EFFECT OF OXYGEN AND CARBON DIOXIDE TENSIONS ON RELEASE OF SUGARS FROM PEANUT ROOTS UNDER GNOTOBIOTIC CONDITIONS

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The quantities of organic compounds exuding from roots is not large, seldom exceeding 0.4% of the carbon photosynthesized. However, these organic compounds exert a very strong influence on soil microorganisms and may be significant in affecting plant nutrient availability and absorption. There is evidence that exudates from the roots of some plants are toxic to roots of neighboring plants and to the germination of some seeds (Rovira, 1969). The microflora of the rhizosphere differs qualitatively and quantitatively from that of the free soil mainly due to differences in the nutritional sources of the microorganisms. Root exudates, which are mostly lower molecular weight substances exuded by the roots of growing plants, provide nutrition for microorganisms living in the rhizosphere. Root exudates furnish substrates for microbial populations of root surfaces and are, from all aspects, the determining factor in the rhizosphere effect (Vancura, 1964).

Root exudation is closely related to permeability and metabolic activity of roots which, in turn, are strongly affected by the balance between aerobic and anaerobic conditions (Grineva, 1961). Variable oxygen tensions change metabolism which, in turn, effects the absorptive activity of roots. However, little is known about the quantitative effects on exudation. CO_2 tension may also play a role (Dubinina, 1961).

The present investigation is part of a continuing study of factors affecting root exudation and sources and modes of exudation. The

quantitative measures of organic loss from root surfaces as affected by environment has not been undertaken very frequently and investigations which have been reported, in addition to being few in number, are difficult to evaluate and compare. No published information is available on exudation from peanut roots. Since exudates may be involved in colonization of roots by toxin producing molds which may colonize the developing underground fruits, information about the kinds and amounts of exudates is needed. The results show that galactose and dihydroxyacetone are the most abundant sugars exuded and that age of plants and the degree of anaerobiosis affect the amounts.

LITERATURE REVIEW

Root Aeration and Plant Growth

A theoretical approach to the problem of O_2 adequacy for normal root respiration in the soil environment has been attempted. Even though emphasis was placed upon O_2 relations, CO_2 may be just as important to the problem. Results were given comparing the relation between plant root demand for O_2 and the supply available in the soil. Generally, in all living cells, increasing metabolic uptake of O_2 is a hyperbolic function of increasing O_2 concentration in the immediate environment. Little attention has been given to the factors influencing O_2 diffusion in the immediate root environment. Depending upon the soil structure and moisture conditions, the root environment in general may be a solid-liquid matrix. This appears valid in view of the fact that soil aeration is believed to be a problem of either high soil moisture or poor soil structure. As far as O_2 relations interior to the root surface are concerned, it was concluded that: (1) in the range of constant respiration intensity, the root cells of the central cylinder are completely supplied with O_2; (2) if the O_2 concentration at the root surface falls to a certain critial point, then O_2 does not diffuse to the inner most cells of the root axis; and (3) at the critical point, O_2 concentration at the root axis is believed to become zero. It was also concluded that in order to characterize soil aeration one needed to know the O_2 concentration at the surface of a root

growing in the soil and the critical value of O_2 concentration for a given root (Lemon, 1962).

The various factors that influence O_2 demand characteristics of plant roots have also been considered. These influencing factors are: (1) the rate of metabolic O_2 uptake by root tissues varies with the genetic background and the physiological age of the tissue; (2) when O_2 is plentiful, the amount of substrate at reaction loci in the roots determines the reaction rate; (3) when the O_2 concentration at the root surface is below the critical level, diffusion controls the rate of O_2 uptake; and (4) the critical O_2 concentration at the root surface is strongly dependent upon the radius of the root and the diffusion of O_2 within the root (Lemon and Wiegand, 1962).

It has been determined that the O_2 content of soil air is usually lower than that of the atmosphere, while the CO_2 content of the soil air is often 100 to 1000 times higher. Forced aeration drives out CO_2 which might otherwise accumulate and the results of growth might be due to CO_2 reduction and not to increased aeration. Root growth is not strongly affected by complete removal of CO_2 from solution and possibly in small amounts CO_2 may have slight stimulating effect on root growth. There may be too a cooling effect in solutions receiving a large volume of moving air. Therefore, in low concentration CO_2 may stimulate cell activity or at high concentration may inhibit cell activity. There is some evidence that plant roots become adapted to the conditions of aeration that exist and that both physiological and morphological changes occur which are biologically advantageous (Carr, 1961).

It has been noted that the root growth of tomato, tobacco, and soybean ceases at root atmospheric O_2 content of 0.5% and then increases in proportion to the O_2 content (Burström, 1953).

Effects of Oxygen and Carbon Dioxide Tensions

Barley roots grown for 60 days in aerated and non-aerated culture solutions showed the following anatomical and histological differences: (1) the non-aerated barley plants had three times as many roots as the aerated plants, (2) the average root length for aerated plants was more than three times that for non-aerated plants, (3) roots of non-aerated plants were about 15% greater in diameter than for aerated plants, (4) roots of aerated plants had uniformly compact cortex while root cortex of non-aerated plants had large air passages, and (5) the concentration of reducing and total sugars was less in roots of aerated plants. These differences were possibly due to the affect of O_2 concentration upon respiratory rate with subsequent change in sugar concentration and growth of root meristems (Bryant, 1934).

The relation of aeration to the growth of soybeans in both sand and solution culture was investigated (Allison and Shive, 1923). When solution cultures were maintained at saturated O_2 conditions and nutrient solution renewed periodically, there was a considerable increase in root development. Renewing the culture solutions continuously produced a considerable increase in the growth of both tops and roots. When corn plants were aerated in solution culture, a better correlation of the rate of root growth was found with the CO_2 content of the solutions, in an inverse ratio, than with O_2 content in a positive ratio.

It was concluded that perhaps CO_2 and not O_2 was the limiting factor in growth of corn plant roots in solution culture. A further conclusion was that no final analysis of the problem could be considered complete until a distinction had been made between the individual effects of O_2 and CO_2 (Knight, 1924).

Maize grown in soil at a low O_2 tension developed roots that were devoid of root hairs (Cannon, 1924). Tomato plants were grown in both aerated and non-aerated solution cultures. The average total dry weight yields of the plants grown in aerated solutions were more than 50% higher than the corresponding yields of those grown in the non-aerated solutions. There was superior development of both tops and roots of aerated plants (Clark and Shive, 1932).

Sunflower and soybean plants were grown in both aerated and un-aerated sand and loam. Aeration was continuous at moderate rates of moist air. The aerated plants were taller and heavier, had larger and more highly branched root systems with larger quantities of reserve carbohydrate and mineral nutrients. When very rapid aeration was used the opposite effects occurred (Loehwing, 1934). Tomato plants were grown in aerated and non-aerated solution cultures at two levels of pH. The O_2 and CO_2 content of the solution cultures was determined and the cation and anion nitrogen absorption after 6 hrs. immersion in the solution culture. The results were: (1) aerated solution cultures showed higher O_2 tensions than non-aerated at both pH levels; (2) a marked increase in rates of absorption of cation, anion, and total nitrogen from aerated solution cultures; and (3) growth yields of

plant material in aerated cultures double that of non-aerated cultures. The CO_2 accumulation in the solution cultures appeared not to effect growth, nitrogen absorption rates, or O_2 content (Arrington and Shive, 1936).

Soybeans, oats, and tomatoes were grown in standard culture solutions with different concentrations of dissolved O_2 at 0.0, 4.0, 8.0, and 16.0 ppm. This was done by using mixtures of O_2 and N_2. Dry weight yields indicated that they may increase with increase in O_2 concentration up to optimum which varies widely for different species. These values also indicated that O_2 saturated nutrient solutions at equilibrium point with the atmosphere are much below optimum required for maximum yields of some species (Shive, 1941). A quantitative study of the effects of five different levels of aeration of nutrient solution as related to fruit production and vegetative growth of tomato was done. The following effects were noted: (1) aeration by natural diffusion of air produced no significant effect on growth, (2) aeration at the rate of 2.5 ml. air per plant per minute greatly increased fruit production and speed of ripe fruit production. Increased aeration rates were without further effects (Durell, 1941). The decrease of O_2 pressure of gas in equilibrium with rooting medium to three fourths of that found in air caused a marked decrease in number and weight of new roots and in top growth of apple, prune, and peach trees. These effects were produced without appreciable increase in CO_2 pressure in the rooting medium above that in the air (Boynton and Compton, 1943). The roots of barley, tomato, and rice plants in nutrient solution were

exposed to air, no aeration, N_2, and CO_2 and were best in that same order as to fresh weights and potassium concentration. There was practically no growth or salt accumulation with just CO_2. A specific lethal action of CO_2 superimposed on the results caused by O_2 deficiency was indicated. The investigators stated that control over experiments of this kind is complicated by the influence of several variables. These variables are CO_2 pressure, accumulation of anaerobic products, and intervention of microbiological activity (Vlamis and Davis, 1944).

The toxicity of CO_2 accumulation as contrasted to O_2 deficiency and the effect of CO_2 on water and nutrient absorption were observed. While air and N_2 did not reduce uptake below that of controls, the CO_2 treatment did affect absorption of wheat, rice, and maize. The indication was that such effects were directly due to the presence of CO_2 and not to the exclusion of O_2. Possibly the CO_2 increased the viscosity of the protoplasm and decreased the permeability of root cell membranes to water (Chang and Loomis, 1945). Another study employed a number of O_2, CO_2, and N_2 gas mixtures supplied by compressed gas cylinders to the roots of cotton plants. The best root growth occurred at a CO_2 concentration of 10% and O_2 range of 7.5 to 21%. It was thought that the optimum O_2 concentration depended upon the range of CO_2 concentrations. In another study of the series, in which the O_2 concentration was held constant at 21%, it was shown that plant growth was good up to nearly 30% CO_2 concentration. Apparently then, the minimum CO_2 concentration can be too low to affect the growth of

the cotton plant when sufficient O_2 is present. This may be due to the fact that all the respiring root cells are themselves producing CO_2 (Leonard and Pinckard, 1946). Another investigation was concerned with the influence of the O_2 content of the nutrient solution upon tomato root growth. There were aerated and non-aerated culture solutions. The aerated culture solutions contained gas mixtures with either the partial pressure of O_2 varies or the partial pressure of CO_2 varied. The growth responses obtained in conjunction with data from other experiments, in which constant levels of O_2 and CO_2 were maintained, indicated that characteristic differences observed between tomato plants in aerated and non-aerated solution cultures were produced by insufficient O_2 in solution and that these differences developed before the concentration of CO_2 reached a value that might have been slightly toxic (Erickson, 1946).

Root respiration in barley seedlings growing aseptically in circulating culture solutions was estimated by measuring the CO_2 liberated from the roots. In a second experiment, the interaction of O_2 tension and nutrient concentration on ion uptake and respiration of the barley plant roots was studied over a wide range of values. It was concluded from the relation of ion uptake to respiration rate at various O_2 levels that over a very limited range of concentration of ions increase in ion absorption is accompanied by increased CO_2 production. However, a steady rate of CO_2 production is reached and its level is dependent upon the O_2 supply. Therefore any further increase in the ion concentration will increase ion uptake, but without any corresponding change

in respiration (Woodford and Gregory, 1948). One study found that in all measured responses, plants getting forced aeration did better than those getting 0.5 to 5.0% O_2. This was in terms of increased plant growth of tomato, tobacco, and soybean. Root growth of all the plants stopped at an O_2 content of 0.5% (Hopkins, Specht, and Hendricks, 1950). Another worker exposed the root systems of soya bean plants to controlled external O_2 tensions as atmospheric air, 5% O_2, and 12% O_2. In nodulated soya bean plants, a progressive reduction in dry weight attained by whole plants and by various plant organs was found on comparison of plants grown with their roots in culture solutions in approximate equilibrium with O_2 and N_2 mixtures containing 21, 12, and 5% O_2 respectively (Bond, 1950). The effect of O_2 supply on the growth of nodulated and non-nodulated red clover was examined. One set was thus dependent on N_2 fixed in nodules and the other set of plants upon ammonium-N_2 in the water culture. The roots were exposed to 21, 12, 5, and 1.0% O_2 concentration. In nodulated plants, each O_2 reduction curtailed growth and the plants receiving ammonium-N_2 were not as marked in their response. This indicated that effective functioning of nodules necessitated higher levels of external O_2 than did roots of red clover (Ferguson and Bond, 1954). The root growth of pea, bean, broad bean, and sunflower was completely inhibited at 6.5% CO_2 in air. Oat and barley was not affected (Stolwijk and Thimann, 1957). It was found that in the case of the alder a high O_2 level around the roots favored infection by nodule organisms and lead to abundant nodulation. The infection organism probably multiplied in the rhizosphere under

the influence of root secretions. Nodulated alder plants proved to be more sensitive to a reduction of O_2 concentration when tested in the same manner. This was probably due to the greater O_2 requirement of the nodules, as evidenced by a N_2 deficiency in the plants (MacConnell, 1959). It has been shown that the degree of O_2 supplied to the roots of pumpkin, tomato, and willow exerts an essential effect on their metabolism. Constant aeration decreased the concentration of organic acids which indicated their utilization in the respiratory cycle. Under O_2 deficient conditions the organic acid concentration increased as a result of the inhibition of the aerobic stage of respiration. Intense aeration decreased the content of most free amino acids in the roots and suggested protein synthesis and rapid root growth (Dubinina, 1961).

