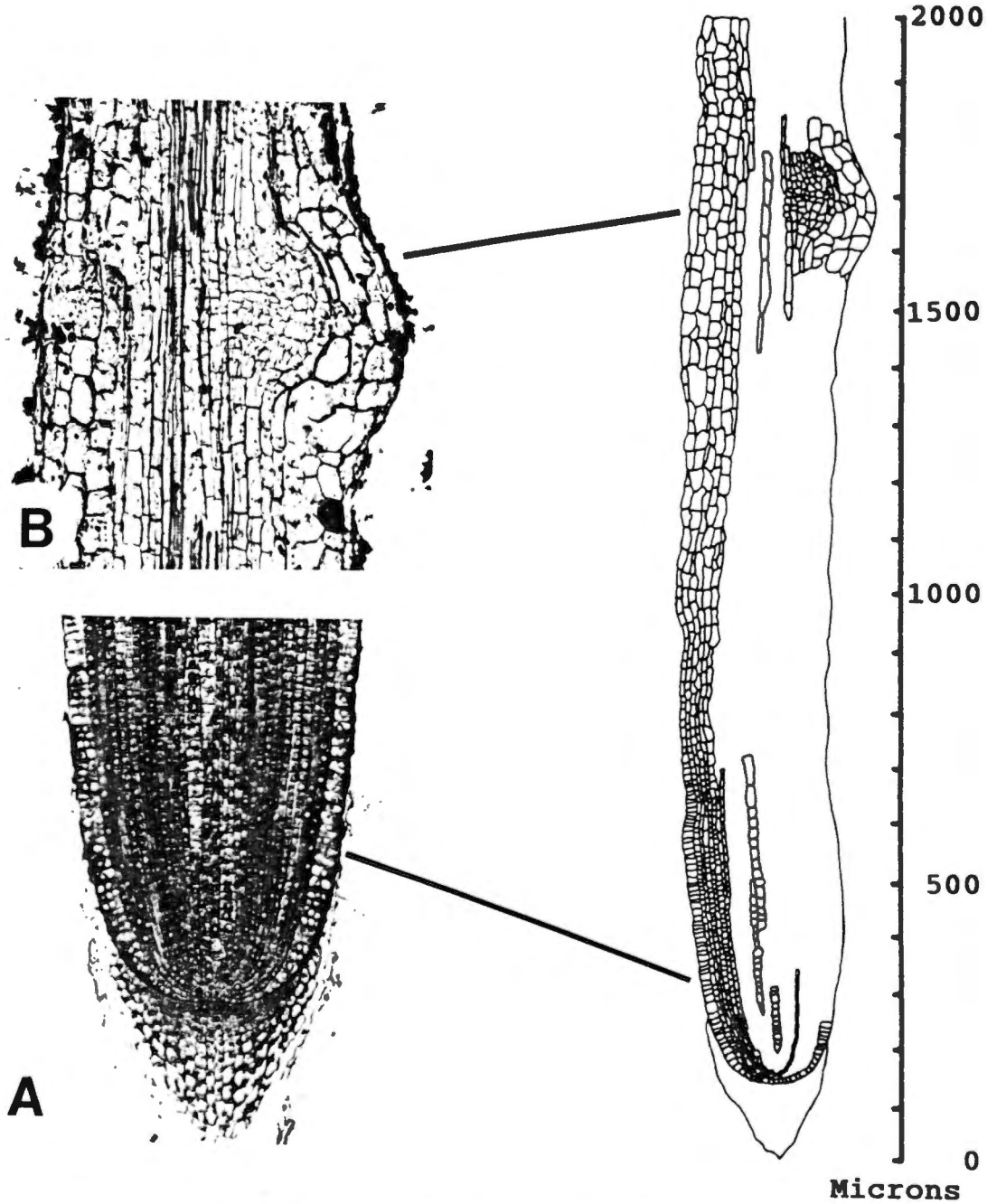

Figure 42. Diagram of a median longitudinal section of the apical 2000 μm of a sethoxydim treated 'Tifgreen' bermudagrass root after four weeks in nutrient culture. Drawing at 80X, photomicrographs A and B at 175X.

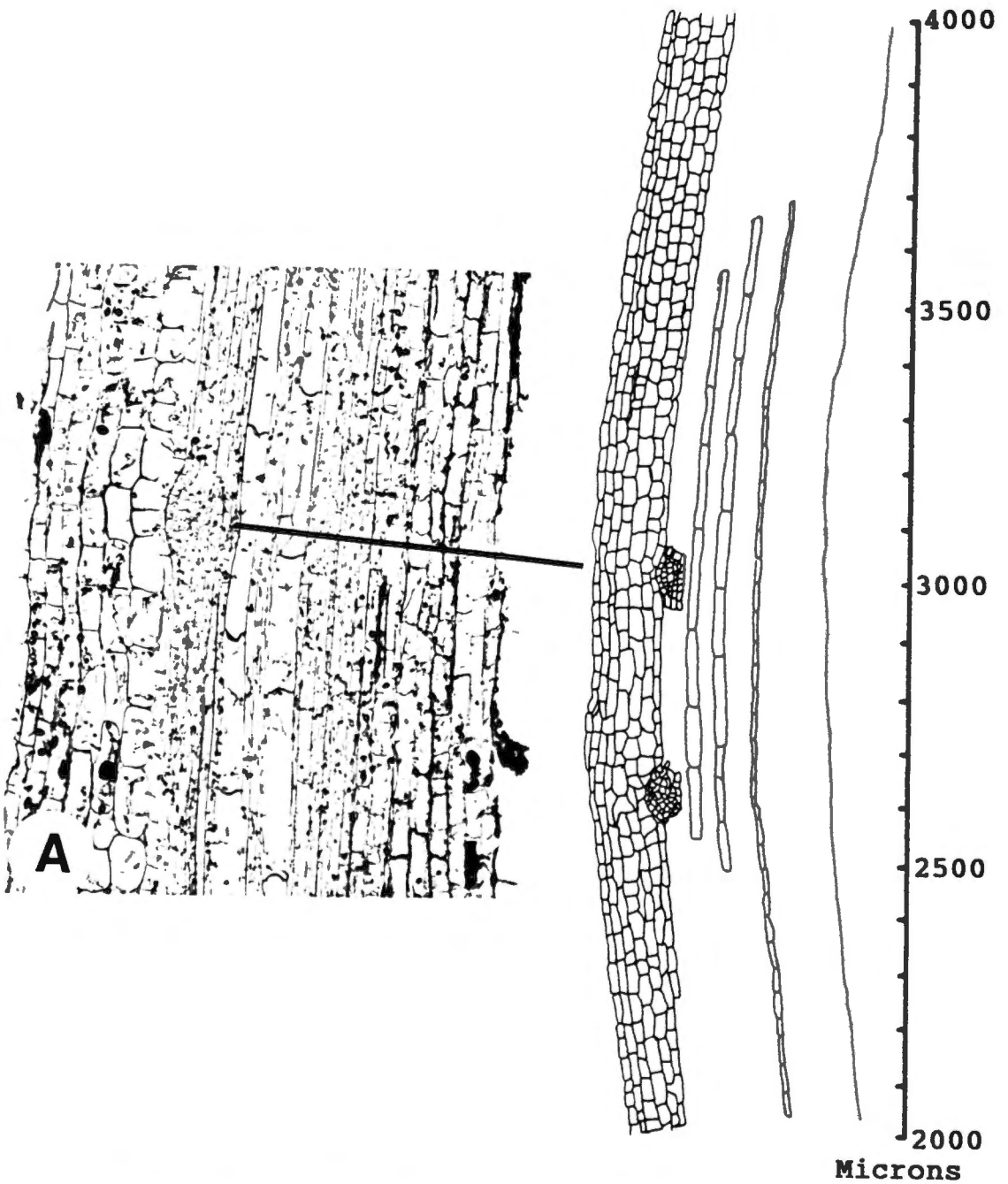

Figure 43. Diagram of an intermittent median longitudinal section of the subapical 2000 μm of a sethoxydim treated 'Tifgreen' bermudagrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

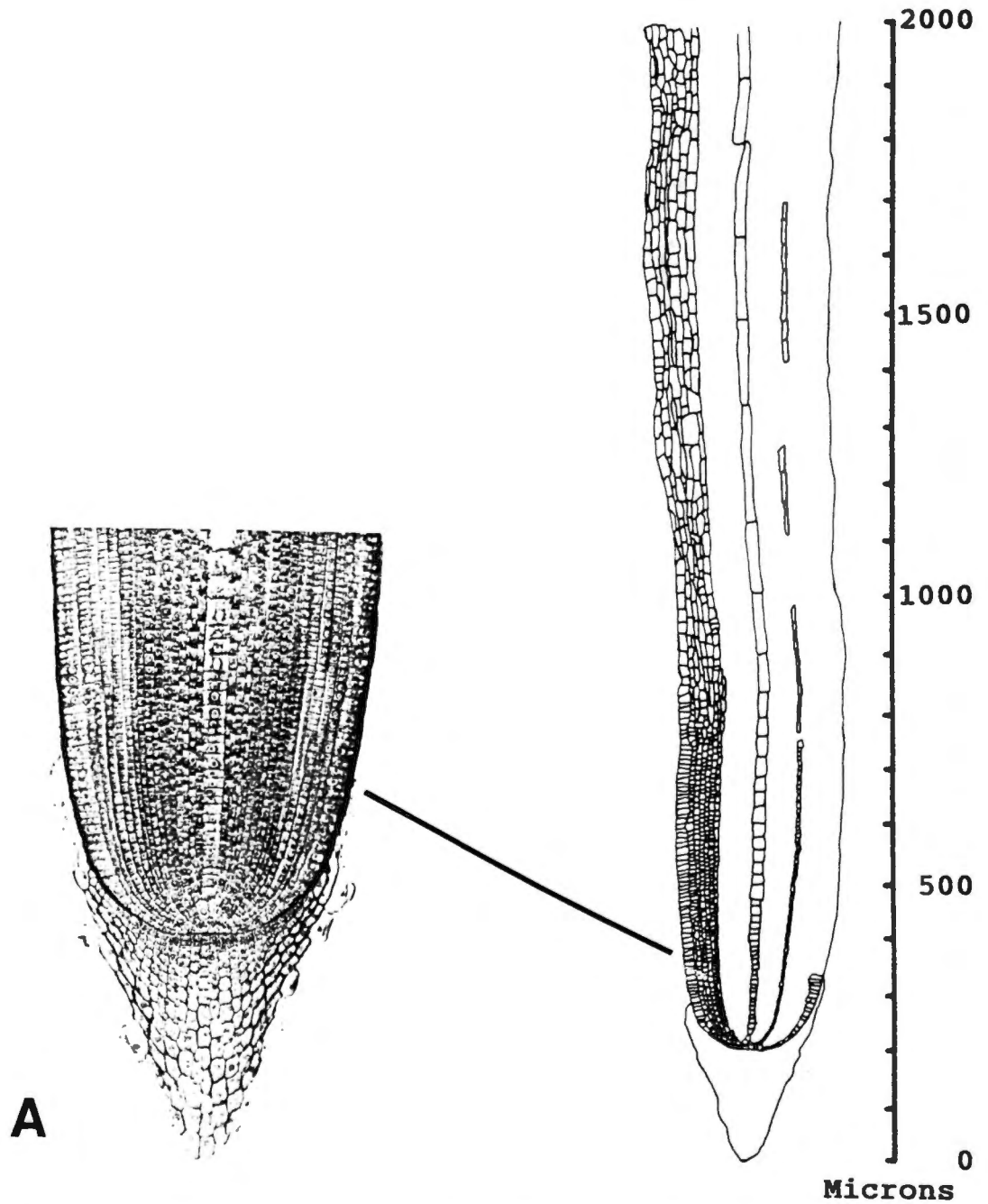

Figure 44. Diagram of a median longitudinal section of the apical 2000 µm of a sethoxydim treated 'Tifgreen' bermudagrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

were well developed, and root cells gained in strength. Stelar definition was good, and roots straightened out in growth. Cortical cells showed good columnar arrangement, and generally good size and shape. Some weakening of cells persisted in subapical sections (Figure 45). Epidermal and outer cortical cells continued susceptibility to shattering during histological processing. Stelar definition continued to be good. Cortical cell size generally appeared good, but arrangement was somewhat inconsistent.

