THE RESPONSE OF PLANT TISSUE TO HIGH VOLTAGE ELECTRIC FIELDS

A Dissertation

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

by

Cynthia Ann Rogers

May, 1975

To

Ken, Shelley,

and Lori

who endured much

that I might pursue

this course in

my life.

ACKNOWLEDGEMENT

I would like to express my appreciation to Dr. Hugh Freebairn, Dr. Jerry Modisette, Dr. S. Venketeswaren, Dr. A. L. Lawrence, and Dr. Glen Aumann for serving as my graduate committee, for their careful reading of this dissertation and for their many helpful suggestions. I especially thank Dr. Freebairn, my advisor and friend, for his excellent direction, stimulating ideas, and generous support throughout this course of my program.

I am very grateful to Dr. Modisette for his assistance in the design of the electric field apparatus, statistical analysis, and his sense of humor which served as a stabilizing factor during many of the crises which arose. I also thank Dr. Venketeswaren for the use of his tissue culture facilities, Dr. Betty Bartschmid and Dr. Doris Warren for their technical direction with the atomic fluorescence and ESR studies, and Dr. Hendricks for attending my defense.

I would like to acknowledge the administration of Houston Baptist University for allowing me to pursue this degree during the working day, to my fellow science faculty for carrying part of my work load during critical periods, and to my students who shared many of my experiences with me.

With sincerest gratitude, I thank my friends Doris Warren, John Hemphill, Jim Ray and Bob Jansen for the tears, laughter, and comradery shared over the past several years.

THE RESPONSE OF PLANT TISSUE TO HIGH VOLTAGE ELECTRIC FIELDS

An Abstract

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

by

Cynthia Ann Rogers

May, 1975

ABSTRACT

Plant material, including seeds of Phaseolus vulgaris, callus tissue of Glysine soja, Nicotiana tobaccum, Pinus elliottii, Pisum sativum, Vicia faba, and Daucus carota, discs of tuber tissue of Solanum tuberosum L, variety Russet, intact roots of Vicia faba, root explants of Pisum sativum, and excised roots of Allium cepa, were placed in an alternating high voltage electric field for designated periods of time, removed from the field, and examined by several methods to ascertain the effect of the electric field on the plant material. The size of mature plants grown from treated seeds were significantly different from the nontreated seeds. The rate of germination, rate of respiration, ethylene production, also appeared to be effected by exposure to the electric field. The chlorophyll content in the leaves of Phaseolus vulgaris was somewhat higher in those plants grown from seeds treated in the electric field. Growth rates of callus tissue demonstrated a response to the electric field with each species exhibiting its own optimum period of exposure to produce the greatest stimulation and depression of growth.

Studies with ^{32}P demonstrated that phosphorus uptake by callus tissue, potato discs, and root tips was clearly effected by the external electric field. Studies with atomic fluorescence and electron spin resonance indicated that the effect on ion uptake may be general in nature.

The utilization of paired samples rather than large groups of samples was critical to the evaluation of the effect of the field. This technique combined with statistical analysis with rank correlation and linear regression coefficients resulted in the formulation of a model that might explain the results observed in the paired samples. The results showed that the electric field was bringing about a move toward an "equilibrium" state in relation to the parameter being examined resulting in a reduced degree of variation in the treated group as compared to the nontreated group.

Finally, the study demonstrated that the effect of the electric field on plant tissue was altered by changes in the environment of the tissue before or after placement in the field. This was shown by changing the pH of the absorption solution for phosphorus uptake, lowering the nighttime temperature during seed germination, changing the osmotic concentration with cells, submerging tissue in liquid, and aging the tissues.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

TABLE OF CONTENTS CONTINUED

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

LIST OF TABLES CONTINUED

LIST OF TABLES CONTINUED

LIST OF FIGURES

INTRODUCTION

INTRODUCTION

Environmental effects on plant growth and development have been the subject of much concern and research for many years. As a result, many of the environmental parameters, such as the effects of light, temperature, pH, water, soil nutrients, etc., have been studied in depth and clearly understood to have an effect on plant growth. In recent years study of environmental influences have received new impetus with the current interest in the effects of pollution, herbicides, and pesticides on plants.

One aspect of the environment that has received very little scientific attention in the last thirty years is that of the effect of electric fields on plants. This lack of interest has not always been the case, however, for there have been periods of time during which this was an area of active research. Unfortunately, the results of the research were not very conclusive so that even the most basic question of whether an external electric field has an effect on plants is still unsettled. A review of the history of electrical studies provides a basis for understanding the controversy associated with the subject.

The first recorded experiment concerning the effect of electricity on plants was performed by Dr. Maimbray of Edinburgh in 1746. According to Solly (1846), Dr. Maimbray applied an electric current to two myrtles in a grove throughout

the month of October. These two myrtles produced several inches of growth and even flowered while the other myrtles did not demonstrate this unseasonable growth. This apparent stimulation of growth by electricity attracted the attention of a large number of people in all parts of Europe. The initial studies of 45 independent electricians during the period of 1746-1845 are reviewed in detail by Solly (1846). During this period the term "electroculture" was applied to the study of applying electricity to plants and became a topic of cyclic favor and disfavor among the electricians studying the problem.

In spite of a century of controversial results, the hope of finding a method of increasing crop production was so appealing that the agricultural scientist continued sporadically for another century to explore the use of electroculture. The early studies utilized atmospheric electricity (Monahan, 1904; Jørgensen and Stiles, 1917; Brezowsky, 1919; and Briggs, Campbell, Heald, and Flint, 1926.) The term eventually came to refer to those practices which maintained an electric charge on a wire network stretched over the plants or those practices which produced an electric current through the soil. The results of these studies in Europe and the United States are reviewed by Monahan, 1904; Monahan, 1905; Priestly, 1907; Dudgeon, 1912; Hendrick, 1918; Blackman, 1924; Blackman and Legg, 1924; Briggs, Campbell, Heald, and Flint, 1926; and

Dorchester, 1937. The analysis of the effects of the electrical treatment usually involved measuring the difference in dry weight of the treated and non-treated plants and/or a change in the yield of a particular crop. While many of the investigators reported a sensational increase in yield, a similar number reported a decrease or no apparent effect due to electrical treatment.

During this period very few attempts were made to seek a physiological explanation of the effect of the electrical treatment. Knight and Priestley (1914) studies the effect of the electrical discharge on plant respiration measured by the output of carbon dioxide. They were not able to show any alteration in respiration with germinating peas whether they used direct current or overhead discharge. Blackman (1924) reported that the mode of action of the overhead discharge was obscure, but that in several cases the electrified field crops showed a deeper green color than the controls implicating the formation of more chlorophyll pigment.

Another popular method of electrical treatment in the early 1900's was the application of electricity to seeds and bulbs and measuring the change in rate of germination. The electricity was applied either directly to the seeds or bulbs or applied by passing a current through the soil in which the seeds were planted. An increase in germination in the treated seeds was reported by Leicester (1892), Kinney (1897), Mercier

(1919) and Lee (1920). Inconsistent results were reported by Darnell-Smith (1920), Sutton (1920), and Russell (1920).

Apparently, electro-culture was not sufficiently successful or consistent to be economically beneficial and the agricultural societies stopped providing financial support for research in the electrical treatment of plants in the late 1930's. However, a closely related field began to emerge which later stimulated a renewed interest in electrical treatment of plants. In 1928, Lund proposed that the continuous electrical current produced by cells may control the magnification and direction of metabolic processes in other cells. Burr (1935) reported: "The pattern of organization of any biological system is established by a complex electro-dynamic field.... Therefore, it must regulate and control living things, it must be the mechanism the outcome of whose activity is 'wholeness,' organization and continuity." Avid interest arose around the issue that cells produce a natural bioelectric current and that an electric field was established around a plant as a result of the bioelectric activity. The early literature in this area has been reviewed by Lund (1947). More current reviews include Scott (1967), Presman (1970), and Higinbotham (1973). Based upon the possibility that a cell or entire plant might use this bioelectric activity as a primitive form of communication and integration of development, interest was regenerated in the effect of applied electric

currents and electric fields that they might alter the integrating mechanisms of the plant.

Using onion roots, Marsh (1930), studied the effect of applied direct current on the inherent electromotive force (E.M.F.) of the onion cells and reported that the E.M.F. is altered by the applied current. Berry and Hoyt (1943 a and b) could also alter the observed electrical potential with both direct and alternating current. The effect of small electric currents on the growth of coleoptiles have been studied by Gregory and Batten (1926), Cholodny and Sankewitsch (1937), Schrank (1950), Schrank and Backus (1951), Webster and Schrank (1953), Weigand and Schrank (1954), and Schrank (1959). The first two papers explored the alternations in growth patterns as a result of applied current while the others were concerned with the relationship of curvature, auxin, and the applied current. The response of the inherent E.M.F. of Phaseolus multiflorus to electrical currents was studied by Rehm. (1939) who reported that the plant demonstrates an increased basal positivity in response to applied current. Rehm also reported that "...there is an intimate relation between the orientation of bioelectric potentials and morphology. When the plant changes from one morphological state to another there is a concomitant reorientation of electrical potential patterns."

Research with applied electric fields did not resume until the 1960's. These more recent papers will be examined in greater detail since they have a direct relationship to our work with electric fields. Murr has published several papers on the effect of electric fields on plants (1963a, 1963b, 1964, 1965, and 1966). Nurr's basic procedure is to place one aluminum wire mesh electrode in the soil and one above the plants with the plates charged with a d.c. potential of 15-80 KV/M. Seeds were germinated within the field and plants allowed to grow from 10 to 30 days within the field. He has reported a reduction in dry weight, leaf tip burning, and chloroplast derangement in orchard grass seedlings grown in this environment. He suggested that ionized salts are mobilized and drawn into the epidermal cells causing large quantities of water to enter and eventually rupturing these cells. He also reported more severe plant-leaf damage using a.c. current although germination rates were not effected by the electric field. Zinc, aluminum, and iron are reported to accumulate in the leaf tips of plants grown in the electric field. Murr suggested that with the a.c. current polarization is not the cause of damage but a type of molecular field fatigue causing a direct elastic rupture of epidermal cells. It is also possible that more complete leaf damage results from overstimulation of the respiratory enzymes. Murr also reported that growth stimulation is possible if the potential

gradient is adjusted to prevent severe leaf damage and that each species of plant would have its own optimum potential for stimulation.

Pickett and Schrank (1965) applied 500 to 2500 volts of d.c. durrent through the longitudinal axis of Avena coleoptiels and noted a significant increase in elongation. This field applied laterally inhibited curvature of the coleoptile. The field was also applied in conjunction with a magnetic field. A magnetic field alone inhibited curvature; the addition of an electric field reduced the curvature further. The curvature inhibition induced by the electric field was stated to be the result of the field altering some aspect of asymmetry which is required for lateral curvature.

Mihalyfi and Serf (1967) used 3, 5, and 10 kv/cm a.c. fields for 5 and 10 seconds to treat maize, pea and cucumber seeds. After exposure to the field the seeds were soaked for 24 hours. Analysis of catalase activity after soaking showed that the catalase activity of the maize and pea seeds have been decreased with 3 and 5 kv/cm for 5 seconds and increased with 5 kv/cm for 10 seconds. The catalase activity of cucumber seeds was increased with all the electric field treatments.

Sidaway has studied effects of electric fields in relation to phytochrome mediated processes. Sidaway (1966) used a d.c. current to produce a field of 90 v/cm with

lettuce seeds in contact with the positive plate for 24 hours. This field appeared to inhibit the germination of lettuce seeds. Seeds placed on the negative plate did not appear to respond to the field. Sidaway also reported the possibility that light might reduce the inhibition seen in a positive field. Sidaway and Asprey (1968) applied a d.c. field of 150 v within respirometer flasks containing thin slices of the spadix tissue of Arum. Tissue that had been "washed" for three hours before being placed in the flasks was much more sensitive to the effects of the electric field than unwashed slices as measured by oxygen absorption. The effects of the field on the respiration of cotyledons of Vicia faba were inconsistent. The inconsistency was attributed to (a) variations in the seeds and (b) variations in the earth's atmospheric electric field. In 1969 Sidaway applied a 75 v/cm d.c. field for two to four minutes to lettuce seeds two hours after imbibition. The treatment increased the level of dark germination with the negatively charged seeds having a higher germination level than those on the positive plate. The same treatment had no effect if applied one hour after a 20-minute far-red irradiation. The treatment also had no effect if given after 4 minutes of red irradiation, an exposure period bringing about almost complete conversion of phytochrome to the P_{fr} state. Far-red irradiation given simultaneously with the electric field brought

about a pronounced antagonism of the far-red inhibition with the positively charged seeds. This experiment provided strong evidence for an interraction of the electric field with the phytochrome system.

In summary, the study of the electrical treatment of plants has been approached by three groups of scientists during three different periods over the past two and a half centuries. The initial work was instigated by electricians, followed by investigations from the agricultural societies, and currently studied by plant physiologists. Although its history is lengthy, very few facts have been established with any certainty about the effects of electric fields on plants. This scarcity of information is due, at least in part, to the lack of a consistent response of plants to the applied field. The current interest in studying the problem has been stimulated by the increased understanding of inherent bioelectric activities of plant materials.

Our present study arose from observations of apparent stimulation of plant growth by an electric field and the conflicting reports in the literature concerning this topic. Our original experiments were patterned after a study by Kozhevnikova and Stanko (1967) in which they treated corn seeds in a 50 hertz field at a voltage of 2 to 4 kv/cm for thirty seconds or one hour. The plants grown from the treated seeds were darker green in color, had an increased photosynthetic

rate, exhibited less of a mid-day photosynthetic slump, and showed an increase in dry weight of the entire plant. Our early experiments with treated Red Kentucky Wonder Bush Bean seeds were sufficiently successful in demonstrating an effect of the field on plant growth to create an increased interest in the study and to warrent further investigation.

We hoped to accomplish the following goals during the course of our study: (1) to establish that there was indeed an influence from an externally applied electric field on plant growth (2) to design an experimental procedure which would produce consistent results and (3) to explore a possible explanation of the interaction of the electric field and the plant material at a cellular level.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

I. Biological Materials

The organisms used in this study include (1) the seeds and mature plant of the Red Kentucky Wonder Bean, bush variety, Phaseolus vulgaris, (2) Callus tissue of soybean, Glysine soja, habituated tobacco, Nicotiana tabaccum, slash pine, Pinus elliottii, Alaskan pea, Pisum sativum, broad bean, Vicia faba, and carrot, Daucus carota, initiated from carrot roots purchased locally, (3) Potato discs from locally purchased tubers, Solanum tuberosum L., variety Russet, (4) seeds of Vicia faba, large variety, with intact roots, (5) root explants from germinated seeds of Pisum sativum, and (6) excised roots from onion bulbs, Allium cepa, purchased locally.

II. Growth Media

The callus tissue and the root explants required the use of complex growth media containing the macronutrients, micronutrients, vitamins, and hormones essential for their growth. The composition of these media and the tissues grown on them are given in Appendix A.

III. Standard Sterile Techniques

Several experiments used in this study required sterile procedures to prevent undue growth of microorganisms and

fungi. Surface sterilization included washing the external surfaces of the plant material with a sodium hypochlorite solution followed by three rinses in sterile distilled water. The concentration of the sodium hypochlorite and the length of time in the solution varied according to the size of the material involved. All equipment used during a sterile procedure were autoclaved at 18 pounds per square inch for fifteen minutes. The transfer and handling of the plant material was performed within a Agnew-Higgins laminar flow chanber Cat. # 000-018 which produced an outward flow of filtered air reducing the possibility of contamination from airborne microorganisms. Before using the chamber, all interior surfaces were wiped with 95% ethancl. Scalpels, spatulas, and the exterior lip of media jars were flamed before and after each use to prevent spread of any contaminant that might be present in a particular sample of tissue. Jars containing the callus tissue were covered with sterile polypropylene and secured with a rubber band before removing from the chamber.

IV. Electric Field Apparatus

The electric field was produced by a Jefferson luminous tube transformer attached to two vertical 45 x 45cm aluminum capacitor plates. In such an arrangement the field is almost normal to the faces of the plates and is

uniform in the space between them although there is some "fringing" or spreading of the field at the edges. The fringing may be considered negligible if the space between the plates is small compared to their linear dimensions (Sears and Zemansky, 1966). The transformer produced a peak potential difference of 15,000 volts at a frequency of 60 hertz. The magnitude, E, of the field is determined by the equation given below:

$$E = \frac{V}{d}$$

where V is the instantaneous voltage and d is the distance between the two capacitors. The distance was varied in this work from 3cm to 5cm producing maximum field strengths from 5000 v/cm to 3000 v/cm. The biological samples were placed at the center of the space between the plates to ensure being within the uniform range of the field. The samples were supported on a wooden shelf in such a manner that neither the samples nor the shelf contacted the aluminum plates at any point. The wooden shelf and the samples are dielectrics, so that in an electric field they produce surface charge distributions tending to modify the field. The effect of the shelf is small for objects placed upon it. Considering the samples to have the same dielectric constant as water, the field inside would be reduced by a factor of about 30.

V. Counting Methods

A. Gas-flow:

The radioactivity in the callus tissue, root tips, and potato discs was counted with a Nuclear Chicago gas-flow counter, Model number 186, utilizing aluminum planchets placed in holders and an automatic sample changer. The counting system had an efficiency of 33%. The range of counts in the samples with callus tissue was 1000 - 9000 cpm; for root tips, the range was 1000 - 5000 cpm; for potato discs, the range was 600 - 9000 cpm. The coincidence loss in this range is negligible. The counts per minute figures were converted to counts per minute per gram tissue to adjust for differences in the weights of the samples. The per cent recovery was determined to be 94% with 0.1% of the activity located within the tissue samples. The remaining activity was in the original solution and the wash water resulting from three one-minute washes after the samples were removed from the radioactive solution. The amount of phosphorus added to the absorption solution was insignificant (1×10^{-12} moles).

B. Autogamma:

Two experiments with callus tissue required the use of a counting assembly capable of counting very high radioactivity. These samples were counted with a Nuclear

Chicago Autogamma Spectrometer, Model 1185, with a well-type two inch NaI and thallium crystal utilizing 16 x 125 mm Amersham plastic sample tubes.

VI. Growth Conditions

Several of the experiments required a period of growth before and/or after treatment in the electric field. Listed below is a description of the physical conditions maintained during the growth period.

A. Seed Germination:

1. Red Kentucky Wonder Bush Bean seeds were germinated in the dark at 24°C in one of two manners: (1) between wet paper toweling or (2) in 250 ml erlenmeyer flasks containing four layers of filter paper on the bottom which were moistened with sterile distilled water as needed. The seeds in both instances were surface sterilized before germination by soaking in 0.11 M sodium hypochlorite for 15 minutes.
2. Alaskan peas were planted in vermiculite and grown in the dark at room temperature.
3. The seeds of broad beans were first placed in aerated tap water for two days until the radicle began to emerge. The seeds were then placed on wet absorbant paper, covered with wet paper towling, rolled into

a cylinder, and stored in a vertical position for three days at room temperature.

B. Mature plants:

Seeds of Red Kentucky Wonder Bush Beans were planted in separate pots and placed in a greenhouse to germinate and produce mature plants. The greenhouse was equipped with fluorescent lighting which remained on continuously. The plants were watered as needed with tap water.

C. Tissue cultures:

Callus tissue was grown on solid medium in a Biotronette Mark III environmental Chamber Cat. #846 equipped with fluorescent lights which remained on continuously. The temperature of the chamber was maintained at 25°C, ± 1°. The stock cultures were grown in 200 ml mason jars containing 30 ml of medium. Experimental samples were grown in 100 ml jars containing 20 ml solid medium. The jars were sealed with polypropylene secured with a rubber band.

D. Root explants:

Root explants of the Alaskan pea were grown on solid medium in the dark at room temperature in 60 x 15 mm plastic petri dishes.

VII. Methods of Measurement of Biological Activity

A. Growth studies:

1. The seeds of Red Kentucky Wonder Bush Beans were selected to ensure uniform color, weight, shape and intact seed coats. Those seeds to serve as the treated group were placed on the shelf between the capacitors positioned such that the longitudinal axes of the seeds were parallel to the electric field. The seeds were subjected to a peak alternating field strength of 4100 v/cm for two minutes. These seeds and those of the control group were stored in separate sealed jars until the day of planting. At the time of planting, the seeds were surface sterilized and planted in separated pots at 7, 14, and 21 days after treatment in the electric field. Observations were made on (1) the rate of emergence of the seedling above the soil, (2) the length of the stem, (3) the length of the petiole, and (4) the length and width of the leaves. Measurements were made every other day and continued until the thirty-eighth day after planting.

