ANAEROBIOSIS AS IT AFFECTS THE EXUDATION AND INFECTION OF PEA ROOTS GROWN IN AN ASEPTIC MIST CHAMBER

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
ALVIN J. M. SMUCKER
1971

This is to certify that the

thesis entitled

Anaerobiosis as it Affects the Exudation
and Infection of Pea Roots Grown
in an Aseptic Mist Chamber

presented by

Alvin J. M. Smucker

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Soil Physics

Major professor

Date June 14, 1971

O-7639

ABSTRACT

ANAEROBIOSIS AS IT AFFECTS THE EXUDATION AND INFECTION OF PEA ROOTS GROWN IN AN ASEPTIC MIST CHAMBER

By

Alvin J. M. Smucker

The effects of anaerobiosis on exudation and infection of pea roots were determined by growing seedlings in an aseptic root chamber filled with mist of a mineral nutrient solution. Gaseous compositions simulating those found in flooded soils were flushed through the mist chamber for periods up to 17 days. Root exudation was related to gas composition and light intensity by measuring the accumulation of plant metabolites in the circulating nutrient solution. Pathogenesis was related to anaerobiosis by ranking the severity of disease on the treated plants.

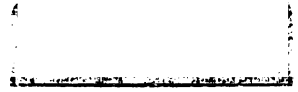
Ethanol, an indicator of cellular oxygen deficiency, was the primary constituent of exudates from roots subjected to anaerobic conditions. Essentially no ethanol was detected in the exudates of aerobic roots. Conversely, up to 295 mg ethanol was produced per gram of dry weight by roots subjected to 70% N_2 with 30% CO_2 for 6 days. There were 125 mg ethanol produced per gram dry weight by roots treated with

N_2 for 6 days. Ethanol was detected in root exudates a few hours after the onset of anaerobiosis.

The silylated derivatives of amino acids and carbohydrates in the root exudates were quantitatively measured by gas chromatography. Anaerobiosis increased the quantity of both amino acids and carbohydrates. An atmosphere of 70% N_2 and 30% CO_2 caused roots to lose the most amino acids and carbohydrates. Air with 30% CO_2 caused the next highest loss. An atmosphere of 100% N_2 appeared to have the least effect upon exudation except for the air control.

Anaerobic treatment of pea roots inoculated with Fusarium promoted spore germination, fungal growth, and disease. There was a 5-fold increase in the fungal growth with a concomitant 4-fold increase in the disease of roots treated with anaerobic as compared to aerobic conditions.

Bioassay experiments indicated ethanol alone had no effect upon spore germination. In contrast, alanine promoted spore germination. Both ethanol and alanine promoted the growth of Fusarium.



ANAEROBIOSIS AS IT AFFECTS THE EXUDATION AND INFECTION OF PEA ROOTS GROWN IN AN ASEPTIC MIST CHAMBER

By

Alvin J. Mc Smucker

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Sciences

1971

TO BETTY

This thesis is dedicated to my wife.
For without her interest, patience
and unfailing support this study
could not have been completed.

ACKNOWLEDGEMENTS

Sincere appreciation is expressed to Dr. A. E. Erickson for his assistance and guidance during this investigation. His enthusiasm in all phases of research and teaching has been a true inspiration.

Special thanks are due to Dr. B. D. Knezek, Dr. J. M. Tiedje, Dr. A. R. Wolcott, Dr. R. S. Bandurski, and Dr. J. L. Lockwood who served on my guidance committee.

Thanks are also due to Dr. C. M. Harrison, Dr. B. G. Ellis, and Dr. M. L. Lacy for their assistance in the preparation of this thesis.

Appreciation is extended to Dr. R. K. Hammond and other members of the mass spectrometer laboratory for their assistance in identifying some of the root exudates.

Financial assistance granted by the Agricultural Experiment Station is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

Table Page

LIST OF FIGURES

Figure | Page

INTRODUCTION

Maximum plant growth requires ample quantities of oxygen available to the root system. Peas, tomatoes, and other vegetable crops are quite sensitive to oxygen stress. Short periods of soil flooding frequently result in root diseases reducing plant growth and yield.

