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THE INFLUENCE OF MECHANICAL IMPEDANCE
AND SHORT TERM ANOXIA ON RESPIRATION,
GROWTH AND STRUCTURE OF *PHASEOLUS VULGARIS*
L. ROOTS

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THE INFLUENCE OF MECHANICAL IMPEDANCE AND SHORT TERM ANOXIA ON RESPIRATION, GROWTH AND STRUCTURE OF *PHASEOLUS VULGARIS* L. ROOTS

By

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ABSTRACT

THE INFLUENCE OF MECHANICAL IMPEDANCE AND SHORT TERM ANOXIA ON RESPIRATION, GROWTH AND STRUCTURE OF *PHASEOLUS VULGARIS* L. ROOTS

By

Thomas Edward Schumacher

Investigations were carried out to study the effect of mechanical impedance on shoot and root morphology, root oxygen uptake and short term anoxia response of *Phaseolus vulgaris*, variety Seafarer. Seedlings were grown in glass chambers containing 1 mm, 3 mm or no glass bead matrices. Nutrient solution was passed through the root and matrix system. Oxygen content of the solution was controlled by equilibrating it with 0.21, 0.10, 0.03, or 0.00 atm of oxygen. Oxygen uptake was measured using a modified biological oxygen monitor. Primary root growth was mechanically inhibited by both bead matrices. Lateral root growth appeared to be limited the most by the smaller glass bead matrix. Porosity, dry matter percentage, weight, length and volume of roots and several shoot parameters were altered by mechanical impedance. Roots grown in the 1 mm matrix had higher oxygen uptake rates per unit fresh weight. Shoot to root ratios were highly correlated with root respiration for non-impeded roots. Short term anoxia responses, when corrected for root system size, were similar among matrix treatments.

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INTRODUCTION

Environmental stresses on crops contribute to the high degree of variability in annual yield which plague many areas of the world. The development of management practices and germ plasm to stabilize fluctuating yields requires an understanding of the effects and mechanisms of the various stresses.

Intensive cropping systems are being used more extensively as a result of increasing demands for greater food production. Such systems under conditions of poor soil management and marginal soils result in compacted soils. These soils are associated with a soil microenvironment which promote smaller root systems. A small root system in addition to imposing a possible stress on the shoot predisposes the plant to damage from other environmental stresses.

Three major functions of the root are anchorage, absorption of water and nutrients, and the synthesis of growth regulators (26). A small root system has a reduced capacity to carry out these functions. Plants with small root systems have lower thresholds for stress resulting from excessive wind, drought and low soil fertility.

Plants with large root systems appear to have greater potentials for absorbing water and nutrients due to the increased soil volume available to plants. A deep root system is able to permeate into soils containing greater quantities of water than small shallow root systems. Deep and dispersed root systems may also provide greater anchorage.

Soil conditions interfering with root growth also appear to reduce growth regulator export to the shoot (26). Consequently, smaller root systems could directly reduce shoot development by inhibiting the production of specific growth regulators.

Compacted soils are both physical and chemical barriers to root growth. Root growth is mechanically impeded by compacted soils when soil particles mechanically resist root elongation. A combination of soil pores smaller than the mean root tip diameter and soil resistance to deformation by the roots form the mechanical barrier.

A second barrier to root growth in compacted soils results from a lowered oxygen supply available to roots. Root anoxia (oxygen deficiency of root tissue) produces a reduction in aerobic respiration and a concomitant increase in anaerobic respiration. Subsequently, root growth is reduced by toxic compounds, lower metabolic energy and changes in hormonal relationships.

Mechanical impedance and anoxia frequently act together to limit root growth and it is difficult to separate the effects these two factors have on root growth. Little is known concerning the interactions of these two factors or their separate influences which limit root growth.

This study was designed to investigate the combined and separate influences of mechanical impedance and anoxia on root growth. Objectives of this study were:

1. To develop a system which could be used to separate and measure the effects of mechanical impedance and anoxia on the whole root system of dry edible beans (_Phaseolus_ _vulgaris_ L.) during the vegetative stage.

2. To determine the effect of mechanical impedance on root and shoot morphological characteristics.

3. To determine the oxygen uptake rates of mechanically impeded and nonimpeded roots under selected oxygen concentrations.

4. To determine the effect of short term oxygen treatments on root respiration and morphology.

LITERATURE REVIEW

Root Anoxia

Root Growth: Much literature is devoted to the adverse effects of anoxia on root growth (41,97,61). Cell elongation and division are retarded by anoxia. Elongation of cotton and soybean seedlings stopped within 2-3 minutes after oxygen was removed from the media (49). Root growth pressure was also reduced (29). Poorly aerated roots were shorter, thicker, more branched and had fewer root hairs than well aerated roots (28,60).

Root elongation was reduced at intermediate oxygen partial pressures (pO_2) of 0.03 - 0.10 atm (28,49,66). Huck (49) found that roots gradually recovered their original elongation rates under continued exposure to intermediate pO_2. Loepez-Soez et al. (66) found that cell division was more sensitive to pO_2 below 0.10 atm than was elongation. Eavis (28), however, demonstrated that between pO_2 of 0.08 and 0.21 atm the rate of cell division was unaffected while cell elongation decreased. At a pO_2 of 0.03 atm the rate of cell division was also reduced.

Root Respiration: The loss of metabolic energy has been proposed as a cause of root inhibition during anoxia (72). During anaerobiosis, when oxygen is unavailable to accept electrons, organic acids from the tricarboxylic acid cycle and other reduced compounds accumulate. This prevents the oxidation of pyruvate in the mitochondria and the reoxidation of cytoplasmic NADH formed during glycolysis. The cell must have a mechanism to reoxidize NADH to continue anaerobic glycolysis. The predominate pathway in most plants is through the decarboxylation and reduction of pyruvate to ethanol. Anaerobic respiration yields two ATP molecules, two carbon dioxide molecules and two ethanol molecules from one glucose molecule. Aerobic respiration yields thirty-six ATP molecules and six carbon dioxide molecules from the degradation of one glucose molecule. Anaerobic cells attempt to adjust to an energy imbalance by increasing the rate of glycolysis (Pasteur effect) (35,62). The amount of energy available to the anaerobic root is 1% of that under aerobic conditions (2).

As oxygen concentrations decline, oxygen uptake by the root declines in a hyperbolic fashion. Oxygen concentrations at which oxygen uptake began to decline, varied from just below 0.21 atm to below 0.05 atm (2,89,110). Based on the Km for cytochrome oxidase and the concentration for oxygen in air-saturated water, oxygen consumption in the cell should approach half the maximum velocity when cell pO_2 approaches 3×10^{-5} to 4×10^{-3} atm (41). The former observations can be explained by the slow diffusion of oxygen in water compared to that in air (97). The diffusion of oxygen may be further reduced in living tissue to one fourth of that in water (63).

Luxmoore _et_ _al_. (71) concluded that the gas filled porosity of the root system and the thickness of the water film around the root have the largest influence on the oxygen relations of maize roots. Thickness of the water film adjacent to roots is dependent on the soil water potential and the distribution of roots in relation to soil pores. Letey and Stolzy (65) calculated the following water film thickness as being growth limiting: 400, 150 and 7 μm for pO_2 of 0.20, 0.10 and 0.02

atm, respectively. Oxygen partial pressure gradients in water-saturated aggregates can range from 0.21 to 0.00 atm in 1 mm if soil respiration rates are high (42). Scotter et al. (89) concluded that decreases in oxygen uptake of 50-60 day old dwarf peas grown in Ottawa sand at low oxygen concentrations, were due to diffusion resistances inside the root and not in the water film surrounding the root. Amoore (2) concluded that respiration at low oxygen concentrations was limited by the slow diffusion of oxygen through the tissue of pea root tips. Lemon (63) suggested that oxygen uptake of stele cells could be decreased at a critical surface oxygen partial pressure. Subsequent decreases in root surface pO_2 resulted in an increase in the size of the oxygen deficient zone and pO_2 dependent oxygen uptake rates. Critical levels increased with the square of the radius of the root for any given respiration rate (64).

Root Porosity: Investigators have observed intercellular gas spaces in roots since the late 19th Century (74). Tracer studies for several species showed that $^{15}O_2$ flow through the gas spaces fit a model of oxygen diffusing down a long narrow porous tube (6,31,52,54). Root porosity is directly related to the longitudinal oxygen diffusion coefficient of roots and is simpler to measure (53). Generally, plants well adapted to waterlogging have larger root porosities. Cultivars of maize, wheat and rice with high root porosities appear to have improved tolerance to anoxia (24,25,112). Simulation models demonstrated that under appropriate conditions root porosity could contribute significant amounts of oxygen to the root (70,110). Jensen et al. (53) reported the following root porosities: 4.2% ± .5%; 7.6% ± 1%; 26.5% ± 3% for *Phaseolus vulgaris* L., *Zea mays* L. and *Oryza sativa* L., respectively. The contribution of this pathway depends on the degree of root porosity.

respiration rate, length, diameter and cell permeability to oxygen (70). Most plant species with root systems larger than that of a seedling may not utilize this pathway to a great extent (41,78).

Limited oxygen content in the rooting medium induced higher root porosities in several species (25,67,75,82,103), although this induction was not found in maize and rice (68,102). High light intensity and temperature were also correlated with greater root porosities in wheat and rice (67,96,102,103).

Shoot Growth: Shoot growth of many plant species are inhibited by root anoxia. Epinasty was usually the first symptom to appear (51) followed by wilting, if conditions were favorable for high transpiration rates (61). Continued anoxia resulted in the progressive yellowing and death of older leaves (57).

Yields of sorghum, soybeans, cabbage, sweet corn and dwarf field corn were drastically reduced when plants were subjected to long term anoxic stress (108). Short term anoxia may also be very detrimental. Pea and tomato yields were severely decreased when plant roots were subjected to soil flooding for 24 hours during critical stages of growth (30).

Root Exudation: Short periods of anaerobiosis increased the exudation of organic compounds (43,85,95). In comparison to controls, anoxic corn and sunflower plants excreted larger amounts of the following amino acids: alanine, aminobutyric acid, proline, glutamic acid, serine, threonine, aspartic acid and histidine (43). In an aseptic mist chamber study using peas, Smucker (95) reported that an atmosphere of 70% nitrogen and 30% carbon dioxide increased the exudation of amino acids four-fold, with alanine being the predominant amino acid exuded under anaerobic conditions.

Ethylene: Ethylene is considered to be a factor in anoxic plant damage (20,51,57,94). Root elongation in a wide variety of plant species were inhibited by ethylene concentrations of 1 ppm or more (59,92). Ethylene concentrations of 1 ppm or higher are frequently found in soils when the pO_2 is below 0.02 atm (93).

Soil ethylene appears to be produced primarily by anaerobic bacteria (20,91). Smith (91) purposed that ethylene is produced in anaerobic microsites in the soil. The rhizosphere is likely to contain a high proportion of microsites because of the rapid utilization of oxygen by plant roots and the rhizosphere bacteria (90).

Ethylene from the soil environment diffused to the shoot through intercellular air spaces (50). The root also appears to transport precursors or stimulators of ethylene formation to the shoot (51).

Growth Hormones: Waterlogging can effect the concentrations of various growth hormones in the plant. Burrows and Carr (18) found that flooding the root system of sunflower plants reduced the translocation of gibberellin like substances. Phillips (81) found that flooded plants contain three times the auxin content of control plants.

Resistance to anoxia in tobacco plants previously grown in saline solution appeared to be caused by increased amounts of abscisic acid in the shoot. Less damage also occurred in plants pretreated with abscisic acid (76).

Cornforth (20) purposed that short term anoxic damage is the result of ethylene interaction with growth regulators in the shoot. This mechanism would be most critical during the reproductive phase due to the important role of growth regulators in reproduction.

Root Resistance: Kramer (61) stated that flooding injury is usually attributed to dessication resulting from increased root resistance to water absorbtion. Resistance to water flow through root tissue was greatly increased by removing oxygen from solution cultures (17,61, 105). Water uptake by tobacco plants was reduced by 50% when subjected to a six hour anaerobic period (105). Shoot dehydration resulting from root anaerobiosis was more prevalent under conditions promoting high transpiration rates (61).

Ethanol Production: Plant damage during anoxia may be due to the accumulation of ethanol. Many cultivated plant species subjected to soil anaerobiosis produce ethanol as an end product of anaerobic metabolism (21). Bolton and Erickson (14) correlated ethanol content in the xylem exudate with the degree of anoxic damage.

Crawford (22) concluded that the ability of a wide variety of tissues and species to tolerate low oxygen environments was due to the limitation of ethanol production. Ethanol production and movement in the plant can be limited in various ways. Alternate pyruvate pathways have been shown to occur in flood tolerant plants. In such cases, a nontoxic compound (eg. malate) is formed from pyruvate in place of ethanol (21). Studies have shown reduced alcohol dehydrogenase (ADH) activities in flood tolerant species of maize and clover (32,72). However, other studies with barley and maize indicated that ADH activities may be higher in flood tolerant cultivars (106,107). John and Greenway (55) suggested that pyruvate decarboxylase (PCD) activities better represent in vivo alcholic fermentation since the activity of PDC is generally rate limiting. Rice adapted to anoxia by producing larger amounts of ethanol (55). Crawford and Baines (23) suggested that this could be explained by the ready diffusion of ethanol from the adventious roots of rice. Plants which have a mechanism of removing ethanol may be able to tolerate flooding. Intact roots have been shown to exude large quantities of ethanol during short term anoxia (44,95).

Mechanical Impedance

Root Growth: Root elongation appears to decrease with increasing mechanical impedance (11). Pressures inhibiting root elongation were much smaller than pressures developed by the root. Soil pressures in the range from 0.2 to 0.7 bar impeded root growth significantly (11,87). This compared to root growth pressures of 5 to 25 bars produced by a wide variety of plant species (29,33,99).

Greacen and Oh (40) postulated that the reduction of root elongation in mechanically impeded soil was due to a reduction in the ability of the root to osmoregulate against an external force. Russell and Goss (87) supported an alternate theory that elongation is controlled by hormonal regulation of cell wall extension.

Root penetration into soil depends upon soil strength and the diameter of soil pores (37). Accordingly, rigid (3,38,56,104) and nonrigid (1,9,10,34,37) systems have been used to study mechanical impedance. Rigid systems demonstrated that roots were unable to penetrate pores smaller than the root tip diameter (37,104). Nonrigid systems were used to determine the minimum pressure which inhibited root elongation (26).

Mechanical impedance generally stimulates the development of lateral roots near the root apex (87). If lateral root formation is prolific, the total weight and ability of the root system to absorb nutrients may not be affected (37).

Morphologically, roots grown in a nonrigid 1 mm bead system had longer root axes and fewer lateral roots per root axis than roots grown in rigid systems. Barley axial roots penetrated up to 6 cm in a rigid system of 1 mm glass beads before the root apex appeared to lose its meristematic activity (36). Barley lateral roots grew twice as long in the 1 mm bead matrix than in the 3 mm bead matrix. This compensated for the lack of growth by the root axes (37).

Cortical cells of impeded roots were reduced in length but greatly increased in width, resulting in a thicker root with essentially no change in the cell volume (11,87). However, Wilson et al. (109) observed great variability in dimensions of cortical cells in impeded barley roots. Radial expansion occurred near the root tip but overall root volume and surface area were reduced. Radial expansion near the root tip may reduce the stress on axial elongation (11).

Root Respiration: Very few studies have measured respiration rates of mechanically impeded roots. Tackett and Pearson (98) found that roots growing in a soil of high bulk density produced more carbon dioxide per root dry weight than did roots grown in low bulk density soil. This was true over a wide range of oxygen concentrations. However, a study by Eavis (28) suggested that mechanically impeded roots need less oxygen per unit root than nonimpeded roots. The larger root diameters of impeded roots (70) and the increased root surface area blocked by the solid phase of the soil (41) could reduce the diffusion of oxygen resulting in lowered oxygen uptake.

Oxygen uptake rates per unit root may not change for a mechanically impeded root. Theoretical calculations based on the energy requirements of an elongating root indicate that energy used in elongation is small compared to the total amount produced. A root 1 mm in diameter elongating at a rate of 1 mm per hour against a resistance of 10 bar requires approximately 0.2% of the energy produced during respiration (13).

Root Porosity: Although root porosities have not been measured for mechanically impeded roots, there are indications that significant changes can occur in impeded roots. Roots grown in the soil generally have less porosity than those grown in nutrient solution (67). Goss and Walter (38) observed distorted cortical cells in barley roots growing in a 1 mm rigid bead system. It appears that root responses to an obstruction result in cell wall relaxation permitting turgor forces to act externally causing cell distortion (12). Barley and Greacen (12) suggested that cell distortion causes compression of the intercellular spaces which decrease internal aeration. The decreased gas filled spacc and perhaps greater tortuousity could reduce the diffusivity of gases in plant tissues resulting in the accumulation of carbon dioxide and volatile growth regulators, such as ethylene (11).

Shoot Growth: Accurate observations on the effect of mechanical impedance on shoot growth are difficult because a plant with an impeded root system is prone to many other stresses. Laboratory investigations which control water availability, nutrient availability and oxygen concentrations around the root indicate that mechanical impedance *per se* may not harm the shoot (26). Cannell and Finney (19) indicated that mechanical impedance of the seminal roots of cereals grown in the field was not harmful when an adequate supply of nutrients and water were available.

Root Exudation: The loss of organic compounds was enhanced when roots were grown in a solid medium (7,8). Barber and Gunn (7) grew barley and maize in nutrient solutions with and without glass beads. Exudation of amino acids and carbohydrates increased from roots grown in the presence of 1 mm glass beads. Exudates from roots grown in 3 mm glass beads were greater than solution cultures and less than the 1 mm glass bead matrix. Hale *et al.* (45) suggested that roots grown in soil may exude more than those grown in solution culture, but questioned the ability of investigators to remove roots from the soil uninjured. Barber and Martin (8), in a soil study using ^{14}C labelled carbon dioxide, estimated root exudation to be at least 10% of the dry matter increment of wheat seedlings. This value is much higher than values obtained in nutrient culture studies.

Ethylene: Ethylene may be a contributing factor in the reaction of roots to mechanical impedance. Kays *et al.* (58) found that roots of *Vicia faba* grown against a barrier produced six times more ethylene than the control. Roots exposed to 100 ppm ethylene for 10-25 days showed enhanced lateral root formation and inhibition of axial root extension (92). This is similiar to responses seen in mechanically impeded roots. Barley (11) purposed that endogenously produced ethylene in mechanically impeded roots may cause elongating cells to become shorter and wider resulting in the inhibition of root elongation.

Growth Hormones: The effects, if any, of mechnical impedance on plant growth hormone regulation in the shoot is unknown. The root apex has been shown to be a major source of auxins, cytokinins, gibberellins and abscisic acid (86). Goss and Ward (39) demonstrated that the root apex was important in determining the rate of elongation. However, the relationship between the synthesis of growth hormones and root response to mechanical impedance has not been investigated. Physical models of root response to mechanical impedance are inadequate and there is increasing speculation that root response to mechanical impedance is mediated by growth hormones (87).

Root Resistance: Resistance to water movement by mechanically impeded roots has not been measured. Mechanical stress resulted in thicker cortical tissue and greater surface area per unit length of root (109). If the main resistance to water flow is at the endodermis, water uptake rates should be proportional to root surface area (77). An increased uptake of water and nutrients resulting from increased root surface area may compensate partly for the smaller root system in mechanically impeded roots. The increased tortuosity of cortical cells of mechanically impeded roots (11) could result in an additional resistance to water uptake if the cortical apoplast (free space) is limiting water uptake.

Mechanical Impedance and Root Anoxia

Root Growth: Several investigators have studied the combined and separate effects of mechanical impedance and anoxia on root growth (4, 9,27,28,34,48,84,88,98). Roots growing at 0.21 atm of O_2 showed decreased growth rates with increasing mechanical impedance. Roots growing at minimum levels of mechanical impedance showed reduced growth rates with decreasing pO_2. Data at intermediate levels of mechanical impedance and oxygen partial pressures generally have been interpreted as demonstrating an interaction between the two factors (12). Examination of the data indicates that the type of interaction taking place varies widely.

A decrease in pO_2 from 0.21 to 0.10 atm resulted in varied root growth rate responses for mechanically impeded and nonimpeded roots. Most studies demonstrated a greater reduction of root growth rates in nonimpeded roots (9,27,34,48). Other studies showed a greater decrease in root growth rates for mechanically impeded roots (9,48); similar increases in root growth rates (4) and no change in root growth rates (98).

A reduction in pO_2 from 0.10 to 0.03 atm also resulted in discrepancies between studies. Studies demonstrated greater reductions in root growth rates for nonimpeded roots (9,48); greater reductions in root growth rates for mechanically impeded roots (4,48,98) and similar reductions in root growth rates (29).

Part of the variance arises from the parameters used to measure root growth. Barley (9) found a larger decrease in root growth at 0.03 atm pO_2 with roots subjected to 0.5 bar pressure compared to 0.0 bar pressure when using root weight as the parameter. When using root length as the parameter, root growth decreased at the same rate at both pressures. Discrepancies also arise from the methods and procedures used. Table 1 outlines principal points from the various investigations. Graphs in the appendix visually depict the interactions outlined previously, Figures 26 to 33.

Root Respiration: Tackett and Pearson (98) measured the combined influence of anoxia and mechanical impedance on carbon dioxide production. Carbon dioxide production per dry weight of root, decreased linearly with decreasing pO_2 for roots grown in high bulk density soil. Carbon dioxide production per root dry weight by roots growing in soils of low bulk density were constant when pO_2 levels were greater than 0.05 atm and then increased sharply at pO_2 levels less than 0.05 atm. They concluded that root respiration was dependent on pO_2 for roots grown in high density soils but not for roots grown in low density soils. However, the microbial background was not measured. Microbial respiration could have masked root response under conditions of limited root growth.

Table 1. Variables used in studies of mechanical impedance-anoxia interactions.

Reference	Species	Length of Treatment	Media	Oxygen Treatments	Mechanical impedance treatments	Root measurements
Aubertin and Kardos (1965) (4)	Corn	14 d	1600 μ glass beads	0.21, 0.10 0.05 and 0.02 atm pO_2 gas	Rigid and nonrigid bead system	Volume, Length, Dry weight, Number, Diameter
Barley (1962) (9)	Corn	70 h	80 μ glass beads	0.20, 0.05 and 0.01 atm pO_2 gas	Variable pressure chamber; 0.5, 1.1, 1.8, 2.8 & 4.5 bars	Length, Dry weight
Eavis (1972)a (27)	Pea	48 h	Sandy loam	None, pO_2 at root surface inferred from "Aeration Deficiency Index"	Root growth in compacted soil; bulk densities = 1.1, 1.4 & 1.6.	Length, Diameter
Eavis (1972)b (28)	Pea	2-7 d	Sandy loam	0.21, 0.15, 0.10, 0.07, 0.03 and 0.01 atm pO_2 by flowing gas around soil columns	Root growth in compacted soil; bulk densities = 1.1, 1.4 & 1.6.	Elongation in terms of "Aeration Deficiency Index"
Gill and Miller (1956) (34)	Corn	2-7 d	50 μ glass beads	0.20, 0.15, 0.10, 0.05 and 0.01 atm pO_2 gas	Variable pressure chamber	Length
Hopkins and Patrick (1969) (48)	Sudan Grass	14 d	Commerce five sandy loam, Commerce silty loam, Mhoon silty loam	0.21, 0.08 and 0.03 atm pO_2 by passing gases over the top of the compacted core	Root growth from noncompacted into compacted soil. ft-lb/in^3 = 1.5, 4.0, 10.0 & 20.0.	Weight of penetrating roots, Time to reach ^{32}P placed 4.5 cm deep in compacted core
Rickman, Letey and Stolzy (1966) (84)	Tomato	7 d	Yolo silt loam	with and without 0.60 atm pO_2 gas to maintain O.D.R. > .20 μg cm^{-2} min.$^{-1}$	Root growth from noncompacted into compacted soil; bulk densities = 1.4 & 1.6.	Depth of penetration, Water uptake
Scott and Erickson (1964) (88)	Alfafa Sugar beets Tomato	16 w 6 w 6 w	Granby sandy loam, dense layer of kaolinite, quartz and sand	with and without calcium peroxide placed in the dense layer	Root growth from noncompacted into dense layer (B.D. = 1.9).	Dry weights
Tackett and Pearson (1964) (98)	Cotton	92.5 h	B$_1$ of Norfolk sandy loam	0.21, 0.10, 0.05 and 0.01 2 atm pO_2 by flowing gas across bottom of compacted core	Root growth from noncompacted into compacted soil; bulk densities = 1.3, 1.5, 1.6, 1.7 & 1.9.	Depth of penetration, Dry weight (% of max.), Soil water extracted (% of max.), CO_2 production

MATERIALS AND METHODS

Pyrex Root Chamber

A glass chamber was designed to hold the root medium and provide a rigid support to the plant while allowing flow of aerated nutrient solution past the root system, Figure 1. Neoprene stoppers were modified by removing the top quarter section and boring three holes into the remaining section. The center hole served as an exit for the circulating nutrient solution. The two plant ports were split diagonally on the side to facilitate the transfer of seedlings. The glass chamber was flared at the top to accept the modified no. 10 stopper. Volumes of the chambers were approximately 150 cm^3. The lower inlet port was fitted with a 6 mm septum through which aerated nutrient solution entered the chamber.

