

THE EFFECT OF MOISTURE STRESS ON
THE ION METABOLISM OF THE
COTTON SEEDLING

By

CHAUNCEY C. SCHALLER

Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
1947


Master of Science
Oklahoma State University
Stillwater, Oklahoma
1960

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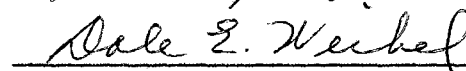
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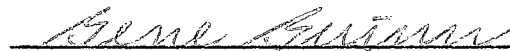
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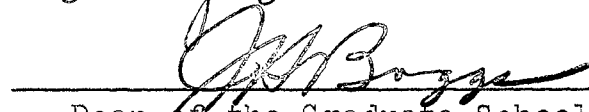
Thesis Adviser











Dean of the Graduate School

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I. INTRODUCTION

The study of moisture stress and its affect on plants is important due to the human population pressure which may require the use of less desirable areas for growing food and fiber. As the population increases the growing of food must be relegated to ever less desirable land. This often means cultivating less fertile areas that are more drouthy due to slope or decreasing rainfall. A better understanding of plants in relation to their environment is essential, if the challenge of the future is to be met successfully.

The absorption of elements by plants and the movement of these materials have received considerable study, resulting in innumerable theories of absorption and translocation. However, most of these studies have been conducted under favorable moisture relations for the plant and root. Relatively little consideration has been given in these studies to the fact that much of the time plants which are often growing normally may be under some moisture stress. Certain stages in the life cycle of the plant may be especially critical for a certain species or variety of plant as far as moisture relations are concerned. Hagan et al. (24)¹ mentioned some unpublished data of Henderson which indicated

¹ Figures in parentheses refer to Literature Cited.

grain sorghum to be especially sensitive to drought in the early stages of growth. The cause of this sensitivity has yet to be determined.

The fact that differences exist in the efficient use of nutrients and moisture for different species of plants is well known. An example offered by Burton et al. (9) shows great differences in varieties of Bermuda grass and other species in use of water under different nitrogen levels. It is possible that a response to stress is involved.

The effect of moisture stress on plants and plant parts has been the subject for considerable research. However, the effect of moisture stress on the absorption and translocation of cations within the plant has been neglected.

The purpose of this study was to determine the effect of short duration moisture stress on cations already distributed throughout the plant, on recently absorbed cations, and on absorption of cations following stress. The duration of the effects initiated by moisture stress were also of interest.

Many of the moisture stress experiments in the literature reviewed have been conducted over extended periods of time and have of necessity included several cycles of soil wetting and drying. An attempt has been made in this study to determine the first effects of moisture stress.

II. LITERATURE REVIEW

The study of the effects of water stress on plants has been of interest to many investigators as indicated by the number of review articles emphasizing one phase or another of the subject. Some of the more recent reviews are by Henckel (26), Stocker (64), Vaadia (69), Kramer (38, 39) and Russell (57).

Brown (6) reported that soil moisture had considerable influence on the effective cation exchange capacity of the soil. He found that a saturated soil was required before the entire exchange capacity was expressed. There was a sudden drop in effective cation exchange capacity as the moisture level dropped below saturation. This work was conducted with resins in soil and he found that the Ca:K ratios were affected by the amount of soil moisture. These effects suggested to him the importance of continuous moisture films for the movement of nutrient elements through the soil. Kramer (36) recognized the role of the moisture film in the soil and believed that little moisture moves toward the roots. He believed that roots contact new zones of soil moisture by elongation.

Kramer (35) conducted experiments with tomato and sunflower plants in an attempt to determine the location of the rate-limiting factor in water uptake within the plant. By

removing the roots of the plants under varying conditions and following the absorption of water in the stems, he found that the roots contained the barrier to free flow of water through the plant. He believed that the protoplasm offered resistance to water flow and that low temperatures were especially effective in reducing water absorption. In another experiment Kramer (34) found the water-supplying power of the roots was only half as great at 0° C. as at 25° C.

Allerup (2) studied the movement of water in young barley plants. If the roots of water-stressed barley plants were cut off in water containing P³², detection of beta activity in the leaves was possible in 15 seconds. Equally stressed plants with roots attached and placed in the same P³²-tagged water required 20 minutes for a similar distribution of the P³². This seems to indicate a positive suction within the vessels of the barley.

Kramer (33) also studied the effect of dead root systems on moisture uptake. He reviewed some of the findings of Strasburger (63) who used copper sulfate to kill the roots. The dead roots were able to absorb large amounts of materials not ordinarily absorbed and the absorption of nutrient elements seemed to be in proportion to those in the solution. Kramer (33) discovered that freshly killed root systems allowed more water to be taken in when a vacuum was applied to the stump than through the live root systems receiving the same treatment. The rapid uptake of water by the dead roots quickly diminished. The diminution seemed to be due to clogging of the vessels by materials from the

dead roots. He concluded that a negative pressure exists in the xylem vessels.

Moisture stress affects the structure as well as the function and properties of the plant root. Aykin (3) studied the effect of osmotic solutions of sucrose on carrot parenchyma. He found that the higher the concentration of the hypertonic solution the slower the loss of water from the carrot tissue, and the slower the reabsorption when returned to water. The high concentrations of the sucrose solutions affected the permeability of the cells both ways. High concentration reduced the flow of water from the cell and the permeability was still impaired when replaced in water. He concluded that the effect of the hypertonic solutions was due to the dehydration of membranes resulting in smaller water passages.

Kramer (37) presumed that loss of turgidity in the tops was indicative of the loss of turgor in the roots which resulted in the destruction of root hairs, increased suberization and reduced elongation of the roots. He found no effect of the rate of drying of sunflower and tomato plants on their subsequent recovery. Kramer found that tomato plants had a greater capacity to regenerate new roots and recover from severe extended wilting than sunflower plants. Also, plants wilted over night, when allowed to recover, exuded only 46% as much water from the stumps as did the control plants not subjected to moisture stress. He suggested a change in the permeability of the protoplasm (possibly dehydration of the cell membrane) to account for

at least a portion of the reduction of water intake but that most of the loss was due to suberization and loss of elongation.

One might question this sudden effect of suberization and loss of elongation that is suggested by Kramer as the processes which reduced the moisture absorption rate 46% by overnight wilting, especially in the light of a paper by Zgwouskaya and Tsel'niker (76). They studied the changes occurring in the roots of a number of trees under drouth conditions. The meristematic regions became covered with fused cells and the exoderm was corked (suberized) except for the meristematic regions, and the protoplasm was separated from the cell wall. These changes required several weeks to occur and four to five days to break following the addition of moisture to the soil. Following a brief drouth, transpiration increased soon after irrigation, but after a long drouth three or four days were required to increase transpiration following irrigation.

Salim² studied the effects of restricted soil moisture on the root growth of small grain varieties. He found differences in response to moisture stress between species but not between varieties of the same species. The varieties of the same species had been picked for their apparent differences in resistance to drouth. In general he found

²

Salim, Muhammad H. Root Development of Wheat, Oats, and Barley Under Conditions of Soil Moisture Stress. Oklahoma State University, Unpublished Masters Thesis. 1962.

decreased elongation and increased branching of roots under moisture deficiency conditions. Barley reduced the soil to a lower moisture content but gave indications of wilting at an early date. Two grass species studied continued to penetrate the soil which was at a moisture percentage considered below permanent wilting.

Stevenson and Boersma (60) studied the growth of the adventitious roots of sunflowers and concluded that new root growth was related to initial soil water content under certain conditions. They found that there was little correlation between the initial water content of sandy soil and the amount of root growth that developed but that there was a more direct correlation between initial water content of a clay-loam soil and the resulting root growth.

The effect of soil moisture tension and osmotic stress on root growth of corn seedlings was studied by Gingrich and Russell (21). They found little effect of osmotic stress between $\frac{1}{2}$ and 1 atmosphere on the seedling roots, either in soil or osmotic pressure media. However, they found a straight line reduction of water in the seedlings between 1 and 12 atmospheres. The effect on the plants was greater in the soil than in the osmotic medium at the same moisture tension.

Gates (19) studied the leaves of tomato plants under stress and concluded that there was a relative increase in the stem weights and decrease in lamina weights of the young tomato plants under stress compared to unstressed plants. Gates also observed that the actively growing portions of the

plant were least affected by drouth and first to recover with improved moisture conditions.

McCune³ concluded that there is a decrease in elongation of plants under increased moisture stress. He found that zylem movement is decreased while the movement of materials through the phloem are increased by moisture stress. He also considered that there is a recirculation between the zylem and phloem tissues.

Gates (18) studied moisture stress of tomatoes growing in pots in a greenhouse. He found that wilting reduced the growth of the plants under moisture stress but after rewatering the growth rate of the previously stressed plants exceeded their controls. He also found that the net assimilation rates and relative growth rates rose above the control after recovery from wilting. He considered the effects of moisture stress on the plants as a tendency towards senescence and a return to more juvenile conditions with watering. No change was found in the economy of water usage by the tomato plants following moisture stress.

Chibnall (11) worked with runner-bean leaves and determined that the presence of roots exerted considerable effect on certain physiological processes in the bean leaves. Leaves separated from plants but with roots induced by indol-acetic acid remained viable for weeks compared to only days

3

McCune, D. L. Effect of Moisture Stress on the Production and Movement of Carbohydrates in Plants. Purdue University, Unpublished Ph.D. Dissertation. 1958. Diss. Abs. 18:1932. 1958.

before rootless leaves turned yellow. He found a rapid breakdown in protein in the leaves removed from the plants but the rate of breakdown was greatly diminished as roots were developed. These results should be considered when working with detached plant parts.

The effect of reduced moisture conditions on apple trees was studied by Magness et al. (42). They found an accumulation of sugars and a reduction of starch in the leaves and bark of stressed trees. However, under ample moisture the carbohydrate accumulated as starch.

* Eaton and Ergle (15) interpreted the accumulation of carbohydrates in the cotton plant under drouth conditions as a greater depression in utilization than of photosynthesis. They found no apparent effect of drouth on polar transport of carbohydrates. Zholkevich (75) reported an accumulation of simple sugars and a decrease in phosphorylated sugars as an early indication of drouth conditions. He suggested an uncoupling of the phosphorylated compounds with no energy transfer and a decrease in effectiveness of respiration.

Petrie and Wood (51) studied nitrogen metabolism of plants and they decided that moisture stress shifts the equilibrium between proteins and amino acids towards the amino acids which was due to an increase in net proteolysis. They suggested a relation between protein synthesis and hydrolysis and water content, and between protein and water content. The net rate of protein formation is reduced when the water content is reduced.

Petrie and Wood (50) found the rate of protein formation reduced when the water content of the plants was reduced. They also found that the supply of ammonium sulfate available to the plants influenced the water content of the leaves. This effect was not noticeable for potassium sulfate and little effect on pH was noted.

In a series of physiological and biochemical studies on plant metabolism Roberts (55) determined that different parts of the wheat leaf reacted differently when subjected to moisture stress as indicated by differences in respiration, water content, protein, nitrogen content and carbohydrates. He found that excessive water also affected leaf constituents.

Meyer and Gingrich (47) studied the effect of moisture stress on wheat through the use of Carbowax 6000 in a split-root experiment and found that the concentration of phosphorus and nitrogen in the roots were decreased when both or only one of the two roots was under one bar of stress. He also found a large decrease in ninhydrin positive material in the wheat plant as a response to moisture stress.

Goldacre (22) suggested the unfolding and folding of proteins as a mechanism for dye, ion and water uptake for cells. Movement of the protein within the cell served as a carrier for the cations. Steward and Millar (61) considered at least a portion of the uptake to be due to ion-binding centers. They found the absorption of cesium to be related to increase in fresh weight for rapidly dividing cells. In more mature cells the increasing size of the

vacuole appeared to influence the great increase in uptake more than did the change in tissue weight. Ling (41) considers ion accumulation into cells as due to an association-induction effect caused by the absorption of such materials as ATP, creatine phosphate and hexose phosphate by the protein in the cell. This action induced an open configuration of the protein exposing charged sites for ion absorption.

Soil moisture can affect the nutrient elements in the plant in several ways. It affects the solubility and movement of the ions within the soil, the amount of carbon dioxide and oxygen in the soil pore space, the growth of roots through the soil mass and possibly the rate of absorption, distribution and translocation of ions within the plant. With as many factors involved it is obvious that conflicting results will be reported as to the effect of moisture stress on plant composition, especially when one considers the wide variety of conditions existing in the experiments and the different test plants used.

Emmert and Ball (16), Janes (31) and McMurtrey et al. (45) found the nitrogen content of plants to increase under moisture stress while Gates (20) reported a decrease. Magness et al. (42) reported erratic results in apple trees. The phosphorus content of plants declined under moisture stress as reported by Mederski and Wilson (46), Gates (20), Emmert and Ball (16), Hibbard (27), Wooley (74) and Williams and Shapter (73), but McMurtrey et al. (45) and Janes (31) found an increase of phosphorus under low moisture conditions.

Moisture stress decreased the accumulation of potassium in certain plants as reported by Mederski and Wilson (46), Stewart (62), Hibbard (27), McMurtrey et al. (45) and Janes (31), but Emmert and Ball (16) and Williams and Shapter (73) reported increased potassium under low moisture conditions.

The calcium content of plants was constant for different levels of moisture as reported by Janes (31). Mederski and Wilson (46) found the calcium content of plants to be erratic but Williams and Shapter (73) and McMurtrey et al. (45) found the calcium content of plants to be increased under dry conditions. Stewart (62) studied the influence of soil moisture on the uptake of cesium by plants. Under low moisture the cesium accumulated more rapidly in the stems than in the leaves compared to plants at field capacity. Also, there was a reduction in cesium in the leaves as compared to the stems as the moisture level decreased. Stewart (62) also found that increasing the soil moisture tension increased the proportion of cesium absorbed compared to potassium and the ratio was as high as four to one for some plants. He found a rapid reversal in the cesium to potassium ratios in the plants when the soil was watered to field capacity.

~~*~~ In general, from the foregoing studies, nitrogen and calcium content of plants was found to increase and the phosphorus and potassium to decrease under moisture stress. The nitrogen increase under reduced moisture conditions is often ascribed to the higher concentration of nitrogen in

the reduced volume of the soil solution. A reduction in volume of the soil solution causes a precipitation of certain soluble phosphorus compounds, reducing the amount available to plants. Likewise, a reduction in volume of the soil solution causes a partial fixation of the potassium. These effects are mostly external and tend to mask any direct physiological effect of moisture stress on the absorption and utilization of nutrient elements by the plant.

Danielson and Russell (14) studied the effect of moisture stress on the absorption of rubidium by corn roots. They found little correlation between rubidium uptake and the osmotic pressure of the solution in which the plants were grown. However, rubidium uptake decreased rapidly with initial increase in soil moisture tension. They found a large decrease in root and shoot hydration from either soil moisture tension or osmotic pressure and that tissue hydration was apparently not a limiting factor to rubidium accumulation by young corn seedlings. It was concluded that a reduction in movement of the rubidium through the soil was the cause of the reduced uptake in the dry soil.

Mason and Maskell (43) studied the movement of phosphorus, potassium and calcium through the cotton plant and concluded that phosphorus and potassium are easily transported and transferred. They found that calcium moves only through the woody part of the plant which excludes its easy transfer. They suggested that nitrogen, phosphorus and potassium could be withheld from the plant at a relatively

early stage but that a constant supply of calcium is needed throughout the life of the plant. A further study by Mason and Phillis (44) suggested that sugars, nitrogen, phosphorus, potassium, magnesium and chlorine are mobile in the phloem while calcium is not. Phillis and Mason (52) studied the movement of calcium and phosphorus and concluded that the phosphorus moved up in the wood and down in the phloem.

Abutalybov and Dzhangirova (1) studied the movement of calcium in the cotton plant and concluded that it moved in the cortex in an upward direction. It would travel through the wood to pass a girdled zone but returned again to the cortex. Phillis and Mason (53) reported that the tops of cotton plants influenced the uptake of ions by the roots. The uptake of bromide by the cotton plants was greatly reduced within two hours following ringing of the stems but there was little effect on the bromide in the tops during this time.

Broyer (8) found that roots have first priority on absorbed nutrient elements. The roots of plants with a low salt content retain more of the absorbed salt than the roots of high salt plants. He also found a notable difference in the uptake of bromine by barley roots between 5° and 20° C. The difference in uptake between 5° and 20° is believed to be due to active uptake and the amount taken up at 5° is due to passive exchange.

Cotton leaves lost dry weight due to export during the night according to Phillis and Mason (54). They also found

a definite diurnal variation for certain nutrient elements with an increase by day but no change or decrease by night. Magnesium and potassium gave the greatest diurnal variation while phosphorus and nitrogen gave the least. The differences were on the order of 15% for the former and 0-5% for the latter. Calcium varied from 0-5% between evening and morning. They decided that the export movement was through the phloem but could not decide the origin of the diurnal mobile elements--veins or chlorenchyma. Potassium was found to be easily lost from the leaves in dew.

Williams (72) presented a review of accumulation and redistribution of elements by different organs of plants during their life cycle. He presented the work of Phillis and Mason (54) as evidence that the elements are being redistributed even as they are accumulating and that as the plant develops there is a redistribution within the organs. Sometimes this redistribution is quite rapid. The growth and physiology of the plant according to Williams are also greatly affected by the nutrients supplied and by the timing of nutrient applications.

Stenlid (59) suggests that the differences in the elements found in tree leaves at different times in the summer are due to redistribution. Some of the losses may be due to leaching from the leaves, redistribution in the plant and losses from the root. He also found potassium to be highly mobile and easily lost by leaching.

Exudation of certain organic and inorganic materials from the root seems to be a natural phenomenon, either by exchange, as suggested by Jenny (32), or by diffusion. Ivanov (30) found an exchange of unidentified root exudate between broadbeans and corn within six hours of supplying carbon 14 to one of the species. Sulfur and phosphorus compounds were found to migrate from the leaves of certain plants to other plants growing in the same container, according to Uzorin (68). At the end of 14 days he reported that untreated plants in the same containers contained 50% as much sulfur 35 as the treated plants. Tolbert and Zill (67) found sedoheptulose, glucose, fructose and sucrose in the water in which plants were standing. Sokolov (58) suggests that a portion of the exchange of elements between plants of the same association is the result of the dying of plant organs or whole plants as well as the utilization of root exudations.

Frenzel (17) studied the accumulation of amino acids about the roots of sunflower seedlings growing in sterile nutrient solution. He considered the possibility that the accumulating amino acids resulted from the enzymatic breakdown of dead cells on the exterior but concluded that the proportions of the materials found did not agree with the composition or contents of the adjoining tissue. When amino acids were added in excess of the amounts normally excreted they were absorbed by the root.

The exudate of pea roots differed quantitatively and qualitatively from that of oats in experiments conducted by Rovira (56). The pea roots excreted 22 amino acids and the oat roots 14 amino acids within 21 days. At the same time the compounds excreted by the pea roots were approximately 16 times more concentrated than those from the oat roots. Both plants excreted sucrose and fructose during the first 10 days but at the end of a 21-day period neither was excreting sucrose or fructose.

In addition to the "normal" continuous loss of materials from the roots to the surroundings, certain conditions may cause an increase in the rate and a change in the composition of the materials lost. Stenlid (59) listed the factors causing the loss of salts by roots as: oxygen deficiency, low carbohydrate content and special toxic substances. He believed the respiratory inhibitors increased loss due to decreased accumulation and the direct effect on the permeability of the protoplasm. Grineva (23) subjected young corn and sunflower plants to periods of anaerobiosis by immersion in water and found the active acidity of the solution increased and the dry weight of material excreted increased. The anaerobic condition induced the release of glucose and fructose but not sucrose. He found that sucrose was decreased in the tissue. He also found a number of Krebs cycle organic acids: oxalic, citric, malic, succinic and fumaric in addition to a number of amino acids. He suggests that the excretion is caused by the shift to a metabolism

that can no longer utilize these materials and that this might be active excretion. This last thought might be questioned. Active metabolism is usually concerned with metabolism utilizing oxygen. The effect of carbon dioxide on the absorption of water and nutrients by roots was studied by Chang and Loomis (10). They found that most plants need about 10% of oxygen for good growth and may have difficulty with amounts as low as $\frac{1}{2}$ to 2%. They also found that carbon dioxide affected the plants. Less than 10% was not damaging but amounts of 15-20% could be lethal. They also found that carbon dioxide could greatly decrease the absorption of water and mineral salts. Potassium was affected more than the other elements. Carbon dioxide was also found to cause the excretion of potassium from the roots of treated plants.

Sutcliff (65) studied the absorption of potassium by plasmolyzed beet tissue and the effect of non-electrolyte plasmolyzing agents on the release of potassium from beet tissue. The greatest release of potassium was obtained when the osmotic pressure of the solution was slightly greater than that of the tissue. In fact he found a decreased release of potassium as the osmotic pressure was increased similar to that mentioned previously for water, by Aykin (3). It was suggested that the potassium was released uniformly from all the cells rather than from just a portion of the cells and release is at a maximum at incipient plasmolysis. Sutcliff found that all plasmolyzing agents, both electrolytes

and non-electrolytes, stimulated the absorption of potassium. He rejected a number of theories as to the cause of this effect but accepted Jacque's theory that the relieved pressure on the cell membrane decreases resistance to diffusion and, conversely, increased pressure forces the membrane into the cell wall. Sutcliff also suggested that plasmolyzing agents orient the water as a hydration shell, increasing the effective concentration of the potassium.

The relation between the transpiration stream and the absorption of salts by tobacco growing in the open was studied by Hasselbring (25). Tobacco plants growing in the open absorbed more water and less salts than plants growing under shade, indicating no direct relation between water and salt absorption.

Smith⁴ found that as the transpiration rate was increased, less absorbed phosphorus remained in the root and more was transported to the shoot in pea seedlings. Brouwer (5) found that at low rates of water and anion uptake a higher percentage was taken up by the tips of the roots of Vicia fabia, but at higher uptake rates more was absorbed by the older portions of the root. Uptake of phosphorus at the root tip resulted in little or no transport. However, uptake in the root hair zone resulted in transport in both directions. He concluded that an upward movement of ions in the cortex was unlikely.