The stem cuttings of carnation were placed in containers with the basal 3 cm in tap water and either O_2 or CO_2 was passed into the water to modify the atmosphere. Decreased levels of O_2 below that in normal air progressively decreased root initiation and growth. Increased levels of CO_2 at the base of the stem decreased root growth linearly. The experiment showed that an undesirable root environment resulted from either an O_2 level as low as 15% or a CO_2 level as high as 2% (Tinga, 1965). Barley and pea plants were grown under several different levels of soil atmosphere with the O_2 concentration varying from 0 to 21% and the CO_2 concentration from 0 to 8%. Barley root growth was good down to 7% O_2 and pea root growth was good down to 14% O_2. The interactive effects of CO_2 and O_2 were characterized by a reduced

susceptibility to CO_2 at O_2 values below 7% and a very deleterious effect of 8% CO_2 at 7% O_2 (Geisler, 1967).

Plant Root Exudation

Nature of Exudates

A number of simple sugars and disaccharides have been identified in the exudates of a wide range of plants. Glucose has been found in exudates of peas, soybean, barley, wheat, oats, and white pine; fructose in peas and oats; and arabinose, xylose, and raffinose in white pine and wheat (Rovira, 1956a; Katznelson, Rouatt, and Payne, 1955; Riviere, 1960; Slankis, 1958; and Vrany, Vancura, and Macura, 1962). All of the twenty common amino acids have been found in the root exudates of as many different plant species (Same references as listed above for the amino acids). A number of vitamins, organic acids, and enzymes have also been recovered from the root exudates of clover, lucerne, tomato, cotton, wheat, maize, and peas (Rovira and Harris, 1961; Sulochana, 1962; Vrany, Vancura, and Macura, 1962; Riviere, 1959, 1960; and Krasil'nikov, 1952).

Ethanol was found in the root exudation of corn and sunflower under anaerobiosis (Grineva, 1963). The root exudates of barley and/or wheat have yielded ten simple sugars, five organic acids, and twenty different amino acids (Vancura, 1964). Other workers found in the root exudates of cucumber, turnip cabbage, red pepper, and tomato a total of twenty-four amino acids, seven organic acids, and ten different sugars (Vancura and Hovadik, 1965). A number of other investigators have also found a considerable number of sugar, amino acid, and organic

acid compounds in the root exudates of pea seedlings, alfalfa, sugar maple seedlings, sorghum, sunnhemp, ragi, and tomato (Boulter, Jeremy, and Wilding, 1966; Richter, Wilms, and Scheffer, 1968; Salasubramanian and Rangaswamii, 1969; and Smith, 1969).

Effect on the Soil Rhizosphere

It has been noted that oat seedling roots exude compounds which inhibit the growth of oats and peas (Borner, 1960). Flax roots excrete enough HCN to retard the growth of several species of pathogenic fungi. Toxic exudates from the roots of citrus, chinquapin, green leaf manzanita, ponderosa pine, redwood, and monterey pine have been found. In the case of grasses, sorghum depresses growth of succeeding crops, rye retards grape plants, and quack grass rhizomes inhibit alfalfa. Aqueous extracts of field bindweed and Canada thistle inhibit germination of flax and wheat seedling (Woods, 1960). It has been concluded that plant root exudates may enter into the pathogenesis of root-attacking fungi by aiding or hindering spore germination, by acting upon the growth of the pathogen, by influencing disease incidence, by becoming a factor in disease resistance, and by influencing the physiological conditions in the soil (Schroth and Hildebrand, 1964). One worker examined the germination and seedling vigor of four clover species as affected by six different grass root extracts. Root extracts of Johnson grass and sorghum cause severe reduction in germination and seedling vigor. The affect of the grass root inhibitor on clover is temporary and can be overcome by placing the sprouted seed in distilled water (Hoveland, 1964).

The root washings of potato will cause the cysts of potato eelworm to hatch (O'brien and Prentice, 1930). During the fruiting stage of red pepper a gibberellin-like root exudate is released into the soil which decreases the rhizosphere microflora to such an extent that equilibrium with the rhizosphere soil is reached (Vancura and Hovadik, 1965). It has been shown that non-nodulating soybean plants exude compounds which alter the morphology of Rhizobium and prevent nodulation of the nodulating soybean line (Elkan, 1961). An investigation of mixed pastures of subterranean clover and lucerne showed that root exudates of clover stimulated Rhizobium trifolii, but not Rhizobium meliloti. Lucerne produced a root exudate that stimulated both species. This is a case of either direct exudate stimulation or indirect by the stimulation of a microflora in the rhizosphere antagonistic to R. meliloti (Robinson, 1967).

The roots of avocado draw zoospores to the zone immediately behind the root tip, while roots of mandarin orange do not attract zoospores to any part of the root (Zentmeyer, 1961). Flax varieties resistant to root rot are known to excrete HCN (Timonim, 1941). It was found that roots of mature asparagus, resistant to stubby root nematode, exuded a nematocide (Rohde and Jenkins, 1958). It has been demonstrated that roots of guayule exude compounds highly toxic to the growth of its own roots, but not necessarily to the roots of other plant species (Bonner and Galston, 1944; and Bonner, 1946). The roots of 3 to 4 yr. old lucerne exuded saponins which retarded the growth of cotton, but not the growth of wheat (Mishustim and Nawnova, 1955).

P^{32}-phosphate was applied to the leaves of one tree species and was transferred through the root system to trees 0.25 to 2.0 m apart (Rah Teenko, 1958). The isotope, C^{14}, applied as $C^{14}O_2$ to the tops of corn and bean plants soon appeared in the roots of neighboring untreated plants (Ivanov, 1962).

After the application of dicamba and picloram to the leaves of Black Valentine beans, very low concentrations of these two herbicides were detected in recipient plants (Hurtt and Foy, 1965a). Pea root exudate stimulates the number of gram-negative bacteria in several types of soil (Rovira, 1956). Root exudates from seedlings of ten plant species influenced the growth of rhizosphere microorganisms (Rovira and Harris, 1961).

Factors Affecting Root Exudation

A. Plant Species. A difference between wheat and barley root exudates was found with respect to certain sugars, whereas other sugars occurred in like amounts in the exudates of both plants (Vancura, 1964).

B. Plant Age. In both pea and oat seedlings larger quantities of amino acids and sugars were exuded during the first 10 days of growth than during the second 10 days (Rovira, 1956).

C. Temperature. Larger quantities of amino acids were found in the exudates of strawberry plant roots grown at 5 to 10 C than at 20 to 30 C (Husain and McKeen, 1963).

D. Light. Both tomato and clover plants showed reduced concentrations of certain amino acids in the root exudates when the plants were

shaded than when they were grown at full sunlight intensity (Rovira, 1959).

E. Plant Nutrition. In pine seedlings, it was demonstrated that plant nutrition in terms of nutrient sufficiency, phosphate deficiency, and nitrogen deficiency produced large differences in the loss of amides and amino acids from the roots (Bowen, 1969).

F. Microorganism. Microorganisms may effect root exudation by: (1) their effect upon root cell permeability, (2) their effect upon root metabolism, and (3) their ability to absorb certain compounds in the root exudates and to excrete other compounds (Rovira, 1969).

G. Medium Supporting Roots. A seven-fold increase was found in the release of certain amino acids when the plant roots were retained in quartz sand instead of solution culture (Boulter, Jeremy, and Wilding, 1966).

H. Soil Moisture. The release of amino acids from plant roots was greatly increased after the plants had experienced temporary wilting (Katznelson, Rouatt, Payne, 1954, 1955).

I. Root Damage. Roots of wheat seedlings were carefully removed from sand and placed on moist filter paper. Those root tips released more amides and amino acids than tips of wheat roots grown in solution culture. If roots were drawn across filter paper surfaces or were air dried for a few minutes, they released quantities of ninhydrin-reacting compounds resembling those from sand grown roots (Ayers and Thornton, 1968).

Influence of O_2 and CO_2 Tensions upon Root Exudation

The roots of young corn and sunflower plants were subjected to periods of anaerobiosis by immersion in water. There was an increase in the dry weight of material excreted from the roots. Sizeable amounts of oxidizing compounds were excreted. There were two sugars, seven amino acids, and five organic acids in the exudates and they were all typical of the compounds found in root tissues. The conclusion advanced was that cessation of aerobic respiration brought a shift in metabolism and therefore promoted excretion of those compounds not utilized in metabolism under the new conditions. This excretion was thought to be active (Grineva, 1961). Bean seeds were incubated for 3 days in water. Under increasingly more anaerobic conditions, seed germination decreased, but exudation increased. Compared with air, pure O_2 decreased seed germination slightly, but increased the amount of exudation. The exudates were tested for effect on fungi in air (Woodcock, 1962).

In corn and sunflower plants induced root anaerobiosis caused the formation and excretion of ethanol. Root analysis showed that sugar consumption and alcohol formation had both increased. This indicated that the rate of glycolysis had increased during anaerobiosis. Lack of O_2 apparently disrupted the directivity of biochemical transformations and fostered accumulation and excretion of toxic metabolic products and metabolites not used by the plants at the new level of metabolism and lowered water conditions (Grineva, 1963). Two herbicides, dicamba and picloram, were foliarly applied to Black Valentine

bean plants. Under aerobic conditions, both compounds were excreted from the root systems into nutrient solution. When anaerobic conditions were imposed by N_2 aeration of the nutrient solution, dicamba was not excreted. O_2 did not increase the excretion of dicamba over that with air. Picloram acted oppositely in both situations (Hurtt and Foy, 1965b). Wheat and pea seedlings were grown aseptically in both sand and solution cultures. The total amino-N_2 and the proportions of several amino acids in the root exudates were influenced by several ratios of O_2 and CO_2 concentrations. Both qualitatively and quantitatively, more ninhydrin-reactive material was released under the soil air series than the other gas mixtures. In fact, approximately 5 times as much N_2 per plant was released from peas grown in the soil air series than those grown in sand under the 10% CO_2 mixture or the 2% O_2 mixture. The values of N_2 for the soil air series were 5 to 10 times that found from peas grown in solution-culture. Wheat roots released extremely low levels of amino-N_2 into the solution culture (Ayers and Thornton, 1968).

The Problem

The objectives of the present investigation were: (1) to explore an area, root exudation of peanuts, in which no previous work has been done; (2) to study the effects of O_2 and CO_2 tensions, both individually and combined, on peanut root exudation; and (3) to determine quantitatively the sugars exuded from the roots of peanut plants grown under gnotobiotic conditions.

