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FIXATION, TRANSLOCATION AND ROOT EXUDATION OF ^{14}C-LABELLED ASSIMILATES BY TWO GENOTYPES OF PHASEOLUS VULGARIS L., SUBJECTED TO ROOT ANAEROBIOSIS

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FIXATION, TRANSLOCATION AND ROOT EXUDATION OF ^{14}C-LABELLED ASSIMILATES BY TWO GENOTYPES OF *PHASEOLUS VULGARIS* L., SUBJECTED TO ROOT ANAEROBIOSIS

By

Martha Mary Shadan

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ABSTRACT

FIXATION, TRANSLOCATION AND ROOT
EXUDATION OF ^{14}C- LABELLED ASSIMILATES
BY TWO GENOTYPES OF PHASEOLUS VULGARIS L.
SUBJECTED TO ROOT ANAEROBIOSIS

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A study was conducted to investigate the effects of short term anaerobic stresses on total root exudation, ethanol accumulation and CO_2 respiration of two dry bean varieties under axenic conditions. Roots were grown in glass mist chambers and shoots were continuously fed $^{14}CO_2$ for 11 days. Stressed plants received two 48 hour anaerobic treatments of 20% CO_2 and N_2. Roots of anaerobic Seafarer and San Fernando respired 2.5 and 2.0 times more CO_2 per unit dry weight root than the aerobic controls. Ethanol was not detected for aerobic controls but accumulated in root assimilates in response to anaerobiosis with a greater accumulation for Seafarer than San Fernando. Of the ^{14}C fixed, 35% and 25% more assimilates were detected for Seafarer and San Fernando, respectively, under anaerobic than aerobic conditions. The exudation potentials, which included ^{14}C exuded and $^{14}CO_2$ respired, for Seafarer and San Fernando, increased under anaerobiosis and were approximately 1.8 and 1.4 times greater than their aerobic controls.

TO GERRY

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INTRODUCTION

An ever increasing world population has led to a greater demand for increased food production and increased tillage practices have followed. However, soil compaction resulting from poor soil structure and promoted by increased tillage practices has become one of the most limiting components of crop yield.

Soil compaction and the resulting increases in soil density decrease the diffusion of soil gases. Greater soil density reduces the oxygen supplying capacity adjacent to plant roots resulting in unfavorable conditions for root growth because of both increased soil resistance and decreased gas exchange (Tackett and Pearson, 1964).

Plant roots consume large quantities of oxygen and at 25C may consume 9 times their volume of oxygen gas each day (Greenwood, 1969). Under oxygen stress, aerobic respiration and the production of plant energy are reduced while anaerobic respiration increases. Anaerobic respiration converts pyruvic acid formed during glycolysis into ethanol which has a toxic effect on plant growth (Grineva, 1963). Soil aeration is also necessary for the active absorption of water and minerals by roots.

Recent evidence suggests that soil compaction and the concomitant periods of oxygen stress could increase root exudation of plants (Grineva 1961, Smucker 1971, Barber and Gunn 1974). It also appears that a large quantity of photosynthates produced by legumes may be

lost through root respiration and exudation, resulting in both reduced plant growth and yields. Preliminary studies of dry beans suggests that soil aeration stress promotes root exudation and that the loss of photosynthates through root exudation may differ among genotypes.

Correlations have been found between root excretions and colonization of the root surface by microorganisms (Rovira 1956, Rovira 1965a and 1965b, Vancura and Hovadick 1965a). Root exudation appears to influence the absorption of inorganic nutrients by plants (Barber, 1968). Although much work has been done on the composition of root exudates little is known of the total quantity which is exuded by dry bean roots and the differences in the total exudation losses among varieties.

This study was designed to quantitatively measure the influence of short periods of anaerobiosis upon photosynthate losses by roots of Phaseolus vulgaris, L. varieties Seafarer and San Fernando. Objectives of this study were:

1. To develop axenic conditions for the growth of dry bean seedlings in solution and mist cultures.

2. To develop a system which would allow shoots, completely sealed from their roots, to receive $^{14}CO_2$ gas. This system would provide a method for sampling the sterile nutrient solution, which continuously washed the roots, for root exudation of carbon-14 assimilates.

3. To determine the effect of short term aeration stresses on total root exudation, ethanol accumulation and CO_2 respiration.

4. To determine the difference in root exudation between two dry bean varieties.

LITERATURE REVIEW

Exudation by Roots

Root exudation, the release of organic compounds from the roots of intact plants, is affected by many factors. Soil aeration as well as soil moisture (Katznelson et al. 1954 and 1955), light (Rovira, 1959), temperature (Husain and McKeen 1963, Rovira 1959, Rovira 1969, Schroth et al. 1966, Vancura 1967), plant species (Vancura and Hovadik 1965, Rovira 1969), age of plant (Rovira 1969, Shay and Hale 1973, Lespinat et al. 1975) and plant nutrition (Rovira, 1969) affect root exudation.

Root exudation appears to be increased by soil anaerobiosis. Plants grown under anaerobic conditions were found to accumulate significant quantities of amino acids in their leaves and roots (Grineva, 1961). Anaerobic conditions enhanced the exudation of these accumulated metabolites as well as sugars and organic acids. Excised roots of barley lost organic acids, amino acids, K^+ and Cl^- during the first fifteen minutes of anaerobic stress. Anaerobic conditions caused organic and amino acids to leak from the roots to the bathing medium (Hiatt and Lowe, 1967). Rittenhouse and Hale (1971) also found that anaerobiosis increased exudation by peanut roots.

Alternate drying and wetting cycles of soybean, tomato and wheat plants resulted in the greater liberation of amino acids from the roots into the nutrient solution (Katznelson et al., 1954). Rovira (1959) found that the light intensity at which plants grew affected the amount as well as the type of root exudates. Clover grown at high

light intensity exuded more serine, glutamic acid and α-alanine than plants grown under 60% shade. Tomatoes produced lower levels of aspartic acid, glutamic acids, phenylalanine and leucine in the root exudates.

The temperature at which plants are grown also appears to affect both the composition and quantity of exudation. Considerable amounts of amino acids, alanine, serine, glutamine, glycine and threonine were present in exudates from strawberry plants grown at 5C and 10C but were absent from strawberry plants grown at 20C and 30C (Husain and McKeen, 1963). This increase in root exudation under lower temperatures could be explained by the effect of temperature on membrane permeability and cellular metabolism. It has been postulated by Hale et al. (1971) that lower temperatures reduce metabolic energy causing assimilates to leak out of cells. However, exudation of sugars and amino acids from germinating seeds of maize and cucumber increased with increasing temperature, but the exudation of maltose and fructose was lower at 28C than at 19C (Vancura, 1967). The amount of exudation for germinating seeds of cotton and bean remained constant from 15C to 33C but increased at 37C (Schroth et al. 1966). Rovira (1959) found that higher temperatures appeared to increase the amounts of amino acids exuded by tomato and clover roots.

Qualitative and quantitative differences in exudates can sometimes be found to correlate with the age of the plant. The roots of older maize plants were observed to excrete more labelled carbon than those of younger plants (Lespinat et al., 1975). In contrast, root exudation from tomato, subterranean clover, and Phalaris was reported to be greater during the first two weeks of growth than in

in the second two-week period (Rovira, 1959). Vancura and Hovadik (1965b) found that tomato and red pepper exuded tyrosine at fruiting and cucumber exuded β-pyrazolylalanine only at the early seedling stage. Vancura and Hovadik (1965a) found that amino acid excretion by cucumber roots increased in parallel with growth while in late fruiting phase it clearly diminished. They found three phases of qualitative changes in root excretion during the growth of a cucumber plant. The first occurred during the transition from seed and cotyledon nutrition to primarily photosynthetic nutrition, the second during flowering and the third during late fruiting. Hamlen et al. (1972) found a decreased concentration of total neutral carbohydrates released with increasing plant age.

Nutrient levels have been found to influence the exudation by plant roots. Four times more sugar was exuded by peanut roots at 10 mg Ca^+ than at 50 mg of Ca^+ per liter (Shay and Hale, 1973). Shay and Hale suggest that the low levels of Ca^+ increased root cell membrane permeability. Bowen (1969) found that phosphate-deficient seedlings of Pinus radiata exuded more amino acids than control plants. He postulated that increased exudation from phosphate-deficient plants might be the result of doubling amino-nitrogen in the roots of the seedlings rather than an increase in permeability.

The loss of organic metabolites from roots occurs primarily at the growing apex, regions of elongation and root hair formation (Street, 1966). The region directly behind the root tip in healthy plants is considered to be the major source of excreted substances (Pearson and Parkinson, 1961). They also found that injury or stress caused small quantities of substances to be released from any region of the

root. Van Egeraat (1975a) found that the tips of both the main and lateral roots of young peas were important sites of exudation. He also demonstrated that ninhydrin positive compounds were released from the roots during the formation of lateral roots, a process which damages the main root. McDougall and Rovira (1970) placed wheat plants labelled with carbon-14 between moist sheets of filter paper and found that exudation determined by spots of high radio-activity occurred either from the root apices or from the points of rupture where lateral roots emerged from the main root. Ayers and Thornton (1968) suggested that root damage caused by experimental methods may be responsible for a large proportion of organic materials released by plant roots. However, there is evidence from other experiments using nutrient solutions as the growth medium that organic substances are lost from undamaged roots (Boulter et al. 1966, Hale 1969, Slankis et al. 1964).

The cultural conditions for roots is very important during exudation studies. McDougall and Rovira (1965) found that 0.1% of carbon-14 assimilated by wheat plants was exuded by the roots into the nutrient solution in which they were growing. But if the roots were immersed in distilled water rather than plant nutrient solution during the course of the experiment, the amount of exudate was increased. Amino acids liberated into the culture medium by the roots of pea seedlings were found to be much greater in sand compared to liquid culture medium (Boulter et al., 1966). Cereal plants grown under sterile conditions in solution culture were found to exude more when their roots grew between glass ballotini than unrestricted (Barber and Gunn, 1974). Martin and Barber (1974) found 6.1% of the carbon-14

assimilated by wheat roots was exuded under sterile conditions. However, more was exuded under nonsterile conditions, suggesting a stimulative effect by soil organisms on the loss of carbon by roots.