Bentgrass

Untreated check. Untreated root tips of 'Penncross' creeping bentgrass exhibited good cortical cell columnar arrangement after four weeks in nutrient culture (Figure 46). Root caps were large and healthy in appearance and meristematic cells typically rounded. Cortical cells evolved to a normal square shape and then rectangular shaped further back from the tip. Stelar cells were normally arranged and well defined, and the root tips were straight allowing for a continuous median longitudinal section. The subapical 2000 μm exhibited cortical cells characteristically elongated (Figure 47). Stelar cell elements appeared good. Bentgrass roots were generally thinner than bermudagrass roots.

Apical and subapical sections of untreated 'Penncross' creeping bentgrass roots showed no changes in cellular

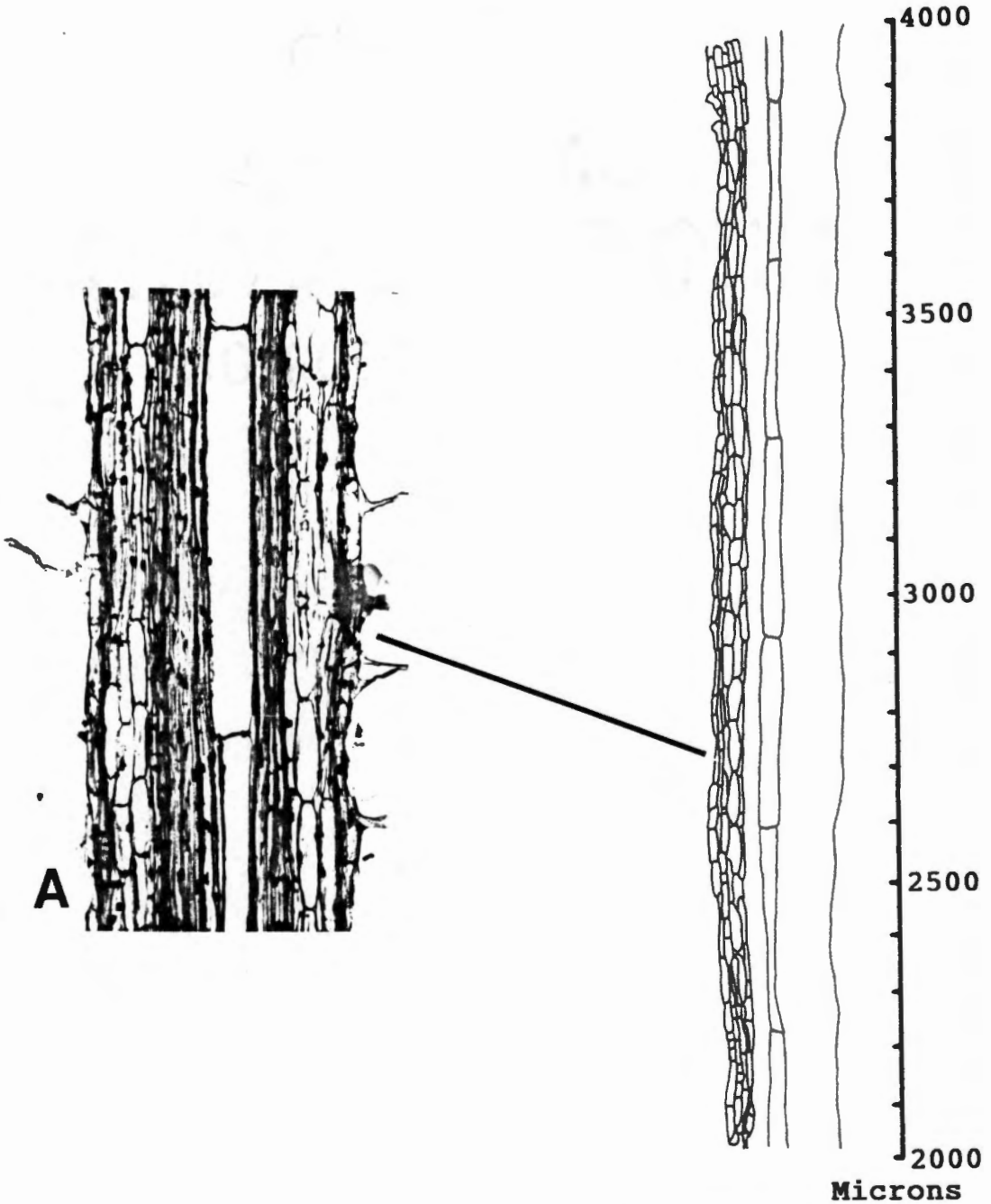

Figure 45. Diagram of a median longitudinal section of the subapical 2000 μm of a sethoxydim treated 'Tifgreen' bermudagrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

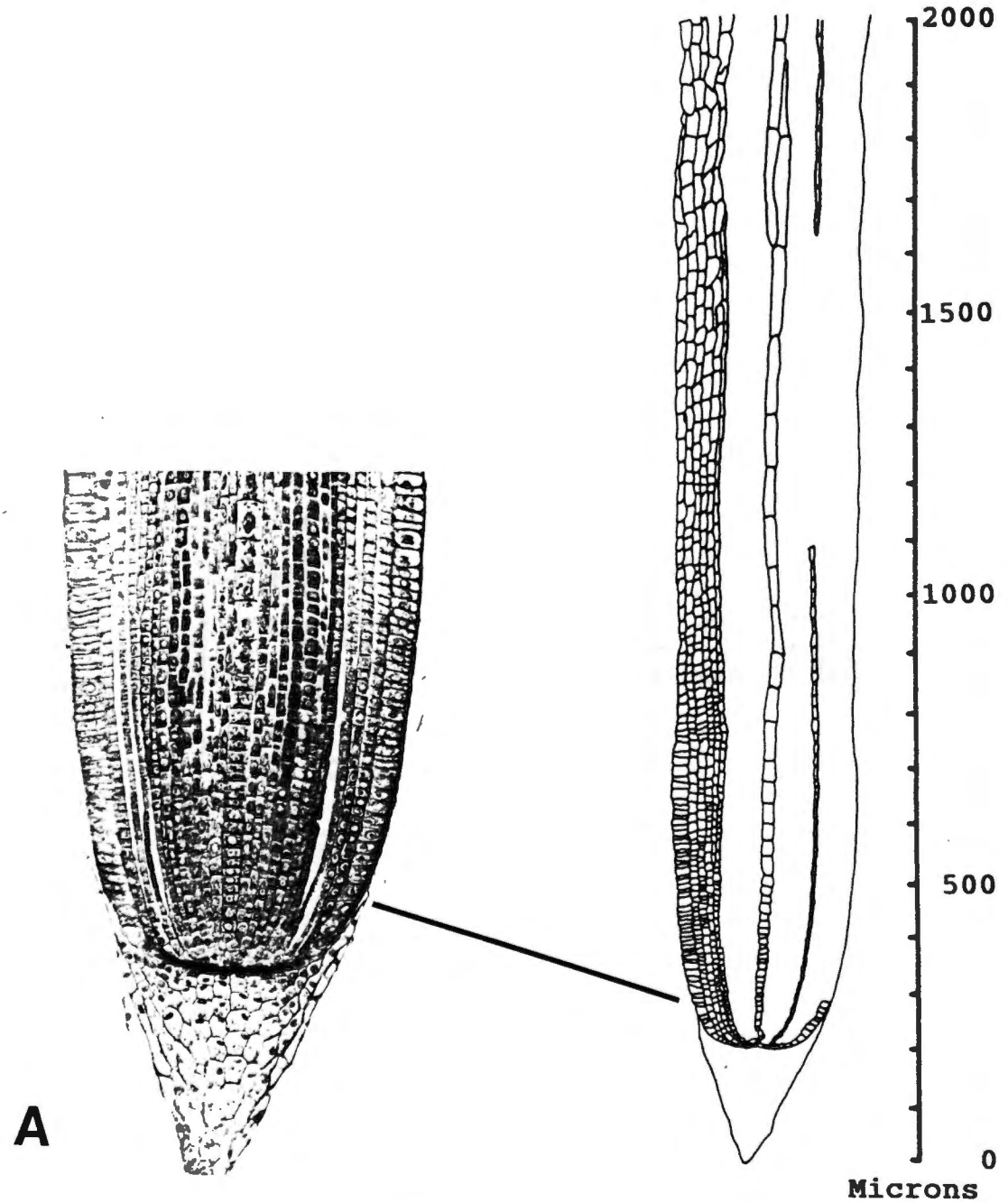

Figure 46. Diagram of a median longitudinal section of the apical 2000 μm of an untreated check of a 'Penncross' bentgrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

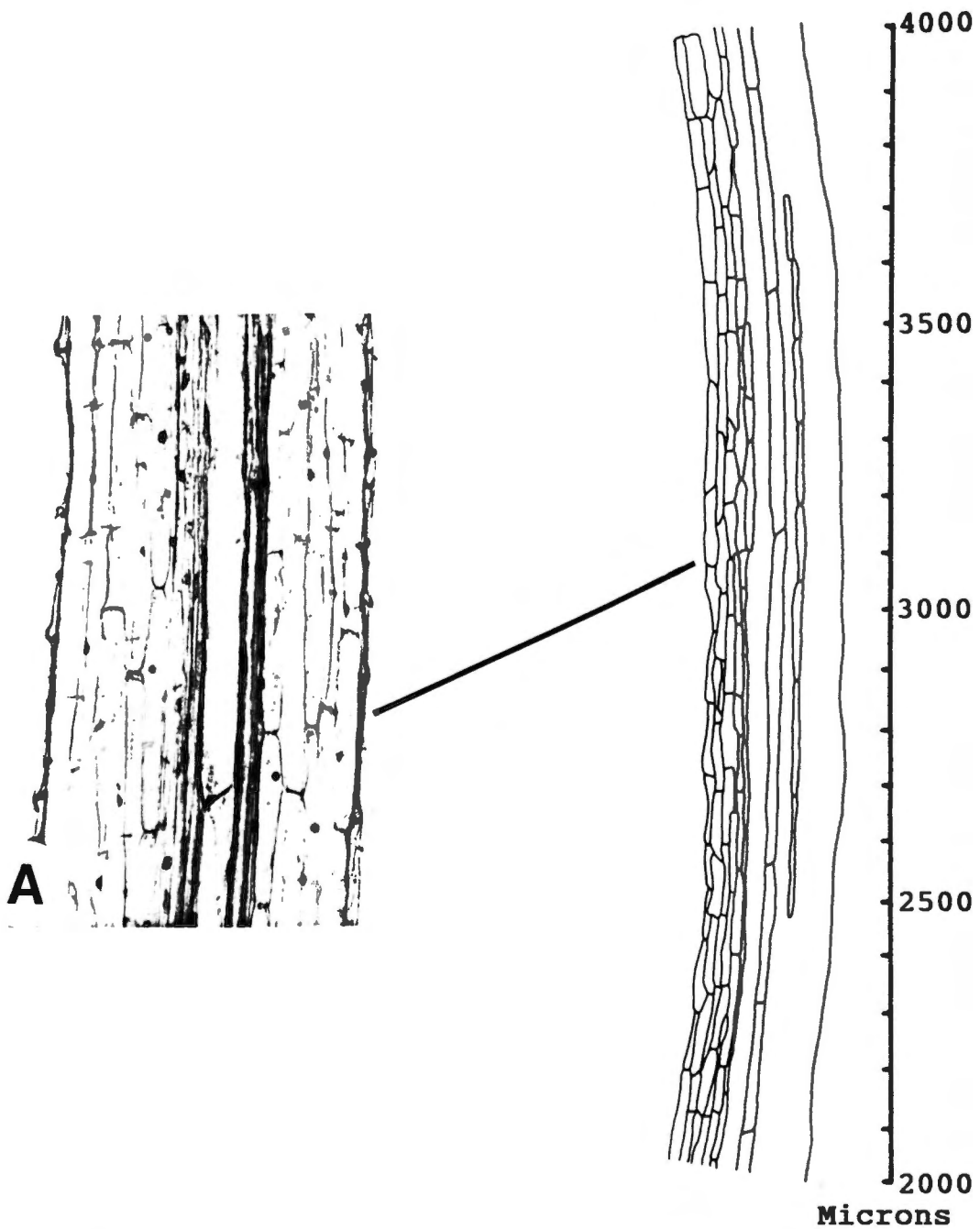

Figure 47. Diagram of a median longitudinal section of the subapical 2000 μm of an untreated check of a 'Penncross' bentgrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

formation or arrangement after eight weeks of growth in nutrient culture (Figures 48 and 49).