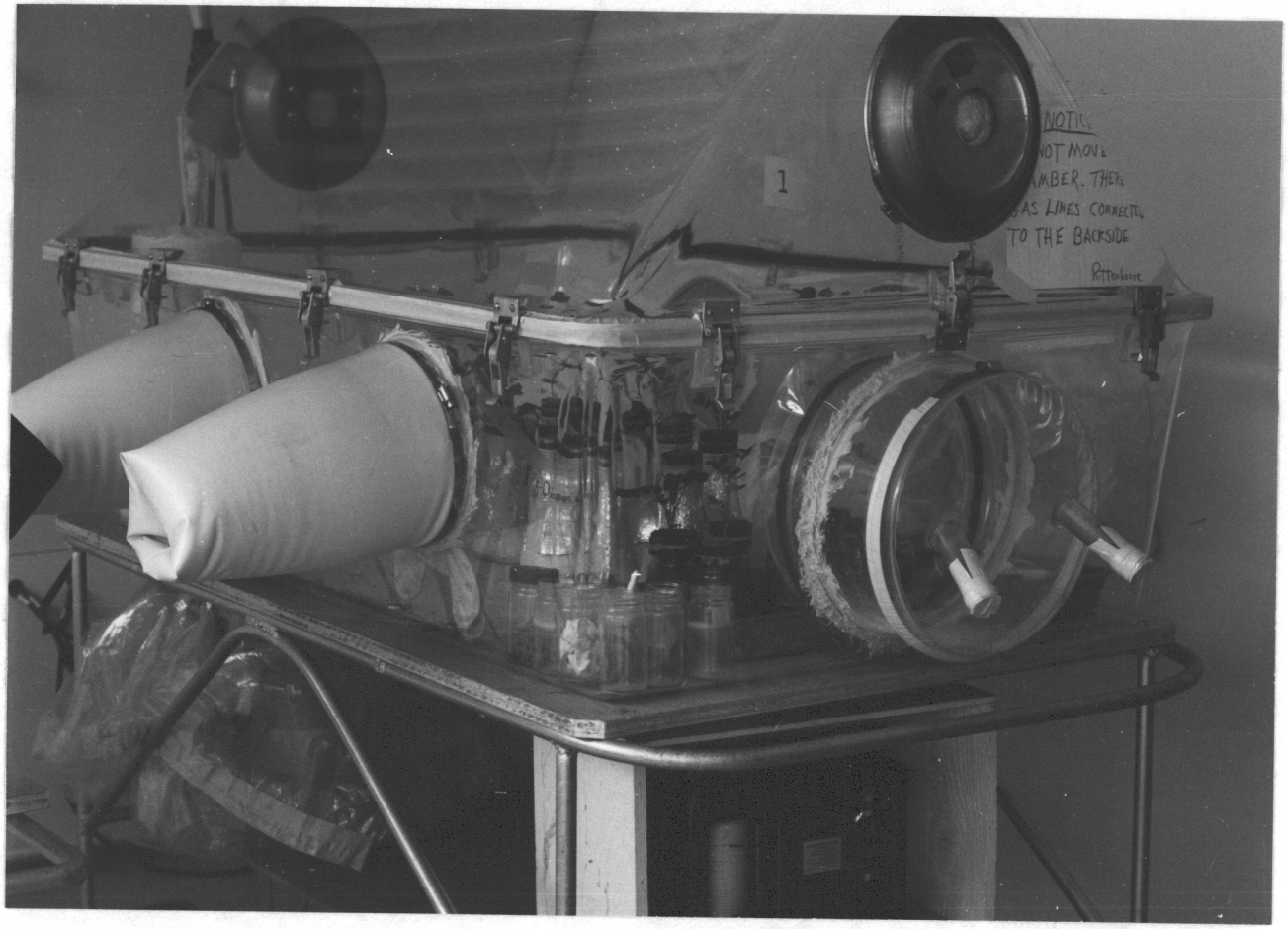

Fig. 1. Plastic isolator chamber mounted on moveable cart in a growth room. Blower and filter on left end and filter on right end provide air circulation. Entry port is on the right end. Rubber gloves on the left side permit work within the chamber. The chamber contains young untreated peanut plants in glass transplanting tubes.

MATERIALS AND METHODS

Seed from the peanut plant, Arachis hypogaea, L. var. NC-2 (Yarbrough, 1949), was obtained from Keel Peanut Co., Greenville, N. C.

Preparation of Plant Materials for Axenic Culture

To obtain axenic peanut plants, cotyledons were carefully removed from peanut embryos (Hale, 1969; Pettit and Taber, 1968; Chang, 1967) which were surface sterilized for five minutes in a 20% solution of commercial sodium hypochlorite and immediately transferred to solidified nutrient medium, five embryos per petri dish. The sterilized nutrient medium consisted of 7.5 g agar, 10.0 g sucrose, and enough Hoagland and Arnon nutrient solution (Hoagland and Arnon, 1938) to make a final volume of one liter. Embryos were allowed to germinate for five days at 37 C in a humidified incubator (Hale, 1969).

Embryos, selected for uniformity, were transferred aseptically to sterile, screw-capped, glass jars which were, in turn, placed aseptically into sterile plexiglass isolator chambers (Fig. 1). Germinated embryos were planted in vermiculite saturated with nutrient solution and contained within transplanting tubes (Fig. 1). The transplanting tube consisted of a 4 oz glass bottle with two Bakelite bottle caps sealed back to back with silastic. A hole drilled through the caps accommodated a Pyrex tube (35 x 70 mm) which was covered at the lower end with a fiberglass screen held in place with silastic. Enough Hoagland and Arnon nutrient solution was placed in the bottle to cover the lower

end of the Pyrex tube so that the vermiculite was kept moist by capillarity. For each experiment the peanut seedlings were maintained in the transplanting tubes for about 90 days until the root systems became large enough to be used in the O_2 and CO_2 gas tension studies. Length of time in transplanting tubes was necessary because of slow embryo and seedling growth without the cotyledons.

Preparation of Gnotobiotic Isolator Chambers

The procedure used to sterilize isolator chambers was as follows. The inlet and exhaust air filters were removed and sterilized with dry heat for 2 hrs at 160 C. After the filters were replaced the inside surfaces of the isolator chamber were thoroughly sprayed with 350 ml of an aqueous solution of freshly prepared 2% peracetic acid (PAA) (Luckey, 1963; Lindsey and Baker, 1967; Hale, 1969). After an hour or more, the air in the sealed chamber was displaced by breaking the mylar seals on the inside openings of the inlet and exhaust filters. Air movement was created by a blower fan attached through the sterile filter on one end of the isolator chamber (Fig. 1). The chambers were vented for five days before plant material was introduced.

All materials which were put into the chambers, with the exception of plants, were autoclaved in a sealed stainless-steel cylinder for 3.5 hrs. The cylinder was attached by means of a plastic sleeve to the entry port of the isolator chamber. The entry port and connecting sleeve were sprayed with PAA. After an hour or more, the mylar seal on the cylinder was broken and the sterile materials were taken into the chamber. All manipulations inside the chamber such as watering,

transplanting, nutrient solution renewal, and collection of root exudate samples were done through glove ports in the sides of the chamber.

Sterility checks were taken every 2 wks throughout the operation of the chamber. Random sampling and then incubation of the samples at 37 C and at room temperature were carried out on the following media: nutrient broth, potato dextrose agar, Levine EMB agar, Sabouraud dextrose agar, brain heart in fusion agar, and thioglycollate medium (Difco Manual, 1953).

Preparation of Water and Gas Systems

Each isolator chamber contained a three liter polypropylene water reservoir with a 5 ft delivery hose which was used to supply water to all the peanut plants in the chamber. One end of a second length of rubber tubing was forced into a hole at the top of the reservoir and the other end was connected to a bulkhead union sealed in the isolator chamber wall. This gave access to the external sterile water supply for refilling the reservoir.

There were also two systems in each of the two chambers that carried gas mixtures to the nutrient solutions in which the peanut roots were growing (Fig. 2). One end of each gas line terminated in a needle valve while the opposite end was attached to a bulkhead union sealed into the wall of the isolator chamber. This gave access to the external gas supply. An in-line valve placed in the system insured one way flow. From the main line of each gas system six lateral lines terminated in a fritted, immersion filter tube which was immersed in nutrient solution in each flask.

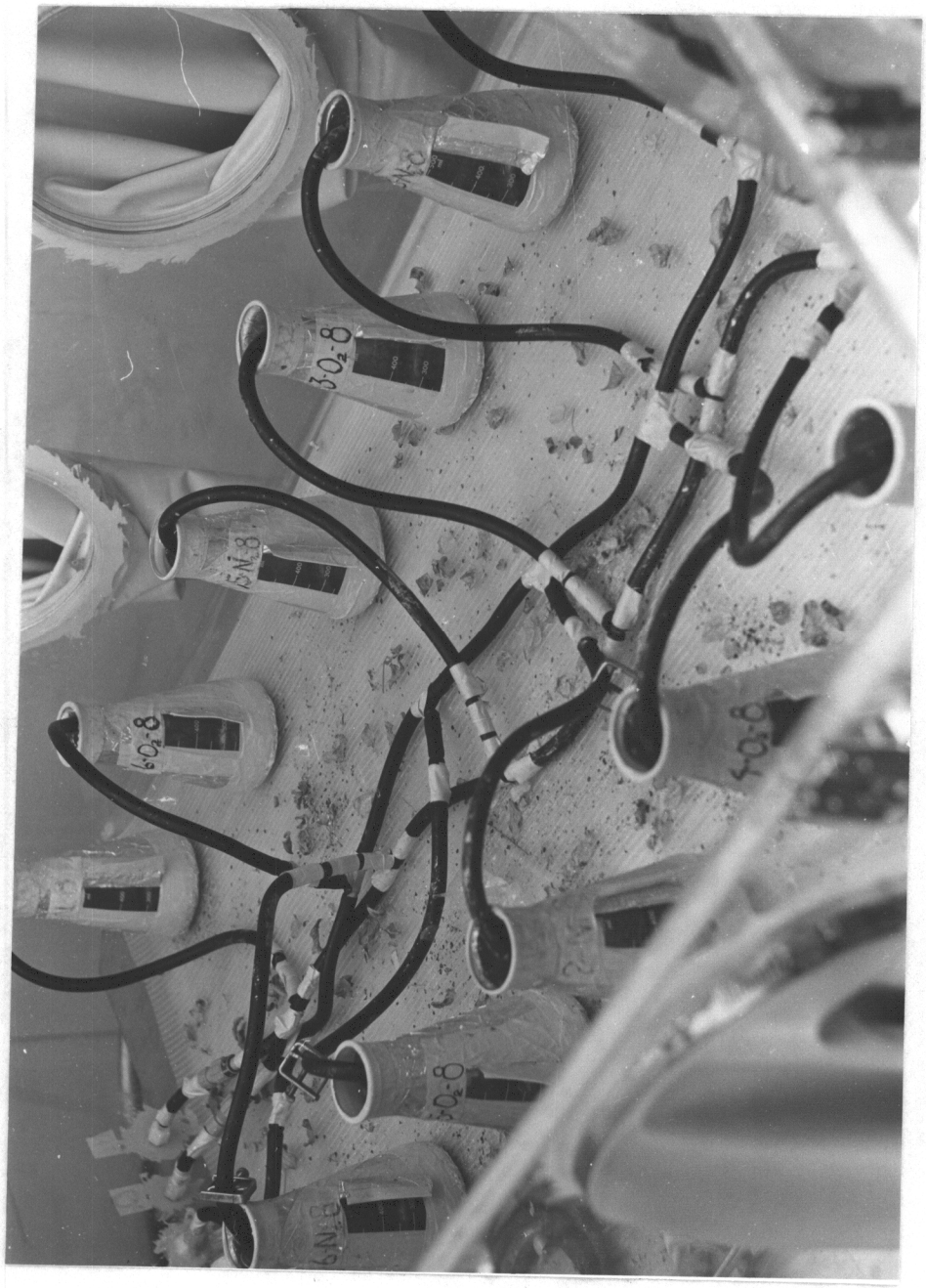

Fig. 2. Arrangement of rubber tubing system used to carry gas mixtures to the roots of peanut plants growing in nutrient solutions contained in wide-mouthed flasks.

The external, sterile, water supply consisted of three polypropylene bottles of one gallon size taped together and connected in series with rubber tubing. To the air inlet was attached both a fiberglass filter and a stainless steel filter holder with a Metricel membrane of 0.2 μ pore size. To the outlet tube was attached a 6 ft length of rubber tubing with a bulkhead union at the end to fasten it through the wall of the isolator chamber. The rubber tubing between each bottle was open to the atmosphere during autoclaving, by way of fiber glass filters, to equalize pressure differences. Before the unit was autoclaved for one hour, the bottles were filled with deionized water and the open tubing was plugged and capped with cotton. The sterilized water supply unit was attached through the wall of the isolator chamber prior to spraying with PAA. The bulkhead union was then sealed with silastic. As needed during the course of an experiment, water was forced from the supply unit into the water reservoir inside the isolator chamber by briefly attaching an air line at low pressure to the sterile system.