2. Growth studies of tissue culture samples were made by measuring the amount of growth, indicated by an increase in weight, which took place during a two week period following treatment in the electric

field. Using sterile techniques, samples of approximately the same size, color, and texture were taken from stock cultures, placed in numbered plastic petri dishes, and weighed on a Mettler Analytical Balance. Samples were matched according to weight and placed in a field of 3000 v/cm for varying periods of time. The treated and control samples were transferred to jars containing the appropriate growth medium and allowed to grow in the growth chamber for two weeks. After that period, the samples were removed from the medium and weighed. This final weight was divided by the initial weight to produce a number termed the "growth ratio". "Mean growth ratios" were obtained by averaging the growth ratios for the entire treated group and for the entire control group.

B. Determination of chlorophyll content:

Leaves of the mature plants of Red Kentucky Wonder Bush Bean from the growth study experiments were used to determine the effect of an electric field on chlorophyll content. Leaves from control and treated plants were cut into 1 mm sections and ground with a mortar and pestle. One hundred ml of 85% acetone were added per gram of tissue and the solution filtered through

glass wool. The filtrate was refrigerated until readings were taken on a Coleman Model GC spectrophotometer. Wavelengths of 644 nm and 663 nm were used to detect chlorophyll a and chlorophyll b. The following equations were used to determine the amount of chlorophyll a and b per gram of tissue (Arnon, 1949; Koski, 1950):

$$\text{mg Ch a/gram} = 1.07\ (D_{663}) - 0.094\ (D_{644})$$

$$\text{mg Ch b/gram} = 1.77\ (D_{644}) - 0.280\ (D_{663})$$

Where D_{663} and D_{644} are the optical density readings at 663 nm and 644 nm, respectively.

C. Respiration studies and ethylene production:

Red Kentucky Wonder Bean seeds were divided into three groups to be germinated: (1) immediately after treatment in an electric field, (2) three days following treatment, and (3) seven days following treatment. Each of these groups contained subgroups with equal numbers of untreated seeds and those which had been treated in an electric field of 4100 v/cm. Dry weights were recorded on all groups. At the time for germination, the seeds were placed between wet paper toweling and kept in the dark for 60 hours. After this period

the seeds were weighed to determine the amount of water imbibed per group. The groups of seeds were each placed in a 500 ml freeze drying flask and sealed. A serum cap placed on the top opening allowed samples of air to be removed from the flasks at five minute intervals for determination of carbon dioxide and ethylene content by gas chromatography. A similar study was conducted to cover the initial stages of germination.

In this case there were four groups, each containing 60 seeds: (1) a control group, (2) a group treated 7 days before germination, (3) a group treated 3 days before germination, and (4) a group treated in the electric field immediately before germination. Dry weights were determined for each group and each group was placed in a 250 ml erlenmeyer flask with moistened filter paper in the bottom of each flask. The flasks were sealed for two hours after which a 10 ml air sample was withdrawn for ethylene determination. Following this initial reading, the serum cap was replaced with cotton and the flasks placed in the dark at 24°C. Readings were repeated at 24, 48, and 72 hours. Before each reading, the flasks were sealed with a serum cap for two hours before the air sample

was withdrawn. Ethylene determinations were made with a Varian Aerograph, Model 500-D, gas chromatograph with a hydrogen flame ionization detector. The gas sampling valve had a 2 ml loop, the carrier gas was nitrogen, and a three-foot 20 to 40 mesh activated alumina column was used. Carbon dioxide determinations were made with a Loenco Thermoconductivity gas chromatograph equipped with a gas sampling value, a 10 ml loop, and a six inch 20-40 mesh silica gel column. The carrier gas was helium.

D. Cytological observations:

1. Soybean tissue culture was selected to study possible cytological effects induced by the electric field. Several methods of preparations and staining were attempted with the following sequence being the most effective with this particular tissue.

   (1) Small sections of tissue were treated with three parts 95% ethanol: 1 part glacial acetic acid overnight to kill and preserve the tissue.

   (2) This "fixed" tissue was hydrolyzed in 1N HCl at 60^{o}C for 10 minutes.

   (3) The tissue was rinsed in distilled water to remove the fixative solution.

(4) The tissue was placed in 3% pectinase at 60°C for 20 minutes.

(5) The tissue was rinsed in distilled water and allowed to remain in the final rinse for 15 minutes.

(6) The tissue was stained in Lacto-Propriono-DMSO Carmine (LPDC) stain at 60°C for 2 hours. The LPDC stain is prepared by dissolving 6 g carmine in 300 ml DMSO and then mixing lactic acid, propionic acid, and the DMSO carmine in a 1:1:6 ratio. (S. Venketeswaran, personal communication.)

(7) The tissue was stored in LPDC at room temperature until ready for use.

A squash preparation was made using standard techniques (Purvis, et al., 1966, pp. 160-162) and the slides were made permanent by resting them on dry ice, removing the coverslip, allowing the slides to air dry overnight and applying a new coverslip with Permount.

2. Root explants were utilized for a study of mitotic frequency and xylem differentiation. Two-day-old Alaskan pea seedlings were surface sterilized in 0.01 M sodium hypochlorite for 15 minutes, rinsed, and the roots excised. The roots were

placed five per dish in sterile 35 x 10 mm plastic petri dishes. Those to be treated were placed in the field so that the long axes of the roots were perpendicular to the field which had a peak strength of 3000 v/cm. Two 1 mm segments were made from each control and treated root in the region located 1 cm back from the root tip. The explants were placed with one of the cut surfaces in contact with the medium and allowed to culture in the dark three to seven days (Fosket and Torrey, 1969). At the end of the culture period, the explants were "fixed" in 3:1 95% ethanol: glacial acetic acid for 24 hours, hydrolyzed in 1N HCl at 60°C for 10 minutes, rinsed in distilled water, and placed in a Fuchsinsulfurous acid mixture (Purvis, et al., 1966, pg. 163-4) for 40 minutes. The explants were then rinsed with distilled water for 10 - 15 minutes until a bright pink color was evident. The tissue was placed on a clean slide, carefully seperated, and squashed using standard techniques. The slides were made permanent and studied microscopically for mitotic frequency and for the effect of the electric field on xylem differentiation. Each slide consisted of the entire root section, and all mitotic figures were counted.

E. Phosphorus uptake:

1. Potato discs were prepared from potato tubers using sterile techniques. After surface sterilization in 0.04% sodium hypochlorite for 15 minutes, both ends of the tuber were cut off with a scapel and cores were removed from the periphery of the tuber with a 9 mm cork borer. The cores were placed in 5×10^{-4} M $CaCl_2$ until all the cores were prepared; this removed the excess starch from the cores and preserves membrane integrity. (Epstein, Rains, and Schmid, 1962.) One mm discs were cut from the cores and placed in fresh $CaCl_2$ until ready for use. The discs were gently blotted with paper towels and placed in numbered plastic petri dishes, seven discs per dish. (The average weight of seven discs was 0.5438 g.) The weights were recorded and those discs to be treated were placed in a peak electric field of 3000 v/cm for selected time periods. After treatment, the treated and control discs were placed in separate 10 x 10 cm squares of cheesecloth ties into teabags as described by Epstein et al. (1963). Each teabag was numbered and contained the seven discs from one petri dish. The teabags were placed in a vigorously aerated solution containing 10^{-5} M KH_2PO_4, 5×10^{-4} M

$CaCl_2$ and phosphorus-32 supplied from New England Nuclear, carrier-free as $H_3{}^{32}PO_4$ in 0.02 M HCl. The pH of this absorbing solution was adjusted to 4.7 with HCl. The volume of the solution varied from 200 ml to 2 liters depending upon the concentration of the KH_2PO_4, the length of the absorption period, and the number of teabags in the container. After the teabags were removed from the absorbing solution they were transferred to distilled water to remove surface radioactivity. The discs were removed from the teabag, blotted gently with paper toweling, and placed in aluminum planchets. They were counted, seven discs per planchet, in a Nuclear-Chicago counter. The resulting counts for each group of seven discs were divided by the weight of the tissue to obtain the counts per minute per gram of potato tissue.

2. The initial work with phosphorus uptake in callus tissue involved the addition of radioactive phosphorus to the solid medium before its solidified. The tissue was transferred to this medium for 24, 48, and 72 hours, removed from the medium, rinsed carefully with distilled water, and counted with the gas-flow counter and the autogamma counter. This method for callus tissue was eventually re-

replaced by the system described above for potato discs in which the samples of tissue were placed in a teabag and submerged in aerated solutions of 10^{-5} M KH_2PO_4, 5 x 10^{-4} M $CaCl_2$ and ^{32}P at a pH of 4.7. The treatment in the electric field was the same as that described for other tissue culture experiments.

3. Intact roots were also used for phosphorus uptake studies. After the seeds of broad bean had germinated for 5 days, the seeds were matched according to the length of the root; half of each group served as controls and the remaining half were treated in a peak field of 3000 v/cm for 15 minutes. The seeds were supported such that the roots were vertical in the electric field. After treatment, the seeds were placed in a cheesecloth teabag and suspended in a solution of 10^{-5} M KH_2PO_4, 5 x 10^{-4} M $CaCl_2$ and ^{32}P at a pH of 4.7. After an absorption period of one hour, the bags were removed, rinsed in distilled water, and the roots excised from the seeds. Measuring from the root tip, 15 mm root segments were removed from each seed, cut into three segments, and counted as described in previous sections.

F. Presence of free radicals and uptake of transition metals:

Callus tissue of pine and soybean were examined for the presence of unpaired electrons which are commonly found in free radicals and in certain transition metal ions such as Mn^{2+}, Cu^{2+}, and Fe^{3+}. This analysis was made with the use of a Varian E - 3 Electron Spin Resonance Spectrometer. For this analysis, the samples were air dried and examined as a dry solid in a quartz sample tube to approximate the effect of an electric field on the final concentration of metal ions in the tissue after a two-week growth period following the treatment in the field.

G. Zinc uptake:

Potato discs were prepared as before and divided into three groups: (1) Set I was placed in a cheesecloth teabag and immersed in 5 x 10^{-4} M $CaCl_2$ solution for three hours. (2) Set II was placed in a teabag and immersed in a solution containing 5.3 x 10^{-5} M of $ZNSO_4$ and 5 x 10^{-4} M $CaCl_2$ for three hours. (3) Set III was placed in an electric field for one hour before being placed in the above $ZNSO_4$ and $CaCl_2$ solution for the three hour absorption period. The pH of all three solutions was adjusted to 5.8. After the absorption period, the discs were blotted and digested

in concentrated nitric acid. The amount of zinc present in the three sets of discs was determined by flameless atomic fluorescence at 2139 Å as described by Murphy, et al. (1973).

RESULTS

RESULTS

I. Growth Studies

A. Red Kentucky Wonder Bush Beans

1. Germination rates: There was no noticeable difference in the rate of germination between the control and treated seeds.

2. Size of plant: A marked increase was apparent in the size of the plants produced from the treated seeds. In all instances the plants produced by the treated seeds were larger than the control plants as indicated by the length of the stem, length of the petiole, and the length and width of the leaves. Table I illustrates the differences between treated and control plants planted 7 days after planting. Figures 1 - 4 show the differences between the treated and controled plants of those seeds planted 7 days after treatment including measurements from the ninth day after planting to the thirty-eighth day. Figure 5 shows the stem measurements of those plants whose seeds were planted 21 days after treatment demonstrating that the effect of the field was still evident without any apparent reduction in the effect after a three week period between treatment and planting.

TABLE I

Effect of an Electric Field on the Growth* of Red Kentucky Wonder Bush Bean Plants Planted Seven Days after a Two Minute Treatment in the Electric Field

	Mean values for Control Samples with standard deviations of the mean	Mean values for Treated Samples with standard deviations of the mean
Stem length	9.9 ± 2.9 cm	14.8 ± 2.4 cm
Petiole length	2.3 ± 1.0 cm	3.5 ± 0.7 cm
Leaf width	4.7 ± 1.7 cm	7.0 ± 1.4 cm
Leaf length	4.8 ± 1.5 cm	6.8 ± 1.3 cm

*Measurements made 17 days after planting.
**Statistical analysis of the data showed that the difference between the means of the control and treated plants were significant at the 95% level.

FIGURE 1

EFFECT OF AN ELECTRIC FIELD ON THE STEM LENGTH OF RED KENTUCKY WONDER BUSH BEANS PLANTED SEVEN DAYS AFTER TREATMENT

○ - Controls

□ - Treated 2 minutes in field

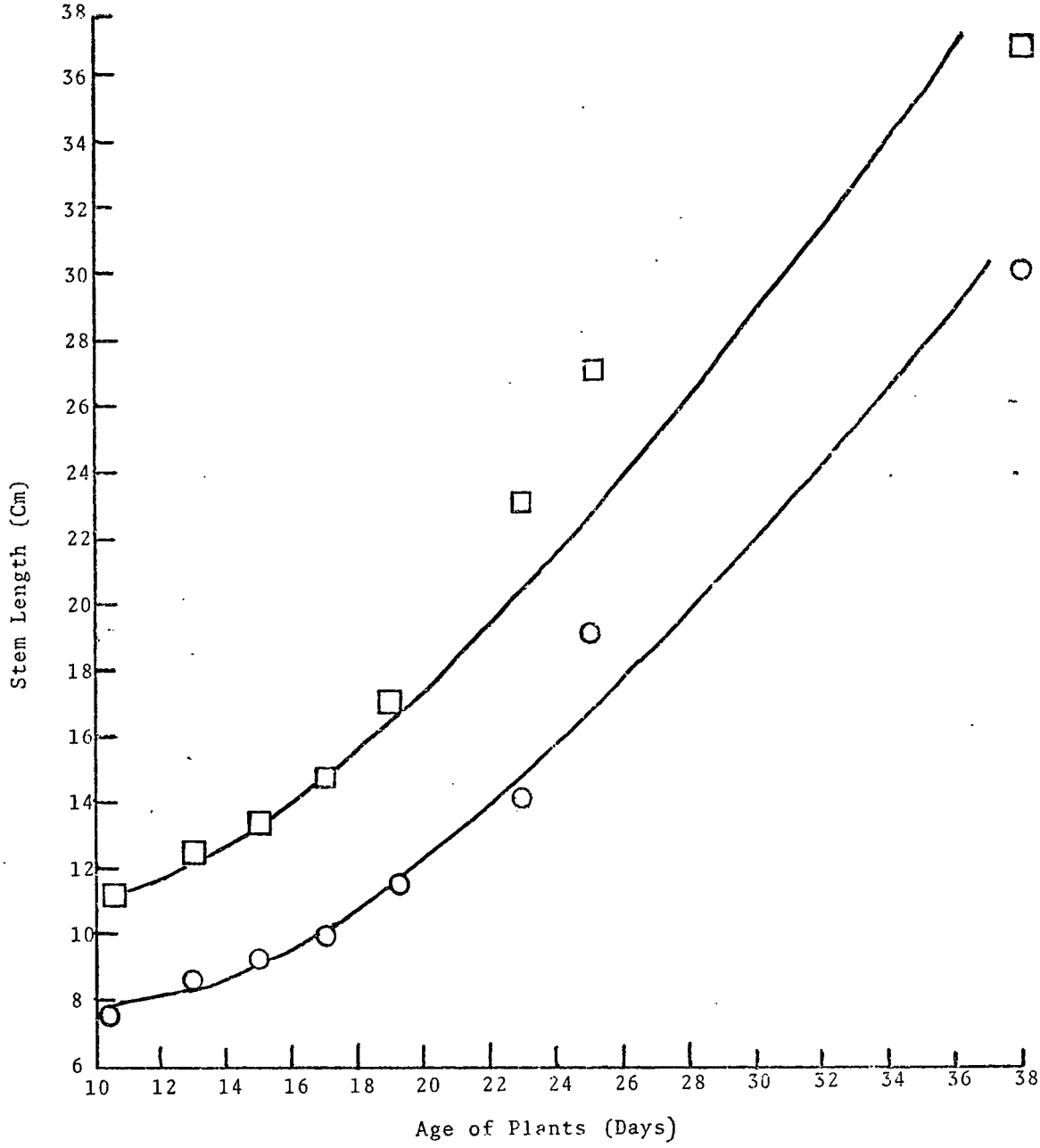

Stem Length (Cm)
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
Age of Plants (Days)

FIGURE 2

EFFECT OF AN ELECTRIC FIELD ON THE LENGTH OF PETIOLES OF RED KENTUCKY WONDER BEANS PLANTED SEVEN DAYS AFTER TREATMENT

○ - Controls

□ - Treated 2 minutes in field

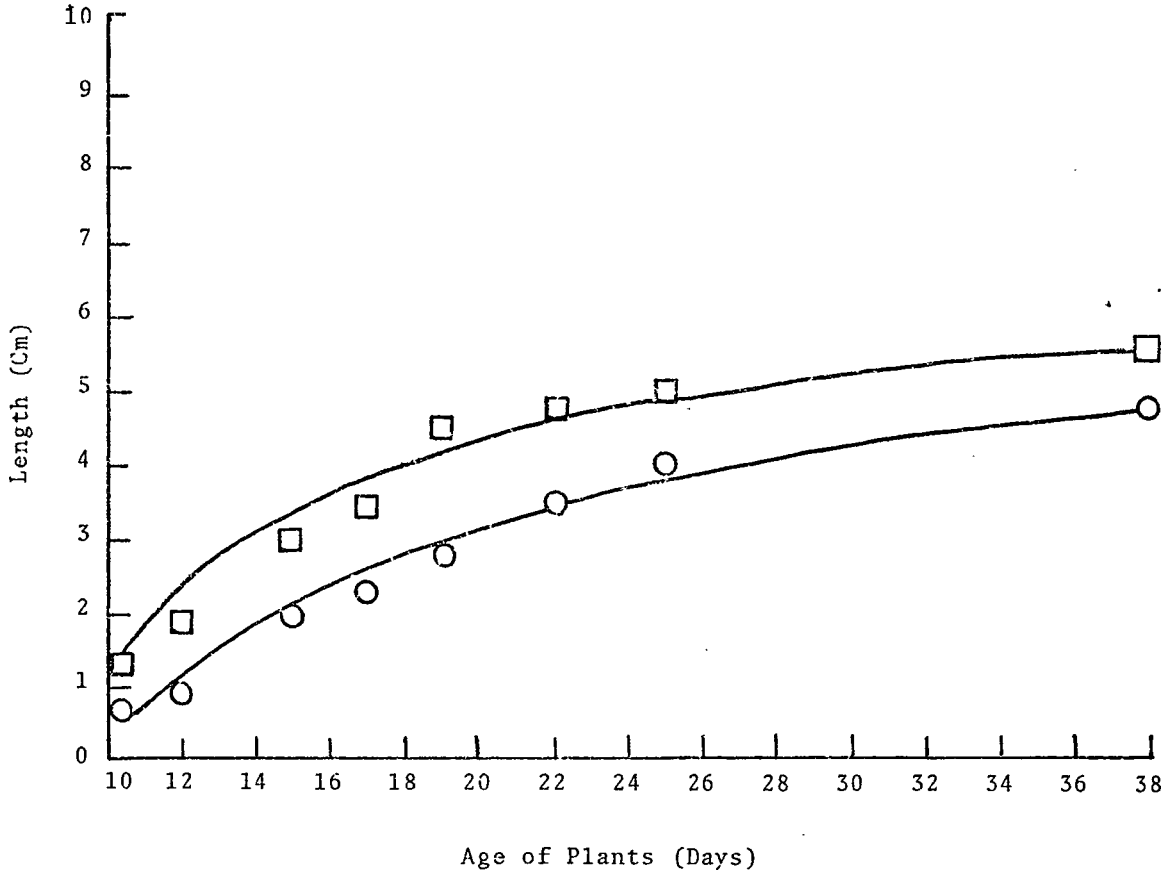

10
9
8
7
6
5
4
3
2
1
0
Length (Cm)
10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Age of Plants (Days)