Asulam. Asulam resulted in severe abnormalities in root apices of bentgrass during the four week treatment period (Figure 50). Root cells steadily degenerated as senescence progressed. Cells in the meristematic zone and root cap were very fragile. Cortical cells were enlarged throughout this region but particularly near the root tip. Mass proliferation of lateral root initials occurred throughout the tip 2000 μm. Stelar definition was poor. Epidermal cells easily sloughed-off. Root tissue was very difficult to process histologically. The subapical root section also showed enlarged cortical cells, multiple lateral root initials, and poor stelar element definition (Figure 51). Epidermal and outer cortical cells easily sloughed off.

Soon after the non-herbicide recovery period began, senescence terminated. No new roots developed as the shoot and root system died.

Bentazon. Bentgrass apical and subapical root segments following four weeks of exposure to bentazon exhibited very little cell injury (Figures 52 and 53). Cell structure and arrangement was generally good with only epidermal and outer cortical cells appearing weak (Figure 53).

After four weeks of non-herbicide exposure in nutrient culture, apical cells continued to exhibit good structure

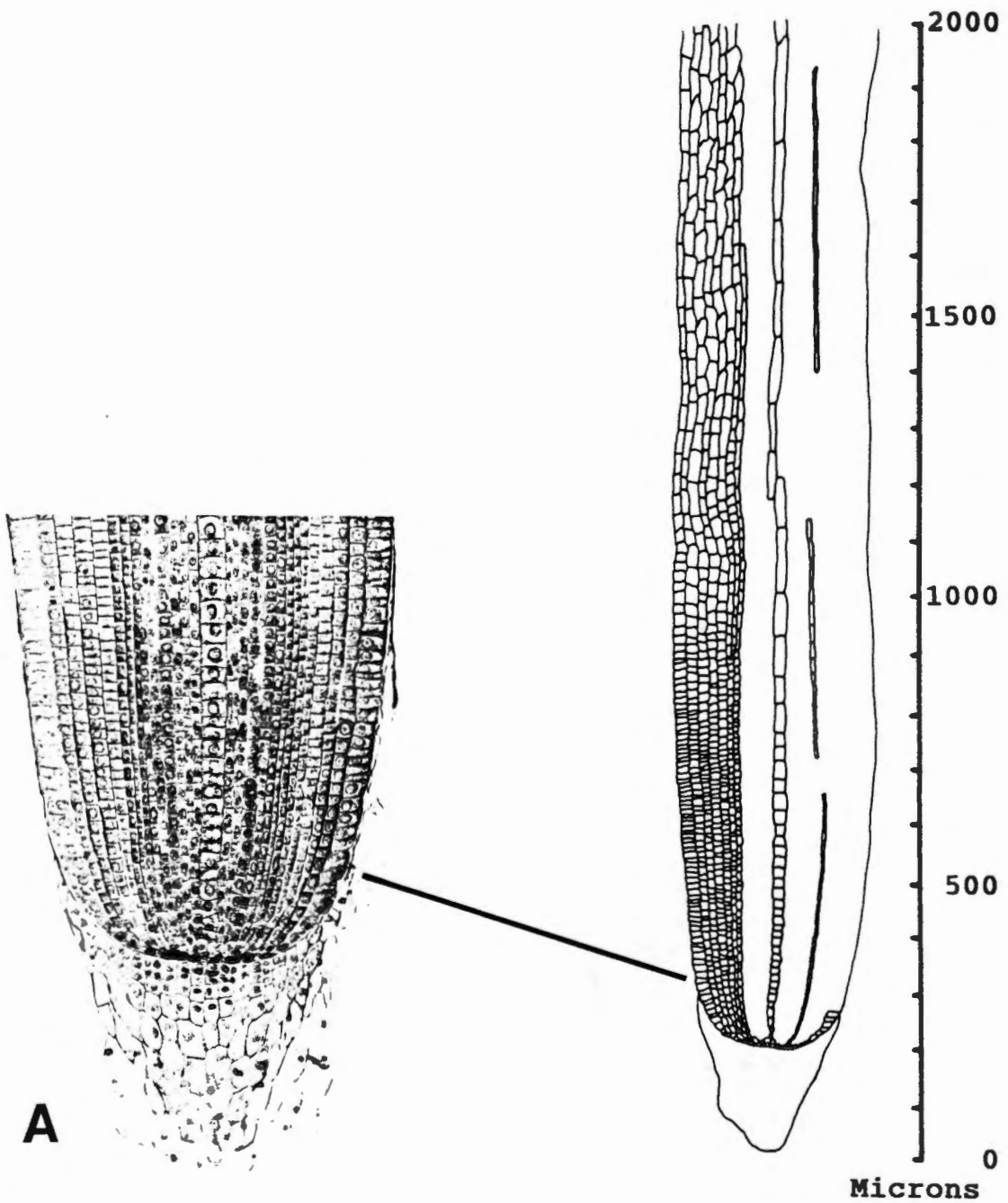

Figure 48. Diagram of a median longitudinal section of the apical 2000 µm of an untreated check of a 'Penncross' bentgrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

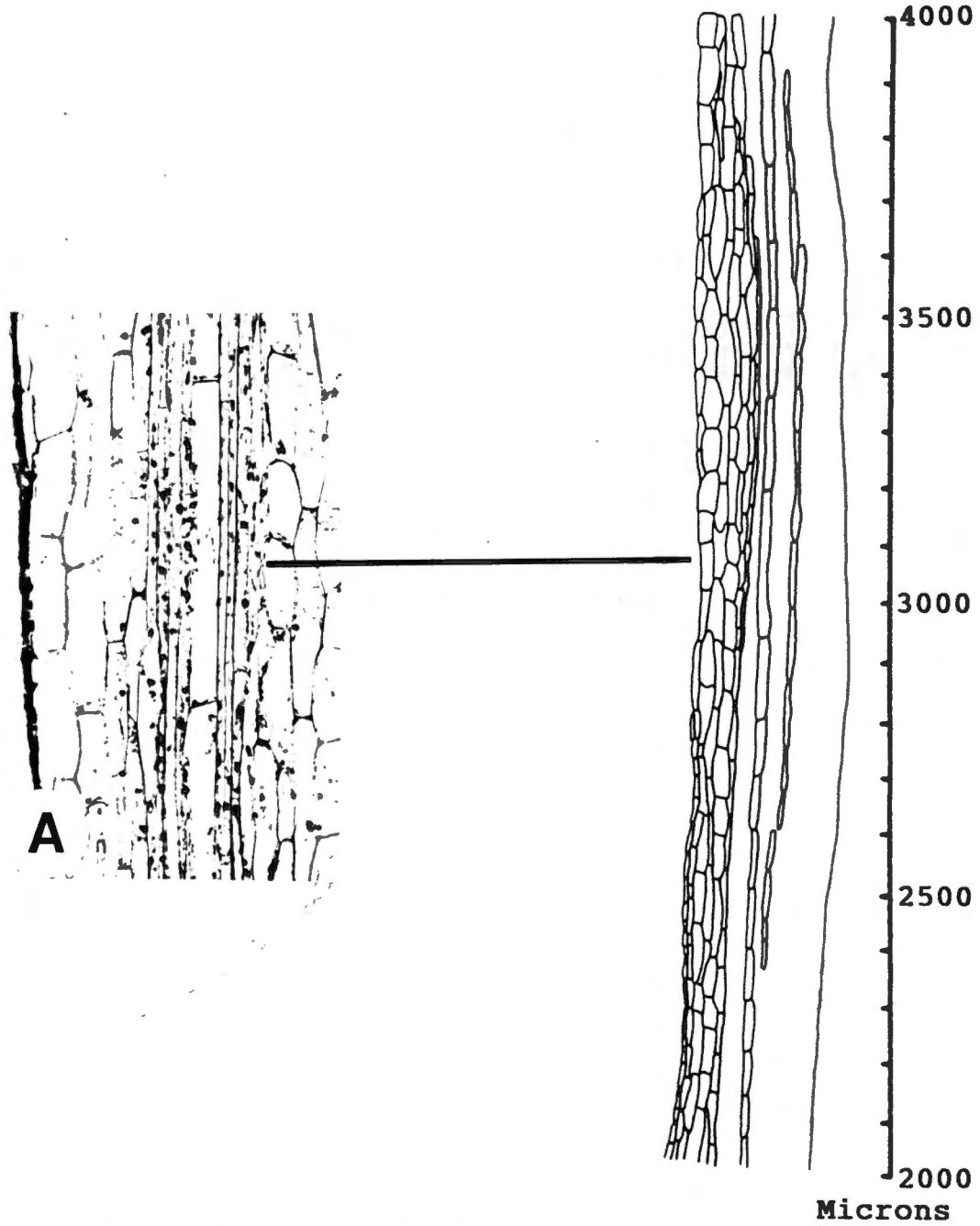

Figure 49. Diagram of a median longitudinal section of the subapical 2000 μm of an untreated check of a 'Penncross' bentgrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