Many of the fine textured soils frequently become saturated after a heavy rainfall or when located in low areas of a field. As the water content of soil approaches the saturation point, soil pores normally occupied by air become filled with water, greatly reducing the supply of oxygen to plant roots.

Root diseases are increased by high soil moisture. Walker (1942) reported that root diseases of peas grown in Wisconsin were invariably greater in wet years and on wet soils. Often soil anaerobiosis inflicts damage to the roots within a few hours after the soil is saturated. The resultant root injury, especially to young seedlings, affects plant growth throughout the growing season. Prolonged soil flooding may also reduce the resistance of plant roots to the surrounding biota.

Most studies have related oxygen stress to nutrient uptake, plant growth, and alterations in plant metabolism.

No effort has been directed toward delineating the role of soil flooding or soil anaerobiosis in root diseases. Since most of the vegetables in this stage are grown on soils which frequently become saturated during the seedling stage, a study should determine whether the major effect of anaerobiosis is on the host, making it more or less susceptible to disease, or on the pathogen making it more or less virulent.

This investigation was designed to determine the effects of soil anaerobiosis on root exudates and the inoculum potential of Fusarium root rot of peas. Knowledge of this interrelationship should lead to a clearer understanding of the role exudates have in host pathogen interactions.

The objectives of this study were: 1) to determine the effects of anaerobiosis on the exudation and respiration of sterile pea roots, 2) to determine the role of anaerobic metabolites and gases on Fusarium spore germination and growth, and 3) to determine the role of anaerobiosis on infection by Fusarium.

LITERATURE REVIEW

Root Exudates

There is much evidence that intact cells of plant roots exude sufficient quantities of organic compounds to support large populations of microorganisms in the root zone or rhizosphere; Schroth and Hildebrand (1964) and Rovira (1962 and 1969). Compounds identified by Rovira (1969) in the exudates included: amino acids, sugars, peptides, organic acids, enzymes, vitamins, nucleotides, and many others which attract, stimulate, or inhibit soil microflora. The quantity of root exudates tended to control the number of microorganisms in the rhizosphere, whereas their quality controlled the specificity of the microorganisms. Many factors of the environment affected the quality and quantity of root exudates. These factors included: 1) plant species, age, and nutrition, 2) temperature and light, 3) presence or absence of microorganisms, 4) supporting medium, 5) moisture content, and 6) chemical and physical damage.

Pearson and Parkinson (1961) and Schroth and Snyder (1961) designed experiments and reported that amino acids are exuded from the zone immediately behind the root tip of beans while most of the sugars are exuded from the hypocotyl. By subjecting older plants to $^{14}CO_2$ and measuring the

radioactivity at the roots, McDougall and Rovira (1969) showed the greatest amount of metabolites was exuded at the tips of lateral roots.

The mechanisms involved in root exudation are essentially unknown. Evidence that exudation is a metabolic process involving cell permeability was demonstrated by Hiatt and Lowe (1967) and McDougall and Rovira (1960). They reported that potassium cyanide and 2,4 denitrophenol, inhibitors of oxidative phosphorylation, increased both the permeability of membranes and the production of root exudates. However, since low concentrations of oxygen have been reported to increase exudation by Brown and Kennedy (1966), root exudation may be primarily controlled by the energy processes within the cell.

Root Physiology

The oxygen supply in flooded soils is greatly reduced to that dissolved in water. Erickson and Van Doren (1960) have shown that short periods of flooding caused a decrease in the diffusion of oxygen to plant roots reducing plant growth and yield. In addition to less growth, the quantity of mineral elements in peas is reduced by soil flooding; Cline and Erickson (1959).

Crawford (1966) reported soil flooding increased the rate of glycolysis in the roots of species susceptible to saturated soil conditions while there was no change in the glycolitic rate of tolerant species. Flooding also

increased alcohol dehydrogenase activity in the susceptible plants producing ethanol; Crawford (1967). After studying the rates of other enzymes, Crawford concluded that the activity of alcohol dehydrogenase was an enzyme induction process which could be controlled by soil flooding.