Vancura (1965a) stated that during plant development, root exudates together with other factors determine the size and composition of the bacterial population in the rhizosphere. Consequently, these losses affect the life processes of the plant, promote the susceptibility or resistance of the plant to pathogenic organisms and possibly influence the relationships between Rhizobia or between saprophytic fungi and plants. Several workers have observed these correlations between root excretions and the colonization of root surfaces by microorganisms (Rovira 1956, Roviera 1965a, Vancura 1965). Currier and Strobel (1976) demonstrated that Rhizobium spp. are attracted to root exudates of both legumes and non-legumes. However, Van Egeraat (1975a) found that the roots of young pea plants, in addition to growth-stimulating substances, exude inhibiting compounds. These exuded materials, whether growth stimulating or inhibitory, are important in the establishment and maintenance of the rhizosphere population of young plants.

Ethanol Accumulation

Ethanol and other reduced metabolites (e.g. DPNH) replace oxygen as the final electron acceptor during anaerobic metabolism. The pyruvic acid formed from the anaerobic decomposition of sugars is decarboxylated forming CO_2 and acetaldehyde. Acetaldehyde is reduced further leading to the formation of ethanol. The accumulation of ethanol in plants under anaerobic conditions makes it an indicator

of the extent of damage caused by anaerobic conditions (Fulton and Erickson, 1964). There are many cases where ethanol has been found to accumulate in plants under such stress conditions (Kenefick 1962, Grineva 1963, Bolton and Erickson 1970, Leblova et al. 1969). Under anaerobic conditions, ethanol production by excised apical root tissues was five times greater than for non-stressed plants (John and Greenway, 1976). Ethanol was not found to be produced under aerobic conditions with either the stressed or non-stressed plants. The use of alcohol dehydrogenase (ADH) activity as a test for flooding tolerance is a practical possibility. Prolonged flooding was found to increase the acvitity of ADH more in roots of flooding sensitive cultivars than in roots of flooding tolerant cultivars (Crawford 1967, Crawford and McManmon 1968, Francis et al. 1974, John and Greenway 1976). Under anaerobic conditions it has been found that alcohol accumulates in the roots of corn, sunflower and peas, which is excreted into the external environment (Grineva 1963, Smucker and Erickson 1976).

Physiology of the Root Under Anaerobiosis

Oxygen is the final electron acceptor in respiration. If oxygen is limiting, electron transfer and respiration are limited and fermentation occurs. Anaerobic respiration of one mole of glucose results in the production of 2 moles each of CO_2 and ethanol with the release of 54 kcal per mole of glucose. Aerobic respiration yields 686 kcal per mole of glucose and 6 moles of CO_2 and H_2O (Grable, 1966). Therefore, fermentation produces only a fraction of the useable energy produced by respiration. Fulton and Erickson (1964) showed that

flooding of tomato roots quickly inhibited respiration and metabolism in all plant parts and inhibited the Krebs citric acid cycle in roots.

Respiration in plants is necessary for mineral and water uptake and the growth and maintenance of plants. Many workers have shown that poor soil aeration will inhibit root growth and elongation (Gill and Miller 1956, Kramer 1965, Geisler 1965). Letey et al. (1962) found that shoot as well as root growth was reduced under low oxygen conditions at early stages of development. Oxygen levels of 1% to 10% caused an 80% reduction in the rate of cell division of Vicia faba after a 24-hour treatment (Williamson, 1968). Anaerobiosis has also been found to inhibit water uptake of tobacco roots by 50% or more (Willy, 1970). Inadequate aeration appears to reduce the absorption of water by reducing the size of the root system which would limit the surface area for absorption of water. Low oxygen also decreases the permeability of roots to water (Glinka and Reinhold, 1962). Root morphology and physiology appear to be modified by anaerobiosis. Root hair development may be suppressed decreasing nutrient uptake (Daubenmire, 1959). When root penetration is restricted, lateral roots occur (Greenwood, 1969). Roots growing in deficient oxygen conditions are usually shorter, thicker, more branched and have fewer root hairs than those which are sufficiently aerated (Kramer, 1965).

MATERIALS AND METHODS

Seed Sterilization and Germination

Phaseolus vulgaris L., varieties Seafarer and San Fernando, were used in this experiment. Seeds were surface sterilized by dual sterile soakings as follows. Seeds of each variety, 150, were selected and washed in Alconox and water and rinsed six times with tap water. The seeds were then surface sterilized in 100 ml of a solution of 1% sodium hypochlorite containing two drops Tween 20, for 5 minutes in a 200 ml Erlenmeyer flask. The seed-slurry was thoroughly agitated, by hand, during the sterilization period. Seeds were rinsed six times with sterile distilled water and then allowed to soak for 4 minutes in sterile distilled water. The water was replaced by 100 ml of 0.54% sodium hypochlorite, seeds were shaken by hand for 2 minutes and then rinsed six times with sterile distilled water. Sodium hypochlorite concentration was reduced because of increased penetration into the seed during the second soaking period resulting in a reduction of viable seeds. With this surface sterilization technique, 98% San Fernando seeds germinated with 50% of these seeds aseptic. For Seafarer, 60% of the seeds germinated of which 90% were aseptic.

Under a sterile Environmental Air lamina flow transfer hood, five seeds were placed in a petri dish containing 2 sheets of Whatman #1 filter paper and 6.0 ml distilled water. A correlation between germination of Seafarer and water added to each petri plate was

determined. The percentage of seeds germinated ranged from 35.0% when 5 ml of water was added to each dish to a maximum of 60.0% germination when 6 ml were added to the petri dishes. San Fernando was more tolerant of the amount of water added than Seafarer, and germination remained constant at all water levels. The petri dish, two sheets of Whatman #1 filter paper, and the water were sterilized by autoclaving and allowed to cool before transfer of sterilized seeds. The glass petri plate cover was secured in place with masking tape. Seeds were germinated in an incubator at 27C for 2 days. Selected germinated seeds were transferred, under sterile conditions, to a sterilized vial (Figure 1) with forceps, sterilized in 70% ethanol and flamed. Seeds remaining in each plate were cultured on Potato Dextrose Agar (PDA), pH of 5.6, and Beef Lactose Agar (BLA), pH of 6.8, to detect surface contamination. Aliquots of 0.5 ml of the nutrient solution was removed from each vial with a syringe, employing sterile techniques, 1 day after seed transfer into the vials. The solution samples were cultured on PDA and BLA to detect surface contamination.

Seedling Culture and Transplanting

Glass storage vials, 25 ml, each fitted with a ground-glass plant port supported sterile seedlings for the first 4 days of growth (Figure 1). A modified Hoagland nutrient solution, 23 ml of 1/2 strength, was added to each glass storage vial. Vials were wrapped in aluminum foil to prohibit light penetration. A ground glass plant port, 18 X 20 mm, the base of which contained a small opening approximately 6 mm in diameter, and a thin section of polyurethane

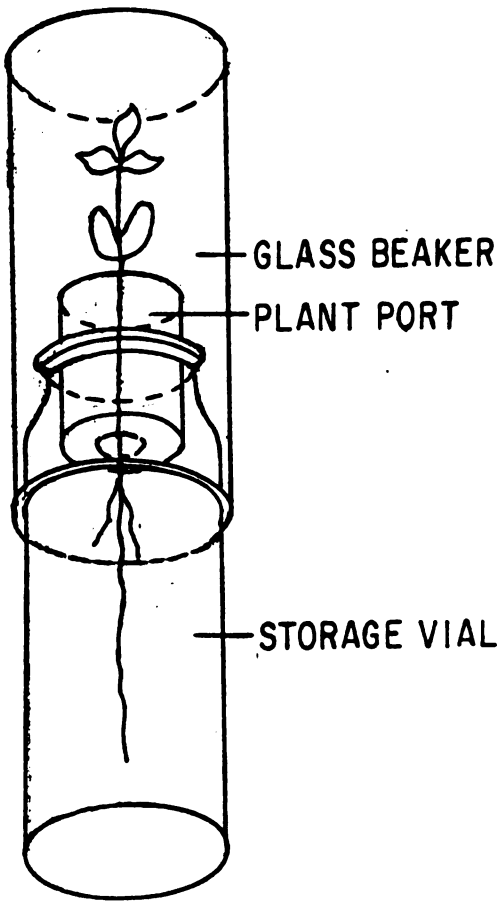

Figure 1. Diagram of Glass Culture Vial for growing sterile seedlings.

stopper was fitted onto each vial. The stopper was provided to support the hypocotyl in early growth stages. To ensure sterility, a 10 ml pyrex glass beaker was secured over the top of each vial with a 2 X 6 cm strip of aluminum foil. The vial, filled with nutrient solution, glass plant port, stopper and beaker were autoclaved for 30 minutes at 15 lbs pressure and 121C and allowed to cool before sterile transfer of terminated seeds. Seedlings were grown in vials for four days after which they were transferred from the vials to the sterilized glass root chambers. Each plant port was filled with sterilized parawax, a 1:8 g/g mixture of hard paraffin and petroleum jelly. The parawax provided a medium which enabled the seedling to expand during growth but prevented gaseous exchange and contamination by microorganisms.

Root and Shoot Chambers

Sixteen plants were grown in modified pyrex glass mist chambers designed by Smucker and Erickson (1976). The brass nozzles were replaced by 0.94 cm stainless steel nozzles attached to the glass access tube of the reservoir with quick-drying epoxy cement. The inline cellulose ester filter, used to filter circulating solution for plant debris which would clog the brass nozzles, was removed. The larger orifice of the stainless steel nozzles allowed flow through the nozzle despite accumulation of plant debris in the nutrient solution. This system was especially advantageous for the study of root responses to changes in rhizosphere gaseous composition. The entire system could be autoclaved, and aseptic conditions maintained throughout the experiment.

Each glass reservoir was filled with 200 ml of a sterilized 1/2 strength modified Hoagland's nutrient solution. The reservoir was connected to the root chamber with a ground glass joint. A plexiglas shoot chamber, 20.6 cm in length and 11.5 cm in diameter, with a wall thickness of 1.3 cm, was fitted over each pyrex mist chamber and secured in place with modelling clay. A strip of foam rubber weatherstriping, 1 X 32 cm, was secured to the base of each plexiglas chamber using Dupont Duco cement. The weatherstripping along with modelling clay created an air tight seal at the junction of plexiglas shoot and glass mist chambers. Gas inlets and outlets each with a 5 mm orifice, were located on opposite sides of the plexiglas chamber, 3 cm from the top and bottom. A thermometer extending halfway into the plexiglas chamber, sealed by a swage fitting, was located approximately 5 cm below the gas inlet. Additional features of the sterile plant chamber system may be observed in Figure 2. After the condensor and condensate separator were attached, the system was carefully transferred to the growth chamber approximately 15 minutes after transfer of seedlings from the glass growth vials to the pyrex mist chamber. A variable speed Gilson minipuls pump continuously recirculated the sterile nutrient solution at a flow rate of 25 ml min^{-1}. Gas flowing into the root chamber through the gas inlet was sterilized by a Millipore filter having a mean pore size of 0.2u. Flow through the gas inlet of each chamber was 30 ml $min^{-1} \pm 5$ ml min^{-1}. Plants were grown in the mist chambers for 13 days.