⁴

Smith, R. C. Studies on the Relation of Ion Absorption to Water Uptake in Plants. Unpublished Ph.D. Dissertation, Duke Univ., Durham, North Carolina, 1957.

The effect of sectioning on the absorption of potassium by the apex of the corn root was tested by Brown and Cartwright (7). There was a rapid loss of potassium from the second 1.5 mm. section of the root the first 24 hours if potassium were not provided in the external solution. There was an increase in potassium and protein per unit cell from the root tip to 9 mm. but absorption per section decreased from the tip to 9 mm. The sectioned meristem portion of the root was found to have the lowest potassium absorption but the meristem portion of the intact roots contained the highest amount of potassium.

Viets (70) studied the effects of polyvalent cations as accelerators of ion absorption and found a number of polyvalent cations to increase the absorption of potassium and bromide. However, he believed calcium to be the most effective accelerator. He proposed that the theory of antagonism between monovalent and divalent cations was due to the experimental procedures used. Over 30 times as much calcium as potassium was needed in his experiments to depress the uptake of potassium and he found mixtures of calcium and magnesium to be as effective as calcium alone in increasing the uptake of potassium.

Sutcliff (65) found all plasmolyzing agents stimulated the absorption of potassium, both electrolytes and non-electrolytes (as mentioned in a previous section).

Bange and Overstreet (4) studied the uptake of cesium by barley roots at different concentrations. At low

concentrations, 0.1 me. per liter, the uptake appeared active but at concentrations 100 times greater there was little effect from lack of oxygen. Potassium, rubidium and, to some extent, ammonium inhibited cesium uptake in the 0 to 0.1 me. per liter range. Lithium and magnesium had little effect, sodium gave a negative effect and calcium a positive effect.

The selective absorption of cations was studied by Collander (13) who used a number of species of plants growing in several different nutrient solutions. The relative uptake of the cations in the different solutions by the various species was determined. The amount of uptake of sodium was found to vary more than any of the other cations: 32 times more in the highest than in the lowest. The maximum and minimum ratios for the different ions between plant varieties growing in the different nutrient solutions were as follows: potassium, 1.9-2.8; rubidium, 2.1-3.4; cesium, 2.5; calcium, 2.8-6.7. He found that potassium, rubidium and cesium were extensively accumulated by plants and at about the same relative amounts. He found no apparent selectivity for monovalent ions, potassium, rubidium and cesium or between the divalent calcium and strontium.

Munzel (49) found rubidium and cesium uptake by millet and corn to be inversely related to the amount of potassium in the soil. However, Cline (12) found that cesium was accumulated largely independently of potassium under certain conditions which suggests some discrimination by plants between these two elements.

III. METHODS AND MATERIALS

Plant Culture

Certified cotton seed⁵, Gossypium hirsutum, variety Parrott, was used in these experiments. The seed had been previously treated with a fungicide and were treated further by washing with 70% ethanol and 10% hydrogen peroxide prior to placement on the germination rack. The germination chamber consisted of a frame supporting parallel glass rods 1 cm. apart and covered with alcohol-rinsed cheese cloth. The seed were spaced evenly between the glass rods about $1\frac{1}{2}$ cm. apart. The rack containing the seed rested 2 cm. inside a straight-sided plastic pan. The seed were covered with wax paper and the top of the container was covered with another layer of wax paper. Water or calcium and magnesium sulfate, $2 \times 10^{-4}M$ total, the same ratio as Hoagland solution (29), came to within $1\frac{1}{2}$ cm. of the seed and aerators provided a mist to maintain the seed at satisfactory moisture level for germination. The pan contained approximately 6 liters of solution. It was found that removal of the non-sprouting seed improved the quality and number of surviving seedlings. The water in the germination chamber was changed several times.

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Provided by Dr. J. C. Murray of the Oklahoma State University Agronomy Department.

The germination chamber was kept in the dark at a temperature of 25° C. On the fifth day the seedlings were removed from the germination rack, graded for size and distributed to the corks. An attempt was made to obtain a similar population in each cork. The corks were $2\frac{1}{4}$ inches in diameter with four $\frac{1}{4}$ -inch holes in each cork and were coated with hot paraffin to prevent moisture absorption. The corks containing two seedlings per hole were inserted in holes in waxed masonite covers which were resting on 3-liter battery jars. The sides of the jars were covered with aluminum sheet metal to exclude light. Aeration was provided in each jar.

The jars containing the plants were placed on an illuminated bench in a constant-temperature room. The room temperature was 25° C. The temperature under the fluorescent lights for daylight time was about 30° C. The ten fluorescent lights furnished about 1800 foot-candles as determined by a Weston light meter calibrated to an incandescent light of 3000° K. The day length was 15 hours, regulated by an automatic timer. The solution in the jars containing the cotton seedlings was $2 \times 10^{-4}M$ calcium and magnesium sulfate described previously.

Apparatus

The moisture stress apparatus consisted of an 800-ml. beaker inverted over a waxed masonite base. The base had a hole in the center of sufficient size to accommodate a

cork containing the plants. The base rested on the top of a large-mouth gallon jar so that the roots of the plants were suspended in the gallon jar and the tops protruded into the stress chamber formed by the inverted beaker. The root jar was covered on the inside with moistened blotter paper and was covered on the outside upper shoulder with paper to exclude light. Air circulation in the upper stress chamber was obtained by an incoming air jet at the bottom of the chamber and an air removal tube at the top. Aquarium cement was used to seal together the parts of the apparatus. A pressure-vacuum pump was used so that the air was recirculated. The air from the pump was bubbled through a sulfuric acid and water solution, or water, to give the desired relative humidity. This version of the apparatus was used in Experiments 1, 6 and 7. The apparatus was then modified so that the jar containing the roots, and the flask of solution through which the air was bubbled were set into a constant-temperature bath regulated at the same temperature maintained in the stress chamber. The jars were surrounded by a styro-foam cover set on the top of the bath. This modification of the apparatus was operated for Experiments 2, 5, 8, 9, 11 and 12. In all cases the blotter paper in the root chamber was saturated with water the day before an experiment and the root chamber kept closed to maintain a moisture-saturated atmosphere. The circulating pump was set to turn on with the lights the morning of an experiment to provide temperature equilibrium.

No other changes in the apparatus were initiated until it was found necessary to increase the number of stressed plants. A larger stress chamber, the bottom half of a four-liter beaker, was inverted over a base containing four holes. The number of air ports into the chamber was increased to four coming into the bottom and one exhaust from the top. The root compartment was a 6" x 12" battery jar. This larger chamber was used for Experiments 3, 13 and 14.

Analyses

Digestion of Plant Samples

Three to four ml. of nitric-perchloric acid (4:1) were added to each root, stem and cotyledon sample in 30-ml. beakers. The samples were covered and allowed to stand all day or over night at room temperature before the temperature was raised to about 90° C. After the organic material was digested the cover glasses were removed and the remaining acids evaporated. The salts were washed into planchets with 2 N hydrochloric acid, dried at 100° C. over night and the radioactivity determined.

Radioactivity Determination

The radioactivity detection device consisted of a model DS5 Nuclear-Chicago scintillation detector, with interchangeable anthracene or sodium iodide-thallium scintillation crystals, attached to a model 1810 Nuclear-Chicago recording spectrometer. This device, using the sodium iodide-thallium crystal, was used to determine the energy of

gamma rays emitted by a sample. A Nuclear-Chicago decade scaler, Model 186, was attached to the gamma ray spectrometer and was calibrated to count the activity. The beta activity of a sample was determined by using the anthracene crystal and making the appropriate adjustments in the radiation analyzer.

Radioactivity Interpretation

Most of the experiments were conducted using cesium 137 as a tracer to determine the movement of cesium in the plant. The cesium in a planchet was determined by comparing the beta or gamma rays emitted by the sample and a known amount of the nutrient absorption solution. The interpretation of the activity of a sample becomes much more difficult when more than one radioisotope is present.

Mulkey⁶ was able to differentiate between strontium 85, strontium 90 + yttrium 90, and strontium 89. A gamma count gave the amount of strontium 85, and a system of five different absorbers was necessary to differentiate between the other two.

A modification of the counting technique of Onken⁷ was used to differentiate between cesium 137 and calcium 45.

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Mulkey, J. R. Preliminary Investigations of a Multiple Radioisotope Technique for the Evaluation of Root Activity. Unpublished M. S. Thesis, Oklahoma State Univ. 1960.

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Onken, A. B. Investigations Concerning Proposed Cation Uptake Mechanism in Plants. Unpublished Ph.D. Thesis, Oklahoma State Univ. 1964.

The cesium emits a .663 Mev. gamma and two beta particles of the energies 0.518 and 1.17 Mev. The calcium 45 emits a beta particle with an energy of 0.255 Mev. The energy levels between the beta particles of the two elements is so similar that an absorber technique would be difficult to use. It would be possible to differentiate between cesium 137 and calcium 45 by using the relatively short half-life of calcium 45. This method would require several months to complete.

The modified method of Onken⁷ required only two separate counts, gamma and beta, and a nominal amount of calculation. The following method was used to calculate the activity of cesium 137 and calcium 45 in each of the samples.

A $\frac{1}{2}$ ml. aliquot of a cesium 137 solution was dispensed accurately into a series of planchets. Several drops of concentrated hydrochloric acid and a disk of lens paper was added to each. The planchets were dried slowly under an infra-red heat lamp so that the paper stuck to the bottom of the planchets. The gamma and beta counts were determined. Salts and digested samples of plants, in the same quantities as used in the experiments, were added as absorbers to the planchets and dried thoroughly. The beta count was repeated. A $\frac{1}{2}$ ml. aliquot of calcium 45 solution was dispensed into each planchet, dried thoroughly, and the beta count repeated.

The beta:gamma ratio of the cesium 137 was determined from the gamma and first beta count of planchets containing

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no absorbers. The difference between the first and second beta counts gave the beta absorption for the cesium for each of the absorbers. The calcium 45 beta absorption was determined by subtracting the cesium 137 beta count from the cesium 137 plus calcium 45 beta count and dividing the difference by the calcium 45 count of the planchets containing no absorber. The calcium 45 beta absorption is represented by A_{Ca} in the equation that follows and may be determined by the appropriate algebraic conversion.

The cesium 137 and calcium 45 in an unknown sample were found by making a gamma and beta count. The gamma count times the beta/gamma ratio times the percent beta transmitted through this sample absorber gave the number of cesium counts found in the machine reading. (The actual amount of cesium 137 in this sample was the gamma times the beta/gamma ratio.) The actual sample beta count minus the actual cesium beta count gave the amount of calcium 45 that was observed by the crystal. This figure times the transmission factor gave the actual calcium 45 present. The calculation of the calcium 45 beta count for an unknown sample may be represented by the equation

$$B_{Ca} = \left[T. - \gamma \left(\frac{B_{Cs}}{\gamma} \right) A_{Cs} \right] \frac{1}{A_{Ca}}$$

where B_{Ca} represents the machine count of calcium 45 with no self-absorption and B_{Cs} represents the machine count of

cesium 45 with no self-absorption. T . represents the total machine beta count. A_{Cs} and A_{Ca} are the percent sample absorptions for cesium 137 and calcium 45 respectively; $B_{Cs/\gamma}$ is the beta/gamma ratio for cesium 137. Gamma absorption is assumed to be negligible.

IV. EXPERIMENTS

A. Effect of Moisture Stress on Relative Turgidity of Cotton Seedlings

PROCEDURES

Experiment 1. Effect of relative humidity in treatment chamber on relative turgidity of cotton seedlings, constant stress time and varying relative humidity.

Six groups of 11-day old plants, each cork containing eight plants, were placed in the stress chamber for 30 minutes. Air circulated through solutions of different concentrations of sulfuric acid and water to give the desired relative humidity (29). The air was then conducted into the stress chamber and from the stress chamber back to the pump. The air flow was at about 800 ml. per minute. The temperature in the stress chamber was about 31° C. with no temperature control on the air. The illumination in the stress chamber was about 1800 foot-candles.

At the end of the treatment time the plants were removed from the stress chamber and immediately separated into cotyledons, stems and roots, weighed separately in tared beakers and placed in distilled water for 24 hours. The excess water was drained and wiped off and the parts were again

weighed. The plant parts were weighed after they had dried over night at 100° C. The relative turgidity was determined according to Weatherly (71) for the plant parts at each of the levels of treatment.

Experiment 2. Effect of relative humidity in treatment chamber on relative turgidity of cotton seedlings, constant relative humidity of circulating air and varying time of stress.

This experiment was similar to the previous experiment except that the relative humidity was constant and the time of stress varied. The seedlings were nine days old and there were eight plants to the cork. The plants were removed from the holding solution, calcium and magnesium sulfate $2 \times 10^{-4}M$, and allowed to drip hanging in a jar lined with wet blotter paper for two minutes. The plants were removed from this jar, the drops of water removed quickly from the roots and the plants placed in the moisture stress chamber for the desired length of time. The stress apparatus had been modified with a water bath to give a more constant temperature for the root zone and circulating air. This temperature was about 31° C., the approximate temperature of the chamber containing the tops. The air flow of about 800 ml. per min. was bubbled through distilled water and the illumination in the stress chamber kept at about 1800 foot-candles. After the appropriate time in the stress chamber each sample was removed and handled in the same manner as those in the

previous experiment. The treatments in both experiments were conducted one time, in random order.

Experiment 3. Recovery of plants exposed to moisture stress.

The cotton seedlings were sprouted and grown to nine days. The stress apparatus had been modified to accommodate four sets of plants. Two sets of plants were treated at once, each pair for a specified length of time in a random order. The air flow through the stress chamber around the stems and cotyledons was at 1600 ml. per min., bubbled through distilled water at a temperature of 31° C. Following the stress treatment one set of plants was sacrificed for relative turgidity determination and the other was returned to the holding solution. Two, four, six and eight days later photographs were made of the plants that had been under stress. The calcium and magnesium sulfate $2 \times 10^{-4} M$ holding solution was changed to Hoagland solution following the first picture. Fresh Hoagland solution and iron, 1 ppm., as Fe EDDHA, were supplied to the plants following the second photograph. The new leaves formed between the second and third photographs had a darker green color but the previous leaves retained a slightly chlorotic color.

RESULTS AND DISCUSSION

The objective of this series of experiments was to determine the effectiveness of two methods of producing controlled

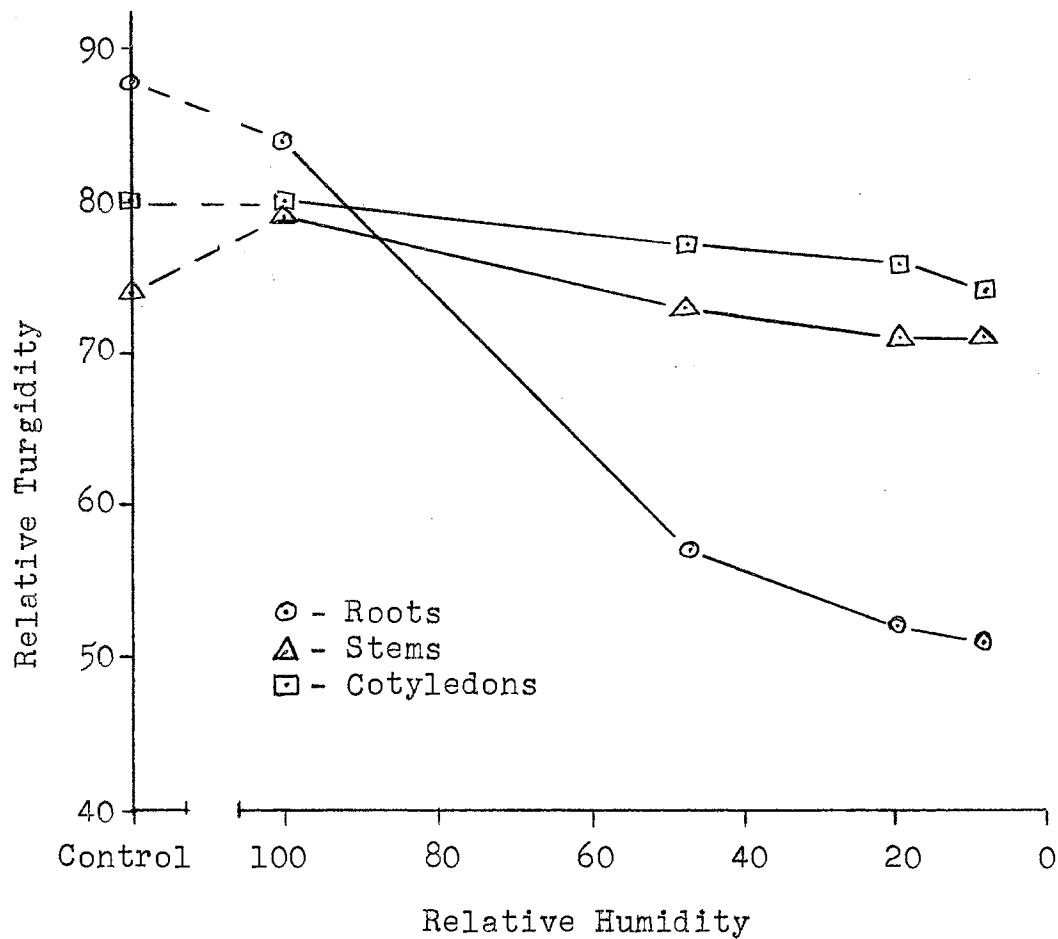


Figure 1. Effect of Relative Humidity of the Air Circulating Through the Stress Chamber on Relative Turgidity of Cotton Seedlings, Experiment 1.

- - Root
 $y = 94.3 - 0.22(x)$; $r = .96$ ** ^a
- △ - Stem
 $y = 86.2 - 0.078(x)$; $r = .96$ **
- - Cotyledons
 $y = 87.9 - 0.048(x)$; $r = .77$ *

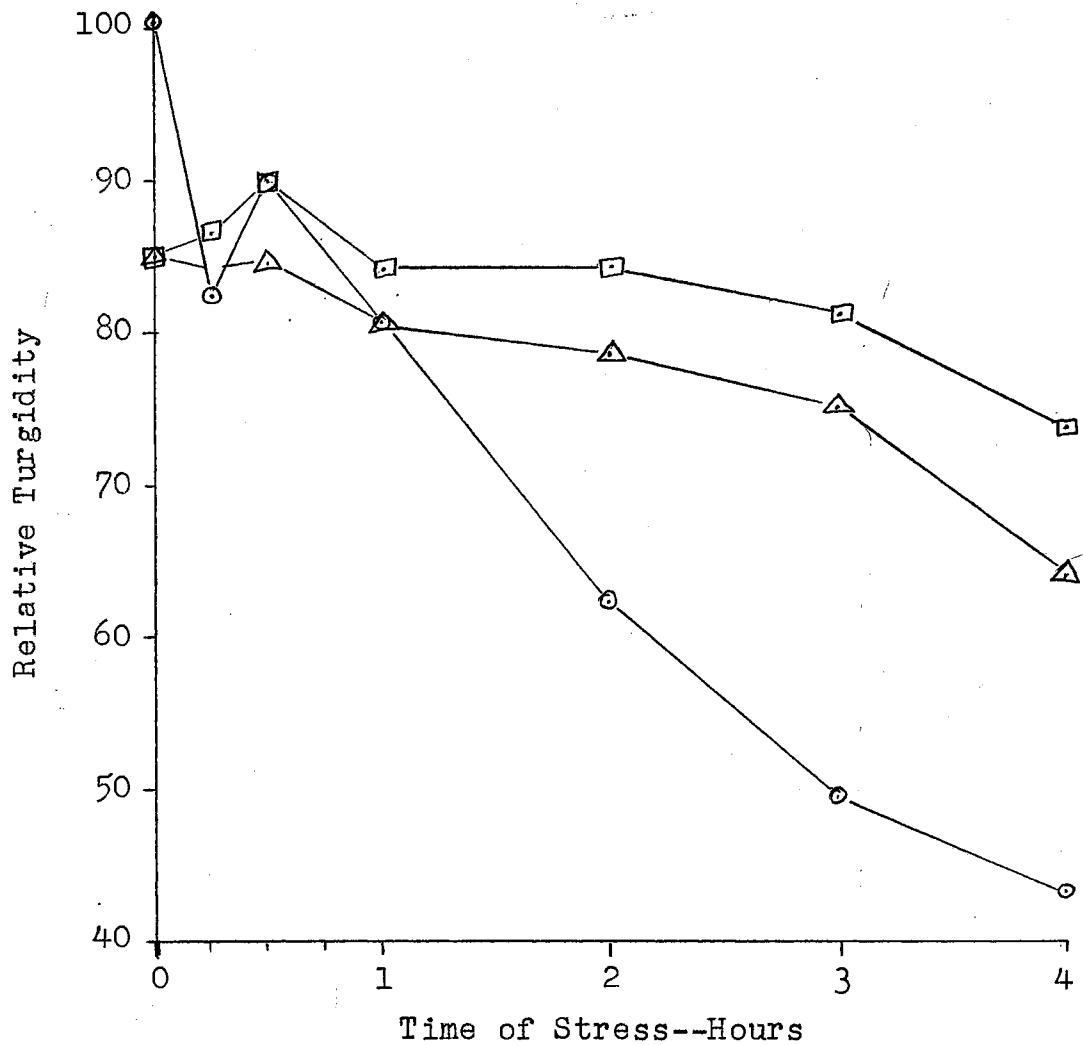


Figure 2. Effect of Duration of Stress with Constant Relative Humidity on Relative Turgidity of Cotton Seedlings, Experiment 2.