FIGURE 3

EFFECT OF AN ELECTRIC FIELD ON THE WIDTH OF LEAVES OF RED KENTUCKY WONDER BUSH BEANS PLANTED SEVEN DAYS AFTER TREATMENT

○ - Controls

□ - Treated 2 minutes in field

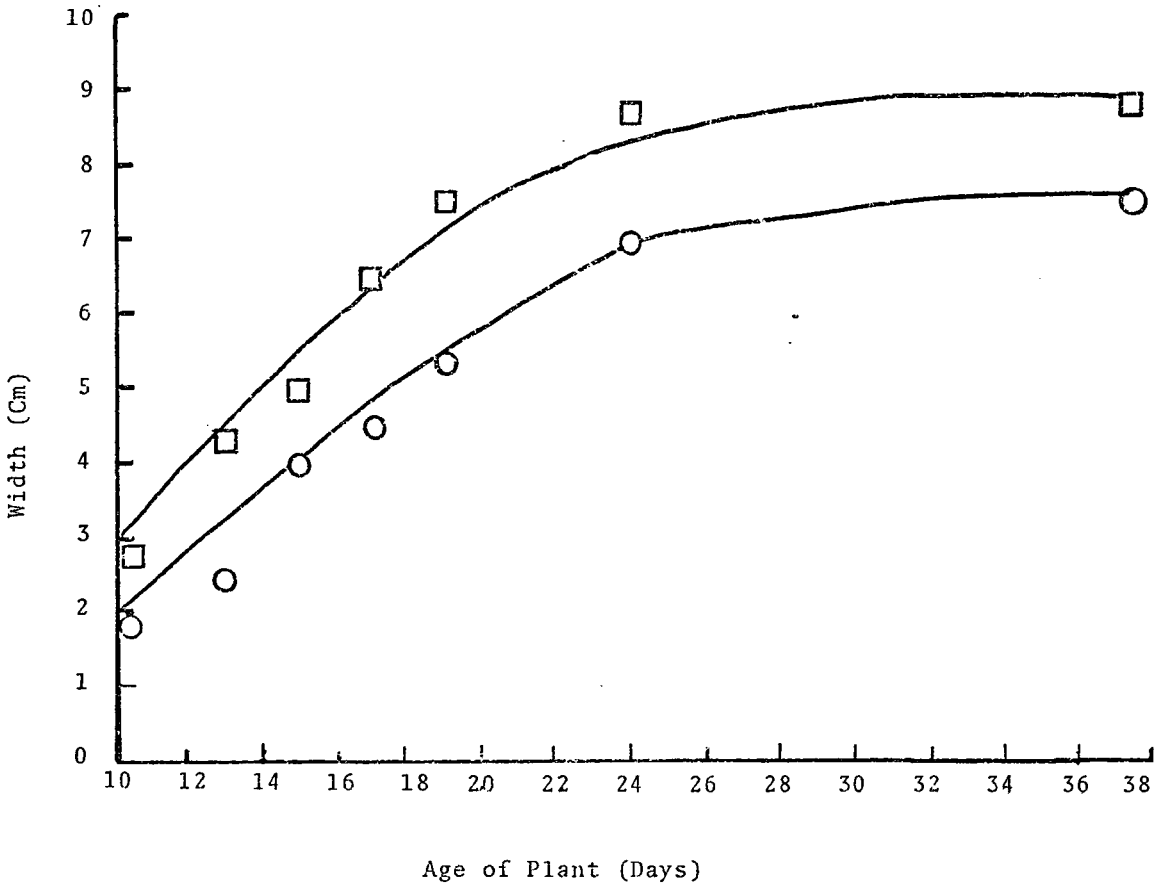

Width (Cm)
0
1
2
3
4
5
6
7
8
9
10
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
Age of Plant (Days)

FIGURE 4

EFFECT OF AN ELECTRIC FIELD ON THE LENGTH OF LEAVES OF RED KENTUCKY WONDER BUSH BEANS PLANTED SEVEN DAYS AFTER TREATMENT

○ - Controls

□ - Treated 2 minutes in field

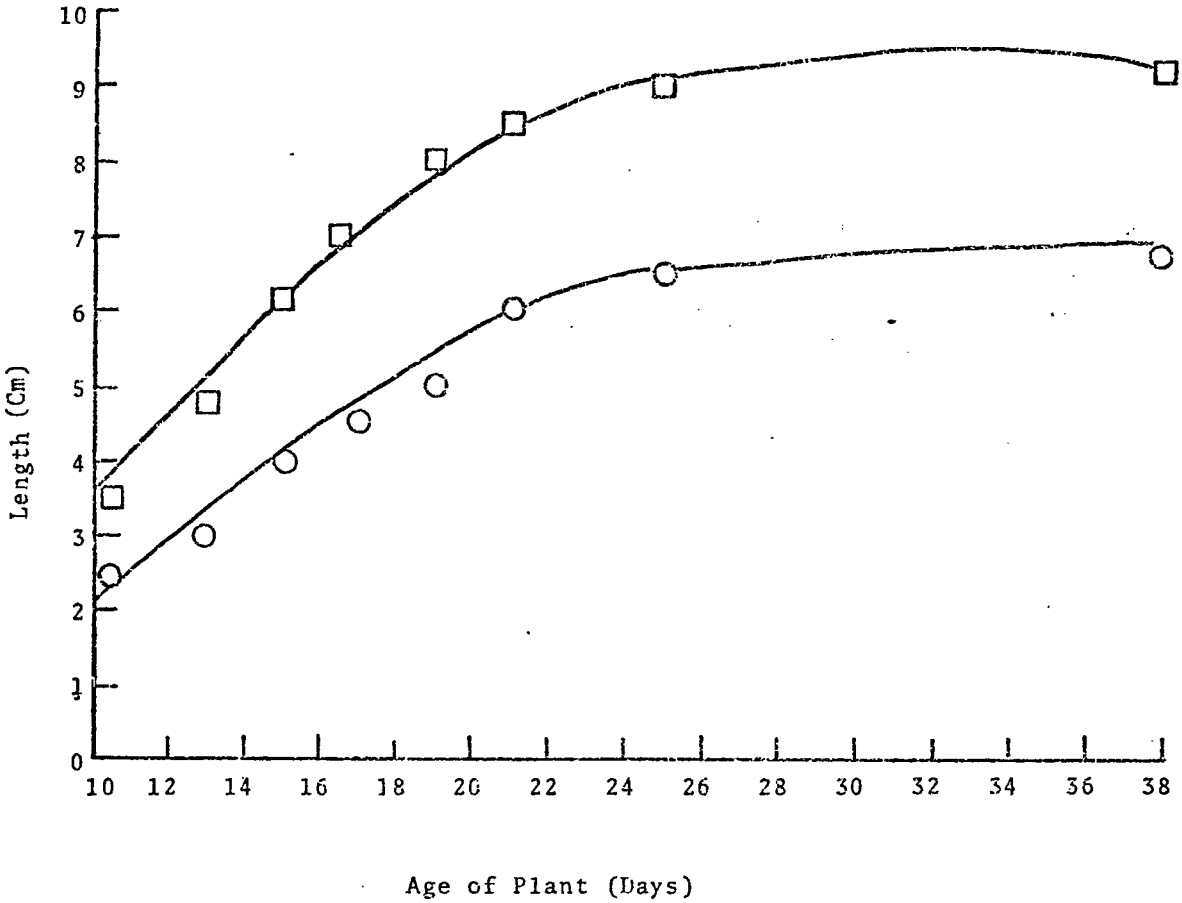
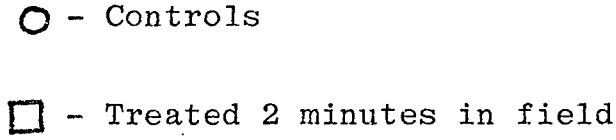
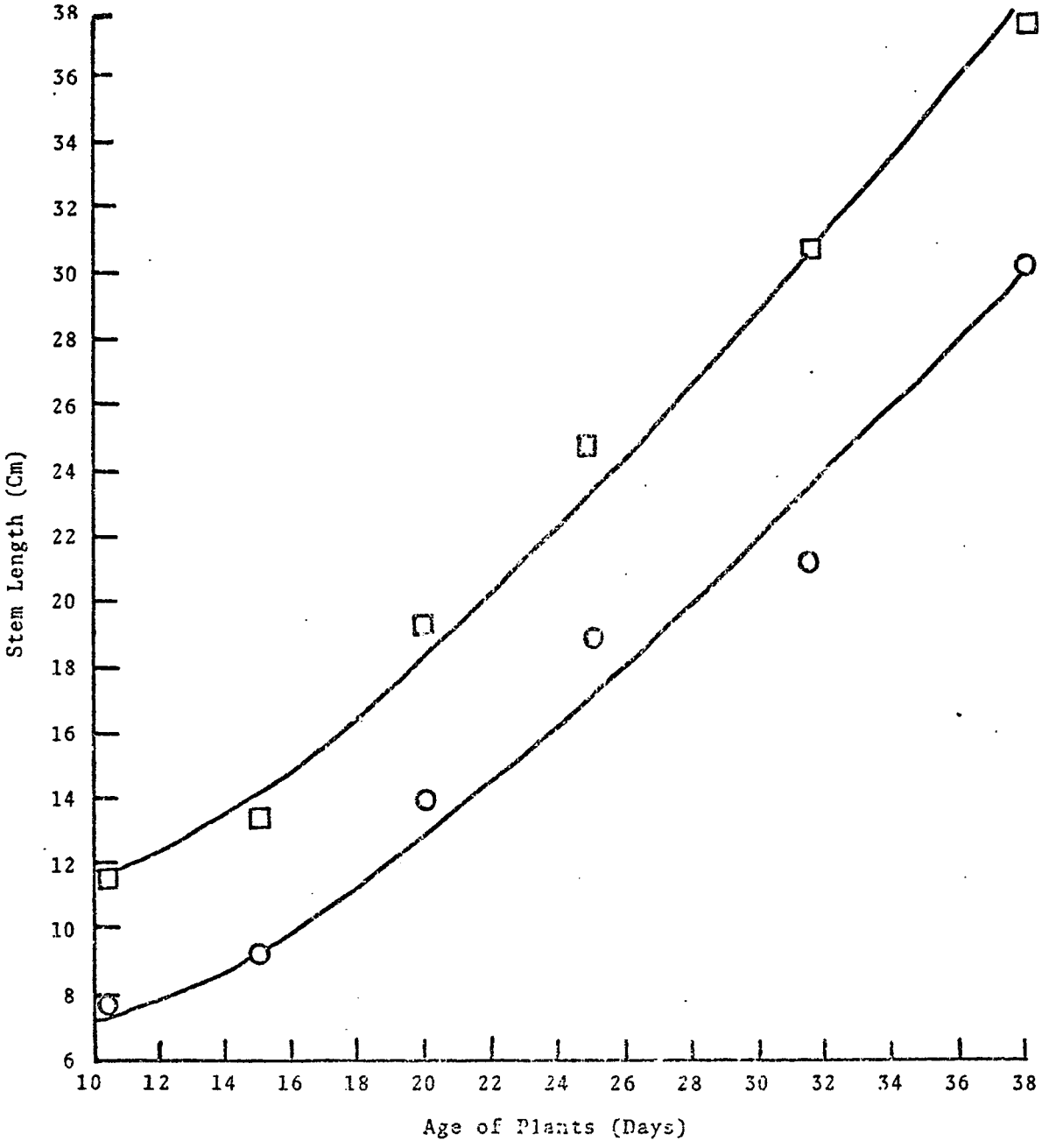

Length (Cm)
0
1
2
3
4
5
6
7
8
9
10
10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Age of Plant (Days)

FIGURE 5

EFFECT OF AN ELECTRIC FIELD ON THE STEM LENGTH OF RED KENTUCKY WONDER BUSH BEANS PLANTED TWENTY-ONE DAYS AFTER TREATMENT




Stem Length (Cm)
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
Age of Plants (Days)

3. Effect of varied treatment periods: The above experiments were repeated with additional periods of treatment in the electric field. Table II shows the results of a 0.5 minute, 2 minute, and 10 minute treatment period on the diameter and length of the stem and length of the first primary leaf. This experiment illustrates two trends which were manifested throughout this study: (1) A treatment period which is effective in producing a particular response during one experiment may not be the most effective period in other, apparently identical, experiments. For example, in the previous experiment a 2-minute treatment period produced plants which were larger than the controls. In this experiment, a 2-minute treatment period produced plants smaller than the controls and a 10-minute treatment period produced plants larger than the controls. (2) During one experiment some periods of treatment reduce growth, some have very little effect on growth, and some stimulate growth.
4. Chlorophyll content: Chlorophyll a and chlorophyll b concentrations were determined in one of the primary leaves from four plants of the treated and control groups. The average concentrations of each

of each type of chlorophyll for the treated and control groups are given in Table III.

B. Callus tissue

The decision to test the effect of an electric field on callus tissue was based on the fact that tissue culture samples offered a possibility for utilization a more uniform system than had been used with the seeds. This is true because: (1) The environment can be carefully regulated and duplicated within a growth chamber. (2) The medium for callus tissue growth can be clearly defined and reproduced throughout a series of experiments. (3) The cells are more homogeneous in a callus sample than in the organs of a mature plant. (4) Differentiation can be controlled to a certain degree by varying the concentration of plant hormones in the medium. (5) Finally, larger numbers of samples can be maintained in a reasonable space.

1. Growth ratios: Tissue culture methods were used to determine the effect of the electric field on growth rates. Treated and nontreated tissue samples were placed on the appropriate growth medium for two weeks after which their growth ratio, the ratio of final to initial weight, was determined. Figure 6 presents the results of this study with

TABLE II

Effect of Varied Treatment Periods in the Electric Field on the Growth of Red Kentucky Wonder Bush Bean Plants

Time in Field	*Mean Stem Diameter with Standard Deviations of the Mean	*Mean Stem Length with Standard Deviations of the Mean	*Mean Leaf Length with Standard Deviations of the Mean
0 minutes	0.30 ± .02 cm	24.89 ± 2.05 cm	4.60 ± .28 cm
0.5 minutes	0.29 ± .02 cm	17.96 ± 2.62 cm**	3.17 ± .44 cm**
2 minutes	0.32 ± .15 cm	21.75 ± 2.30 cm	4.20 ± .53 cm
10 minutes	0.36 ± .02 cm	27.81 ± 2.36 cm	5.70 ± .33 cm**

*Each figure represents an average of 6 - 9 plants measured 21 days after planting. The seeds were planted 7 days after treatment in the electric field.

**Statistical analysis of the data showed that the difference between the means of the control and treated plants were significant at the 95% level.

TABLE III

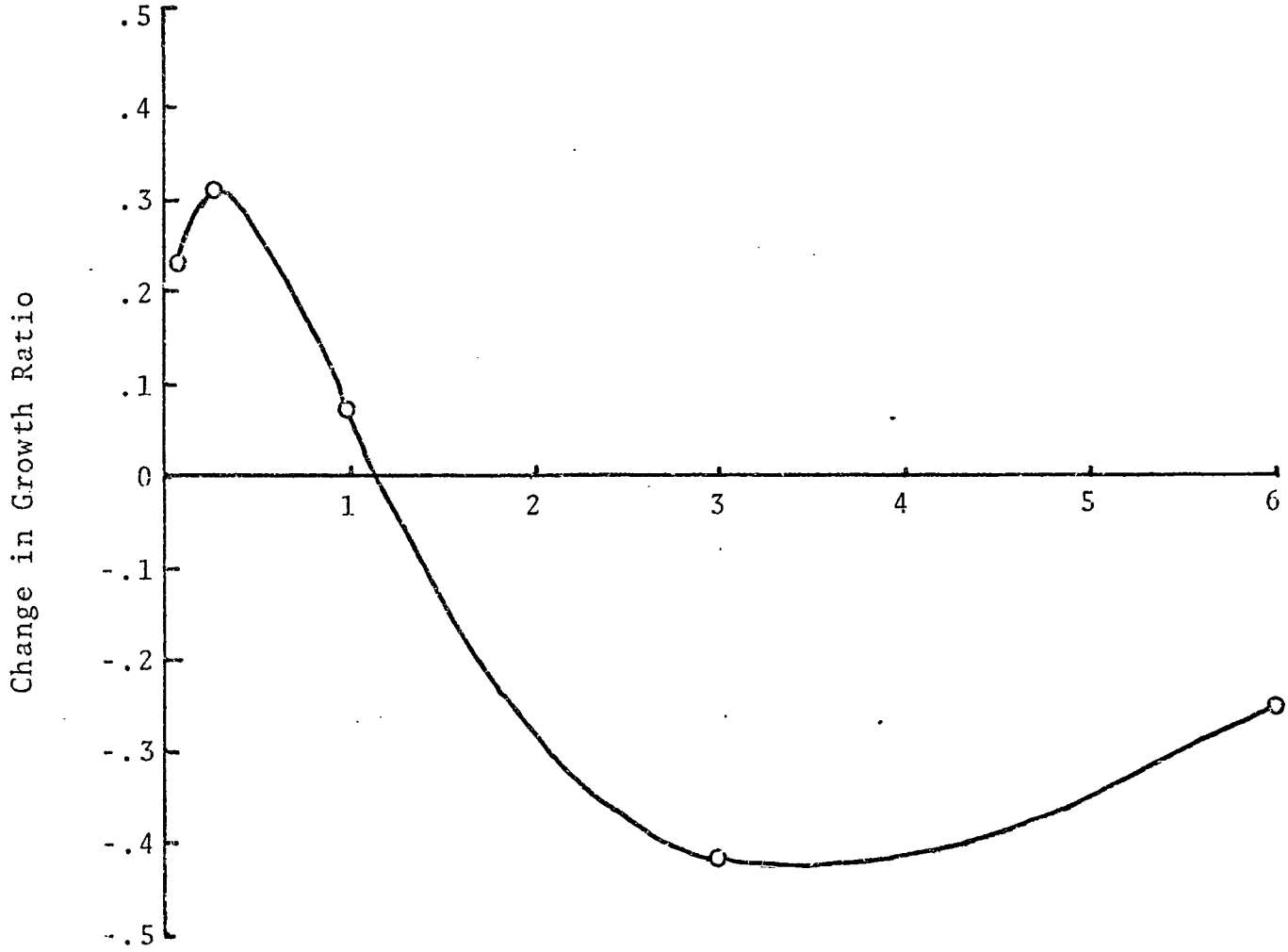
CHLOROPHYLL CONTENT IN PRIMARY LEAVES OF RED KENTUCKY WONDER BUSH BEAN PLANTS

Days between treatment and planting	Mean % increase in length of stem between control and treated plants	Chlorophyll a	Chlorophyll b
7	25	Treated: 0.68 mg Controls: 0.50	Treated: 0.82 mg Controls: 0.54
*14	0	Treated: 0.38 Controls: 0.45	Treated: 0.46 Controls: 0.44
21	23	Treated: 0.59 Controls: 0.47	Treated: 0.64 Controls: 0.47

*These seedlings experienced freezing temperature at emergence.

FIGURE 6

CHANGE IN GROWTH RATIO OF SOYBEAN CALLUS TISSUE AS A FUNCTION OF TREATMENT TIME


.5
.4
.3
.2
.1
0
-.1
-.2
-.3
-.4
-.5
1
2
3
4
5
6
Change in Growth Ratio
Time (in hours) in Electric Field

callus tissue of soybean. The ordinate of each point on the graph is the difference between the mean growth ratios of the treated and nontreated groups. The abscissa is the treatment period which ranged from 2 minutes to 6 hours. Points above the horizontal axis, which represents zero difference between treated and control samples, indicate that the treatment in the field stimulated growth, while points below the line represent groups for which the field retarded growth. Table IV presents the standard deviations for the difference between the means for this data.