Figure 2. An overview of the aseptic mist root chamber and plastic shoot chamber system for measuring the losses of ^{14}C-labelled assimilates by dry bean plants.

Environmental Conditions

The environmental conditions were controlled by a Warren-Shearer model CEL-36-10 growth chamber. The light source in the growth chamber consisting of eight 100-watt incandescent bulbs and twelve 39-watt General Electric High Output Warm White fluorescent bulbs produced 800 uEinsteins $sec.^{-1}$ m^{-2} for full light at the plant surface as measured by a Lambda LI-170 quantum/radiometer/photometer. The photoperiod was 16 hours consisting of 14 hours of full light preceded and followed by 1 hour of incandescent light only. The growth chamber was 70% relative humidity while the humidity in the sealed plexiglas shoot chambers was 100% as measured by a Bacharach Industrial relative humidity detector. Temperatures were 18C during the dark period and 21C during the light period in the growth chamber. However, due to heat absorption by the plexiglas shoot chambers, temperature within these chambers was approximately 22C during the dark period and 26C during the light period.

Measurement of Carbon Dioxide

Carbon dioxide concentrations of the root and shoot chambers were monitored by the rapid method of CO_2 analysis described by Clegg et al. (1978). The method involved injecting a 10 ml or less gas sample with a 10 ml syringe through a modified glass tube fitted with a rubber septum into the flowing carrier gas that passed through the infrared analyzer. The flow rate of the nitrogen carrier gas was maintained at 600 ml min^{-1}. The gas sample passed through a 1.5 cm X 11 cm tube of Hammond drierite before entering the analyzer to remove any moisture in the sample. A strip chart recorder,

Linear Instruments Corporation Model 261, was attached to the infrared analyzer so that peak response was obtained which was proportional to the carbon dioxide concentration. The sample and reference outlets were each connected to glass tubes containing 50 ml ascarite which was used to trap $^{14}CO_2$ contained in the gas sample.

Root Gas Treatments

Experiments were duplicated and repeated twice. Two of the root chambers received two 48 hour stress treatments of 20% carbon dioxide with the balance of nitrogen. The two control chambers at all times received air delivered by bottled gas with a CO_2 concentration of 340 ppm. The first 48 hour stress treatment was introduced 3 days after transplanting and again on day 8. Between the two stress treatments, the two root chambers received air from the same gas source as the aerobic controls. To maximize air displacement by the treatment gas at the beginning of each stress period, flow of the treatment gas was introduced into each of the two root chambers at the rate of 275 ml min^{-1} for 20 minutes. At the end of each 48 hour stress period, air flow into each treatment root chamber was increased to 275 ml min^{-1} for 20 minutes before returning to the normal flow of 30 ml $min^{-1} \pm 5$ ml min^{-1}. Gas displacement rate in the root chambers was determined by Smucker and Erickson (1976). Gas flow through the control root chambers was maintained at 30 ml $min^{-1} \pm 5$ ml min^{-1} throughout the experiment.

Radioisotope Feeding of Shoots

A diagram of carbon dioxide flow to the shoots is shown in Figure 3. On day 3, the four plexiglas shoot chambers were connected with a series of t-tubes and tygon tubing, 0.63 cm I.D., to a 20 liter polypropylene carbon (A) which served as a gas mixing vessel. A small electric fan (B) attached to the bottom of the carboy by epoxy glue, mixed the $^{14}CO_2$, having a specific activity of 5.47 millicuries per 137 liters, with air in the carboy. The $^{14}CO_2$ was added through injection port (C). Injection and sample ports were modified glass t-tubes fitted with a rubber septum.

At 0800 hours on day 3, all carbon dioxide was removed from the carboy by pumping air in the carboy through bypass (D) and through a tube of ascarite (E). A diaphram pump (F) replaced peristalic pump (G) during carbon dioxide removal from the carboy. Carbon dioxide was depleted to less than 5 ppm within 10 minutes. Air was pumped from the carboy and through the ascarite tube at a rate of 10 liters min^{-1}. Gas samples were removed through port (H) and injected into the infrared gas analyzer to determine carbon dioxide concentration in the carboy. When carbon dioxide concentrations were less than 5 ppm, the minimum detection limit, the ascarite bypass was closed. A 10 ml sample of $^{14}CO_2$ was removed from the bottled radioactive gas and injected into port (C) to bring the concentration in the carboy to 340 ppm CO_2. The gas was recycled through the carboy for 5 minutes to allow sufficient time for thorough mixing. Gas samples were removed through port (C) and injected into the infrared analyzer to monitor CO_2 concentration in the carboy throughout the experiment.

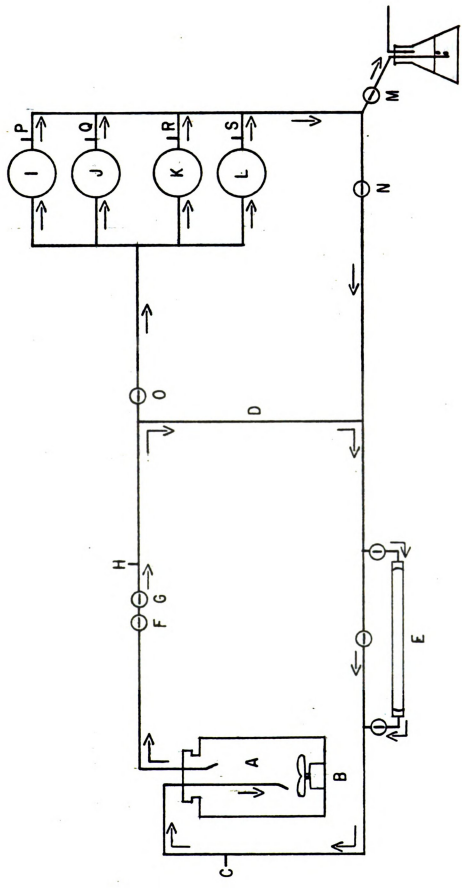

Figure 3. Diagramatic representation of system for carbon-14 dioxide flow through root chambers.

When the desired $^{14}CO_2$ concentration had been reached in the carboy, the diaphram pump was replaced with the variable speed peristalic pump (G) which was used for the duration of the experiment. Line (D) was once again closed and gas was pumped from the carboy at a rate of 130 ml min^{-1} $\pm$ 8 ml min^{-1} into the four plexiglas shoot chambers (I-L) and back into the carboy giving a distributed rate of 33 ml min^{-1} per chamber. Several times per day gas flow rates into the four chambers was determined by redirecting flow through line (M) which was connected to a flow meter and closing flow into the carboy at valve (N). The flow meter was attached to an erlenmeyer flask containing the solution of ethanolamine and methanol used to trap $^{14}CO_2$ lost during flow rate determination. This measurement took approximately 30 seconds, line (M) was closed and valve (N) opened to resume flow into the carboy.

Gases circulating through the shoot chambers were sampled several times daily and the CO_2 concentration monitored. When the CO_2 concentration approached 340 ppm, valves (O and N) were closed, line (D) opened, and gas in the carboy was recycled by passing the four shoot chambers. Between 5 and 10 ml $^{14}CO_2$ were injected into port (C) to bring the CO_2 concentration in the carboy to 340 ppm. The gas was recycled within the carboy for 2-3 minutes to ensure thorough mixing of the added gas. At the end of this time, a 10 ml gas sample was removed through port (H) and injected into the infrared analyzer. When a gas equilibrium was reached, line (D) was closed and valves (O and N) opened. This procedure was followed whenever injection of $^{14}CO_2$ was required; the time and amounts being quite variable. Gas samples, 10 ml each, were removed through ports (P-S) and injected into

the infrared analyzer to determine the rate of carbon dioxide used by the shoots in each chamber at 0800, 1200, 1600, 2000, and 2300 hours on day 1 through day 11.

Measurement of Root Respiration

Carbon dioxide respired by the roots for all treatments was measured every 4 hours during the light period from day 1 through day 11 using the following procedure. Tygon tubing; 0.63 cm I.D., approximately 76 cm in length, each containing a sampling port, were attached to the four glass condensate separators. The four tygon lines were connected to two 500 ml erlenmeyer flasks each containing 300 ml of the solution of ethanolamine and methanol, used to trap any $^{14}CO_2$ respired by the roots (Kobaysashi and Maudsley, 1969). Gas samples, 10 ml each, were removed from the sampling ports and injected into the analyzer to determine carbon dioxide respired by the roots at 0800, 1200, 1600, 2000 and 2300 hours. Also, during these sampling periods, the four tygon tubes were disconnected from the two 500 ml erlenmeyer flasks and each was attached to one 20 ml erlenmeyer flask containing the solution of ethanolomine and methanol. Gas-respired from the roots of each root chamber flowed into the appropriate flask for 15 minutes after which the four tygon lines were reconnected to the larger, 500 ml, erlenmeyer flasks. Solution, 1 ml aliquots, was sampled from the 20 ml erlenmeyer flasks and together with 9 mls of Beckman Ready-Solv Ep Scintillation fluid, was added to scintillation vials. The vials were then shaken for 30 minutes on a Forma Scientific model 2563 shaker. Carbon-14 activities were determined by counting each vial in a Beckman 8100 System Scintillation Counter for 10 minutes. The

remaining solution in the 20 ml flasks was replaced with fresh solution every sampling period.

Measurement of Root Exudates

At 0800, 1200, 1600, 2000, and 2300 hours on day 1 through day 11, 2 ml samples were extracted from each reservoir through the access port by a 3 ml syringe and needle. Each access port was fitted with a rubber septum and covered with aluminum foil to avoid contamination. The aluminum foil was lifted only during sampling. Of the extracted sample, 1 ml was immediately injected into 3 ml glass vacutainer tubes and frozen for analysis of ethanol using a Beckman GC 72-5 gas chromatograph. The remaining sample was added with 9 ml Beckman Ready-Solv EP scintillation fluid to a scintillation vial. The vials were shaken for 30 minutes to ensure proper mixing of sample and scintillation fluid. Carbon-14 activity was determined using a Beckman Scintillation counter. Each sample was counted once for ten minutes. Every other day, after sampling, modified 1/2 strength, sterilized Hoagland's nutrient solution was injected into each reservoir via the access port to bring the level to 200 ml.