^a
 * = Significant at 5% level
 ** = Significant at 1% level (40)

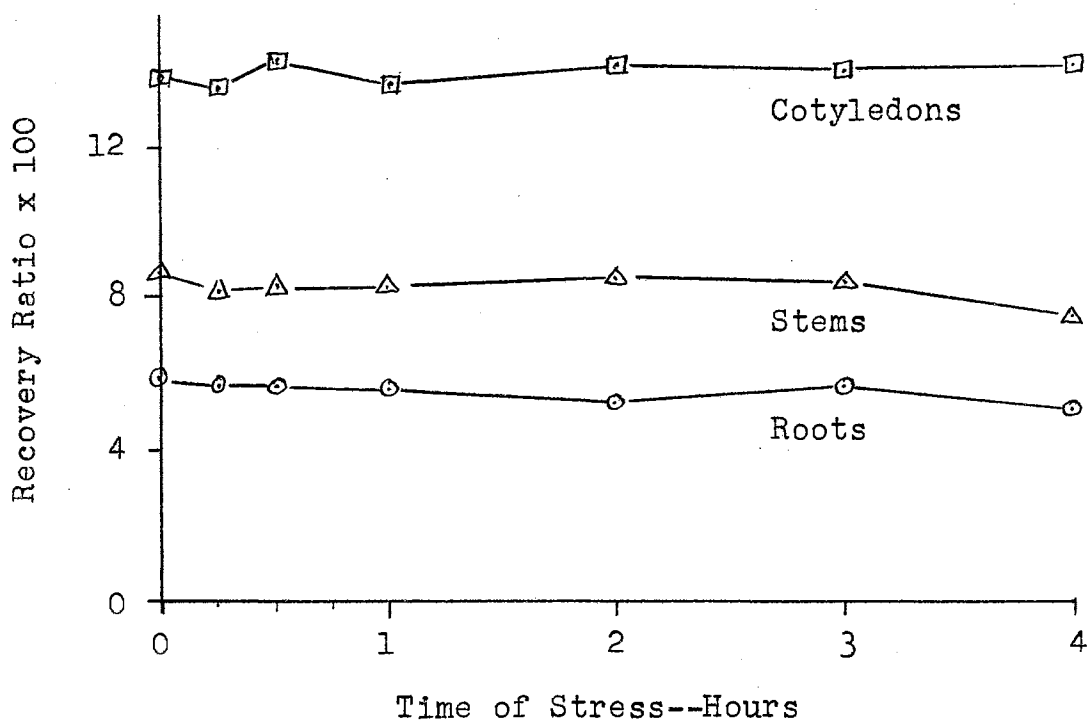


Figure 3. Effect of Duration of Stress on Recovery Ratio, Experiment 2.

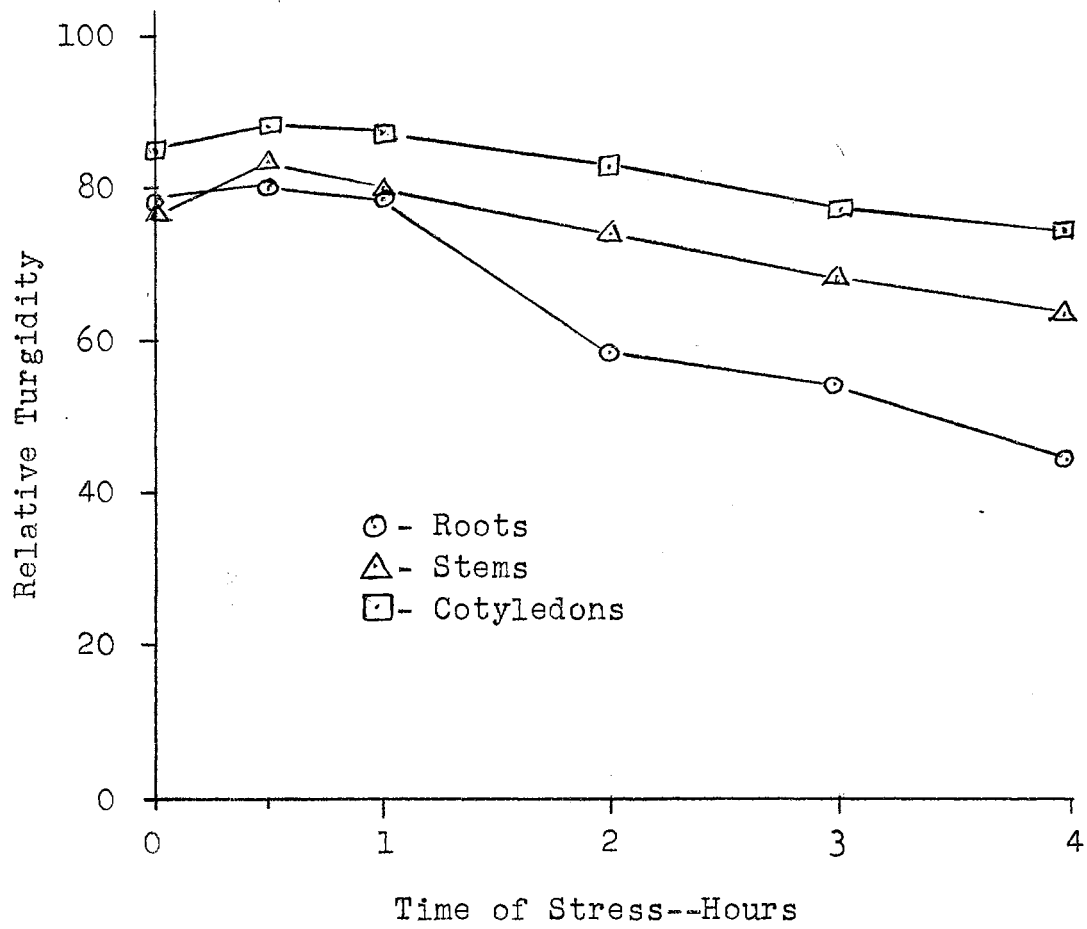
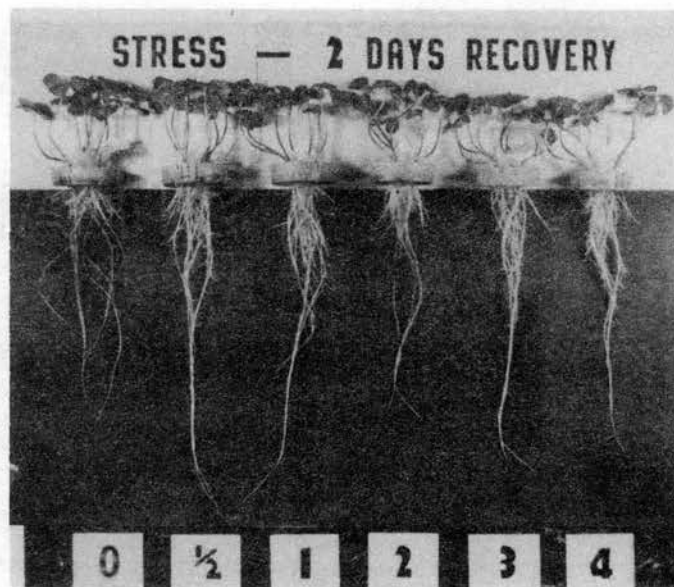
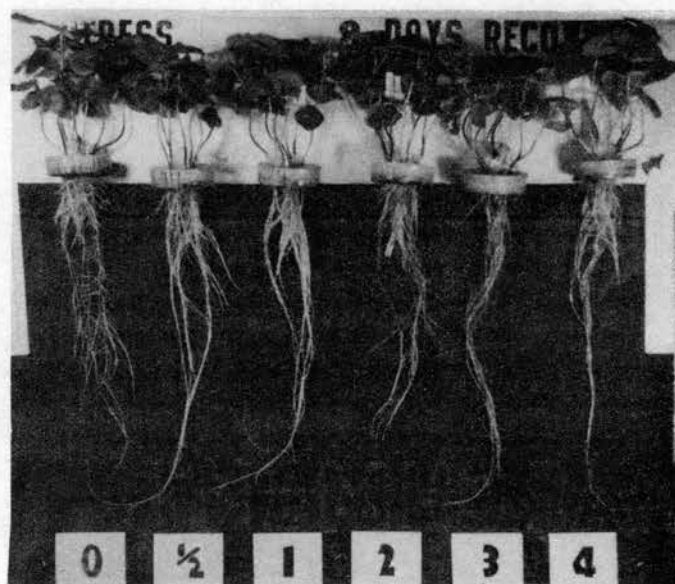


Figure 4. Effect of Duration of Stress with Constant Relative Humidity on Relative Turgidity of Cotton Seedlings, Experiment 3.



2 days



8 days

Figure 5. Recovery of Cotton Seedlings Two Days and Eight Days Following Moisture Stress Treatments.

moisture stress in cotton seedlings. The two methods involved the suspension of the cotton seedling roots in water-saturated air and circulating air past the cotyledons. The treatments for the one method consisted of circulating air of different relative humidities past the cotyledons of the plants. In the other method the groups of plants were treated for different periods of time with air of the same relative humidity. The second method seems to have more application and was used a number of times in this series of studies.

The moisture content of the cotyledons appeared to be a poor criterion for estimating the water content of the root system under these conditions as was shown in all three experiments (Figures 1, 2, 4). The moisture content of the roots was greatly reduced before there was any noticeable reduction in moisture in the cotyledons or stems.

The "recovery ratio", $\text{dry weight}/(\text{turgid wt.} - \text{dry wt.})$, was calculated as a possible indication of plant recovery following severe moisture stress in Experiment 2 (Figure 3). There was no indication in the values obtained of any change in the amount of water absorbed by the plant segments when placed in distilled water. A sudden change in values might indicate permanent damage.

The four-hour stress period caused severe wilting of the seedlings in Experiments 2 and 3. In spite of the water loss to about 45% relative turgidity the cotton seedlings in Experiment 3 recovered visually as indicated by a maintenance

of a light color of the old roots and the initiation of new lateral roots within a few days following the stress treatment (Figure 5). One seedling lost its pair of cotyledons following the four-hour moisture stress but new leaves appeared by the sixth day and the root system showed no visible damage.

Todd et al. (66) studied the moisture percent at which small grains failed to recover when rewatered. They found that 25% relative turgidity was the approximate minimum from which small grain plants could recover. They found little difference between species of the small grains: rye, Secale cereale, L.; barley, Hordeum vulgare, L.; oat, Avena sativa, L.; winter wheat, Triticum aestivum, L.

B. Factors Affecting Absorption and Movement of Ions

PROCEDURES

Experiment 4. Effect of accompanying ions and aeration on uptake and distribution of cesium.

The cotton seedlings were grown as previously described. The cotton seedlings, eight to the cork, were allowed to absorb cesium from solutions containing several different accompanying ions, with and without aeration. The absorption solutions consisted of deionized water, calcium and magnesium sulfate $2 \times 10^{-4}M$ total (2.5:1), and 0.057 Hoagland solution. The same calcium and magnesium sulfate concentrations were in the latter two solutions. Each absorption

solution contained 4.75 me. per liter of cesium tagged with cesium 137. One hundred and sixty ml. of each solution was poured into each of two 200-ml. tall-form beakers and the plants were placed with their roots in the solutions for two hours.

The plants were rinsed three times, in their respective solutions containing no cesium, for one minute each. The plants were then placed in solutions identical to the absorption solutions but containing five me. of potassium chloride per liter and mannitol to equal two atmospheres of osmotic stress. The mannitol solution was made according to Morse (48). The plants remained in the stress-exchange solution for 30 minutes and were then rinsed in distilled water, separated into roots, stems and cotyledons and placed in tared beakers. The plant parts were dried at 100° C., weighed and digested. The salts remaining following digestion were washed into planchets and the beta count determined. One set of plants in each treatment was aerated during absorption and exchange, and the other set of plants was not. The activity of cesium in each of the plant segments was compared to the activity in the absorption solutions and the total amount of cesium calculated.

Experiment 5. Effect of moisture stress, root removal and exchange solution on uptake and distribution of calcium and cesium within the cotton seedling.

The three treatments--moisture stress, root removal and exchange solution, and a control treatment for each of them--were imposed on groups of cotton plants in different combinations. The designated groups of plants were exposed to one hour stress in the moisture stress apparatus with controlled root and air temperature of 31° C. and the air circulating at 900 ml. per minute. The plants were exposed to 1800 foot-candles of light during growth and stress.

The plants were placed in solutions containing five me. per liter each of cesium and calcium, both in the chloride form, for two hours. The activity was about 26,000 beta counts per minute for each element.

The plants were removed from the absorption solution and washed for one minute each in two containers of distilled water. The roots were cut from the designated plants and placed in magnesium sulfate 10^{-2} M or in distilled water as were the intact plants. The stems and cotyledons of the plants which had their roots removed were placed in tared beakers. After one hour in the exchange or diffusion solutions the roots and plants were removed and the intact plants were separated and placed in tared beakers. The plant parts were digested and prepared for beta and gamma determination.

RESULTS AND DISCUSSION

Experiment 4 was conducted to determine an absorption solution for the study of movement of cations within the cotton seedlings. The results indicate a difference in the amount of cesium moving out of the roots and into the stems and cotyledons for the three solutions (Figure 6). The total uptake of cesium or cesium plus potassium for the entire plant per gram of roots is surprisingly constant, as shown in Figure 8. However, when the concentration of cesium in the different plant parts per gram of tissue is considered, considerable variation occurs. The plants that absorbed cesium from the distilled water retained most of the cesium in the roots. The plants that absorbed from the calcium-magnesium sulfate solution transported a relatively large proportion of cesium to the stems and cotyledons as compared to the former treatment. The cotton plants in Hoagland solution absorbed and transported cesium to the stems and cotyledons at concentrations intermediate to the other two treatments.

The so-called aeration seemed to be quite consistent in its effect on the uptake of the cesium. However, it is possible that the increase was due largely to agitation of the solutions. The roots were quite long and curled together around the bottom of the beaker and without agitation there could be little change in the solution at the surface of the roots. Onken⁷ found practically no increase in absorption

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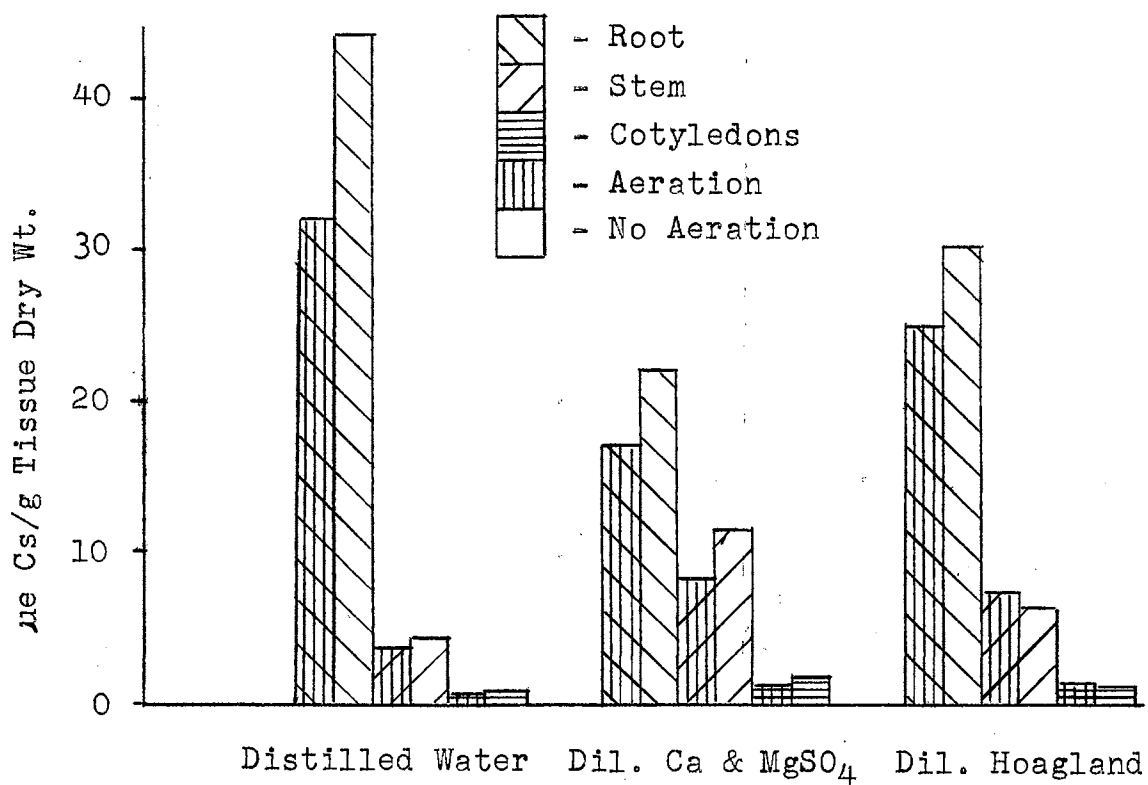


Figure 6. Effect of Accompanying Ions and Aeration on Uptake and Distribution of Cesium by Cotton Seedlings.

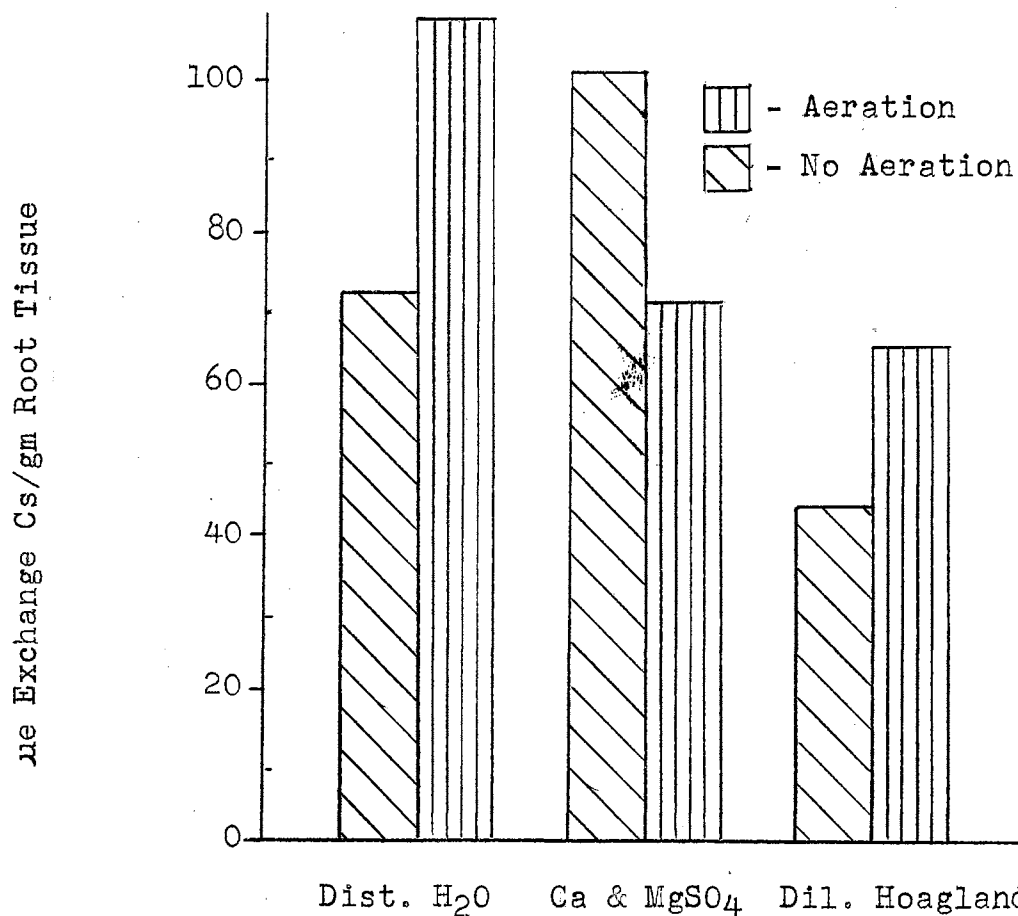


Figure 7. Effect of Accompanying Ions and Exchange-Moist. Stress on Release of Cesium from Cotton Roots.