2. Effect of initial sample size: It was observed the initial size of the sample had considerable effect on the growth ratio of the sample. Figure 7 illustrates the relation between initial sample size and the resulting growth ratio. Each point on the graph represents the average initial weight and the mean growth ratio for a group. Circles indicate control groups and squares indicate treated groups. It was apparent that overall correlation was independent of the electric field treatment and that a larger growth ratio would be expected from a smaller sample. Hereafter, careful attempts were made to have the initial weight of the sample

TABLE IV

DIFFERENCES IN MEAN GROWTH RATIOS OF SOYBEAN CALLUS TISSUE AS A FUNCTION OF TREATMENT TIME IN THE ELECTRIC FIELD

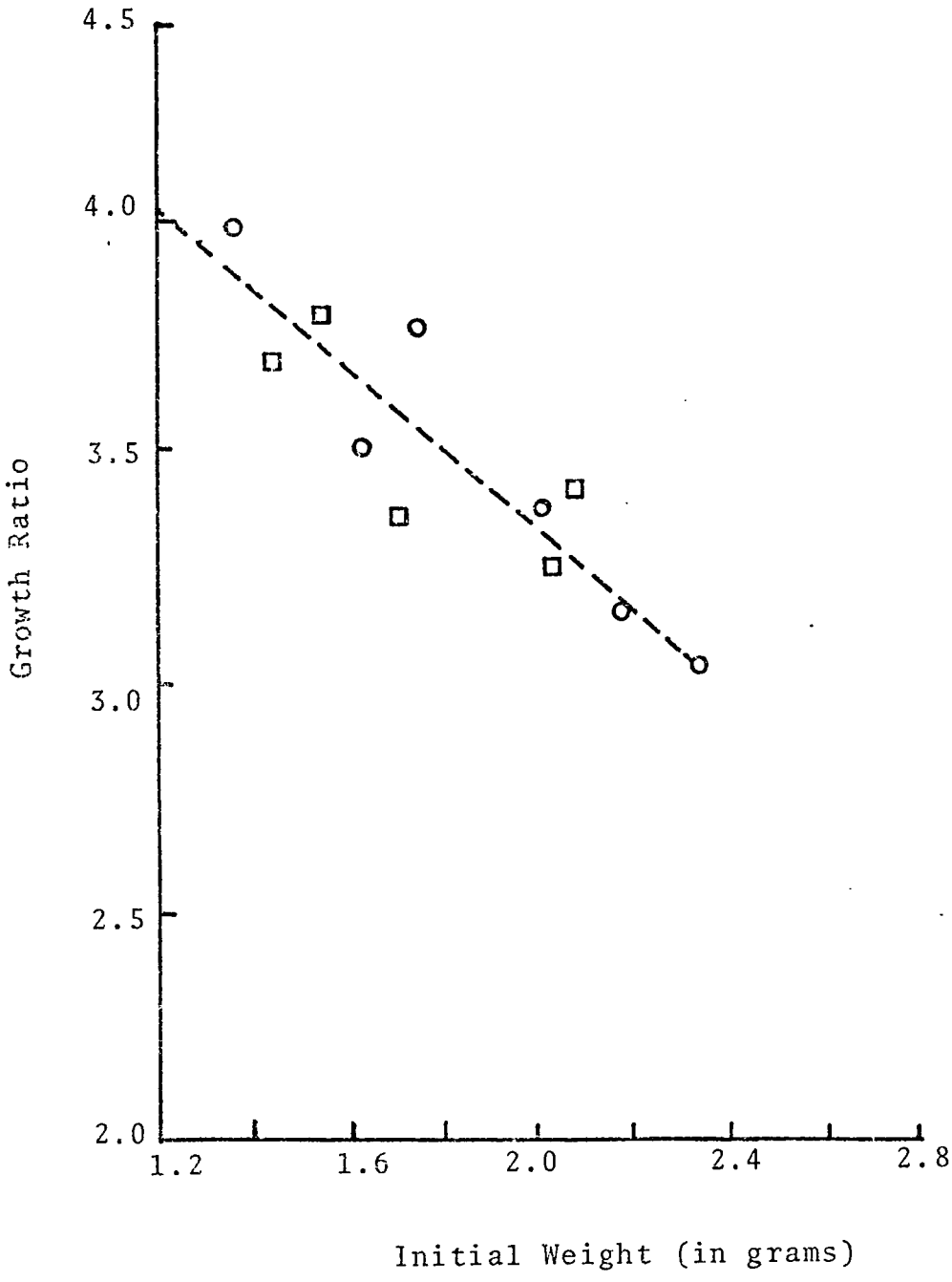
Treatment Period	Mean of Treated Samples	Mean of Control Samples	Difference Between Means with Standard Deviations of the Mean
2 minutes	3.376	3.135	+ 0.24 ± .186
15 minutes	3.793	3.494	+ 0.30 ± .349
60 minutes	3.209	3.135	+ 0.06 ± .252
180 minutes	2.547	3.041	- 0.49 ± .335
360 minutes	3.700	3.921	- 0.24 ± .346

FIGURE 7

GROWTH RATIOS AS A FUNCTION OF SAMPLE SIZE--
BY GROUPS

○ - Control groups

□ - Treated groups


4.5
4.0
3.5
3.0
2.5
2.0
Growth Ratio
1.2
1.6
2.0
2.4
2.8
Initial Weight (in grams)

as similar as possible, and Appendix B demonstrates how the difference due to initial weight was removed from the data in order to observe the effect of the electric field.

3. Lethal period in the electric field: The treatment period was lengthened to 20 and 24 hours to determine if an extended exposure to the electric field would cause the death of the callus tissue. Following a twenty hour treatment period, the change in the mean growth ratios of the treated and control groups was -0.344 indicating that a 20 hour period of exposure depressed the growth of the tissue but not to the extent that had been evident with a three-hour treatment period as seen in Figure 6 which was -.49. The samples treated for twenty-four hours had a mean growth ratio value of -2.7, a 680% decrease in growth when compared to the samples treated 20 hours. In fact, after the two-week growth period none of the samples treated 24 hours gave any visible evidence of growth while four of the control samples had patches of bright yellow-green cells indicating the production of new cells.

II. Survey of Plant Species

One of the major phases of this study was to survey the effect of the electric field on the callus tissue of several different plant species in order to see if the response of soybean tissue could be repeated with other species and if another species might be more responsive to the field. The survey included callus tissue of carrot, pine, tobacco, broad bean, and pea. The broad bean tissue was treated three hours and the pea tissue for two minutes; neither of these tissues showed a difference in mean growth ratios as a result of being exposed to the electric field for these time periods. The mean growth ratios of carrot, pine, and tobacco are given in Figure 8 for various treatment periods. While all three of these tissues demonstrated an effect from the field soybean remained the most consistent in its response.

III. Respiration and Ethylene Studies

A. Treated and control seeds which had been germinating for 60 hours were placed in sealed flasks. The headspace gases in the flasks were analyzed for carbon dioxide and ethylene formation after a thirty minute collection period. Table V shows the percent increase in carbon dioxide content per gram of tissue per hour. Table V also shows the change in ethylene production. A comparison of the amount of water imbibed by the control

FIGURE 8

CHANGE IN GROWTH RATIOS OF VARIOUS SPECIES AS A FUNCTION OF TREATMENT TIME

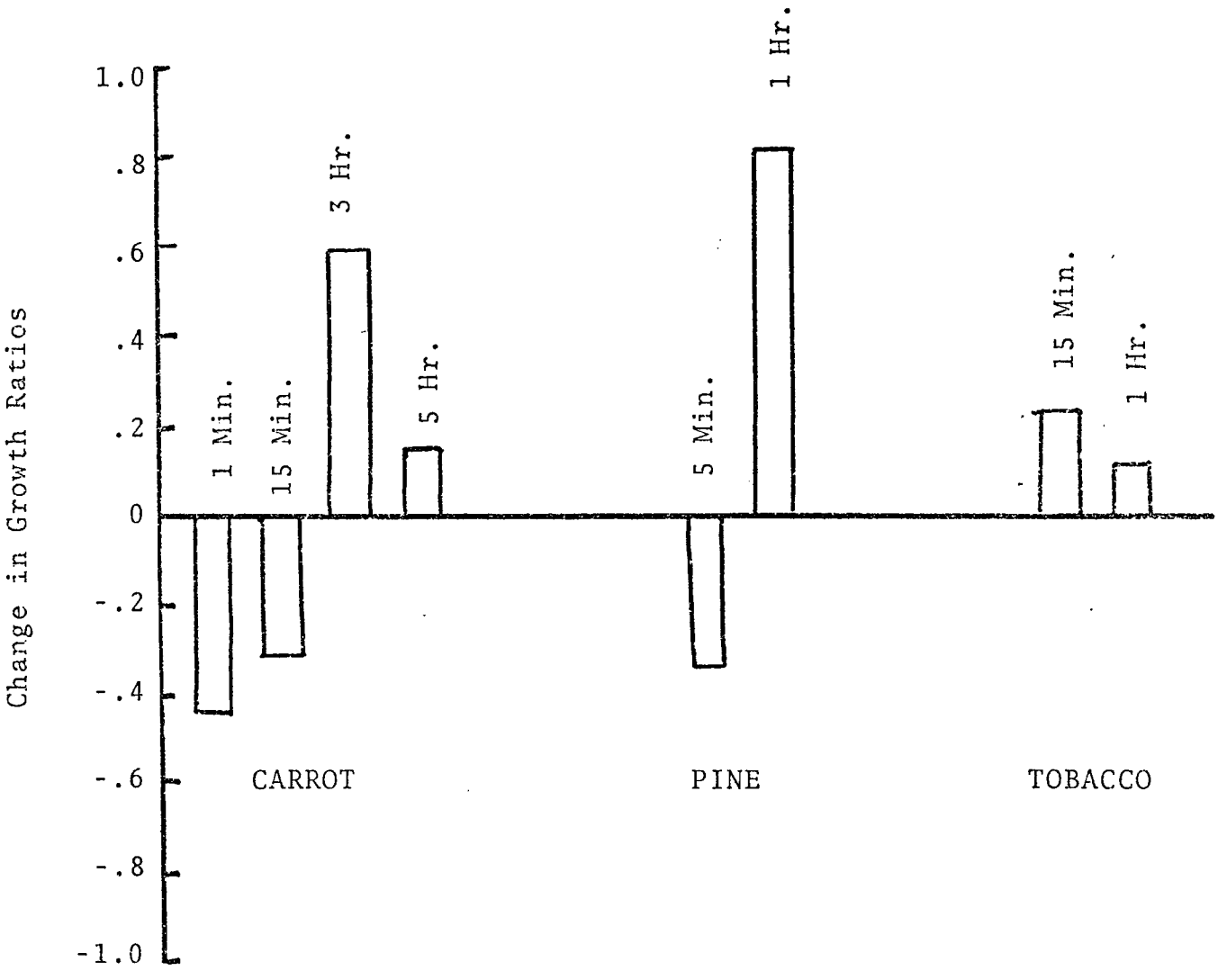

Change in Growth Ratios
1.0
.8
.6
.4
.2
0
-.2
-.4
-.6
-.8
-1.0
1 Min.
15 Min.
3 Hr.
5 Hr.
5 Min.
1 Hr.
15 Min.
1 Hr.
CARROT
PINE
TOBACCO

TABLE V

PER CENT INCREASE IN CARBON DIOXIDE AND ETHYLENE PRODUCTION IN GERMINATING RED KENTUCKY WONDER BUSH BEAN SEEDS DUE TO TREATMENT IN AN ELECTRIC FIELD

Days Between Treatment and Germination	Per Cent Increase* in CO_2 Concentration with 2-Minute Treatment in Field	Per Cent Increase* in Ethylene Production with 2-Minute Treatment
0	167 %	30 %
3	7 %	108 %
7	6 %	38 %

*Determinations made after a 60-hour germination period.

and treated seeds during the 60-hour germination period showed that the electric field had no apparent effect on water uptake during this period.

B. In the second study oxygen and ethylene readings were begun on the first day of germination, and measurements were made at 24-hour intervals for four days. At 24 hours all flasks showed evidence of a small amount of ethylene production. These two flasks contained seeds which had been treated 3 days before the onset of germination. At 48 hours, all were producing ethylene with no apparent difference between control and treated seeds. The oxygen supply was nearly depleted in the two hour period in which the flasks were sealed before the sample was removed indicating that the seeds were respiring rapidly. The respiration rate appeared to be the same for treated and control seeds. On the third day all flasks showed evidence of fungal growth. Analysis of both oxygen uptake and carbon dioxide evolution indicated no difference in respiration rates due to treatment of the seeds in the electric field. The results for ethylene production were eratic, with some treated seeds producing more ethylene than the controls and some much less. Due to the fungal contamination this experiment was terminated. Subsequent attempts to determine an effect of the electric field

on respiration and ethylene production of germination seeds failed to yield consistent results although there was a slight trend for the treated seeds to produce more ethylene.

IV. Cytological Observations

A. Squash preparations of soybean callus tissue were prepared for microscopic examination. The slides were carefully studied for evidence of any cytological damage created by exposure of the tissue to an electric field. Special attention was focused on evidence of swelling or rupturing of cells or cell organelles, distortion of organelles, and unusual alignment of organelles. None of these appeared in the slides examined in tissue treated for one minute or for one hour.

B. Two factors were important in the decision to use root explants rather than soybean tissue culture for a study of mitotic frequency: (1) Considerably difficulty was experienced in preparing slides consisting of only one layer of cells with soybean callus tissue. The use of liquid culture helped to some degree, but the separation was still not sufficient. (2) The percentage of cells undergoing mitosis in tissue culture is very low, usually only six percent (Henshaw, et al., 1966). Root explants of the Alaskan pea were chosen for this study using the well established system of Torrey and Fosket (1970)

in which cell separation is easily attained and the rate of mitosis very high. The results of three replications of this study are given in Table VI; the electric field appears to reduce the frequency of mitosis with this system.

C. In the presence of kinetin, the polyploid cells of the cortex are also stimulated to resume mitotic activity. Torrey and Fosket (1970) report that in the presence of kinetin the cortical cells which begin division on the third day after initiation quickly begin cytodifferentiation at 5-7 days to form xylem tracheary elements. Without kinetin no new tracheary elements are observable within the first several weeks of culture. The system of root explants used in this study exhibited all of these features, and the treatment of the pea roots in the electric field before the initiation process did not appear in any manner to create a change in the system in relation to onset of mitotic activity, stimulation of diploid or polyploid cells to divide, or the differenentation of tracheary elements.

V. Ion Accumulation

The possibility of an electric field changing the uptake ability of plant tissue was pursued by three methods: Atomic fluorescence, electron spin resonance, and ^{32}P uptake.

TABLE VI

EFFECT OF THE ELECTRIC FIELD ON MITOTIC FREQUENCY ON PEA ROOT EXPLANTS

Experiment Number	Treatment Period	Average Number of Mitotic Figures per Slide	Per Cent Difference Between Treated and Control Samples
1	0	27	- 26 %
	1 minute	20	
	0	26	
	60 minutes	20	- 23 %
2	0	31	
	1 minute	30	- 3 %
	0	39	
	60 minutes	34	- 13 %
3	0	28	
	1 minute	25	- 11 %
	0	33	
	60 minutes	32	- 3 %

A. Accumulation of zinc

Three sets of potato discs were prepared and tested to see if treatment in the electric field would alter their uptake of zinc. The results of this experiment are given in Table VII and show that the electric field did cause the potato to have a greater concentration of zinc after the three hour absorption period.

B. Accumulation of other metal ions

Callus tissue of pine and soybean were examined by electron spin resonance spectroscopy to measure the concentration of metal ions in control and treated samples after they had been on their appropriate growth medium for two weeks. Although these results were not highly significant a trend was evident which indicated that the treated samples took up more metal ions than the nontreated ones and that there was a possible correlation between the metal concentration and the growth ratio of the sample. These results are tabulated below:

(1) Pine

(a) Samples treated both one minute and six hours in the electric field accumulated more metal ions than the nontreated samples.

(b) Those samples treated for six hours accumulated considerably greater amounts of metal ions than those treated for one minute.

TABLE VII

EFFECT OF THE ELECTRIC FIELD ON ZINC ACCUMULATION IN POTATO DISCS

Experiment Number	Absorption Solution	Treatment Period	Zinc Present in Tissue Expressed in Parts per Millon
1	$CaCl_2$	0	0.142
	$ZnSO_4$	0	0.279
	$ZnSO_4$	1 hour	0.294
2	$CaCl_2$	0	0.147
	$ZnSO_4$	0	0.290
	$ZnSO_4$	1 hour	0.305

(c) The mean growth ratio for the control samples was 2.42. The mean growth ratio for the samples treated for one minute was 2.67 and that for the samples treated for six hours was 1.97. This represents a 10% increase in growth with a one minute treatment period and a 19% decrease in growth with a six hour treatment period.

(2) Soybean

(a) Samples treated three hours in the field exhibited considerably greater metal ion accumulation than the control samples.

(b) Those samples treated 15 minutes showed the same or somewhat less ion accumulation than the control samples.

(c) The mean growth ratio for the samples treated 15 minutes was 6.54 and for those treated 3 hours, 5.53. This represents a 25% increase in growth with a 15 - minute treatment period and a 5% increase with a 3 - hour treatment period.

C. Phosphorus uptake

The initial experiments with ^{32}P were carried out by placing callus tissue of carrot on S. V. Medium (Appendix A) to which ^{32}P had been added. Forty samples

were weighed and placed on the solid medium. Twenty of these samples had been placed in a 60 hertz electric field with a peak strength of 3000 v/cm for three hours. Treated and control samples were removed from the medium after 24 and 48 hours, weighed, and counted with an autogamma counter. This experiment was repeated reducing the time of contact with the medium to only one hour. The results of these two experiments are presented in Table VIII. To further explore the use of ^{32}P as a monitor of the effect of the electric field, potato discs were substituted for the callus tissue for the following reasons: (1) The system for phosphorus uptake in storage tissue is well established (Laties, et al., 1964; Loughman, 1960; Osmond and Laties, 1968; and Barber, 1972.) (2) The large size of a potato tuber would provide all the discs needed for a single experiment; hopefully this would eliminate some of the variability experienced with callus tissue.

1. Determination of Optimum period in the electric field: Preliminary experiments established that the greatest absolute per cent change in uptake between treated and nontreated samples occurred with a treatment period of one hour. This is illustrated in Figure 9. Note that there was a change in direction of the effect of the field at 90 minutes in the field; the

TABLE XIII

EFFECT OF THE ELECTRIC FIELD ON THE PHOSPHORUS UPTAKE OF SOYBEAN CALLUS TISSUE TREATED 15 MINUTES IN THE FIELD

Counts per minute/gram tissue		Per Cent Difference Between Samples
Controls	Treated	
64,073	26,070	-59
59,486	34,160	-43
57,035	30,825	-46
51,026	30,045	-41
Mean		
57,750 $\pm$ 28,917	30,000 $\pm$ 3,266	

Rank Correlation Coefficient: -0.200

Significance Level of Correlation: None

Regression Correlation Coefficient: 0.2746

Significance Level of Correlation: None

difference between the uptake of control and treated samples was not further increased by extending the treatment period beyond 180 minutes.

2. Determination of optimum ^{32}P absorption period: For potato discs, absorption periods less than two hours produced eratic results. Figure 10 illustrates that absorption periods of 3, 6, 12, 24, 48, and 72 hours produced more consistent results; again note the change in the direction of the effect of the field when the absorption period was extended beyond 6 hours. An absorption period of three hours was selected for subsequent experiments.

3. Determination of optimum pH of absorption solution: Hagen and Hopkins (1955) and Welch (1973) state that a change in the pH of the external solution will alter the rate of anion uptake by plant tissue. The change in uptake is exemplified by a decrease in uptake as the pH of the external solution increases. Figure 11 shows that the system used in this study followed their same pattern. The points on the graph represent the averages of triplicate experiments. Further experimentation illustrated that the pH of the external solution greatly changed the response of the potato discs to the electric field in relation to their ability to take up phosphorus.