The design of the gas system which automatically controlled the volume flow to the roots of peanut plants in the isolator chambers is shown in Figure 3. The entire gas system was constructed in duplicate with half metering one kind of gas to both chambers and the other half metering a second kind of gas to both chambers and containing mixtures of O_2, CO_2, and N_2 in one and N_2 in the other were positioned on both sides of a board holding control devices. Flow of gas from the pressure regulator nozzle on a cylinder to the inlet of a one way, normally

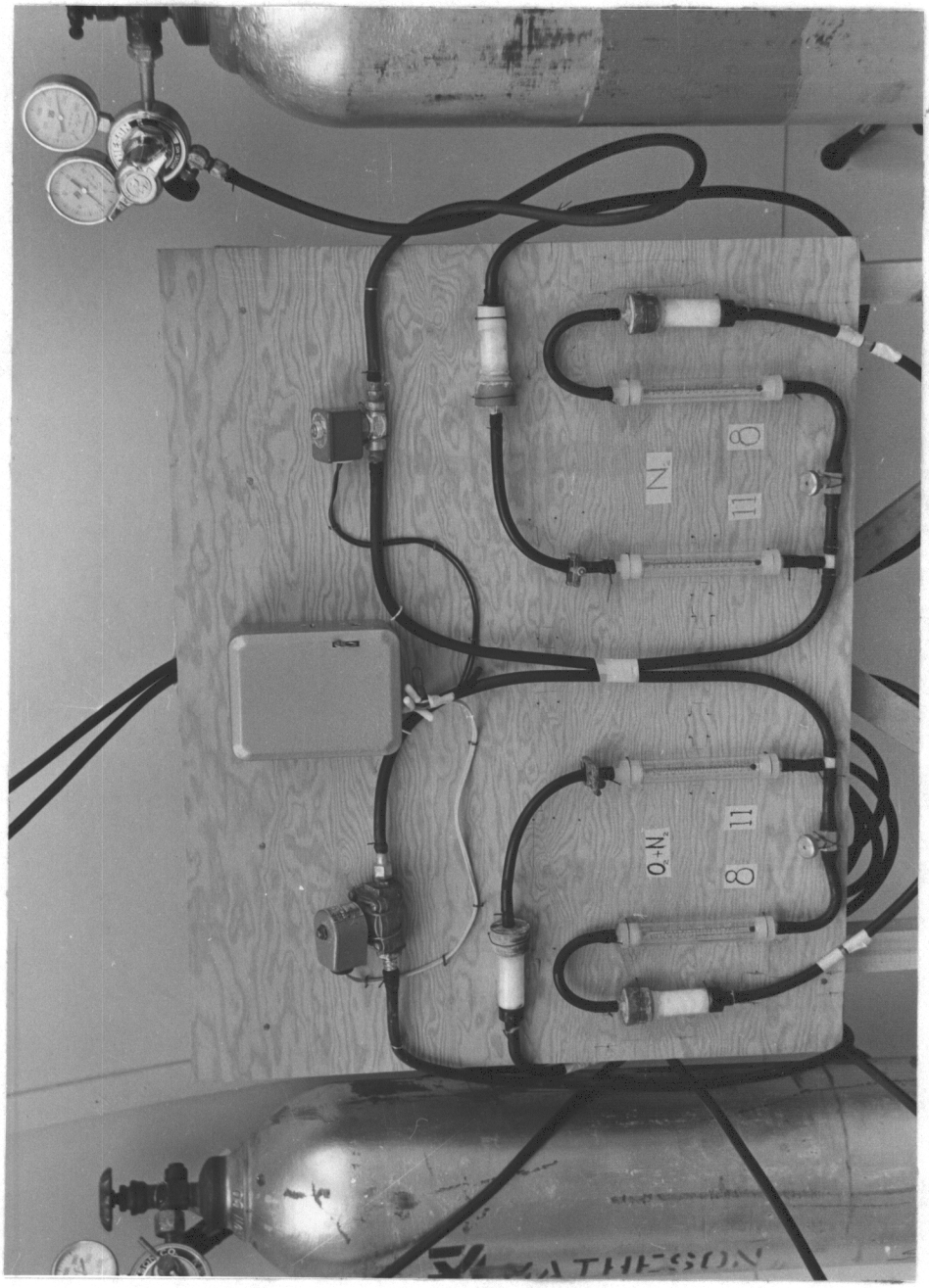

Fig. 3. Metering system designed to deliver automatically measured volumes of gases to the roots of peanut plants growing in plastic isolator chambers.

closed, electrically operated, solenoid valve then through a plastic tee to the openings of two shielded, compact flowmeters with flow rates of 200 to 12,500 ml of gas per min. The gas volume was divided equally between the two flowmeters by placing a stainless steel needle valve in the line between them. Each flowmeter was connected through a fiberglass filter, a millipore filter, and a one gallon polypropylene gas expansion bottle to a bulkhead union sealed into the isolator chamber wall. That portion of the gas system from the fiberglass filter to the bulkhead union was always sealed and autoclaved as a unit. The solenoid valves were wired to a 12 min interval timer which was set so that the solenoid valves were open 15 to 20 sec every 12 min. Rate of gas flow was approximately 2.5 liters per minute.

Gas Experiments with Peanut Roots

Three separate experiments were conducted. In all three, N_2 gas was metered to the roots of six plants in each of two isolator chambers. In addition, in the first study, roots of six plants in each of two isolator chambers received a gas mixture composed of 21% O_2, 10% CO_2, and 69% N_2; in the second study, they received a gas mixture composed of 21% O_2 and 79% N_2; and in the third study, they received a gas mixture composed of 10% CO_2 and 90% N_2.

Each experiment was conducted in the following manner. Twenty four wide-mouthed, graduated, 500 ml Erlenmeyer flasks were wrapped in heavy duty aluminum foil. The flasks contained 350 ml of nutrient solution at one half normal concentration and with chelated iron

at one eighth normal concentration (Wallace, 1962). Flasks were autoclaved 3.5 hrs and then twelve of them were placed in each of two isolator chambers. A fritted, immersion filter-tube was placed in each flask. Peanut plants were transferred in transplanting tubes to flasks (Fig. 4). Duration of each experiment was 6 wks. Every 2 wks the flasks were carefully emptied and 350 ml of fresh nutrient solution was added. Each collected sample consisted of nutrient solution containing peanut root exudates from three plants. The same three plants were always pooled together at each of three collection periods. In this manner twenty four samples of nutrient solution with peanut root exudates in them were collected for each experiment. Experimental design was complete randomization of plants within each isolator chamber.

The peanut plants received continuous light throughout the studies. The light intensity at the top of the chambers was 1,100 ft-c and approximately 900 ft-c at the level of the dense peanut foliage (Fig. 5). The chamber temperature was 27 to 29 C. After harvest, fresh weights; dry weights; and lengths of both shoots and roots separate were obtained.

Root Exudate Analysis

All insoluble material was removed from the seventy two nutrient solutions containing peanut root exudates collected from three experiments. The solutions were filtered through Whatman No. 1 filter paper and then through Millipore filters with a pore size of 0.22 μ. The residue consisted of bits of vermiculite and sloughed peanut root cells

Fig. 4. Closeup view showing how transplanting tubes hold the peanut plants in place on top of flasks of nutrient solution. Rubber tubing to each flask carries the gas treatment.

Fig. 5. Closeup view through wall of isolator chamber showing dense foliage of peanut plants receiving either nitrogen or oxygen gas treatment.

and tissues. The 700 to 1000 ml filtrate containing the exudates was reduced in volume to 10 ml under reduced pressure at 45 C in a Bucher portable flash evaporator.

Each 10 ml sample was transferred to a 50 ml Erlenmeyer flask and freeze dried (8 hrs) in a Cryochem freeze-dryer. Sample flasks were then stoppered with plastic caps and stored at 10 C.

Inorganic salts interfere with the separation of sugar compounds by thin-layer chromatography. Salts were removed by cation and anion exchange resins. The exudates were desalted 12 at a time on ion exchange columns containing Rexyn 201 (OH^-) which is a strong-base anion exchange resin and Rexyn 101 (H^+) which is a strong-acid cation exchange resin (Fisher Scientific Co.) and deionized water was used as the eluent. A rubber tubing manifold system connected the water reservoir to the top of all columns. The system functioned as a single unit and all 12 pairs of exchange resin columns could be eluted at the same time. The rate of flow, which was set at 2 ml per minute, was controlled by a screw clamp setting.

Freeze-dried peanut root exudate samples were dissolved in 5 ml of deionized water. Each sample was gently applied to the top of a column and a volume of water six times the combined void volumes of both columns was passed through. An effluent of 220 ml was collected. This contained the desalted peanut root sugar exudates (Mueller et al. 1955).

The next step was to obtain standard curves for sugar mixtures. A mixture of glucose, galactose, fructose, xylose, ribose, and arabinose

in equal amounts was dissolved in deionized water. By making a dilution series, concentrations of 10, 20, 30, 40, 50, and 60 ug of sugar per 2 ml volume were obtained. These sugar solutions were treated with phenol and concentrated sulfuric acid to produce an orange-yellow color (Dubois et al. 1956). Absorbancy readings were taken at 480, 485, and 490 nm on a Beckman DU II spectrophotometer. The final absorbancy value for each sugar concentration at each wavelength was an average of three samples. The standard curves were determined by the method of least squares (Ostle, 1963). The slope of each curve was calculated. The concentration in micrograms of any sugar was found by dividing the absorbancy reading by the slope of the curve (Fig. 6).

Eight sugar mixtures of known concentration were passed through the exchange resin columns to determine the percent recovery that could be expected. The average recovery at 480 nm was 31.0%, at 485 nm it was 32.4%, and at 490 nm it was 34.2% (McDougall, 1968). It was necessary to reduce the large effluent volumes from the exchange resin columns to a manageable size by flash evaporating as previously described. All eluted sample volumes were reduced to 5 ml which were freeze dried and stored at 10 C.

Thin-Layer Chromatography (TLC)

A slurry was made of 15 g of cellulose powder MN 300 (Macherey, Nagel and Co.) and 90 ml of deionized water-methanol solution (5:1 v/v) by mixing in a blendor for 30 seconds. This homogeneous slurry was spread over five 20 x 20 cm glass plates at one time with an adjustable

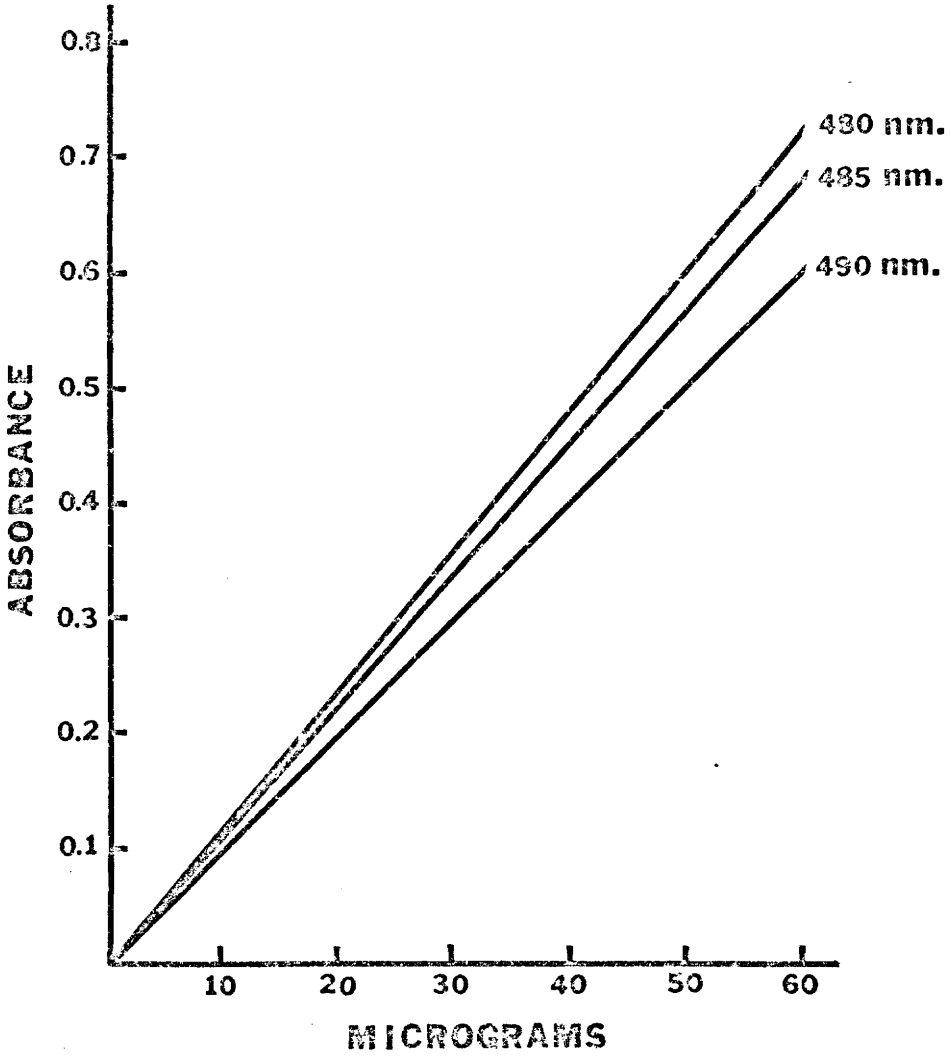

Fig. 6. Standard curves of a known mixture of sugars at three wavelengths.