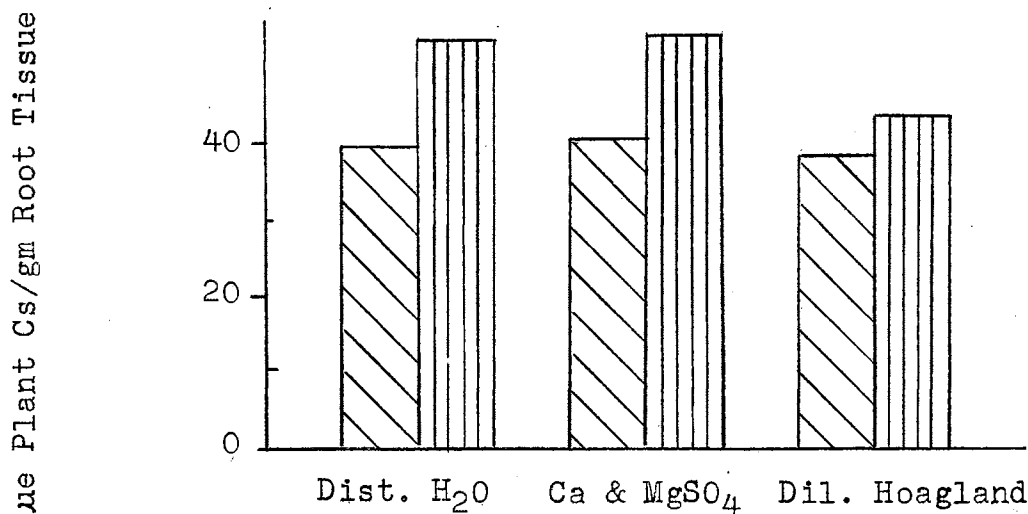
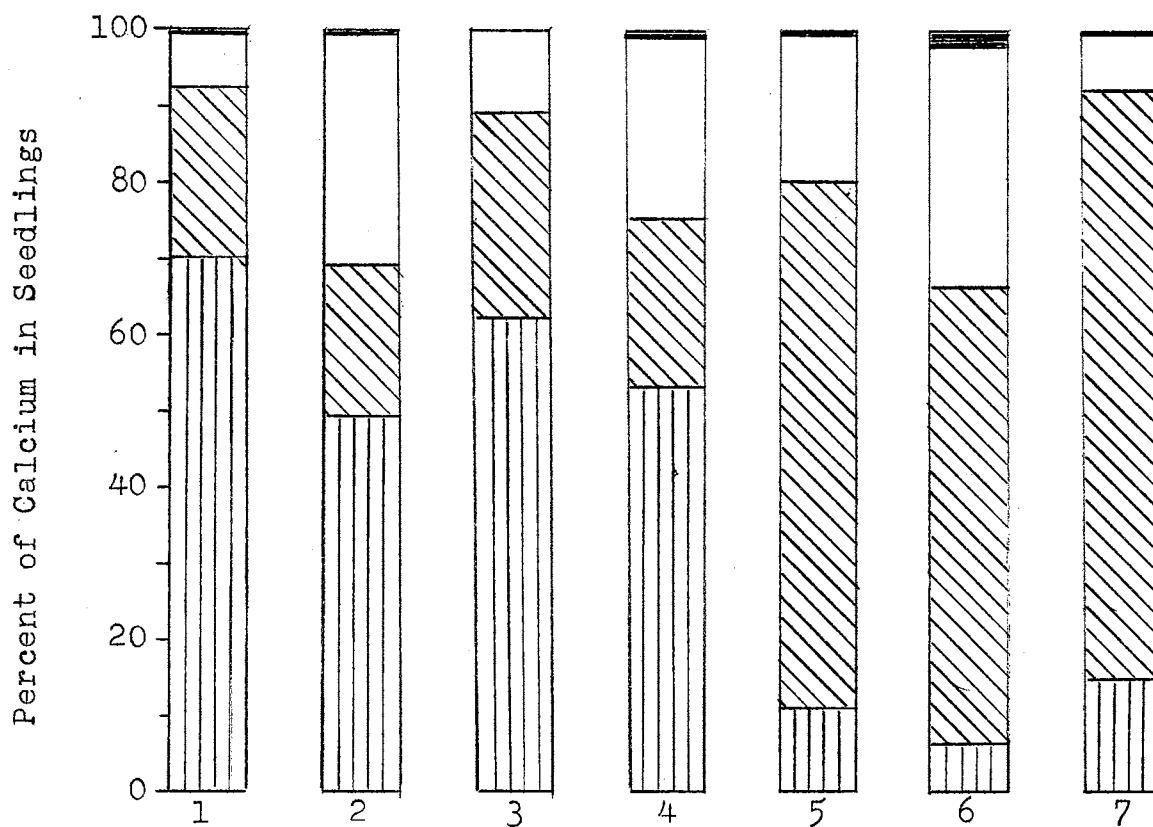


Figure 8. Effect of Accompanying Ions on the Total Cesium Absorbed per Gram of Roots.



1 - Stress, Intact, MgSO₄

2 - Control, Intact, MgSO₄

3 - Stress, Cut Roots, MgSO₄

4 - Control, Cut Roots, MgSO₄

5 - Stress, Intact, H₂O

6 - Control, Intact, H₂O

7 - Stress, Cut Roots, H₂O

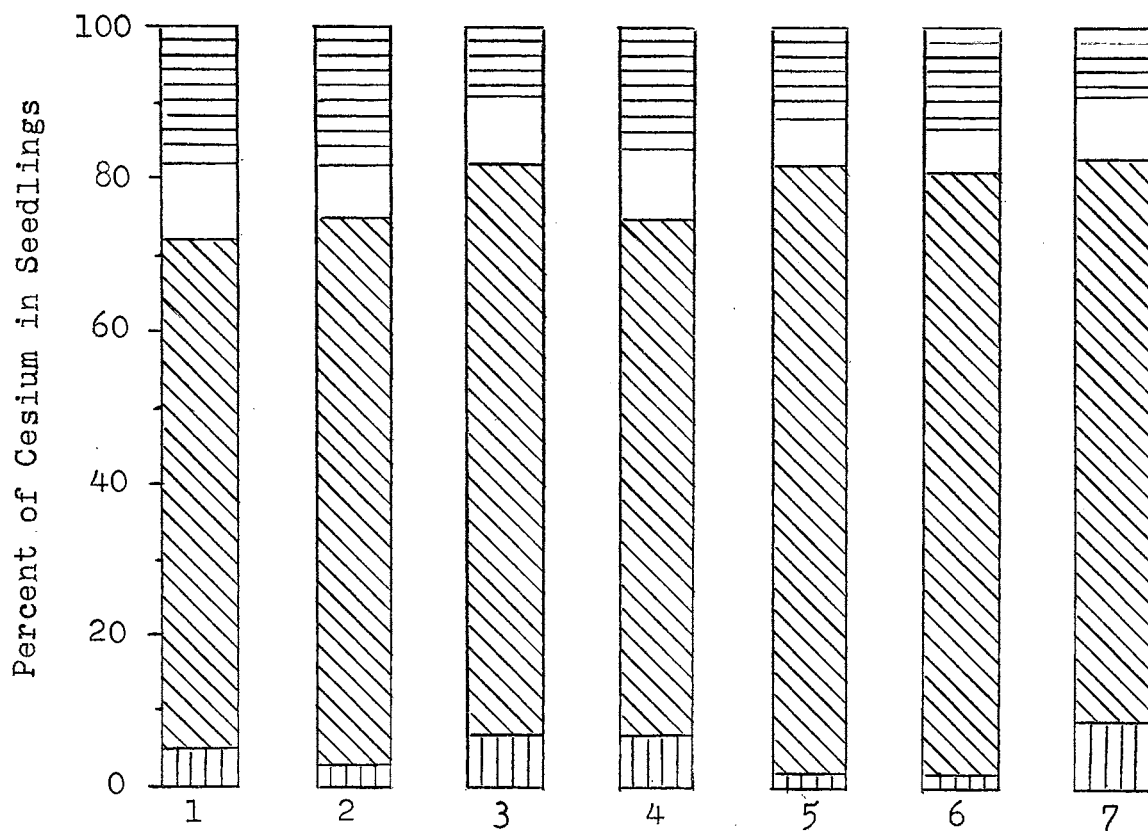
■ - Cotyledons

□ - Stems

▨ - Roots

▤ - Exchange

Figure 9. Effect of Moisture Stress, Removal of Roots and Exchange on Uptake and Movement of Calcium in Cotton Seedlings.



- 1 - Stress, Intact, MgSO₄
 2 - Control, Intact, MgSO₄
 3 - Stress, Cut Roots, MgSO₄
 4 - Control, Cut Roots, MgSO₄
 5 - Stress, Intact, H₂O
 6 - Control, Intact, H₂O
 7 - Stress, Cut Roots, H₂O

- ☐ - Cotyledons
 □ - Stems
 ▨ - Roots
 ▩ - Exchange

Figure 10. Effect of Moisture Stress, Removal of Roots and Exchange on Uptake and Movement of Cesium in Cotton Seedlings.

of cesium due to aeration of grain sorghum seedling roots suspended in test tubes. These roots did not touch the sides of the tubes or each other. The level of the nutrient solution was several cm. below the seed.

The stress-exchange treatment in Experiment 4 was included to determine the effect of stress on the release of cesium by the roots but the results were inconclusive, as shown in Figure 7.

Calcium was more responsive to the moisture stress and exchange treatments of Experiment 5 than was cesium (Figures 9, 10). The greatest response of calcium was between the water and the magnesium sulfate exchange solution following uptake. Considerably more calcium was exchanged from the roots into the magnesium sulfate solution than was lost by diffusion into water under the same conditions.

The effect of stress on the movement of calcium was noticeable both in the amount of calcium exchanged or diffused out of the roots into the solutions and in the amount moved into the stems. Within the groups of plants whose roots were diffused in water or exchanged in magnesium sulfate the amount of calcium in the root tended to remain constant. If a larger amount were lost outward from the root due to stress the percentage in the stem decreased, thus tending to maintain a constant percentage of calcium in the root within either group. Only a trace of calcium was indicated to have moved into the cotyledon during this experiment.

Cesium was very unresponsive to all treatments when compared to calcium. The magnesium sulfate exchange solution produced little observable difference when compared to water

in the amount of cesium exchanged or diffused from the root. There appeared to be a tendency for a constant percentage of cesium in the stem, rather than in the root as was found for calcium. The effect of moisture stress was not consistent on cesium lost from the root by diffusion or exchange into the surrounding solution.

There were some differences in movement of the calcium and cesium caused by severing the roots following absorption. The most consistent effect for the severed roots was the percent increase of cesium in the exchange solutions.

C. Effect of Moisture Stress on Movement of Cesium Within the Cotton Seedling

PROCEDURES

Experiment 6. Constant time of stress at varying relative humidities, 8 to 100%.

The purpose of this experiment was to determine the effect of short-duration moisture stress on the movement of cesium distributed within the cotton seedling.

Eight-day old cotton seedlings that had been sprouted and grown in the holding solution were allowed to absorb cesium at five me. per liter for two hours from a solution also containing the same calcium and magnesium sulfate as the holding solution. The solution was tagged with cesium 137, about 28,000 beta counts per minute per ml. The roots were rinsed three times and returned to the holding solution.

Twenty-four hours later the plants were removed from the holding solution and were rinsed one minute in a solution of the same composition. The excess water was blotted gently from the roots and the roots were then placed in the moisture stress chamber. The treatments were conducted one at a time with no replication. The same levels of moisture stress obtained by circulating air at different relative humidities were used as in Experiment 1, and the same conditions of temperature and air flow were used. Following air stress the roots were separated and placed in magnesium sulfate $10^{-2}M$ exchange solution for one hour. The roots were rinsed in distilled water and placed in tared beakers as were the stems and cotyledons at the time of separation. The plant parts were digested with nitric-perchloric acid and the cesium activity was determined. The activity of the exchange solution and absorption solution was also determined.

The root:exchange ratio was calculated by dividing the total cesium activity in the roots by the total cesium activity in the exchange solution.

Experiment 7. Constant time of stress at varying relative humidities, 80 to 100%.

The purpose of this experiment was to determine whether or not the release of absorbed cesium from the cotton seedling root under stress was sensitive to slight differences in moisture stress in the area of 100% relative humidity.

This experiment was practically the same as the previous experiment except that the relative humidity treatments in the stress chamber varied from 80 to 100% and the cesium was absorbed for three and one-half hours instead of two hours.

Experiment 8. Constant relative humidity for varying periods of time, 0 to 60 minutes.

The previous experiments indicated that any movement of cesium within the cotton seedlings would likely occur at low amounts of moisture stress over a longer period of time. This experiment was conducted with this in mind. Cotton seedlings were subjected to moisture stress in the stress apparatus for various lengths of time at two different relative humidities, 95 and 100%, 24 hours following the absorption of cesium 133 and 137. The stress apparatus used in this experiment was arranged so that the constant-temperature bath regulated the root temperature and the temperature of the air flowing through the stress chamber.

Cesium was absorbed into the cotton seedlings, starting 24 hours prior to the experiment. The plants were placed for three hours in holding solution containing five me. per liter of cesium chloride tagged with cesium 137. They were then rinsed and returned to the calcium and magnesium sulfate holding solution.

The following day the corks of plants were removed from the holding solution and rinsed one minute in fresh holding solution. The clinging drops were removed with soft absorbent

tissue and the corks of plants were placed one at a time in the stress chamber for the required length of time. Following the stress period the plants were removed and separated into roots, stems and cotyledons. The roots were placed in 100 ml. of aerated $10^{-2}M$ magnesium sulfate for one hour and rinsed. They were then placed in tared beakers as had been the stems and cotyledons. The plant parts were dried at $100^{\circ} C.$, weighed, wet-ashed and rinsed into planchets. The beta activity was determined for the plant parts as well as for the exchange solution and absorption solution.

Experiment 9. Constant relative humidity for varying periods of time, 0 to 240 minutes.

The previous experiment indicated that 60 minutes of stress was insufficient to cause discernible movement of cesium within the cotton seedling. A longer experiment, of four hours duration, was conducted.

Cotton seedlings in corks were allowed to absorb cesium for three hours, from a solution containing five me. per liter tagged with cesium 137 dissolved in the calcium and magnesium sulfate holding solution. The seedlings were returned to the calcium and magnesium sulfate solution for 24 hours or until the following day. The seedlings were removed from the holding solution, rinsed one minute in the calcium and magnesium sulfate solution and allowed to drip in a closed, wet blotter-lined jar for one minute. The collected drops of solution were blotted from the roots and

the plants were treated in the stress chamber for the desired lengths of time, from 15 minutes to 4 hours. Two controls were included, (1) direct from the holding solution and, (2) following the "drip" treatment. As in the previous experiment the roots for each treatment were removed and placed in 100 ml. of a $10^{-2}M$ magnesium sulfate solution for one hour, then rinsed with distilled water. The roots were placed in tared beakers as had been the stems and cotyledons. The plant parts were dried at $100^{\circ} C.$, digested and the beta activity determined.

Experiment 10. Effect of mannitol-induced moisture stress on movement of cesium in the cotton seedling.

Cotton seedlings were allowed to absorb cesium and were then subjected to osmotic stress to observe the effect on the movement of the cesium within the plant and the stress solution.

Cotton seedlings were grown in the manner described and on the ninth day allowed to absorb cesium for two hours from a solution containing five me. per liter of the holding solution tagged with cesium 137. The seedlings were then exposed to one of three treatments: control, two atmospheres or eight atmospheres of osmotic stress produced by mannitol. The stress solutions contained potassium chloride, ten me. per liter of the calcium and magnesium sulfate holding solution. The three treatments were duplicated and the whole experiment was repeated one time.

The cotton seedlings remained in the osmotic stress solution for four hours during which time samples were taken at intervals to follow the accumulation of cesium. Following the four-hour stress period the plants were removed and the roots washed in distilled water. The plants were separated into roots, stems and cotyledons, dried in tared beakers at 100° C., digested in nitric-perchloric acid and the beta activity determined. Samples taken from the stress solution were dried and the beta activity determined.

This experiment was conducted under fluorescent lights with about 1800 foot-candles. A fan was directed toward the cotton plants from across the room to facilitate transpiration of water from the cotyledons. The roots were aerated during absorption of the cesium and during the osmotic stress treatment.

RESULTS AND DISCUSSION

The percentage distribution of cesium within the segments of the cotton plants was relatively constant following the 30-minute moisture stress treatments with air at different relative humidities (Figures 11, 13). However, there was a noticeable amount of cesium released to the magnesium sulfate solution either by exchange or simple diffusion for all increments of stress. This cesium release by the roots is indicated by Figures 12 and 14. The increase in the number of relative humidity treatments near 100% did not detect or indicate a gradation in the release of cesium.

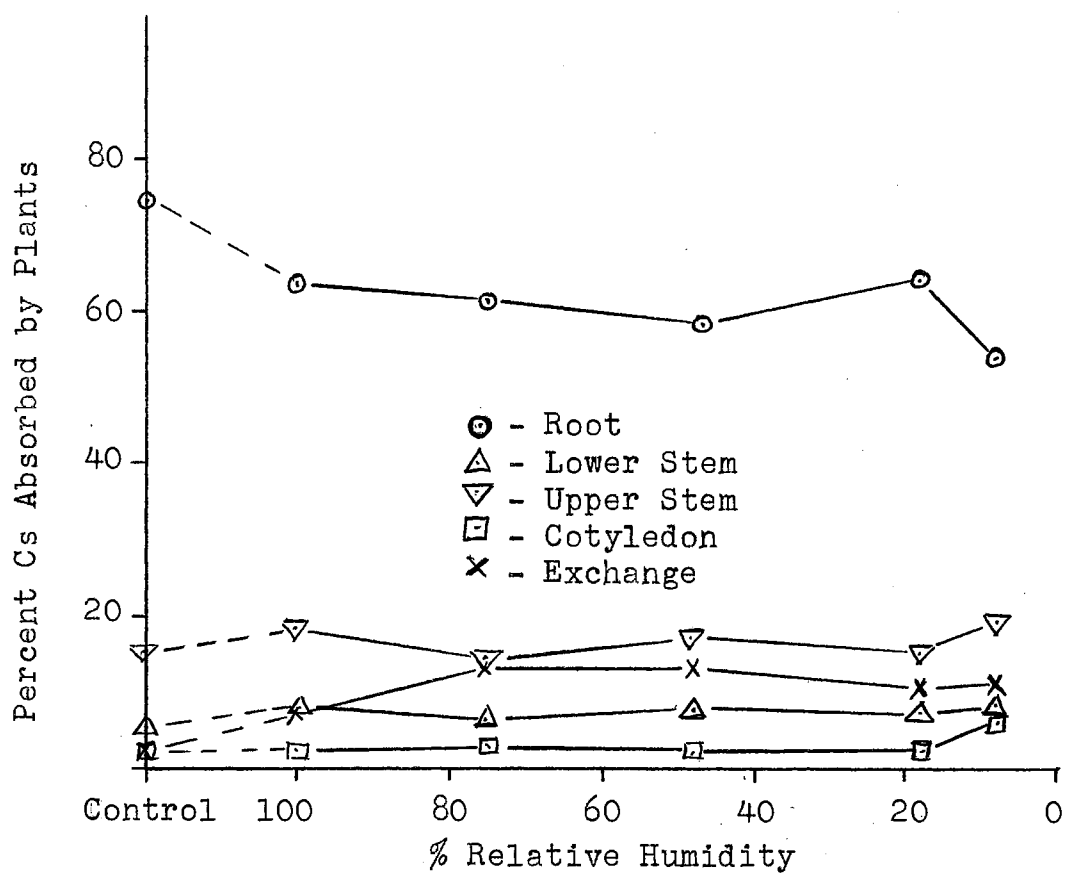


Figure 11. Effect of 30-Minute Moisture Stress on Movement of Cesium Within the Cotton Seedling.

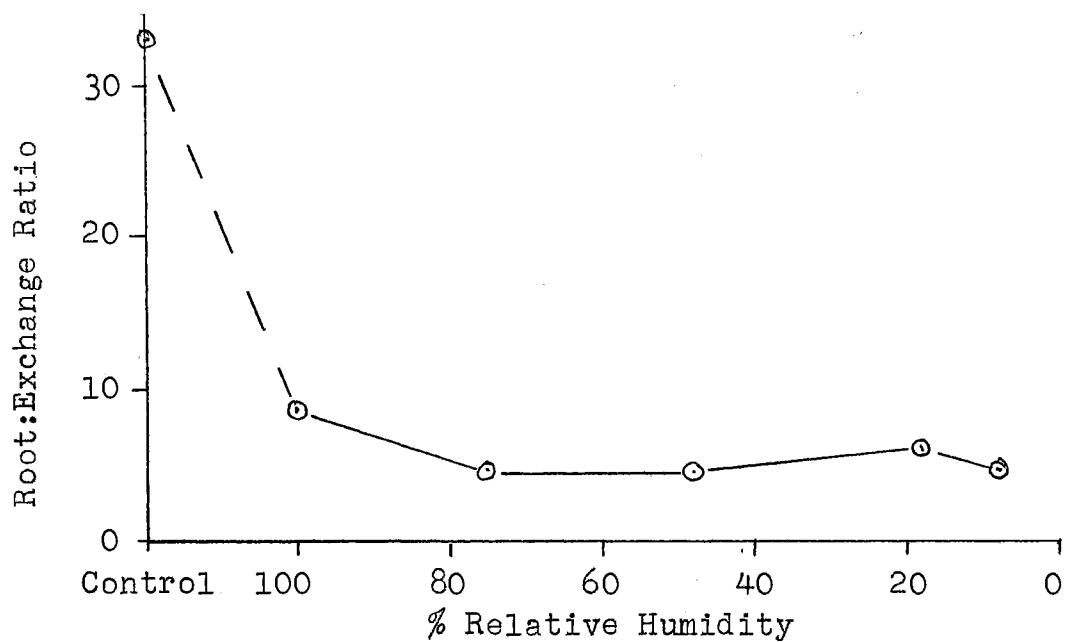


Figure 12. Effect of 30-Minute Moisture Stress on Loss of Cesium to External Solution.

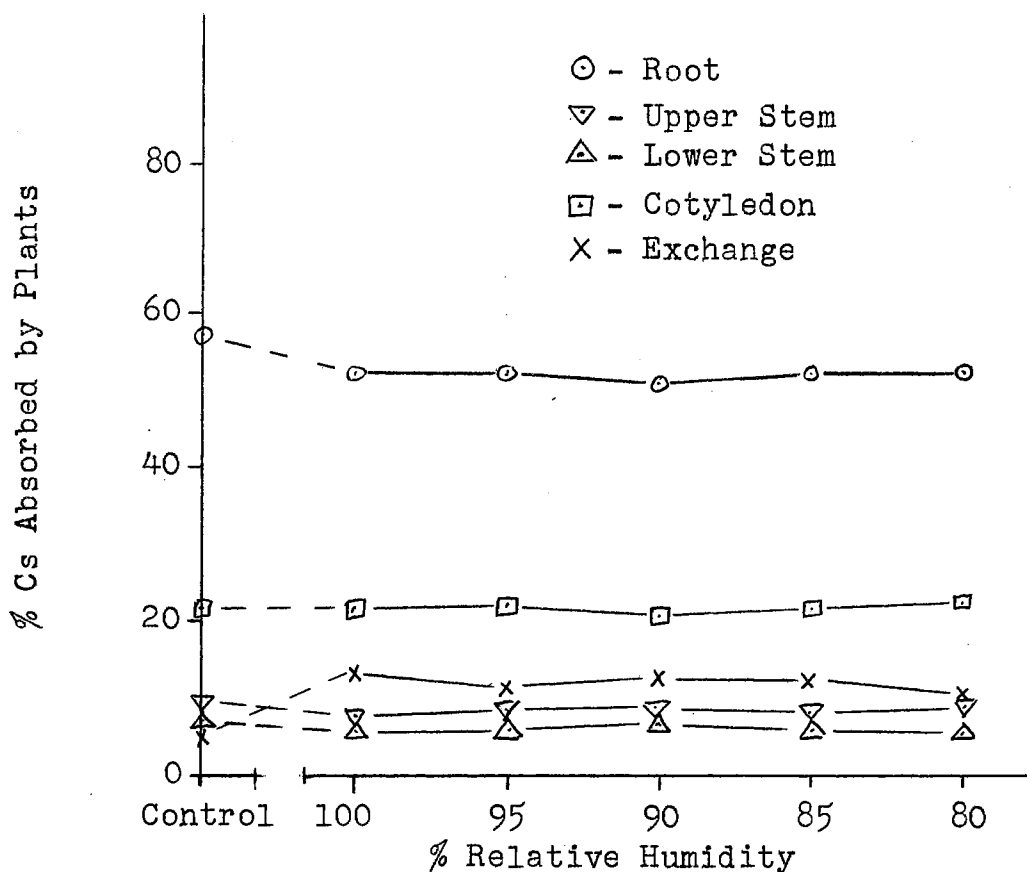


Figure 13. Effect of 30-Minute Moisture Stress on Movement of Cesium Within the Cotton Seedling.