Ethanol accumulation in plants has been determined as an index of anaerobic soil conditions. Kenefick (1962) showed that the ethanol content of sugar beets increased under anaerobic conditions. Fulton and Erickson (1964) reported the concentration of ethanol in the xylem exudates of tomatoes increased during short periods of soil flooding. Bolton and Erickson (1970) reported a 100-fold increase in the ethanol content of root exudates of tomato plants during anaerobiosis. A concurrent five-fold increase in the ethanol content of the roots suggests that most of the ethanol produced during anaerobiosis is exuded at the site of production.

Cossins and Turner (1959 and 1963) and Lin et al. (1965) presented evidence that a functional alcohol dehydrogenase complex is active in pea roots. They reported that during anaerobiosis ethanol was produced and exuded during germination. When the seedlings were aerated they oxidized exogenous ethanol to acetaldehyde.

Most of the amino acids and two monosaccharides have been identified in the exudates of pea roots. Rovira (1956) and Boulter et al. (1966) reported 21 amino acids were exuded into the culture medium of 14-day-old pea roots.

Paper and column chromatography revealed that 26.1 mg of amino acids were exuded per g of dry root during the first 14 days. During the first seven days, glucose and fructose were the primary sugar exudates. Twenty-five micrograms of each monosaccharide was exuded from each plant; Rovira (1956). Information on the effects of anaerobiosis upon the qualitative or quantitative exudation of amino acids and sugars is unavailable. There is, however, contradictory evidence concerning the role of oxygen in root exudation. Ayers and Thornton (1968) reported more material reactive to ninhydrin was released from roots of wheat and peas when grown in solution culture saturated with air as opposed to a solution culture saturated with gas containing 2% O_2. In contrast, Brown and Kennedy (1966) reported increased production of sucrose, fructose, galactose, glucose, and substances reactive with ninhydrin occurred in exudates collected from soybean seedlings germinated at low oxygen concentrations. They also reported bacterial contamination favored increased sugar production in exudates collected at all oxygen concentrations.

The evolution of volatile organic compounds such as ethylene has been noted in diseased, senescing, and wounded tissue by Burg (1962). Other volatile organics in the soil atmosphere have been neglected.

Fusarium Physiology

The need of macroconidia of Fusarium species for exogenous nutrients in germination has been established for several species and may be fairly general; Sisler and Cox (1954) and Marchant and White (1966). Cochrane et al. (1963) found that macroconidia of F. solani f. phaseoli required exogenous carbon and nitrogen and a growth factor present in yeast extract, which later could be replaced completely by ethanol or acetoin and partly by acetaldehyde or one of several amino acids.

Nutrient requirements for infection are higher than those for spore germination and so the nutrients supplied by root exudate are generally essential for infection; Garrett (1970). Toussoun, Nash, and Snyder (1960) reported that glucose was essential for the penetration stage of infection of bean hypocotyl by macroconidia of F. solani f. phaseoli, but glucose without a nitrogen source delayed host penetration and the onset of pathogenesis. Nitrogenous compounds did favor early penetration and pathogenesis with the organic forms being more effective than the inorganic forms.

Root exudates provided the necessary energy to increase the inoculum potential, Garrett (1970), of selected root saprophytes making them pathogenic. In turn, adverse soil conditions, such as soil flooding, degraded the integrity of root cells making them more susceptible to infection.

Fusarium solani f. *pisi*, an important component of the pea root rot complex, is a good example of the conversion of a soil saprophyte to a pathogen by root exudates. In a seedling test, Lockwood (1962) reported that the root rot of peas increased significantly when the sand was saturated immediately after inoculating with *F. solani* f. *pisi*. Payne *et al.* (1966) designed experiments and reported that the permeability of root cells was altered by anaerobiosis as more amino acids leaked from the roots of peas growing in saturated sand. These and others, Geisler (1965) and Cook and Flentje (1967), suggested that soil anaerobiosis played an important role in the root rot complex of peas.

Soil flooding also affects the survival of soil fungi. Newcombe (1960) demonstrated the survival of chlamydospores was dependent upon soil aeration. More recently, Griffin (1968) designed an experiment which demonstrated the gaseous requirements for the production of spores by *F. roseum* f. *cerealis*. He showed less air was required for the production of macroconidia than for the production of chalmydospores. Macroconidia were produced within the water film while chlamydospores were produced only in the air space of a simulated soil matrix. The report by Cochrane *et al.* (1963) agrees with Griffin as they demonstrated macroconidia germinated best at oxygen concentrations of 3-4%. However, there was essentially no germination of macroconidia under zero oxygen concentrations.