FIGURE 9

DETERMINATION OF THE OPTIMUM TREATMENT PERIOD IN THE ELECTRIC FIELD FOR POTATO DISCS

Three hour absorption period, pH of external solution 4.7,

● - Control samples

○ - Treated samples

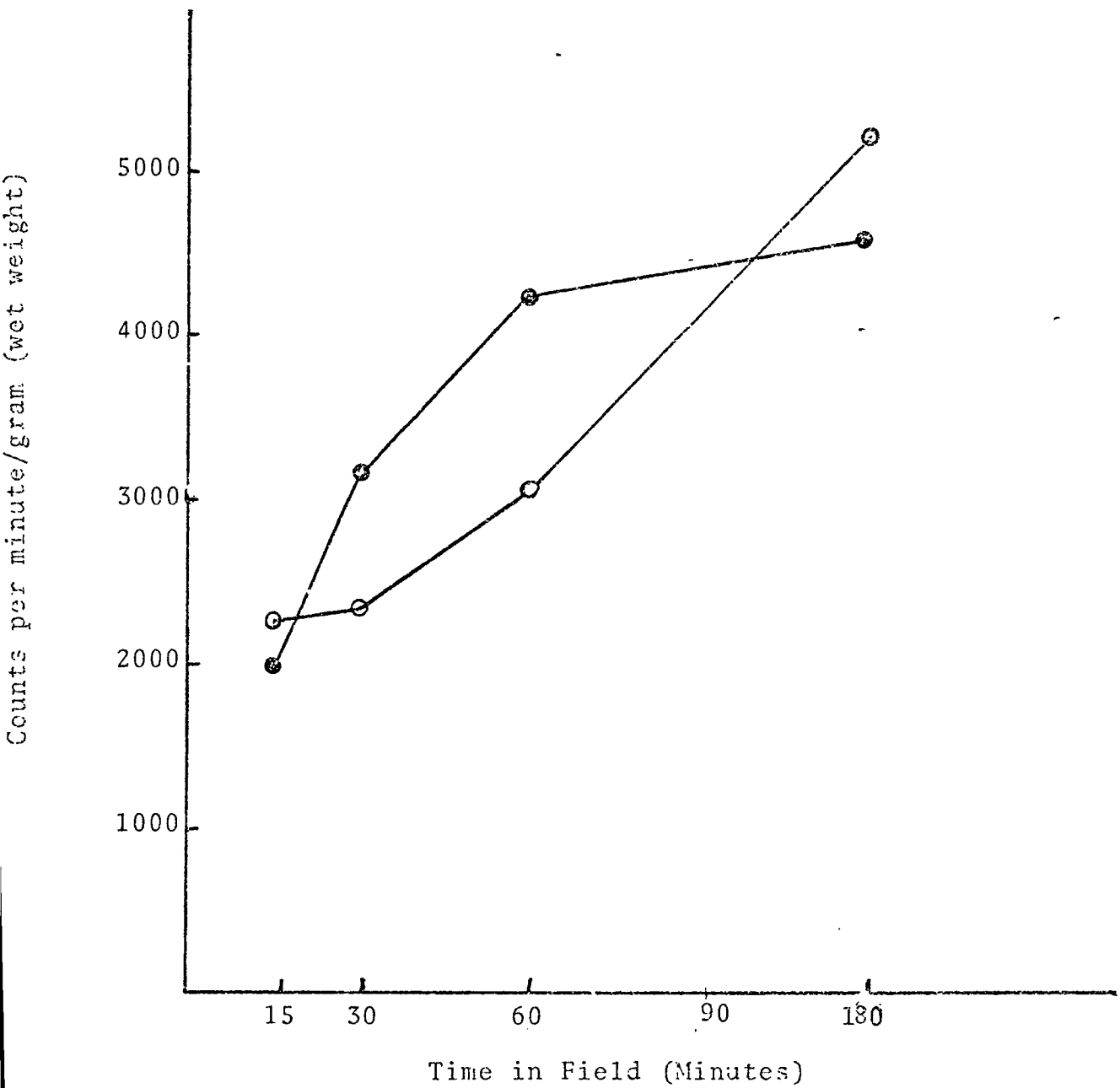
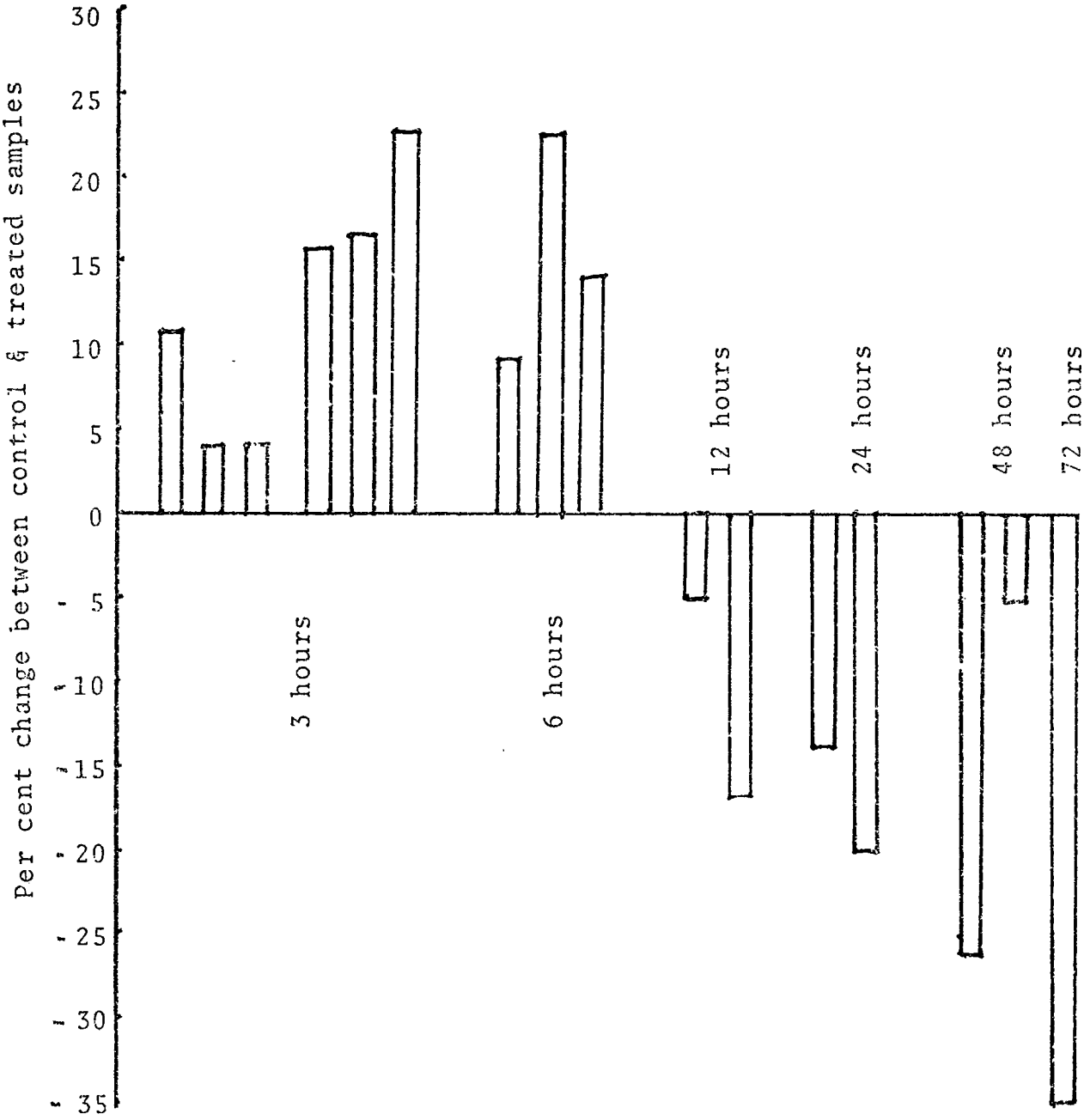

Counts per minute/gram (wet weight)
5000
4000
3000
2000
1000
15
30
60
90
180
Time in Field (Minutes)

FIGURE 10

DETERMINATION OF THE OPTIMUM PHOSPHORUS ABSORPTION PERIOD FOR POTATO DISCS

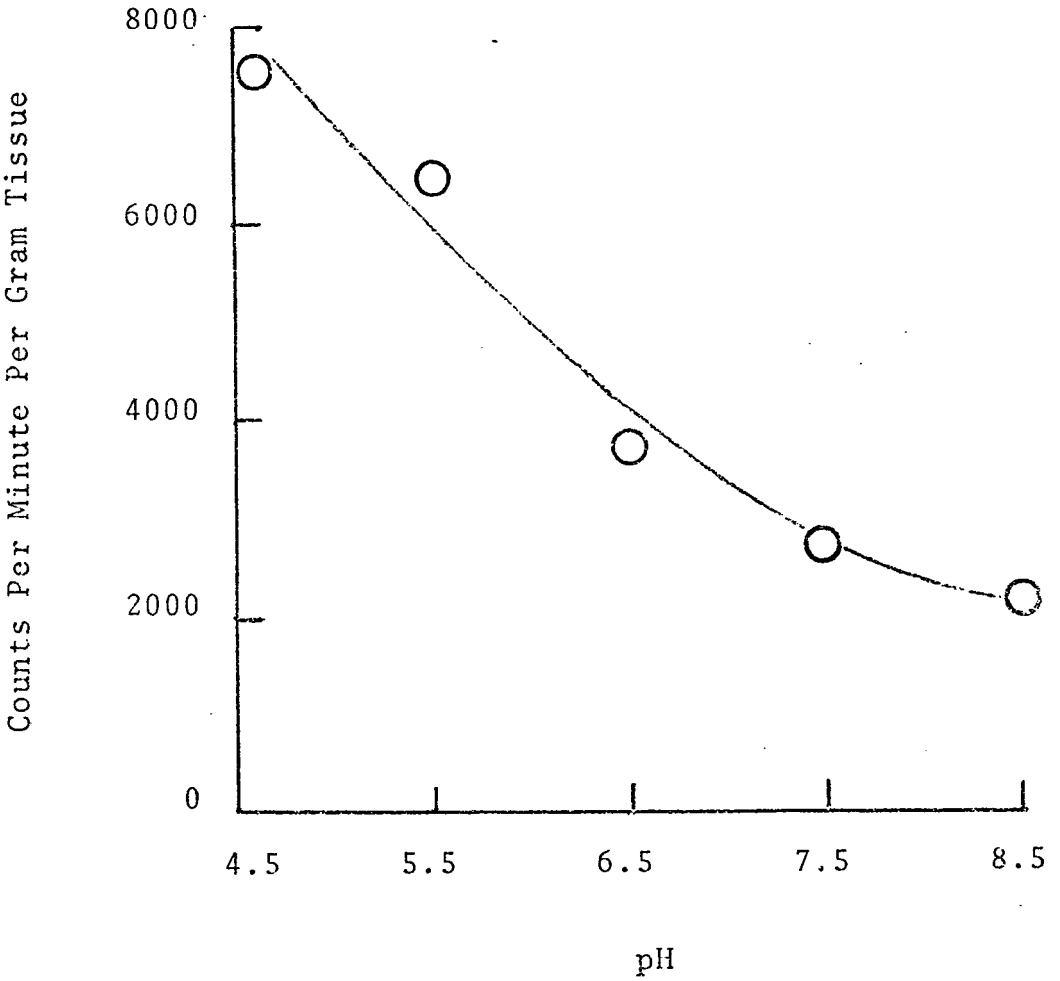
Bars extending above the horizontal line indicate that the treated samples absorbed more ^{32}P than the control samples; bars extending below the horizontal line indicate that the treated samples absorbed less ^{32}P than the control samples.



Time in field one hour, pH of external solution 4.7

FIGURE 11

CHANGE IN UPTAKE OF PHOSPHORUS BY POTATO DISCS RELATED TO CHANGES IN pH


Counts Per Minute Per Gram Tissue
8000
6000
4000
2000
0
4.5
5.5
6.5
7.5
8.5
pH

This change is shown in Table IX. Table X shows that the pH of the external KH_2PO_4 solution changed during the absorption period. The remaining experiments were conducted at a pH of 4.7 for the following reasons: (1) The basic system reported in the literature for ^{32}P uptake studies employ a pH of 4.7 since at that pH 99% of the KH_2PO_4 is in the $H_2PO_4^-$ form (Hagen and Hopkins, 1955). (2) The effect of the electric field in this pH range caused a considerably difference in uptake capacities between the treated and control samples (26 - 50%). (3) The change in the pH of the external solution during the absorption period at higher pH values would extend into a range where the effect of the field would be reversed during the absorption period. At pH 4.7 the final pH is near 5.7, a pH in which the direction of the effect of the field is the same as at pH 4.7.

4. Determination of the optimum volume of the absorption solution: The results of this study indicated that for a three hour absorption period one liter of solution was a minimum volume which would prevent an excessive change in pH and a depletion of phosphorus at a concentration of 10^{-5} M KH_2PO_4. A volume of 250 ml was used for those experiments using a 10^{-2} M KH_2PO_4 absorption solution.

TABLE IX

EFFECT OF CHANGING THE pH OF THE EXTERNAL ABSORPTION SOLUTION ON PHOSPHORUS UPTAKE BY POTATO DISCS TREATED IN AN ELECTRIC FIELD

Experiment Number	pH of the external solution	% difference in ^{32}P uptake between treated* and control samples
1	4.5	- 22**
	5.5	- 50
	6.5	+ 10
	7.5	+ 61
2	4.5	- 29
	5.5	- 20
	6.5	+ 29
	7.5	+ 16
	8.5	+ 7
3	4.5	- 26
	5.5	- 35
	6.5	+ 20
	7.5	+ 42

*Samples treated one hour in the electric field.

**A negative sign indicates that the uptake of the treated samples was less than that of the controls; a positive sign indicates that the uptake of the treated samples was more than that of the controls. These signs will be used in the same manner throughout this study.

TABLE X

CHANGE IN pH OF THE EXTERNAL SOLUTION DURING THE THREE HOUR ABSORPTION PERIOD WITH POTATO DISCS

Experiment Number	Initial pH	Final pH
1	4.5	5.7
	5.5	6.9
	6.5	7.7
	7.5	8.0
2	4.5	5.2
	5.5	7.0
	6.5	7.7
	7.5	8.1
	8.5	8.0
3	4.5	5.6
	5.5	6.8
	6.5	7.3
	7.5	7.8

VI. Use of Paired Samples

Throughout all of the various methods of investigations reported in this study thus far, there has been sufficient evidence to indicate that an externally applied field altered many of the parameters measured. Those altered include growth ratios of callus tissue, size of mature plants, chlorophyll content in leaves, uptake of zinc, metal ions, and phosphorus. The major difficulty encountered in each of these was that the effect on the factor being measured was not consistent from one experiment to another, and at times not consistent within the same experiment. This problem led to a continuing search for a more reliable system, refined techniques, and better methods for ensuring that the biological materials used were as similar as possible in all respects. A careful review of the data led to the conclusion that the use of "paired samples" might provide the system needed. This involved taking a small section of callus tissue, aseptically dividing it into halves, treating one half and allowing the other to serve as the control. The parameter measured was the amount of ^{32}P incorporated during a three-hour absorption period. As many paired samples as possible were prepared from one stock callus with careful precautions being made to select the small clumps so that the tissue within each clump appeared to be uniform in color and texture. Although visually alike, it was recognized that this did not ensure

that all areas of a single clump were behaving alike metabolically. It is well established that callus tissue has different centers of activity scattered throughout (Caplin, 1947). The reason for using very small clumps was to decrease the possibility of including areas of different metabolic activities. Over a period of several months, this type of experiment was repeated numerous times using the callus of various plant species. The results obtained from these experiments confirmed that the parent callus contained tissues with very different activities. There was such a large variation in phosphorus uptake from one small clump to another that to view the data in groups made it impossible to obtain statistically significant differences between the control and treated groups. Table XI illustrates this phenomenon. However, a closer scrutiny of this experiment revealed some interesting trends including:

(1) The greatest amount of variation appeared among the control samples. In the experiment presented in Table XI, a difference of 892,145 cpm per gram existed between the control having the highest and the lowest uptake of phosphorus. This represented a difference of 241%. In comparison, a difference of 141,441 cpm per gram existed among the treated samples, a difference of 20.4%.

(2) Any attempt to average the control and treated groups to obtain a mean cpm per gram or per cent difference tended to erase or greatly reduce the apparent effect of the field that is evident by examining the paired samples individually.

(3) If the data were rearranged in order of decreasing amount of phosphorus taken up according to the uptake of the control sample of a pair, a definite trend was evident; the electric field tended to have more effect on those samples at the extreme ends of the absorption range and very little effect on those in the middle range. This is illustrated by rearranging the data from Table XII.

This trend was further verified by the experiment presented in Table XIII in which all of the controls had similar uptake capacities for phosphorus, and the effect of the field on all four samples was very similar. Thus the electric field appeared to have a consistent effect on phosphorus uptake if the samples were similar initially, as indicated in this experiment by the similarity in phosphorus uptake by the control samples. Tables XIV through XX illustrate that this phenomena was apparent with the callus tissue of several plant species which adds considerably to the significance of this approach.

TABLE XI

EFFECT OF THE ELECTRIC FIELD ON THE PHOSPHORUS UPTAKE OF SOYBEAN CALLUS TISSUE

Counts per minute/gram tissue		Per Cent Difference Between Samples
Controls	Treated*	
639,628	604,478	- 6
522,024	576,570	+10
1,262,693	607,545	-52
439,815	692,980	+58
608,919	600,453	- 1
370,448	551,429	+49
789,714	583,682	-26
Mean		
661,892 ± 112,802	602,448 ± 16,800	
Per Cent Deviation		
17%	3%	

*Treated 15 minutes in electric field.

TABLE XII

DATA FROM TABLE XI REARRANGED IN ORDER OF DECREASING AMOUNTS OF PHOSPHORUS UPTAKE BY THE CONTROL SAMPLES

Counts per minute/gram tissue		Per Cent Difference Between Samples
Controls	Treated	
1,262,693	607,545	-52
789,714	583,682	-26
639,628	604,478	- 6
608,919	600,453	- 1
522,024	576,570	+10
439,815	692,980	+58
370,448	551,429	+49

Rank Correlation Coefficient: 0.3714

Significance Level of Correlation: None

Regression Correlation Coefficient: -0.0112

Significance Level of Correlation: None

TABLE XIII

EFFECT OF THE ELECTRIC FIELD ON THE PHOSPHORUS UPTAKE OF SOYBEAN CALLUS TISSUE TREATED 15 MINUTES IN THE FIELD

Counts per minute/gram tissue		Per Cent Difference Between Samples
Controls	Treated	
64,073	26,070	-59
53,486	34,160	-43
57,035	30,825	-46
51,026	30,045	-41
Mean		
57,750 $\pm$ 28,917	30,000 $\pm$ 3,266	

Rank Correlation Coefficient: -0.200

Significance Level of Correlation: None

Regression Correlation Coefficient: 0.2746

Significance Level of Correlation: None

TABLE XIV

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF CARROT CALLUS TREATED THREE HOURS IN THE FIELD AND IN CONTACT WITH ^{32}P-CONTAINING SOLID MEDIUM FOR FORTY-EIGHT HOURS

Counts per minute/gram tissue		Per Cent Difference Between Pairs
Controls	Treated	
767,201	602,973	-21
765,988	534,928	-30
670,257	604,861	-10
568,355	517,241	- 9
434,518	536,586	+23
420,422	698,188	+66
Mean		
604,000 ± 156,000	581,833 ± 67,801	

Rank Correlation Coefficient: -0.26

Significance Level of Correlation: None

Regression Correlation Coefficient: -0.2694

Significance Level of Correlation: None

TABLE XV

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF CARROT CALLUS TREATED THREE HOURS IN THE FIELD AND IN CONTACT WITH ^{32}P-CONTAINING SOLID MEDIUM FOR ONE HOUR

Counts per minute/gram tissue		Per Cent Difference Between Pairs
Controls	Treated	
83,212	75,358	- 9
44,142	60,738	+38
32,039	37,027	+16
30,931	39,129	+27
Mean		
47,250 + 24,622	52,750 $\pm$ 18,118	

Rank Correlation Coefficient: 0.8

Significance Level of Correlation: 95%

Regression Correlation Coefficient: 0.9335

Significance Level of Correlation: 95%

TABLE XVI

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF PEA CALLUS TREATED FOR ONE HOUR AND IN ^{32}P-CONTAINING ABSORPTION SOLUTION FOR THREE HOURS

Counts per minute/gram tissue		Per Cent Difference Between Pairs
Controls	Treated	
149,254	59,837	-60
125,200	33,643	-73
32,002	33,636	+ 5
17,438	27,801	+ 6
Mean		
80,973 $\pm$ 32,981	38,729 $\pm$ 7,169	

Rank Correlation Coefficient: 1.00

Significance Level of Correlation: 99%

Regression Correlation Coefficient: 0.7631

Significance Level of Correlation: 95%

TABLE XVII

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF PEA CALLUS TREATED FOR ONE HOUR AND IN ^{32}P-CONTAINING ABSORPTION SOLUTION FOR THREE HOURS

Counts per minute/gram tissue		Per Cent Difference Between Pairs
Controls	Treated	
36,687	33,613	- 8
23,519	24,677	+ 5
20,133	21,274	+ 6
Mean		
26,779 $\pm$ 5,049	26,521 $\pm$ 3,679	

Rank Correlation Coefficient: 1.0

Significance Level of Correlation: 99%

Regression Correlation Coefficient: 0.9979

Significance Level of Correlation: 95%

TABLE XVIII

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF PEA CALLUS TREATED FOR ONE HOUR AND IN ^{32}P-CONTAINING ABSORPTION SOLUTION FOR ONE HOUR

Counts per minute/gram tissue		Per Cent Difference Between Pairs
Controls	Treated	
23,993	7,328	-70
10,349	5,247	-49
4,117	3,365	-18
Mean		
12,820 ± 10,165	5,313 ± 1,982	

Rank Correlation Coefficient: 1.0

Significance Level of Correlation: 95%

Regression Correlation Coefficient: 0.9833

Significance Level of Correlation: 95%

TABLE XIX

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF TOBACCO CALLUS TREATED 30 MINUTES AND IN ^{32}P-CONTAINING ABSORPTION SOLUTION FOR ONE HOUR

Counts per minute/gram tissue		Per Cent Difference Between Pairs
Controls	Treated	
11,753	3,448	-71
2,204	3,684	+67
1,791	2,467	+38
1,437	2,562	+78
Mean		
4,296 ± 4,981	3,048 ± 615	

Rank Correlation Coefficient: 0.6

Significance Level of Correlation: Name

Regression Correlation Coefficient: 0.4886

Significance Level of Correlation: None

TABLE XX

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF TOBACCO CALLUS TREATED 2 MINUTES AND IN ^{32}P-CONTAINING ABSORPTION SOLUTION FOR THREE HOURS

Counts per minute/gram tissue		Per Cent Difference Between Pairs
Controls	Treated	
12,171	4,931	-59
3,944	4,348	+23
3,848	4,014	+ 4
2,676	3,394	+27
Mean		
5,660 ± 4,379	4,172 ± 642	

Rank Correlation Coefficient: 1.0

Significance Level of Correlation: 99%

Regression Correlation Coefficient: 0.8591

Significance Level of Correlation: 95%

VII. Alteration of Phosphorus Uptake by Changing the Environment of the Tissue

The study with paired samples revealed that samples from a single parent plant callus had a wide range of phosphorus uptake capacities. Evidently the microenvironment within the callus was not the same throughout so that all parts of the callus were not alike metabolically. This metabolic difference was manifested through the wide range in phosphorus uptake capacities. This led to the speculation that a change in the environment would induce different metabolic activities within the tissue which would be reflected by different phosphorus uptake capacities. If the environmental change caused the tissue to have greatly increased or decreased abilities of phosphorus uptake, the tissue should respond to the electric field to a greater extent.