Plant Growth and Treatment Procedures

Phaseolus vulgaris L., variety Seafarer, seeds were germinated in an incubator at 25°C. Fifteen seeds were placed on each set of two towels and saturated with 22 ml distilled water. On day five, when hypocotyls were approximately 2.5 cm, seedling pairs of similar size were transferred to the modified neoprene stoppers. Parawax, a 1:6 mixture (g/g) of paraffin and petroleum jelly, was used to support the seedling in each plant port and prevent gaseous exchange and loss of nutrient solution. Care was taken during the transfer process to

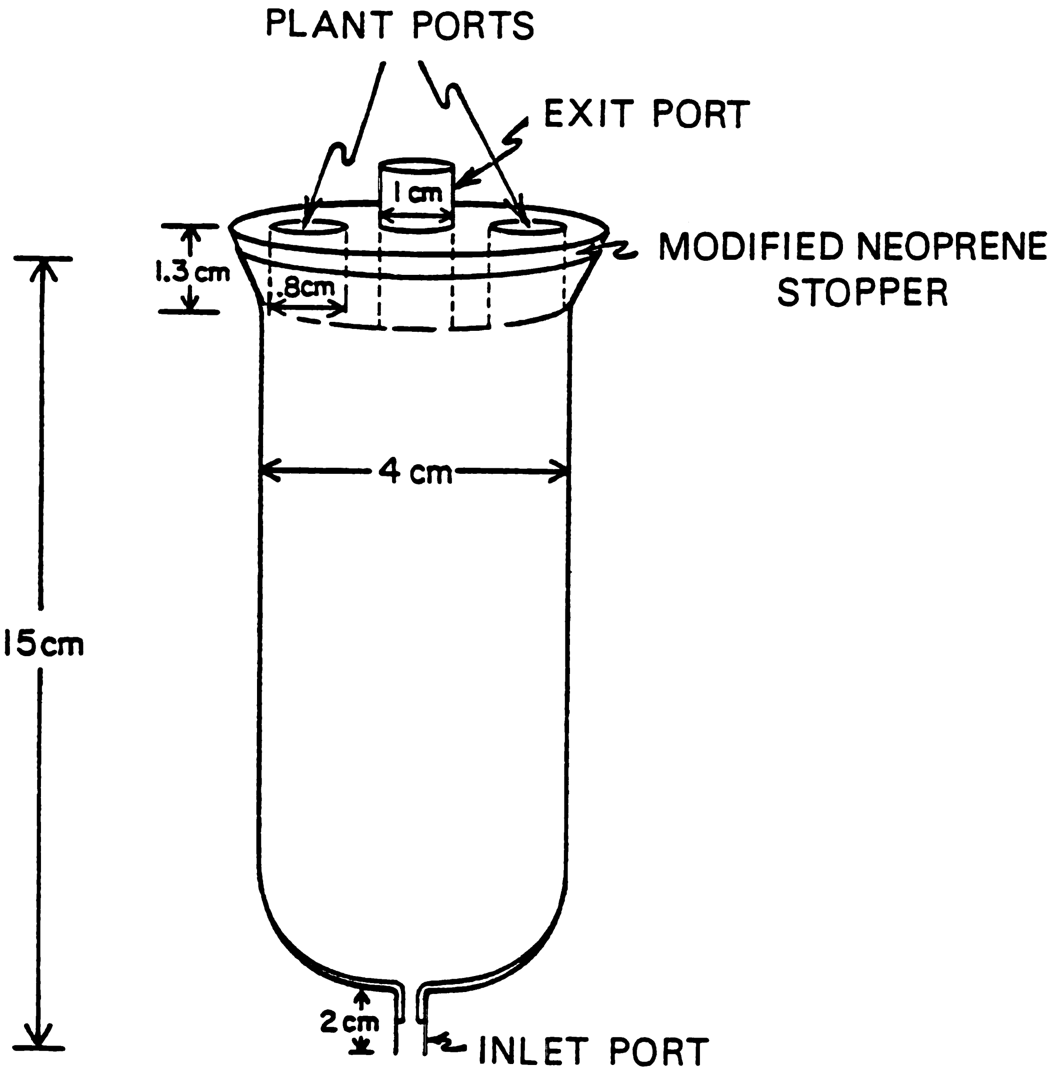

Figure 1. Pyrex root chamber construction.

prevent damage from dehydration and to minimize mechanical damage to the root system.

Stoppers containing the seedlings were transferred to a small jar containing nutrient solution and placed in the growth chamber. This served as a means of assessing transfer injury before the introduction of matrix treatments. Seedling pairs were again selected for uniformity after 24 hours. The seedling and stopper were both fitted to the top of a pyrex root chamber, filled with nutrient solution and a strip of parafilm was used to seal the stopper to the glass chamber. Two rubber bands aided in retaining the stopper and helped to prevent nutrient solution loss.

Matrix treatments consisting of 1 mm glass beads, 3 mm glass beads and no glass beads (control) were randomly chosen within each replication and introduced into the pyrex root chambers through the center port. The glass beads were carefully added to the solution to minimize damage to the root system and prevent the entrapment of air. Displaced solution was drained through a needle inserted in the bottom septum. Glass beads were tightly packed by intermittent vibrations from a Cole Palmer supermixer. Table 2 provides information for each matrix treatment. Individual replications were randomly allocated positions within one unit of the plant growth system.

Experiments having an objective of determining the effects of mechanical impedance on shoot and root parameters were conducted at a constant pO_2 of 0.21 atm. Plants were grown for 8 days in the plant growth system, described in the next section, using a randomized complete block design. Experiments designed to determine the combined effects of mechanical impedance and anoxia on root growth

Table 2. Relation of matrix treatments to pore size, surface area and porosity.

Treatment	Matrix mean pore diameter (cm)	Matrix and root chamber surface area (cm^2)	Matrix porosity (%)
Control	--	180	100
3 mm matrix	0.045	1,950	40
1 mm matrix	0.015	5,870	36

were exposed to pO_2 below 0.21 atm for specific periods of time. One experiment examined the effect of root exposure to pO_2 of 0.210, 0.105, 0.032 and 0.00 atm for one hour on oxygen uptake by roots. Selected pO_2 were introduced in decreasing order to the treatments and oxygen uptake monitored. The 0.00 atm pO_2 treatment was followed by a return to 0.210 atm pO_2 for two of the replications. These oxygen treatments were introduced 14 days after placing seeds in the incubator. Another experiment examined the effects of a 24 hour exposure to randomly selected pO_2. Levels of pO_2 were the same as in the experiment described above. The 0.00 atm pO_2 treatment was achieved by equilibrating the circulating nutrient solution with nitrogen gas. The remaining pO_2 treatments were achieved by equilibrating the circulating nutrient solution with prepurified standards from Matheson. A waterlogging treatment in which the nutrient solution was not circulated through the pyrex root chambers was also applied and analyzed separately. Gas treatments were introduced on the 13th day. Oxygen uptake measurements were made before and after the 24 hour treatment period. The order of measurement among treatments was determined randomly within each replication.

Limitations of space and time resulted in the application of the various oxygen treatments separated by time. A split plot design was used. The main treatments consisted of oxygen levels and the subtreatments were two replications of the matrix levels.

The inability to measure other parameters before treatment and the variability between treatment periods precluded the use of other parameters as indicators of effects of short term anoxia. An exception was the waterlogging treatment in which root exudation (ethanol and amino acids) and water uptake data were also taken during the 24 hour treatment period.

Description of Plant Growth System

The objective of the plant growth system was to maintain a controlled environment for the shoot and root systems. The differing environments of the shoot and root required separate control systems.

The shoot environment was controlled by a Warren-Shearer model CEL-36-10 growth chamber. The growth chamber light source consisted of eight 75 watt incandescent bulbs and twelve 39 watt fluorescent bulbs which produced 680 μEinsteins sec^{-1} m^{-2} for full light. Incandescent light produced quanta of 86 μEinsteins sec^{-1} m^{-2} as measured by a Lambda LI-170 quantum/radiometer/photometer. The 15 hour photoperiod consisted of 13 hours of full light preceded and followed by 1 hour of incandescent light only. Temperature was maintained at 20°C for 11 hours during the dark period and at 30°C for 13 hours during the light period. Relative humidity in the growth chamber was maintained at 40-60%. Pyrex root chambers were clamped on a stand with the top of the stopper 45 cm from the light source.

The root environment was controlled in part by pumping aerated nutrient solution past the root system, Figure 2. The circulation system consisted of 3 units each with a separate nutrient solution reservoir and pump channel. Each unit had the capacity to service 6 pyrex root chambers (two replications of matrix treatments). Temperature of the root system was slightly lower than the shoot temperature.

A variable speed Masterflex pump continuously circulated a modified half-strength Hoagland's solution at a flow rate of 15 to 25 ml/min. Nutrient solution from a common 6 l reservoir was pumped into the pyrex root chamber through a syringe needle at the base of the chamber and flowed past the root system. Nutrient solution in the reservoir was vigorously

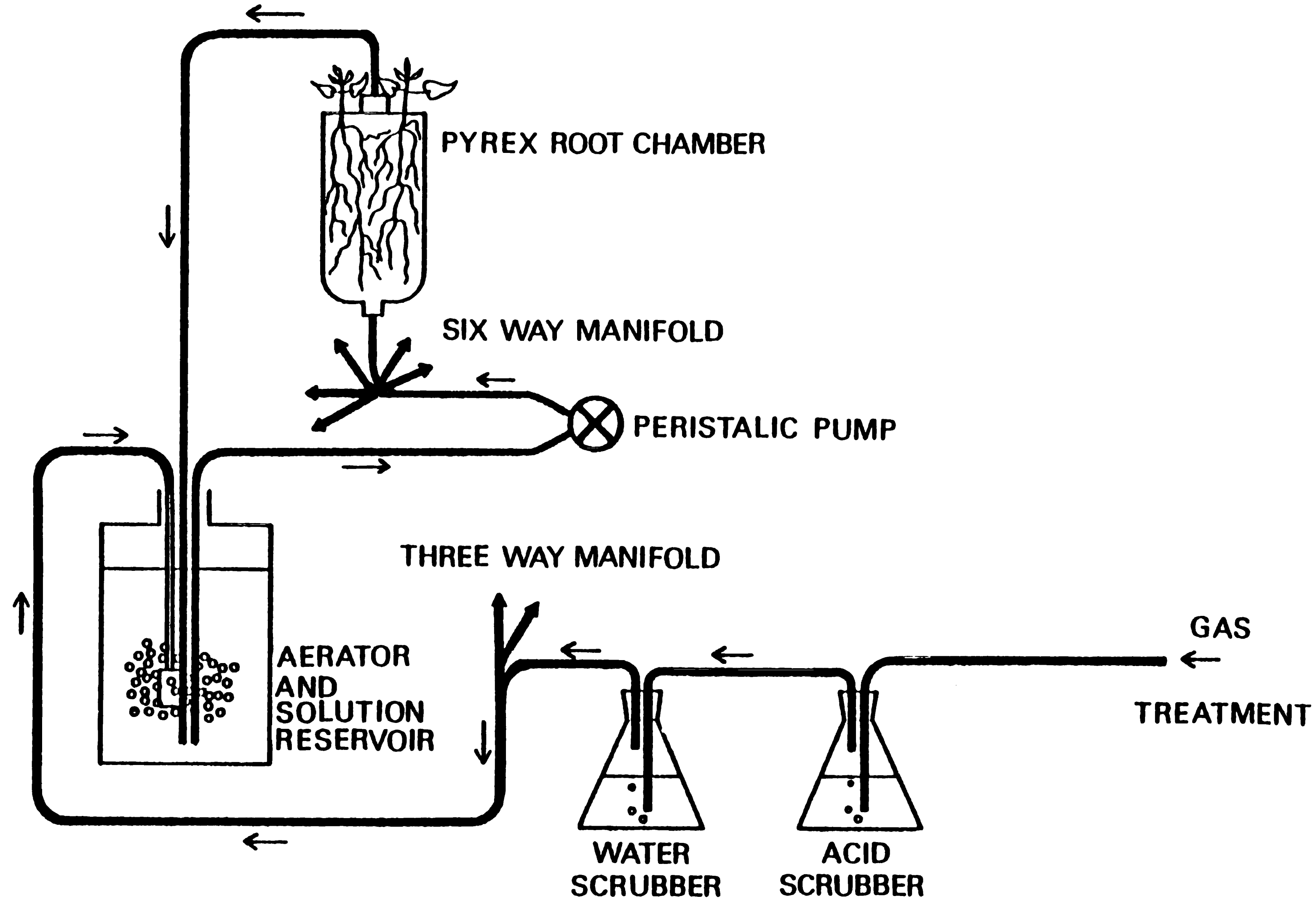

Figure 2. Diagram of the plant growth system.

aerated with acid washed compressed air flowing through cindered gas dispersion tubes. Oxygen treatments less than 0.210 atm pO_2 were introduced through gas dispersion tubes from the appropriate gas tanks for the experiment involving 24 hour oxygen treatments. A photograph of the actual plant growth system is shown in Figure 3.

The pH and conductivity of the nutrient solution were monitored daily. The pH was maintained at 6.5 ± 0.5 pH units by the addition of either 0.1 M H_2SO_4 or 0.1 M NaOH. Nutrient solution was replaced every seven days. All tubing, glass chambers and reservoir flasks were covered with aluminum foil to prevent algal growth.

The primary difficulty of this system was the need to closely monitor the system for leaks. Tubing in the peristalic pump needed to be replaced every three to four days. The system could be improved by installing an air entrapment device between pump and the pyrex root chambers. This would prevent air entry into the root chamber and oxygen monitor system.

Description of Root Respiration Monitor

Root oxygen uptake measurements were made using a modified YSI model 53 biological oxygen monitor. The system is based on polarographic oxygen sensors. Other investigators of root respiration have incorporated the use of polarographic oxygen sensors in their studies. A brief summary of their studies follows.

Scotter *et al.* (89) determined, using polarographic methods, the oxygen concentration in gas circulated through a sand column containing pea roots. Root oxygen uptake was measured in a closed circulating system by monitoring the change in gaseous oxygen concentration with

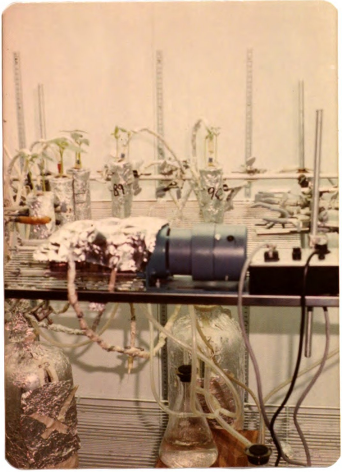

Figure 3. An overview of the plant growth system in the growth chamber.

time and measuring the gas pressure increase when a known amount of gas was added to estimate the gas volume. Willey (105) used a similar method in a mist chamber study. An additional oxygen electrode was used to determine if equilibrium existed between the misting solution and the treatment gas phase.

Higgins et al. (47) employed a polarographic oxygen sensor to measure the respiration rate of excised root tips in stationary solution. Boogie (15) measured the oxygen concentrations of aliquots of nutrient solution using a polarographic oxygen sensor for a root growth study.

The requirements of the present study determined the need for a system which could monitor the oxygen concentrations in solution before and after flowing past a root system. The respective lack and presence of a solid medium in the control and bead treatments prevented the use of a mist chamber or a gas permeated solid medium study. Inability to control the water film thickness around the root between the different matrix treatments prevented the use of directly supplying oxygen, as a gas, to the roots. The supply of oxygen to the root is dependent upon the distance oxygen must diffuse through water to the root (65). This study required a method of minimizing differences in oxygen gradients around the root surface arising from the different matrix treatments. The use of a solution flowing past the root system to supply oxygen, water and nutrients was an attempt to minimize gradient differences of these components among the matrix treatments.

The designed root respiration monitor measured the difference in oxygen partial pressures in solutions before and after passage through the root system. Root oxygen uptake was calculated using the differential oxygen concentration and solution volume determined from the nutrient solution flow rate.

A diagram of the root respiration monitor used to measure the root oxygen uptake is shown in Figure 4. A four liter flask modified as a Mariotte flask (a) served as the reservoir for the one-half strength modified Hoagland's solution. The siphon from this flask delivered solution to a 500 ml thermos bottle serving as the gas equilibrium chamber (c). Treatment gases from bottled gas (b) were equilibrated in the nutrient solution by vigorous mixing through gas dispersion tubes. A magnetic stirring bar aided in bringing the solution to gas equilibrium. The gas equilibrated solution was pumped (d) from the gas equilibrium chamber to a smaller chamber (e) within the water bath (i) housing the oxygen probe chambers. This chamber allowed the solution to reach temperature equilibrium with the water bath while gas was dispersed through the solution. Siphons (not shown in diagram) were connected to this chamber from the gas equilibrium chamber as well as the two chambers containing the oxygen probes. The siphons maintained the level of solution to the level of the air inlet tube of the Mariotte flask. Temperature equilibrated solutions were pumped through the oxygen probe chamber I (f) via access holes (2.4 mm) which were drilled through the acrylic oxygen probe plunger. The chamber contained 5 ml of solution. A magnetic stirring bar at the base of the chamber prevented oxygen diffusion gradients from developing around the electrode membrane. The solution was then transferred to the glass chamber containing the root system (j). Solution exiting the root chamber flowed through a vertical float type flow meter (l). Temperature of the returning solutions were equilibrated by a glass thermal exchange coil (m)

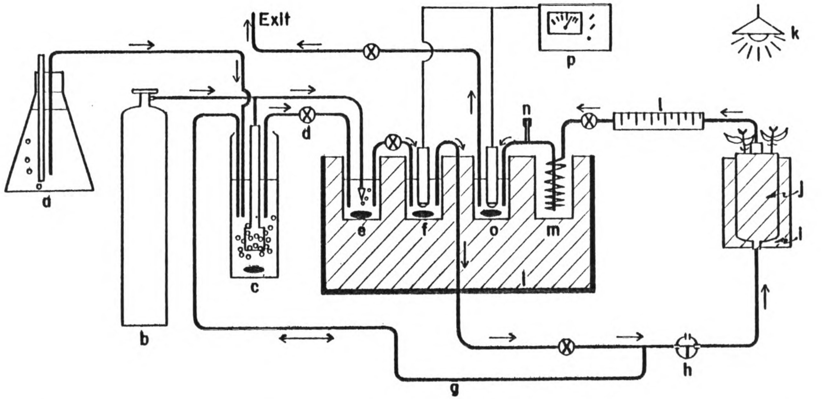

Figure 4. Diagram of the root respiration monitor. a. Mariotte flask, b. treatment gas, c. aerator flask, d. peristalic pump, e. equilibrium chamber I, f. oxygen probe chamber I, g. pressure relief line, h. three way valve, i. water bath, j. root chamber, k. mercury halide lamp, l. flow meter, m. equilibrium chamber II, n. access port, o. oxygen probe chamber II, p. YSI oxygen monitor.

in the oxygen monitor water bath. The solution next flowed through the oxygen probe chamber II (o) as described for the first probe. This chamber had a slight positive pressure preventing the entry of solution from the siphon tube. The solution was then pumped into a reservoir for waste or sampling.

A small line (g) (ID 1 mm) served as a shunt between (j) and (c) to minimize pressure build up within the root chamber. This line also served to trap air bubbles introduced into the system. A three-way valve (h) was also positioned near this line providing the possibility of obtaining solution from another source in the event of air introduction in the first half of the system. A Y tube (n) fitted with a septum was placed between the oxygen probe chamber II and glass thermal exchange coil and served as an air entrapment device. A similar tube (not shown in the diagram) was placed between the temperature equilibrium chamber I and oxygen probe chamber I.

Flow through the oxygen monitoring system was regulated with an eight channel Miniplus II HP8 peristalic pump. Flow rates from 6 to 12 ml/min were achieved. Oxygen partial pressures were recorded on a Linear Instruments Corp. Model 282/MM recorder from either of the two oxygen probe chambers by means of a switch on the oxygen monitor control unit (p). Temperature was maintained in the oxygen probe chambers at 20 ± 0.05°C and the glass chamber at 21 ± 0.1°C by a Lauda K-2/R circulating water bath. A mercury halide lamp (k) provided 680 μEinsteins sec^{-1} m^{-2} of light for the seedlings at 70 cm from the light source. The photosynthetically active radiation at this distance was similar to that in the growth chamber, although the radiation intensity gradient was three times greater.

All measurements were made in a constant temperature room maintained at 20 ± 2°C. All of the solution carrying lines and the oxygen monitoring system except for the part containing the glass chamber were shielded from the light source. Photographs of the system used to monitor oxygen uptake by roots are shown in Figure 5.

Measurement of Oxygen Uptake

The oxygen monitoring system was calibrated daily with circulating 0.210 atm oxygen saturated nutrient solution. Calibration curves based on standard oxygen concentrations indicated similar linear responses for static and circulating systems, Figure 6. A slight deviation from the linear for the circulating system was noted at the lowest oxygen concentrations. During the initial calibration procedure the current output - applied voltage relationship of the oxygen probes were investigated. Sensor performance was determined to be satisfactory if the relationship indicated a departure from linearity of less than 0.5% (111). The probe membranes were inspected daily for air bubble inclusion. Abnormalities were corrected before proceeding with measurements. Calibration with the gas used in the measurement was carried out before and after each measurement. After calibration, the pyrex root chamber was connected to the system. An initial drop in oxygen concentration was followed by a recovery until equilibrium was reached. This was caused by the consumption of oxygen occurring during the time of transfer from the growth chamber to the oxygen measuring system. Measurements were continued at least fifteen minutes after reaching apparent equilibrium as indicated by a steady flat trace on the recorder. Measurement time generally took from 1 to 2 hours. The time to reach equilibrium was variable and

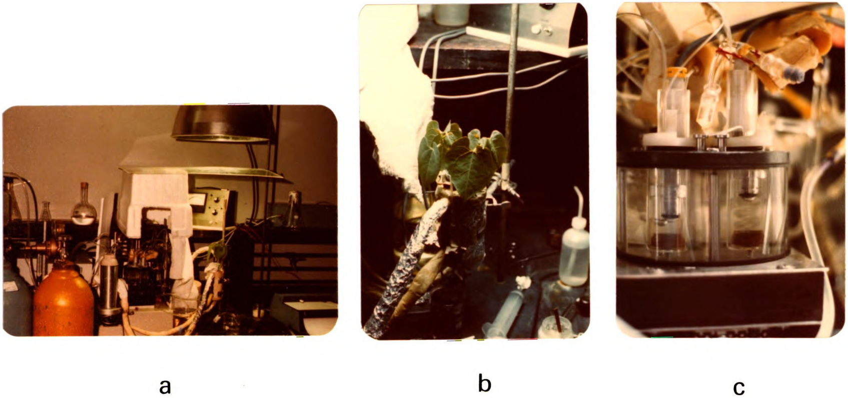

Figure 5. System used to monitor root oxygen uptake. a. An overall view of the system. b. Water bath containing the root chamber. c. Water bath containing polarographic oxygen sensors.