Desaga applicator. The plates were dried in a hood for 2 hours and then stored in a desiccator cabinet overnight before using (Vomhof and Tucker, 1965).

The freeze-dried sugar exudate samples were dissolved in 80 ul of 10% isopropanol. Sixty to 80 ul of six sugar exudates were applied, 2 ml at a time, to one end of a TLC plate with a microliter syringe. A warm air blower was used to dry the spots. Five spots which contained a total of eight standard sugars at known concentrations were also applied in like manner. The solvent system was composed of formic acid: methyl ethyl ketone: tert.-butanol: water (15:30:40:15, v/v). The Gelman chambers, containing TLC plates were allowed to equilibrate for 30 minutes. After solvent front advanced 15 cm, plates were removed from the chambers and air dried for 2 hours. The separated sugar compounds were detected by spraying with a solution of diphenylamine and aniline in acetone to which 85% phosphoric acid was added just prior to spraying (De Stefanis and Ponte, Jr. 1968). The plates were then placed in an oven at 70 to 80 C until colors developed. Rf values and spot colors were recorded. Representative TLC plates of all sugar exudate samples were photographed.

Densitometry

Quantity of sugar in each spot on the freshly developed plates was determined with a Photovolt multiplier photometer which read the spots by white light transmission through a 0.1 x 6 mm slit aperature (Huber et al. 1966). Quantity of sugar in each TLC spot was recorded on chart paper by the instrument as a curve. The area under each sugar

curve was also determined on the chart as number of counts. These counts were translated into micrograms of sugar in the following manner. Five different sugar compounds were spotted on a TLC plate at concentrations of 5, 10, 20, and 40 ug. This was done on three plates. The sugars were then put through the same TLC procedures as previously described. The sugar spots were read on the Photovolt multiplier photometer and number of counts was determined. Counts for the three spots for each sugar at each concentration were averaged. Average values were used to construct standard curves for galactose, mannose, xylose, ribose, and dihydroxyacetone by the method of least squares (Fig. 7). The slope of each standard curve was calculated. Micrograms of sugar in each TLC spot obtained from peanut root exudates was determined from the product of the slope and number of counts produced by the sugar exudate spot.

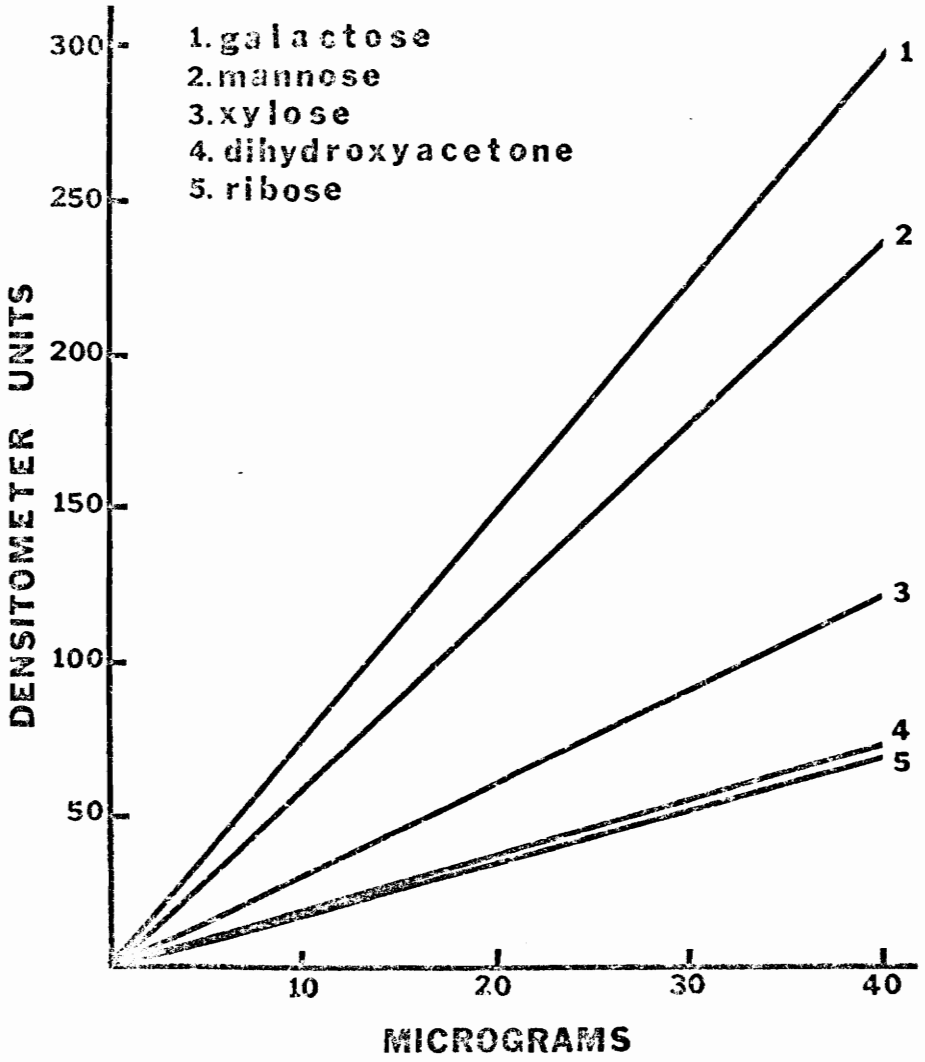

Fig. 7. Standard curves of densitometer readings for five sugars chromatographed on TLC.

RESULTS

At the end of each experiment, peanut plants were harvested to obtain fresh and dry weights, and lengths of shoots and roots. Half the plants in all three experiments received N_2-gas. Nitrogen was an internal standard whereby corrections for plant growth differences caused by small environmental differences between the three experiments could be made. Therefore, all harvest values were adjusted by bringing them into alignment with the smallest N_2 value for each kind of measurement (Table I, see Table VII in Appendix for original values).

Analysis of variance showed for all adjusted harvest values no significant difference as a result of treatment or replication (Tables IX through XX in Appendix). For all measured growth parameters, Duncan's multiple range test showed no significant differences within replicates caused by treatment.

Sugars in peanut root exudates were separated and identified by thin-layer chromatography. Comparison of Rf's and colors of root exudate sugar spots with standard sugars indicated the presence of galactose (blue-grey), mannose (light blue), xylose (blue-grey), ribose (blue-grey), and dihydroxyacetone (pink). Residue buildup within root exudate spots, due to large volume application, caused a lag in movement and resulted in lower Rf's than for standard sugars. Therefore, Rf's for root exudate sugar spots were adjusted by bringing them into alignment with the Rf's for the galactose standard (Tables II, III, IV, and Figures 8, 9, 10).

Table I. Fresh weight (F. W.), dry weight (D. W.), and length (L.) of peanut shoots and roots at end of treatment.[1]

Treatment	Shoot			Root			Total	
	F.W.(g.)	D.W.(g.)	L.(cm.)	F.W.(g.)	D.W.(g.)	L.(cm.)	F.W.(g.)	D.W.(g.)
$O_2 + CO_2 + N_2$	62.0 a*	10.8 a	95.6 a	12.6 a	1.7 a	24.5 a	74.6	12.5
$O_2 + N_2$	48.2 a	8.7 a	77.5 a	12.4 a	1.7 a	22.1 a	60.6	10.4
$CO_2 + N_2$	48.1 a	8.9 a	89.2 a	12.1 a	1.7 a	20.7 a	60.2	10.6
N_2	48.1 a	8.7 a	81.8 a	10.3 a	1.4 a	20.7 a	58.4	10.1

[1] Average of four replicates

* Values in the same column having different lower case letters are significantly different at the 0.05 level using Duncan's multiple range method.

Table II. Rf's on TLC for peanut root sugar exudates.

Sugar	Treatment					
	$O_2 + CO_2 + N_2$	N_2	$O_2 + N_2$	N_2	$CO_2 + N_2$	N_2
Galactose	20 (4)	19 (4)	20 (4)	19 (4)	20 (4)	18 (3)
Mannose	--	--	--	--	--	33 (1)
Xylose	--	--	35 (1)	33 (1)	--	--
Ribose	45 (3)	38 (2)	43 (1)	45 (1)	44 (2)	37 (2)
Dihydroxy-acetone	67 (4)	53 (4)	69 (4)	60 (3)	71 (4)	64 (2)

Number of replicates in average in parenthesis

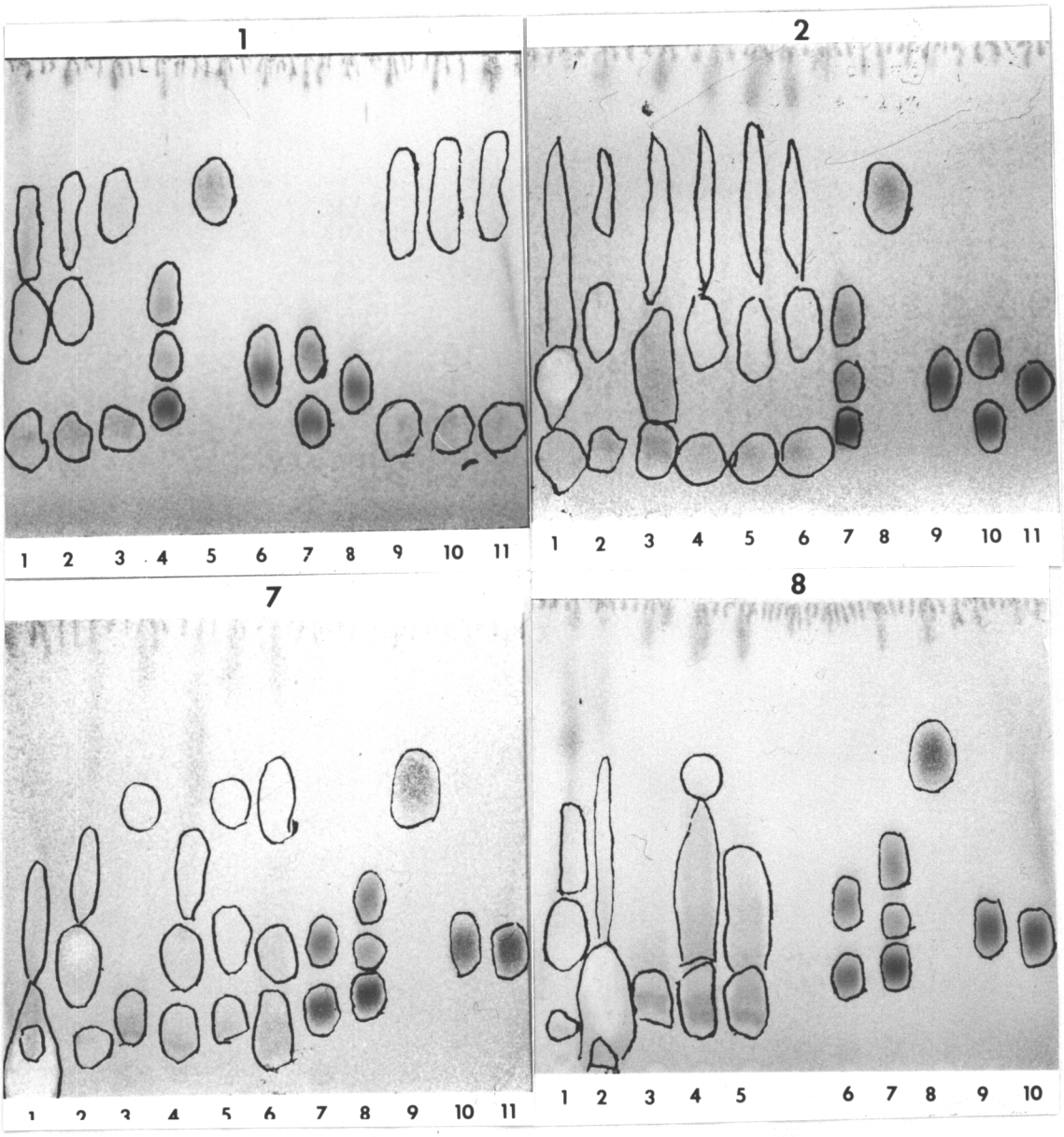

Fig. 8. Chromatograms of sugar exudates from first 2 wks collection period. There are four replications of each treatment.
Treatments:

$O_2 + CO_2 + N_2$	Plates 1 and 2; Columns 1, 2 and 1, 2
$O_2 + N_2$	Plates 1 and 2; Columns 3, 9 and 3, 4
$CO_2 + N_2$	Plates 1 and 2; Columns 10, 11 and 5, 6
N_2	Plates 7 and 8; Columns 1-6 and 1-5

All other columns are sugar standards.