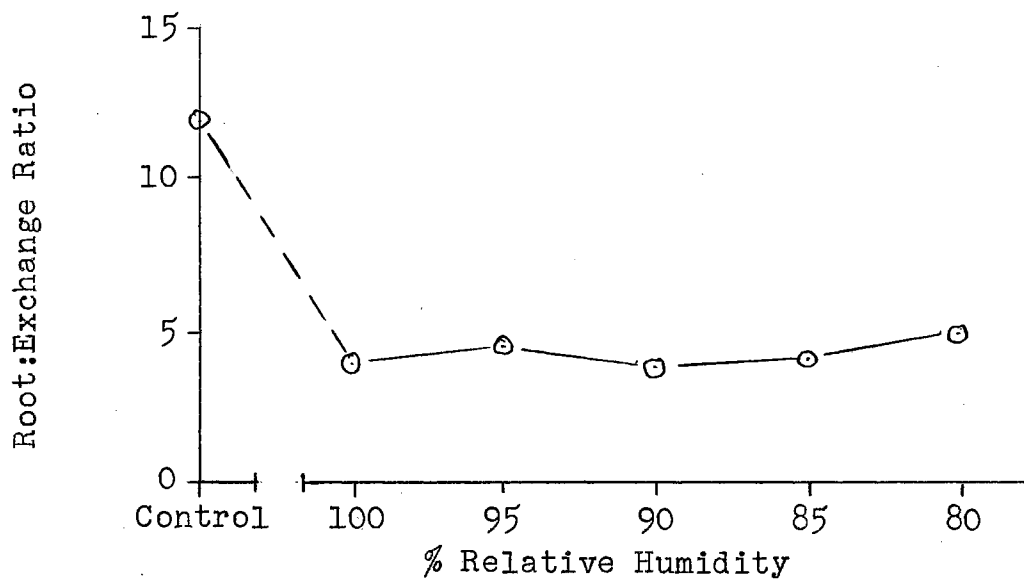


Figure 14. Effect of 30-Minute Moisture Stress on Loss of Cesium to External Solution.

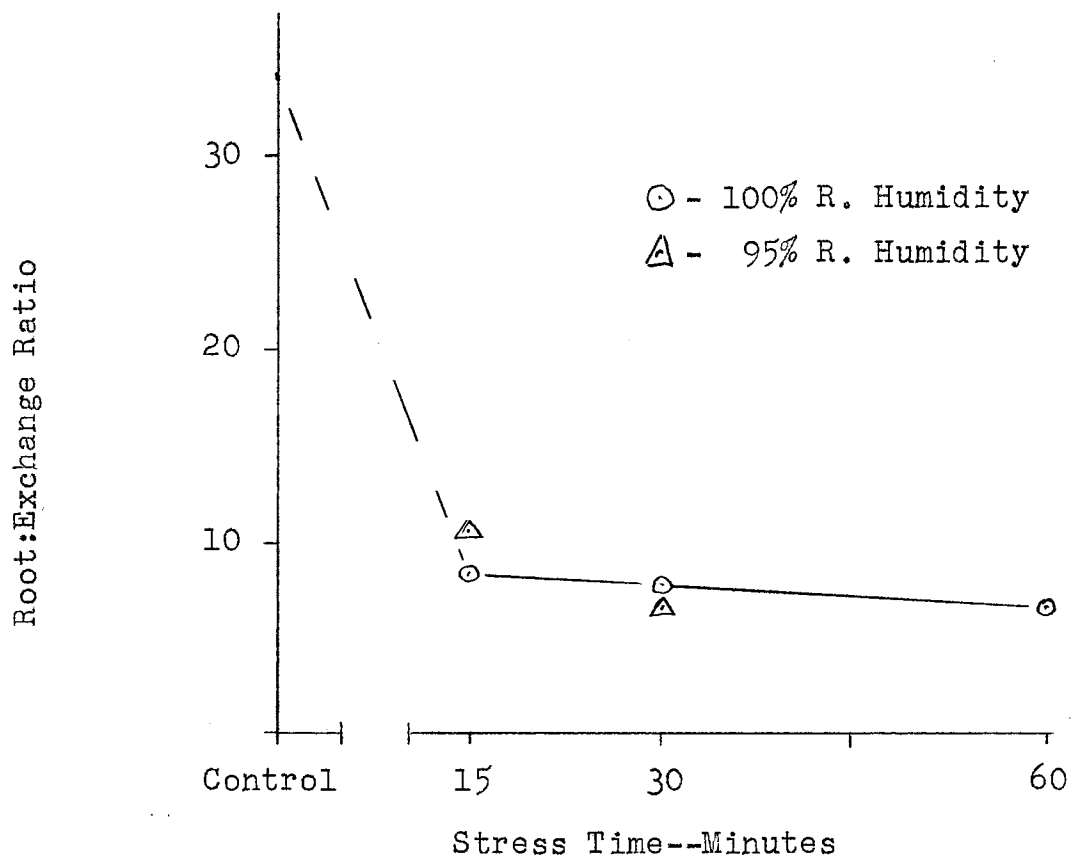


Figure 15. Effect of Moisture Stress on Release of Cesium from Roots to External Solution.

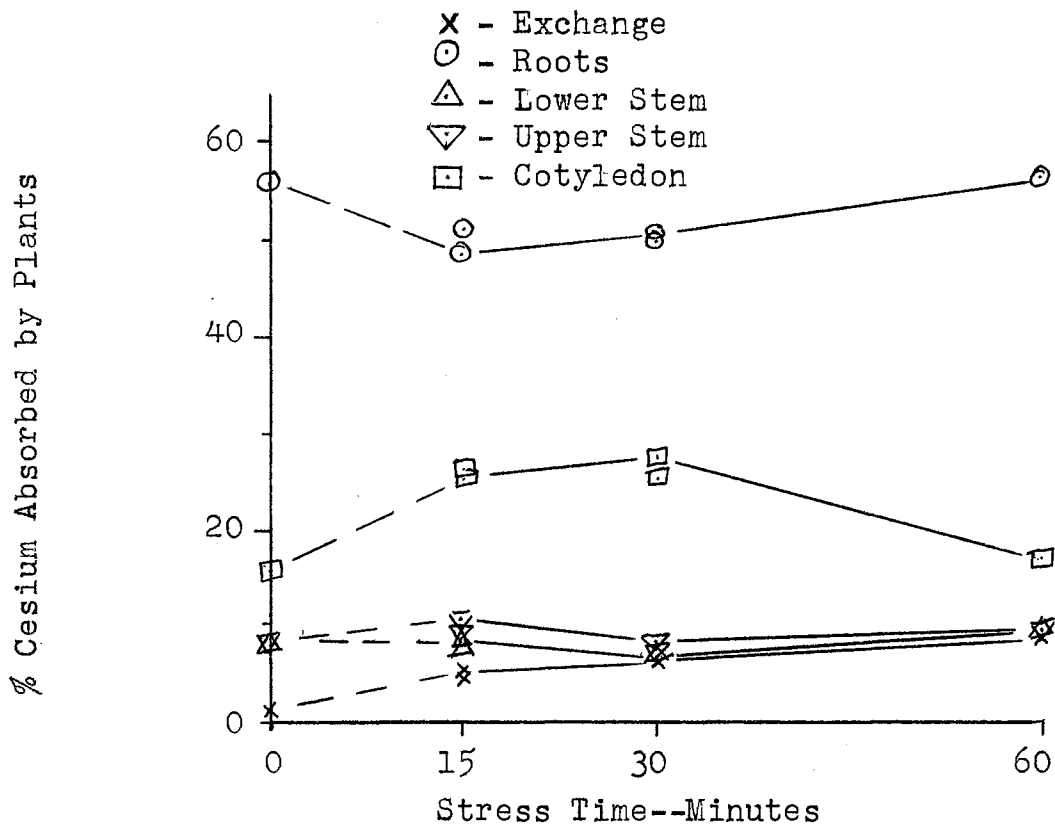


Figure 16. Effect of Moisture Stress on Movement of Cesium Within Cotton Seedlings.

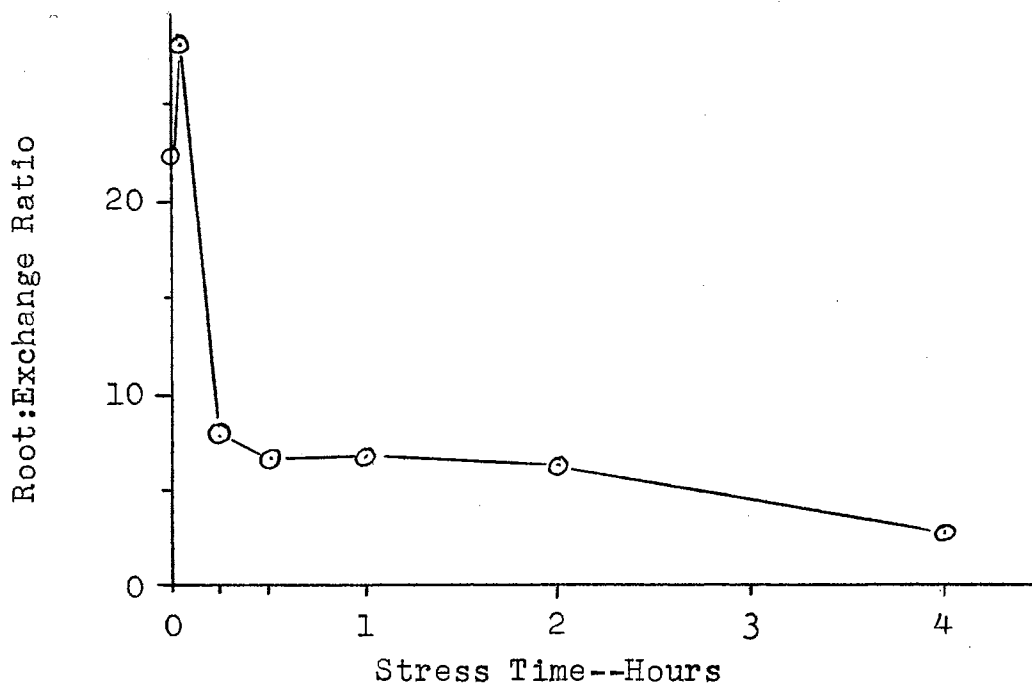


Figure 17. Effect of Moisture Stress on Release of Cesium From Root to External Solution.

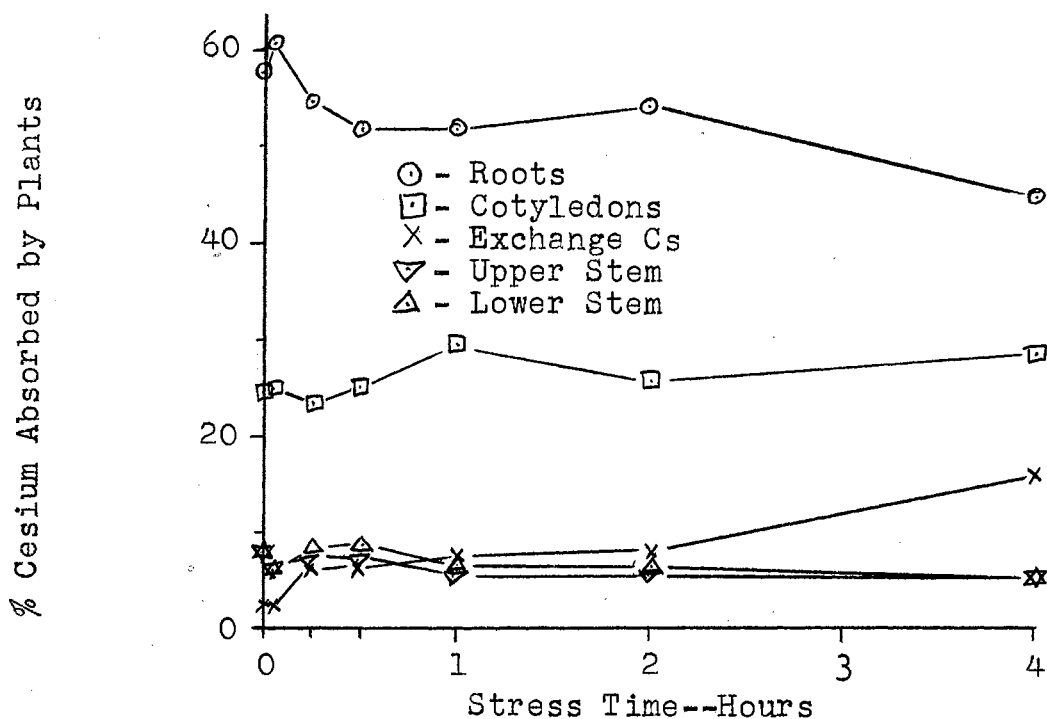


Figure 18. Effect of Moisture Stress on Movement of Cesium Within Cotton Seedlings.

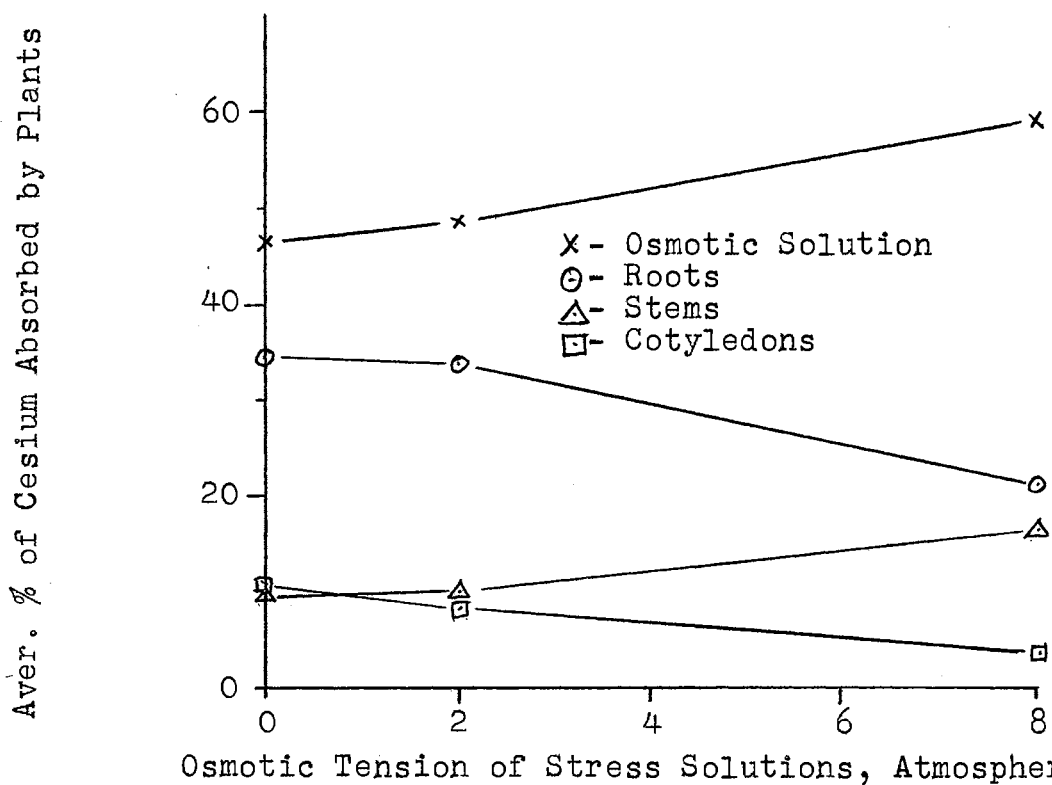


Figure 19. The Effect of Moisture Stress on Distribution of Cesium Absorbed Immediately Prior to Stress.

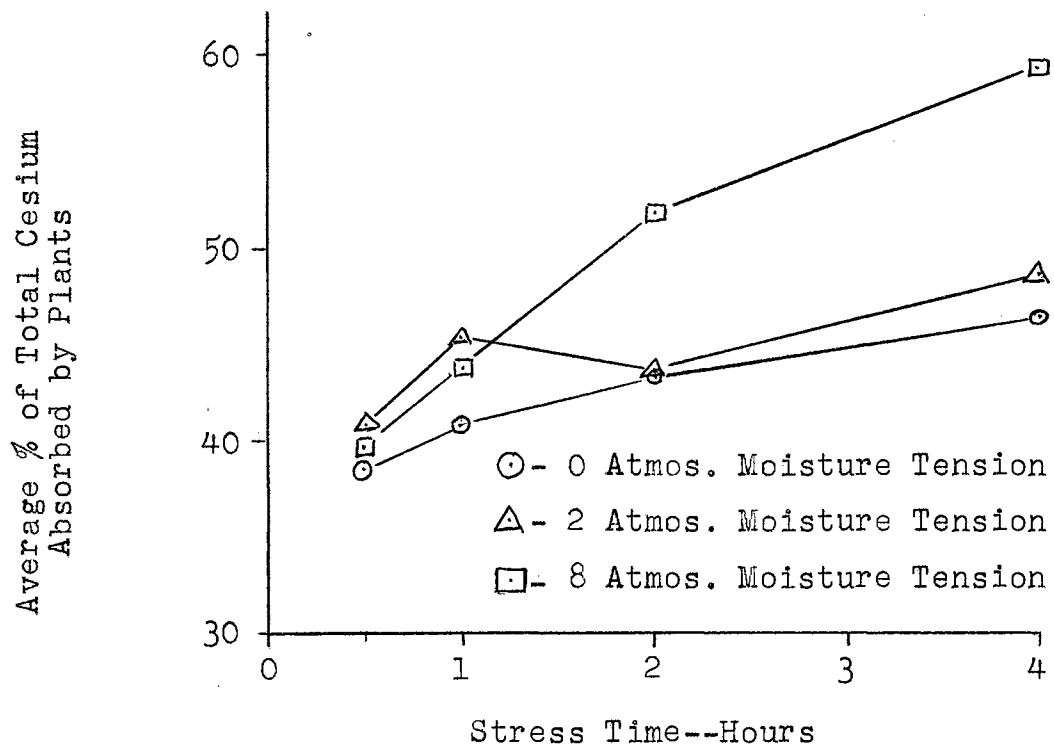


Figure 20. Effect of Osmotic Stress on Desorption of Cesium from the Plant Roots.

The greatest difference in the results of Experiments 6 and 7 was in the amount of cesium taken up by the cotyledons. There was nothing observed in the two experiments which would suggest this difference except the increased time of cesium uptake for Experiment 7. The increased cesium possibly moved through the conductive tissue and accumulated in the cotyledons with little lateral movement into the stems.

The results from Experiment 8 indicate that moisture stress of as little as 15 minutes duration, with the air circulating at practically 100% relative humidity, resulted in a loss of cesium from the root to the external solution (Figure 15). There appeared to be a decrease of cesium in the cotyledons during the time shown (Figure 16) but there was no confirmation of this in the other experiments.

Moisture stress induced considerable loss of cesium from the root to the external solution in all treatments except the two controls in Experiment 9. A relatively large percent of cesium was lost by the root to the exchange solution in the four-hour treatment as shown in Figures 17 and 18. The results obtained for the movement of cesium within the plant are shown in Figure 18. Again, the points were erratic. A somewhat similar pattern for cesium movement in the cotton seedlings was indicated for the one-hour stress in Figure 16 and the first two hours of Figure 18, although the similarity may be fortuitous.

Experiment 10 differs from the preceding experiments in the method of causing stress, the time the cesium had been

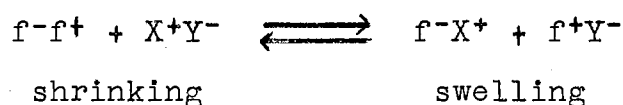
absorbed into the plants and the measurement of cesium released by the roots. In Experiments 6 through 9 the cesium had been absorbed 24 hours prior to stress and the location of cesium in the plants was determined at the end of the moisture stress period. The cesium released from the severed roots was determined by placing them in a dilute magnesium sulfate solution. However, in Experiment 10, the cesium was absorbed just prior to exposure to mannitol-induced moisture stress: cesium was allowed to exchange or diffuse into the surrounding solution during stress.

The differences in procedures of the experiments produced differing results as shown in Figures 19 and 20. The total percentage of cesium diffusing from the roots was quite high, even for the control plants in Experiment 10. The two-atmosphere osmotic stress treatment produced results similar to the control. The eight-atmosphere osmotic stress treatment seemed to cause an increased percent of cesium in the external solution, a decrease in the percentage of cesium in the root, an increase in the percentage of cesium in the stem and a decrease in the percentage of cesium in the cotyledons. The increased percentage of cesium in the stem is possibly due to a reduced movement of cesium to the cotyledons caused by a decreased upward movement of water from the root.

Stewart (62) conducted experiments with kidney beans growing in soil. He found relatively more cesium in the

stems of moisture-stressed plants than in the leaves, as compared to that found in control plants.