To test for axenic conditions of each reservoir, 0.5 ml samples of the nutrient solution were removed from each reservoir through the access port on days 1, 4, 7, 10 and 11. PDA and BLA were used to test for aseptic conditions.

$^{14}CO_2$ Fixation

On day 12 after initiation of $^{14}CO_2$, the gas mixing carboy was closed and gas from the four shoot chambers was cycled through the

ascarite tube and back into the shoot chambers at a rate of 130 ml min^{-1} $\pm$ 8 ml min^{-1} for approximately 2 hours. This was done to remove carbon-14 in the shoot chambers before the chambers were removed. Gas samples, 10 ml, were extracted periodically during the two hours from port (H) and injected into the infrared analyzer. When carbon dioxide could no longer be detected, plants were removed from the chambers. The shoot and root of each plant was separated, weighed, and dried for 5 days at 70C.

Carbon-14 activity in the shoots and roots was determined by homogenizing the entire dried plant sample with 4 mls of distilled water. Of the resulting homogenate, 3-0.35 ml aliquots were each added to 1.5 ml Beckman BTS-450 and allowed to digest over night. Aliquots of 15 ml of Ready-Solv-EP was added to the digested material. The sample was shaken in the scintillation for 1/2 hour and carbon-14 activity was determined using a Beckman scintillation counter.

A 2 X 2 factorial was used. The main effects were varieties X aeration. Plants were replicated 4 times and physiological measurements (i.e. DPM, ethanol, respiration, etc.) were measured per chamber. Each experiment was run twice.

RESULTS AND DISCUSSION

Plant Growth

Shoot and root growth appeared to be good during the 11 days of treatment. However, plants showed visual symptoms of slight wilting and chlorosis, mainly on the older leaves, after 6 days that persisted throughout the remainder of the experiment. Humidity in the shoot chambers approached saturation 4 days into the experiment. Although concentrations of gases other than CO_2 were not measured, these symptoms may have resulted from the accumulation of oxygen in the shoot chambers as well as the high humidity.

Roots of all treatments appeared healthy during the first 3 days of the experiment and displayed extensive branching (Figure 4A). Aerobically treated roots remained white throughout the experiment as did anaerobic Seafarer roots. San Fernando roots subjected to anaerobic treatments turned dark 2 or 3 days after the initiation of the first 48-hour anaerobic stress and displayed very few white tipped branches upon termination of the experiment (Figure 4B). Fresh and dry weights of shoots and roots for all treatments were not significantly different (Table 1).

Root chambers were monitored after introducing $^{14}CO_2$ on days 1, 4, 7, 10 and 11 for root contamination of bacteria or fungus (Table 2). Sterile conditions were maintained for 80% of the experiments. Aerobic Seafarer control appeared to become contaminated between day 7 and day

Table 1. Fresh and dry weights of Seafarer and San Fernando shoots, roots and entire plants on day 11 of the experiment. Values are an average of 8 plants in two separate experiments.

Treatment	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Total plant fresh weight	Total plant dry weight
			(g/plant)			
Aerobic control						
Seafarer	1.50_{A}	0.13_{A}	0.77_{A}	0.04_{A}	2.20_{AB}	0.17_{AB}
San Fernando	1.66_{A}	0.15_{A}	1.18_{A}	0.05_{A}	2.83_{B}	0.19_{B}
Anaerobic stress						
Seafarer	1.41_{A}	0.13_{A}	0.59_{A}	0.04_{A}	2.00_{AB}	0.16_{AB}
San Fernando	0.84_{A}	0.12_{A}	0.35_{A}	0.04_{A}	1.19_{A}	0.16_{A}

Those values within columns having different subscripts represent statistical difference at the .05 level according to Duncan't Multiple Range Test.

A

B

Figure 4. Root systems displayed in pyrex glass root chambers.
A. Roots of variety Seafarer grown in glass mist chambers.
B. Root system of anaerobically stressed San Fernando plants.

Table 2. Sterile conditions of Seafarer and San Fernando root systems as determined by plating 0.5 ml aliquots of nutrient solutions from mist chambers onto Potato Dextrose Agar (PDA) and Beef Lactose Agar (BLA) for 14 days. Each symbol represents the results of two experiments containing 100-150 seeds each.

	Day 1		Day 4		Day 7		Day 10		Day 11	
Treatment	PDA	BLA	PDA	BLA	PDA	BLA	PDA	BLA	PDA	BLA
Aerobic control										
Seafarer	+	+	+	+	+	+	-	-	-	-
San Fernando	+	+	+	+	+	+	+	+	+	+
Anaerobic stress										
Seafarer	+	+	+	+	+	+	+	+	+	+
San Fernando	+	+	+	+	+	+	+	+	+	+

(+) axenic

(-) contamination by bacteria and fungi

10. Multiple samplings of the mist chamber nutrient solution or addition of nutrient solution could have caused the detected contamination. Microorganisms have been reported to increase the carbon-14 assimilates found outside of the roots (Barber and Martin 1976, Vancura and Hovadick 1965, Martin 1976). However, in this experiment, there was no significant change in carbon-14 assimilates detected in the nutrient solution of anaerobic Seafarer controls between days 7 and 10.

Carbon-14 Activity in Shoot and Root

Total fixation of ^{14}C by dry bean plants was determined by measuring the radioactivities of roots and shoots of Seafarer and San Fernando. Neither anaerobic stress nor variety had a significant effect at the .05 level on total fixation of ^{14}C (Table 3). Aerobic Seafarer contained 41% and 27% more ^{14}C activity per unit dry weight of shoot and root, respectively, than aerobic San Fernando. Anaerobic Seafarer contained 21% more ^{14}C activity per unit dry weight shoot than anaerobic San Fernando. Both anaerobic stressed varieties contained approximately the same amount of ^{14}C activity per unit dry weight of root.

^{14}C activity found per unit dry weight shoot was greater than that contained in a unit dry weight of root for each of the treatments. Between 3 and 5 times more ^{14}C activity was found in the shoots than the roots. Martin (1976) also found a difference in the amount of ^{14}C activity detected in the shoots compared with the roots of the same plant. Rye grass, wheat and clover had 2.4, 4.8, and 5.6 times more ^{14}C activity in the shoots than roots, respectively. It is possible

Table 3. Influence of anaerobic stress and dry bean genotype on the total fixation and translocation of ^{14}C assimilates. Each value represents the ^{14}C activity detected in chambers containing 4 plants.

Treatments	Shoot	Root
	DPM/gram dry weight	
Aerobic controls		
Seafarer	$8,347,461_A$	$2,246,605_A$
San Fernando	$4,927,910_A$	$1,650,979_A$
Anaerobic stress		
Seafarer	$6,859,312_A$	$1,352,632_A$
San Fernando	$5,385,917_A$	$1,533,421_A$

Those values within columns having different subscripts represent statistical difference at the .05 level according to Duncan's Multiple Range Test.

that carbon compounds translocated to the roots were lost by root exudation or respiration. Grineva (1961) found that organic compounds typical of root tissues were excreted by the roots into the growth medium.

Carbon Dioxide Assimilated by Shoots

$^{14}CO_2$ fixed in the light by the shoots of each treatment was measured several times daily throughout the experiment. Both aerobic and anaerobic Seafarer plants fixed more $^{14}CO_2$ than San Fernando on day 11 (Table 4). Seafarer plants of aerobic and anaerobic treatments fixed 80% to 100% and 250% to 550% more $^{14}CO_2$ than San Fernando, respectively. Both aerobic treatments (Figure 5) and anaerobic treatments (Figure 6) experienced a diurnal fluctuation in CO_2 fixed with a decreasing trend. It would be expected that as the plants grew and leaf surface area increased, more carbon dioxide would be fixed per unit leaf area. The deteriorating condition of plants due to a possible oxygen toxicity in the shoot chambers could explain the decrease in CO_2 fixed by the shoots.

Root Respiration

$^{14}CO_2$ evolved from the roots of the 4 treatment plants was determined at 0800, 1200, 1600, 2000, and 2300 hours from day 1 through day 11. Carbon-14 dioxide respired by the roots of aerobic San Fernando plants ranged from 3.5 ul CO_2/plant/minute on day 3 to 1.0 ul CO_2/plant/minute on day 11 (Figure 7). Aerobic Seafarer plants showed a gradual decrease in ^{14}C evolved by its roots, with a range of approximately 2.3 ul/plant/minute on day 1 to less than 0.5 ul/plant/

Table 4. Influence of anaerobic stress and dry bean genotype on the $^{14}CO_2$ assimilated by four 17-day-old dry bean plants. Each value represents the average of 2 experiments.

Treatment	Shoot		Total plant	
	ul CO_2/gram fresh weight/min	ul CO_2/gram dry weight/min	ul CO_2/gram fresh weight/min	ul CO_2/gram dry weight/min
Aerobic controls				
Seafarer	0.39	4.95	0.27	3.63
San Fernando	0.22	2.48	0.15	1.87
Anaerobic stress				
Seafarer	0.45	5.18	0.32	3.9
San Fernando	0.12	0.84	0.08	0.6
LSD .05	.54	6.68	.38	4.72