Table III. Rf's on TLC for peanut root sugar exudates.

Sugar	Treatment					
	$O_2 + CO_2 + N_2$	N_2	$O_2 + N_2$	N_2	$CO_2 + N_2$	N_2
Galactose	20 (4)	19 (4)	20 (4)	19 (4)	20 (4)	19 (4)
Mannose	--	29 (1)	--	29 (2)	--	--
Xylose	--	33 (1)	--	--	--	--
Ribose	--	43 (2)	42 (2)	40 (1)	39 (3)	40 (2)
Dihydroxy-acetone	68 (3)	67 (4)	66 (2)	70 (1)	65 (3)	62 (2)

Number of replicates in average in parenthesis

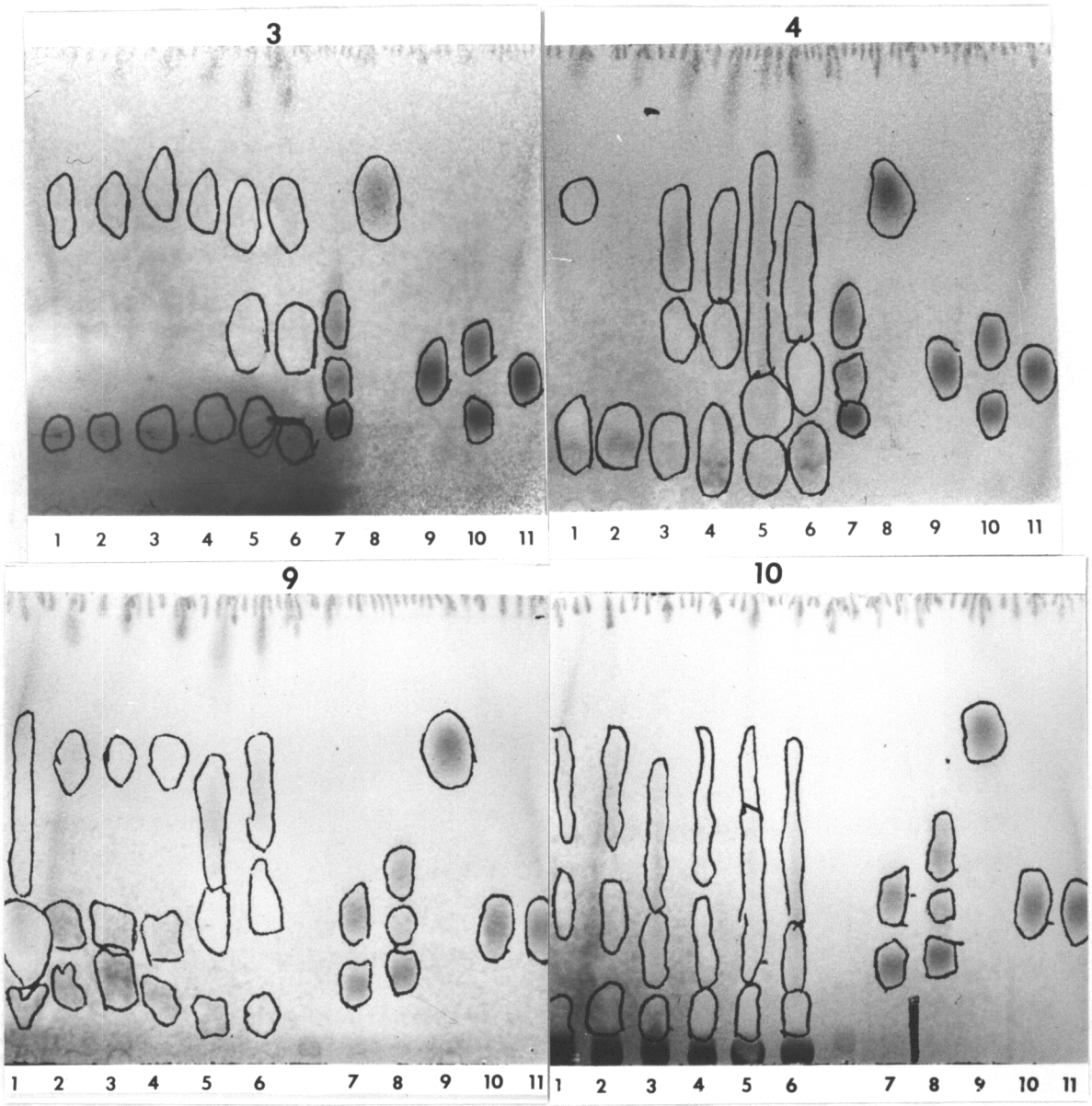

Fig. 9. Chromatograms of sugar exudates from second 2 wks collection period. There are four replications of each treatment.
Treatments:

$O_2 + CO_2 + N_2$	Plates 3 and 4; Columns 1, 2 and 1, 2
$O_2 + N_2$	Plates 3 and 4; Columns 3, 4 and 3, 4
$CO_2 + N_2$	Plates 3 and 4; Columns 5, 6 and 5, 6
N_2	Plates 9 and 10; Columns 1-6 and 1-6

All other columns are sugar standards.

Table IV. Rf's on TLC for peanut root sugar exudates.

Sugar	Treatment					
	$O_2 + CO_2 + N_2$	N_2	$O_2 + N_2$	N_2	$CO_2 + N_2$	N_2
Galactose	21 (4)	18 (4)	21 (4)	18 (3)	21 (4)	18 (4)
Mannose	30 (2)	--	35 (1)	--	31 (1)	--
Xylose	--	--	--	--	--	37 (2)
Ribose	53 (2)	44 (1)	45 (3)	42 (2)	46 (3)	40 (1)
Dihydroxy-acetone	73 (4)	70 (4)	65 (4)	72 (3)	66 (4)	73 (4)

Number of replicates in average in parenthesis

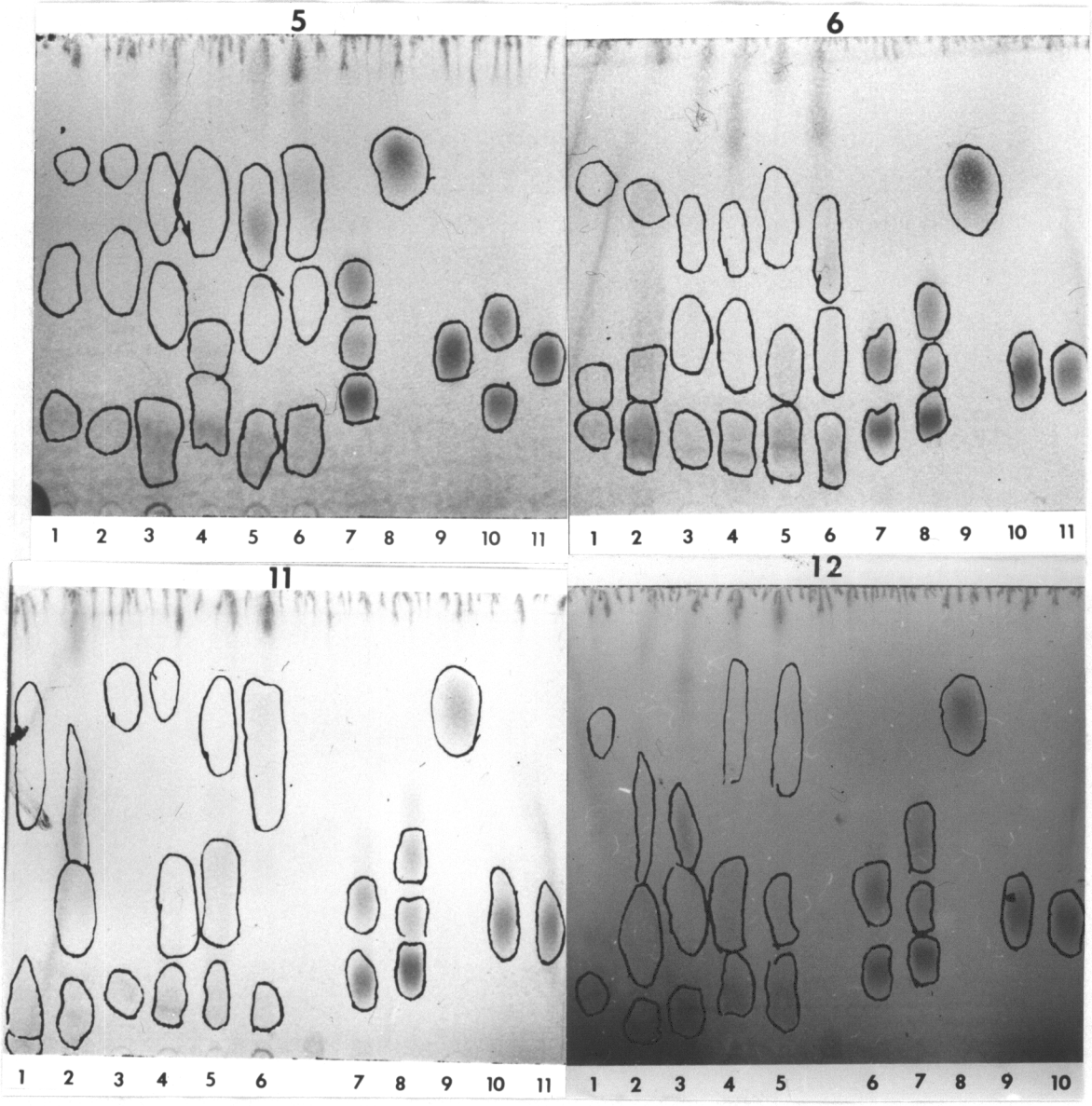

Fig. 10. Chromatograms of sugar exudates from third 2 wks collection period. There are four replications of each treatment.
Treatments:

$O_2 + CO_2 + N_2$	Plates 5 and 6; Columns 1, 2 and 1, 2
$O_2 + N_2$	Plates 5 and 6; Columns 3, 4 and 3, 4
$CO_2 + N_2$	Plates 5 and 6; Columns 5, 6 and 5, 6
N_2	Plates 11 and 12; Columns 1-6 and 1-5

All other columns are sugar standards.

Galactose was present in all 72 root exudate samples and dihydroxyacetone was detected in 69. Ribose was present in 4 to 8 samples in every gas treatment while mannose and xylose were each found in 8 and 5 samples respectively, scattered evenly among the treatments (Table V).

Galactose and dihydroxyacetone were present in peanut root exudates in sufficient quantity to be determined by densitometry. All values were adjusted to bring them into alignment with the smallest N_2 value for each collection period (Table VI, see Table VIII in Appendix for original values).

Analysis of variance showed a significant difference at the 20% level in the total amount of galactose exuded as a result of treatment and a significant difference at the 25% level in the amount of galactose exuded as a result of plant age (Tables XXI and XXII). Duncan's multiple range test showed an interaction between treatment and time which was significant at the 5% level. Plants were subjected to four different root aeration treatments ranging from aerobic to anaerobic. Young plants (2 wks old) showed a significantly greater amount ($p=0.05$) of galactose exuded under aerobic conditions. As the plants matured (4 and 6 wks old) there was a trend toward greater amounts of galactose exuded under anaerobic conditions than under aerobic conditions.