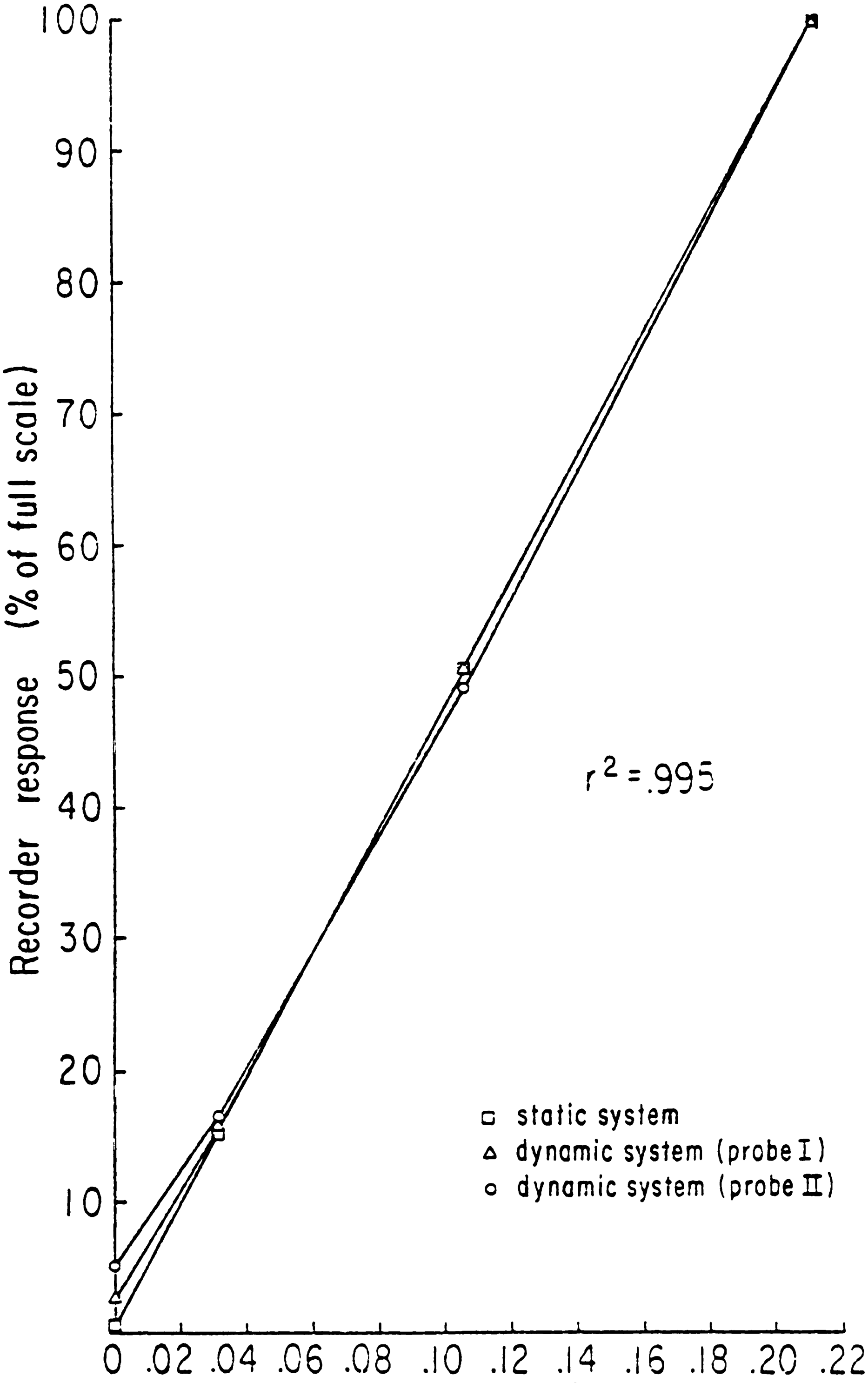

Figure 6. Calibration curves for the root respiration monitor.

depended on solution flow rate, time taken to transfer, and oxygen sink of the rhizosphere.

Samples of nutrient solution which had passed the root system were taken at the termination of the measurement period. Samples were placed into 10 ml vacutainers acidified with sulfuric acid to a pH of 2 to prevent microbial growth and release carbon dioxide from the solution. These samples were stored in a refrigerator for possible analysis at a future time. Solution flow rates were also taken at the termination of the experiment by determining the time for 6 to 8 ml of solution to collect in a pipette. The pyrex root chamber was either placed back in the growth chamber or the plants were harvested depending on the experiment conducted.

Oxygen uptake per hour was determined by the following equation:

$$x = (6.234)(r)(60)(d)$$

where x is the oxygen uptake per hour ($\mu l\ O_2\ hr^{-1}$); d is the differential pO_2; r is the flow rate ($cm^3\ min^{-1}$); and 6.234 is the solubility of oxygen in solution at 21°C.

Microbial respiration was estimated by removing the root system, replacing the solution and matrix treatment and measuring the resulting oxygen uptake. Chambers with root systems removed were stored at 4°C for 1 day before microbial respiration could be measured. Comparisons of experimental units before and after refrigerated storage indicated that the period of storage had little or no effect upon respiration rates.

Transmission Electron Microscopy of Treated Roots

Roots were removed from each of the three matrix treatments at 14 days. Four to six 3 mm sections were taken from representative axial root tips, lateral root tips and 1 cm above the lateral root tip. The sections were placed in vials containing 5% gluteraldehyde in 0.1 M Sorenson buffer. The vials were placed in an ice bath and carried to the Center for Electron Optics at Michigan State University for further processing. The samples were stained with osmium, embedded, sectioned and placed on a grid for observation under the transmission electron microscope. Samples were taken from roots treated with 24 hour treatments of 0.210 atm pO_2 and 0.00 atm pO_2 (nitrogen gas saturated solution).

Measurement of Root Porosity

Root porosity was determined by the procedure outlined by Jensen et al. (53). The root porosity was determined using the following formula:

$$\text{root porosity} = 100(Wh-Wr+w)/(Ww+Wr-Wr+w)$$

where W_h = weight of homogenated root and pycnometer

W_{r+w} = weight of root, water and pycnometer

W_r = fresh weight of root

W_w = weight of water and pycnometer

The denominator when multipled by the density of water at 20°C is the fresh root volume. The numerator when multiplied by the density of water at 20°C is the volume of air spaces in the root. The fresh root weight and root volume were used to calculate the root tissue density. Root porosity determinations were tedious especially during tissue homogenation. At times mishaps would occur resulting in the loss of homogenate. This was noted and the root porosity determinations for these samples were discarded.

Measurement of Shoot and Root Morphology and Growth

Slides were taken of the intact plants in the pyrex root chambers before harvesting. Plants were removed from the root chambers by removing the shoot at the base and carefully removing the stopper of the chamber. Leaf area was measured using a Lambda LI-3000 leaf area meter. Shoot fresh weights and dry weights were also measured. The roots were carefully tapped out of the chamber and removed from the matrix. The roots were then washed and carefully laid out on a grid in a tray containing water, Figures 14 and 15. The roots were separated and photographed on the grid. The grid consisted of squares with lengths of 0.75 cm. Slides were used to document root morphological differences among treatments, and to measure root length and diameter.

Root length was measured from the slides by projecting them onto a translucent screen and tracing roots with an inch counter (83). Measurement of root length was made in square areas consisting of 10 grid squares per side (100 grid squares). The measurement areas were given a vertical and horizontal vector designation. Figure 7 designates the measurement areas used. Root length was determined using a scaling factor based on the known length of the grid squares. Total root length was determined by summing the root lengths of the measurement areas.

Root diameter was determined by randomly selecting at least 30 roots per measurement area and measuring the diameter from the projected slide with a ruler. A scaling factor was also used to obtain actual diameters. The length and diameters of primary roots were measured separately.

Two methods were used in this study to measure root volume. Root volume was estimated by the calculation method using the root length (h)

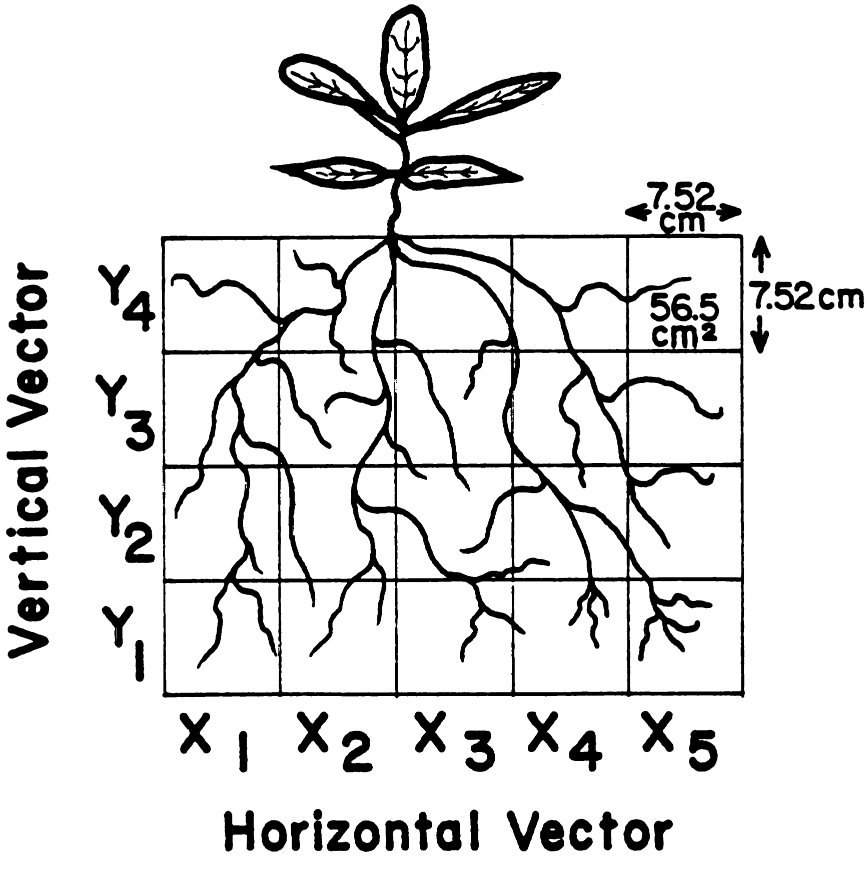

Figure 7. Root length measurement grid.

and diameter (d) measurements. Estimates were made by approximating the roots to a cylinder (69). The volume of a cylinder = $\pi(d/2)^2h$. Root volume was also determined by water displacement from the root porosity measurements (53). This method was a more precise and less time consuming method than by measurement and calculation. These two methods were highly correlated, Figure 8. Root volume measurements tended to be lower for the calculation method. The differences between these methods of estimating root volume were similar among matrix treatments, Figure 9. A major source of error in the calculation method is the use of representative root diameters. This results in the treatment of the root system as a uniform cylinder. Any error in the diameter representation is magnified when finding the volume due to the squaring of the radius.

Root systems were measured for fresh weight and placed in a drying oven. Roots dried at 70°C were weighed to obtain root dry weights.

Measurements of Waterlogged Treatments

Waterlogging involved a 24 hour treatment period during which nutrient solution was not circulated past the root system. Since nutrient solution remained in the root chamber this treatment lent itself to a number of measurements which could not be made on the other treatments. Measurement was made of water uptake and root exudation of ethanol and amino acids during the 24 hour period of waterlogging.

Water uptake was measured by connecting the exit siphon from the pyrex root chamber to a parafilm sealed test tube containing 40 ml of nutrient solution. The amount of water removed from the test tube was measured after the 24 hour period.

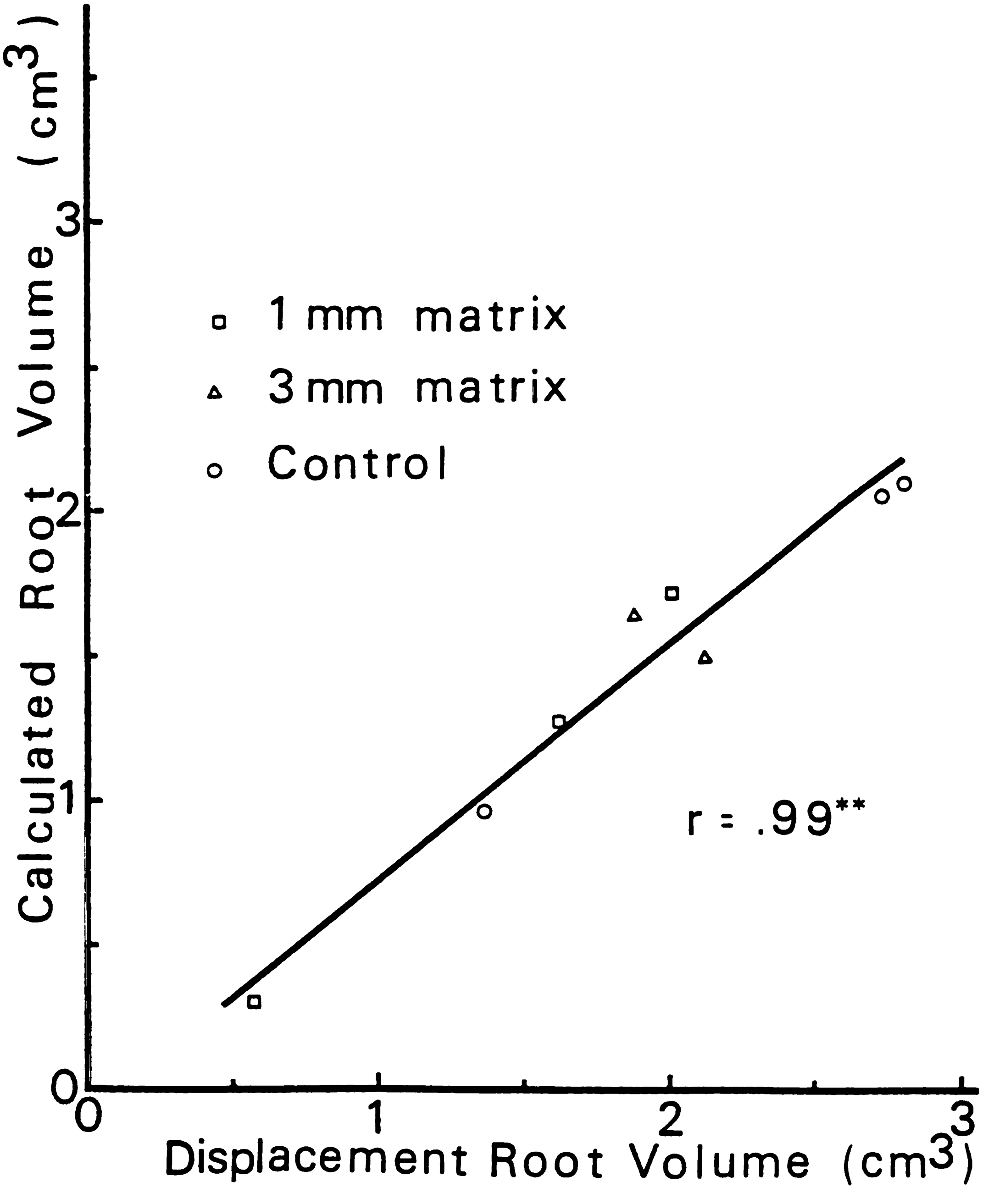

Figure 8. Comparison of methods for determining root volume.

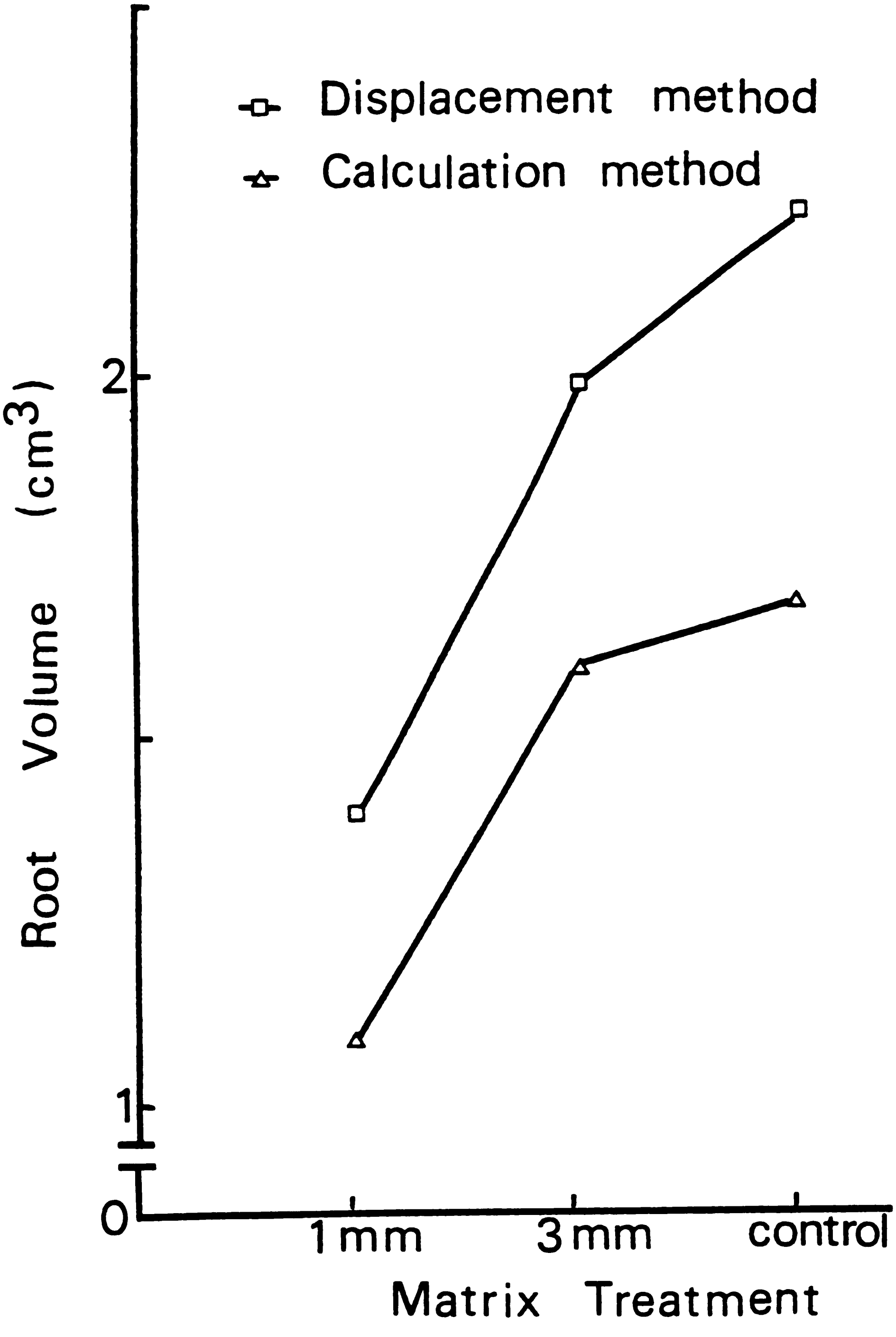

Figure 9. Comparison of methods for determining root volume responses to matrix treatments.

Samples of solution were taken from the chamber at the end of the 24 hour period, stored in a freezer, and analyzed for ethanol and amino acid contents. Ethanol content was measured by a GC2-A Beckman gas chromatograph equipped with a hydrogen flame detector. Samples, 1 µl, were eluted through a 6 foot x 1/16 inch stainless steel column containing Porapak QS, 100/120 mesh. The carrier gas, N_2, flowed 80 ml/min through the column at isothermal conditions of 180°C. Ethanol content of the nutrient solution was determined by comparing the area under sample peaks with the area under peaks produced by injections of ethanol standards.

A modified Durrum HPLC amino acid analyzer was used to analyze solution for amino acid composition. A 0.3 x 25 cm column containing Durrum DA-X4-11 resin was used for amino acid separation. A Durrum femto - buffer under a column pressure of 300 to 330 psi and flowing at a rate of 12 ml/min was used to elute amino acids from the column. The column temperature was programmed to change from 45°C at 27 min to 65°C at the end of 49 minutes. Fluoropa (o-phthalaldehyde) was used as the detection agent. Amino acid amounts were determined by comparing standard chromatograms to the sample chromatogram.

RESULTS AND DISCUSSION

Oxygen Monitoring System

The oxygen monitoring system provided a means of continually monitoring oxygen uptake by roots. Root response could be accurately measured to ± 5 μl O_2 hr^{-1}. Over short intervals this measurement accuracy could be greatly increased by expanding the recorder scale through the use of an offset zero and increased recorder attenuation.

Preliminary measurements were made to determine the effects of nutrient solution flow rate on oxygen uptake by Phaseolus vulgaris roots. A root chamber containing two bean plants grown in nutrient solution for 17 days were used as the experimental unit. Oxygen uptake by this experimental unit was greater than those in succeeding experiments which used 14 day old plants. Figure 10 depicts the effect solution flow rate has on the change which occurs in the pO_2 of the nutrient solution after passing through the root chamber (exit pO_2). Also shown is the effect of solution flow rate on the oxygen uptake rate of the bean roots. Decreasing the nutrient solution flow through the root chamber resulted in lowered exit pO_2 for both the 0.210 and 0.105 atm pO_2 treatments (treatment pO_2). The slower the flow rate the greater was the difference in pO_2 between the lower and upper parts of the root system. The largest oxygen gradient which developed for a treatment pO_2 of 0.210 atm resulted in an exit pO_2 of 0.067 atm and this apparently did not affect the oxygen uptake of the root system.

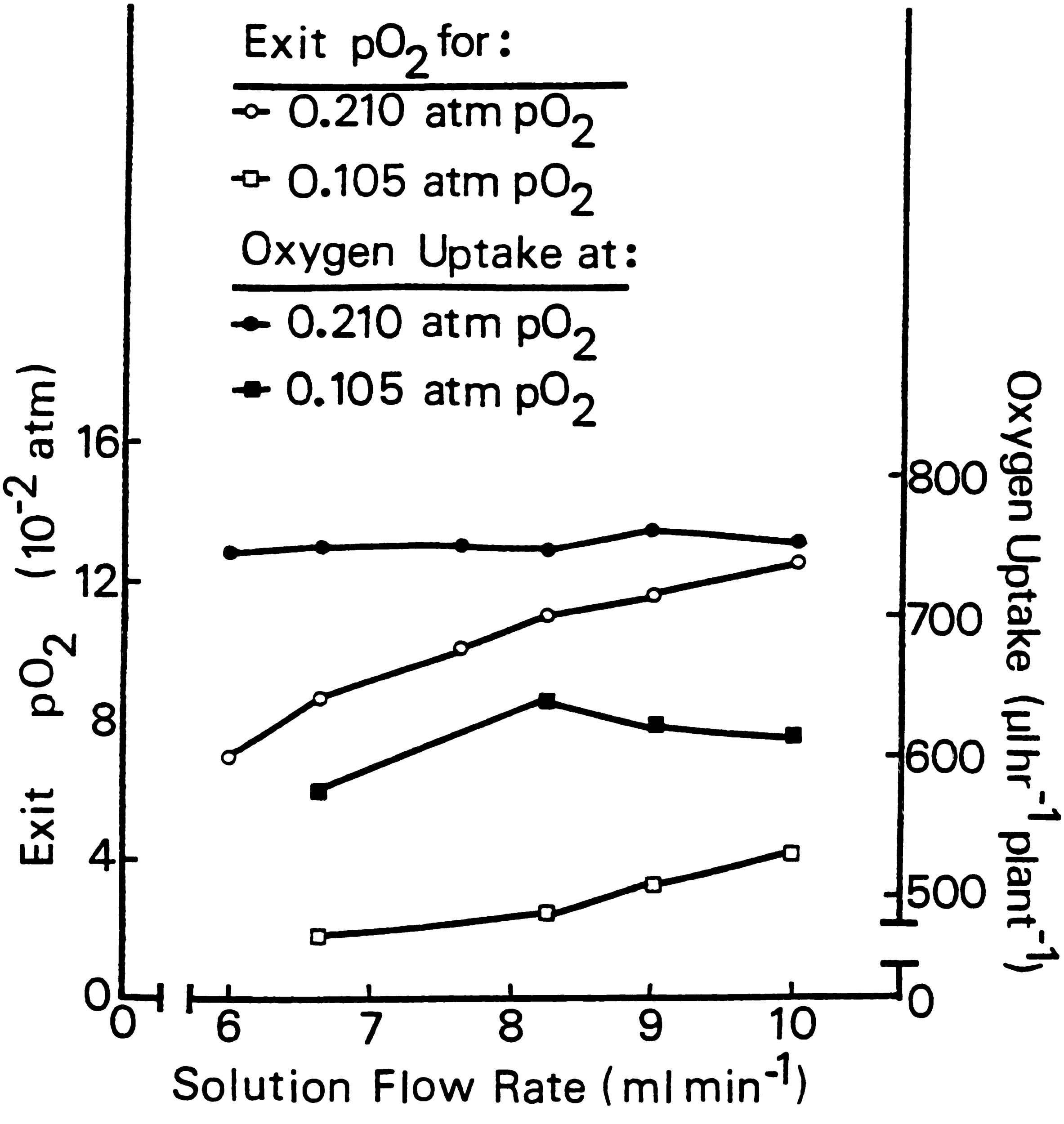

Figure 10. The effect of solution flow rate on exit pO_2 and oxygen uptake measurements. Results were obtained from the roots of two 17 day old *Phaseolus vulgaris* plants.