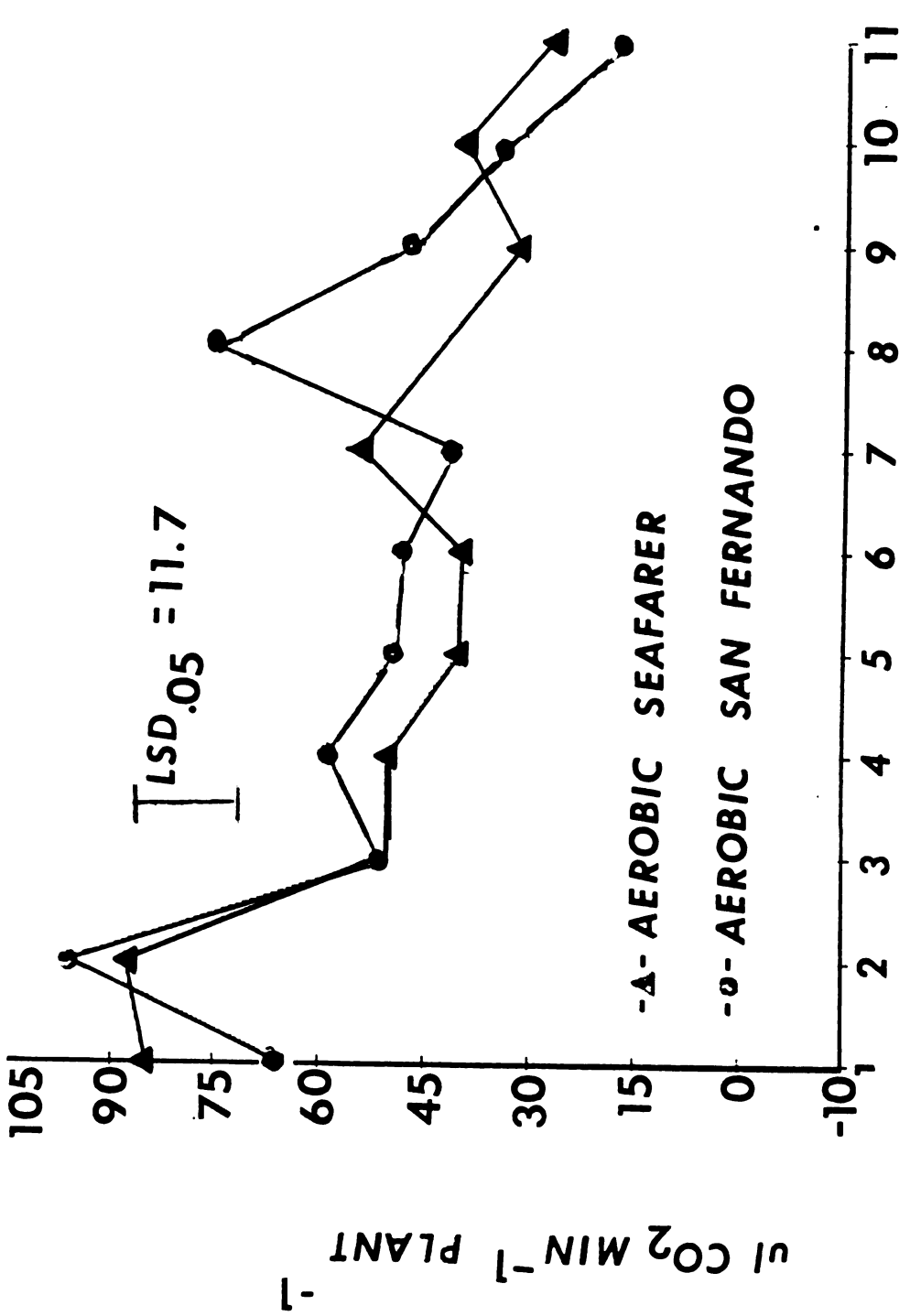

Figure 5. Carbon dioxide assimilated per day for nonstressed plants of Seafarer and San Fernando. Each point represents the average CO_2 concentration for each chamber of four plants taken between 0800 hours and 2300 hours from day 1 through day 11.

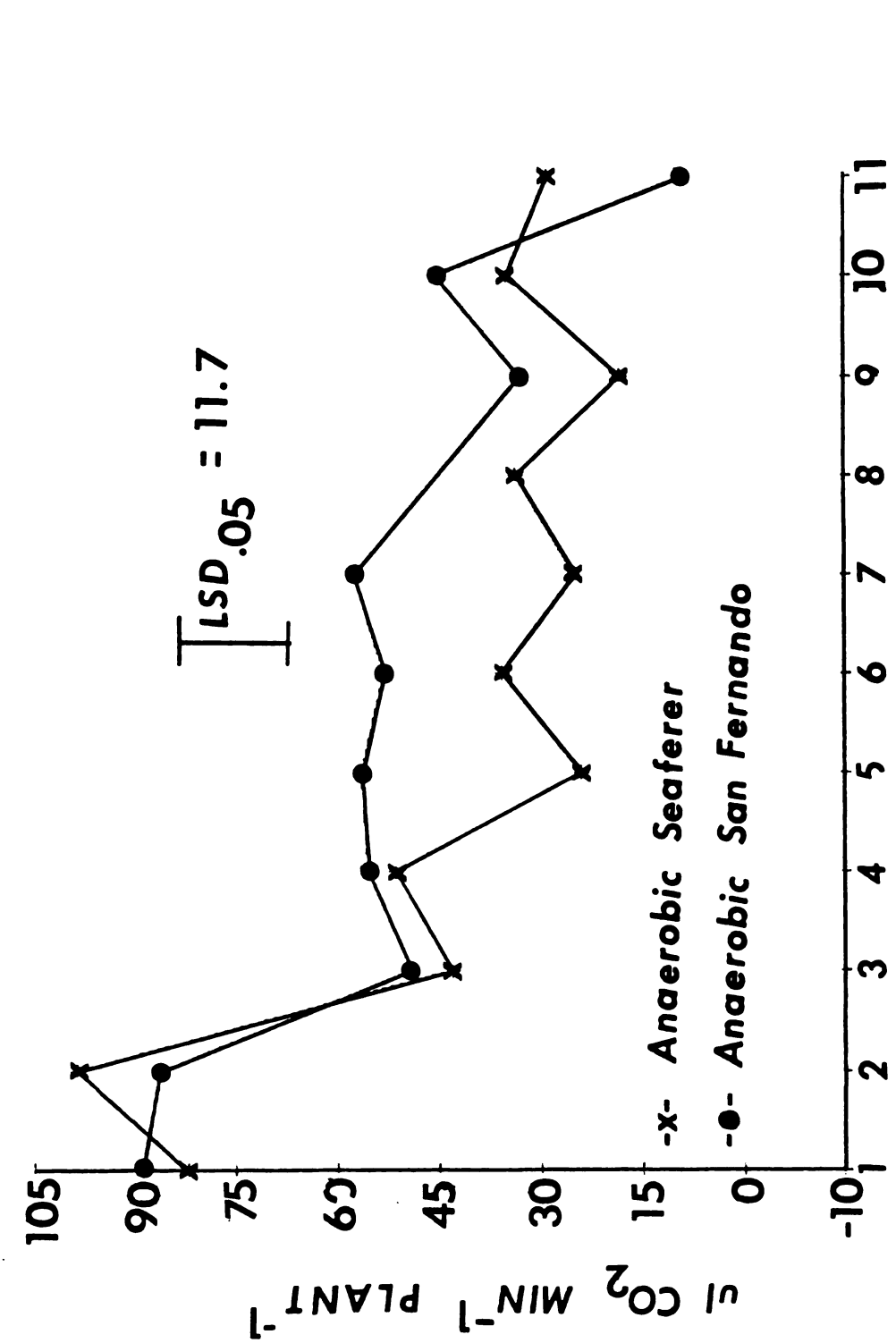

Figure 6. CO_2 assimilated per day for anaerobically stressed plants of Seafarer and San Fernando. Each point represents the average CO_2 concentration for each chamber of four plants taken between 0800 hours and 2300 hours from day 1 through day 11.

minute on day 8. Daily carbon dioxide respired by San Fernando roots was approximately twice that of Seafarer (Figure 7). It is possible that the declining condition of the shoots affected Seafarer more adversely than San Fernando which would explain the decrease in $^{14}CO_2$ evolved throughout the experiment. $^{14}CO_2$ respired by the roots of anaerobic San Fernando ranged between 2.5 ul/plant/minute on day 2 to approximately 3.8 ul/plant/minute on day 10 (Figure 8). Anaerobic Seafarer plants respired between 0.8 ul/plant/minute on day 7 to a high of 3.5 ul/plant/minute on day 10.

On day 11, roots of the variety San Fernando evolved 1.6 times and 1.4 times more $^{14}CO_2$ per unit dry weight root than Seafarer for the aerobic and anaerobic treatments, respectively (Table 5). Anaerobic San Fernando and Seafarer respired approximately 2.0 and 2.5 times more $^{14}CO_2$ per unit dry weight root, respectively, than the aerobic controls. Oxygen stress enhances anaerobic respiration which could account for the increase in $^{14}CO_2$ evolved by the oxygen stressed plants in the experiment. Street and Cockburn (1972) have found that between 1 to 9% oxygen there is a minimum rate of carbon dioxide evolved and below this range, the CO_2 evoluation rises steeply due to glycolytic fermentation.

Carbon-14 Exuded

Total ^{14}C losses by stressed and control roots were determined by taking daily measurements of the ^{14}C accumulation in the nutrient solution. Roots of both Seafarer and San Fernando exuded some ^{14}C-labelled assimilates even under aerobic conditions. However, anaerobic conditions greatly enhanced the root exudation of both varieties