Greater partial pressures of CO_2 in flooded soils frequently approach levels which promote growth of certain fungi. Durbin (1955) reported the growth of fungi and certain other microorganisms was directly related to CO_2 concentration even at levels toxic to plant roots. In contrast, Cochrane et al. (1963) reported the germination of macroconidia accelerated when metabolic CO_2 was removed from the atmosphere. The report by Papavizas and Davey (1962) agrees with Durbin in that Fusarium growth increased proportionally to CO_2 concentrations up to 30%.

Cook and Flentje (1967) reviewed earlier studies and presented evidence that exudates from flooded seeds and not water alone influenced germination and subsequent growth of chlamydospores of F. solani f. pisi in soils contiguous to germinating peas. They also reported mycelial growth near the host was greatest in soil with an intermediate water content. This evidence implies that during anaerobiosis the nutrient content in pea root exudate approached the critical level required for germination and exceeded the level for fungal survival; Cochrane et al. (1963) and Cook and Snyder (1965).

Exudate Measurements

Gas liquid chromatography (GLC) has attained great sophistication with great speed, sensitivity, and versatility. GLC methods now encompass a wide range of analytical applications; examples include analytical

methods for carbohydrates and related polyhydroxy compounds, amino acids, and amino sugars; Sweeley et al. (1963), Zumwalt (1971), and Karkkainen and Vihko (1960). The analysis of these compounds by GLC techniques has been advanced through extensive studies involving numerous derivatization methods and chromatographic systems; McBride et al. (1968) and Pierce (1970). GLC analysis of trimethylsilylated (TMSi) derivatives of the above compounds is the preferred method. This method is simple in its formation, quantitative in yield (95-100%), and forms sufficiently volatile compounds that give good resolution in chromatography without losses during sample concentration by evaporation.

MATERIALS AND METHODS

Description of Plant Growth Chamber

A Sherer growth chamber model CEL 25-7 was used for controlling the environmental conditions. The unit was equipped with temperature and photoperiod controls. The relative humidity was controlled by evaporation of free water.

The unit was modified to provide a range in quality and intensity of light. Low light conditions of 0.63 g cal cm^{-2} min^{-1} (Langleys) were achieved by using 6 fluorescent bulbs, 4-25 watt, and 5-75 watt incandescent bulbs. High light conditions of 1.1 Langleys were achieved by 6 fluorescent bulbs, 4-100 watt, 4-150 watt, and 1-200 watt incandescent bulbs. The energy emitted for each light intensity was measured by a Beckman and Whitley thermal radiometer and an inline Sargent recorder.

Temperatures were maintained at 18.3±.5 and 23.3±.5 C for the low and high light intensities. These air temperatures simulated the temperature of field conditions in Michigan during the spring growing season. Temperatures inside the mist chamber were 18.0±.5 C. The conditioned air was filtered through eight layers of moist cheesecloth and circulated through the growth chamber.

Description of the Root Chamber System

A pyrex glass mist chamber was designed and constructed to produce fine water droplets which continuously bathed the plant roots. Figure 1 shows the details of the root chamber. The chamber was equipped with a variable flow Masterflex pump* model 7545-15 which recirculated the sterile nutrient solution. The inline pressure was 15±5 psi producing a flow of 25 ml min^{-1} at the orifice of the nozzle. The circulating solution was filtered by an inline cellulose ester filter+ which had a mean pore size of 0.22μ. The fine droplets of spray were produced by a small brass nozzle with an orifice of 0.5 mm which formed a full cone spray. The brass nozzle was secured to the glass access tube with epoxy cement, Figure 1.

The gas exhaust apparatus was designed to remove water from the escaping gases without resisting flow. The dry barrier prevented contamination of the system by micro-organisms. A U-shaped gas exhaust tube collected and returned the condensate to the solution reservoir via the condensate return, Figure 1. Gas flowing into the chamber via the gas inlet was sterilized by filtration. Filtration was achieved by flowing the gas through a Millipore filter having a mean pore size of 0.22μ. Treatment gases flowed through the chamber at the rate of 25±5 ml min^{-1}.