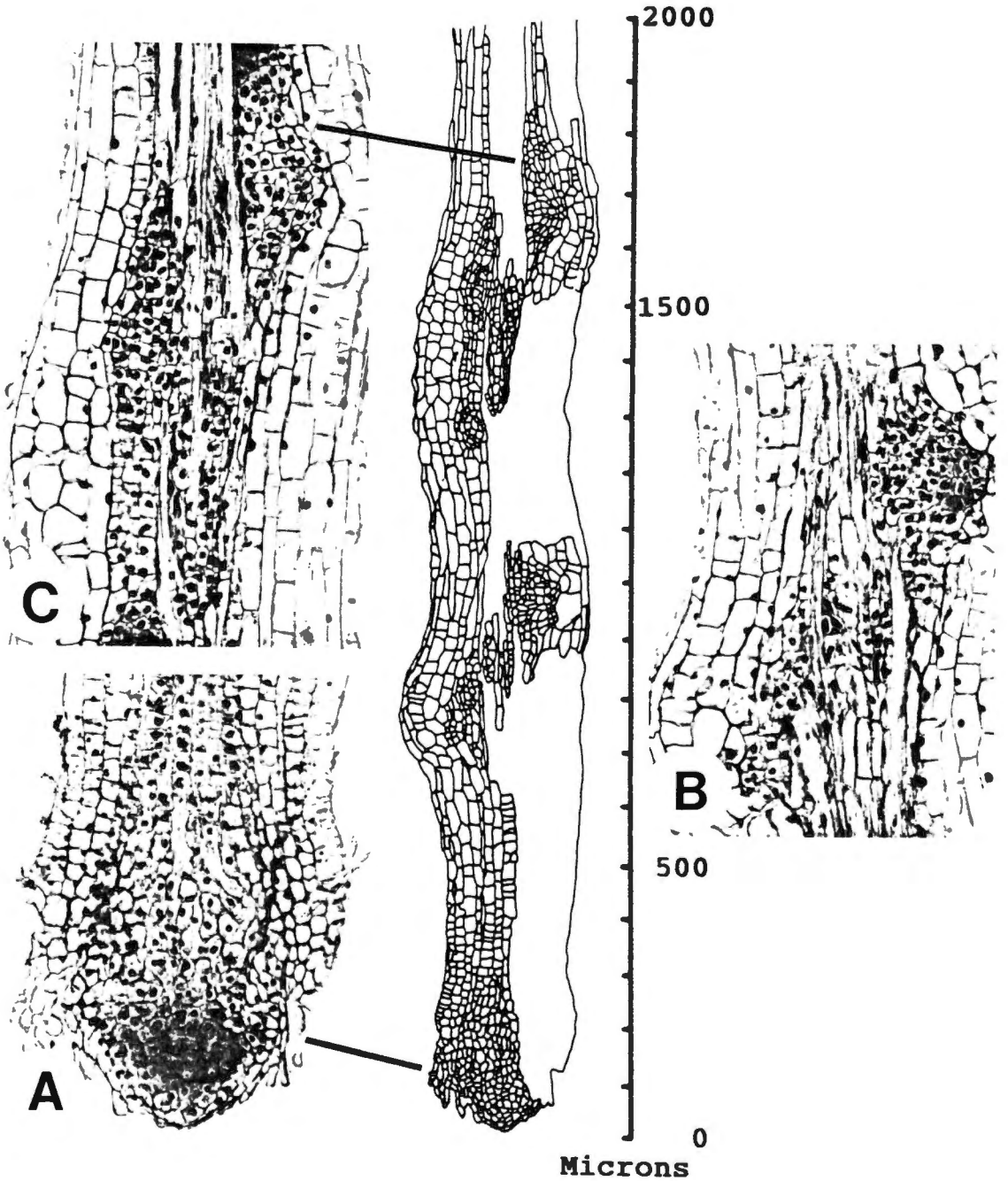

Figure 50. Diagram of an intermittent median longitudinal section of the apical 2000 µm of an asulam treated 'Penncross' bentgrass root after four weeks in nutrient culture. Drawing at 80X, photomicrographs A, B, and C at 175X.

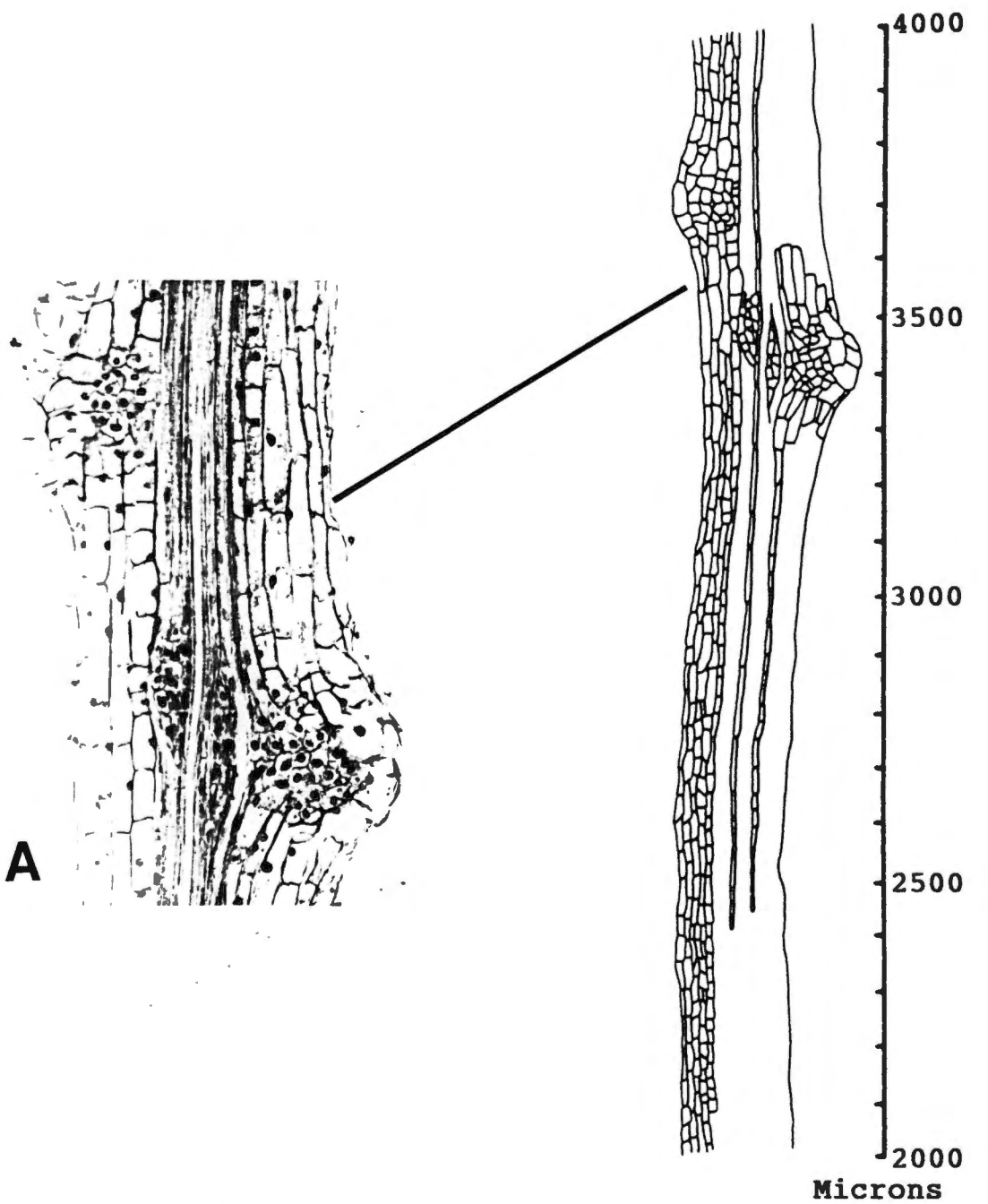

Figure 51. Diagram of a median longitudinal section of the subapical 2000 µm of an asulam treated 'Penncross' bentgrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

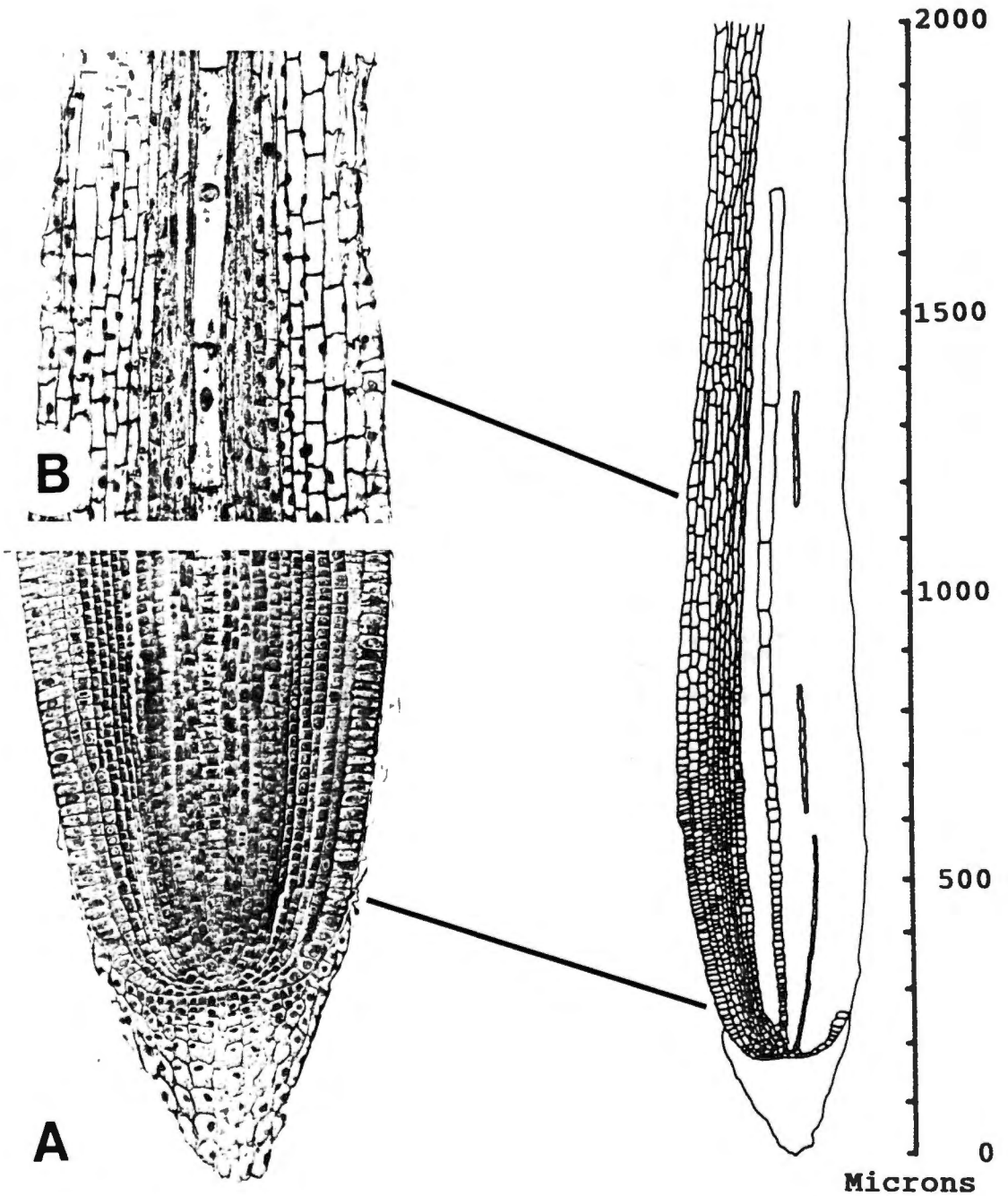

Figure 52. Diagram of a median longitudinal section of the apical 2000 μm of a bentazon treated 'Penncross' bentgrass root after four weeks in nutrient culture. Drawing at 80X, photomicrographs A and B at 175X.