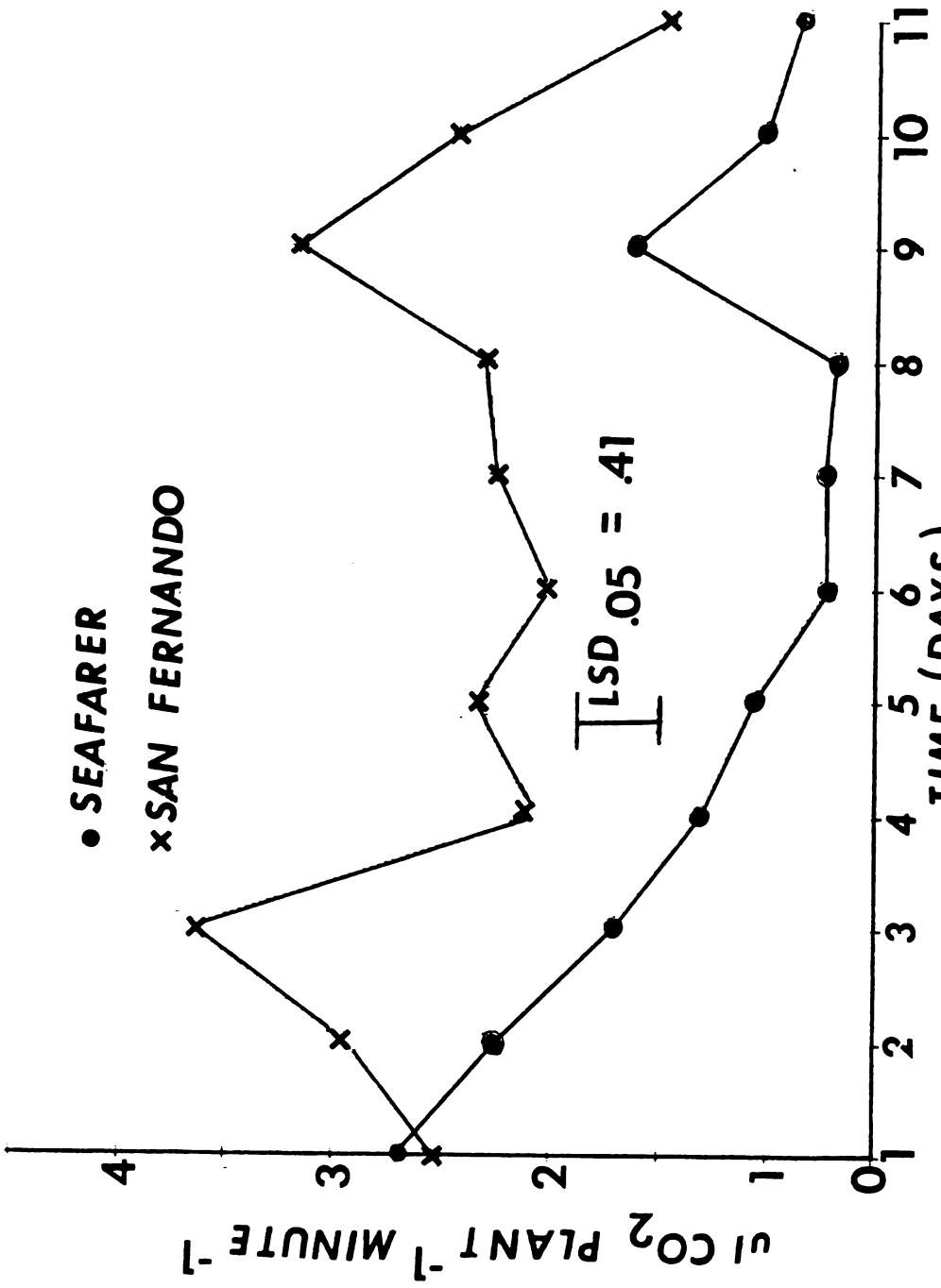

Figure 7. Carbon dioxide respired by individual aerobic Seafarer and San Fernando plants. Each point represents the average CO_2 concentration for each chamber of 4 plants taken between 0800 hours and 2300 hours from day 1 through day 11.

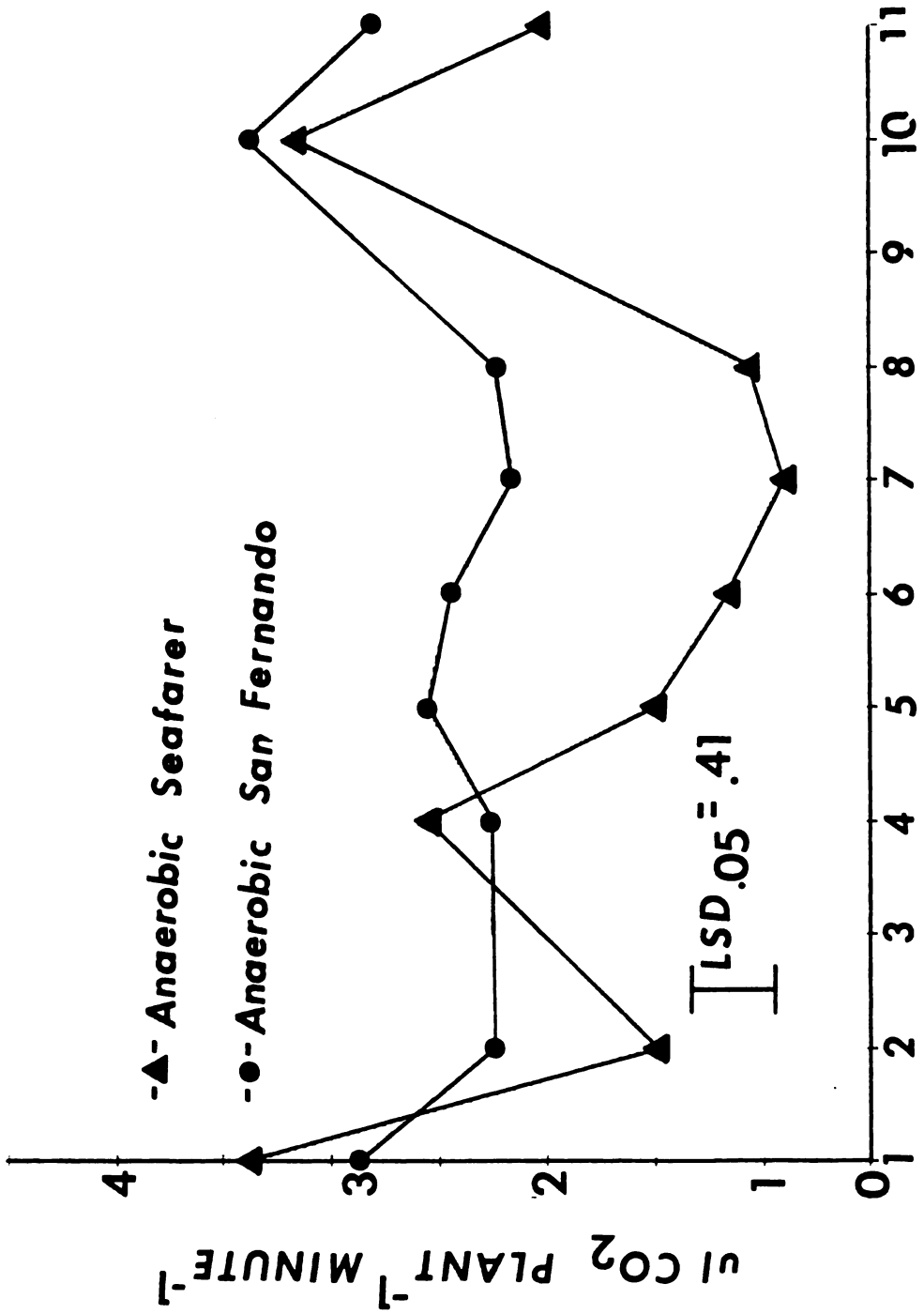

Figure 8. Carbon dioxide respired by individual anaerobic Seafarer and San Fernando plants. Each point represents the average CO_2 concentration for each chamber of 4 plants taken between 0800 hours and 2300 hours from day 1 through day 11.

Table 5. Carbon-14 dioxide evolved by roots of four plants of 17-day-old dry beans. Each value represents the average of two experiments.

Treatment	Root		Total plant	
	ul CO_2/gram fresh weight/min	ul CO_2/gram dry weight/min	ul CO_2/gram fresh weight/min	ul CO_2/gram dry weight/min
Aerobic controls				
Seafarer	0.9	16.32	0.32	3.92
San Fernando	1.67	29.57	0.55	7.38
Anaerobic stress				
Seafarer	2.57	40.12	0.72	8.4
San Fernando	6.60	56.49	1.75	13.33
LSD .05	2.0	25.0	.95	7.89

(Table 6). San Fernando and Seafarer exuded 30 and 19 times more ^{14}C-labelled materials per unit dry weight root, respectively, than the aerobic controls for each variety. These results agree with others who have reported enhanced exudation under oxygen stress (Grineva 1961, Hiatt et al. 1967 and Rittenhouse and Hale 1971).

Under anaerobic conditions, an increase in ^{14}C assimilates, detected in the mist chamber nutrient solution, were found to be directly related to the time of treatment for both Seafarer and San Fernando (Figure 9). As much as 19 X 10^3 disintegrations per minute (DPM) were detected in the nutrient solution during the anaerobic stress for Seafarer and 7 X 10^3 DPM for San Fernando. After cessation of the first 48-hour stress period, between days 2 and 4, ^{14}C activity decreased in the nutrient solution to approximately 7 X 10^3 DPM for anaerobic Seafarer and 1 X 10^3 DPM for anaerobic San Fernando; representing a 2.5 and 7 fold decrease for Seafarer and San Fernando, respectively. The root exudates could have volatilized or were respired off by the roots. Since volatile ^{14}C assimilates may or may not have been trapped by the ethanolamine, there can be no definite conclusions. Another possible explanation is that material exuded during oxygen stress is reabsorbed upon return to aerobic conditions. Cossins and Turner (1959) have shown that previously exuded ethanol was reabsorbed by the root tissue.

Exudation increases during anaerobic conditions appear to be varietal dependent. Anaerobic Seafarer exuded 42% more ^{14}C labelled assimilates than San Fernando. Figure 10 demonstrates the ratio of ^{14}C assimilates which accumulated in the nutrient solution to root respiration throughout the experiment. The ratio for anaerobic

Table 6. Effect of anaerobic stress on the root exudation of 4 plants of dry bean varieties Seafarer and San Fernando. Each value represents the average of two experiments.

Treatment	Final DPM gram fresh root	Final DPM gram dry root	Final DPM gram fresh plant	Final DPM gram dry plant
Aerobic control				
Seafarer	354.5_A	6328.0_A	123.1_A	1708.4_A
San Fernando	114.6_A	2276.5_A	40.5_A	577.0_A
Anaerobic stress				
Seafarer	7616.5_B	$119,091.5_B$	2148.5_B	$25,814.5_C$
San Fernando	8217.0_B	$69,299.5_B$	2109.6_B	$16,051.9_B$

Those values within columns with different subscripts are significantly different at the .05 level according to Duncan's Multiple Range Test.