One explanation of the loss of cations from the roots as the result of moisture stress might be an interpretation of a proposal of Ling (41). He suggested that the breaking or making of salt linkages affects the osmotic behavior of proteins causing swelling or shrinking respectively. He presented the equation



where f^+ and f^- represent fixed cations and anions and the X^+ and Y^- represent free counterions. He also suggested that a change in pH is produced to account for the change in protein structure. Ling presented data of Jordan-Lloyd, and Alfrey and Gurnee to show that the pH of a solution affects the osmotic properties of protein, whether in living or artificial systems.

The present author considered that a lowering of the moisture content of cells should drive Ling's equation for the structure of the proteins to the left and affect the retention of the cations within the cells, in addition to any change in pH. A sudden increase in the activity of the cations within the cells should reverse their concentration gradient. Should this event occur in the root of a plant, the effect should be the release of cations to the external solution, in addition to any internal change in pH that might occur within the root. The consistent release of

cesium to the surrounding solutions from cotton seedling roots as the result of moisture stress might be explained in the light of this interpretation of Ling's proposal.

The pH determinations were obtained for a series of solutions for one of the stress experiments. The results indicated differences but insufficient data were obtained for presentation.

D. Effect of Moisture Stress on Uptake of Cesium and Calcium by Cotton Seedlings

PROCEDURES

Experiment 11. Effect of varying intervals of moisture stress on uptake of Cesium and Calcium.

The cotton seedlings used in this experiment were 11 days old. The groups of plants were removed from the holding solution, allowed to drip with their roots in a closed jar for two minutes and placed in the moisture stress apparatus for various lengths of time, from 15 minutes to 4 hours. The two control treatments were, (1) direct from the holding solution and, (2) following the two-minute drip period. Following the stress treatments the groups of plants were then placed in beakers containing 5 me. of cesium chloride and 3.6 me. of calcium chloride per liter for 2 hours with aeration except for the 30-minute stress group. The plants were removed, washed two minutes in distilled water, and the roots severed and placed in 100 ml. of magnesium

sulfate $10^{-2}M$ for one hour. The roots were removed, washed with distilled water and placed in tared beakers. The stems and cotyledons had been placed in tared beakers when the roots were removed. The plant parts were dried, weighed and digested. The beta and gamma counts were determined.

Experiment 12. Effect of moisture stress on uptake of cesium and calcium for varying time intervals following moisture stress.

The purpose of this experiment was to determine the effect of a brief period of moisture stress on the uptake of cesium and calcium and the duration of any effects. The plants were allowed to absorb calcium and cesium for varying periods of time following the moisture stress.

The groups of nine-day old cotton plants were selected at random for the different treatments and a random order of conducting the experiment was used. Each group of the stressed plants was exposed to 30 minutes of moisture stress in the chamber. The air flow was about 1200 ml. per minute but the other conditions were the same as in previous experiments. The plants were removed from the stress chamber and placed in an aerated solution containing 5 me. of cesium chloride and 1.8 me. of calcium chloride per liter of solution tagged with cesium 137 and calcium 45. The absorption treatments ranged from 15 to 240 minutes. Each group of plants was washed two times, one minute each, in distilled water, separated and the roots placed in an exchange solution

of magnesium sulfate, $10^{-2}M$, for one hour. The roots were rinsed and placed in tared beakers.

Other groups of plants were taken from the holding solution and allowed to absorb the calcium and cesium mixture with no prior stress. The two portions of the experiment were conducted concurrently. The plant segments were dried at $100^{\circ}C.$, weighed and digested with nitric-perchloric acid. The beta and gamma activities were then determined and the calcium and cesium contents of plant tissues were calculated.

Experiment 13. Effect of recovery time following moisture stress on the uptake of cesium and calcium by cotton seedlings.

Procedures similar to those used in the previous experiments for producing plants and moisture stress upon them were followed for this experiment except that the larger air stress chamber, accommodating four sets of plants, was used. The water bath was set at $31^{\circ}C.$ and water-saturated air at the same temperature was circulated through the top of the stress chamber at about 1600 ml. per minute. Four groups of plants were removed from the holding solution and placed in the stress chamber for the desired length of time. Where applicable, the plants were then immediately returned to the holding solution of calcium and magnesium sulfate for the specified length of time: 0, $\frac{1}{2}$, 2 or 8 hours. The plants were then placed in a solution containing five me. per liter

each of calcium chloride and cesium chloride tagged with calcium 45 and cesium 137 . Following the two-hour absorption the plants were washed two times, one minute each, in distilled water and separated into roots, lower and upper stems and cotyledons. The oven dry-weight was obtained prior to digestion and the beta and gamma activities of the plant parts were determined. One sample accidentally remained in the absorption solution for 30 minutes beyond the intended two hours.

Experiment 14. Effect of moisture stress on subsequent rate of uptake from solutions of varying concentrations of cesium by cotton seedlings.

Cotton seedlings, nine days old and grown similarly to those used in the previous experiments, were also used in this study. Two lots of three groups of plants were exposed to three-hour moisture stress in the four-hole stress chamber with the root and circulating air temperature set at 30° C. and with a flow rate of about 1600 ml. per minute of water-saturated air. Immediately following moisture stress the groups of plants were allowed to absorb cesium for two hours from one of the solutions containing cesium chloride at 2, 4, 6, 8 or 10 me. per liter of calcium and magnesium sulfate holding solution. The absorption solutions were tagged with cesium 137 , about 23,000 counts per minute per ml.

At approximately the same time non-moisture stress plants were exposed to similar absorption solutions. The

plants were then rinsed in distilled water in two different containers for one minute each, dried, weighed and digested.

RESULTS AND DISCUSSION

The results of Experiment 11 indicate that moisture stress affects the subsequent absorption and distribution of calcium and cesium. The data indicate increased stress causes an increase in total plant uptake of calcium per gram of roots. At the same time they also indicate that a greater proportion of calcium remains in an exchangeable position within the roots (Figures 21 and 22). The non-exchangeable calcium was almost constant for the different treatments. However, the total plant uptake of cesium per gram of roots declined at about the same rate as the non-exchangeable cesium (Figures 21 and 22). This resulted in an almost constant total plant cesium to total non-exchangeable cesium ratio (Figure 23).

About 75% of the absorbed calcium was found in the exchange solution following the absorption period and only about 25% of the cesium was released to exchange; however, three times more calcium than cesium was absorbed from a solution containing 3.6 me. and 5 me. per liter respectively. Almost the entire amount of calcium was found in the roots or exchange solution. Therefore, the same results were obtained for calcium whether considering total uptake or the portion found in the roots (Figure 24). The conditions were somewhat different for cesium as a noticeable amount was

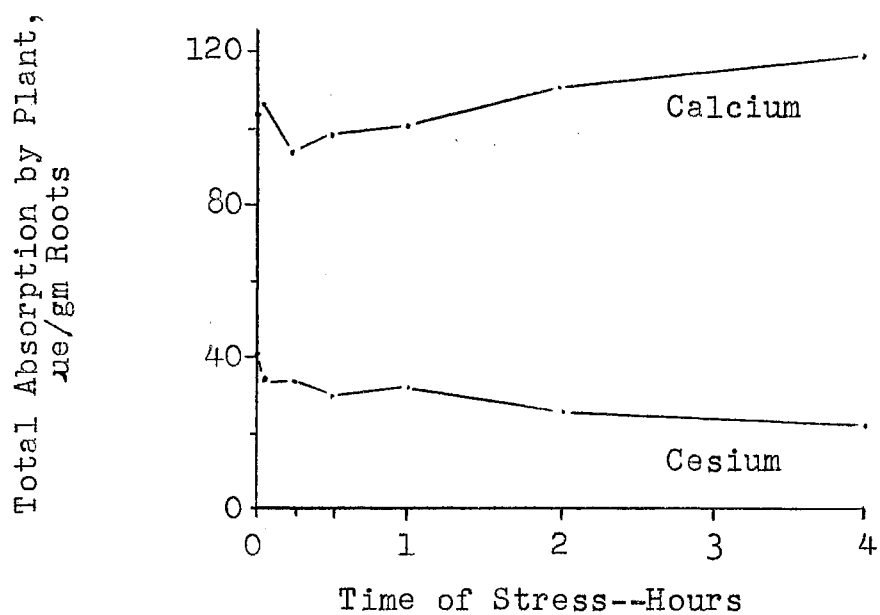


Figure 21. Total Calcium and Cesium per Gram of Roots, Absorbed by Cotton Seedlings Following Moisture Stress.

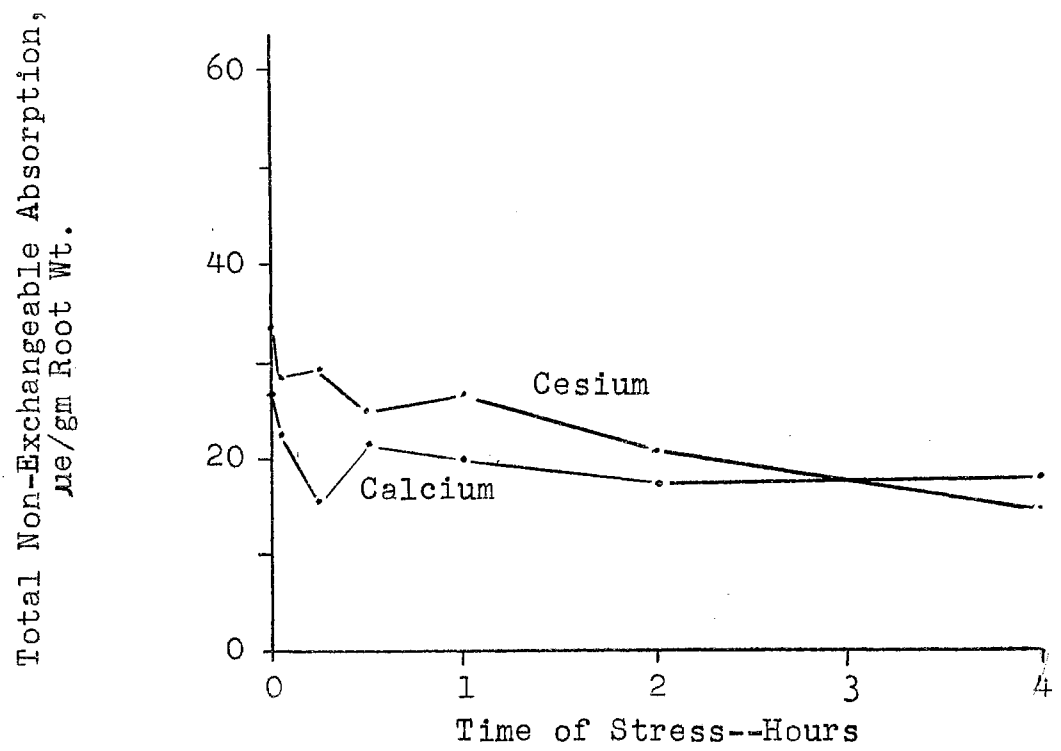


Figure 22. Effect of Moisture Stress on Subsequent Total Non-Exchangeable Ca and Cs Uptake.

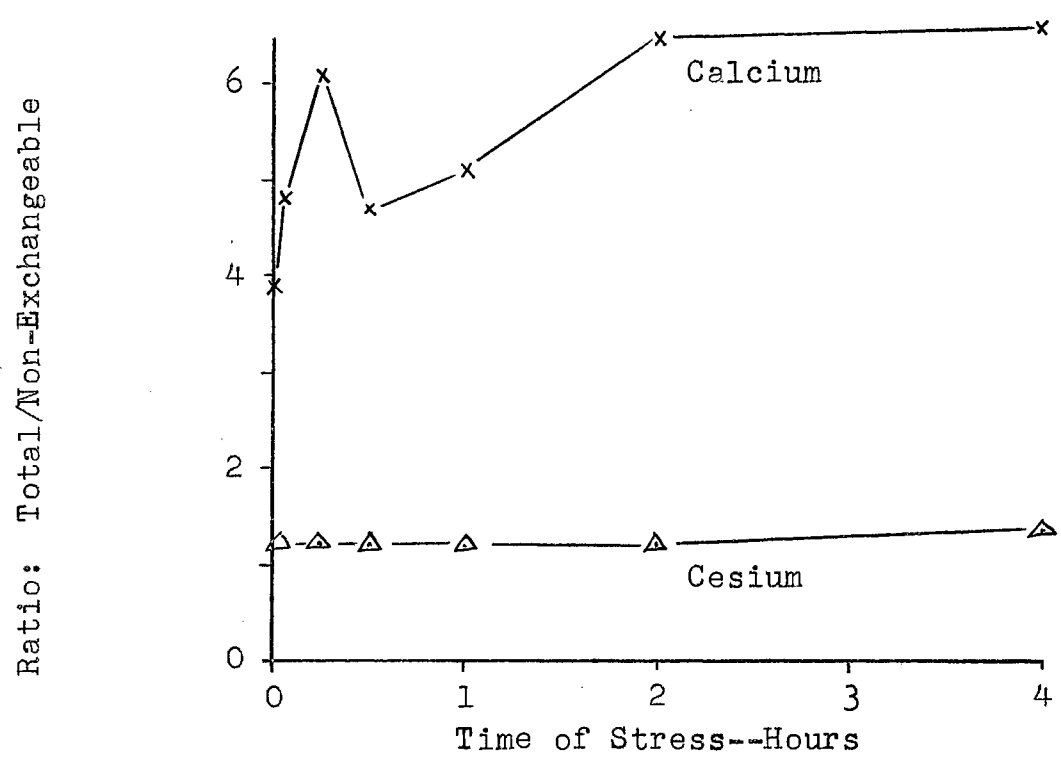


Figure 23. Effect of Moisture Stress on Ratio of Subsequent Total Uptake and Non-Exchangeable Accumulation of Calcium and Cesium.

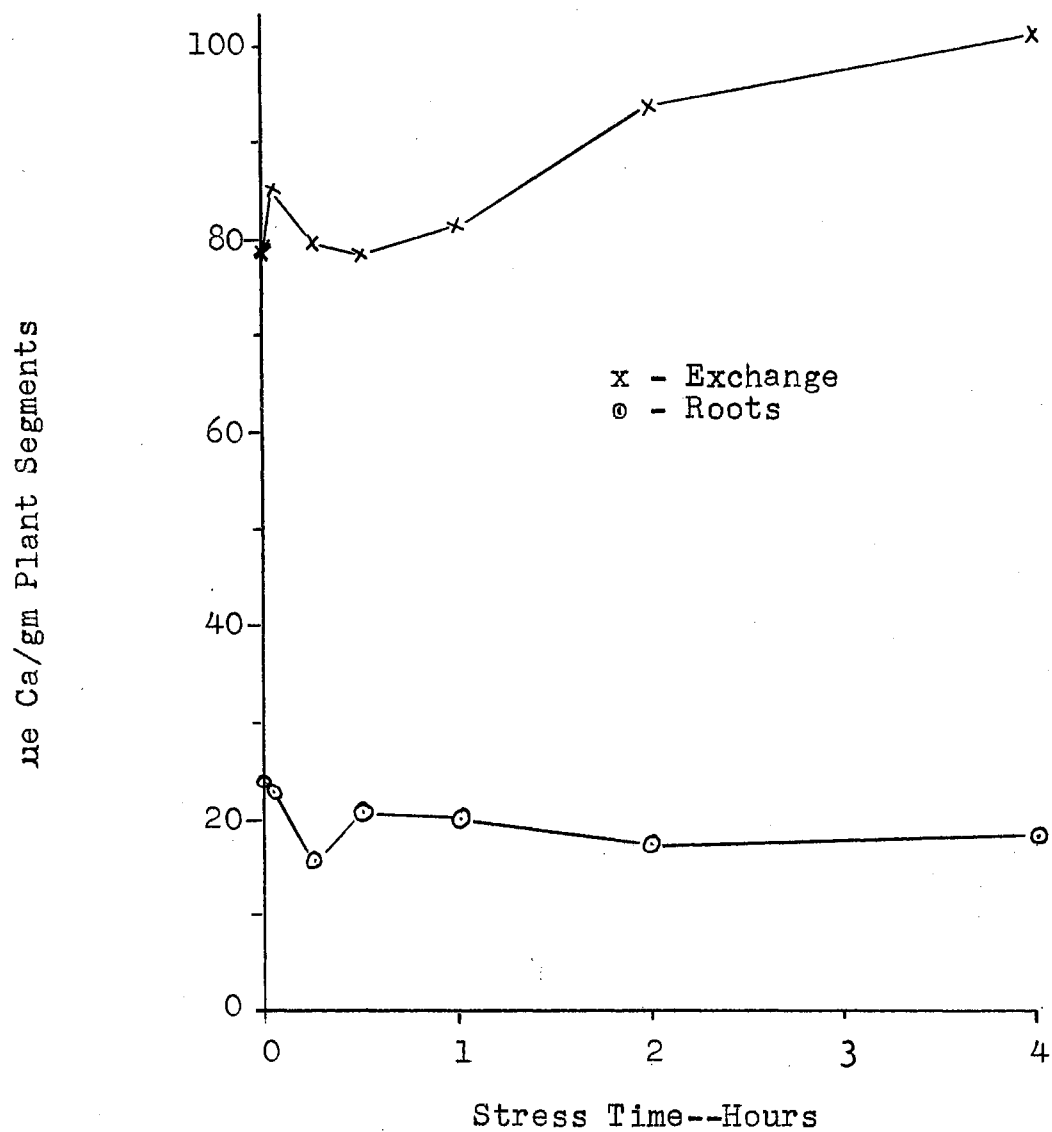


Figure 24. Effect of Duration of Moisture Stress on Subsequent Absorption of Calcium.

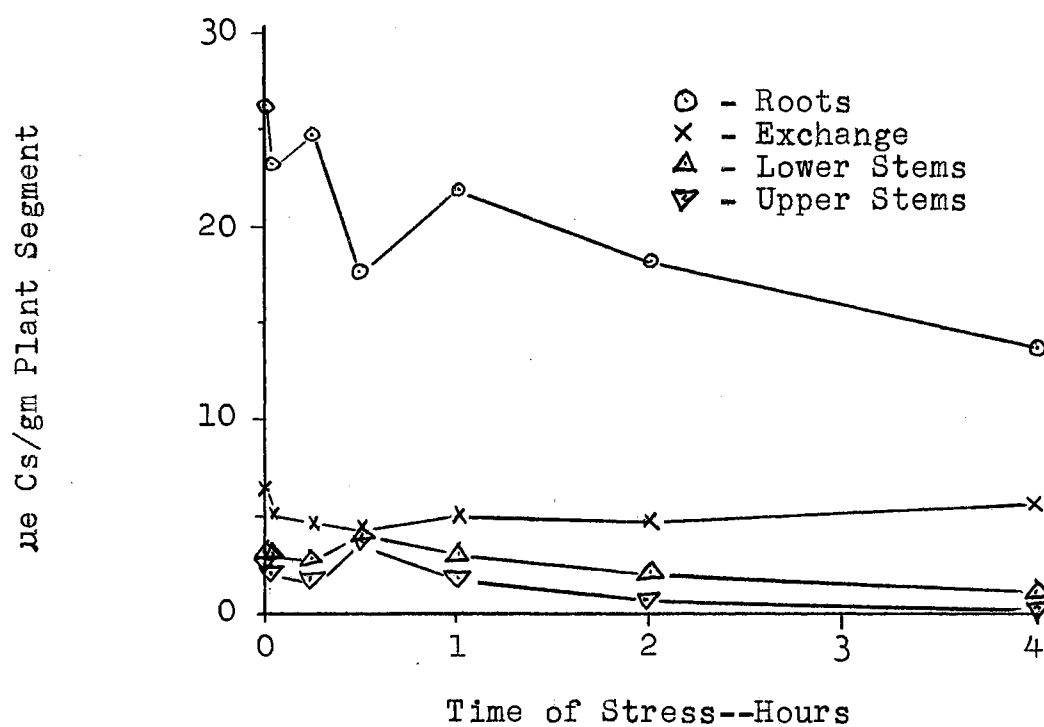


Figure 25. Effect of Duration of Moisture Stress on Subsequent Absorption of Cesium.

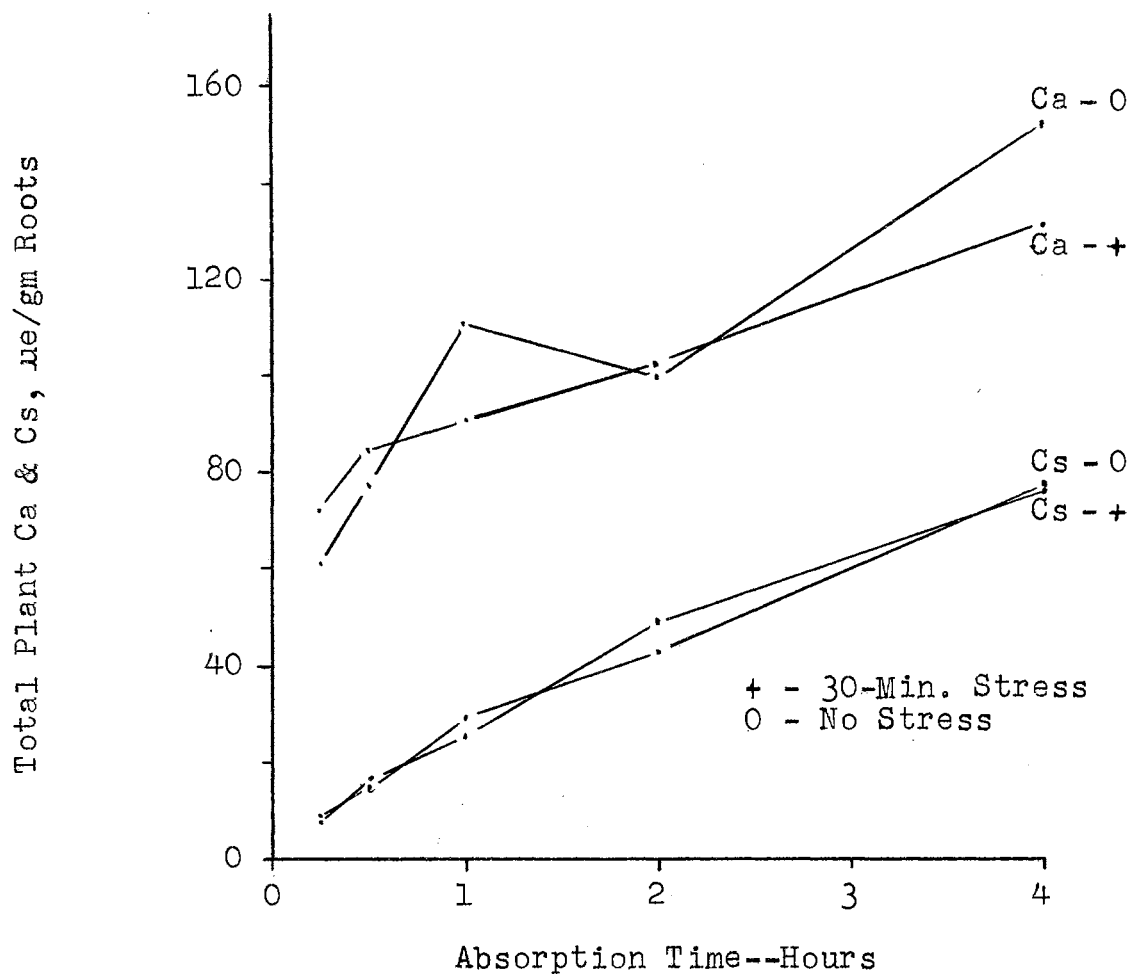


Figure 26. Effect of 30-Minute Moisture Stress on Subsequent Total Uptake of Calcium and Cesium.