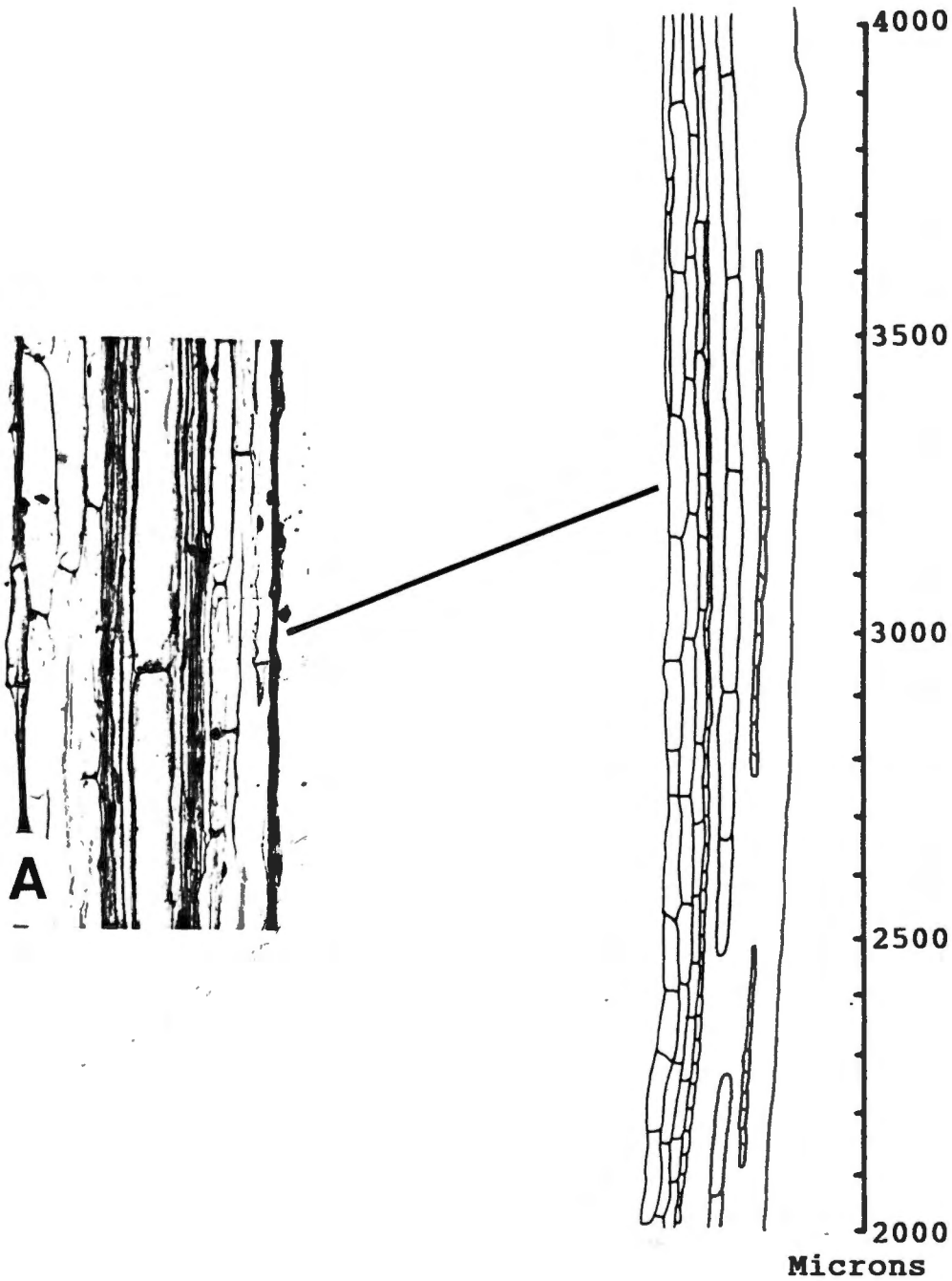

Figure 53. Diagram of a median longitudinal section of the subapical 2000 μm of a bentazon treated 'Penncross' bentgrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

and form (Figure 54). However, subapical sections exhibited numerous abnormal lateral root formations (Figure 55). Some cortical cells adjacent to lateral roots were enlarged. Epidermal and cortical cells tended to be fragile. The undulating curvature of the roots prevented continuous median longitudinal sectioning.

Bromoxynil. After four weeks of bromoxynil exposure, cellular shape, size and structure of the apical 2000 μm of bentgrass roots generally appeared good (Figure 56). Epidermal and outer cortical cells did appear weak. In the subapical region several lateral roots initiated which were abnormal for this region (Figure 57). Stelar cellular elements were not well defined. Cortical cells appeared enlarged next to lateral root initials. Roots also exhibited curvature.

Bentgrass root apices following four weeks of growth in herbicide-free nutrient culture appeared generally good (Figure 58). Root caps were well developed and stelar elements close to the tip were well defined. Cortical cells appeared normal in size and reasonably well arranged. Compressed epidermal cells were in evidence near the root tip, and some root curvature persisted. Subapical sections continued to exhibit several abnormal lateral root proliferations (Figure 59). Weakened epidermal and outer cortical cells easily shattered during histological processing.

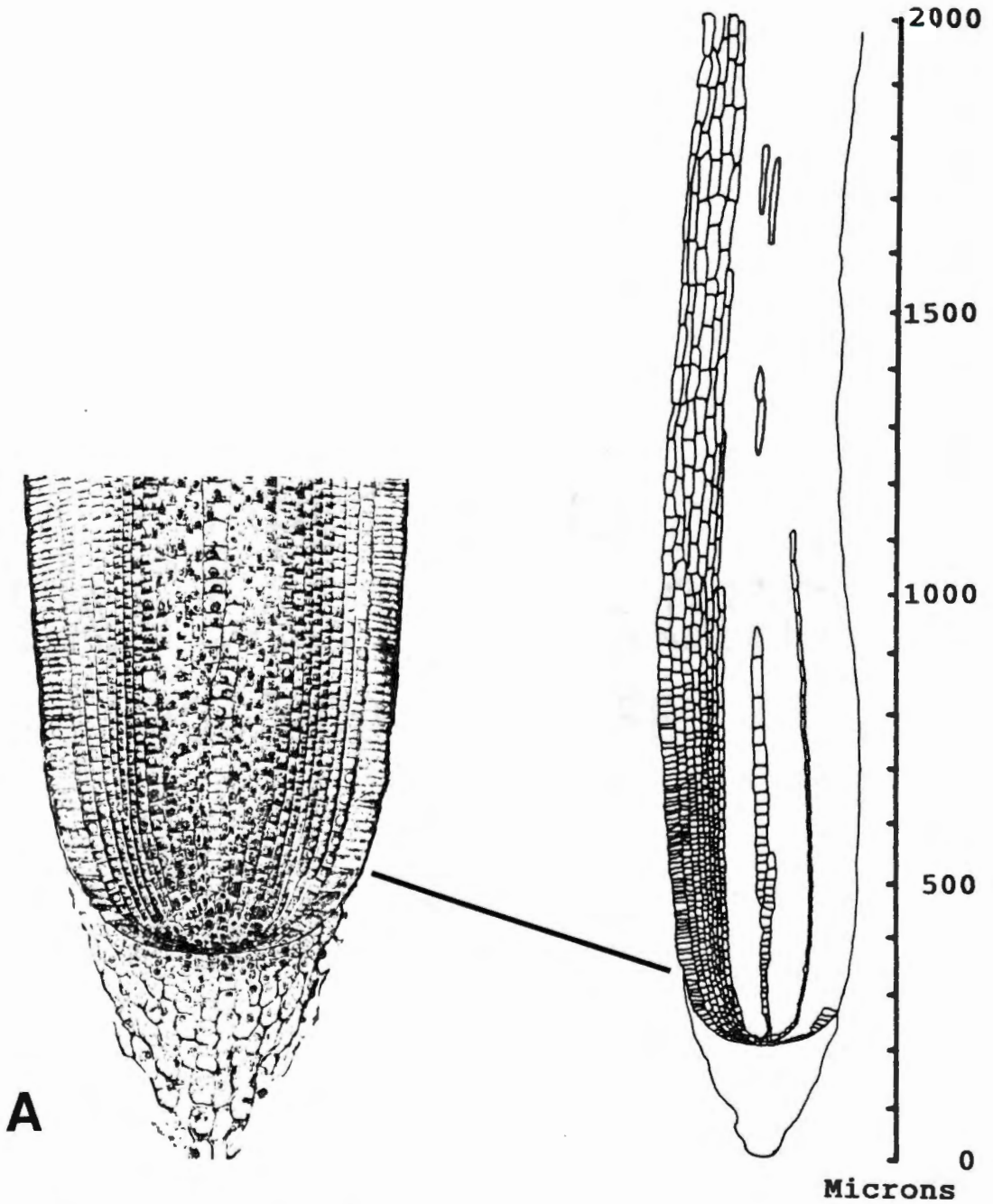

Figure 54. Diagram of an intermittent median longitudinal section of the apical 2000 μm of a bentazon treated 'Penncross' bentgrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

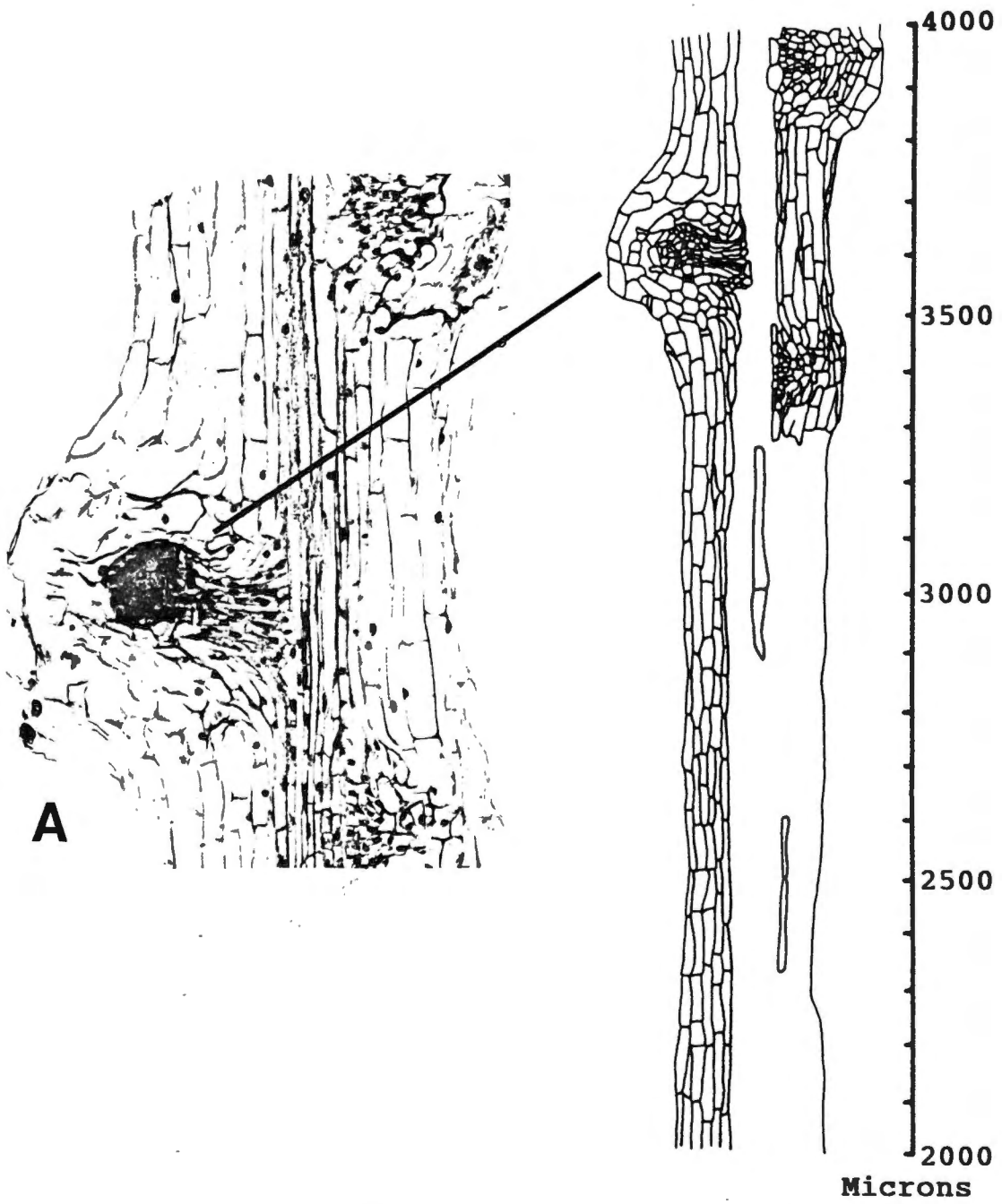

Figure 55. Diagram of an intermittent median longitudinal section of the subapical 2000 µm of a bentazon treated 'Penncross' bentgrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