---

*Obtained from the Cole Parmer Co., Chicago, Ill.

+Obtained from the Millipore Corp., Bedford, Mass.

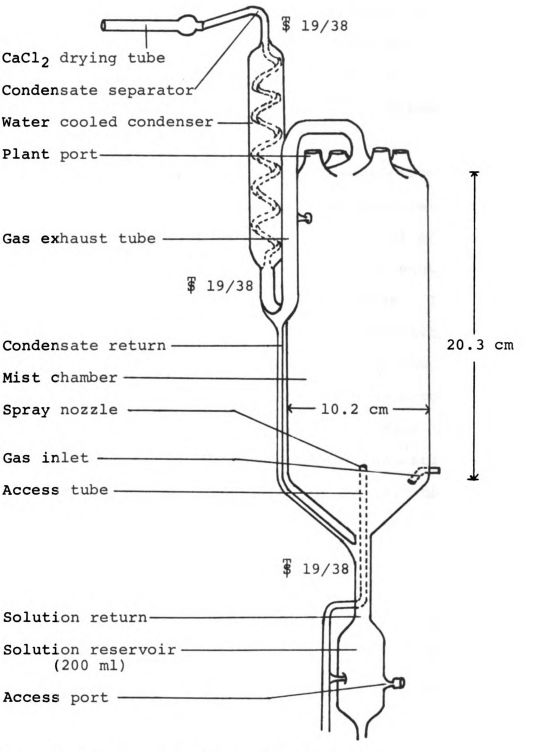


Figure 1. Diagram of pyrex glass mist chamber system.

A glass reservoir was connected to the root chamber with a ground glass joint (₹ 19/38), Figure 1. This connection functioned as the drain to the mist chamber through which the nutrient solution flowed, by gravity, to the reservoir. The reservoir was equipped with an access port that was fitted with a silicone rubber septum.

It is believed the sterile mist chamber removed essentially all the biochemical, microbiological, and physical factors which obscure the true quality and quantity of actual root exudates. This system is especially suited for studying root response to the composition of rhizosphere gases. This system provided a method of manipulating the gas composition of the rhizosphere without changing the thickness of the water film on the roots. Rapid changes in the gaseous composition of the rhizosphere are also possible with this apparatus. The advantage of this system is that the entire apparatus can be sterilized by autoclaving and aseptic conditions maintained for prolonged periods of time.

Preparation of Seedlings

Peas (Pisum sativum L.; variety, Miragreen*) were used throughout this investigation. Mature seeds were surface sterilized by immersing in 50% ethanol for 30 seconds followed by six washings with distilled water. Then the seeds were immersed in 100 ml of 0.2% $HgCl_2$ containing two drops of "Tween 80". After five minutes the seeds were washed

---

*Obtained from the Ferry-Morse Seed Co., Buffalo, N.Y.

free of $HgCl_2$ with six washings of sterile redistilled water and soaked in redistilled water for 12 hours. Sterilization time was determined by the experiment reported in Table 1. The seeds, imbibed with water, were transferred to 500 ml gas diffusion vessels where they germinated in the dark in sterile redistilled water.

Table 1. The effects of time on germination, tissue injury, and sterilization of pea seeds soaked in 0.2% $HgCl_2$ plus Tween 80.

Soaking time - min	Germination %	Lesions per seed	Aseptic seeds %
5	100	6.0	100
10	100	8.0	100
15	84	8.0	100
20	90	7.8	100

The water was vigorously aerated by compressed air scrubbed by acid, base, and water baths and sterilized by filtration. After 72 hours, 48 of the most uniform seedlings were transferred to sterile glass storage vessels 80 x 100 mm. Approximately 35 ml of a 50% modified Hoagland's solution were added to the storage vessels and sterilized by autoclaving. The radicle of each seed was placed in the nutrient solution and the seedlings were held in position by placing them between two glass rods. These vessels were placed in the dark for 48 hours, then placed in the growth chamber for 48 hours.