Two experiments already completed demonstrated this change in response to the field as the environment of the tissue was changed. These experiments are listed below.

A. Change in Temperature

In the growth studies of the Red Kentucky Wonder Bush Bean a 20°F lower nighttime temperature during the germination period altered the effect of the field in two respects:

1. Germination rates: The electric field did not effect the germination rate when the nighttime low was between 55 - 61°F. With a nighttime low of 33° C for three consecutive nights during the germination period, the treated seeds began to germinate five days before the first nontreated seed began to emerge from the soil.

2. Size of mature plants: The plants grown from treated seeds were considerably larger than those grown from untreated seeds for both sets of seeds which were germinated with nighttime temperatures between 55 - 61° one set being planted 7 days following treatment and the other 21 days following treatment. The third set, which was planted 14 days after treatment, experienced the low nighttime temperatures and the size of the mature plants from the treated seeds in this set were essentially the same size as the plants from untreated seeds.

B. Change in pH of the Absorption Solution

With potato discs a change in the pH of the absorbing solution completely reversed the effect of the field on the uptake of radioactive phosphorus. Preliminary work with the effect of pH on phosphorus uptake from a 10^{-5} M KH_2PO_4 solution with untreated potato discs showed that as the pH of the solution changed from 4.5 toward neutrality, the amount of ^{32}P taken up greatly

decreased. The effect of the electric field on phosphorus uptake through this pH range was to reverse this trend. At a pH of 4.5 the field caused a 26% reduction in uptake. At a pH of 6.5 the effect was reversed so that the treated discs showed an increased uptake of about 20%, and at a pH of 7.5 the treated discs were absorbing 50% more phosphorus. At a pH of 8.5 the stimulation of the field was reduced and the treated discs absorbed only 5% more phosphorus than the controls.

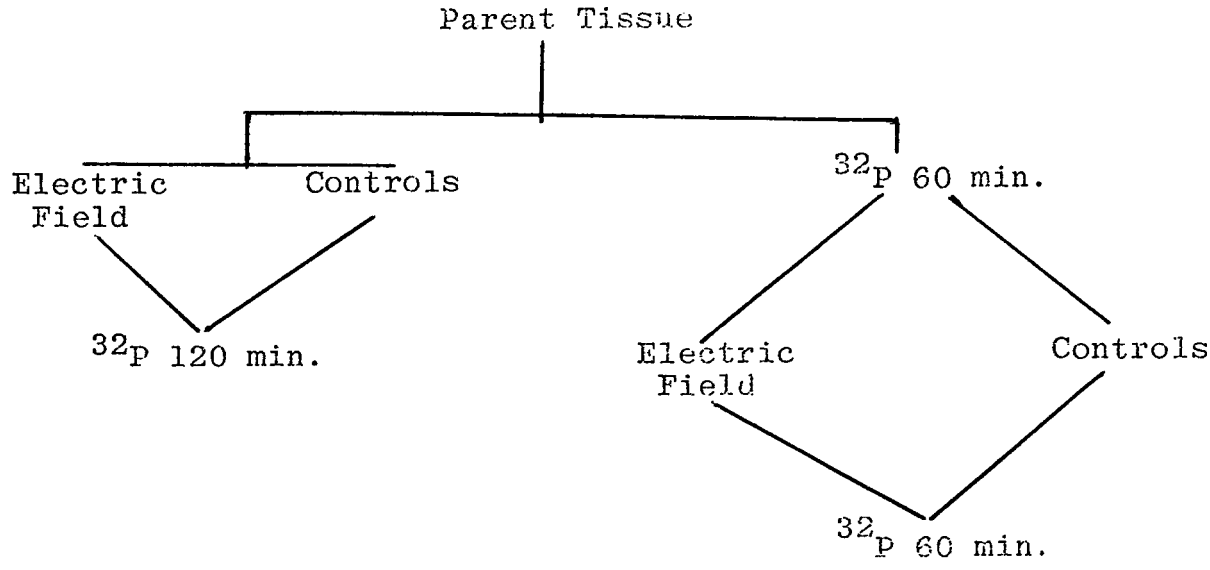
Two additional experiments were designed to confirm the fact that a change in the environment of the tissue would induce a change in response of the tissue to the electric field.

C. Change in Osmotic Pressure

Potato discs were soaked 60 minutes in solutions of differing salt concentrations to induce different states of turgidity within the cells. All solutions contained 0.5 mM $CaCl_2$ to maintain membrane integrity. The treated discs placed in the distilled water plus $CaCl_2$ showed a 10% decrease in phosphorus uptake when compared to the controls. Those treated discs placed in a 1% salt solution demonstrated an 11% decrease in uptake. Those treated discs placed in a 10% salt solution showed only a 2% decrease in uptake capacity when compared to control discs in a 10% salt solution.

C. Submerging the Tissue in Liquid

Two parallel series of experiments were carried out. In Series I the tissue was treated in the electric field, placed in the 10^{-5} M KH_2PO_4 absorption solution for a selected period, and then the counts per minute per gram tissue determined. In series II, the tissue was first placed in the absorption solution for half of the selected absorption period, removed and treated in the electric field, returned to the solution to complete the absorption period, and finally counted in a gas-flow counter. The treatment period and the total absorption period were the same for series I and II. This scheme is diagrammed below:



1. Carrot callus: Four parent pieces of carrot callus (A,B,C, & D) were divided into four samples each, one pair of samples followed series I and the other pair followed series II of the above scheme. Table XXI presents the results of series I with a one hour treatment in the electric field occurring before the 60 minute submersion in the absorption solution. Table XXII presents the results from series II in which the tissue was submerged 30 minutes before the one hour field treatment, and then returned to the absorption for the final 30 minutes. The results in Table XXI reveal that the four parent callus tissues had cpm/g phosphorus uptake in the range of 2000-3000 cpm/g. The difference in response to the field of all four samples was very similar. The results in Table XXII showed that if the tissue was submerged in liquid before treatment in the field, the tissue changed its response to the field and became much more sensitive to the electric field.
2. Potato discs: This experiment was repeated using potato discs in place of callus tissue. Four separate tubers were used (A,B,C, & D). A and B were visually alike; potato C was much greener in color; and potato D was considerably dehydrated. A core was removed from each tuber and four sets of discs (seven

TABLE XXI

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF CARROT CALLUS

WITHOUT INITIAL SUBMERSION PERIOD

Sample	Controls	Treated 1 hour	% Difference between control and treated samples
A - 1	3,157 cpm/g	3,193 cpm/g	+ 1.0
A - 2	2,437	2,456	+ 0.8
B	2,406	2,155	-10.0
C	3,296	2,879	-13.0
D	2,015	1,688	-16.0

TABLE XXII

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF CARROT CALLUS WITH INITIAL SUBMERSION PERIOD

Sample		Initial 30 minute absorption period	Treatment period	Total counts from initial and final absorption periods	% Difference between the pair
A	1	1,188 cpm/g	Control	2,621 cpm/g	
	2	1,806	1 hour	4,709	+80
B	1	1,626	Control	3,049	
	2	2,050	1 hour	5,982	+96
C	1	1,905	Control	4,642	
	2	2,297	1 hour	3,843	-17
D	1	1,351	Control	3,960	
	2	2,368	1 hour	7,115	+80

discs per set) were prepared from each core. Two sets from each core followed series I of the scheme and two sets from each core followed series II. With the potato tissue the treatment period in the electric field was kept at one hour, but the absorption periods were increased from 30 minutes each to 60 minutes each. Table XXIII gives the results from the series I experiments and Table XXIV gives the results from series II. The discs from all four tubers responded in the same manner to the electric field when it was applied before submersion in liquid; the tissues showed an increased phosphorus uptake ability as a result of the electric field treatment. However, if the field was not applied until after the tissue had been placed in the absorption solution for one hour, the effect of the field was reversed and the treated samples demonstrated a greatly reduced uptake ability when compared to the nontreated samples.

VIII. The results from tissue culture and the potato disc experiments suggested that tissues undergoing different physiological activities might respond differently to an externally applied electric field. To further explore this possibility, three experiments were done selecting tissue known to be undergoing different metabolic activities. The results of these experiments are given below.

TABLE XXIII

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF POTATO DISCS

WITHOUT THE INITIAL SUBMERSION PERIOD

Tuber	Controls	Treated 1 hour	% Difference between the control & treated samples
A	712 cpm/g	976 cpm/g	+37
B	829	914	+10
C	457	610	+33
D	581	763	+32

TABLE XXIV

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF POTATO DISCS WITH THE INITIAL SUBMERSION PERIOD

Sample		Initial 60 minute absorption period	Treatment period	Total counts from initial and final absorption periods	% Difference between the pair
A	1	443 cpm/g	Control	1,308 cpm/g	
	2	417	1 hour	770	-41
B	1	286	Control	761	
	2	203	1 hour	897	+18
C	1	384	Control	670	
	2	154	1 hour	448	-33
D	1	191	Control	742	
	2	181	1 hour	583	-21

A. Fresh and Aged Potato Tissue

There are numerous reports in the literature that the washing, or aging, of plant storage tissue changes greatly the metabolic activities of the tissue including the capacity of that tissue to absorb ions (Tang and Castelfrance, 1968; Leonard and Hanson, 1972; Beileski, 1966; Luttge, 1967; Rains and Floyd, 1970; Loughman, 1960; and Beileski and Laties, 1963). The tissue was aged by washing the potato discs for 24 hours in 5 x 10^{-4} M $CaCl_2$ at room temperature with continuous aeration. The washing was carried out with sterile techniques to prevent contamination of the discs. (Table XXV confirms that the system used in this study produced this same increase in phosphorus uptake with aging.) The application of an electric field to both fresh and aged tissue from the same potato tuber showed that the electric field produced different effects on fresh and aged tissue. This is shown in Table XXVI.

B. Meristematic and Elongating Root Tissue

Intact roots of germinated broad bean roots were treated 15 minutes in an electric field. The last 15 mm of the root tip from treated and nontreated seeds were excised and divided into two segments -- a d mm segment containing meristematic tissue and a 12 mm segment containing predominantly elongating tissue. Both types of tissue

TABLE XXV

EFFECT OF AGING ON PHOSPHORUS UPTAKE WITH POTATO DISCS

Experiment Number	Tissue	Counts per minute/mg
1	Fresh	9
	Aged	45
2	Fresh	9
	Aged	33
3	Fresh	7
	Aged	36
4	Fresh	9
	Aged	43

TABLE XXVI

EFFECT OF THE ELECTRIC FIELD ON PHOSPHORUS UPTAKE OF FRESH AND AGED POTATO TISSUE

Experiment Number	Tissue	% Difference in ^{32}P uptake between control & treated samples
1	Fresh	+ 0.1
	Aged	+18.0
2	Fresh	- 6.0
	Aged	+13.0
3	Fresh	- 1.6
	Aged	-23.0
4	Fresh	-11.0
	Aged	- 6.0

responded to the electric field with an increase in phosphorus uptake, but the meristematic tissue was stimulated to a much greater extent. This is illustrated in Table XXVII.

C. Tissue from Roots of Different Lengths

Experiments were designed to measure the uptake of phosphorus by the last 15 mm of the roots of germinated broad beans to determine if the length of the intact root had an effect on the ability of this 15 mm segment to take up phosphorus. The speculation that this might be true was based upon the fact that depending upon the original length of the root, the last 15 mm of the root tip would be composed primarily of meristematic and elongating tissue or meristematic, elongating and differentiating tissue. Table XXVIII gives the results of this experiment in which the roots were in the radioactive solution for one hour before counting the activity of the excised 15 mm segments. After determining that the length of the root did make a difference in the ability of the last 15 mm of the root to take up the phosphorus, the experiment was extended to find out if the response to the electric field would be different in roots of different lengths. The results from four experiments revealed that the length of the root did not seem related to the response to the electric field

TABLE XXVII

EFFECT OF THE ELECTRONIC FIELD ON PHOSPHORUS UPTAKE OF MERISTEMATIC AND ELONGATING TISSUE OF INTACT BROAD BEAN ROOTS

Experiment Number	Tissue	% Difference in ^{32}P uptake between control and treated samples
1	Meristematic	+259
	Elongating	+ 42
2	Meristematic	+240
	Elongating	+ 59

TABLE XXVIII

PHOSPHORUS UPTAKE BY BROAD BEAN ROOTS

OF DIFFERENTIAL LENGTHS

Experiment Number	Average Length of Root	Counts per minute per gram tissue
1	long	43,456
	short	9,936
2	long (6.7 cm)	109,469
	medium (4.0 cm)	46,622
	short (1.7 cm)	10,344
3	medium (3.8 cm)	53,617
4	long (4.8 cm)	146,958
	medium (2.6 cm)	57,654
	short (1.5 cm)	41,882

measure by phosphorus uptake unless the original length of the intact root was less than 1.9 cm. In these extremely short roots, there was consistently a reduction of 52%.

DISCUSSION

DISCUSSION

The results obtained in this study strongly support the hypothesis that an electric field does effect the growth of plants and plant tissue. Reproducibility of results, however, has been a most difficulty standard to achieve. This obstacle has appeared not only in our study but has been reported in the very earliest literature and has probably been one of the greatest deterrents for a continuing research effort in this area. Two major books which review the research on the effects of electric fields emphasize this factor. In his historical review in BIOELECTRIC FIELDS AND GROWTH (1948) Lund states: "Since this first work many investigators have studied the effects of electricity, making use of a variety of experimental techniques and reporting a variety of results, many of them conflicting." In ELECTROMAGNETIC FIELDS AND LIFE (1970) edited by Presman, Parin states in the foreward: "Of course, not all the views expressed in this book are sufficiently well substantiated and some are quite controversial." Individual papers almost always indicate, either indirectly through presentation of their data or directly by statements from their authors, that problems of inconsistent effects from the electric field existed somewhere within their work. (Dudgeon, 1912; Hendrick, 1918; Shrank, 1959; Murr, 1963; Sidaway, 1966; Murr, 1966; Mihalyfi & Serf, 1967; Sidaway & Asprey, 1968; and Sidaway, 1970.)

After becoming convinced that an external electric field did in fact effect the growth of plants, the emphasis of this study was directed toward developing a system which would produce consistent results. The basic assumption was that the effects of the field were being masked and distorted by flaws in the experimental design and that a more controlled environment and reduction in variability of the biological materials would clarify the effect of the field on the plant tissue. The work with Red Kentucky Wonder Bush Beans had demonstrated that a change in temperature could change the effect of the electric field on germination of seeds and the final size of the mature plant. Sidaway and Asprey (1968) report that part of their inconsistent results in measuring the effect of an electric field on the respiration of Vicia faba cotyledons might be due to day-to-day variation in weather conditions. Thus the study was moved to the laboratory with a more controlled environment than the greenhouse. Within the lab, work continued with respiration and ethylene studies on germinating seeds of Red Kentucky Wonder Bush Bean. Inconsistency of the effect of the electric field on these parameters was still evident and attributed to the genetic variability existing from seed to seed.

The decision to replace whole plants with plant callus tissue was an attempt to improve both environmental control and biological variation. Tissue culture samples were grown in a growth chamber with temperature and light control. Callus

cultures were initiated from a single plant organ and were subcultured to produce a large amount of callus stock from the same parent stock. Although definite trends could be observed as a result of exposing the callus tissue to the electric field, the response of the tissue was not always consistent from one experiment to another or even within one experiment.

The next step was to survey the effect of the electric field on the callus tissue of several plant species with the hope of finding a species which would respond more consistently to the field than the soybean which was used initially. Although, the various species responded to the field, the response was not more consistent than the soybean callus tissue.

Another attempt to increase the reproducibility of the experiments was to reduce the initial size of the callus sample to eliminate the necessity of using three or four jars of parent callus tissue. With smaller samples one parent callus would provide sufficient material for ten treated and ten control samples which was expected to reduce the variation from one sample to another. Unfortunately, no significant improvement in consistency was gained with this measure.

Increasing the number of samples in each group in order to improve the significance of the results statistically seemed the next logical step. However, increasing the number of individuals in each group to 20 and even 30 did not increase the significance level in the difference between the mean of the

control and treated groups. A close examination of the growth ratios of invididual samples revealed that the large variation was found not only in the treated groups, but was present within the control group as well and even to a greater extent than in the treated group. Such variation made it virtually impossible to statistically show that a change in the mean growth ratio between the treated and control groups was due to the effect of the electric field. Yet it was clearly evident that some individuals within the treated group were being strongly effected by the external electric field.

A re-examination of the experimental procedure was definitely in order. Although callus tissue is a more homogeneous mixture of plant cell types (predominantly parenchymtous cells (Street, 1966) than a whole plant, one section of callus tissue will not necessarily be identical throughout. The heterogeneity occurs because, with time, some cells of the callus will become palyploid (Partanen, 1963). Some will form centers of meristematic activity (Gautheret, 1957), and some will differentiate into more mature plant cell types--such as xylem vessels (Halperin, 1973). Unfortunately, it is presently impossible to visually detect these areas of special activity. The large variation in phosphorus uptake and growth ratios within the control group indicated that the samples were not similar physiologically even though visually they appeared alike.

To correct this situation paired samples were used in which a small section of callus tissue was separated from the parent callus. Hopefully, the sample would be small enough to contain essentially the same physiological type of tissue throughout. This sample was divided into two pieces--one to be treated in the electric field and the other to serve as the control. Thus, results from an average of a large number of individuals were replaced with a comparison of one treated sample with its matched control.

The results obtained with the use of paired samples in studying phosphorus uptake brought about a major revision in the approach to our problem. The paired samples technique allowed a most reliable fact to be recognized--the multiple effects of the field and the inconsistency of the results were the key to the effect of the field rather than the problem to be removed. Thus, while the field did stimulate other samples, the effect was not at all haphazard. Those samples which demonstrated an extremely high capacity for phosphorus uptake had this capacity greatly reduced by exposure to the electric field. Those treated samples whose controls had an extremely low capacity for phosphorus uptake had their uptake capacities stimulated. Those control samples with uptake capacities at neither extreme were not effected by the electric field.

The observation of the varied effects of the field on the tissue led to the formulation of a hypothesis of how the electric field might be interacting with the biological tissues. It appeared that there was an "equilibrium" state in the parent callus for phosphorus uptake, the term "equilibrium" being used to indicate those samples not displaying extremely high or low uptake capacities. From studies with the paried samples, the majority of the callus tissue appeared to be in this equilibrium state, and the electric field altered the phosphorus uptake abilities of the samples only to a small degree. The effect of the field on these samples with extremely high or low uptake capacities was to drive them toward this equilibrium state. The overall effect was to reduce the amount of variation seen within the treated group; this is illustrated in Table XXIX. Table XXIX emphasizes again that the effect of the field was not always apparent if the data is presented as an average of the group; the effect of the field was to cause the samples to move toward this average uptake capacity, thus reducing the variation within the group. Rank correlation analysis revealed that there was no significant correlation between the rank of the highest cpm/g to the lowest cpm/g of the control groups and that of the treated group. The only cases in which a significant correlation existed occurred in experiments containing only 3 or 4 paired samples. Determination of the regression correlation coefficient also showed no linear corre-

TABLE XXIX

EFFECT OF THE ELECTRIC FIELD ON DECREASING THE VARIABILITY OF PHOSPHORUS UPTAKE BY CALLUS TISSUE AS A RESULT OF DRIVING THE EXTREME SAMPLES TOWARD THE EQUILIBRIUM REGION

*Table in Results chapter	Range of uptake in cpm/g tissue	Mean Uptake	Standard Deviation of the mean	Percent deviation for each group
XI. Controls	1,262,693 370,448	661,892	112,802	17.04%
Treated	692,980 551,429	602,448	16,800	2.79
XIV. Controls	767,201 420,422	604,000	156,000	10.50
Treated	698,188 534,928	581,833	67,801	4.55
XVI. Controls	149,254 17,438	80,973	32,981	40.73 18.51
Treated	59,837 27,801	38,729	7,169	18.51
XVII. Controls	36,687 20,133	26,779	5,049	18.85
Treated	33,613 21,274	26,521	3,679	13.87

TABLE XXIX CONTINUED

EFFECT OF THE ELECTRIC FIELD ON DECREASING THE VARIABILITY OF PHOSPHORUS UPTAKE BY CALLUS TISSUE AS A RESULT OF DRIVING THE EXTREME SAMPLES TOWARD THE EQUILIBRIUM REGIONS

*Table in Results chapter		Range of uptake in cpm/g tissue	Mean Uptake	Standard Deviation of the mean	Percent deviation for each group
XX.	Controls	12,171 2,676	5,660	4,379	38.68
	Treated	4,931 3,394	4,172	642	7.91

*Table XI represents data from soybean tissue.
Table XIV represents data from carrot tissue.
Table XVI represents data from pea tissue.
Table XVII represents data from pea tissue.
Table XA represents data from pea tissue.

lation between the count rates of the control and treated samples, except in those experiments containing only 3 or 4 paired samples. (The procedure for determining rank correlation coefficients and linear regression coefficients are presented in Appendix C.) The results of the rank correlation analysis and the regression analysis were interpreted to indicate that the variation present in the treated group represented random scatter about a mean and that the uptake capacity of the tissue after treatment was not dependent upon the original uptake capacity of the tissue.