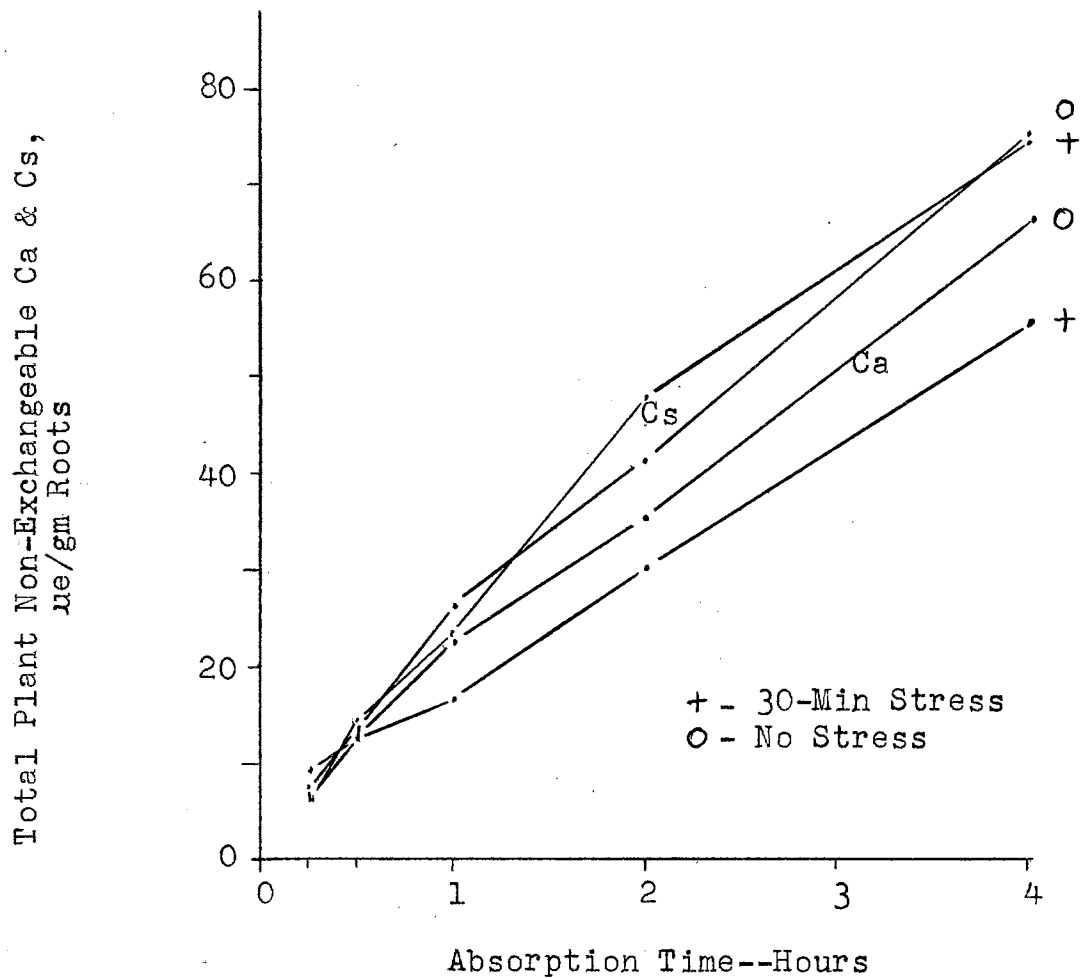


Figure 27. Effect of 30-Minute Moisture Stress on Subsequent Total Non-Exchangeable Uptake of Calcium & Cesium.

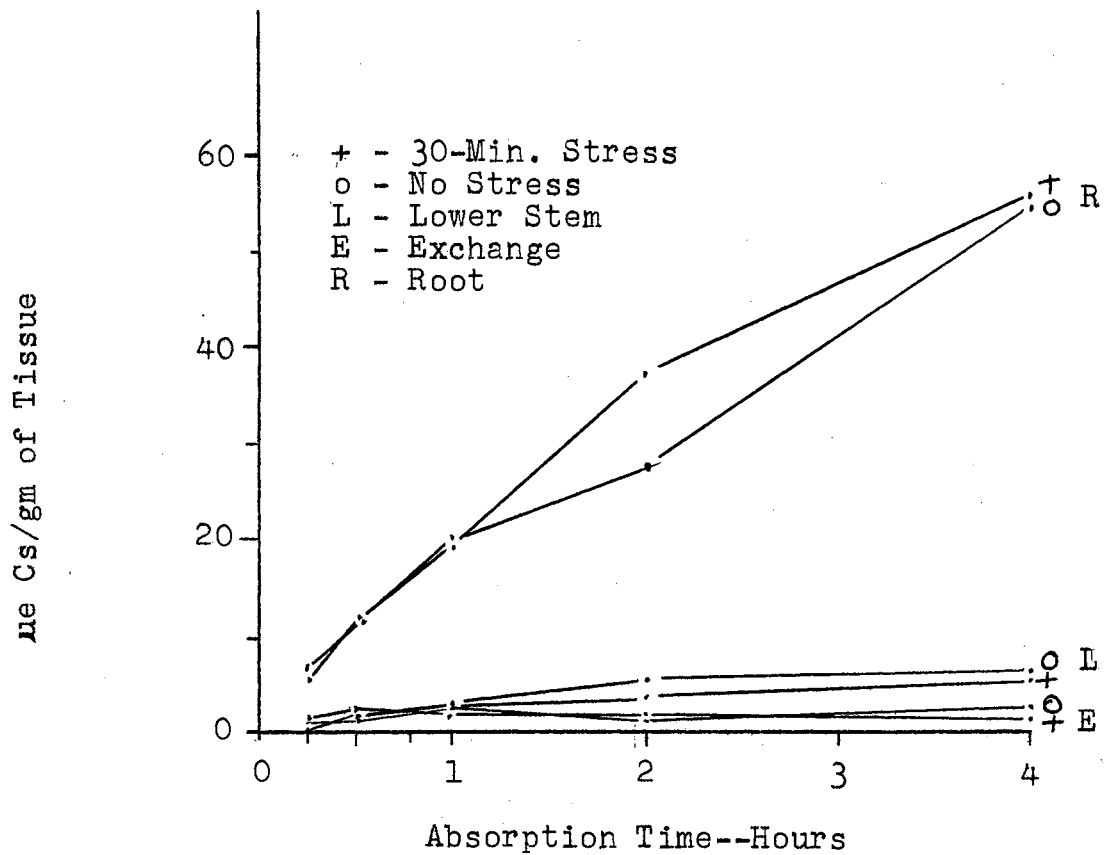


Figure 28. Effect of 30-Minute Moisture Stress and Varying Absorption Time on Uptake and Distribution of Cesium in Cotton Seedlings.

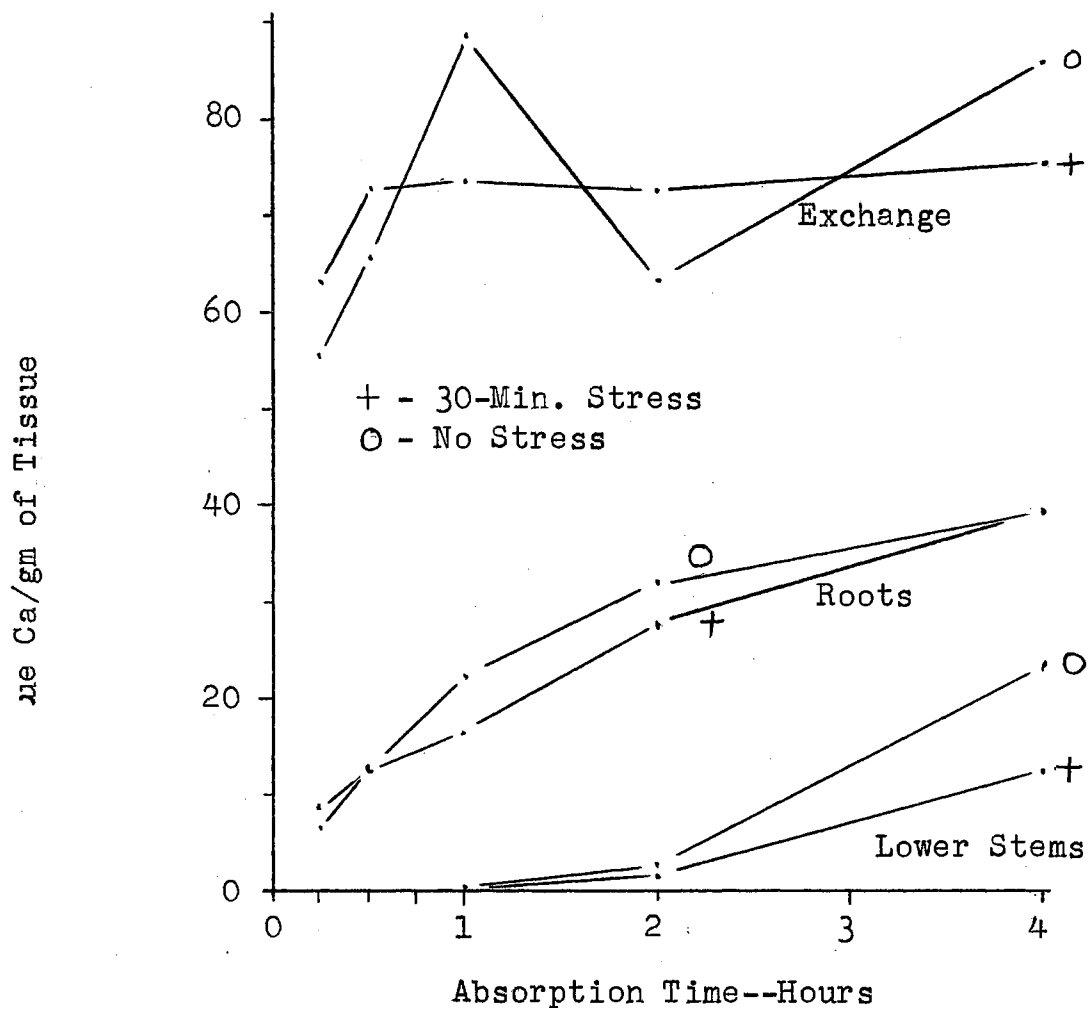


Figure 29. Effect of 30-Minute Moisture Stress and Varying Absorption Time on Uptake and Distribution of Calcium in Cotton Seedlings.

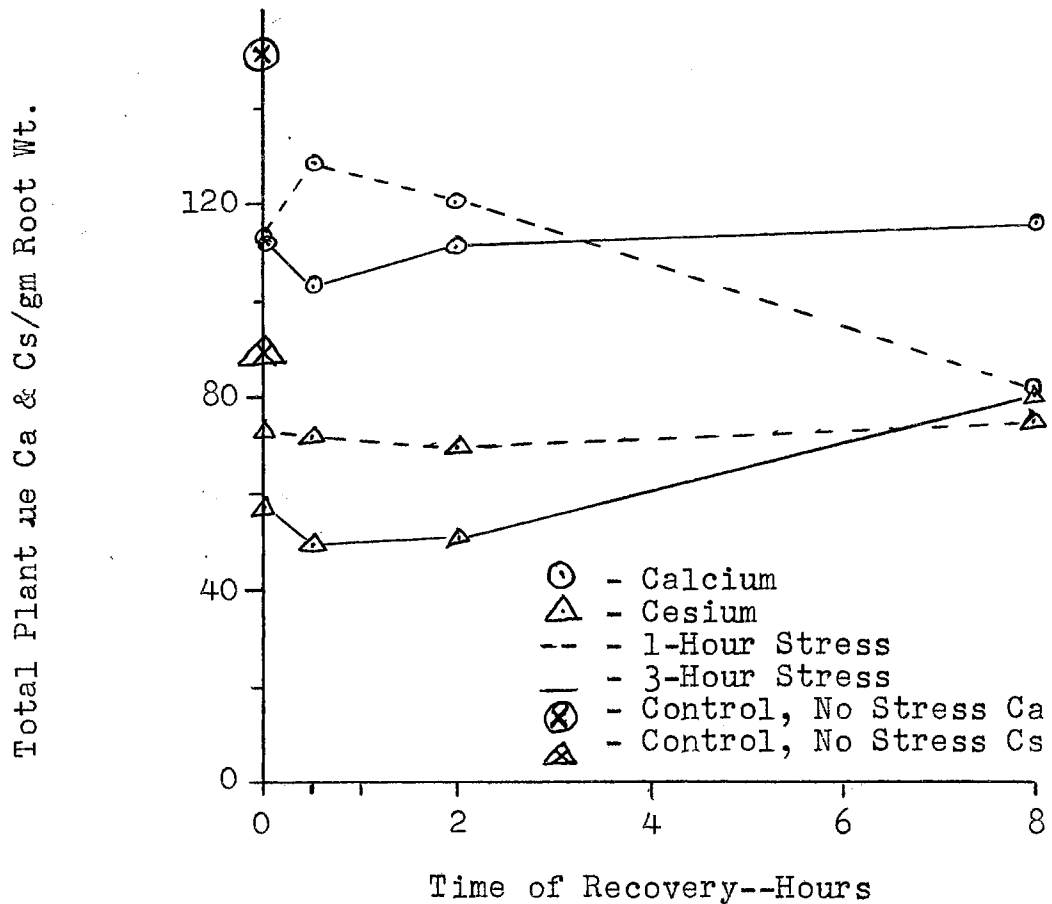


Figure 30. Effect of Recovery Time Following Stress on Total Calcium and Cesium Uptake per Gram of Root Weight.

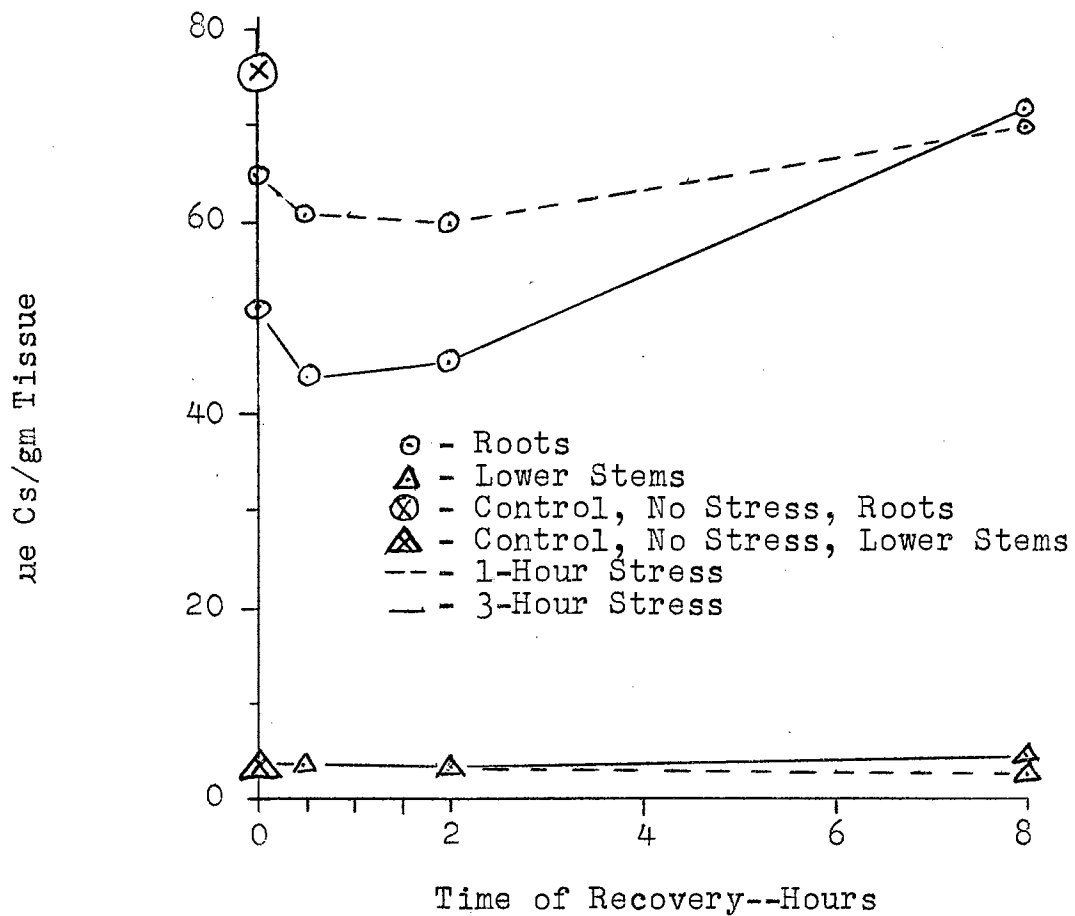


Figure 31. Effect of Recovery Time Following Stress on Uptake of Cesium per Gram of Dry Tissue.

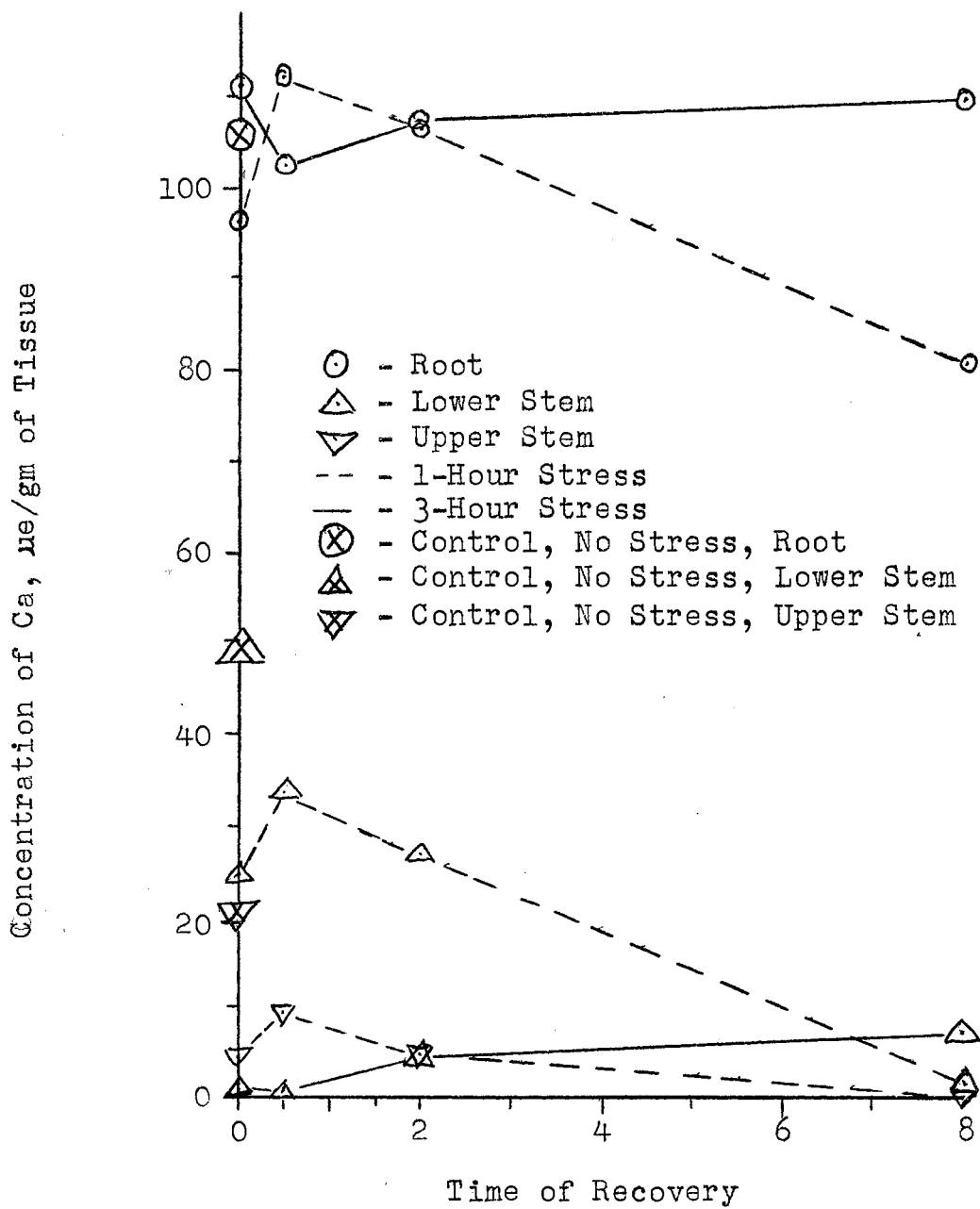


Figure 32. Effect of Recovery Time Following Stress on Uptake and Distribution of Calcium per Gram of Tissue.