Analysis of variance showed no significant differences at the 25% level in the total amounts of dihydroxyacetone exuded as a result of treatment and no significant difference at the 25% level in the amounts of dihydroxyacetone exuded as a result of plant age (Tables

Table V. Frequency of occurrence of sugars in exudates from twelve samples per treatment.

Sugar	Treatment					
	$O_2 + CO_2 + N_2$	N_2	$O_2 + N_2$	N_2	$CO_2 + N_2$	N_2
Galactose	12	12	12	12	12	12
Mannose	2	1	1	2	1	1
Xylose	--	1	1	1	--	2
Ribose	5	5	6	4	8	5
Dihydroxy-acetone	10	12	12	11	12	10

Table VI. Quantity of sugar exuded (ug.) per treatment for each collection period.

	Weeks	Treatment[1] $O_2 + CO_2 + N_2$	$O_2 + N_2$	$CO_2 + N_2$	N_2
Galactose	2	82.3 f*	60.9 cde	48.8 bc	19.6 a
	4	56.6 bcd	76.3 def	79.2 ef	48.8 bc
	6	45.2 bc	55.7 bc	39.2 ab	37.4 ab
Total		184.1	192.9	167.2	105.7
Dihydroxy-acetone	2	143.6 bcd	90.2 abc	5.0 a	5.0 a
	4	5.0 a	198.2 cd	214.8 d	5.0 a
	6	T[2] a	5.0 a	79.5 ab	51.6 ab
Total		148.6	293.4	299.3	61.6

[1] Average of four replicates

[2] Trace of sugar

* Values for the same compound having different lower case letters are significantly different at the 0.05 level using Duncan's multiple range method.

XXIII and XXIV). Duncan's multiple range test showed in young plants (2 wks old) a significantly greater amount ($p=0.05$) of dihydroxyacetone exuded under aerobic conditions. As the plants matured (4 and 6 wks old) there was a trend toward greater amounts of dihydroxyacetone exuded under anaerobic conditions than under aerobic conditions.

DISCUSSION

The series of three experiments had these objectives:

(1) to examine the effects of O_2 and CO_2 tensions on the exudation of sugars from peanut roots;

(2) to determine the kinds and amounts of sugars released from peanut plants as root exudates;

(3) to devise gas and water systems which would be sterile, which could be attached to the isolator chambers, but which would function outside the chambers.

To obtain axenic peanut cultures, three sterile transfers of embryos and seedlings were necessary before plants were established in sterile isolator chambers. On one attempt fungal contamination occurred which necessitated starting an experiment anew. One deceptive feature of excised peanut embryos germinated on solid agar nutrient was the release of a yellow pigment-like substance thought to be a bacterium. However, the substance was always present regardless of the type of sterility procedure used and did not grow on any of the media used for sterility checks.

It is sometimes essential that certain plant physiological processes be studied in the absence of microorganisms. Some particular reasons are to: (1) obtain the most valid results possible, (2) determine the nature and amount of substances excreted by plant roots, (3) check ability of plant to absorb and utilize organic compounds,

and (4) compare axenic and nonaxenic plants such as in the determination of the effect of microorganisms upon respiration of roots or on the availability of inorganic nutrients (Reuszer, 1962).

The present investigation, among others, has demonstrated the feasibility of growing germ-free plants inside plexiglass isolator chambers (Linsey, 1967; and Hale, 1969). The isolator chamber permits a great variety of gnotobiotic experimental conditions such as: (1) axenic growth of the entire plant, (2) growth of a number of plants, (3) sufficient space for handling plants, (4) use of different kinds of rooting media, (5) growth of successive generations of plants, (6) introduction of various microorganisms in association with plants, and (7) growth of two or more plant species for interaction studies (Reuszer, 1962; Lindsey, 1967; Lindsey and Baker, 1967; Lukezic, et al., 1969; and Hale, 1969).

The external sterile gas and water systems connected to the isolator chambers in the present investigation required a small amount of trial and error adjustment after which they functioned reliably with little or no additional care. Such an external system could be used to meter many kinds of gases and liquids in automatically measured amounts to entire plants or just to the roots or shoots within isolator chambers. Even living organisms could be introduced into the chamber by means of a slightly pressured air line attached through the wall. The time required to replace an empty external water system with a sterile, filled water system was just one half to one third as much time as required to introduce sterile bottles of water into the isolator chambers.

Another salient feature of external systems was the fact that the plant growing space in the chamber was not excessively limited by equipment or large numbers of water bottles. Excessive equipment sterilization was avoided because if trouble developed in the external system, the isolator chamber could be closed off to prevent contamination and the system repaired without removing it from the chamber or sterilizing and putting tools and parts into the chamber.

The analytical procedure employed to obtain small volumes of desalted root exudate samples was inadequate in the following ways: (1) there were too many opportunities for microbial contamination to occur, (2) there were too many steps involved, each of which retained a small amount of each sample, and (3) an excessive amount of time was required to process 72 samples. The average time required to reduce the volume of a root exudate sample from 900 to 1000 ml to 5 ml by flash evaporation was 3.5 hrs. The ion exchange resin columns used to desalt the exudate samples retained, irreversibly, two thirds of the total sugar. It was not determined what proportion of each individual sugar was actually retained. Perhaps some exudate sugars, present in very small amounts, were totally retained by the resin columns. This should be examined further. More than one hour was required to apply six root exudate samples, 2 ml at a time, to each thin-layer chromatography plate. Sugar spot concentrations at or below approximately 5 ug could not be detected by the densitometer so it was not possible to quantify all of the sugar spots on the TLC plates.

It has been shown that low concentrations of root atmospheric O_2 inhibit plant growth, while plant growth increases in proportion to the O_2 content up to approximately 21% (Burstrom, 1953). Low concentrations of root atmospheric CO_2 may stimulate root growth, while plant growth may decrease in proportion to the CO_2 content up to 20 to 30% (Erickson, 1946). Numerous other investigators have studied the effects of O_2 and CO_2 on root and plant growth. Some conclusions were: (1) no growth effects due to CO_2 accumulation, (2) decrease of O_2 pressure in rooting medium below atmospheric decreased plant growth when CO_2 concentration remained unchanged, (3) specific lethal action of CO_2 superimposed on the results caused by O_2 deficiency, (4) direct effect on plant growth due to the presence of CO_2 and not to the exclusion of O_2, (5) optimum O_2 concentration depended upon the range of CO_2 concentration, and (6) plant growth differences were produced by insufficient O_2 before CO_2 concentration reached a toxic value (Arrington and Shive, 1936; Boynton and Compton, 1943; Vlamis and Davis, 1944; Chang and Loomis, 1945; Leonard and Pinckard, 1946; and Erickson, 1946).

Reports are somewhat contradictory concerning the levels of O_2 and CO_2 which are limiting for root growth. This is understandable because different species of plants in different stages of development, and growing in different media ranging from fine-textured soil to solution cultures have been used. The methods used to aerate root systems vary widely and thus produce different results. Most likely the actual O_2 concentrations at the root surfaces are different in water culture, in soil aerated by forced circulation of a gas mixture, and in soil aerated

by diffusion. It does appear likely, however, that O_2 concentrations above 10% are adequate for plant growth. There is more uncertainty concerning the effects of high CO_2 on root growth. From 1 to 20% or more of CO_2 has been shown to be injurious to plant roots and overall growth. It appears that CO_2 concentration in the soil is seldom high enough to cause injury, but the concentration of O_2 is often low enough to be inhibitory (Kramer, 1969).

In the present experiments since there were no significant growth differences produced by varying the O_2 and CO_2 concentrations, the O_2-CO_2 balance in the nutrient solutions in which all the peanut roots were growing must have been sufficient. No O_2 was added to treatments receiving either $CO_2 + N_2$ or N_2 and consequently, the air above the nutrient solutions (these flasks were open to the isolator chamber atmosphere) could have supplied enough O_2 to maintain adequate growth.

However, under the treatment conditions of the present investigation, greater anaerobiosis produced a trend toward more exudation in older plants than in younger plants. Perhaps in the $CO_2 + N_2$ and N_2 treatments the peanut plant roots were creating, in part, their own O_2 deficits by growing beyond the capability of the dissolved atmospheric O_2 to supply increasing respirational needs.

Root exudation has been shown to increase when anaerobic conditions prevail. Many of the excreted compounds reflected the change in root respiration which accompanied the O_2 to CO_2 shift (Grineva, 1961; Woodcock, 1962; and Grineva, 1963). In both pea and oat seedlings larger quantities of amino acids and sugars were exuded during the first 10

days than during the second 10 days (Rovira, 1956). The results of this present investigation also show that age of plants and the degree of anaerobiosis affect the amount of sugars in plant root exudations. However, others found that, qualitatively and quantitatively, more ninhydrin-reactive material was released under 0.5% CO_2-enriched air than from 10% CO_2-enriched air, or 2% O_2, 0.5% CO_2, and 97.5% N_2 gas mixture (Ayers and Thornton, 1968).

Perhaps the trend toward greater release of root exudates in older plants under anaerobic conditions can be correlated with a gradual decrease in O_2 and/or increase in CO_2 which lowers the respiration rate of the root cells. Root cell membrane permeability then increases due to the lack of sufficient respiration energy to maintain its integrity (Salisbury and Ross, 1969).

After an initial review of the literature, glucose, fructose, galactose, xylose, ribose, and arabinose were thought to be the most likely five and six carbon sugars to be found in peanut plant root exudations. Glucose, fructose, and arabinose were not present. Surprisingly, the disaccharide, sucrose, was not found in any of the root exudate samples. However, this correlated with the lack of glucose and fructose. The quantities of galactose, which as a galactan is a component of the plant cell wall, were larger in these root exudates than in any previously reported in the literature. Dihydroxyacetone, a 3 carbon sugar which is an essential respiratory intermediate in the glycolytic metabolic pathway, was an unexpected find which has not been reported previously in the literature. Nor could this investigator find

any previous report of mannose in plant root exudations. Mannose, as a mannan, is a constituent of plant cell walls. It was speculated that the more anaerobic conditions created in nutrient solutions receiving $CO_2 + N_2$ and N_2 may have inhibited the aerobic portion of cell respiration with the subsequent blockage and buildup of glycolytic intermediates such as dihydroxyacetone which then exuded from the root cells. Larger numbers of sugars have been found in plant root exudations than reported in this present investigation. This may be due to: (1) plant species tested, (2) kind of treatment applied, or (3) methodology used.

This investigator believes that the objectives of this present investigation have been met and accomplished.

SUMMARY

The effects of O_2 and CO_2 tensions, both individually and combined, on peanut root exudation and the amount of sugars exuded from roots of peanut plants grown in nutrient solutions under gnotobiotic conditions were measured.

At the end of each experiment, peanut plants were harvested and fresh and dry weights and lengths of shoots and roots obtained. There were no effects on plant growth, but there were effects on root exudation.

Sugars in peanut root exudates were separated and identified by thin-layer chromatography. Comparison of Rf's and colors of root exudate sugar spots with standard sugars indicated the presence of galactose (blue-grey), mannose (light blue), xylose (blue-grey), ribose (blue-grey), and dihydroxyacetone (pink). Galactose was present in all 72 root exudate samples, dihydroxyacetone in 69, ribose in 33, mannose in 8, and xylose in 5.

Galactose and dihydroxyacetone were present in peanut root exudates in sufficient quantity to be determined by densitometry. All values were adjusted to bring them into alignment with the smallest N_2 value for each collection period. Analysis of variance showed a significant difference at the 20% level in the total amount of galactose exuded as a result of plant age. Duncan's multiple range test showed an interaction between treatment and time which was significant at the 5% level. Young plants (2 wks old) showed a significantly greater amount of

galactose exuded under the less anaerobic conditions. As the plants matured (4 and 6 wks old) there was a trend toward greater amounts of galactose exuded under the more anaerobic conditions than under the less anaerobic.

Analysis of variance showed no significant differences at the 25% level in the total amounts of dihydroxyacetone exuded as a result of treatment and no significant difference at the 25% level in the amounts of dihydroxyacetone exuded as a result of plant age. Duncan's multiple range test showed in young plants (2 wks old) a significantly greater amount of dihydroxyacetone exuded under aerobic conditions ($p=0.05$). As the plants matured (4 and 6 wks old) there was a trend toward greater amounts of dihydroxyacetone exuded under anaerobic conditions than under aerobic.