While the electric field may interact with the cells of the plant tissue in many aspects, the data from our study of phosphorus uptake using ^{32}P, zinc uptake measured by atomic fluorescent spectrophotometry, and metal ion uptake measured by electron spin resonance spectrophotometry indicated that membrane permeability is one of the factors effected. That an electric field might alter membrane permeability is not a new idea. Research with artificial membranes (Hill, 1957; Klotz and Horowitz, 1957; Friedenberg, 1967; Adekman, 1971; and Babakov, Ermishkin and Liberman, 1966) and with biological membranes (Hamilton and Sale, 1967) have demonstrated that this phenomena occurs.

The data from our study provided the basis for the construction of a model which describes how the electric field might be interacting with the constituents of the membrane to produce the effects seen in this study. The model is based on

immediately adjacent to the lipid bilayer. He assumes with his model that the dipoles are free to rotate within certain constraints. He stated: "thus the polar groups may be perpendicular or may rotate into an external field depending on constraints." Friedenberg further stated that with sheets of artificial membranes, there are points of high field intensity at various locations under the sheet and that "these points would move if any motion were imparted to the membrane. Thus, a vibrating membrane would move the points of symmetry in a regular manner." He speculated that in cells these points of symmetry are due to charged molecules located near the phospholipid layer and that the dipoles of the phospholipids are orientated toward the center nearest to them. Thus under naturally occurring conditions within the cell, the charge centers may change causing rearrangement of the orientation of the dipoles.

This dipole model provides a good basis for explaining the actions of the electric field in driving phosphorus uptake toward an equilibrium state as seen in this study. The equilibrium state would correlate with the state in which the activities of the tissue create a symmetrical arrangement of "charge centers" due to the regular distribution of macromolecules and electrolytes near the membrane. This would establish the "equilibrium" uptake rate. The electric field would have very little effect on this tissue because the "charge centers" are already regularly arranged and the agitation of the dipoles would not create any great change in

two factors: (1) The field drives the permeability of the membrane for phosphorus toward an "equilibrium" uptake capacity, and (2) the force of the a.c. field used in this study was sufficient to cause an agitation of dipole groups.

The effect of an a.c. field on a dipolar molecule is to cause the molecule to "flip" itself 60 times per second in order to maintain the proper alignment with the changing polarity of the field. Within the membranes, however, the dipoles are not free to "flip" since their movement is constrained by their attachment to a larger molecule. The resulting compromise is a tilting, or rotating, of the dipoles in response to the field. Studies on the orientation of the lipid molecules within artificial monolayer and bilayer-phosphate lipid membranes have produced theoretical calculations that the polar phosphate group on their hydrocarbon chains have room to tilt in alignment with an externally applied electric field (Friedenberg, 1967, p. 24). Goldman (1964) formulated a membrane model for axons of nerve cells in which the phospholipids change their orientation under the influence of an electric field. His model included the orientation effects of the dipoles in the polar end groups of the phospholipid bilayer. Friedenberg proposed that Goldman's model could be extended to all cells, and thus proposed a dipole model for cell membranes. According to this model (Friedenberg, 1967, p. 31) the dipoles of the phospholipids orient themselves toward a charge center created by the proteins and electrolytes

the redistribution of these centers. However, we speculate that tissue exhibiting extremely high or low uptake abilities has the "charge centers" distributed in a very irregular manner which changes the membrane's permeability to phosphorus and also cuases it to be most sensitive to the external electric field. The effect of the field is to cause motion, or agitation, within the membrane due to the rotation of the dipoles; this motion in turn drives the "charge centers" toward a more symmetrical distribution thus causing the tissue to move toward the equilibrium range of phosphorus uptake.

The effect of the electric field--to drive a system toward an equilibrium status--is clearly seen with phosphorus uptake in callus tissue and potato discs. Table XXX demonstrates that the same effect can be seen in the growth rate of callus tissue. As with phosphorus uptake, statistical analysis shows no rank correlation between the growth ratios of the treated and control pairs, no linear correlation between the percent difference between the pairs and the growth ratios of the treated samples, but there is an inverse correlation between the percent difference between the pairs and the growth ratios of the control samples significant at the 1% level.

In a review article "Permeability of the Plant Cell" (Stadelman, 1969) the statement is made that: "Permeability changes may be caused by endogenous conditions within cells or by natural or artificial changes in the environment." We propose

TABLE XXX

EFFECT OF THE ELECTRIC FIELD ON THE GROWTH RATIOS OF UNPAIRED SAMPLES OF BROAD BEAN CALLUS TISSUS

Pair number	Growth ratio of control samples	Growth ratio of treated samples	Per-cent difference between the pair
1	2.00	1.49	-26
2	1.87	1.62	-13
3	1.83	1.65	-10
4	1.59	1.64	+ 3
5	1.22	1.51	+23
6	1.08	1.45	+33

that the externally applied electric field produces its effect by agitation of the dipolar components of the membrane which causes the redistribution of the charge centers created by macromolecules adjacent to the membrane. This artifically produced redistribution of the charged macromolecules in turn brings about a change in the alignment of the dipole within the membrane once the tissue is removed from the electric field. The realignment of the dipoles in the membrane has a consequent influence on membrane permeability. Two macromolecules which might be involved in the charge centers are the proteins in ribosomes which are attached to the membrane (Tanada, 1968) and the phytochromes localized within or on the membrane (Jaffe, 1968, and Tanada, 1968). In reference to the phytochromes Tanada suggests that "...changes in the molecular conformation of phytochrome may induce local changes in the electric charges of the membrane consequently effecting membrane permeability."

Therefore, it should be possible to demonstrate that the effect of the field can be altered by (1) changing the environment of the tissue bringing about a different relation to the "equilibrium" state of uptake and (2) selecting tissue known to be in different stages of development that would require different phosphorus uptake capacities. Four experiments in this study created a change in the external environment which brought about a correpsonding change in the effect produced by the electric field. These are described below:

1. Change in temperature: Thirty Red Kentucky Wonder Bush Bean seeds were all treated at the same time but were planted 7, 14, and 21 days after treatment. Both groups of plants grown from treated seeds planted 7 and 21 days following treatment were considerably larger than the control plants. However, those treated seeds planted 14 days after treatment were essentially the same size as the control plants in all respects. The only significant variable during this study as a temperature change during the germination period of the 14-day group. The night temperature during this period was about 20°F lower than that of the 7 and 21-day groups during their corresponding period of germination. Another difference between the 14-day and the 7- and 12-day groups was in the germination rate. The germination rate was the same for treated and nontreated seeds in the 7- and 21-day groups. In those seeds plants 14-days after treatment the treated seeds began to emerge five days before the first control seed. Thus the reduced temperature altered two aspects of this experiment. It eliminated any effect of the field as far as the final size of the plant was concerned, but it altered the effect on germination rate.
2. Change in pH: With potato discs a change in pH of the absorbing solution completely reversed the effect of the field on the uptake of the radioactive phosphorus. At a low

pH (4.5) the field caused a 26% reduction in uptake. At a pH of 6.5 the field effect was reversed so that the treated discs showed an increased uptake of about 20% above that of the untreated discs. At a pH of 7.5 the treated discs were absorbing 50% more. At 8.5 the stimulation of the field dropped so that the treated discs absorbed only 7% more ^{32}P than the controls.

3. Change in osmotic pressure: Potato discs were soaked 60 minutes before treatment in the electric field in solutions of differing salt concentration to induce different states of turgidity within the cells. All solutions contain 5 x 10^{-4} M $CaCl_2$ to maintain membrane integrity. The treated slices placed in distilled water showed a 10% decrease in phosphorus uptake when compared to the controls. Those treated discs placed in a 1% salt solution demonstrated a 11% decrease and those treated discs placed in a 10% salt solution showed only a 2% decrease in uptake capacity.

4. Submerging the tissue in liquid: Carrot callus and potato discs were submerged in an absorbing solution before treatment in the electric field and then returned for a final absorption period. Similar samples of both tissues were treated in the field before being placed in the absorbing solution. The total amount of time in the absorbing solution was the same for the continuous and interrupted absorption periods. Comparing the callus samples that were treated before submersion and those that were treated after

submersion showed that the latter had an increased absorption capacity averaging 146% above those treated before submersion. With the potato discs the uptake capacities were reduced among the treated samples by an average of 24% if the discs were first submerged before treatment. Thus, the submersion of both of these tissues increased the sensitivity of the tissue to the electric field but the field effected them oppositely.

Three experiments can be cited in which tissues of different physiological states were selected.

1. Potato discs which were "aged" by washing 24 hours in an aerated solution of 5×10^{-4} M $CaCl_2$ showed an increased sensitivity to the field when compared to fresh discs. In two cases the aged tissue demonstrated an increased uptake of 18% and in two other experiments the uptake was decreased 22% and 5% in the aged tissue. This is in agreement with Sidaway and Asprey (1968) who found an increased sensitivity to the effect of a d.c. field on oxygen absorption by washed spadix tissue slices of Arum as compared to unwashed slices.
2. Roots of germinated broad bean seeds were grouped according to total root length after 5 days of germination. Untreated root tips taken from roots less than 1.9 cm in length consistently demonstrated a greatly reduced ability to take up phosphorus as compared to those roots longer than 1.9 cm. Treatment of these short roots

caused an average 52% decrease in the absorption capacity.

3. That portion of the root tip which is engaged in meristematic activity have their capacity to absorb phosphorus effected by an electric field to a much greater extent than that portion primarily concerned with activities associated with elongation. The meristematic tissue exposed to an electric field demonstrated 245% increased uptake above the controls while the elongating regions were stimulated to take up 51% more phosphorus than the controls.

SUMMARY

SUMMARY

The controversial issue of the effect of externally applied high voltage electric fields on plant tissue was explored. After exposure to an alternating field with a peak potential of 15,000 volts, the plant material was removed from the field and examined to determine the response of the tissue to the field. Parameters measured throughout the course of the study included growth rate, chlorophyll content, respiration rate, ethylene production, cell differentiation, chromosome number, organelle distortion, germination rate of seeds, metal ion uptake, mitotic frequency, and phosphorus uptake.

The results indicated that the growth rate of plants grown from seeds placed in an electric field was effected by this treatment. Most studies concerning growth rates had been carried out with continued exposure of the plants to the field for several days or weeks. In this study the seeds of Red Kentucky Wonder Bush Beans were treated, removed from the field, sowed, and allowed to grow under standard greenhouse conditions. The effect of the field was evident even when the seeds were planted 21 days after treatment in the electric field.

For the first time callus tissue of plants were treated in an electric field and examined for alterations in growth rate. Several species were studied and all exhibited some response to the field. Each species, however, had its own optimum treatment

period to produce the greatest response. The callus tissue of the following species were studied: Glycine soja (soybean), Nicotiana tabaccum (habituated tobacco), Pinus elliotti (slash pine), Pisum sativum (Alaskan pea), Vicia faba (broad bean), and Daucus carota (carrot). Within the design of the experimental system employed with callus tissue, cell differentiation, changes in chromosome number, and organelle integrity did not appear to be significantly effected by the electric field. There was an indication, however, that the electric field might cause a reduction in the rate of mitosis in root explant tissues. With seeds, the rate of germination, respiration rate, ethylene production, and chlorophyll content of the leaves grown from treated seeds were somewhat effected by the field, but the results did not permit a conclusive statement about the field effect on these processes.

Phosphorus uptake by callus tissue, potato discs, and root tips was clearly effected by the electric field. Studies with atomic fluorescence and electron spin resonance indicated that the effect on ion uptake may be extended to other ions as well.

A major contribution of our study was the establishment of the fact that the reported contradictory effects of the electric field on plants were a true reflection of the interaction of the plant material and the electric field. The interaction brings about a move toward an "equilibrium" state in relation to the parameter being examined, i.e. phosphorus uptake

and growth rate in our study. Whether the field brought about a stimulation or reduction in uptake or growth depended on the relationship of the sample to its equilibrium state at the time of treatment. Because of the difficulty in determining the state of the sample before treatment, the paired samples techniques proved to be the most effective method for this study in which the control exhibited a high degree of variation. Paired samples allowed the detection of those tissues being effected by the field and consequently allowed the formulation of the hypothesis that the field caused a movement toward an equilibrium state.

A model was constructed to describe at the molecular level the manner in which the electric field might interact with the polar phosphate groups within the membrane to bring about a movement toward an equilibrium uptake of phosphorus. Further experiments demonstrated that the effect of the field on phosphorus uptake could be altered by changing the environment of the tissue before or after treatment. Examples of altered environments causing changes to response to the field and later to growth included studies with pH, nighttime temperatures during germination, osmotic concentration within cells, submerging tissues in liquid and aging tissues. The response to the field also differed only if those tissues of the same callus or plant were selected which were known to be engaged in different metabolic activities. These experiments further supported our supposition that the variation in response to an

electric field seen in our work as well as that of others is a reflection of variable physiological activities of the tissue at the time of treatment. The effect of the electric field appeared to cause the tissues to become more homogeneous in growth rate or phosphorus uptake resulting in a reduction of variability in the treated samples.

APPENDIX A

WHITE'S 10X MEDIUM*

Tissues cultured on medium: Habituated Tobacco pH: 5.5-6.0

	Stock Solution, gm/liter	Stock Solution ml/1 liter of medium	Final concentration, mg/liter
(1) Macroelements			
$Ca(NO_3)_2 \cdot 4H_2O$	6.0		3000.0
Na_2SO_4	4.0		2000.0
KNO_3	1.6		800.0
KCl	1.3	500.0	650.0
$NaH_2PO_4 \cdot H_2O$	0.33		165.0
$MgSO_4 \cdot 7H_2O$	14.4		7200.0
Microelements			
(2) $ZnSO_4 \cdot 7H_2O$	3.0		30.0
$MnSO_4 \cdot 4H_2O$	7.0		70.0
H_3BO_3	1.5		15.0
KI	0.75	10.0	7.5
$CuSO_4 \cdot 5H_2O$	0.001		0.01
$Na_2MoO_4 \cdot 2H_2O$	0.25		2.5
(3) $FeCl_3$	1.5	1.0	1.5
Vitamins and Amino acids			
(4) Glycine	3.0		3.0
Thiamine HCl	0.5		0.5
Nicotinic acid	0.5	1.0	0.5
Pyridoxine	0.5		0.5

Thirty grams of sucrose and ten grams of agar were added for a final concentration of 3% and 1% respectively for every liter of medium prepared.

*(White, 1943).

S. V. MEDIUM

Tissues cultured on medium: Daucus carota, Glysine soja, Vicia Faba — pH: 5.5-6.0

		Stock Solution, gm/liter	Stock Solution, ml/liter of medium	Final concentration mg/liter
Macronutrients				
(1)	NH_4NO_3	82.5	20.0	1650.0
	KNO_3	95.0		1900.00
(2)	$CaCl_2 \cdot 2H_2O$	88.0	5.0	440.0
(3)	$MgSO_4 7H_2O$	74.0	5.0	370.0
	KH_2PO_4	34.0		170.0
(4)	Na_2EDTA	7.45	5.0	37.3
	$FeSO_4 \cdot 7H_2O$	5.57		27.8
Micronutrients				
(5)	$MnSO_4 \cdot H_2O$	4.5		4.5
	$ZnSO_4 \cdot 7H_2O$	1.5		1.5
	H_3BO_3	1.5		1.5
	$CuSO_4 \cdot 5H_2O$	0.04		0.04
	$Na_4MoO_4 \cdot 2H_2O$	0.25	1.0	0.25
	$CoCl_3 \cdot 6H_2O$	0.005		0.005
	$AlCl_3$	0.003		0.003
	$NiCl_2 \cdot 6H_2O$	0.003		0.003
	KI	0.001		0.001
Vitamins and Amino acids				
(6)	Nicotinic acid	0.5	1.0	0.5

		Stock Solution, gm/liter	Stock Solution, ml/liter of medium	Final concentration mg/liter
	Thiamine HCl	0.1	1.0	0.5
	Pyridoxine HCl	0.1		0.1
	Glycine	3.0		3.0
(7)	NAA	0.175	1.0	0.175
(8)	2.4-D	0.221	1.0	0.221
(9)	Ascorbic Acid	50.0	1.0	50.0

In addition, each liter of medium contained 1 gram of yeast extract, 100 ml of coconut milk, 30 grams of sugar and 10 grams of agar.

MODIFIED MURASHIGUE AND SKOOG MEDIUM (MM-1)

Tissue cultured on medium: *Pinus Elliottii* pH: 5.7-5.8

	Major Solution	Stock Solution, gm/liter	Stock Solution, ml/liter of medium	Final concentration mg/liter
(1)	NH_4NO_3	16.5		1650.0
	KNO_3	19.0	100.0	1900.0
	$CaCl_2 \cdot 2H_2O$	3.31		440.0
	$MgSO_4 \cdot 7H_2O$	3.7		370.0
	KH_2PO_4	1.7		170.0
(2)	Na_2-EDTA	14.90	10.	37.3
	$FeSO_4 \cdot 7H_2O$	11.16		27.8
	Minor Solution			
(3)	HBO_3	6.2		6.2
	$MnSO_4 \cdot 4H_2O$	22.3		22.3
	$ZnSO_4 \cdot 4H_2O$	8.6	1.0	8.6
	KI	0.83		0.83
	$Na_2MoO_4 \cdot 2H_2O$	0.25		0.25
	$CuSO_4 \cdot 5H_2O$	0.025		0.025
	$CoCl_2 \cdot 6H_2O$	0.025		0.025
	Vitamins and Amino Acids			
(4)	Nicotinic acid	0.5		0.5
	Thiamine HCl	0.1		0.1
	Pyridoxine HCl	0.1	1.0	0.1
	Kinetin	0.5		0.5

	Stock Solution, gm/liter	Stock Solution, ml/liter of medium	Final concentration, mg/liter
(5) 2,4-D			
(6) Inositol			
(7) Asparagine			
(8) Ascorbic acid			

Thirty grams of sucrose and ten grams of agar were added for a final concentration of 3% and 1% respectively for every liter of medium prepared.

MODIFICATION OF MEDIUM BY TORREY AND FOSKET (1970)

Tissue cultured on medium: Pisum sativum pH: 5.5

Major Solution		Stock Solution, gm/liter	Stock Solution, ml/liter of medium	Final concentration mg/liter
(1)	KNO_3	95.0	20	1900
	NH_4NO_3	82.5		1650
(2)	$CaCl_2 \cdot 2H_2O$	88.0	5	440
(3)	KH_2PO_4	34.0	5	670
	$MgSO_4 \cdot 7H_2O$	74		370
(4)	Na_2EDTA	7.45	5	37.3
	$FeSO_4 \cdot 7H_2O$	5.58		27.8
	Minor Solution			
(5)	$MnSO_4 \cdot H_2O$	16.9		16.9
	H_3BO_3	6.2		6.2
	$ZnSO_4 \cdot 7H_2O$	8.6		8.6
	KI	0.83		0.83
	$CoCl_2 \cdot 6H_2O$	0.025	1	0.025
	$Na_2MoO_4 \cdot 2H_2O$	0.25		0.25
	$CuSO_4 \cdot 5H_2O$	0.025		0.025
	Vitamins & Amino acids			
(6)	Inositol	20	5	100
(7)	Nicotinic acid	0.5		0.5
	Thiamin HCl	0.1		0.1
	Pyridoxine HCl	0.1	1	0.1
	Glycine	3.0		3.0

		Stock Solution, gm/liter	Stock Solution, ml/liter of medium	Final concentration mg/liter
(8)	NAA*	0.175	1	0.175
(9)	2,4-D	1.1	1	1.1
(10)	Kinetin	.500		0.5

In addition, each liter of medium contained 40 grams of sucrose and 10 grams of agar.

*Substitute IAA in the same concentration to use this medium for initiating pea cultures and for root explants.