Four uniform seedlings were transferred to each root chamber. The green plumule of each plant measured 25±5 mm and the primary root 35±5 mm in length. During this transfer the seedcoat was removed and the seed and root were washed with a sterilized nutrient solution. Methods of sterile technique were executed during all transfers including the use of sterile gloves while installing seedlings in the plant holder, Figure 2. The plant port and holder were constructed from the lower half of a female and male ground glass joint, respectively. The base of the male was partially closed forming a small opening 6 mm in diameter. The plumule of the pea seedling was inserted through the bottom of the plant holder to the seed and secured to the holder with sterile parawax (a mixture, by weight, of 1 g hard filtered paraffin : 8 g petroleum jelly). This seal proved to be a barrier to gas, water, and microorganisms, yet was plastic, enabling the plant to expand during growth. Plant leaves were protected from desiccation by a polyethylene bag removed 12 hours after the root chamber was installed in the plant growth chamber. The root chambers were wrapped with aluminum foil simulating the dark conditions of the soil.

Treatment of Seedlings

Seedlings were transplanted 7 days after seed sterilization. Seedlings were treated for the following 6 days and harvested. Preliminary studies indicated this stage of

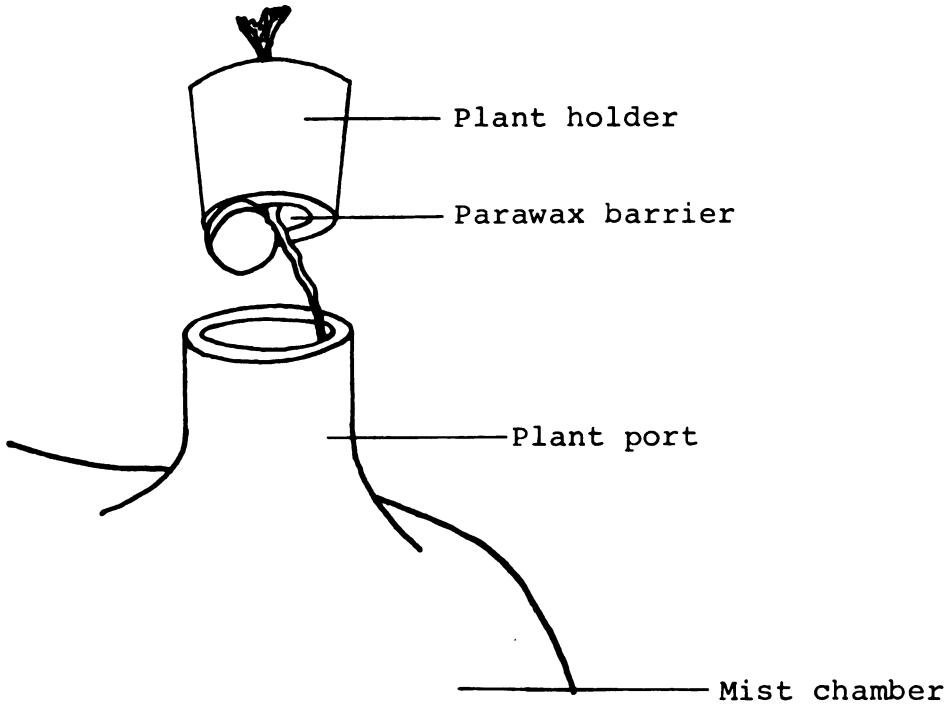


Figure 2. Diagram of apparatus used to mount seedling into mist chamber.

plant growth was the most dynamic for pea roots. During this period the secondary root initials emerge rupturing the primary root, exposing the young root tip. It is believed this phase of root development is most sensitive to the gas composition of the rhizosphere.

The treatments of this investigation were:

1) Air control,
2) N_2 without CO_2,
3) Air for 48 hours, then N_2 without CO_2 for 48 hours, then air for 48 hours--low light,
4) 70% N_2 with 30% CO_2,
5) 70% air with 30% CO_2,
6) Air for 48 hours, then N_2 without CO_2 for 48 hours, then air for 48 hours--high light,
7) Air plus inoculum,
8) 70% N_2 with 30% CO_2 plus inoculum for 10 days, then air for 7 days.