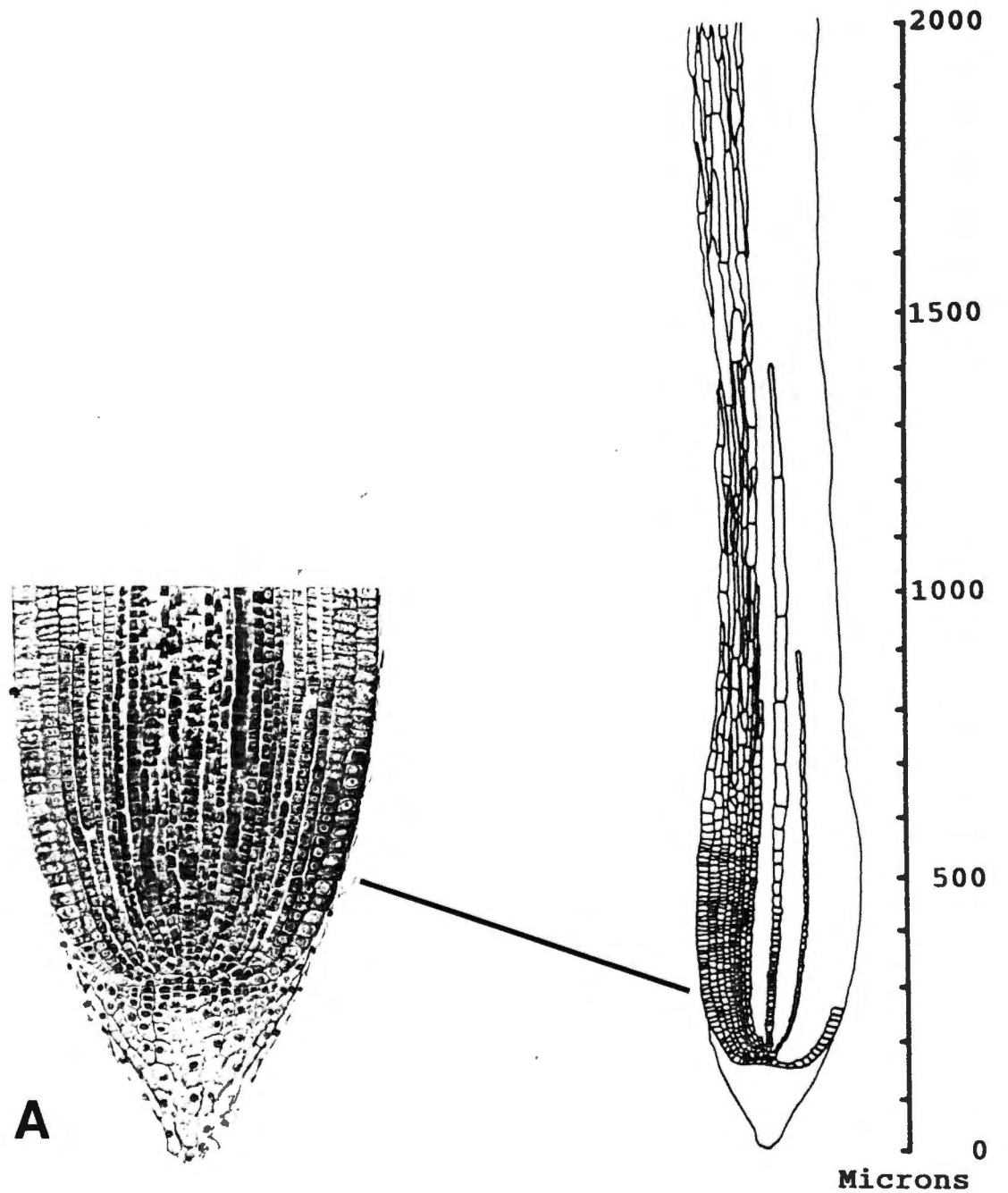

Figure 56. Diagram of an intermittent median longitudinal section of the apical 2000 µm of a bromoxynil treated 'Penncross' bentgrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

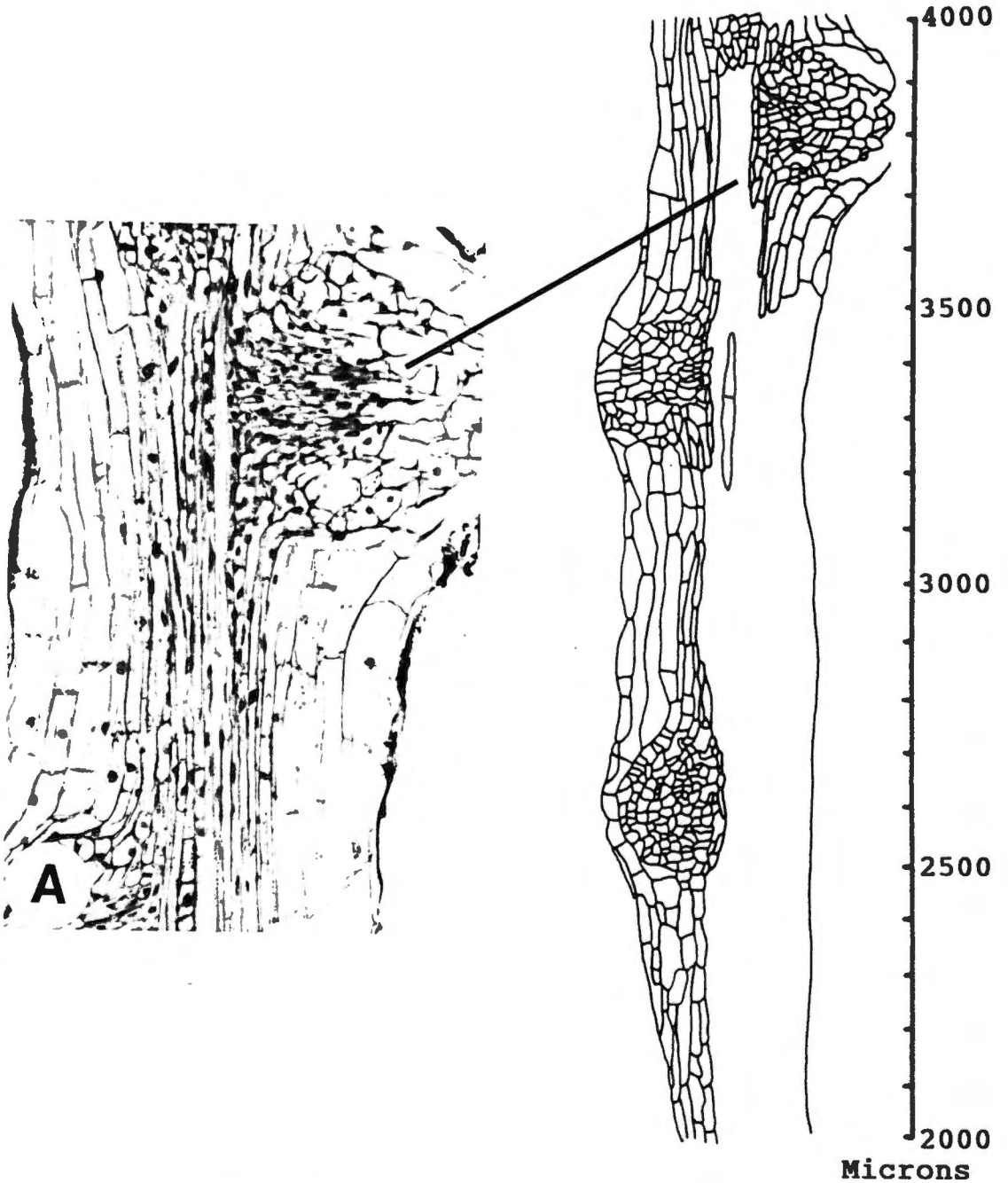

Figure 57. Diagram of an intermittent median longitudinal section of the subapical 2000 µm of a bromoxynil treated 'Penncross' bentgrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

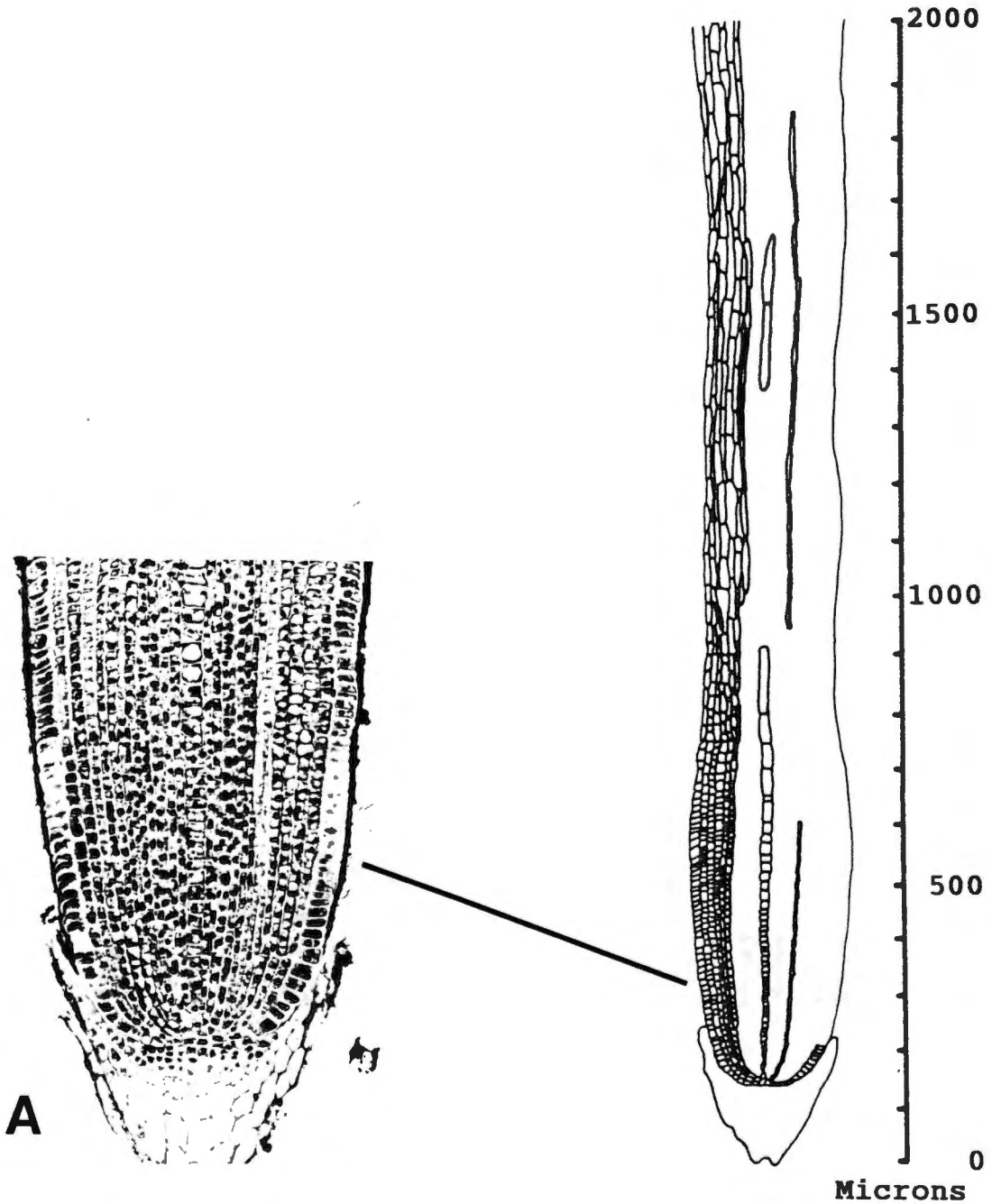

Figure 58. Diagram of an intermittent median longitudinal section of the apical 2000 μm of a bromoxynil treated 'Penncross' bentgrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