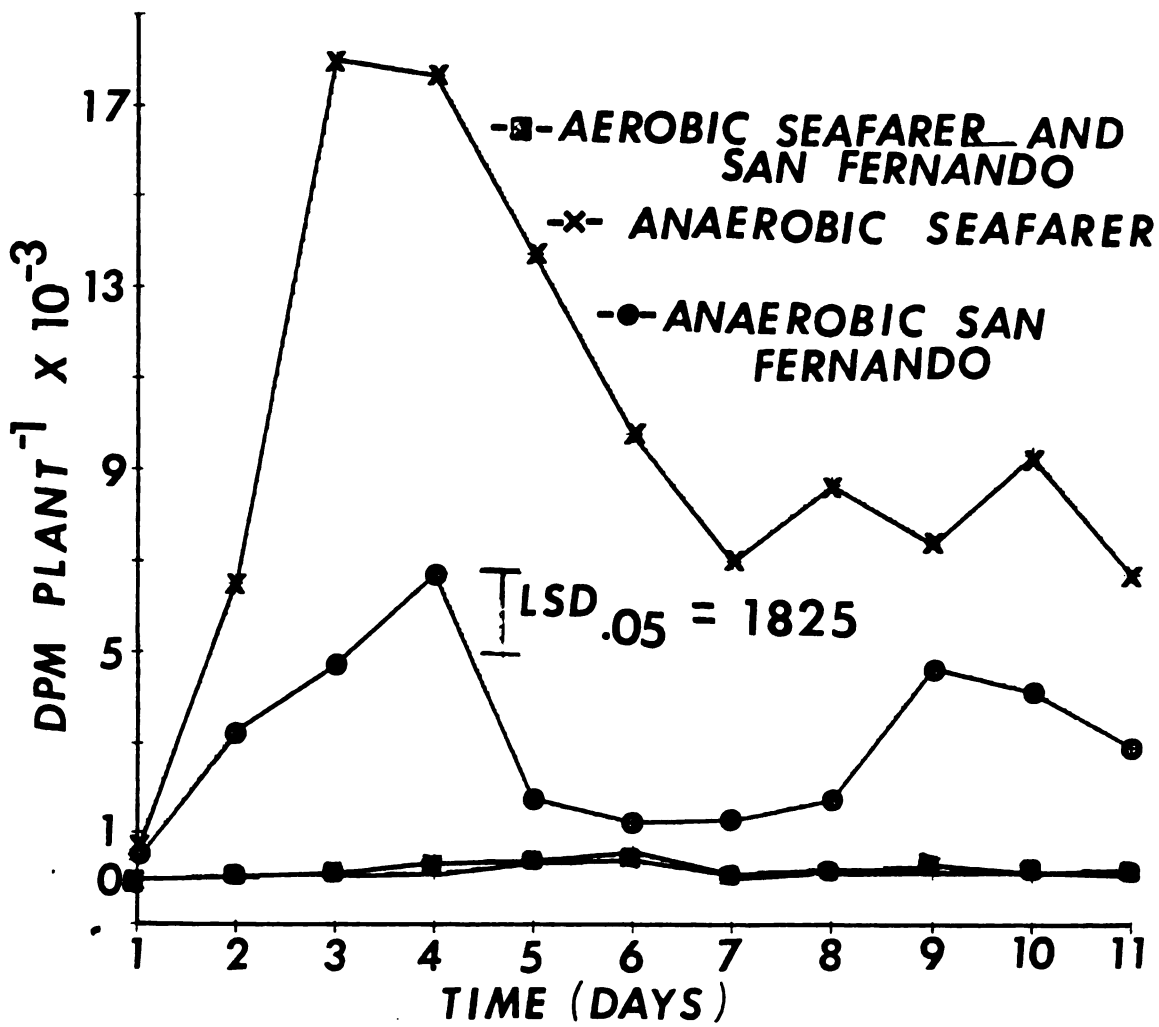

Figure 9. Carbon-14 accumulated per day in the mist chamber nutrient solution by individual aerobically and anaerobically treated Seafarer and San Fernando plants. Each point represents the average measurements for each chamber of 4 plants taken between 0800 hours and 2300 hours from day 1 through day 11.

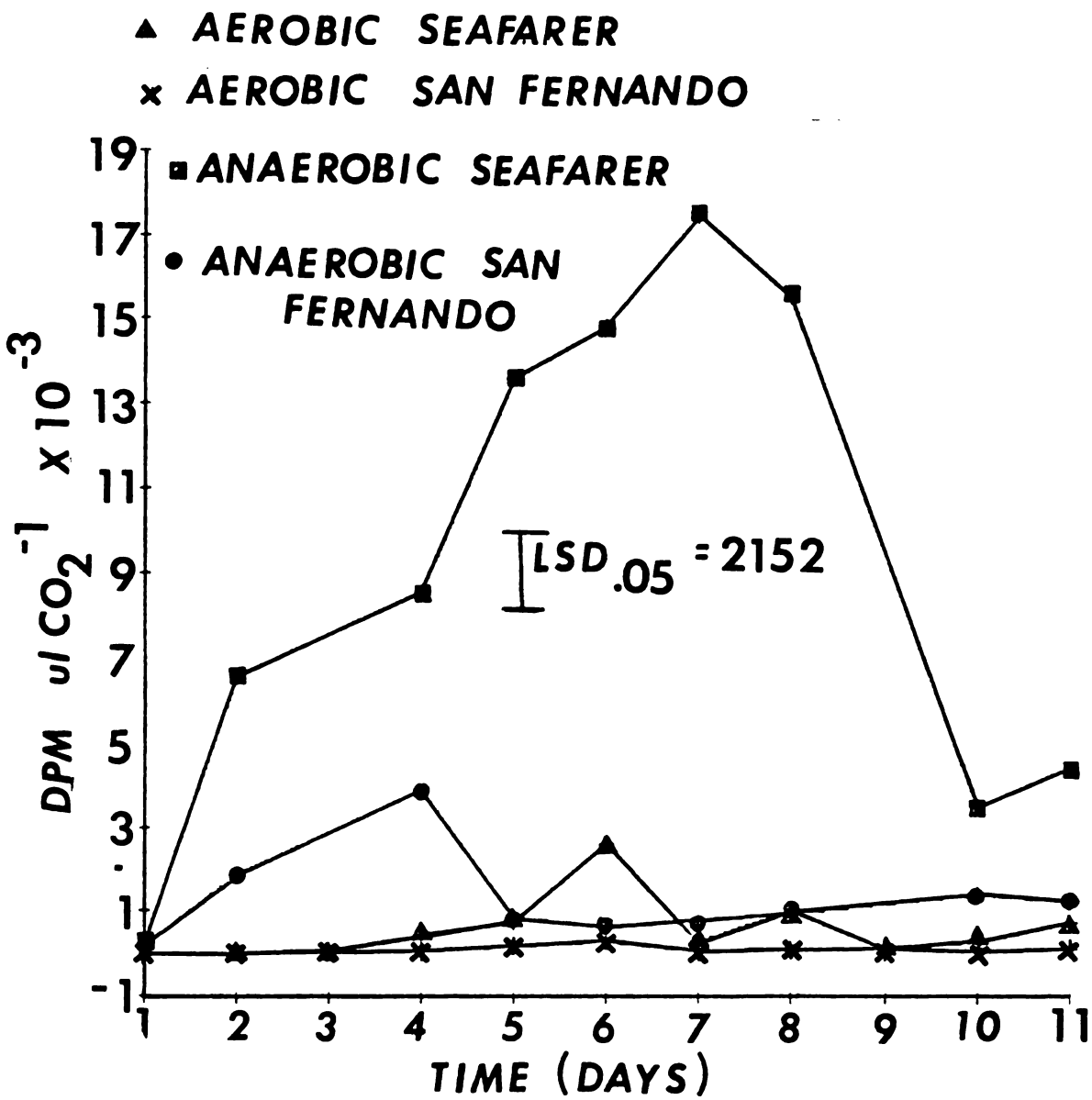

Figure 10. Ratio of carbon-14 assimilates detected in the mist chamber nutrient solution to root respiration for aerobic and anaerobic Seafarer and San Fernando plants. Each point represents the average measurements for each chamber of 4 plants taken between 0800 hours and 2300 hours from day 1 through day 11.

Seafarer is considerably higher each day than for anaerobic San Fernando. The greater exudation for anaerobic Seafarer could explain the higher ratio in Figure 10. Another possibility is that the respiration rate of Seaferer could be lower than for San Fernando throughout the experiment. The last day's measurements indicate that anaerobic San Fernando respired 1.4 times more $^{14}CO_2$ per unit dry weight root than stressed Seafarer roots. There appears to be a varietal difference in the rate of exudation to root respiration between anaerobic Seafarer and San Fernando plants.

Ethanol Metabolism, Loss and Accumulation

A trace of ethanol (0.25 to 0.29 ppm) was detected in the nutrient solutions of 4 day old seedlings. The accumulation of ethanol suggests that the oxygen content may have been depleted by the growing roots during the four day period. There were no significant differences in ethanol concentrations found between varieties. However, since there were no significant differences between plants of either variety, the differences in ethanol accumulation measured during each experiment are attributed only to the stresses imposed.

Ethanol loss and accumulation by roots of plants in the mist chambers were determined at 0800, 1200, 1600, 2000 and 2300 hours in the reservoir nutrient solution. Ethanol accumulation in root exudates during the first 48-hour anaerobic stress was 55% and 86% greater than the accumulation of ethanol during the second 48-hour oxygen stress for Seafarer and San Fernando, respectively (Figure 11). Part of the root system may have been destroyed after imposing the first stress (Smucker et al., 1978) and the decrease in surface area caused by root