APPENDIX B

REMOVAL OF EFFECT OF INITIAL SAMPLE SIZE FROM CALLUS SAMPLES

Figure 8 in Chpater Three illustrates that the initial size of the callus sample has a direct effect on the final growth ratio of the sample at the end of the two week growth period. In order to ascertain if the change in growth ratios was due to the electric field or differences in initial sample size a theoretical predicted growth ratio (Y) was determined for each sample in the experiments producing Figure 7 in Chapter Three. This predicted growth ratio was calculated from the following equation:

$$\frac{y - y_1}{y_2 - y_1} = \frac{x - x_1}{x_2 - x_1}$$

The values for x_1, x_2, y_1, and y_2 were obtained from the graph in Figure 8 so that

$$x_1 = 1.2 \qquad x_2 = 2.4$$
$$y_1 = 4.0 \qquad y_2 = 3.0$$

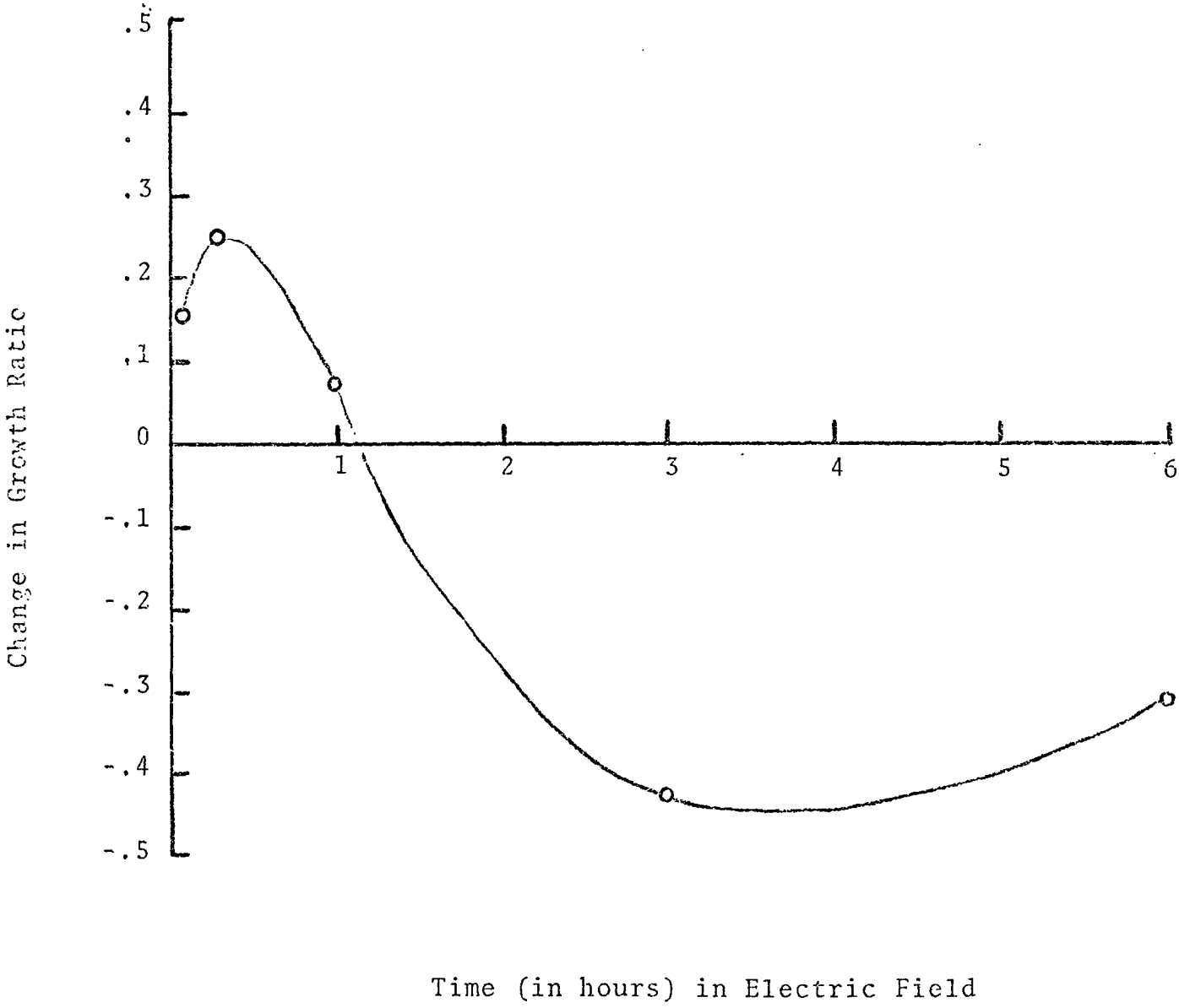
With these values, the value of Y was determined by the equation

$$Y = \frac{X}{1.2} + 5$$

where X represents the original sample size of each sample. The predicted growth ratio was substracted from the actual growth ratio to remove the effect of sample size from the data. Figure 1[illegible]

FIGURE 12

CHANGE IN GROWTH RATIOS OF SOYBEAN CALLUS TISSUE AS A FUNCTION OF TREATMENT TIME AFTER REMOVAL OF THE EFFECT OF INITIAL SAMPLE SIZE


Change in Growth Ratio
.5
.4
.3
.2
.1
0
-.1
-.2
-.3
-.4
-.5
1
2
3
4
5
6
Time (in hours) in Electric Field

APPENDIX C

FORMULAS FOR STATISTICAL ANALYSIS*

I. Standard Deviation

$$S = \frac{(X - \bar{X})^2}{n - 1}$$

where X is value of a single sample, $\bar{X}$ is the arithmetic mean of the group, and n is the number of samples.

II. Standard Deviation of the Mean

$$S_{\bar{X}} = \frac{S}{n}$$

III. Test of Significance for the Difference Between Two Means

$$t = \frac{X_{tr} - X_c}{S_{X_{tr}}^2 + (S_{X_c})^2}$$

$$df = n_t + n_c - 2$$

IV. Rank Correlation

$$r_r = 1 - \frac{6\ d^2}{n(N^2 - 1)}$$

where d is the difference between the ranks for the paired observations and n is the number of paired observations.

*(Hamburg, 1974)

V. Test of Significance (t test) for Rank Correlation

$$t = \frac{r_r}{\frac{(1 - r^2_r)}{n-2}}$$

VI. Equation of the Regression Line

$$Y_c = a + bX$$

where $\underline{Y}_c$ is the computed value of the dependent variable, $\underline{a}$ is the computed value of Y when X = 0, $\underline{b}$ is the slope of the line, and $\underline{X}$ is the observed value of Y when X = 0.

VII. Coefficient of Correlation Equation

$$r = r^2$$

where $\underline{r}$ is the regression coefficient.

VIII. Test of Significance for Coefficient of Correlation

$$t = \frac{r}{\frac{1 - r^2}{(n - 2)}}$$

where $\underline{r}$ is the regression coefficient and n is the number of samples.

BIBLIOGRAPHY

Adelman, W. J. Jr., (Editor), BIOPHYSICS AND PHYSIOLOGY OF EXCITABLE MEMBRANES, Van Nostrand Reinhold Co., New York, pp. 343-344, 1971.

Arnon, D. I., "Copper Enzymes in Isolated Chloroplasts; Polyphenoloxidase in Beta vulgaris," PLANT PHYSIOL. 24:1 - 15, 1949.

Ayscough, P. B., ELECTRON SPIN RESONANCE IN CHEMISTRY, Methuen & Co., Ltd, London, pp. 423-434, 1967.

Babakov, A. V., L. N. Ermishkin, and E. A. Liberman, "Influence of Electric Fields on the Capacity of Phospholipid Membranes," NATURE 210 (5093):953-955, 1966.

Berry, L. J., and R. C. Hoyt (a), "Polarization and Stimulation of the Onion Root by Direct Current," PLANT PHYSIOL. 18:372-396, 1943.

Berry, L. J., and R. C. Hoyt (b), "Stimulation of the Onion Root by Alternating Current," PLANT PHYSIOL. 18:570-587, 1943.

Bieleski, R. L., and G. G. Laties, "Turnover Rates of Phosphate Esters in Fresh and Aged Slices of Potato Tuber Tissue," PLANT PHYSIOL. 38:586-594, 1963.

Blackman, V. H., "Field Experiments in Electro-culture," JOURNAL AGRICULTURAL SOCIETY 14:240-267, 1924.

Blackman, V. H., and A. T. Legg, "Pot-culture Experiments with an Electric Discharge," JOURNAL AGRICULTURAL SOCIETY 14:268-276, 1924.

Brezowsky, H., "On the Effect of Atmospheric Conditions on Seed Germination," ARCH. METEOROL. GEOPHYS. BIOKLIMATOL. SER. B. ALLG. BIOL. KLIMATOL 13 (4): 521-530, English Summary, 1919.

Briggs, L. J., A. B. Campbell, R. H. Heald, and L. H. Flint, "Electroculture," United States Department of Agriculture, Department Bulletin No. 1379, 1926.

Burr, H. S. and F. S. C. Northrop, "Electrodynamic Theory of Life," QUARTERLY REV. BIOL. 10 (3):322-333, 1935.

Caplin, S. M., "Growth and Morphology of Tobacco Tissue Cultures in Vitro," BOTANICAL GAZETTE 108:379-393, 1947.

Darnell-Smith, G. P., "The Electrolytic Treatment of Seeds (Wolfryn Process) Before Sowing," AGRICULTURAL GAZETTE OF NEW SOUTH WALES, 31:393-395, 1920.

Dorchester, C. S., "The Effect of Electric Current on Certain Crop Plants," IOWA COLLEGE RESEARCH BULLETIN 210, 1937.

Dudgeon, E. C., "Growing Crops and Plants by Electricity," JOURNAL BOARD OF AGRICULTURE (London) 18:862-863, 1912.

Epstein, E., D. W. Rains, and W. E. Schmid, "Course of Cation Absorption by Plant Tissue," SCIENCE 136:1051-1052, 1962.

Epstein, E., W. E. Schmid, and D. W. Rains, "Significance and Technique of Short-term Experiments on Solute Absorption by Plant Tissue," PLANT AND CELL PHYSIOL. 4:79-84, 1963.

Gautheret, R. J., "Histogenesis in Plant Tissue Cultures," J. NATIONAL CANCER INST. 19:555-573, 1957.

Goldman, D. E., "A Molecular Structural Basis for the Excitation Properties of Axons," BIOPHYSICAL J. 4 (3):167-169, 1964.

Gregory, F. G. and L. Batten, "A Critical Statistical Study of Experimental Data on the Effect of Minute Electric Currents on the Growth Rate of the Coleoptile of Barley," PROC. ROY. SCO. LONDON B 99:122-130, 1926.

Hagen, C. E. and H. T. Hopkins, "Ionic Species in Orthophosphate Absorption by Barley Roots," PLANT PHYSIOL. 30:193-199, 1955.

Halperin, W., "The Use of Cultured Tissue in Studying Developmental Problems," CAN. J. BOT. 51:1801-1806, 1973.

Hamburg, M., BASIC STATISTICS: A MODERN APPROACH, Harcourt Brace Jovanovich, Inc., New York, pp. 450, 1974.

Hamilton, W. A. and A. J. H. Sale, "Effects of High Electric Fields on Micro-organisms II. Mechanisms of Action of the Lethal Effect," BIOCHIM. BIOPHYS. ACTA 148:789-800, 1967.

Hendrick, J., "Experiments on the Treatment of Growing Crops with Overhead Electric Discharges," SCOTTISH JOURNAL OF AGRICULTURE 1:160-171, 1918.

Henshaw, G. G., K.. K. Jha, A. R. Mehta, D. J. Shakeshaft, and H. E. Street, "Studies on the Growth in Culture of Plant Cells I. Growth Patterns in Batch Propagated Suspension Cultures," J. EXP. BOT. 17 (51):362-377, 1966.

Higinbotham, N., "Electropotentials of Plant Cells," ANN. REV. OF PLANT PHYSIOL. 24:25-46, 1973.

Hill, T. L., "Some Possible Biological Effects of an Electric Field Acting on Nucleic Acids or Proteins," J. AM. CHEM. SOC. 80:2142-2146, 1957.

Jaffe, M. J., "Phytochrome-mediated Bioelectric Potentials in Mung Bean Seedlings," SCIENCE 162:1016-1017, 1968.

Jenkinson, I. S., "Bioelectric Oscillations of Bean Roots: Further Evidence for a Feedback Oscillator," AUST. J. BIOL. SCI. 15:115-125, 1962.

Jenkinson, I. S. and B. I. H. Scott, "Bioelectric Oscillations of Bean Roots: Further Evidence for a Feedback Oscillator," AUST. J. BIOL. SCIENCE 14:231-247, 1961.

Jørgensen, I. and W. Stiles, "Atmospheric Electricity as an Environmental Factor," JOURNAL OF ECOLOGY 5:203-209, 1917.

Knight, R. C. and J. H. Priestley, "The Respiration of Plants under Various Electrical Conditions," ANNALS OF BOTANY 28:135-163, 1914.

Koski, V., "Chlorophyll Formation in Seedlings of Zea mays L.," ARCH. BIOCHEM. BIOPHYS. 29:339-343, 1950.

Kozhevnikova, N. F. and S. A. Stanko, "The Effect of Treating Corn Seeds in an Alternating Electric Field before Sowing on some Physiological Processes in the Plant," ELKETRON OBRAB MATER 2:70-76, 1966 (in Russian). English Abstract in BIOLOGICAL ABSTRACTS 49 Number 53197, 1968.

Laties, G. G., N. R. MacDonald, and J. Dainty, "Influence of the Counter-ion on the absorption Isotherm for Chloride at Low Temperatures," PLANT PHYSIOL. 39:254-62, 1964.

Lee, S. C., "Electrical Treatment of Seed," THE AGRICULTURAL GAZETTE OF CANADA 7:248-249, 1920.

Leicester, J., "The Action of Electric Currents upon the Growth of Seeds and Plants," CHEMICAL NEWS 65 (1680):63, 1892.

Loughman, B. C., "Uptake and Utilization of Phosphate Associated with Respiratory Changes in Potato Tuber Slices," PLANT PHYSIOL. 35:418-424, 1960.

Lund, E. J., "The Theory of Bioelectric Currents," JOUR. EXP. ZOOL. 51:265-290, 1928.

Lund, E. J. and Collaborators, BIOELECTRIC FIELDS AND GROWTH, The University of Texas Press, Austin, Texas, 1947.

Marsh, Gordon, "The Effect of Applied Electric Currents on Inherent Cellular E. M. F. and Its Possible Significance in Cell Correlation," PROTOPLASMA 11:447-474, 1930.

Mercier, C. A., "The Electrification of Seeds," SCIENTIFIC AMERICAN 120:142-143, 1919.

Mihalyfi, J. P. and L. Serf, "Catalase Activity of the Seeds as Affected by Electric Fields," ACTA AGRONOMICA ACADEMIAE SCIENTIARUM HUNGARICAE TOMUS 16:335-338, 1967.

Monahan, N. F., "The Influence of Atmospheric Electrical Potential on Plants," Hatch Experiment Station of Massachusetts Annual Report 16:31-36, 1904.

Monahan, N. R., "The Influence of Electric Potential on the Growth of Plants," Hatch Experiment Station of Massachusetts 17:14-31, 1905.

Murphy, M. K., S. A. Clyburn, and C. Veillon, "Comparison of Lock-in Amplification and Photon Counting with Low Background Flames and Graphite Atomizers in Atomic Fluorescence Spectrometry," ANAL. CHEM. 45 (8):1468-1473, 1973.

Murr, L. E., "Plant Growth Response in a Simulated Electric Field Environment," NATURE 200:490-491, 1963.

Murr, L. E., "The Microscopy Investigations of Plant Cell Destruction in an Electrostatic Field," PENNSYLVANIA ACADEMY OF SCIENCE 37:109-121, 1963.

Murr, L. E. "The Mechanism of Plant-Cell Damage in an Electrostatic Environment," NATURE 201:1305-1306, 1964.

Murr, L. E. "Biophysics of Plant Growth in an Electrostatic Field," NATURE 206:467-470, 1965.

Murr, L. E. "Physiological Stimulation of Plants Using Delayed and Regulated Electric Field Envoronment," INT. J. BIOMETEOR. 10 (2):147-153, 1966.

Osmond, C. B. and G. G. Laties, "Interpretation of the Dual Isotherm for Ion Absorption in Beet Tissue," PLANT PHYSIOL. 43:747-753, 1968.

Partanen, C. R., "Plant Tissue Culture in Relation to Developmental Cytology," INTERN. REV. CYTOL. 15:215-243, 1963.

Pickett, J. M. and A. R. Schrank, "Responses of Avena Coleoptile to Magnetic and Electrical Fields," THE TEXAS JOURNAL OF SCIENCE 17:245-258, 1965.

Presman, A. S., ELECTROMAGNETIC FIELDS AND LIFE, Plenum Press, New York, 1970.

Priestly, J. H., "The Effect of Electricity Upon Plants," BRISTOL NATURALISTS SOCIETY PROCEEDINGS, FOURTH SERIES 1:192-203, 1907.

Purvis, M. J., D. C. Collier and D. Walls, LABORATORY TECHNIQUES IN BOTANY Second Ed., Butterworth & Co., Ltd, Washington, 1966.

Rains, D. W. and R. A. Floyd, "Influence of Calcium on Sodium and Potassium Absorption by Fresh and Aged Bean Stem Slices," PLANT PHYSIOL. 46:93-98, 1970.

Rehm, W. S., "Electrical Response of Phaseolus multiflorus to Electrical Currents," PLANT PHYSIOL. 14:359-363, 1939.

Russell, E. J., "Report on the Proposed Electrolytic Treatment of Seeds (Wolfryn Process) Before Sowing," JOURNAL OF THE MINISTRY OF AGRICULTURE, January, pp. 971-981, 1920.

Schrank, A. R., "Control of Phototropic Bending of the Avena Coleoptile by Longitudinally Applied Direct Current," J. CELL. AND COMP. PHYSIOL. 35:353-369, 1950.

Schrank, A. R., "Electronasty and Electrotropism," ENCYCLOPEDIA OF PLANT PHYSIOLOGY 17 (1):148-163, 1959.

Schrank, A. R. and G. E. Backus, "The Relationship of Auxin to Electrically Induced Growth Responses in the Avena Coleoptile," J. CELL. AND COMP. PHYSIOL. 38:361-376, 1951.

Scott, B. I. H., "Electric Oscillations Generated by Plant Roots and a Possible Feedback Mechanism Responsible for Them," AUST. J. BIOL. SCI. 10:164-179, 1957.

Scott, B. I. H., "Electric Fields in Plants," ANN. REV. PLANT PHYSIOL. 18:409-418, 1967.

Sidaway, G. H., "Influence of Electrostatic Fields on Seed Germination," NATURE 211:303, 1966.

Sidaway, G. H., "Electrostatic Influence on Phytochrome-mediated Photomorphogenesis," INT. J. BIOMETEOR. 13 (3 & 4):219-230, 1969.

Sidaway, G. H., "Electrostatic Sensitivity of the Photoreceptive Mechanism in Germinating 'Grand Rapids' Lettuce Seed," PLANTA 90:295-298, 1970.

Sidaway, G. H. and G. F. Asprey, "Influence of Electrostatic Fields on Plant Respiration," INT. J. BIOMETEOR. 12 (4):321-329, 1968.

Solly, E., "The Influence of Electricity on Vegetation," JOURNAL OF THE HORTICULTURAL SOCIETY (London) 1:81-109, 1846.

Street, H. E., (Editor), PLANT TISSUE AND CELL CULTURE, Botanical Monographs Volume II, University of California Press, Berkeley pp. 503, 1973.

Sutton, M. H. F., "Seed Electrification," NATURE Bulletin Number 11, May 13, 1920.

Tanada, T., "Substances Essential for a Red, Far-Red Light Reversible Attachment of Mung Bean Root Tips to Glass," PLANT PHYSIOL. 43:2070-2071, 1968.

Torrey, J. G., "Kinetin as a Trigger for Mitosis in Mature Endomitotic Plant Cells, EXP. CELL RESEARCH 23:281-299, 1961.

Torrey, J. G. and D. E. Fosket, "Cell Division in Relation to Cytodifferentiation in Cultured Pea Root Segments," AM. J. BOT. 57:1072-1080, 1970.

Trifonova, M. F., "Influence of Preplanting Treatment of Seeds with a Weak Direct Current on the Uptake of Trace Elements by Barley Plants," FIZIOLOGIYA RASTENII 17 (1):103-106, 1970.

Webster, W. W. and A. R. Schrank, "Electrical Induction of Lateral Transport of 3-Indol-Acetic Acid in the Avena Coleoptile," ARCH. BIOCHIM. AND BIOPHYS. 47:107-117, 1953.

Van't Hoff, J. and B. McMillan, "Cell Population Kinetics in Callus Tissues of Cultured Pea Root Segments," AM. J. BOT. 56:42-51, 1969.

Wertz, J. E. and J. R. Bolton, ELECTRON SPIN RESONANCE, ELEMENTARY THEORY AND PRACTICAL APPLICATIONS, McGraw-Hill, Inc., New York, pp. 378-390, 1972.

White, P. R., A HANDBOOK OF PLANT TISSUE CULTURE, Tempe Ariz.: The Jaques Cattell Press, Inc., 1943.

Wiegand, O. F. and A. R. Schrank, "Curvature Repsonses of Electrically Stimulated Avena Coleoptiles to 3-Indolacetic Acid," ARCH. OF BIOCHIM. AND BIOPHYS. 56:459-468, 1955.