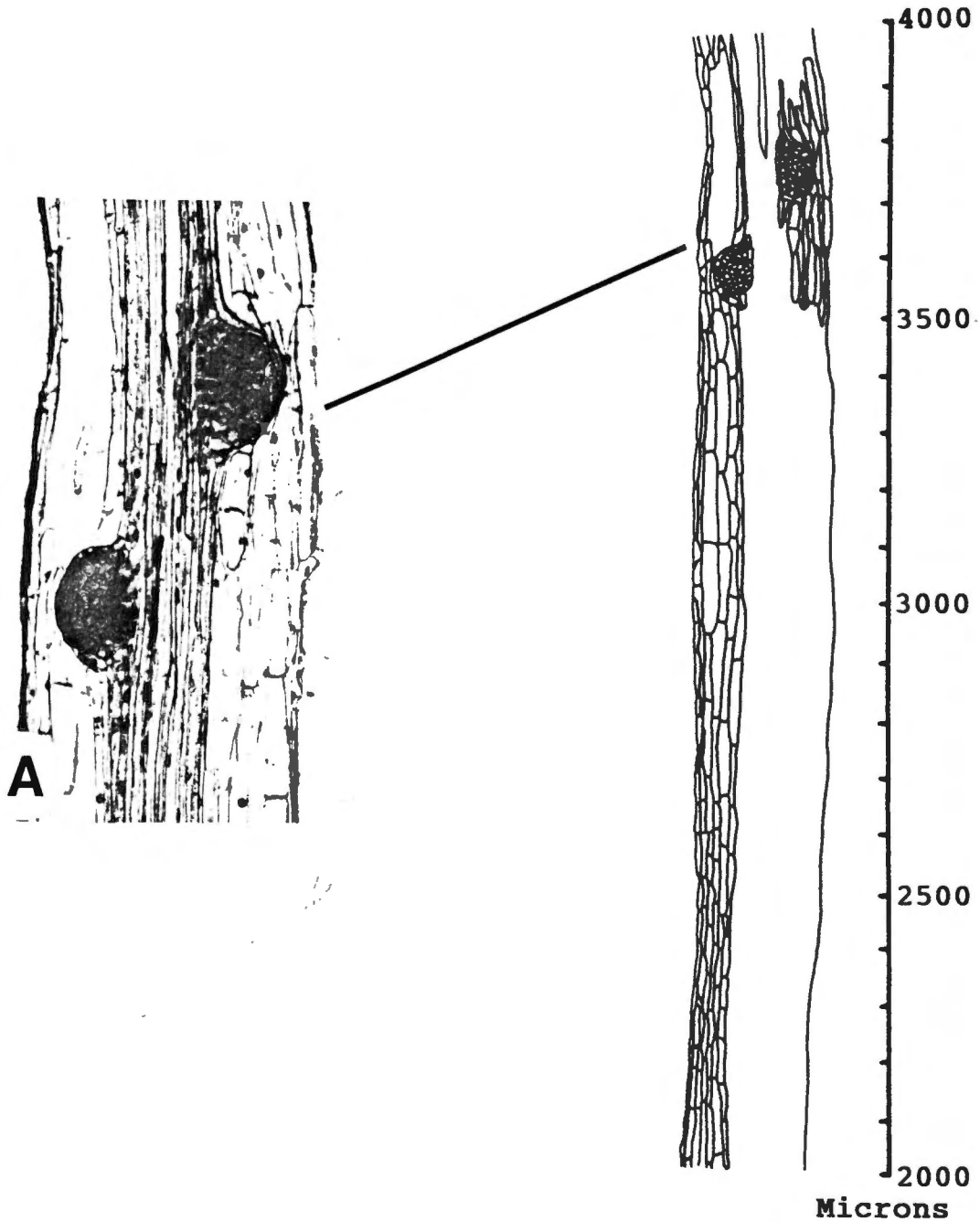

Figure 59. Diagram of an intermittent median longitudinal section of the subapical 2000 μm of a bromoxynil treated 'Penncross' bentgrass root after eight weeks in nutrient culture. Drawing at 80X, photomicrograph A at 175X.

Pronamide. Pronamide treatments killed plants quickly during the herbicide exposure period. During the short time shoots remained alive, no new root tissue was formed.

Sethoxydim. Very little bentgrass root tissue was available for histological processing after four weeks of exposure to sethoxydim. Apical tissue showed severe cellular enlargements and late stage senescence (Figure 60). Root cap cells had already decayed and cell formation had ceased. Stelar element definition was inconsistent due to severe root curvature. Enlarged cortical cells were readily visible in the first 1000 μm. Most epidermal cells and some outer cortical cells had already sloughed off. Tissue collected in the subapical 2000 μm apparently had developed before the full effects of the herbicide had manifested (Figure 61). Cells were weak, very fragile, and some had already sloughed off. Several abnormal lateral root initials had already occurred. Hypertrophic cortical cells occurred extensively.

Bentgrass shoots and roots died during the sethoxydim four week exposure period. No recovery period tissue was available for histological processing.

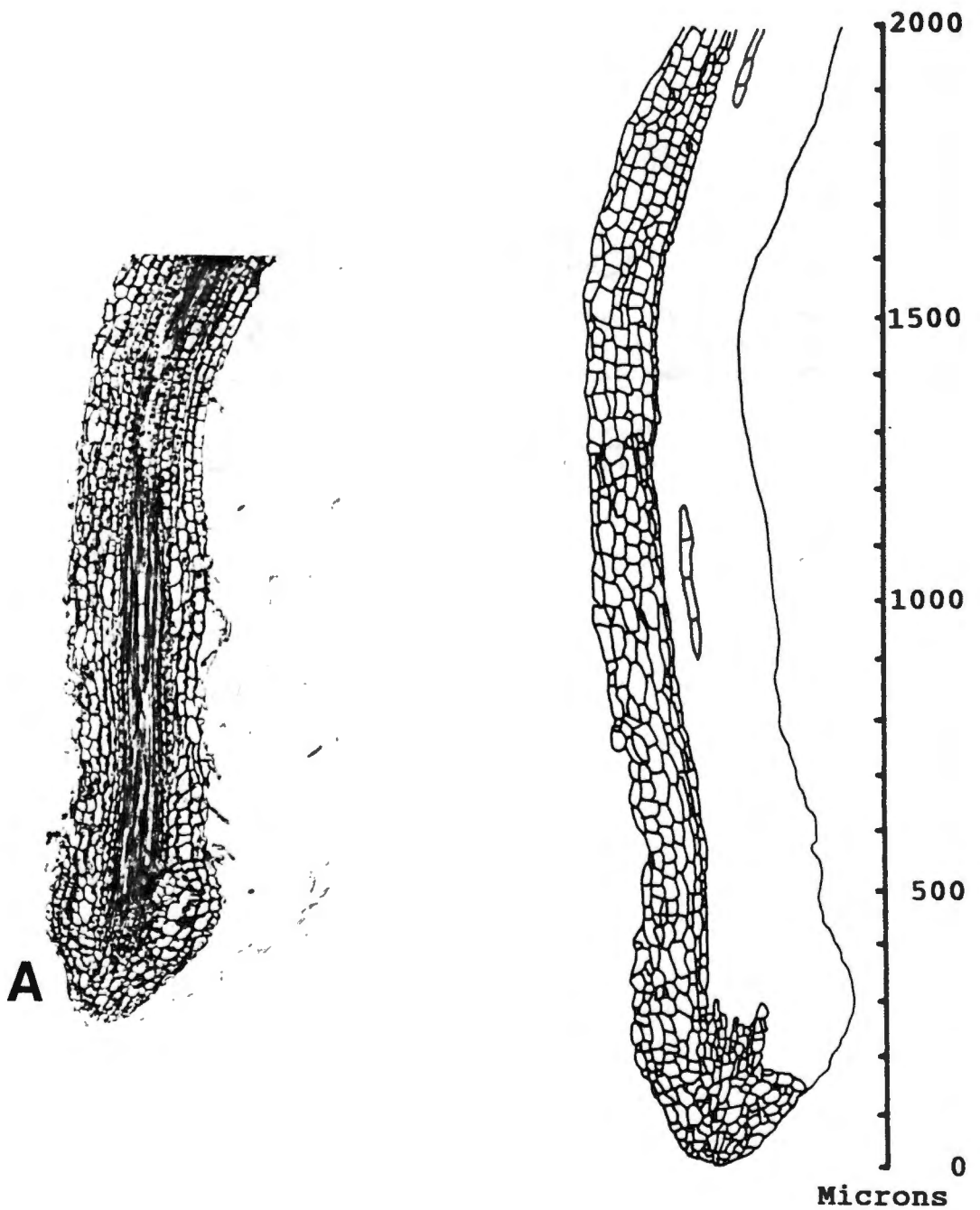

Figure 60. Diagram of an intermittent median longitudinal section of the apical 2000 μm of a sethoxydim treated 'Penncross' bentgrass root after four weeks in nutrient culture. Drawing at 80X, photomicrograph A at 70X.

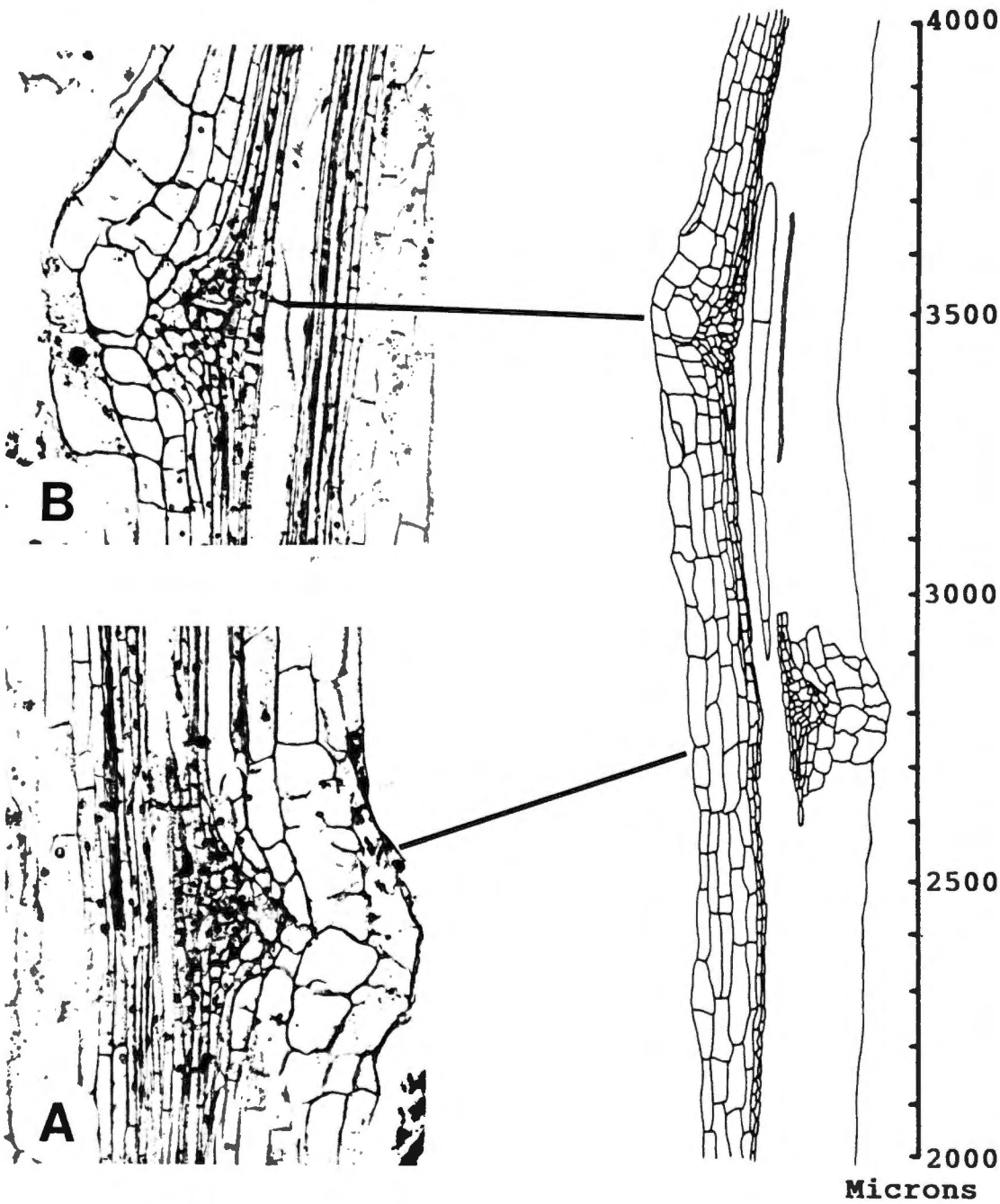

Figure 61. Diagram of an intermittent median longitudinal section of the subapical 2000 μm of a sethoxydim treated 'Penncross' bentgrass root after four weeks in nutrient culture. Drawing at 80X, photomicrographs A and B at 175 X.