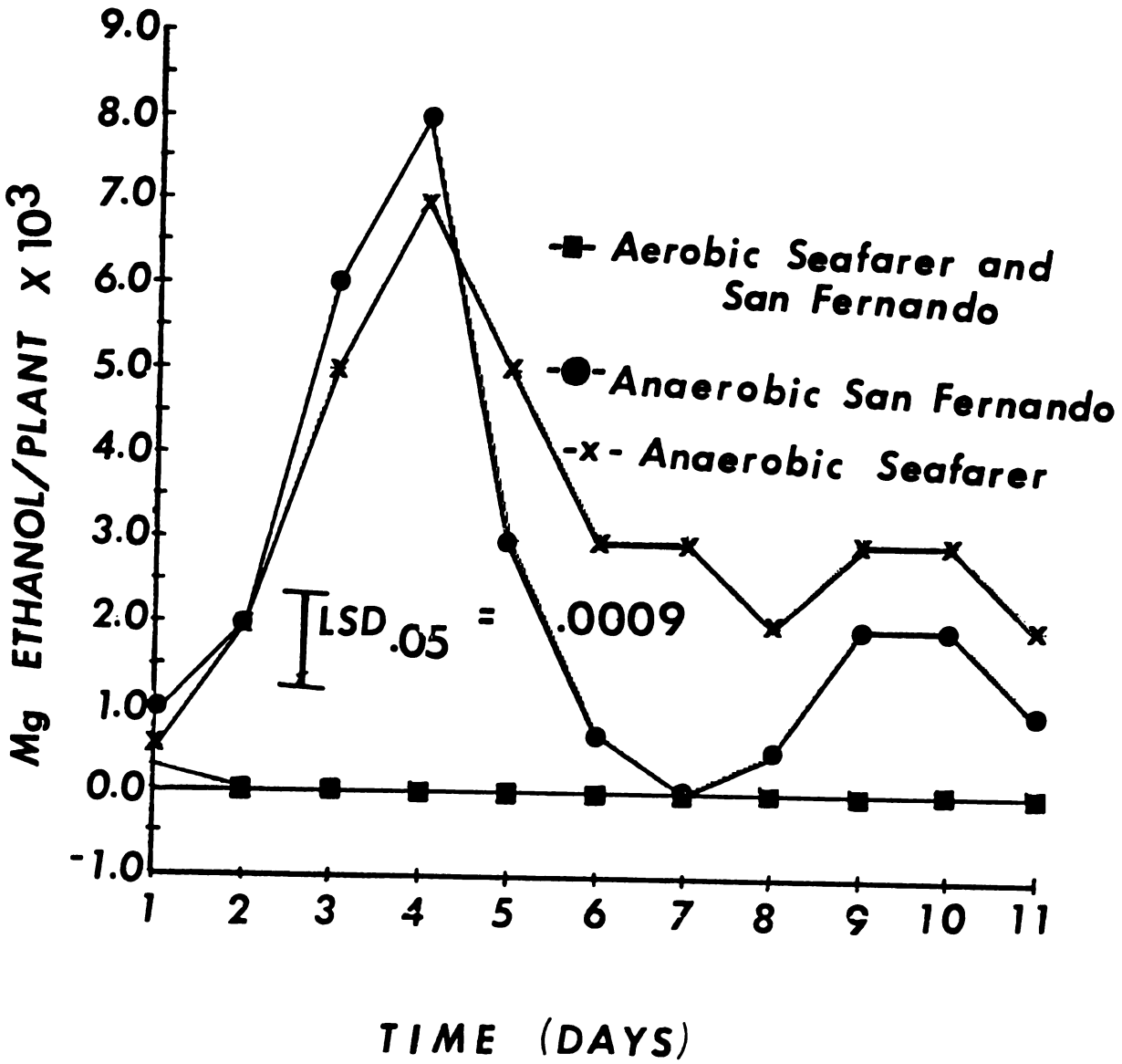

Figure 11. Ethanol accumulation in the mist chamber nutrient solution by individual aerobic and anaerobic treated dry bean varieties. Each point represents the average of measurements for each chamber of 4 plants taken between 0800 hours and 2300 hours from day 1 through day 11.

tissue death could account for the decrease in ethanol lossed by the roots and the accumulation in the nutrient solution. Another possible explanation is that between the first and second stress an aromatic alcohol dehydrogenase was activated resulting in greater root suberization. Davies et al. (1973) have postulated that the aromatic alcohol dehydrogenase functions in the metabolic route from cinnamic acid to lignin. The postulated route involves the reduction of ρ-hydroxycinnamic acid, ferulic acid and sinapic acid to the corresponding alcohols and subsequent incorporation into lignin. If the activity of an aromatic alcohol dehydrogenase increases then it could lead to an increase in lignin formation and suberization, resulting in a decrease in root exudation. A decrease in root exudation could account for the decrease in ethanol lossed through the roots during the second 48-hour anaerobic stress.

Ethanol did not accumulate throughout the duration of the experiment in the nutrient solution of aerobically treated Seafarer or San Fernando (Figure 11). A slight oxygen stress was probably imposed on the plants during seedling development in the vials which would account for the initial ethanol concentrations found in the nutrient solution of the mist chambers. In contrast to the controls, ethanol accumulated in root assimilates under anaerobic conditions. As much as 8×10^{-3} mg/plant and 7×10^{-3} mg/plant was detected at one time for anaerobic stressed Seafarer and San Fernando, respectively. Similar results have been reported by Smucker and Erickson (1980), Smucker and Erickson (1976) and Bolton and Erickson (1970). The increased production of alcohol could indicate an increase of glycolysis during anaerobiosis. Grineva (1961) has postulated that oxygen

deficiency causes an increase in membrane permeability. An increase in permeability could account for the increase in ethanol detected for Seafarer and San Fernando under anaerobic stress.

Ethanol accumulation in the root assimilates under anaerobic conditions was greater for Seafarer than San Fernando throughout the experiment which suggests a varietal difference in ethanol exuded. Noor (1979) found that alcohol dehydrogenase (ADH) activity which controls ethanol production was higher in Seafarer roots than in San Fernando. This greater ADH activity could explain the greater ethanol concentrations in Seafarer exudates.

Ethanol accumulation in the mist chamber nutrient solution for both Seafarer and San Fernando was found to be in direct proportion to the time of anaerobic treatment (Figure 11). Nordheim (1961) also found that ethanol lossed through the roots coincided with the time of treatment. The decrease in ethanol accumulation between stress periods indicates that ethanol may have been reabsorbed by the root tissue and/or respired by the roots. Cossins and Turner (1959) also showed that in a variety of germinating pea seedlings previously accumulated ethanol was reabsorbed by the tissues and by providing ethanol-2-C^{14} (Cossins, 1962) to pea cotyledons, they were able to show extensive conversion to a variety of products, including acetaldehyde, acids of the tricarboxylic acid cycle and amino acids.

Carbon-14 Partitioning

Carbon-14 contained in the treatment plants ranged between 81.3% for aerobic Seafarer to 65.7% for anaerobic San Fernando of the $^{14}CO_2$ fixed (Table 7). The aerobic controls, Seafarer and San Fernando,

Table 7. Total ^{14}C activity recovered per 4 plants and its distribution between ^{14}C fixed, exuded and respired.

Treatment	^{14}C retained in plant	^{14}C exuded	^{14}C respired
	%	%	%
Aerobic controls			
Seafarer	81.3	.14	18.6
San Fernando	75.1	.09	24.8
Anaerobic stress			
Seafarer	66.7	1.5	31.8
San Fernando	65.7	1.3	33.0

contained 25% and 12.5% more ^{14}C, respectively, than the anaerobically stressed treatments.

Anaerobiosis also had a considerable effect on ^{14}C assimilates lost by roots. A total of 1.52% and 1.3% of the ^{14}C fixed was exuded by the anaerobic treatment plants, Seafarer and San Fernando, respectively. This was 35% and 25% more assimilates detected in the nutrient solution of anaerobic Seafarer and San Fernando, respectively, than for each of the aerobic control plants. McDougall and Rovira (1965) found that approximately 0.1% of ^{14}C fixed was exuded by the roots. Martin (1974) found that wheat plants exuded 6.1% and 8.3%. ^{14}C assimilated under sterile and nonsterile conditions, respectively. If these values were included with the carbon dioxide respired by the roots, 7.6% and 15.7% of the total ^{14}C assimilated was lost through the roots. Minchin and Pate (1973) found that 47% of the total carbon fixed by peas was lost through the roots.

Respired ^{14}C, interpreted for the 11 days of the experiment, is shown in Table 7 to be between 18.6% of the ^{14}C fixed for aerobic Seafarer and 33% for anaerobic San Fernando. The exudation potential, ^{14}C assimilates detected outside of the roots at any one time which included ^{14}C assimilates accumulated in the nutrient solution and CO_2 respired, for 11 days, ranged between 18.8% for aerobic Seafarer and 34.3% for anaerobic San Fernando. Aerobic Seafarer and San Fernando had exudation potentials of 18.8% and 24.9% of the ^{14}C fixed, respectively. Anaerobic stressed Seafarer and San Fernando demonstrated exudation potentials of 33.3% and 34.3%, respectively. The exudation potentials for both anaerobically stressed varieties, Seafarer and San Fernando, increased under anaerobic conditions and were 1.8 and

1.4 times greater, respectively, than either their aerobic controls.

Improved soil management techniques would be the most important step in reducing soil compaction which may cause reduced growth and yield. Plant breeding programs designed to develop varieties more tolerant of anaerobic conditions are also needed. The exudation potential could be used to indicate the degrees of tolerance dry bean varieties have to root anaerobiosis. Improved soil management techniques as well as appropriate breeding programs must be implemented before yields of dry beans are to be improved on fine textured soils in Michigan.

Additional research utilizing ^{14}C labeling techniques should be conducted which would be designed to fractionate the organic components (e.g. volatile, soluable, etc.) exuded by roots. Automated sampling procedures as well as a large number of experimental replications would be needed for conclusive results.

SUMMARY AND CONCLUSIONS

A system was designed to determine short-term aeration stresses on total root exudation, ethanol accumulation and CO_2 respiration under axenic conditions employing ^{14}C feeding to plant shoots. The system allowed independent changes in the gaseous composition of the roots and shoots as well as multiple sampling of the sterile nutrient solution for ^{14}C activity. The system was effective in maintaining sterile conditions of the root systems. Sterile conditions were achieved for 80% of the experiments.

Aerobic Seafarer was found to contain 41% and 27% more ^{14}C activity per unit dry weight of shoot and root, respectively, than aerobic San Fernando. Anaerobic Seafarer contained 21% more ^{14}C activity per unit dry weight shoot and approximately the same ^{14}C activity per unit dry weight of root than anerobic San Fernando. ^{14}C activity found per unit dry weight of shoot was 3 to 5 times greater than ^{14}C activity contained in the roots for each of the treatments. ^{14}C assimilates translocated to the roots may have been exuded into the nutrient solution or respired by the roots.

Oxygen stress enhances respiration. Anaerobic conditions increased CO_2 evolved by the roots of Seafarer and San Fernando. Anaerobically stressed Seafarer and San Fernando respired 2.5 and 2.0 times more CO_2 per unit dry weight root, respectively, than the aerobic controls.

Roots of dry bean varieties Seafarer and San Fernando exuded

^{14}C assimilates even under aerobic conditions. However, anaerobic stress enhanced root exudation of both dry bean varieties. San Fernando and Seafarer exuded 30 and 19 times more ^{14}C assimilates per unit dry weight root, respectively, than the aerobic controls. Anaerobic Seafarer exuded 42% more ^{14}C-labelled assimilates than anaerobic San Fernando. Anaerobiosis as well as varietal differences seem to influence total root exudation.

Aerobic controls did not exude ethanol into the nutrient solution. However, ethanol accumulated in root assimilates in response to anaerobiosis with a greater accumulation for Seafarer than San Fernando. Ethanol exudation as well as production by dry bean roots also seems to be varietal dependent.

Of the ^{14}C fixed, 35% and 25% more assimilates were detected for Seafarer and San Fernando, respectively, under anaerobiosis than in aerobic conditions. Aerobic Seafarer and San Fernando had an exudation potential of 18.8% and 24.9%, respectively. Anaerobic stressed Seafarer and San Fernando demonstrated exudation potentials of 33.3% and 34.3% respectively . The exudation potentials for both anaerobic varieties Seafarer and San Fernando increased under anaerobiosis and were approximately 1.8 and 1.4 times greater, respectively, than for either of their aerobic controls.

Anaerobic conditions promoted by soil compaction appear to enhance the root exudation potential of dry beans which may cause a reduction in growth and yield. Improved soil management techniques, as well as plant breeding programs designed to develop varieties more tolerant of anaerobic conditions, are needed if yields of dry beans are to be improved on fine-textured soils in Michigan.

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