At a treatment pO_2 of 0.105 atm, the root system became more sensitive to changes in solution flow rate. Compared to the same root system exposed to a flow rate of 10 cm^3 min^{-1}, a solution flow rate of 6.7 cm^3 min^{-1} resulted in an exit pO_2 of 0.006 atm and a decrease in the oxygen uptake rate of approximately 60-80 μl O_2 hr^{-1}. This is small in comparison to the decrease of approximately 150 μl O_2 hr^{-1} in root oxygen uptake caused by a change of the treatment pO_2 from 0.210 to 0.105. This is expected since the whole root system is subjected to a substantially lower oxygen partial pressure when the aerating gas is changed from 0.210 to 0.105 atm. When the flow rate is altered only a small change in oxygen partial pressure results to the whole root system. However, at low oxygen partial pressures the flow rate may have an effect on oxygen uptake because of the hyperbolic relationship of oxygen uptake to oxygen partial pressure (110). As the oxygen partial pressure approaches zero, small changes in the gradient can cause a greater amount of inhibition than at higher oxygen partial pressures.

A preliminary study was conducted to determine the influence of pO_2 on oxygen uptake. Oxygen gradients encountered when measuring oxygen uptake of 14 day old bean roots indicate that there was very little loss of oxygen in solution when running the system without roots, Figure 11. A comparison of the response without plants and with plants indicates a substantial uptake of oxygen by the root system. The tendency of the two lines to converge is an indication that less oxygen is being used at low pO_2. Figure 12 indicates that oxygen uptake rates are lower for the lower treatment pO_2. The large range for each treatment pO_2 includes response due to different sized root systems, different

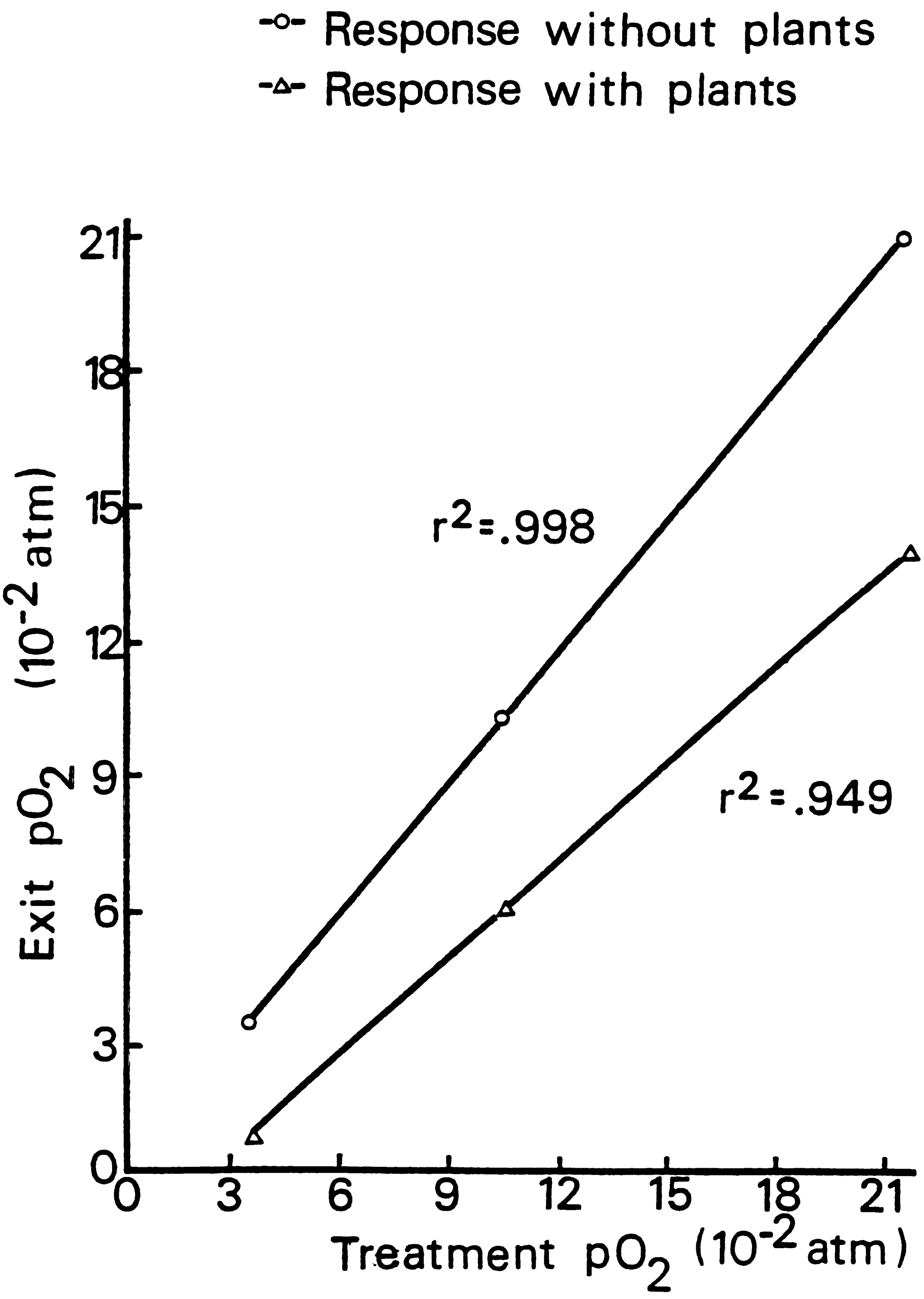

Figure 11. Relationship of exit pO_2 to treatment pO_2 for the root respiration monitor.

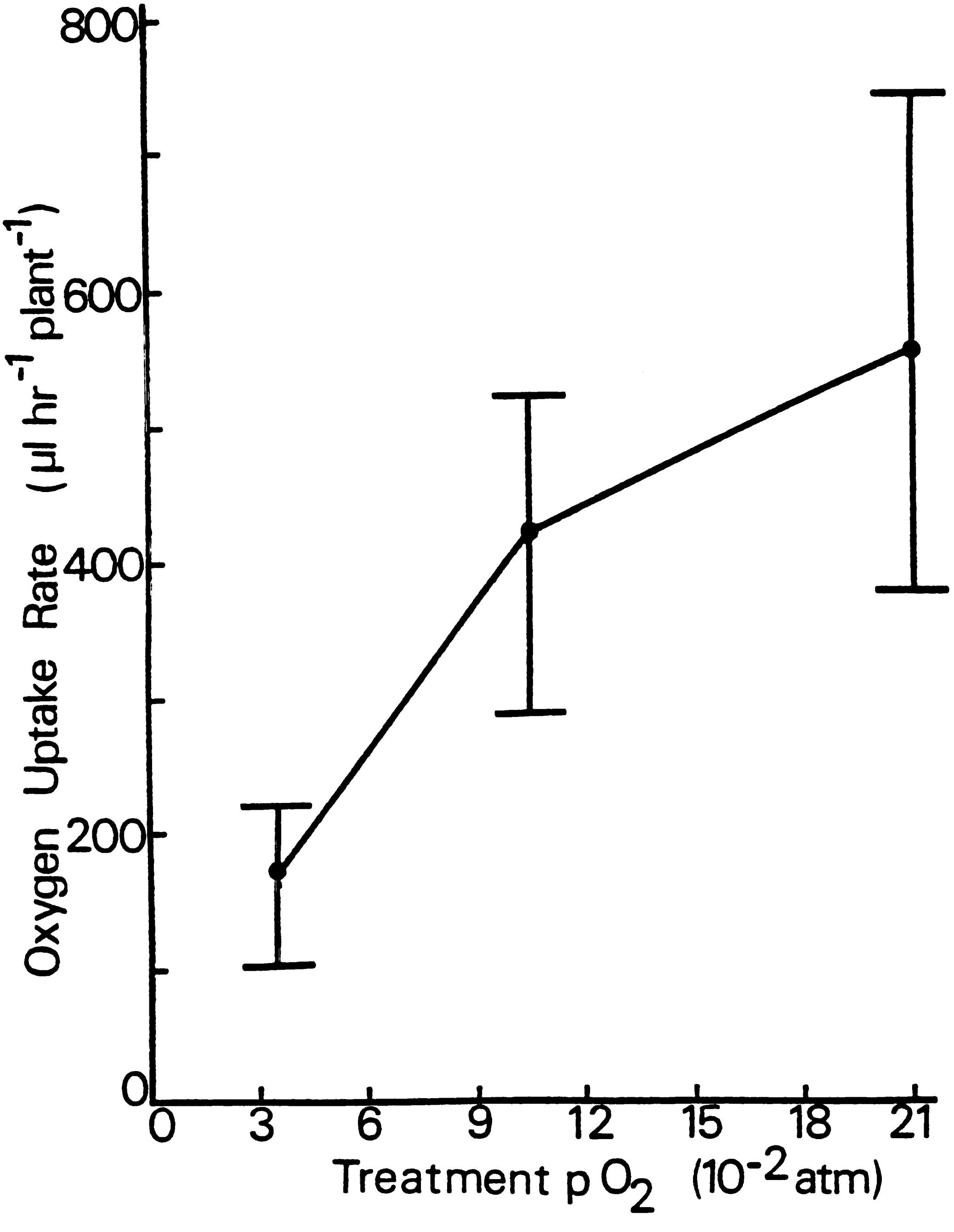

Figure 12. Relationship of oxygen uptake to treatment pO_2 for 14 day old dry bean roots. Brackets indicate the range of response.

levels of microbial background, difference among matrix treatments and experimental variability.

The form of the curve in Figure 12 indicates a hyperbolic relationship for pO_2 levels below 0.21 atm which is similar to other reports (110). When interpreting data resulting from oxygen treatments it is important to note that the oxygen treatment represents a pO_2 gradient within the root chamber. Treatment of 0.210, 0.105 and 0.032 atm of oxygen produced exit pO_2 as low as 0.140, 0.030 and 0.000 atm of oxygen, respectively. Comparisons of oxygen uptake with flow rate demonstrated that the 0.210 atm pO_2 treatment did not have oxygen uptake rates which were dependent on the oxygen gradient within the chamber, Figure 10. As a result, measurements were made using an entry solution equilibrated with 0.210 atm pO_2. In addition to minimizing the effects of an oxygen gradient in the chamber, this removed the recorder response from the non-linear region of the calibration curve, Figure 6.

The effects of ionic strength on oxygen uptake by roots of corn seedlings were measured using nutrient solutions. There was a trend for an increase in oxygen uptake with solution conductivity, Figure 34. Consequently, all measurements were made using one-half strength Hoagland's solution which was passed by the root system once and then discarded.

Diurnal changes in oxygen uptake were negligible during the period from 9:00 am - 9:00 pm, Table 3.

Table 3. Influence of diurnal measurements on root respiration.

Values are average of six replications.

Time	Oxygen uptake rate per root dry wt ($\mu l\ O_2 hr^{-1} g^{-1}$)	Relative oxygen uptake rate	Coefficient of variation (%)
10:00 AM - 3:00 PM	5503	1.00	37
3:00 PM - 8:00 PM	5527	1.01	37
8:00 PM - 1:00 AM	5922	1.09	35
LSD .10	N.S.	N.S.	

Mechanical Impedance and Root Growth

Data from Tables 4 and 5 demonstrate that during the eight days of treatment root systems grew extensively for all matrix treatments. Figure 35 gives an indication of the size of the root system at the initiation of the matrix treatments.

Matrix treatments differentially affected the rate and form of growth. Roots grown without glass beads accumulated fresh weight at twice the rate of the 1 mm bead matrix, 18 mg h^{-1} compared to 9 mg h^{-1} during the treatment period. Roots grew at an intermediate rate of 15 mg h^{-1} in the 3 mm bead matrix. Root growth rates were less in the matrix treatments as shown by the reduced root weight, length, volume and surface area for these treatments, Table 5. Table 6 compares primary root growth with lateral root growth. Primary root length was impeded by the 3 mm and 1 mm matrices. Primary root tip cells were plasmolysed for roots grown in 1 mm matrices but not for the other matrix treatments, Figure 13. This suggests that the primary roots grown in 1 mm matrices have lost meristematic ability while the primary roots grown in 3 mm matrices have retained this ability. Root growth appears to continue at slow rates if meristems subjected to high external pressures are not injured (86). This has important implications for roots growing through hard pans. Presumably a slow growing root could eventually penetrate the pan if mechanical impedance was not so great as to damage the meristem.

Total lateral root length was also reduced by the glass bead matrix treatments, Table 6. Comparisons of lateral root length per unit primary root length indicate a direct relationship between relative lateral root

Table 4. Fresh and dry weights of Phaseolus vulgaris shoots and roots on the day of transplanting to pyrex root chambers. Values are an average of six replications.

	Root fresh weight	Root dry weight	Shoot fresh weight	Shoot dry weight
	(g/plant)			
Mean	0.19	0.013	0.66	0.145
Standard deviation	0.05	0.003	0.13	0.030

Table 5. Influence of matrix treatment upon root growth of 14 day old *Phaseolus vulgaris* L. (var. Seafarer).

Treatment	Root fresh wt (g/plant)	Root dry wt (g/plant)	Root volume† (cm^3/plant)	Root surface area (cm^2/plant)	Root length (cm/plant)	Root volume‡ (cm^3/plant)
Control	1.93	0.101	2.02	143	1018	1.68
3 mm matrix	1.62	0.082	1.81	92	582	1.21
1 mm matrix	1.07	0.063	1.17	70	445	0.89
Number of replications	13	13	10	5	5	5
LSD .01	0.35	0.021	0.61	N.S.	N.S.	N.S.
LSD .05	0.35	0.015	0.44	70	522	N.S.

†Measured by the displacement method.

‡Calculated from length and diameter.

Table 6. A comparison of lateral and primary root growth of beans (var. Seafarer) for the three matrix treatments. Each value is a mean of five replications.

Treatment	Total lateral root length (cm/plant)	Primary root length (cm/plant)	$\frac{\text{Total lateral root length}}{\text{Primary root length}}$ (cm/cm)	Primary root diameter (cm)	Lateral root diameter (cm)	Primary percentage of total root surface area (%)
Control	950	68	14	0.08	0.04	13.2
3 mm matrix	536	46	12	0.08	0.05	14.1
1 mm matrix	406	40	10	0.08	0.05	19.4
LSD .05	507	20	N.S.	N.S.	N.S.	N.S.

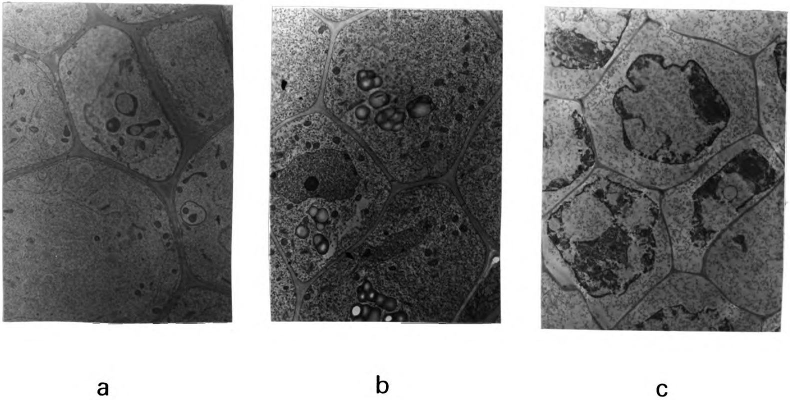

Figure 13. TEM micrographs of representative primary root tip cross sections (x3200). a. Control, b. 3 mm matrix, c. 1 mm matrix.

growth and matrix pore space, Table 6. The reduction in lateral root growth per primary root length accounts for 22 and 29% of the lateral root length reduction for the 3 mm and 1 mm matrices, respectively. The remaining reduction in lateral root length results from the smaller primary roots being unable to initiate or support as many lateral roots as a longer primary root. Compensatory lateral root growth, reported by Goss with barley (37), was not found in this study. These differences could be due to the different root diameters of the two species, reported in these two studies.

Phaseolus vulgaris root diameters, Table 6, were found to be twice as large as barley roots. A comparison of lateral root diameter and the matrix pore diameter, Table 2, indicate that both lateral and primary bean roots should encounter a considerable mechanical impedance to growth by the 1 mm matrix treatment and less for the 3 mm matrix treatment. Wiersma (104) demonstrated that the rigidity of a matrix system decreases as the width of the chamber used increases. Lateral roots growing in the 1 mm matrix treatments may have been able to elongate by deforming the matrix.

Primary root diameters were unchanged by matrix treatment since primary root growth was limited by both the 3 mm and 1 mm matrices. Lateral root diameters increased for the glass bead matrix treatments. This increase in root diameter agrees with other reports (86) relating root responses to mechanical impedance. Ethylene may be responsible for the increased root diameters caused by mechanical impedance (11).

The contribution of primary roots to the total surface area is shown in Table 6. Roots grown in the 1 mm matrix appeared to have a greater percentage of surface area from primary roots than the control and 3 mm matrix treatments. Lateral root growth in the 1 mm matrices also differed considerably from the other treatments. Lateral roots from the 1 mm matrix were considerably shorter in length but more numerous than for the other matrix treatments, Figure 14. Lateral roots grown in the 1 mm matrix were initiated closer to the root apex and were clumped together more than the other treatments. A close up of a primary root with associated lateral roots depicts the contrast between treatments, Figure 15a. Increased lateral root clumping for the 1 mm matrix treatment appears to be the result of increased lateral root initiation, smaller roots and the formation of secondary and tertiary lateral roots. Figure 15b compared representative lateral roots from the three treatments demonstrating secondary lateral root formation on the 1 mm matrix lateral root. Three mechanisms have been postulated to explain the increased formation of lateral roots close to the root apex, these are: i. increased promoter from mature tissues (73), ii. decreased inhibitor production in the root apex (100) and iii. ethylene stimulated root formation (100). Tissue maturing closer to the root apex resulting from the slower growing roots (86) could have resulted in the greater production of a promoter. Damage to the root apex may have reduced inhibitor synthesis. Wounding from mechanical impedance (58) and bending around obstructions (i.e. glass beads) (73) could have resulted in ethylene formation. All three mechanisms may be acting together to increase lateral root formation in the 1 mm matrix treatment.

Influence of the matrix treatment on lateral root formation is apparent in the amount of curvature and distortion observed, Figure 15b. Flattened regions were formed on the lateral roots grown in glass beads.

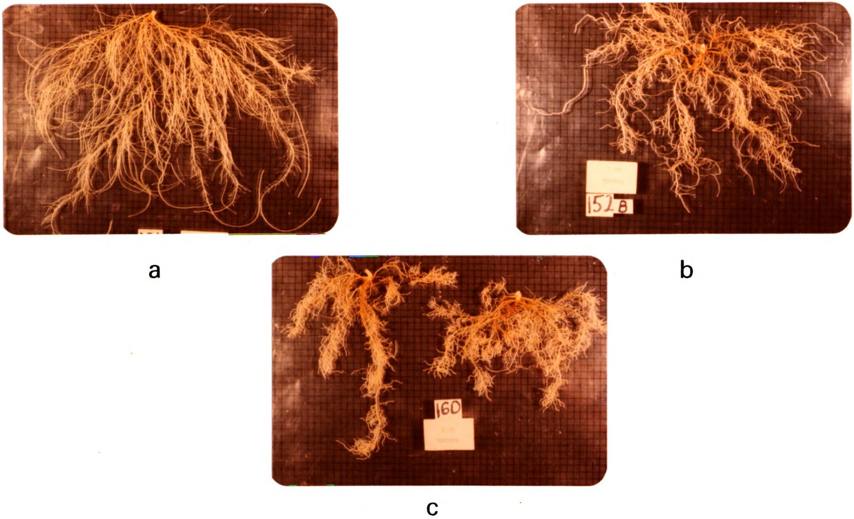

Figure 14. Root systems of the three matrices displaced on grid used to measure root length and diameter. a. Control, b. 3 mm matrix, c. 1 mm matrix.

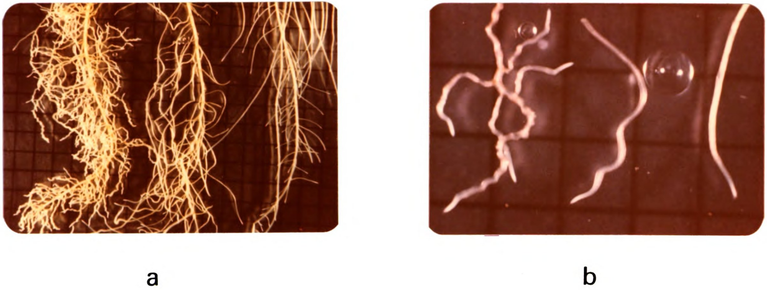

Figure 15. Comparisons of the three matrices effect on root morphology. a. Primary roots, b. Lateral roots. In each figure roots from left to right represent the 1 mm matrix, 3 mm matrix and control treatments, respectively.

Trouse (101) observed similar flattened regions in roots grown in dense soil. He suggested this may occur when roots move into a fracture and the elongating cells are allowed to expand ahead and parallel to the fracture but not perpendicular to the fracture. The effect this has on the association of the roots with the glass beads is demonstrated in Figures 16 and 17. The 1 mm beads were tightly associated with the root system and many of the beads could only be removed through the careful use of small tweezers. Presumably, the root surface area in contact with beads was unavailable for nutrient and gas exchange with the surrounding solution. Greenwood (41) suggested that such contact acts in soil to reduce oxygen availability to the root.

Distortion of lateral roots was also observed at the cellular level, Figure 18. The cortical cells from the control were spherical in shape and surrounded by abundant intercellular spaces. Cells from the 3 mm matrix treatment appeared to be intermediate in character. Cells from the 1 mm matrix treatment were distorted and generally appeared smaller than the other two treatments.

These observations agree with Goss and Walter (38) who observed distortions in older cortical cells of barley roots growing in a matrix of 1 mm glass beads. Barley and Greacen (12) suggested that cellular distortions could lead to a compression of intercellular spaces resulting in the loss of internal aeration.

Root porosity determinations were carried out to determine if the compression of intercellular spaces resulted in significant losses of internal air space. Matrix treatments significantly affected root porosity, root tissue density and root dry matter percentage, Table 7. The 1 mm matrix treatment significantly lowered root porosity, increased

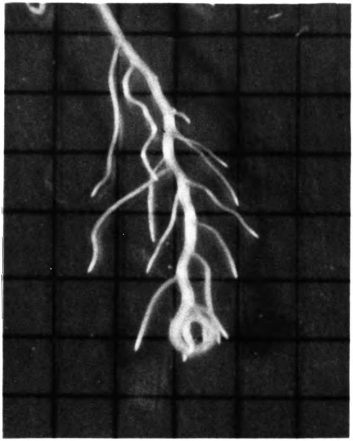

Figure 16. Root growth around a 3 mm glass bead.

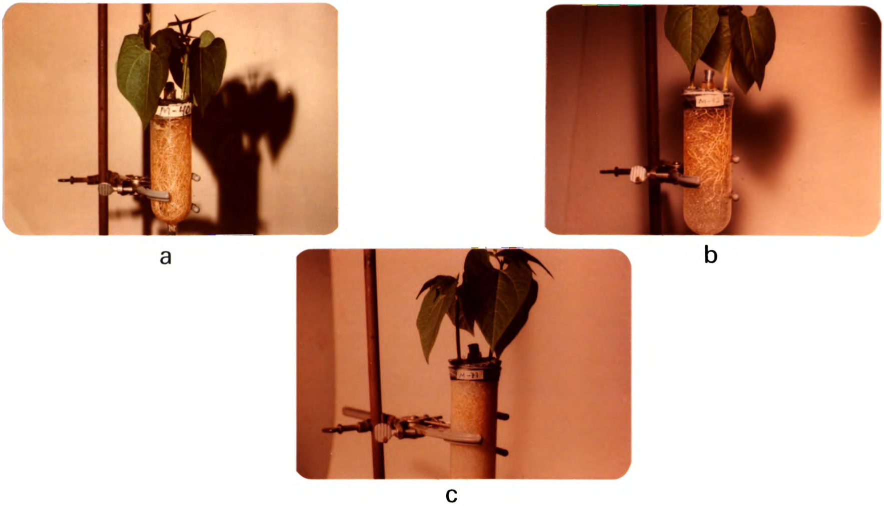

Figure 17. Root chambers containing 14 day old Phaseolus vulgaris plants growing in different matrices.

a. Control, b. 3 mm matrix, c. 1 mm matrix.

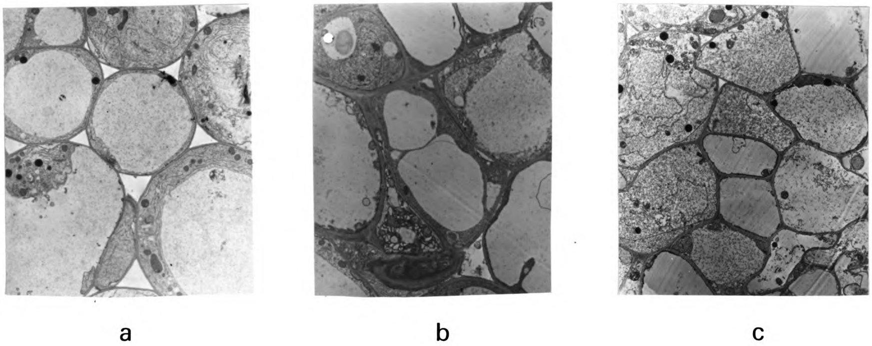

Figure 18. TEM micrographs of cortical cell cross sections. Samples were taken 1 cm above the lateral root tip for all matrix treatments (x3200). a. Control, b. 3 mm matrix, c. 1 mm matrix.