CHAPTER V

SUMMARY

The effects of selected postemergence herbicides on root meristem cells, shoot growth, and root growth of 'Tifgreen' bermudagrass and 'Penncross' creeping bentgrass were investigated. The recuperative capacity of the two cultivars was also evaluated.

A. BERMUDAGRASS

Bentazon and bromoxynil treatments resulted in only slight reductions in shoot and root growth of 'Tifgreen' bermudagrass. Asulam and sethoxydim severely reduced shoot and root growth. Plants exposed to pronamide died shortly after the herbicide exposure test period.

Abnormal lateral root initials occurred within the apical 2000 μm sections of roots treated with asulam, bentazon, pronamide, and sethoxydim. Columnar arrangement of cortical cells was disrupted by asulam, bentazon, and pronamide, and hypertrophic cortical cells resulted following treatments with asulam, bentazon, bromoxynil, and pronamide. Pronamide, asulam, and sethoxydim appeared to result in a general weakening of cell walls.

B. BENTGRASS

Bentazon and bromoxynil treatments resulted in slight reductions of shoot and root growth of 'Penncross' creeping bentgrass plants. However, treatments with asulam, pronamide, and sethoxydim killed all test plants.

Extensive lateral root abnormalities occurred in the apical and subapical 2000 μm sections exposed to asulam. Abnormal lateral root initiations were also noted in the subapical sections of roots treated with bentazon and bromoxynil. Hypertrophic cortical cells wre observed in roots exposed to asulam, bentazon, bromoxynil, and sethoxydim. Weakening of epidermal and outer cortical cells resulted from bentazon and bromoxynil treatments. Root tissue exposed to sethoxydim was very fragile. No root tissue was available for histological processing from pronamide treatments.

CHAPTER IV

CONCLUSIONS

Results of this study showed that of the herbicides tested, bentazon and bromoxynil resulted in slight injury to shoots and to root meristems of 'Tifgreen' bermudagrass. Asulam and sethoxydim severely reduced shoot growth and resulted in severe injury to root meristems. Plants treated with pronamide died shortly after treatment.

Bentazon and bromoxynil resulted in only slight injury to shoots and root meristems of 'Penncross' creeping bentgrass. Plants treated with asulam, pronamide, and sethoxydim died soon after treatment.

Additional research is needed to determine cellular response of these chemicals following treatment of only the shoots of 'Tifgreen' bermudagrass and 'Penncross' creeping bentgrass. Information is needed also on the effects of these chemicals on root meristems of other turfgrass species.

LITERATURE CITED

LITERATURE CITED

1. Arnold, C. E. and J. B. Aitken. 1973. Perennial grass control in pecan orchards. Proc. Southern Weed Sci. Soc. 26:231-235.

2. Ashton, F. M. and A. S. Crafts. 1981. Mode of Action of Herbicides. John Wiley and Sons, New York, p. 183.

3. Audus, L. J. 1976. Herbicides: Physiology, Biochemistry, Ecology. Academic Press, Orlando, p. 73.

4. Bingham, S. W. 1983. Goosegrass control in bermudagrass. Abs. Weed Sci. Soc. p. 36.

5. Blevins, C. L. and C. E. Rieck. 1978. Selectivity of asulam on johnsongrass in fescue. Proc. Southern Weed Sci. Soc. 31:67.

6. Burt, E. O. and S. L. Carylyle. 1977. Asulam in St. Augustinegrass. Proc. Southern Weed Sci. Soc. 30:145-146.

7. Burton, J. D., J. W. Gronwald, D. A. Somers, B. G. Gengenbach and D. L. Wyse. 1988. Inhibition of acetyl-coenzyme A carboxylase by sethoxydim and Haloxyfop. Abs. Weed Sci. Soc. 28:63-64.

8. Callahan, L. M. 1972. Phytotoxicity of herbicides to a Penncross bentgrass green. Weed Sci. 20:387-391.

9. Callahan, L. M. 1976. Phytotoxicity of herbicides to a Tifgreen bermudagrass green. Weed Sci. 24:92-98.

10. Callahan, L. M. and R. E. Engel. 1965. Tissue abnormalities induced in roots of colonial bentgrass by phenoxyalkylcarboxylic acid herbicides. Weed Sci. 13:336-338.

11. Callahan, L. M. and R. E. Engel. 1986. A compact continuous flow and constant level solution culture renewal system. Agron. J. 78:547-549.

12. Callahan, L. M., R. E. Engel, and R. D. Ilnicki. 1968. Environmental influence on bentgrass treated with silvex. Weed Sci. 16:193-197.

13. Cisar, J. L. and J. A. Jagschitz. 1984. Postemergence control of smooth crabgrass in lawn turf. Proc. Northeastern Weed Sci. Soc. 38:276-280.

14. Corbett, J. R., K. Wright, and A. C. Baillie. 1984. The Biochemical Mode of Action of Pesticides. Academic Press, Orlando, p. 39.

15. Corbett, J. R., K. Wright, and A. C. Baillie. 1984. The Biochemical Mode of Action of Pesticides. Academic Press, Orlando, p. 80.

16. Dernoeden, P. H. and J. D. Fry. 1985. Control of annual lespedeza, oxalis and other broadleaf weeds in turf. Proc. Northeastern Weed Sci. Soc. 39:290-294.

17. Ebdon, J. S. and J. A. Jagschitz. 1982. Chemical control of spurge and other broadleaf weeds in turfgrass. Proc. Northeastern Weed Sci. Soc. 36:307-313.

18. Gilbreath, J. P. 1983. "Postemergence grass herbicides", Weeds Today. 14:3-5.

19. Gill, G. W., J. K. Frost, and K. A. Miller. 1974. A new formula for a half-oxidized hematoxylin solution that neither overstains nor requires differentiation. Acta Cytologica. 18:300-311.

20. Hall, J. R. and J. V. Parochetti. 1975. Control of yellow nutsedge in cool-season turf. Proc. Northeastern Weed Sci. Soc. 29:387-391.

21. Harper, C. A. 1982. Unpublished Data. Root histology of two turfgrass cultivars in response to selected herbicides. M. S. Thesis, The University of Tennessee, Knoxville, December 1982.

22. Hartwig, N. L. 1983. Delay in dry matter production in cool season grasses with pronamide. Proc. Northeastern Weed Sci. Soc. 37:81-82.

23. Hawf, L. R. 1975. Nutsedge control in turf. Proc. Northeastern Weed Sci. Soc. 29:382-386.

24. Hewitt, E. J. 1966. Sand and water culture methods used in the study of plant nutrition. Commonwealth Bull. of Hort. and Plant Crops, Tech. Comm. No. 22, 2nd. ed.

25. Hicks, C. P. and T. N. Jordan. 1984. Response of bermudagrass, quackgrass, and wirestem mulhy to postemergence grass herbicides. Weed Sci. 32:835-841.

26. Higgins, J. M., L. B. McCarty, and T. Whitwell. 1986. Response of bermuda and bentgrass to postemergent applied grass herbicides. Proc. Southern Weed Sci. Soc. 39:129.

27. Jagschitz, J. A. 1972. Evaluation of herbicides for the control of broadleaved weeds in seedling and mature turfgrass. Proc. Northeastern Weed Sci. Soc. 26:217-223.

28. Jagschitz, J. A. 1975. Postemergence crabgrass and nutsedge control in turfgrass with herbicides. Proc. Northeastern Weed Sci. Soc. 29:376-380.

29. Jagschitz, J. A. 1976. Herbicidal control of nutsedge in Kentucky bluegrass. Proc. Northeastern Weed Sci. Soc. 30:351-355.

30. Jagschitz, J. A. 1979. Postemergence herbicides for crabgrass and nutsedge control in turfgrass. Proc. Northeastern Weed Sci. Soc. 33:274-279.

31. Jagschitz, J. A. 1980. Control of fall panicum and nutsedge in Kentucky bluegrass with postemergence herbicides. Proc. Northeastern Weed Sci. Soc. 34:368-371.

32. Jagschitz, J. A. 1981. Prostrate spurge control in turfgrass using herbicides. Proc. Northeastern Weed Sci. Soc. 35:288-293.

33. Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Co., Inc., New York, p. 130.

34. Johnson, B. J. 1974. Effects of pronamide treatments on the establishment of centipedegrass. Weed Sci. 22:508-511.

35. Johnson, B. J. 1975. Herbicides for weed control during establishment of warm season turfgrass. Ga. Agric. Res. Bul. 177:3-26.

36. Johnson, B. J. 1975. Purple nutsedge control and tolerance of turfgrasses to herbicide treatments. Ga. Agric. Res. Bul. 176:3-26.

37. Johnson, B. J. 1976. Transition from overseeded cool-season grass to warm-season grass with pronamide. Weed Sci. 24:309-311.

38. Johnson, B. J. 1978. Renovation of lawns with chemicals. Ga. Agric. Res. Bul. 210:3-10.

39. Kern, A. D., W. F. Meggitt, and D. Penner. 1978. Yellow nutsedge control in Kentucky bluegrass with bentazon, cyperquat, and perfluidone. Weed Sci. 26:280-283.

40. Lewis, W. M. and J. M. DiPaola. 1986. Release of centipedegrass from bahiagrass competition. Proc. Southern Weed Sci. Soc. 39:148.

41. McCarty, L. B., J. Higgins, and L. C. Miller. 1986. Centipedegrass tolerance to postemergence applied herbicides. Proc. Southeastern Weed Sci. Soc. 39:147.

42. Morrow, L. A. and C. L. Canode. 1982. First crop seed yield of selected cool-season grasses as influenced by weed control. Agron. J. 74:964-966.

43. Murdoch, C. L. and D. Iked. 1964. Goosegrass control in bermudagrass turf with combinations of MSMA with s-triazines. Agron. J. 66:712-714.

44. Neel, P. L. and E. O. Burt. 1979. Tolerance of 5 warm-seasoned turfgrasses and 36 ornamental plant species to asulam. J. Amer. Soc. Hort. Sci. 104:129-132.

45. Sass, J. E. 1952. Botanical Microtechnique. Third Edition. The Iowa State University Press, Ames, Iowa, p. 18.

46. Seagle, E. D. 1978. Unpublished Data. Cellular responses in roots of bentgrass and bermudagrass to selected herbicides. M.S. Thesis, The University of Tennessee, Knoxville, June 1978.

47. Troutman, B. C., R. E. Frans, and J. W. King. 1974. Postemergence weed control in dormant bermudagrass. Proc. Southeastern Weed Sci. Soc. 27:57.

48. Yahres, R. D. and J. A. Jagschitz. 1982. Pre- and postemergence herbicides for the control of crabgrass in lawn areas. Proc. Northeastern Weed Sci. Soc. 36:292-297.

VITA

The author, John M. Bogert, was born in Knoxville, Tennessee on January 19, 1964. He was raised in Hendersonville, Tennessee. He graduated from Hendersonville High School in 1982 and entered The University of Tennessee at Knoxville. In June 1986, he received a Bachelor of Science degree in Ornamental Horticulture and Landscape Design. Also in June 1986, he entered The Graduate School at The University of Tennessee. The author received a Master of Science degree in Ornamental Horticulture and Landscape Design, specializing in Turfgrass Management, in December 1988.