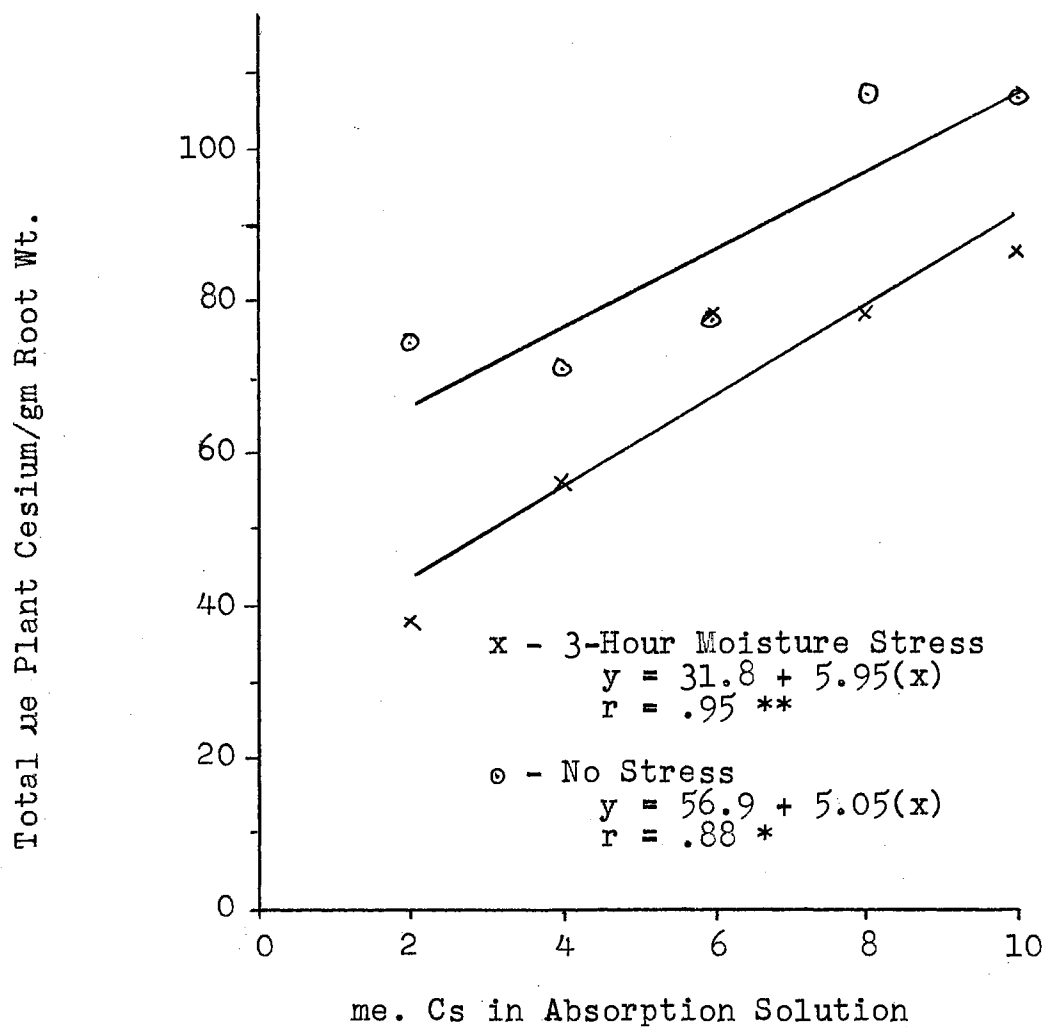


Figure 33. Effect of 3-Hour Moisture Stress on Total Uptake of Cesium by Cotton Seedlings from Solutions of Varying Cesium Concentrations.

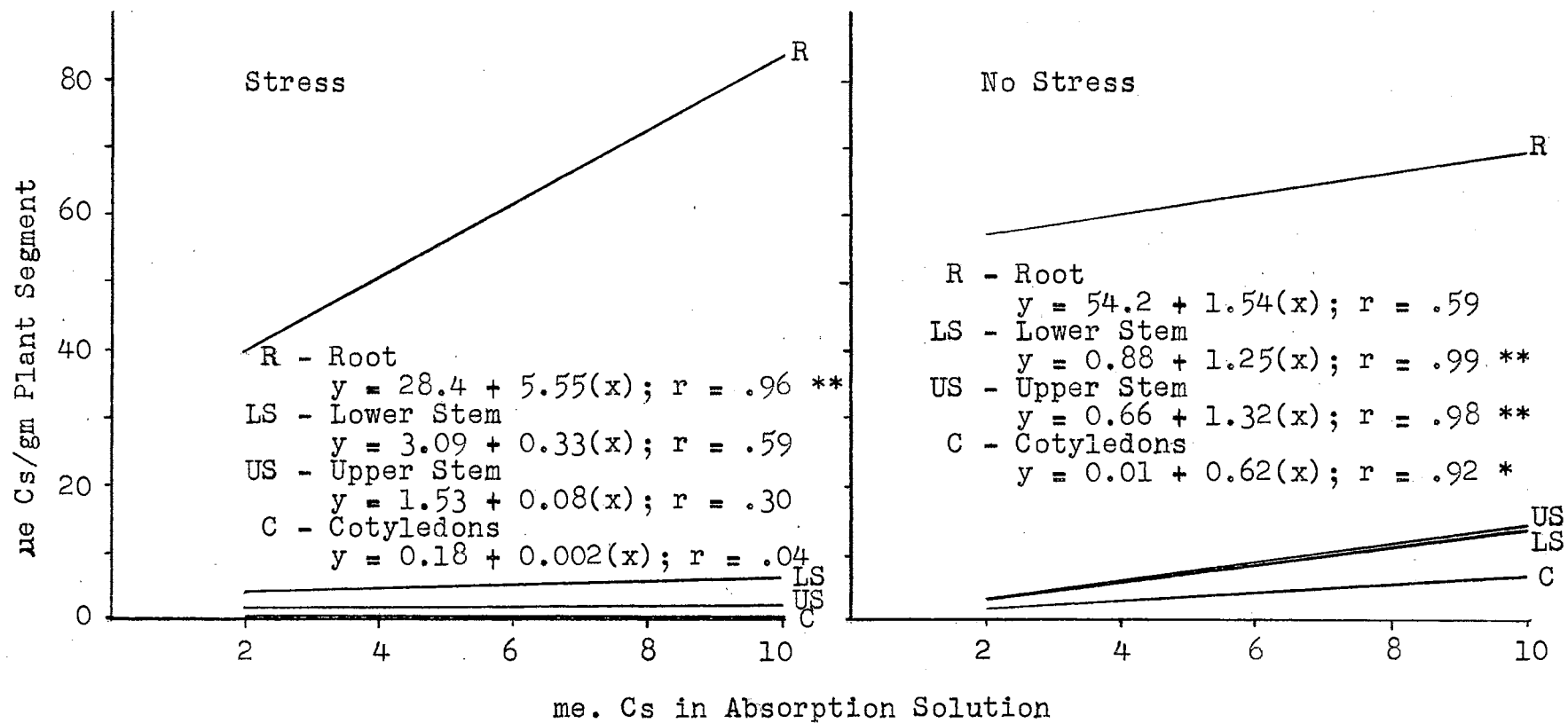


Figure 34. Effect of 3-Hour Moisture Stress and Concentration of the Absorption Solutions on the Distribution of Cesium Within the Cotton Seedlings.

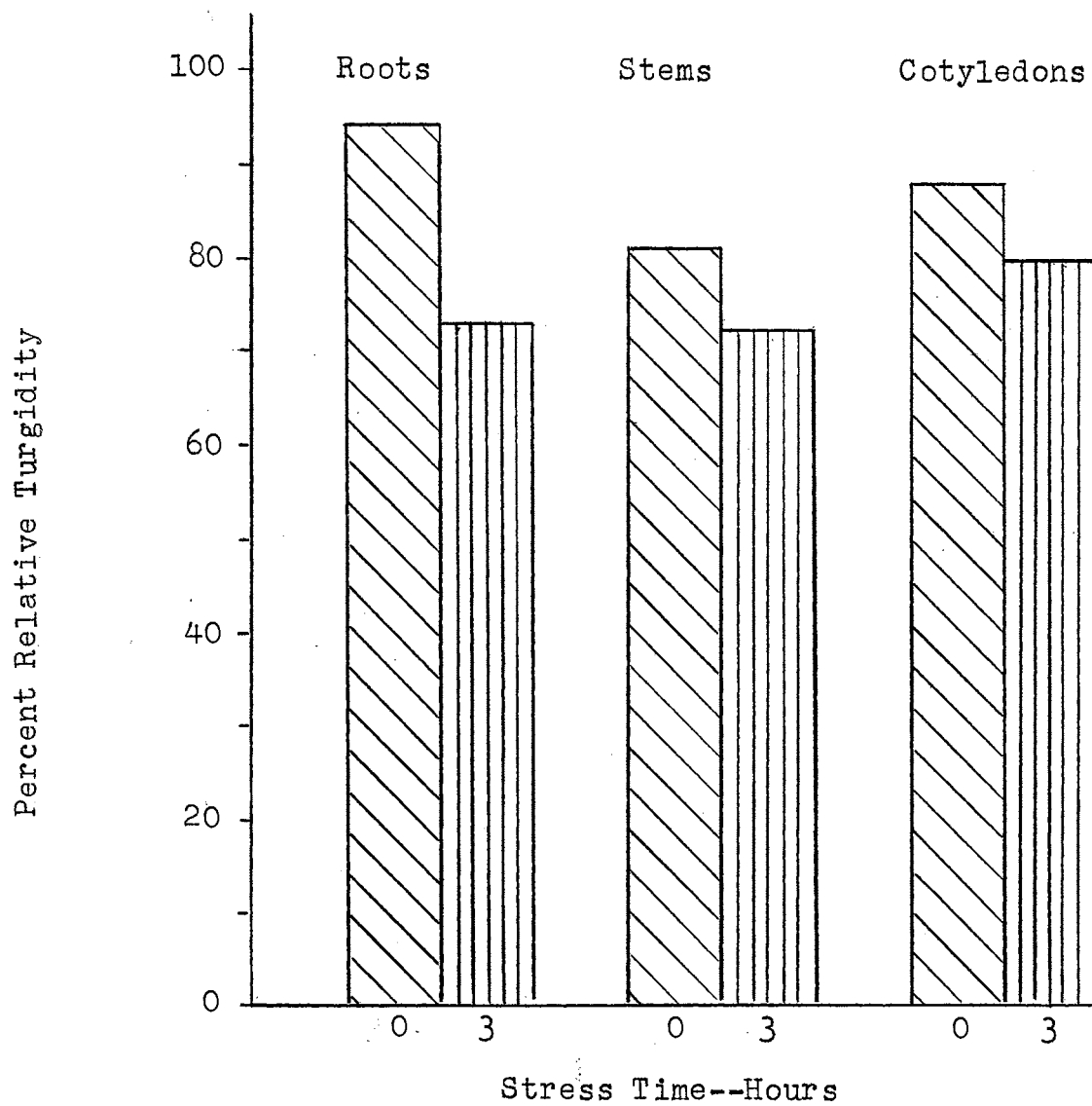


Figure 35. Effect of 3-Hour Moisture Stress on Relative Turgidity of Cotton Seedling Segments.

transferred into the stems and cotyledons (Figure 25), although the amount in the cotyledons was too low to graph effectively. The concentration of non-exchangeable cesium in the root continued to decrease with the increased time of stress, and the concentration of cesium in the exchange solution (per gram of root weight) remained almost constant during this time. The net effect seems to have been that under increasing stress the same total amount of non-exchangeable calcium was accumulated with an increasing amount of exchangeable calcium. At the same time less total cesium was accumulated in the plants and the concentration in the roots and stems was decreased. With the increasing moisture stress a greater proportion of the calcium and cesium held by the root was exchangeable.

The 30-minute stress period in Experiment 12 resulted in little difference in the subsequent total plant uptake of cesium and calcium (Figure 26) or non-exchangeable cesium (Figure 27). The total non-exchangeable calcium was reduced, apparently by the stress period. The amount of exchangeable cesium per gram of roots was almost constant for the different absorption periods and stress treatments. The data for the calcium exchanged from the roots were too erratic to draw any conclusions except that the amount per gram of roots was high and possibly constant for the different absorption periods.

The difference between the absorption and distribution patterns of the two elements seems to deserve comment. The

total absorption of non-exchangeable cesium and calcium was practically linear (Figure 27). The movement of cesium into the root and into the stem seemed to follow two different absorption patterns (Figure 28). It is possible that the concentration of cesium in the absorption solution was too low to satisfy the accumulation in the root tissue and the transfer to the xylem tissue at the same time. Smith⁴ proposed that the absorption of phosphate by plants was concerned with the four interrelated processes: accumulation by the cells of the root, radial diffusion across the root, active transport or secretion into the xylem, and mass movement in the transpiration stream. He found that low-salt roots failed to transport phosphate into the xylem when the root cells were deficient in phosphate or the concentration of phosphate in the nutrient solution was limiting.

The pattern of cesium absorption in the lower stems, upper stems, and cotyledons suggested that cesium had little attraction for the tissue bordering the conductive tissue, as the increase in concentration of cesium in the stem was limited.

The accumulation of the non-exchangeable calcium in the root appeared to follow a different absorption pattern than cesium and is possibly related to a surface phenomenon with a rapid initial absorption followed by a gradually declining

⁴Smith, p. 19

rate of absorption (Figure 29). The appearance of the calcium in the lower stem was gradual at first but accelerated rapidly after the first hour of absorption. No appreciable calcium had appeared in the upper half of the stem at the end of the four-hour absorption. These results seem to indicate that the zone between the surface of the roots and the calcium-conducting tissue in the stem is almost saturated before appreciable calcium moves up the stem. The movement of calcium within the stem was decidedly different from that of cesium. Whereas the cesium appeared early at low concentrations throughout the plant, the calcium appeared as a wave in the lower stem at a relatively high concentration. There was of course no measure of the distance traversed by the calcium into the lower half of the stem.

The results from this experiment also indicate that the cesium and calcium were moving in two different areas within the stem. One might expect a wave of exchanged cesium ahead of the calcium if the calcium were moving in the same conductive tissue but this was not evident. This seems to support some of the experimental work in the literature review that calcium is transported by a unique pathway, possibly the cortex.

The results of Experiment 13 seem to indicate that the effects of brief moisture stress affected uptake for more than just a few hours following recovery. There appeared to have been an over-all decrease in total uptake of calcium and cesium per gram of roots, somewhat in proportion to the duration of stress (Figure 30).

The 3-hour stress plants with the 8-hour recovery received $2\frac{1}{2}$ hours of absorption time instead of the desired 2 hours, which might explain part of the inconsistency of these absorption values. An interpolation downward of these values results in little or no consistent increase in absorption of calcium or cesium from recovery time.

The effect of the duration of moisture stress is more evident again with the calcium uptake. The concentration of calcium in the roots is similar for the two stress treatments and, in spite of the added absorption time for the 3-hour-stress-8-hour-recovery-treatment, the movement of calcium into the lower stem is considerably higher for the 1-hour stress treatment than for the 3-hour stress treatment (Figure 32).

The effect of the duration of moisture stress on the concentration of cesium in the root was more pronounced than might be expected from the results of previous experiments. Considerably more cesium was absorbed by the roots of the 1-hour stress plants than by the roots of the 3-hour stress plants (Figure 31). However, the different periods of stress did not appear to have much effect on the movement of cesium within the stems or cotyledons.

There might be several possible explanations for the failure of the plants to recover the ability to absorb cations. If Zholkevich (75) is correct that available simple phosphorylated sugars and high energy compounds are lost with short periods of moisture stress, this might be

one explanation of the loss of absorption capacity. Another explanation might be the dehydration of the protoplasm in root cells such as suggested by Aykin (3). Transpiration has been pointed out by many plant physiologists, such as Smith⁴, to play an important role in the movement of certain elements within the plant. The behavior of the stomata which largely control transpiration could have been affected by the stress period or by the time of day as suggested by Skidmore⁸. The time of day also plays an important role in the permeability of the root for water according to other experiments by Skidmore and could have been a factor in the failure of the seedlings to recover their full capacity for ion absorption and transport.

A comparison of the cesium uptake for the two stress treatments (Experiment 14) indicates the non-stressed cotton seedlings to have absorbed considerably more total cesium per gram of roots than the stressed plants from the same absorption concentrations (Figure 33). The slopes of the two lines were similar. The correlation coefficient for the stressed plants was high while that for the non-stressed plants was significant only to the 5% confidence level with the t-Test (Figures 33, 34). The concentration of cesium in the plant segments (Figure 34) indicates that practically all

⁴Smith, p. 19

⁸Skidmore, E. L. Physiological Role in Regulating Transpiration Rate of the Cotton Plant. Oklahoma State University, Unpublished Ph.D. Dissertation, 1963.

of the cesium in the stressed plants remained in the roots, resulting in a very high r^2 value. However, the transport of cesium to the various parts of the stressed plants was almost constant from the different absorption solutions. A study of the percent distribution of the cesium resulted in an almost constant value for each plant segment, in spite of the different absorption treatments.

The concentration of cesium in the roots of the non-stressed plants was almost constant but the movement into the plant segments was highly correlated with the concentration of the absorption solutions. A study of the percent distribution of the cesium within the parts of the non-stressed plants resulted in a negative correlation of cesium in the roots for the different absorption solutions. The percent cesium in the stems and cotyledons of the non-stressed plants was correlated positively with concentration of the absorption solutions.

The widely differing pattern of cation transfer to the stems and cotyledons between stressed and non-stressed seedlings seems to add credence to the suggestion of Smith⁴ that the accumulation of ions in the roots and the transfer of ions to the other portions of the plant, by way of the transpiration stream, are separate but related processes.

The loss of water from the cotton seedlings was greater from the roots than from the stems and cotyledons (Figure 35)

⁴Smith, p. 19

although not to the extent as in the first three experiments. Some of these differences in response to moisture stress are possibly due to previous history of the plants, although an attempt was made to keep the plants uniform.

V. SUMMARY AND CONCLUSIONS

The technique of suspending cotton seedlings with their roots in water-saturated air and circulating air past their cotyledons provided a moisture-stress condition that seemed to be satisfactory for the study of a number of problems concerning absorption and distribution of nutrient elements. The plants seemed to grow satisfactorily when returned to a nutrient solution. The one inconvenience of the procedure was the inability to study the direct effect of stress on ion absorption as the stress was occurring. Slight modifications in the procedure might resolve this difficulty.

It was found that the cotyledons were a poor indicator of the moisture conditions in the roots of the plants. Both the cotyledons and the stems retained considerably more moisture than the roots when subjected to moisture stress.

Moisture stress was found to affect the amount of uptake and distribution of cesium and calcium in the nine-day old cotton seedling. Increasing amounts of stress caused a corresponding decrease in total uptake of both elements and affected their movement from the roots into the stems and cotyledons. In most cases the calcium uptake and calcium transfer were more noticeably affected than were cesium uptake and cesium transfer. Although the cotton seedlings exposed to moisture stress took up less cesium than did the

non-stressed plants, the relative rate of uptake from solutions of different concentrations appeared to be practically the same for both treatments.

Moisture stress also affected the amount of cesium and calcium actually accumulated into the plants and the amount of these elements that remained subject to exchange in the roots. It was found that the stressed plants did not fully recover the ability to absorb cations equal to the non-stressed plants for at least eight hours following treatment.

There was a tendency for cesium and calcium to accumulate in the roots when absorption followed moisture stress, which resulted in a reduction in transfer to the other parts of the plant. Moisture stress also caused the loss of cesium from the roots to the surrounding solution. Only a relatively small amount of moisture stress was required to trigger this effect. However, moisture stress under these conditions did not noticeably affect, in a four-hour period, the cesium already distributed throughout the rest of the plant. The calcium and cesium seem to have different accumulation and transfer patterns.

The over-all conclusion from this study is that moisture stress affected the uptake and transfer of calcium and cesium in the nine-day old cotton seedling independently of the influence of growth medium.

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VITA

Chauncey Cox Schaller

Candidate for the Degree
of
Doctor of Philosophy

Thesis: THE EFFECT OF MOISTURE STRESS ON THE ION METABOLISM
OF THE COTTON SEEDLING

Major Field: Soil Science

Biographical:

Personal data: Born Jan. 15, 1921, at Clark, Penna.,
son of Chauncey W. and Emma E. Schaller.

Education: Graduated from New Castle High School,
New Castle, Penna., 1939. Undergraduate work at
Oklahoma Agricultural and Mechanical College of
Stillwater, Oklahoma, 1939-1942, 1946-1947.
Graduate study at Oklahoma State University, 1958-
1964; completed requirements for Master of Science
Degree in May, 1960. Completed requirements for
the Doctor of Philosophy Degree in November, 1964.

Experience: Farm labor during the summer and vaca-
tions, 1935-1939. U. S. Army, 1942-1945. Farm
labor, summer 1946. Soils laboratory technician,
part time, 1946. Wilson & Company, 1947-1948.
S. R. Noble Foundation, Ardmore, 1948-1958.
Graduate research assistant, 1958-1959, Oklahoma
State University. Graduate assistant in charge
of Agronomy Department Soil, Plant and Water
Testing Laboratory, Okla. State Univ., 1959-1964.

Organizations: Agronomy Club, Alpha Zeta, Phi Kappa
Phi, Farm House Fraternity, Soil Science Society
of America, American Society of Agronomy, Society
of Sigma Xi.

Date of Final Examination: November, 1964.