Table 7. Influence of matrix treatment upon root structural parameters of 14 day old <u>Phaseolus vulgaris</u> L. (var. Seafarer).

Treatment	Root† tissue density (mg/cm^3)	Root porosity (%)	Root dry matter percentage (%)	Root‡ cellular density (g/cm^3)
Control	994	3.41	5.3	1.026
3 mm matrix	993	3.61	5.1	1.023
1 mm matrix	1010	2.17	6.2	1.030
Number of replications	10	8	13	8
LSD .01	12	1.16	0.7	N.S.
LSD .05	9	0.83	0.5	N.S.

†Based on root volume measured by the water displacement method and root fresh weight.

‡Calculated by subtracting root air volume from total root volume.

root dry matter percentage and increased density of root tissue. These parameters were similar for the 3 mm matrix and control treatments because the majority of new growth of these treatments occurred as the result of relatively unimpeded lateral root growth. The 3 mm matrix primary root was mechanically impeded and the amount of new growth from this source was less than 14% of the total new growth (primary roots accounted for 14% of the total root surface area, most of which occurred before treatments were installed).

Root porosity is an important factor in the internal aeration of roots. Decreased root porosity could increase tortuosity and interrupt interconnecting intercellular spaces resulting in reduced diffusion to the root from the shoot (31) and reduced radial aeration from the media surrounding the root. Diffusion of oxygen through a water film at the root and soil interface and into intercellular gas spaces could eliminate much of the very slow diffusion of oxygen through living tissue (64). It is suggested that the reduced root porosity of 1 mm matrix treatments reduced the availability of oxygen to internal root cells.

Reduced root porosity may also lead to greater accumulations of ethylene or other volatile growth regulators within the root tissue (11). Since mechanically impeded roots may produce six times more ethylene than nonimpeded roots (58), high local ethylene concentrations could develop. Decreased root porosity could result in the accumulation of ethylene within the root and change growth regulation patterns. Greater concentrations of localized ethylene could increase lateral root formation (73,87), change cortical cell growth (11), and inhibit root elongation (92).

The higher root tissue density for the 1 mm bead matrix treatment could be a result of decreased air volume of the root and/or an increase in the protoplasmic density of the root. Since overall root volume is composed of solid, liquid and air volumes a decrease in root air volume would result in an overall decrease in root volume. This would result in a decrease in the denominator of the root tissue density term (g/cm^3) and an apparent increase in root tissue density. An increase in solute concentration in the cell cytoplasm could also account for an increase in root tissue density through increasing the numerator much more than the denominator of the density term.

Root tissue density correlations with other root parameters, Table 8, indicate that the decrease in root air volume may be a greater factor than an increase in root dry matter percentage. One might expect the increased dry matter percentage to result in an increased root tissue density. The correlation coefficient for root tissue density with root dry matter percentage indicates some degree of association for combined treatments and for the 1 mm matrix, but not nearly as much as root porosity. The root tissue density with root porosity correlation coefficients indicate a highly significant association of increasing root tissue density with decreasing root porosity (i.e. decreasing air volume) for all matrix treatments. The nonsignificant relationship of root tissue density with root fresh weight is indicative of root tissue density (and root porosity) association with all other root growth parameters.

Relative contributions of the two factors contributing to the decrease in root tissue density was more closely examined by calculating the density of the solid and liquid portion of the root (root cellular density). The volume of the solid and liquid portion of the root was

Table 8. The effect of the three matrix treatments on the correlation of root tissue and cellular densities with selected root parameters.

Parameters correlated	Treatment r values			Combined r values
	Control	3 mm matrix	1 mm matrix	
Root tissue density with root porosity	-.86**	-.86**	-.88**	-.90**
Root tissue density with root fresh weight	.00	.15	-.40	-.42
Root tissue density with root dry matter percentage	.47	.50	.67*	.62**
Root cellular density with root dry matter percentage	.10	.63	.55	.46*

*,**Significant at .05 and .01 levels of probability, respectively.

found by calculating the air space volume from the root porosity and total root volume data and subtracting this from the total root volume. The root cellular density was found by dividing the root fresh weight by this volume.

Results shown in Table 7 indicate that the root tissue density increase for the 1 mm matrix treatment resulted from the reduction in air space volume. There is a trend suggesting increased root cellular density of the 1 mm matrix roots. Most of the difference in root dry matter percentage (70-90%) can be accounted for by the differences in root cellular density. The increased dry matter percentage for roots subjected to 1 mm matrix treatments could be the result of an increase in the proportion of cell wall material per cell. Tackett and Pearson (98) inferred from their thin section analysis of mechanically and non-mechanically impeded roots that the increase of root dry matter percentage was due to an increase in the percentage of root volume occupied by cell wall material.

Mechanical Impedance and Shoot Growth

Table 4 provides data on shoot fresh and dry weights before beginning the matrix treatments. The primary leaves were beginning to appear when matrix treatments were allocated to the bean plants. Figure 17 illustrates the shoot growth at a plant age of 14 days (8 days after beginning the matrix treatment). A considerable amount of shoot growth occurred in the 8 days of treatment. Generally, by the end of the treatment period the first trifoliate had appeared and was rapidly increasing in area.

The 1 mm matrix caused significant modification in shoot fresh weight and dry matter percentage, Table 9. Leaf area was related to the shoot fresh weight, Table 10, but the differences between treatments, Table 9, were not significant until the 10% probability level.

Total plant dry weight and leaf area ratio (LAR), Table 11, indicate that mechanical impedance had little or even a slight positive effect on the photosynthetic capabilities of the leaves. There was a significant shift in the distribution of plant photosynthates as shown by greater shoot to root ratios, Table 11. The lack of change in shoot dry weight, Table 9, between treatments indicates that photosynthate is being diverted from the root system to the shoot for the 1 mm matrix treatment. Aung (5) reviewed evidence demonstrating the interdependence of root and shoot growth on photosynthate supply. Studies were cited which demonstrated that as the root growth rate was slowed, photosynthate accumulates in the leaf. This is a possible explanation of the relatively high dry weight level in the shoot and the higher shoot dry matter percentage for the 1 mm matrix treatment.

Another explanation of the higher shoot dry matter percentage and shoot fresh weight is the effect of a small root system on water uptake. Brouwer (16) showed that leaf growth can be inhibited by the removal of a small part of the root system with only a small effect on the rate of transpiration. This explanation is given some credence by the correlation data in Tables 10, 12 and 13. Leaf area is highly correlated with root surface area and root dry weight for the 1 mm matrix treatment,

Table 9. Effect of matrix treatment upon shoot growth of 14 day old *Phaseolus vulgaris* L. (var. Seafarer). Values are means of eleven or more replications.

Treatment	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Leaf area (cm^2/plant)	Shoot dry matter percentage (%)
Control	2.91	0.337	82	11.8
3 mm matrix	2.51	0.331	77	13.1
1 mm matrix	2.27	0.341	66	14.9
LSD .01	0.59	N.S.	N.S.	1.0

Table 10. Separate and combined correlation of leaf area with selected plant parameters for the three matrix treatments.

Parameters correlated	Treatment r values			Combined r values
	Control	3 mm matrix	1 mm matrix	
Leaf area with root dry weight	.62*	.30	.80**	.57**
Leaf area with root surface area	.64	.02	.98**	.73**
Leaf area with shoot fresh weight	.93**	.94**	.98**	.98**

*,**Significant at .05 and .01 levels of probability, respectively.

Table 11. Influence of matrix treatments upon selected plant parameters for <u>Phaseolus vulgaris</u> L. (var. Seafarer). Values are means of eleven or more replications.

Treatment	Shoot/root (fresh weight)	Shoot/root (dry weight)	Plant fresh weight (g/plant)	Plant dry weight (g/plant)	LAR (Leaf area/ plant dry weight) (cm^2 g^{-1})
Control	1.51	3.41	4.89	0.438	184
3 mm matrix	1.59	4.30	4.18	0.414	178
1 mm matrix	2.41	5.73	3.20	0.404	153
LSD .01	0.37	1.62	0.89	N.S.	N.S.

Table 12. The effect of the three matrix treatments on the correlation of shoot with root weight parameters.

Parameters correlated	Treatment r values			Combined r values
	Control	3 mm matrix	1 mm matrix	
Shoot dry weight with root dry weight	.55	.35	.49	.36
Shoot fresh weight with root fresh weight	.50	-.22	.77**	.53**
Shoot dry matter percentage with root dry matter percentage	.32	.53	.40	.53**

*,**Significant at .05 and .01 levels of probability, respectively.

Table 13. The effect of the three matrix treatments on the correlation coefficients of selected root parameters.

Parameters correlated	Treatment r values			Combined r values
	Control	3 mm matrix	1 mm matrix	
Root fresh weight with root dry weight	.80**	.81**	.94**	.90**
Root length with root dry weight	.93*	.93*	.96*	.93**
Root surface area with root fresh weight	.94*	.92*	1.00**	.97**
Root volume† with root fresh weight	1.00**	1.00**	1.00**	1.00**

*,**Significant at .05 and .01 levels of probability, respectively.

†Measured by displacement method.

Table 10. The correlation with root surface area is especially significant since a larger root surface area can result in less resistance to water uptake (77). The correlation of shoot and root fresh weight, Table 12, is probably due to the association of shoot fresh weight with leaf area, Table 10, and root fresh weight with root surface area, Table 13. The shoot dry matter percentage was apparently not related to the the root dry matter percentage for individual experimental units, Table 12.

A third possible explanation is that growth hormone production is disrupted or changed by the matrix treatments. The resulting change in growth hormone concentrations may trigger a shoot response which results in a higher dry matter percentage.

Mechanical Impedance Effects on Root Respiration

Oxygen uptake rates were measured at a plant age of fourteen days. Measurements were made on Phaseolus vulgaris roots grown in nutrient solution equilibrated with a pO_2 of 0.210 atm, all data reported in this section are for the three different matrix treatments replicated five times.

Microbial oxygen uptake rates were measured by removing the root system and measuring the resulting oxygen uptake by the media without roots. Microbial oxygen uptake rates were increased in the bead matrices compared to the control, Table 14. Microbial populations could be greater in the bead matrices due to increased root exudates of mechanically impeded roots (7). The larger microbial populations of the bead matrices may reflect the increased surface area of the matrices. Table 2 indicates that the surface area available for microbial attachment was 30 and 10 fold the control for the 1 mm and 3 mm matrices, respectively.

Table 14. Effect of matrix treatments on microbial and root respiration rates of *Phaseolus vulgaris* L. (var. Seafarer). Values are means of five replications.

		Root respiration rate - µl O_2 hr^{-1} $unit^{-1}$					
Treatment	Microbial respiration (plant)	Plant (plant)	Root fresh weight (g)	Root dry weight (g)	Root surface area (cm^2)	Root length (cm)	Calculated root volume (cm^3)
Control	109	509	270	5429	3.98	0.58	332
3 mm matrix	164	342	252	5052	4.33	0.68	255
1 mm matrix	175	330	326	5122	5.53	0.87	435
LSD .05	48	N.S.	68	N.S.	N.S.	N.S.	N.S.
LSD .10	39	162	55	N.S.	N.S.	.26	179

Oxygen uptake rates for Phaseolus vulgaris roots grown in the three matrix treatments are reported in Table 14. There were no differences between oxygen uptake rates per unit dry weight for all treatments. Oxygen metabolism was apparently unchanged by mechanical impedance. Observed differences in per plant oxygen uptake among treatments should be a function of the amount of dry matter found in the root systems. The amount of dry matter found in the root system was dependent on the size of the root system, the percentage of dry matter of the root system and the supply of photosynthate to the root system.

The larger oxygen uptake rates per plant found for the control roots was a result of the larger root system associated with nonimpeded roots. Standardization of the oxygen uptake rates for root system size results in oxygen uptake rates which are generally greater for roots grown in the 1 mm matrix. The difference in oxygen uptake rates was partly the result of different distributions of dry matter in the root, Table 15. The 1 mm matrix roots contained relatively greater amounts of dry matter for the root dimensional parameters as well as for the fresh weight parameter.

The increased diameters of the matrix grown roots, Table 5, also contributed to the differences in oxygen uptake rates. Average fresh weight per unit length of root was 2.7 g cm^{-1} for the 1 mm and 3 mm matrix treatments and 2.2 g cm^{-1} for the control treatment. This indicates a contribution from the changed root geometry corresponding to the larger diameters of matrix grown roots. A larger diameter results in more root tissue per unit length and per unit surface area of root. This resulted in a trend in which the 3 mm matrix treatment oxygen uptake was of an intermediate character for these two root parameters.

Table 15. Dry matter distribution ratios for roots used in respiration studies. Values are means of five replications.

	Root dry matter			
Treatment	Surface area (mg cm^{-2})	Length (mg cm^{-1})	Volume (mg cm^{-3})	Fresh weight (mg g^{-1})
Control	0.711	0.117	82	49
3 mm matrix	0.759	0.124	74	50
1 mm matrix	1.043	0.221	126	65
LSD .05	N.S.	0.082	N.S.	10

Dimensional root respiration parameters best describe root interaction with the microenvironment surrounding it. Oxygen uptake per root dry weight does not take into account any of the root structural parameters which affect the intensity of oxygen uptake in the immediate vicinity of the root. In this study, mechanical impedance apparently resulted in root structural changes which increased the oxygen needed from the microenvironment surrounding the root. Root fresh weight was highly correlated with volume and other dimensional root parameters, Table 13.

Another factor influencing root respiration may be competition for photosynthate. Hatrick and Bowling (46) demonstrated a complete dependence of root respiration on photosynthate flow to sunflower roots. They concluded that for young plants root metabolism was immediately dependent on shoot metabolism. A source-sink relationship can be postulated to exist between the shoot and root. Photosynthates are rapidly utilized in the root for the synthesis of complex compounds for the cell wall, cytoplasm and organelles. Sucrose and other simple organic compounds are kept at a low level and if not used, are converted to starch and stored in the meristem (79). In this study, shoot to root ratios were greatest for the 1 mm matrix and lowest for the control treatment. Previous mention has already been made of possible accumulation of photosynthates in 1 mm matrix shoots resulting from the smaller root sink. The oxygen uptake per unit fresh weight rates were found to be significantly correlated with shoot to root ratios for the control and 3 mm matrix, Table 16. For both treatments a larger proportion of shoot compared to root should result in a larger supply of photosynthate being made available per unit root. An increase in photosynthates for a rapidly growing root system which is serving as a strong assimilate sink should result in the

Table 16. Separate and combined correlation of root respiration with selected plant parameters for the three matrix treatments.

Parameters correlated	Treatment r values			Combined r values
	Control	3 mm matrix	1 mm matrix	
l O_2 hr^{-1} g^{-1} (FW) with				
Root length	-.82	-.66	-.52	-.58*
Root fresh weight	-.90*	-.87	-.62	-.73**
Root dry weight	-.87	-.62	-.67	-.66**
Shoot dry weight	-.14	.08	-.69	-.39
Shoot fresh weight	-.05	.74	-.79	-.33
Leaf area	-.24	.38	-.67	-.43
Shoot/root (fresh weight)	.90*	.96*	.55	.76**

*,**Significant at .05 and .01 levels of probability, respectively.

stimulation of oxygen uptake. Roots in the 1 mm matrix grew at a reduced rate and constitute a weaker assimilate sink. The larger shoot to root ratio for the 1 mm matrix roots indicates a relatively larger source of photosynthate for the root system. The greater source and weaker sink for the 1 mm matrix roots could have resulted in the reduced association of oxygen uptake rates with shoot to root ratios. Large root systems were associated with smaller rates of oxygen uptake per root fresh weight, Table 16. This may be due to a decrease in the respiration rates of older root tissues. Root respiration rates have been shown to vary with distance from the root apex with greater respiration rates associated with the elongating root tip region (69). Large root systems have smaller proportions of the roots consisting of apical or meristematic tissue which may result in lower oxygen uptake rates per unit root parameter. The noncorrelation of shoot parameters with oxygen uptake rates results because supply of photosynthate was a function of both sink (root) and source (shoot).

Mechanical Impedance and Response to Short Term Anoxia

A preliminary experiment was conducted in which root respiration was measured concurrently with selected oxygen treatments. Figure 19 represents oxygen uptake rates of the entire rhizosphere at selected oxygen levels. Two hour treatments of 0.105 atm and less pO_2 lowered oxygen uptake rates. Little interaction between the matrix and oxygen treatments were noted. The Pasteur effect upon root systems was measured by returning the root systems to 0.210 pO_2 after a two hour treatment at 0.00 pO_2. Oxygen uptake rates were increased 10%, 15% and 1% for the 1 mm matrix, 3 mm matrix and control treatments, respectively.

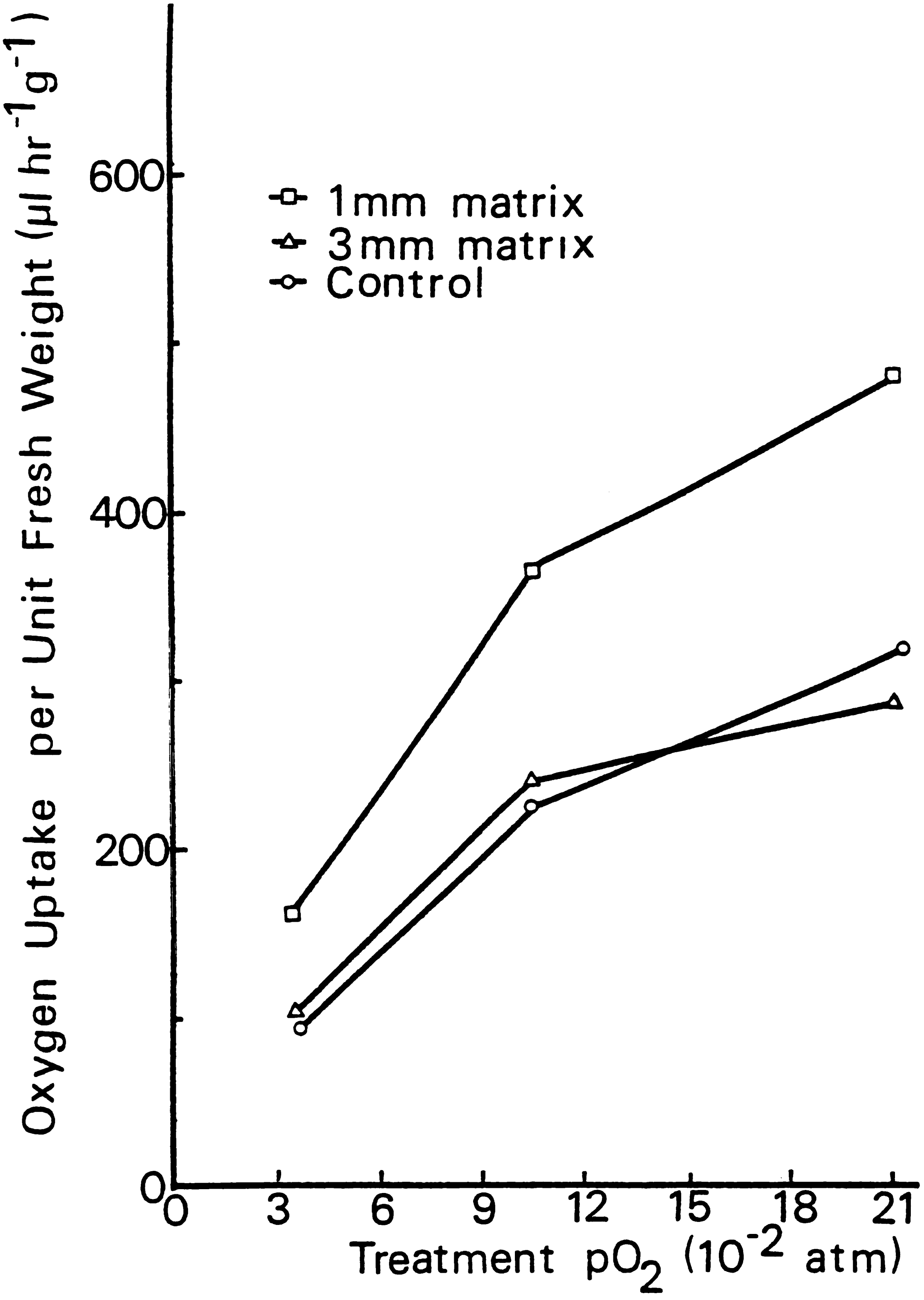

Figure 19. Oxygen uptake by 14 day old *Phaseolus vulgaris* roots and microbes as affected by matrix treatments during 2 hour oxygen treatments.

Oxygen uptake rates were difficult to measure at the lower pO_2 for reasons discussed in the oxygen monitor section.

The remaining experiments reported in this section consisted of a 24 hour treatment with randomly selected oxygen treatments given in the growth chamber. All oxygen uptake measurements were made at 0.210 pO_2 before and after the 24 hour treatment period.

Figure 20 represents the change in oxygen uptake rates (ΔOU) which occurred over the 24 hour treatment period. The decrease in ΔOU with lowered oxygen treatments reflects the lowered oxygen uptake rates observed in Figure 19. The Pasteur effect was not observed after 24 hours of anoxia. Oxygen uptake rates per plant were greater for the larger control roots when measured on day 13 and 14, Table 17. The increase in oxygen uptake between day 13 and 14 results from root growth which occurred during the 24 hour treatment period. Microbial respiration followed trends observed in the last section.

Figure 21 represents the ΔOU which occurred per plant during the 24 hour treatment period for each matrix treatment. The matrix systems interacted with the oxygen treatments, Table 18. When compared to the other treatments the control roots had the greatest increase in oxygen uptake rates at 0.210 atm pO_2 and the greatest decrease at 0.00 atm pO_2. Much of the matrix differences in ΔOU were due to the size of the root system. Figure 22 corrects for root system size resulting in response curves which are very similar for the three matrix treatments. Table 18 indicates that when corrected for root system size no interaction occurs between matrix and oxygen treatments.

Table 19 compares ΔOU and root growth rates for the 24 hour period of 0.210 atm pO_2. Root weights on day 13 were calculated assuming

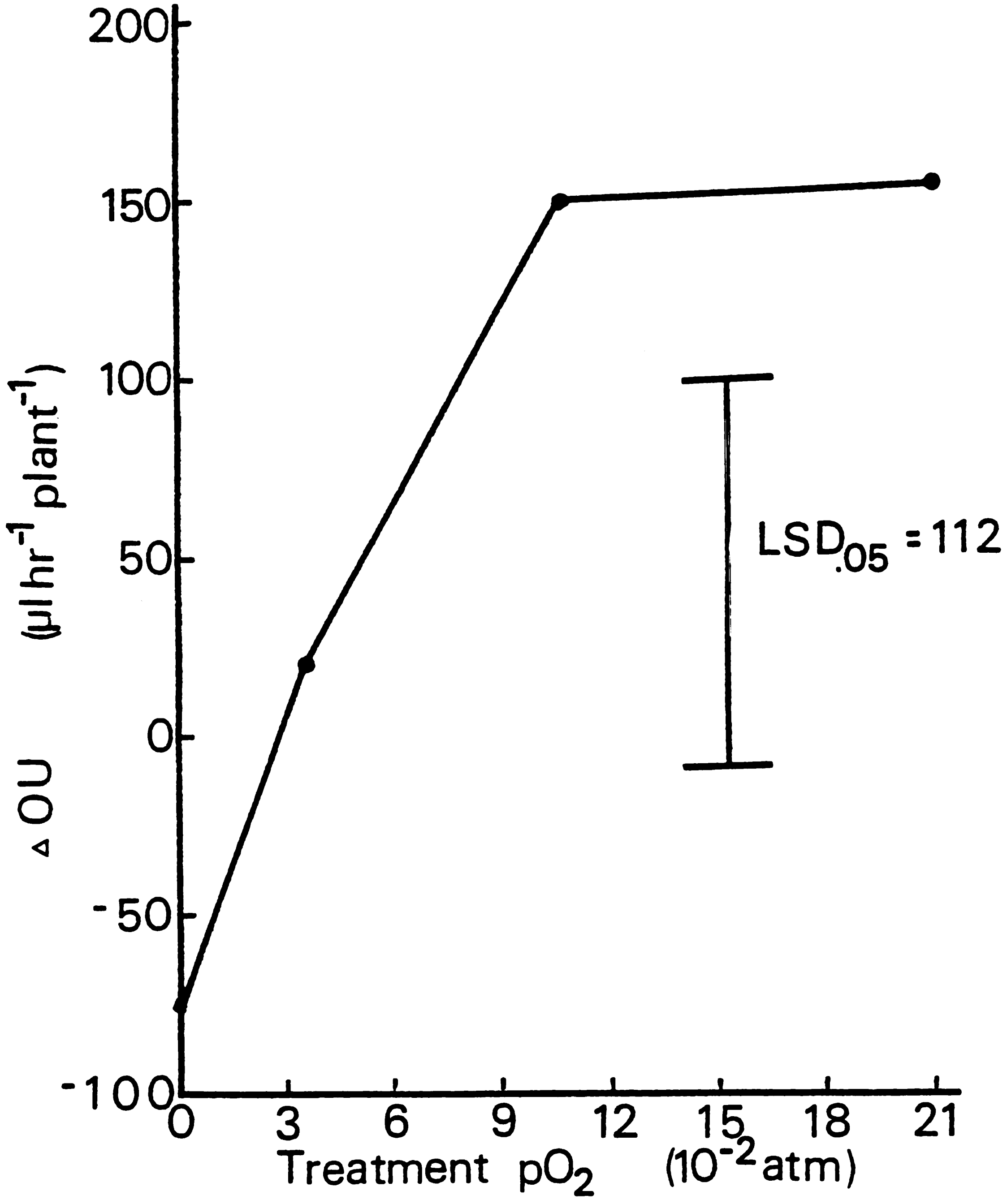

Figure 20. Change in oxygen uptake rates (ΔOU) during 24 hour oxygen treatments. Values are averages of the three matrix treatments.