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APPENDIX

Table VII. Fresh weight (F. W.), dry weight (D. W.), and length (L.) of peanut shoots and roots at end of treatment.[1]

Treatment	Shoot			Root			Total	
	F.W.(g.)	D.W.(g.)	L.(cm.)	F.W.(g.)	D.W.(g.)	L.(cm.)	F.W.(g.)	D.W.(g.)
$O_2 + CO_2 + N_2$	62.0	10.8	104.7	13.0	1.9	24.5	75.0	12.7
N_2	48.0	8.7	93.7	10.7	1.6	20.7	58.7	10.3
$O_2 + N_2$	89.2	15.4	77.5	12.4	1.7	26.7	101.6	17.0
N_2	89.6	15.4	81.3	10.3	1.4	20.3	99.9	16.8
$CO_2 + N_2$	58.4	10.8	104.5	13.7	2.1	22.8	72.1	12.8
N_2	57.7	10.6	104.2	12.0	1.8	22.8	69.7	12.4

[1] Average of four replicates

Table VIII. Quantity of sugar exuded (ug.) per treatment for each collection period.

	Weeks	Treatment[1]					
		$O_2 + CO_2 + N_2$	N_2	$O_2 + N_2$	N_2	$CO_2 + N_2$	N_2
Galactose	2	82.3	19.6	85.6	44.3	82.9	53.8
	4	60.1	52.3	79.0	58.1	79.2	48.8
	6	52.6	44.8	55.7	37.4	70.1	68.2
Total		195.0	116.7	220.3	139.8	232.2	170.7
Dihydroxy-acetone	2	143.6	5.0	90.2	5.0	5.0	5.0
	4	5.0	5.0	198.2	5.0	269.8	60.0
	6	5.0	62.2	5.0	51.6	97.9	70.0
Total		153.6	72.2	293.4	61.6	372.7	135.0

[1] Average of four replicates

Table IX. Preliminary calculations for shoot fresh weight.

Type of Total	Total of Squares	Number of Items Squared	Number of Observations per Squared Item	Total of Squares per Observation
GRAND	680,790.01	1	16	42,549.38
TREATMENTS	172,518.89	4	4	43,129.72
REPLICATIONS	172,039.53	4	4	43,009.88
OBSERVATIONS	44,309.41	16	1	44,309.41

Table X. Analysis of variance for shoot fresh weight.

Source	SS	DF	MS	F
BETWEEN TREATMENTS	580.34	3	193.44	2.42*
BETWEEN REPLICATIONS	460.50	3	153.50	1.92*
ERROR	719.19	9	79.91	
TOTAL	1760.03	15		

* Not significant at the 5% level

Table XI. Preliminary calculations for shoot dry weight.

Type of Total	Total of Squares	Number of Items Squared	Number of Observations per Squared Item	Total of Squares per Observation
GRAND	22,290.49	1	16	1,393.16
TREATMENTS	5,619.05	4	4	1,404.76
REPLICATIONS	5,629.65	4	4	1,407.41
OBSERVATIONS	1,443.99	16	1	1,443.99

Table XII. Analysis of variance for shoot dry weight.

Source	SS	DF	MS	F
BETWEEN TREATMENTS	11.60	3	3.86	1.39*
BETWEEN REPLICATIONS	14.25	3	4.75	1.71*
ERROR	24.98	9	2.77	
TOTAL	50.83	15		

* Not significant at the 5% level

Table XIII. Preliminary calculations for shoot length.

Type of Total	Total of Squares	Number of Items Squared	Number of Observations per Squared Item	Total of Squares per Observation
GRAND	1,894,476.96	1	16	118,404.81
TREATMENTS	476,695.10	4	4	119,173.78
REPLICATIONS	474,378.46	4	4	118,594.62
OBSERVATIONS	120,857.72	16	1	120,857.72

Table XIV. Analysis of variance for shoot length.

Source	SS	DF	MS	F
BETWEEN TREATMENTS	768.97	3	256.32	1.54*
BETWEEN REPLICATIONS	189.81	3	63.27	0.38*
ERROR	1,494.13	9	166.01	
TOTAL	2,452.91	15		

* Not significant at the 5% level

Table XV. Preliminary calculations for root fresh weight.

Type of Total	Total of Squares	Number of Items Squared	Number of Observations per Squared Item	Total of Squares per Observation
GRAND	35,986.09	1	16	2,249.13
TREATMENTS	9,049.27	4	4	2,262.32
REPLICATIONS	9,008.65	4	4	2,252.16
OBSERVATIONS	2,310.57	16	1	2,310.57

Table XVI. Analysis of variance for root fresh weight.

Source	SS	DF	MS	F
BETWEEN TREATMENTS	13.19	3	4.39	0.87*
BETWEEN REPLICATIONS	3.03	3	1.01	0.20*
ERROR	45.22	9	5.02	
TOTAL	61.44	15		

* Not significant at the 5% level

Table XVII. Preliminary calculations for root dry weight.

Type of Total	Total of Squares	Number of Items Squared	Number of Observations per Squared Item	Total of Squares per Observation
GRAND	665.64	1	16	41.60
TREATMENTS	167.42	4	4	41.85
REPLICATIONS	167.16	4	4	41.79
OBSERVATIONS	43.68	16	1	43.68

Table XVIII. Analysis of variance for root dry weight.

Source	SS	DF	MS	F
BETWEEN TREATMENTS	0.25	3	0.083	0.456*
BETWEEN REPLICATIONS	0.19	3	0.063	0.346*
ERROR	1.64	9	0.182	
TOTAL	2.08	15		

* Not significant at the 5% level

Table XIX. Preliminary calculations for root length.

Type of Total	Total of Squares	Number of Items Squared	Number of Observations per Squared Item	Total of Squares per Observation
GRAND	123,552.25	1	16	7,722.02
TREATMENTS	31,046.41	4	4	7,761.60
REPLICATIONS	30,910.57	4	4	7,727.64
OBSERVATIONS	7,835.51	16	1	7,835.51

Table XX. Analysis of variance for root length.

Source	SS	DF	MS	F
BETWEEN TREATMENTS	39.58	3	13.19	1.74*
BETWEEN REPLICATIONS	5.62	3	1.87	0.24*
ERROR	68.29	9	7.58	
TOTAL	113.49	15		

* Not significant at the 5% level

Table XXI. Preliminary calculations for weight of galactose.

Type of Total	Total of Squares	Number of Items Squared	Number of Observations per Squared Item	Total of Squares per Observation
GRAND	422,500.00	1	12	35,208.3
TREATMENTS	109,961.81	4	3	36,653.9
AGES	144,319.62	3	4	36,079.9
OBSERVATIONS	39,007.96	12	1	39,008.0

Table XXII. Analysis of variance for weight of galactose.

Source	SS	DF	MS	F
BETWEEN TREATMENTS	1445.6	3	481.8	1.95[a]
BETWEEN AGES	871.6	2	435.8	1.76[b]
ERROR	1482.5	6	247.0	
TOTAL	3799.7	11		

[a] Significant at the 20% level p=0.80

[b] Significant at the 25% level p=0.75

Table XXIII. Preliminary calculations for weight of dihydroxyacetone.

Type of Total	Total of Squares	Number of Items Squared	Number of Observations per Squared Item	Total of Squares per Observation
GRAND	652,702.41	1	12	54,391.87
TREATMENTS	203,061.57	4	3	67,687.72
AGES	258,248.44	3	4	64,562.11
OBSERVATIONS	123,312.09	12	1	123,312.09

Table XXIV. Analysis of variance for weight of dihydroxyacetone.

Source	SS	DF	MS	F
BETWEEN TREATMENTS	13,295.85	3	4,431.9	0.585N.S.*
BETWEEN AGES	10,170.24	2	5,085.1	0.671N.S.*
ERROR	45,454.13	6	7,575.7	
TOTAL	68,920.22	11		

* N.S. means not significantly different at the 0.25 level

VITA

Richard Lee Rittenhouse was born February 15, 1931 in Piqua, Ohio, the son of Mr. and Mrs. George L. Rittenhouse. He was graduated from Piqua High School, Piqua, Ohio in June, 1949. In September, 1949, he attended Wittenberg College, Springfield, Ohio for a period of two years. In September, 1951, he became a member of the United States Air Force and served for a period of four years. Beginning September, 1955, he attended Ohio University, Athens, Ohio. In June, 1957, he was graduated cum laude and was awarded a Bachelor of Science degree with a major in botany. In September of that year, he began graduate studies under the supervision of Dr. B. S. Meyer of the Ohio State University, Columbus, Ohio. In July, 1960, he accepted a position as research chemist with the Research and Development Department of the Nitrogen Division of the Allied Chemical Corporation in Hopewell, Virginia. In August, 1960, Mr. Rittenhouse was awarded a Master of Science degree with a major in plant physiology. In August, 1963, he accepted a position as assistant professor of biology at Radford College, Radford, Virginia. He continued his graduate studies at Virginia Polytechnic Institute, Blacksburg, Virginia under the supervision of Dr. M. G. Hale.

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EFFECT OF OXYGEN AND CARBON DIOXIDE TENSIONS ON RELEASE OF SUGARS FROM PEANUT ROOTS UNDER GNOTOBIOTIC CONDITIONS

Richard L. Rittenhouse

ABSTRACT

The objectives of the present investigation were: (1) to explore an area, root exudation of peanuts, in which no previous work has been reported; (2) to study the effects of O_2 and CO_2 tensions, both individually and combined, on peanut root exudation; and (3) to determine quantitatively the sugars exuded from the roots of peanut plants grown under gnotobiotic conditions.

Seed from the peanut plant, Arachis hypogaea, L. var. NC-2 was used. Peanut embryos, with the cotyledons removed, were surface sterilized; placed aseptically into sterile plexiglass isolator chambers; and planted in vermiculite saturated with Hoagland and Arnon nutrient solution contained within transplanting tubes. Because of slow embryo and seedling growth without the cotyledons, plants were not transplanted into 500 ml flasks until after 90 days.

Three separate experiments were conducted. In all three, N_2 gas was metered to the roots of six plants in each of two isolator chambers. In addition, in the first study, roots of six plants in each of two isolator chambers received a gas mixture composed of 21% O_2, 10% CO_2, and 69% N_2; in the second study, they received a gas mixture composed

of 21% O_2 and 79% N_2; and in the third study, they received a gas mixture composed of 10% CO_2 and 90% N_2.

Duration of each experiment was 6 wks. Samples consisting of nutrient solution containing peanut root exudates from three plants were collected every 2 wks. Peanut plants were harvested and fresh weights, dry weights, and lengths of both shoots and roots (separate) were obtained.

Root exudate analysis consisted of filtration to remove insoluble materials, flash evaporation to reduce the volume, salt removal by cation and anion exchange resins, freeze-drying, thin-layer chromatography to separate and identify the various sugars, and densitometry to obtain the quantity of each sugar.

Analysis of variance showed for all adjusted harvest values no significant difference as a result of treatment or replication at the 5% level. For all measured growth parameters, Duncan's multiple range test showed no significant differences within replicates caused by treatment. Five sugars, galactose; mannose; xylose; ribose; and dihydroxyacetone, were identified in peanut root exudates. Galactose was present in all 72 root exudate samples, dihydroxyacetone in 69, ribose in 33, mannose in 8, and xylose in 5. Analysis of variance showed a significant difference at the 20% level in the total amount of galactose exuded as a result of treatment and a significant difference at the 25% level in the amount of galactose exuded as a result of plant age. These results are indicative of consistent trends which need further investigation. Duncan's multiple range test showed an interaction

between treatment and time which was significant at the 5% level. Young plants (2 wks old) showed a significantly greater amount of galactose exuded under aerobic conditions. As the plants matured (4 and 6 wks old) there was a trend toward greater amounts of galactose exuded under anaerobic conditions than under aerobic. Analysis of variance showed no significant differences at the 25% level in the total amounts of dihydroxyacetone exuded as a result of treatment and no significant difference at the 25% level in the amounts of dihydroxyacetone exuded as a result of plant age. These results are indicative of consistent trends which need further investigation. Duncan's multiple range test showed in young plants (2 wks old) a significantly greater amount of dihydroxyacetone exuded under aerobic conditions at the 5% level. As the plants matured (4 and 6 wks old) there was a trend toward greater amounts of dihydroxyacetone exuded under anaerobic than under aerobic conditions.