Table 17. Means of matrix treatments at day 13 and 14, before and after 24 hour oxygen treatment, averaged over all oxygen levels.

		Root respiration rate per†	
Treatment	Microbial respiration ($\mu l\ O_2\ hr^{-1}$/ plant)	Plant at day 13 ($\mu l\ O_2\ hr^{-1}$/ plant)	Plant at day 14 ($\mu l\ O_2\ hr^{-1}$/ plant)
Control	85	434	461
3 mm matrix	127	329	358
1 mm matrix	176	263	295
LSD .05	18	118	105

†Microbial oxygen uptake is subtracted from each root respiration measurement.

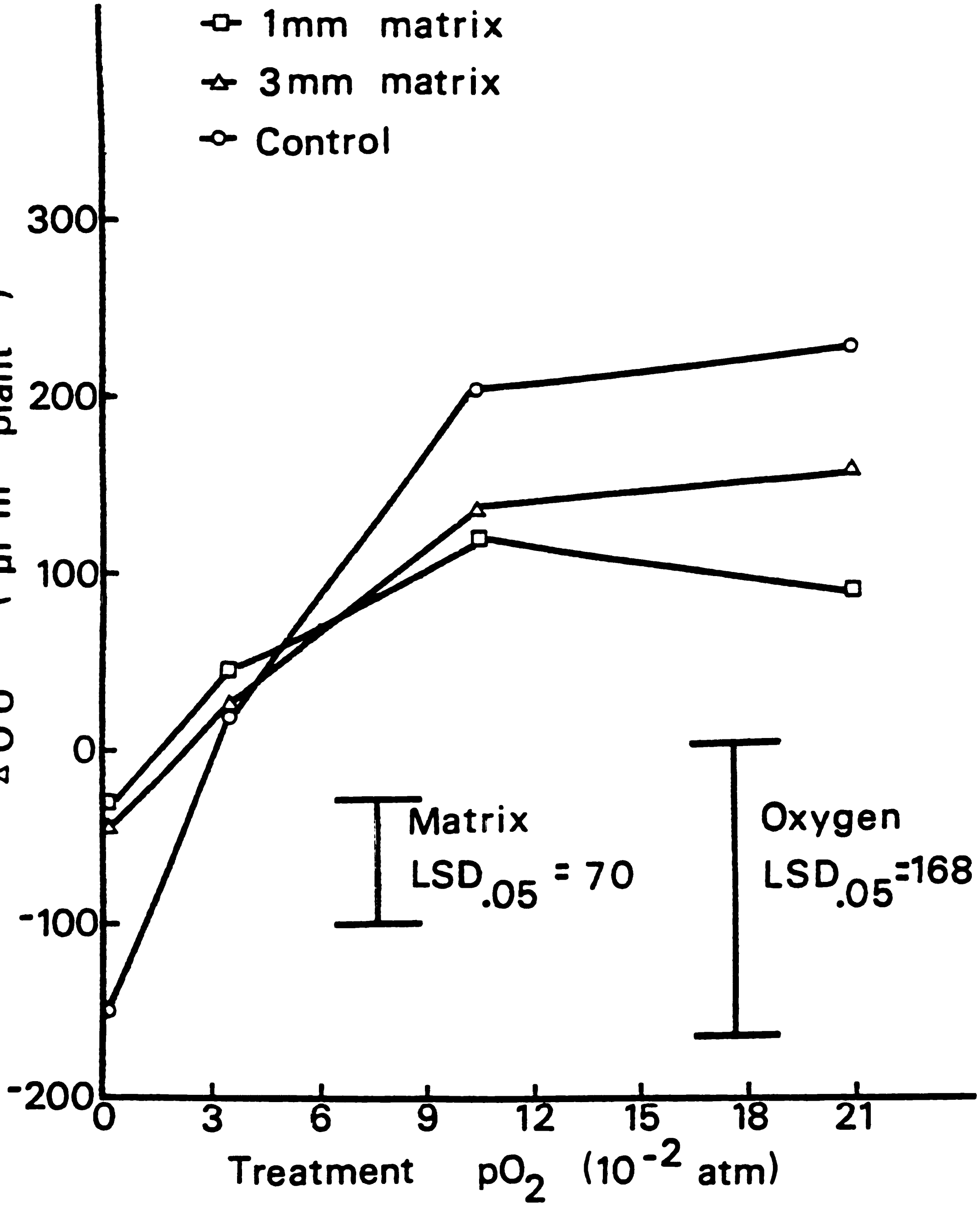

Figure 21. Change in oxygen uptake (ΔOU) during 24 hour oxygen treatments of Phaseolus vulgaris roots for the three matrix treatments.

Table 18. Analysis of variance for the change in oxygen uptake rates of bean roots during 24 hour treatments with 4 oxygen levels.

Source of variation	df	ΔOU† per plant (µl O_2 hr^{-1}/ plant)	ΔOU† per root fresh weight (µl O_2 hr^{-1} g^{-1})
Replications	1	6580	864
O_2 levels	3	148659*	41803*
Error 1	3	7453	1613
Matrix levels	2	1191	967
Matrix x O_2	6	15050**	816
Error 2	8	1764	1704

*,**P < 0.05 and 0.01, respectively.

†ΔOU signifies change in oxygen uptake rate.

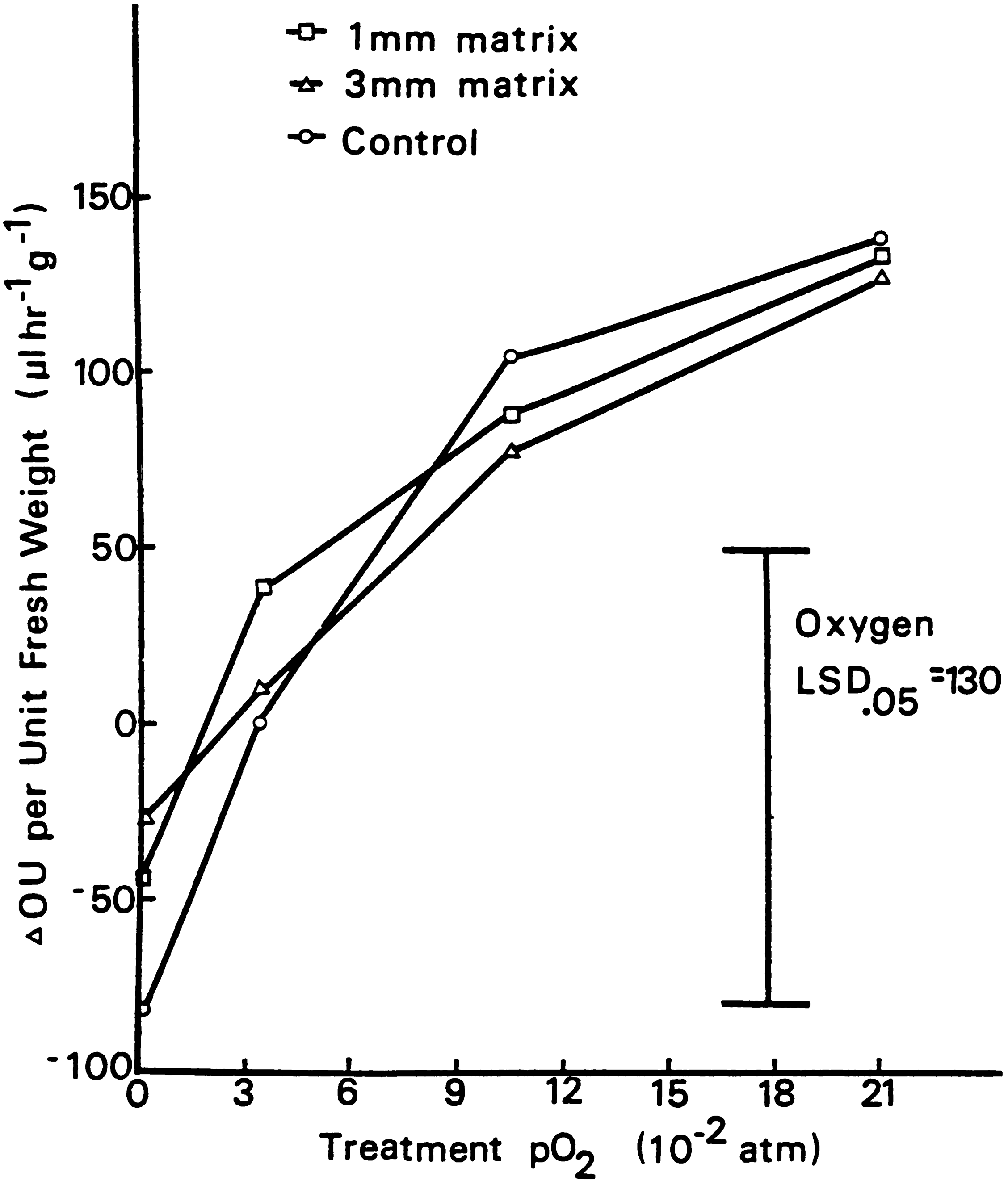

Figure 22. Change in oxygen uptake (ΔOU) during 24 hour oxygen treatments standardized against root fresh weight of *Phaseolus vulgaris* for the three matrix treatments.

Table 19. The relationship of plant size to root growth rates and the change in oxygen uptake rates (ΔOU) during the 0.210 atm pO_2 treatment.

Treatment	ΔOU per plant ($\mu l\ O_2\ hr^{-1}/24\ h$)	ΔOU per root fresh weight ($\mu l\ O_2\ hr^{-1}\ g^{-1}/24\ h$)	Root growth rate (fresh weight) ($mg\ hr^{-1}$)	Relative root growth rate (fresh weight) ($mg\ hr^{-1}\ g^{-1}$)
Control	228	70	29.5	17.5
3 mm matrix	154	66	20.0	17.1
1 mm matrix	86	68	8.5	12.5
LSD .05	70	N.S.	--	--

similar oxygen uptake per unit fresh weight rates on day 13 and 14. Absolute root growth rate during the 24 hour period was over three times greater for the control root system compared to the 1 mm matrix root system. Root growth rates and ΔOU were dependent on the size of the root system as shown by the relative root growth rates (root growth rate per unit root fresh weight) and ΔOU, Table 19. The relative root growth rate was lower for the 1 mm matrix even though relative ΔOU was similar for all matrix treatments.

The 1 mm matrix root system was restricted by both its initial size resulting from the primary root impedance and by a slower growth rate of the lateral root system as demonstrated by the lower relative root growth rates. The reduction in initial size from the primary root impedance was the major factor in the impedance of the 3 mm matrix root system as shown by the similar relative root growth rates of the 3 mm and control treatments. The lateral root growth reduction indicated by lower lateral root length per unit primary root length, Table 6, could have been the result of lag in adaptation to the new environment.

The effect of root size on root growth may explain impedance-anoxia interactions, found in earlier studies, of greater reductions in root growth by nonimpeded compared to impeded root systems. Nonimpeded root growth should be affected more because of the greater number of rapidly growing roots even if impeded and nonimpeded roots respond similarly to low oxygen partial pressures.

The lowered ΔOU resulting from decreasing pO_2 treatments was caused by a decrease in root growth and a reduction of the oxygen uptake rates per unit fresh weight. Figure 23 demonstrates that oxygen uptake rates per unit fresh weight for all matrix treatments generally appeared to

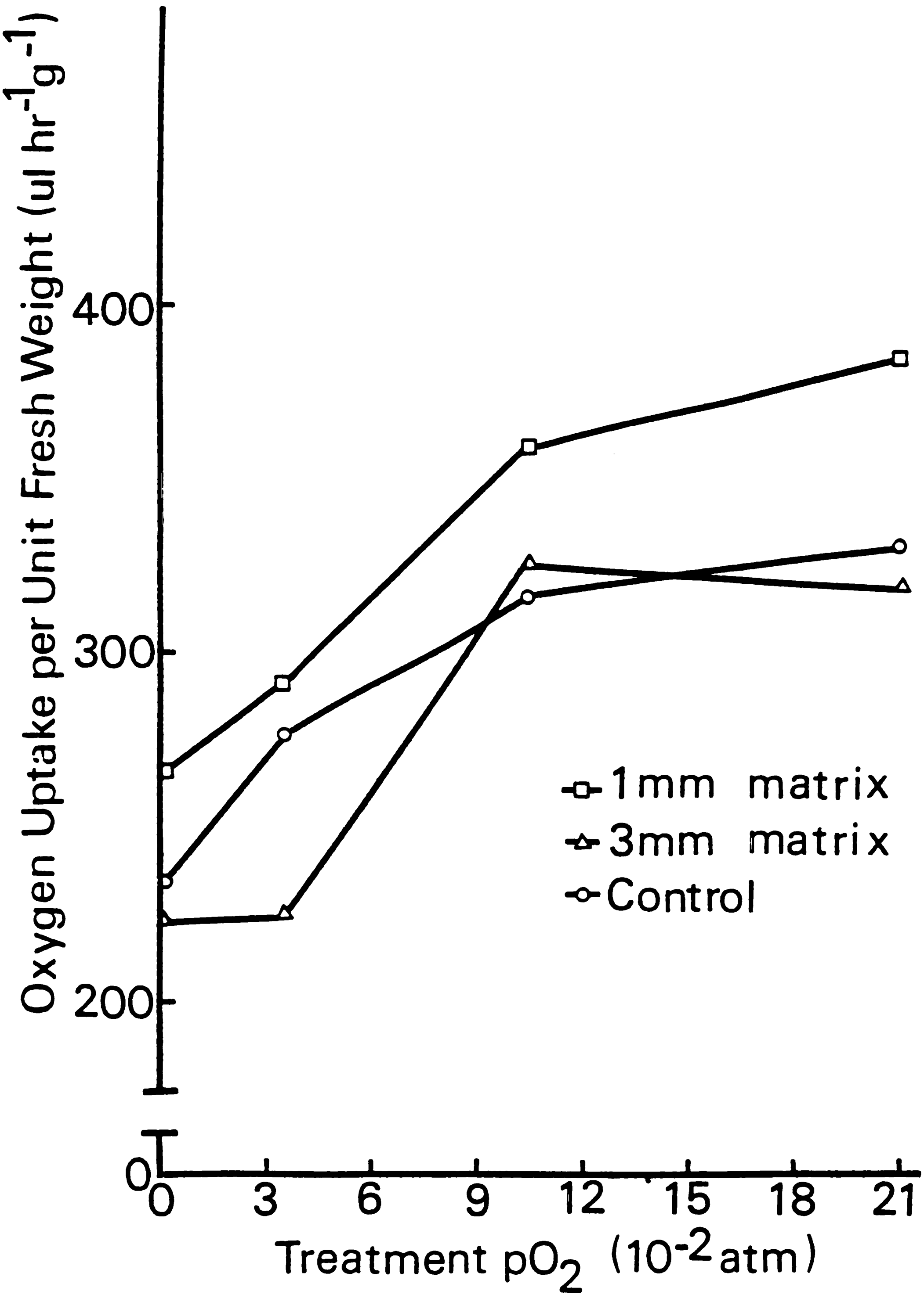

Figure 23. Influence of 24 hour oxygen treatments on oxygen uptake per unit fresh weight of _Phaseolus_ _vulgaris_ roots for the three matrix treatments.

decrease in a similar pattern to lowered pO_2. The 1 mm matrix treatment appeared to have higher oxygen uptake rates per unit fresh weight compared to the other matrix treatments at all levels of pO_2. Root and shoot parameters measured on day 14, after the oxygen treatment, indicate similar results as found in the previous sections, Tables 27, 28 and 29.

An experiment was conducted to measure the effect of two consecutive 24 hour periods of anoxia on oxygen uptake rates. The majority of the reduction of root respiration appears to occur during the first 24 hours of anoxia, Table 20. A large part of the differences between matrix treatments is accounted for by the more rapidly growing root systems of the control and 3 mm matrix treatments. The increase in oxygen uptake noted for the 1 mm matrix treatment was probably due to an increased microbial population. The microbial oxygen uptake was larger compared to results found in Table 17. The larger surface area of the 1 mm glass beads provided a greater area for microbial growth.

TEM micrographs were used to observe the root ultrastructure of samples taken at 0 and 1 cm from the root tip of roots subjected to the 24 hour 0.00 pO_2 treatment. TEM micrographs demonstrate the apparent plasmolysis of cells and a general disorganization of the cytoplasm for all matrix treatments, Figure 24. The granular nature of cells in Figure 25 indicates the marked change which occurs during a 24 hour period of anoxia. The change in cytoplasmic structure occurring during short term anoxia likely results in changed cellular activities as indicated by the observed decrease in oxygen uptake per unit fresh weight during this stress period.

A waterlogging treatment containing 0.00 atm pO_2 was imposed by ceasing the circulation of the nutrient solution through the root

Table 20. The effect of 24 and 48 hours of anoxia on root and microbial respiration for *Phaseolus vulgaris* plants grown in three matrices. Values for microbial respiration represent one replication; all others are means of two replications.

Matrix treatment	Change in O_2 uptake: First 24 hr period ($\mu l\ O_2\ hr^{-1}$/ plant)	Change in O_2 uptake: Second 24 hr period ($\mu l\ O_2\ hr^{-1}$/ plant)	Microbial respiration after 48 hr ($\mu l\ O_2\ hr^{-1}$/ plant)
Control	-266	- 2	131
3 mm matrix	-155	-41	184
1 mm matrix	- 27	+17	245

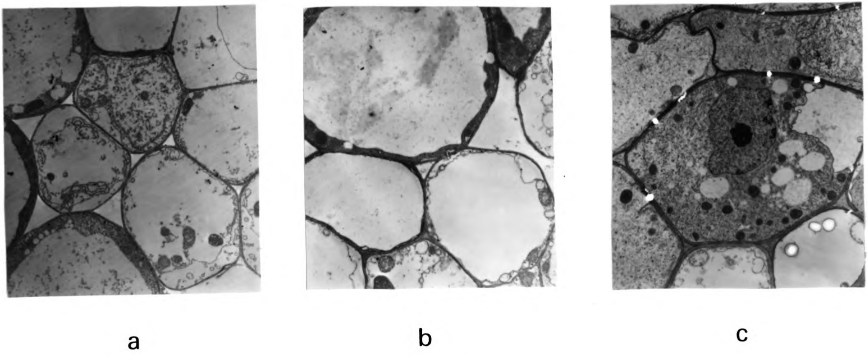

Figure 24. TEM micrographs showing the effects of a 24 hour anoxic period on cortical cells located 1 cm above the lateral root tip. The root cross sections are from 14 day old Phaseolus vulgaris grown in three matrix treatments (x4500). a. Control, b. 3 mm matrix, c. 1 mm matrix.

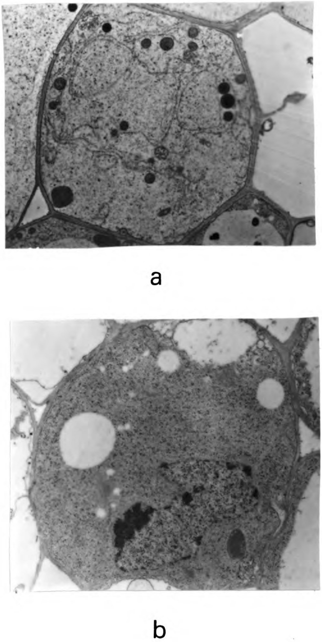

Figure 25. TEM micrograph comparing cytoplasmic organization of 24 hour anoxic stressed and nonstressed 14 day old <u>Phaseolus vulgaris</u> plants. The root cross sections are taken from control matrix plants 1 cm above the lateral root tip. a. Nonstressed cortical cell (x4500), b. 24 hour anoxic stressed cortical cell (x10,000).

chambers. This treatment enabled us to complete several measurements which could not be made on previous treatments. In this treatment it was assumed that volatile and nonvolatile metabolites accumulated in a manner similar to a field situation. When possible, results from the waterlogging treatment (W) were compared to the nitrogen gas equilibrated treatment (N).

Table 21 indicates that the reduction in oxygen uptake during the 24 hour treatment period tended to be greater for the W treatment. A comparison of microbial oxygen uptake rates also indicates a lower value for the waterlogging treatment, Table 21. A possible explanation is the production of a toxic compound (e.g. ethylene) during the waterlogging treatment. Ethylene has been shown to be a powerful inhibitor of microbial growth (90).

The control treatment resulted in the greatest absolute and relative reductions in oxygen uptake for both the N and W treatments. The response of the nonimpeded root may have been a response of its relatively faster growing root system and a lack of storage carbohydrates. Since the root growth relative to root weight was higher for the nonimpeded root system its response to anoxia could also have been proportionally as severe. Less storage carbohydrate may have been available in the control root system compared to the other matrix treatments. Anoxia around a sink for photosynthates (e.g. root) has been shown to reduce photosynthate translocation (80). Oxygen uptake after the anoxic period could be much lower if the control root was low on storage carbohydrates because of its rapid growth before an anoxic period. The control root may also be low on storage carbohydrates because of loss through exudation.

Table 21. The effect of 24 hour treatments of waterlogging (W) and nitrogen gas equilibrated solution (N) on the oxygen uptake of the three matrix treatments.

Treatment	ΔOU†/plant		ΔOU†/root fresh weight		Microbial respiration	
	W	N	W	N	W	N
	($\mu l\ O_2\ hr^{-1}\ plant^{-1}$)		($\mu l\ O_2\ hr^{-1}\ g^{-1}$)		($\mu l\ O_2\ hr^{-1}\ plant^{-1}$)	
Control	-162	-152	-94	-86	19	77
3 mm matrix	- 95	- 43	-60	-31	76	153
1 mm matrix	- 64	- 33	-84	-41	83	192

†ΔOU signifies change in oxygen uptake rate during the 24 hour treatment period.

Ethanol exudation data indicate that the control matrix exuded more ethanol than the other matrix treatments, Table 22. This was true even when the data was expressed on a per root weight basis. Amino acid exudation data shows a similar pattern, Table 23. The confinement of the larger control root system to a limited volume may have resulted in the formation of anoxia earlier than the other matrix treatments. This could also have contributed to the greater exudation observed in the control root system during the waterlogging treatment.

Large starch granules were observed in the 3 mm matrix primary root tip, Figure 13. The 1 mm matrix root system has been shown in the previous section to have a greater dry matter percentage and this may be due to increased storage of carbohydrates. The low Pasteur effect reported earlier for the control root system may have been the result of low levels of carbohydrate reserves.

The difference in oxygen uptake rates are not necessarily related to root damage or the ability of the root to recover. However, the generally lower ΔOU for the W treatment are indicative of a more severe treatment.

During the period of waterlogging the humidifying system malfunctioned resulting in relative humidity values of 10%. All waterlogged plants wilted, however, those plants not subjected to waterlogging but maintained at 0.210 pO_2 did not wilt. In contrast only one plant wilted during the N treatment when the relative humidity was at 40-60%.

The wilting occurred with different degrees of severity. The most severely wilted plants resulted in chlorophyll degradation in the primary leaves, leaving irregular yellowish patches. All plants eventually resumed turgidity. The most severely wilted leaves had portions of

Table 22. Ethanol root exudation by 14 day old Phaseolus vulgaris plants subjected to a 24 hour waterlogging stress and three matrix treatments. Values are means of two determinations.

	Ethanol exuded			
Treatment	Plant (μmol/plant)	Root fresh weight (μmol g^{-1})	Root dry weight (μmol g^{-1})	Leaf area (μmol cm^{-1})
Control	33	20	479	492
3 mm matrix	15	10	234	236
1 mm matrix	12	16	274	238

Table 23. Amino acid exudation by Phaseolus vulgaris roots waterlogged for a 24 hour period. Data for the 1 mm matrix treatments are the means of 3 replications. All others represent 1 replication.

Treatment	Total amino acid exudation per Plant (nmol/plant)	Root fresh weight (nmol g^{-1})	Root dry weight (nmol g^{-1})
Control	2,110	1,310	32,560
3 mm matrix	180	110	2,580
1 mm matrix	690	880	16,440

their blade which were structurally damaged and could not regain turgidity. Generally, those plants showing the greatest wilting damage were of the control treatment. The one plant which wilted during the N treatment was from the control treatment.

The regaining of turgidity may have been simply the result of stomatal closure resulting from severe leaf dehydration. A cycle of loss and gain of turgidity was observed on a plant grown at 0.210 atm pO_2, whose root system was under tension as a result of an improperly connected plant chamber. Figure 36 illustrates a sequence which repeated itself every forty minutes over a period of eight hours.

An examination of the pH and conductivity data indicates a trend to decrease the pH and increase the concentration of the nutrient solution during the W treatment, Table 24. The pH appears to be decreased most by the control treatment. The larger root system would be expected to excrete more organic acids than the smaller root systems of the bead matrices.

Water uptake by the waterlogged plants appears to be controlled by leaf area, Table 25. The 1 mm matrix treatment appeared to absorb more water through its root system per unit weight of root. This was probably due to the increased root surface area per unit weight of root resulting from the increased diameter of the impeded roots and the relatively greater demands for water from the shoot.

Generally, a similar pattern of response to low pO_2 was observed when results were corrected for size of the root system. There was a trend for the control treatment to have lower ΔOU than other matrix treatments when exposed to 0.00 atm pO_2 even when corrected for root size.

Table 24. Solution pH and conductivity of matrix treatments after waterlogging treatment. Each treatment mean represents two measurements.

	Treatment				
Parameter	Control	3 mm matrix	1 mm matrix	Control at 0.21 atm pO_2	Half strength Hoagland's solution
pH	6.30	6.43	6.60	6.88	6.60
Conductivity (µmhos)	1043	1043	1043	1001	991

Table 25. Water uptake by 14 day old <u>Phaseolus vulgaris</u> plants subjected to a 24 hour waterlogging stress and three matrix treatments. Treatment data for the 1 mm matrix are the means of 3 measurements. Other treatment data are the means of 2 measurements.

Treatment	Volume of water absorbed per plant (ml 24 h^{-1}/plant)	Volume of water absorbed per		
		Root fresh weight (ml g^{-1} 24 h^{-1})	Root dry weight (ml g^{-1} 24 h^{-1})	Leaf area (ml cm^{-2} 24 h^{-1})
Control	8.5	5.0	120	0.136
3 mm matrix	8.5	5.3	130	0.132
1 mm matrix	6.0	9.3	140	0.129

Low storage carbohydrate, greater exudation and confinement of the root system may have contributed to this response.

Potential Effects of Mechanical Impedance on Rhizosphere Oxygen Utilization

The rhizosphere is an oxygen sink which can cause the formation of anaerobic microsites (91). Several results indicate that the rhizosphere of a mechanically impeded plant may be a greater oxygen sink than that of a nonimpeded unconfined rhizosphere. The unconfined nonimpeded root system simulates root growth under ideal soil physical conditions. The mechanically impeded root system simulates root growth in relatively uniform compacted soil. The compact morphology, slower growth rate, increased oxygen uptake per root dimension, increased root diameter, decreased root porosity and larger water films around the root could result in a more rapid development of anoxia in the field to mechanically impeded roots.

The morphology of mechanically impeded roots could enhance oxygen depletion in the rhizosphere. Mechanically impeded roots were found to have a denser formation of lateral roots along the length of the impeded primary root. This resulted in a smaller more compact root system in which roots are closely associated with each other and competing for rhizosphere oxygen.

Table 26 indicates the vertical distribution of the root system. The impeded root systems are more compact and a greater proportion of the root system was located in the top 7.5 cm of the media. Under field

Table 26. Percentage of root system located at various depths as approximated from root length measurements.

Treatment	Percentage of root system located at			
	0-7.5 cm (%)	7.5-15 cm (%)	15-22.5 cm (%)	22.5-30 cm (%)
Control	44.1	40.7	12.7	2.7
3 mm matrix	58.9	35.9	5.3	0
1 mm matrix	63.3	32.2	4.5	0

conditions in which anoxic conditions are beginning to form the impeded root system could enhance the formation of anaerobic microsites in the root microenvironment because of the increased demand for oxygen associated with its rhizosphere. Since the impeded root system is confined to a smaller portion of the soil, localized anoxic zones could affect a greater proportion of the root system. The damage done to the plant may also be proportionally greater since impeded root systems could be limiting plant growth.

The increased oxygen uptake per unit root dimension of the mechanically impeded root system should create steeper oxygen gradients around the root. This could compound the effect of relatively larger water films surrounding the root which occur in compacted soils (65). Solid contacts surrounding the root are also increased and represent reduced area available for oxygen exchange.

The slower growing root tip of the impeded root could increase opportunities for microbial colonization near the root tip. This could add an additional demand on oxygen in the root environment. Microbial colonization of dead root tip cells would also have a similar effect.

Root porosity may also adversely affect anoxia survival under certain conditions. The root length (L) which could be supported by plant aeration alone was calculated using a simple plant aeration model developed by Wooley (110) for nonaerated conditions:

$$L = \sqrt{(2CDa)(W(1-a)b)^{-1}}$$

where C equals concentration of oxygen (.206 ml/ml); D equals the diffusion coefficient of oxygen in air (.178 cm^2/sec); b equals oxygen uptake rates per root fresh weight; W equals root tissue density and a equals root porosity. Root lengths that the 1 mm, 3 mm and control matrices could theoretically support were, respectively, 6.0, 8.9 and 8.4 cm. Assuming oxygen diffusion from the shoot is not limiting and that oxygen will diffuse first through the primary roots, 60-90% of the primary roots could be supplied with oxygen by plant aeration. The 1 mm and 3 mm matrix treatments could supply a larger percentage of their primary roots because the primary roots are not as long as the control roots.

These estimates of plant aeration are likely to be high because tortuousity of the intercellular spaces is not included in the calculations (54). Tortuousity may be of greater importance for the 1 mm matrix and 3 mm matrix roots than the control roots. A comparison of electron micrographs, Figure 18, indicate that the intercellular spaces appear more distorted in the bead matrix treatments.

The larger root diameters of mechanically impeded roots could result in larger critical oxygen concentrations. Critical oxygen concentration is defined as the pO_2 at the root surface which produces a pO_2 of 0.00 in the center of the root (63). Calculations were made using Lemon's model for radial aeration:

$$c_R' = qF^2/4D_i$$

where c_R' is the critical oxygen concentration; q is the oxygen uptake per unit root volume; R is the root radius; and D_i is the root diffusivity (9.0×10^{-6} cm^2 sec^{-1}). Diffusivity estimates were taken from Lemon's paper (64). The calculations indicate a critical pO_2 of 0.23, 0.14 and 0.15 for the 1 mm matrix, 3 mm matrix and control root systems, respectively. The greater critical oxygen concentration was due to the greater diameter and oxygen uptake rate of the 1 mm matrix root.

During short term anoxia, the reduction of O_2 uptake per unit fresh weight tended to be greater for roots subjected to mechanical impedance indicating a possible greater c_R' for the matrix treatments when compared to the control, Figure 23. Figure 19 indicated a trend for a greater reduction in oxygen uptake during the oxygen treatment which includes the microbial respiration. A treatment of 0.150 pO_2 would provide a better test of this model since 0.105 pO_2 was past the predicted critical pO_2 for all treatments.

Mechanical impedance can be predicted to enhance oxygen demand in the rhizosphere and increase the number of anaerobic microsites which may synergistically increase the detrimental affects of mechanical impedance upon plant root growth in the field. Mechanical impedance-anoxia interaction in the field probably results from the increased oxygen demand which occurs in an impeded rhizosphere resulting in anoxic conditions occurring earlier and to a greater percentage of the impeded root system compared to a nonimpeded unconfined root system. The greater part of the interaction is probably not due to a synergistic physiological effect.

The ability of a nonimpeded root system to withstand anoxia in the field should decline as the root system is confined. Confinement of roots to a small volume of noncompacted soil in the field could result when there is a plow sole or hardpan and heavy inter-row traffic. Under conditions of confinement individual roots of the nonimpeded root system will be growing densely in a localized area. This should result in an increased oxygen sink per volume of soil and increased damage from localized anoxic zones. In the case of a confined nonimpeded root system anoxia may be more severe than for a mechanically impeded root system growing under similar conditions.

SUMMARY AND CONCLUSIONS

A system was designed to grow plants and monitor root respiration under conditions in which the chemical and physical environment of the root could be controlled independently of each other. The system was used to determine the effect of mechanical impedance and short term anoxia on root respiration. A comparison of static and dynamic calibration curves indicated that the circulation of solution had little effect on the measurement of oxygen. Root response could be measured to ± 5 µl O_2 hr^{-1}. Flow rate had no effect on root respiration at 0.210 pO_2. The root respiration monitor could be adapted for more detailed studies of intact root system response to changes in the root chemical environment and the shoot environment.

The 1 mm matrix severely impeded the primary and lateral root systems resulting in an overall growth rate 50% of the control. The 3 mm matrix impeded the primary root but unlike the 1 mm matrix the root tip remained intact. The size, morphology and structure of the mechanical impeded root systems contrasted sharply with the control. The 1 mm matrix root system was smaller with lateral roots clumped along the primary root. All lateral roots of the 1 mm matrix were distorted. Secondary lateral roots were only formed in the 1 mm treatment. The localized nature of the severely impeded root system could predispose plants to damage from transient localized soil conditions such as low water and oxygen availability. The smaller slower growing root systems result in less uptake of immobile nutrients in the soil. As a result the efficiency of banding fertilizer relative to broadcasting should be increased in compacted soils.

Root system growth depended on the size of the root system. A large root system could grow faster because of the greater number of growing roots. Impedance of the primary root or a temporary inhibition of root growth in the field may result in a much greater overall loss of root growth because of the smaller root system.

The 1 mm matrix resulted in distorted cortical cells and reduced intercellular air spaces reflected in lower root porosity values. The reduced root porosity has two effects on the oxygen relations of the root. The lowered root porosity results in a smaller contribution of oxygen from the shoot. This result of compaction may have more significance in a plant species such as rice which acquires a major portion of its oxygen from the shoot. A second effect of reduced root porosity is possible decreased radial diffusivity of oxygen and ethylene. Wounding caused by cellular distortion from mechanical impedance could be the stimulus for ethylene production and combined with reduced radial diffusivity could result in changes in root morphology. The effects of plasmalemma distortion and possible damage to plasmadesmata on nutrient and water uptake is an area of possible investigation.

The 1 mm matrix root system had larger root dry matter percentages and tissue densities than the other matrix treatments. The increased root tissue density was largely due to the decreased root porosity. A greater proportion of cell wall material or storage of surplus photosynthate could have resulted in increased root dry matter percentage. It may be worthwhile to investigate the feasibility of using the root dry matter percentage or root tissue density as a field measurement of mechanical impedance since the entire root system does not need to be recovered for these measurements.

Shoot fresh weight but not dry weight was decreased by mechanical impedance. Leaf area tended to be reduced but photosynthetic capabilities appeared to not be reduced. The association of leaf area and root surface area for the 1 mm matrix indicated that the reduced leaf area may have resulted from the limitation of a small root system on water uptake. A build up of a surplus of photosynthate in the leaf may produce the increased dry matter percentage found in the shoot. This illustrates that under conditions of severe mechanical impedance shoot growth may be affected under otherwise near optimal growth conditions. Further investigations are needed to study these effects in greater detail over the life of the plant.

Shoot to root ratios were larger for 1 mm matrix grown plants. Root respiration rates were correlated with shoot to root ratios of the control and 3 mm matrix plants indicating that the availability of photosynthate may control root respiration. The 1 mm matrix root system may be less correlated because of surplus photosynthate. The relationship of the shoot to root ratio with root respiration suggests that any factor which increases photosynthesis in the shoot could also result in an increase in root respiration. This could be a partial explanation for increased plant damage from anoxia under high light conditions. Another factor affecting shoot and root relationships could be the changes in growth hormone distribution.

Oxygen uptake rates per unit dry weight were similar for all matrix treatments indicating that metabolism of photosynthate in nonimpeded and mechanically impeded roots uses similar amounts of oxygen. The differential distribution of dry weight between matrix treatments resulted in larger oxygen uptake rates per unit fresh weight for the 1 mm matrix root system. This relationship held true for root dimensional parameters as well. Under field situations, the capacity for oxygen deficiences to develop in the mechanically impeded root may be increased by the larger uptake of oxygen per unit dimension of root. The resulting lower oxygen partial pressure in the rhizosphere may change the character of the microbial population near the root and could increase possibilities of pathogen infection.

Short term anoxia reduced oxygen uptake rates per root system the most for control roots. Root system size and rate of growth was primarily responsible for the control response. Correcting the change in oxygen uptake for root weight resulted in no statistically significant differences between matrix treatments in response to short term anoxia. There appeared to be a trend for the control root to have the greatest decrease in oxygen uptake even when corrected for the size of the root system. This may indicate a proportionally greater reduction in relative root growth than the other treatments or a slower recovery rate due to low supply of carbohydrate. Root growth relative to root weight was highest for the control root and a period of 0.00 atm pO_2 should result in a larger decrease in oxygen uptake even when corrected for root weight. The control root because it was growing the fastest probably had the lowest supply of carbohydrates at the initiation of the period of 0.00 atm pO_2. The control root also exuded the greatest amount of root exudates during the waterlogging treatment. The control root may have been depleted of carbohydrate upon ending the 0.00 atm pO_2 treatment.

Waterlogging generally tended to result in greater decreases in oxygen uptake than circulating nitrogen equilibrated solution. The microbial respiration was also much reduced during waterlogging. Both results may be explained by the accumulation of volatile toxic substances particularly ethylene.

The majority of inhibition of oxygen uptake occurred during the first 24 hours of 0.00 atm pO_2.

The overall response to short term anoxia indicated that there was little physiological differences in response between the matrix treatments. Mechanical impedance-anoxia interactions observed in the field or in other laboratory studies which indicate relatively greater inhibition of mechanically impeded roots may have resulted from a combination of factors which could cause more rapid depletion of oxygen around mechanically impeded roots compared to nonimpeded roots. The smaller and denser root system, greater oxygen uptake rates per unit root dimension, the probability of increased microbial uptake, decreased root porosity and thicker water films surrounding the root in compacted soils, all could contribute to a more oxygen deficient zone around a mechanically impeded root. These characteristics of mechanically impeded roots are proposed to predispose the impeded root system to anoxia under marginally oxygenated field environments.

The mechanical impedance-anoxia interaction in compacted soils could be reduced, i. by reducing the oxygen deficiency around the root, ii. by modifying the characteristics of the mechanically impeded root which cause its greater oxygen uptake, iii. by increasing the tolerance of the root system to anoxia, or iv. by improving the compacted nature of the soil. Scott and Erickson (88) used the first strategy to overcome the mechanical impedance-anoxia interaction by using calcium peroxide as a source of oxygen. The application of calcium peroxide to a compacted field in sufficient quantities to alter the root environment could entail considerable expense. Concerning the root modification strategies, very little is known of the physiological genetics which pertains to root response to mechanical impedance. Considerably more is known about the mechanisms and genetics of root response to anoxia and presumably this knowledge could be used to develop plant varieties which are less sensitive to anoxia. Ideally, the best strategy for a compacted soil is to improve soil structure through improved soil management techniques. In lieu of reducing soil compaction, the most practical strategy for overcoming the mechanical impedance-anoxia interaction would be the development of a Phaseolus vulgaris variety which is more tolerant of anoxia than varieties currently in use.

Solution studies have not taken into account the changes which occur in roots when growing in a solid medium. Conclusions from solution studies should be analyzed to determine if changes in root morphology and structure observed in roots grown in solid media could affect results observed.

APPENDIX

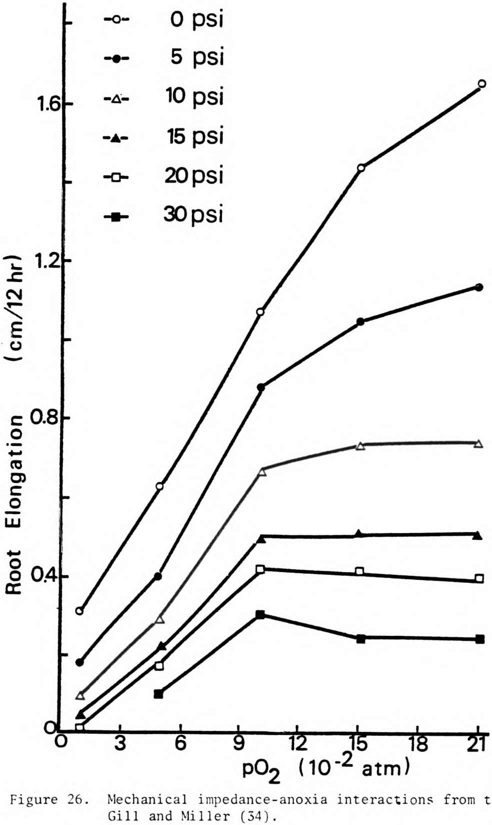

Figure 26. Mechanical impedance-anoxia interactions from the study of Gill and Miller (34).

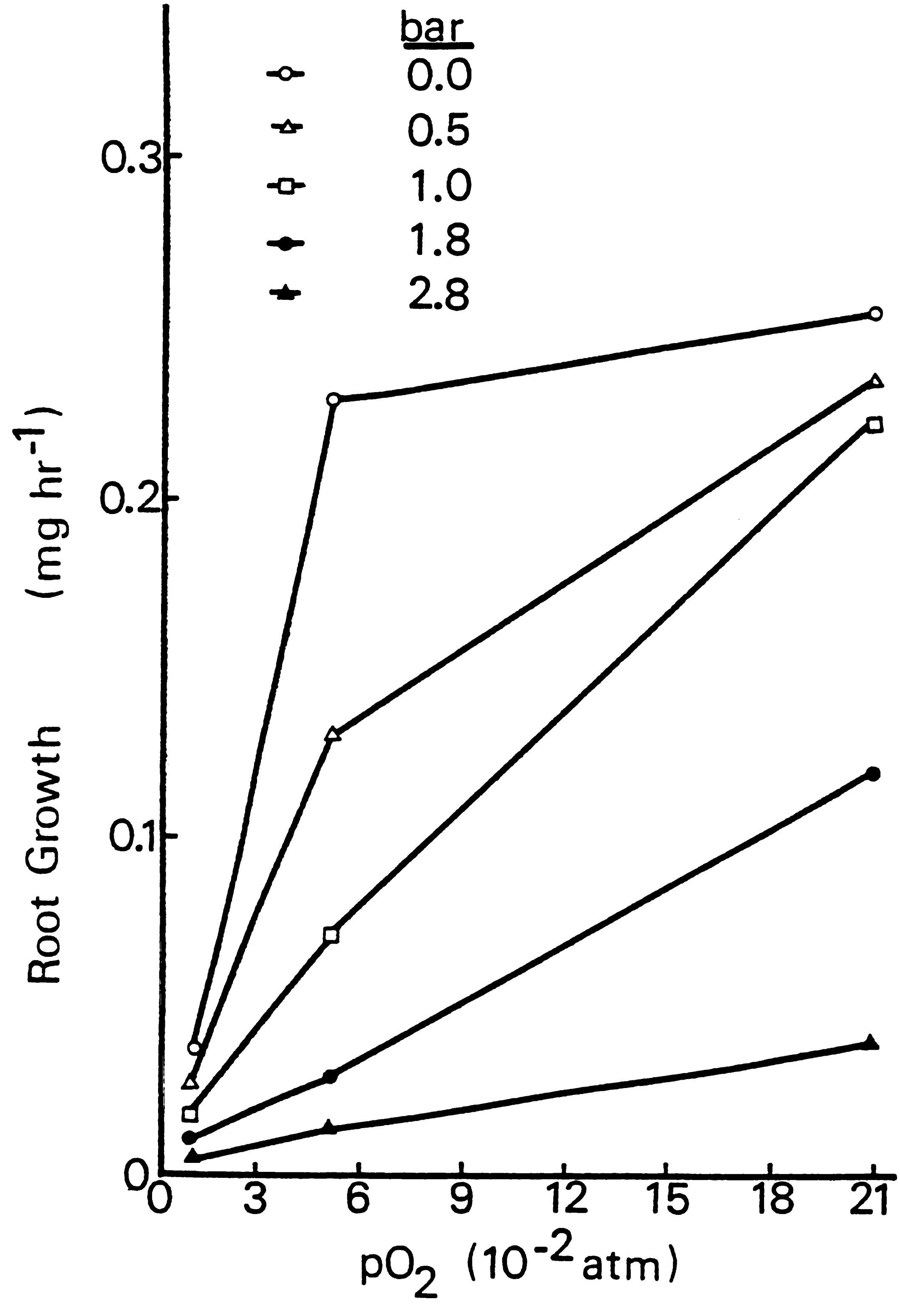

Figure 27. Mechanical impedance-anoxia interactions affecting root weight production from the study of Barley (9).

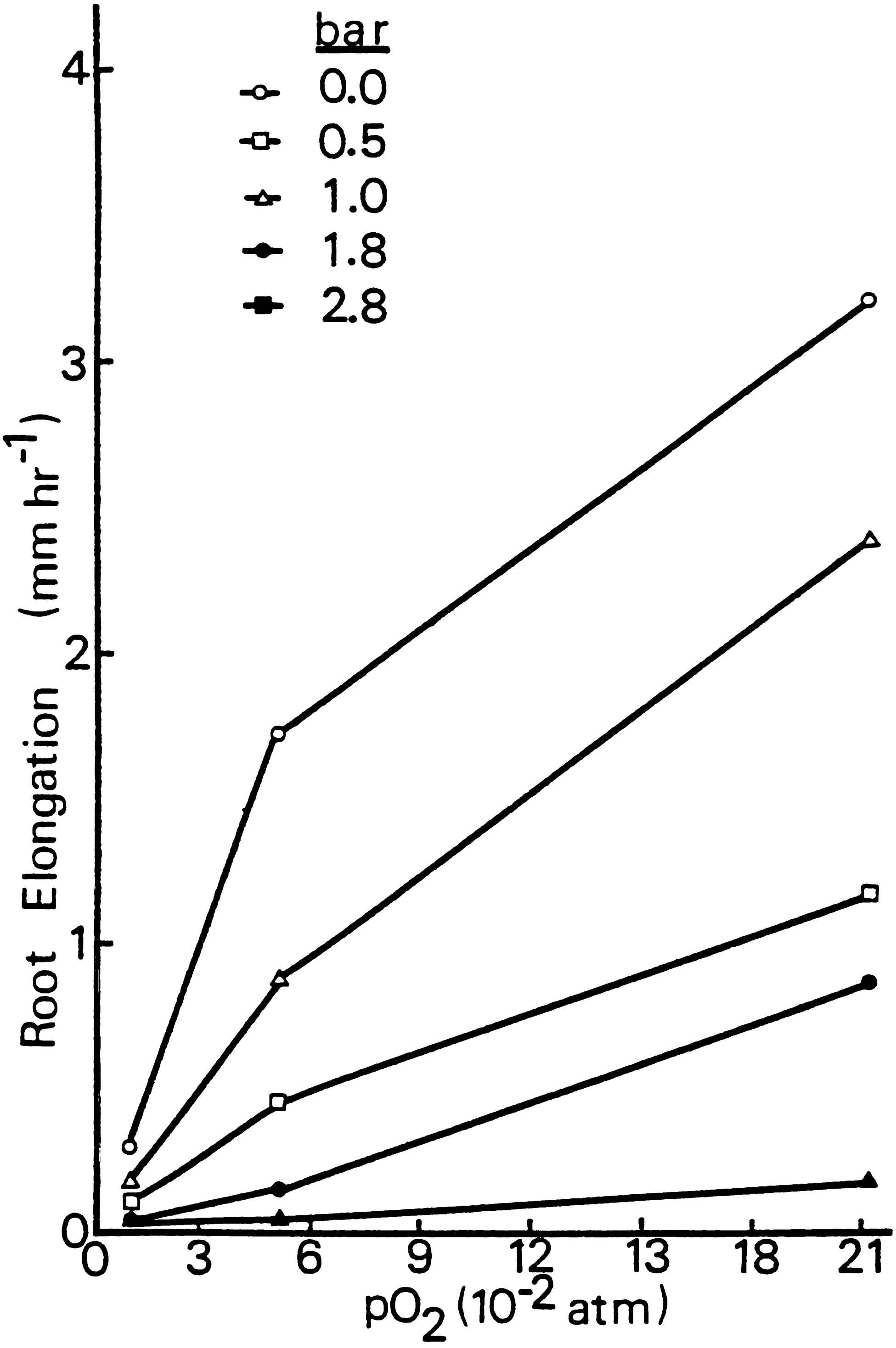

Figure 28. Mechanical impedance-anoxia interactions affecting root elongation from the study of Barley (9).

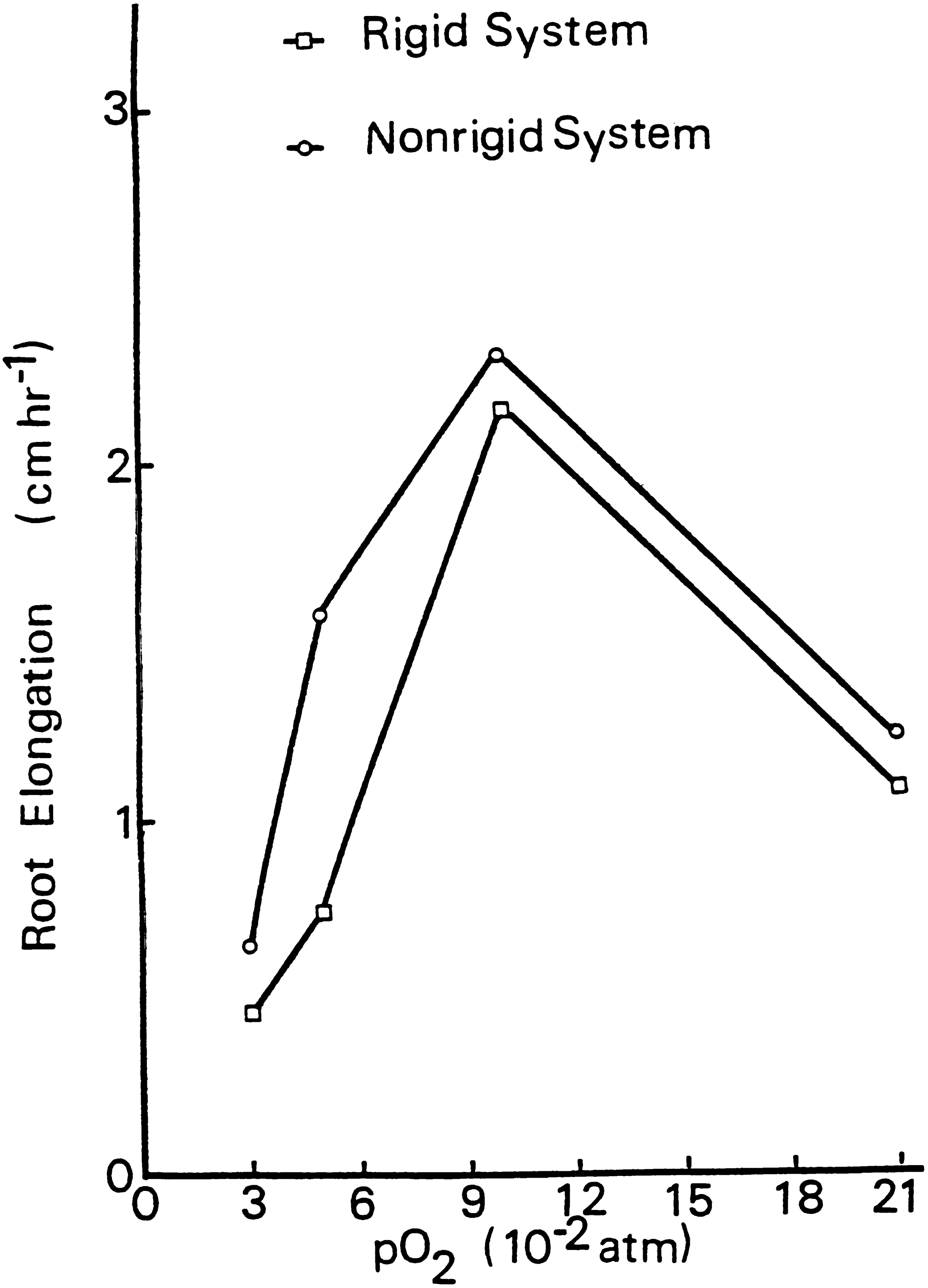

Figure 29. Mechanical impedance-anoxia interactions affecting root elongation from the study of Aubertin and Kardos (4).

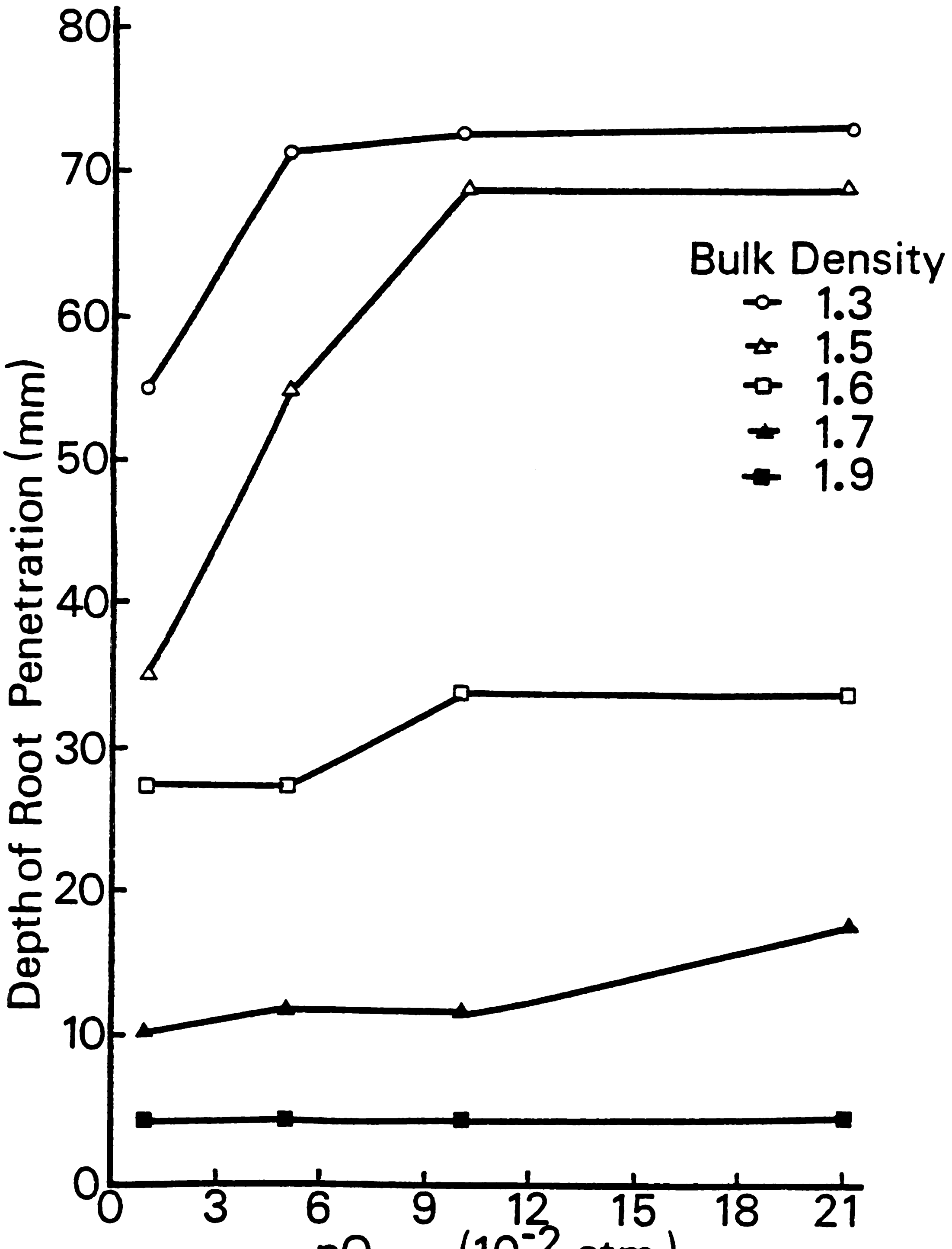

Figure 30. Mechanical impedance-anoxia interactions affecting depth of root penetration from the study of Tackett and Pearson (98).

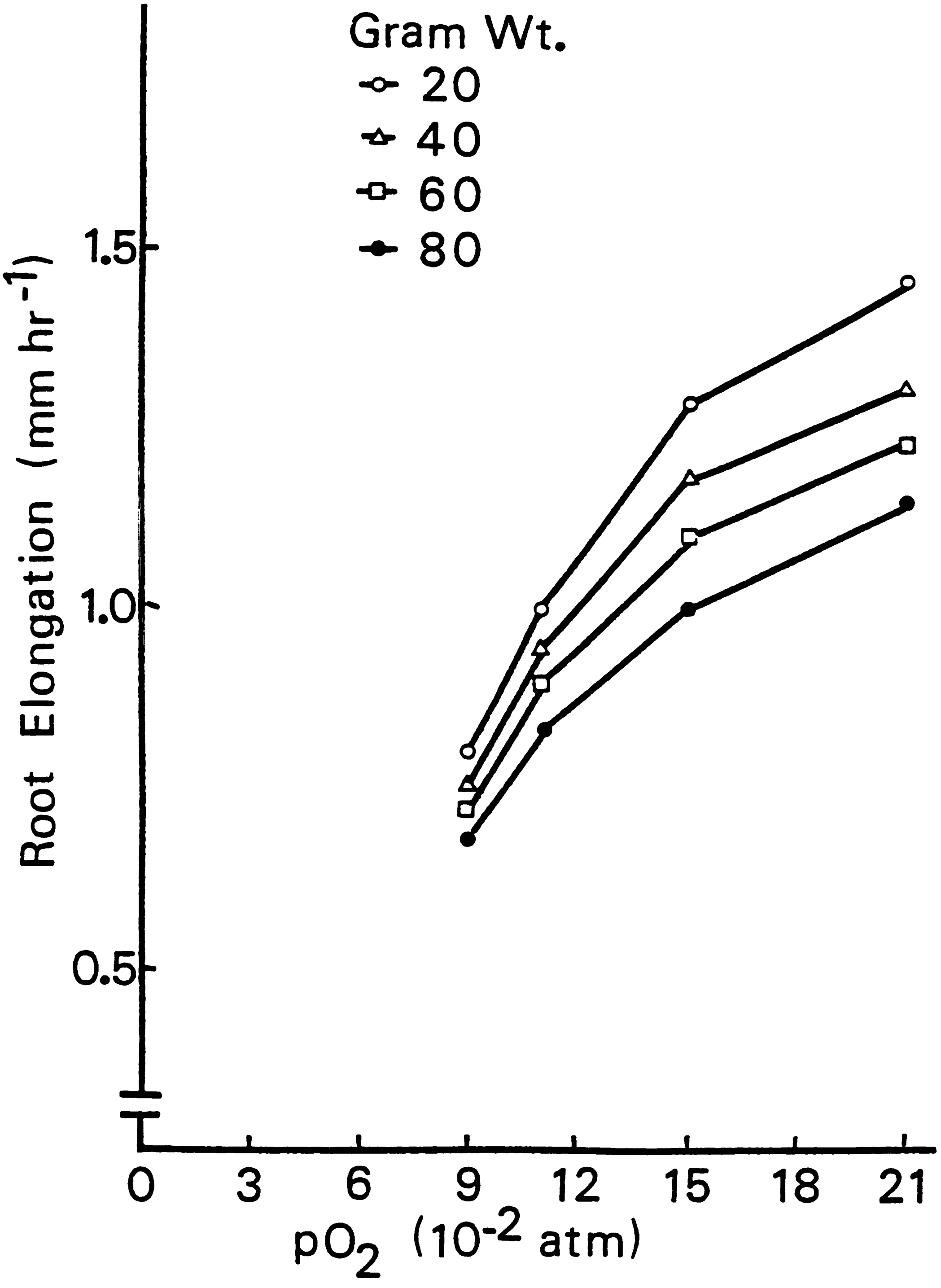

Figure 31. Mechanical impedance-anoxia interactions affecting root elongation from the study of Eavis (28).

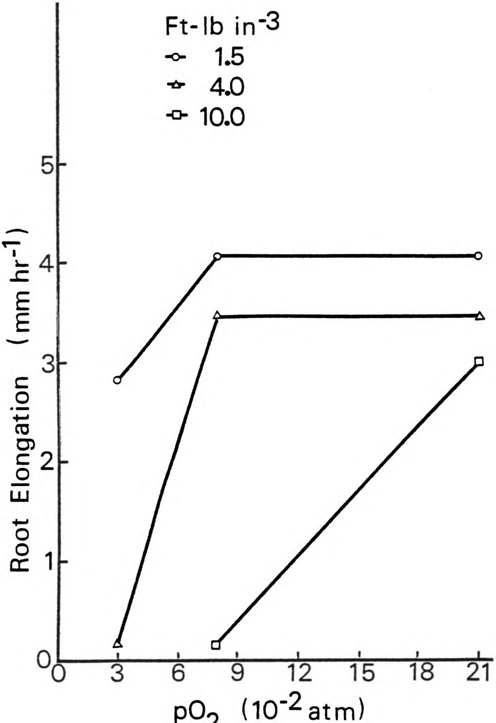

Figure 32. Mechanical impedance-anoxia interactions affecting time to reach ^{32}P in compacted Commerce silty loam from the study of Hopkins and Patrick (48). *Based on time to reach ^{32}P at a depth of 4.5 cm.

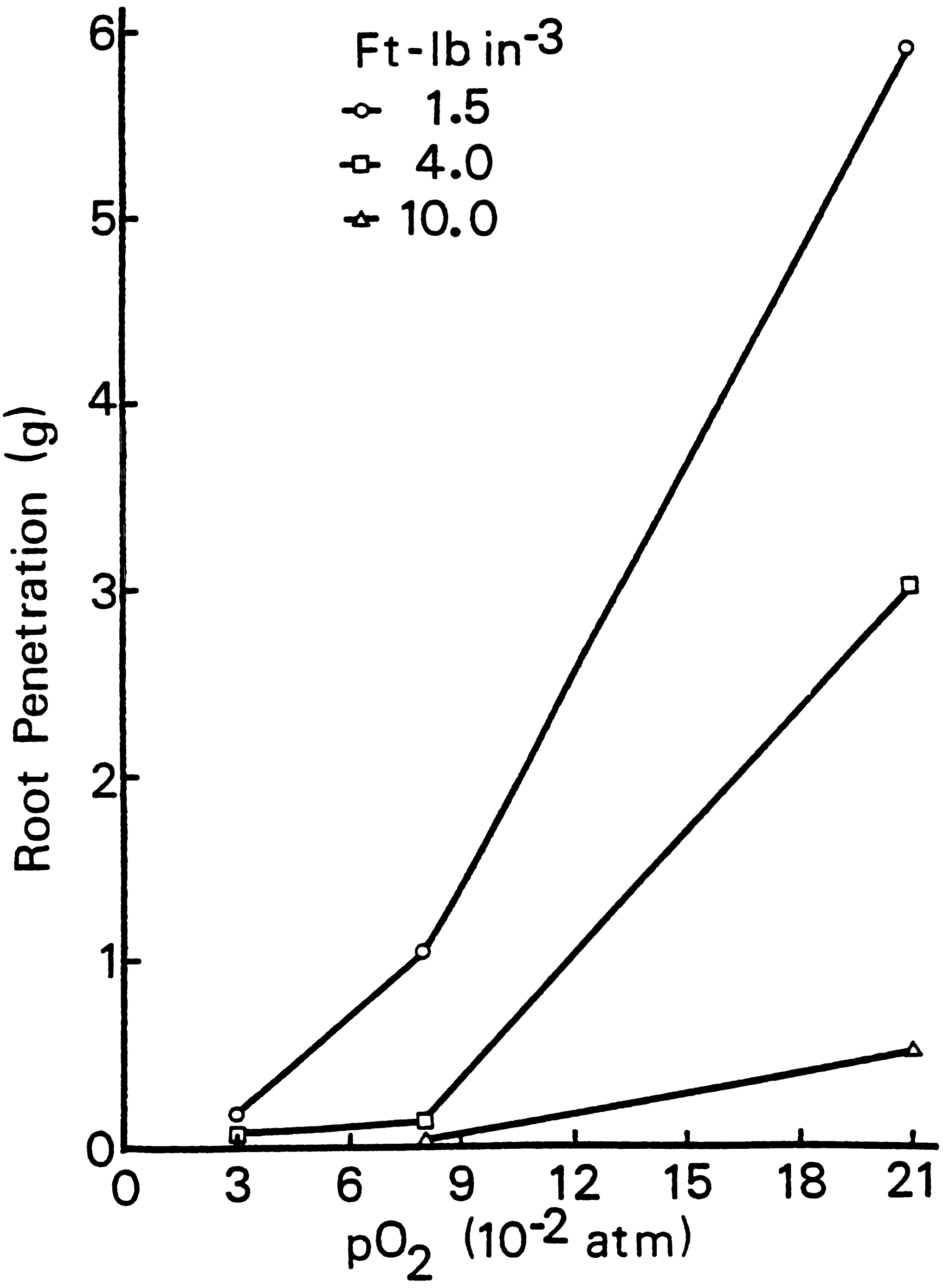

Figure 33. Mechanical impedance-anoxia interactions affecting root penetration into compacted cores of Commerce silty loam during a 14 day period from the study of Hopkins and Patrick (48).

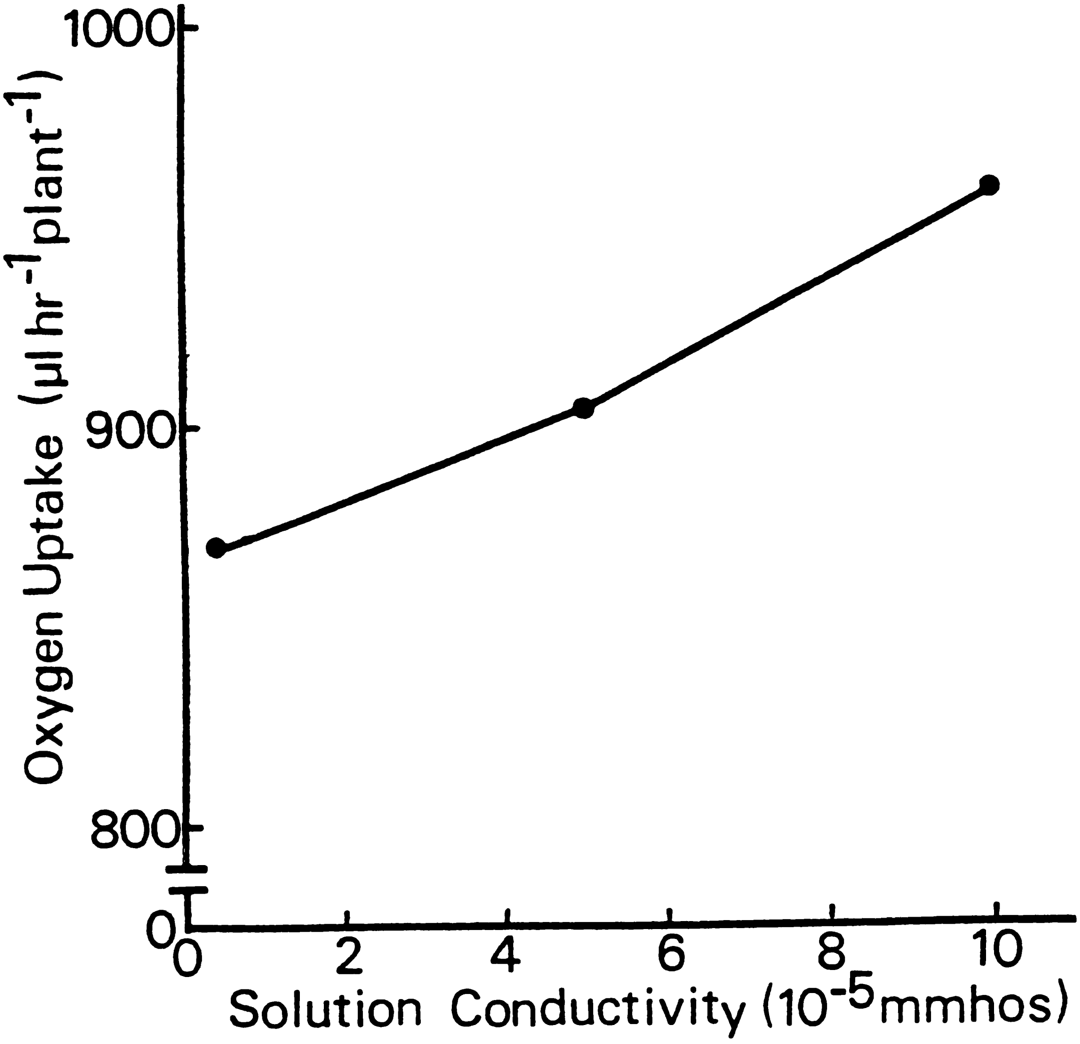

Figure 34. Influence of Hoagland's solution conductivity on the oxygen uptake of 30 day old Zea mays roots. Each value is the mean of two replications.

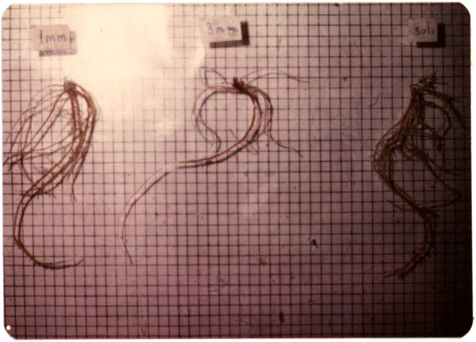

Figure 35. Inhibition in 14 day old <u>Phaseolus vulgaris</u> root growth caused by an acid nutrient solution (pH 2). Root size is similar to that at time of transplantation to root chamber.

Table 27. Means of matrix treatments averaged over oxygen treatments for 14 day old *Phaseolus vulgaris* root parameters. Values are means of ten measurements.

Treatment	Root fresh weight (g/plant)	Root fresh weight (g/plant)	Root dry weight percentage (%)
Control	1.74	0.081	4.7
3 mm matrix	1.49	0.068	4.6
1 mm matrix	0.94	0.056	6.2
LSD .01	0.41	0.019	0.6
LSD .05	0.29	0.014	0.4

Table 28. Means of matrix treatments averaged over oxygen treatments for 14 day old Phaseolus vulgaris plant parameters. Values are means of ten measurements.

Treatment	Shoot/root (fresh weight)	Shoot/root (dry weight)	Plant fresh weight (g/plant)	Plant dry weight (g/plant)
Control	1.83	4.51	4.88	0.443
3 mm matrix	1.95	5.07	4.34	0.410
1 mm matrix	2.68	5.67	3.38	0.368
LSD .01	0.40	N.S.	1.16	N.S.
LSD .05	0.28	0.92	0.81	0.072

Table 29. Means of matrix treatments averaged over oxygen treatments for 14 day old *Phaseolus vulgaris* shoot parameters. Values are means of ten measurements.

Treatment	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Leaf area (cm^2/plant)	Shoot dry matter percentage (%)
Control	3.14	0.362	87.8	11.5
3 mm matrix	2.85	0.342	80.4	12.2
1 mm matrix	2.44	0.313	67.6	13.1
LSD .01	0.55	N.S.	17.3	N.S.
LSD .05	0.44	N.S.	14.0	1.4

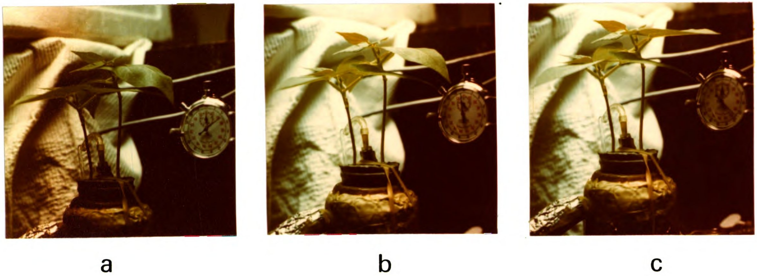

Figure 36. Sequence demonstrating response of *Phaseolus vulgaris* to conditions of low humidity (5% relative humidity) and a negative pressure in the root chamber (-20 cm H_2O). a. Time: 0 min. - right plant severely wilted, b. Time: 5 min. - right plant recovering, c. Time: 8.75 min. - right plant fully recovered. The wilting-recovery cycle was repeated every 40 min. for 12 cycles. Left plant was out of phase with right plant and not as severely affected.

LITERATURE CITED

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