Experiments 1-5 were run under low light conditions and experiments 6-8 were conducted under high light conditions. Treatments 1-6 were run for 6 days. Carbon dioxide was removed from the N_2 gas by flowing the treatment gas through a 35 x 2 cm plexiglass cylinder filled with ascarite.* The pH of the circulating nutrient solution was 6.5 at the beginning of each experiment and changed very little during each experiment except in those treated with 30% CO_2 where

---

*Registered trademark for CO_2 absorber from Arthur H. Thomas Co., Philadelphia, Pa.

the pH dropped to 5.5±.3, 12 hours after the initiation of the experiment.

Seedlings in experiments 7 and 8 were treated for 17 days. Those in experiment 8 were treated with 30% CO_2 + 70% N_2 for 10 days then switched to air (352 ppm CO_2) for the remaining 7 days. Experiment 7 was treated with air for the duration of the experiment. Macroconidia of Fusarium solani f. pisi were injected into the nutrient solutions of these two experiments 5 days after the onset of the experiments. The spore concentration of the circulating nutrient solution was 1.0×10^6 macroconidia ml^{-1} for both experiments.

Collection of Root Exudates

Aliquots, 0.5 ml per chamber, were extracted from the reservoir with a hypodermic needle and syringe via the rubber septum. Samples were taken at 4, 6, 12, or 24 hour intervals and analyzed for ethanol. At the end of each experiment the nutrient solution containing the root exudates was measured and frozen. Water was removed from the solution by lypholization. The lypholized samples were weighed, dried further at 105 C for 60 min, derivatized and analyzed for amino acid and sugar content.

Collection and Measurement of Root Gases

Gases evolved by pea roots were determined by analyzing the gas composition before and after passing it through the chamber. Oxygen and CO_2 were monitored by a magnetic moment

Beckman oxygen analyzer and a Beckman model 215A infrared CO_2 analyzer. Organic volatiles were collected on an organic compound trap and removed, by heating, onto a gas chromatograph column for analysis. The trap was constructed of two QD plastic connectors joined together by a tygon tube 5 x 1.27 cm. The trap was filled with 0.5 g of Porapak type Q* having a mesh size of 50-80 with essentially no resistance to gas flow.

Plant Measurements

The shoot, root, and seed of each plant were separated, measured, dried, and weighed at the end of each experiment. The mean value of 12 plants was considered representative of that component.

All debris from the roots of treated plants was determined by taking the difference between the dry weight of the inline prefilter before and after each experiment.

Bioassay

In this experiment the germination of Fusarium macroconidia in ethanol was tested in the presence of five gaseous atmospheres. Sterile redistilled water containing 0, 10, 100, 1000 ppm of ethanol, by weight, was saturated with air, 30% CO_2 + 70% N_2, 30% CO_2 + 70% air, N_2 without CO_2, and N_2 passed through a heated column filled with elemental copper to remove residual oxygen. The dissolved oxygen concentrations of these solutions were 8.23, 0.13, 4.71, 0.18, and

---

*Obtained from Waters Associates Inc., Farmington, Mass.

0.41 ppm, respectively. Analysis of the dissolved oxygen content was by the Winkler method.

Stoppered Erlenmeyer flasks (125 ml) were used for this experiment. Each vessel contained 100 ml of the treatment solution. The pathogen concentration used was 3.0×10^6 spores, mostly macroconidia, per ml. Aliquots, 1 ml per sample, were removed periodically for spore germination and ethanol consumption analysis.

Preparation of Inoculum

Fusarium solani f. *pisi*, obtained from Dr. Lockwood's laboratory, was the pathogen used in this investigation. The pathogen was cultured on slants of potato dextrose agar (PDA). Spores were obtained from 15-day agar cultures incubated in the dark at 22 C.

Spores were washed from the agar surface, centrifuged, and resuspended four times in sterile redistilled water. The concentration of spores, mostly macroconidia, were standardized by the hemacytometer method. Germination was determined by counting the germ tubes of 100 spores from each replicate. The criterion for germination was the formation of a microscopically visible germ tube. Length of germ tubes was measured by an ocular micrometer.

Tests for Aseptic Conditions

Samples from all experiments were cultured on PDA to detect contamination. The pH of PDA was adjusted to 7.0

with 40 ml of 0.1 M K_2HPO_4 l^{-1} PDA to provide a medium conducive to fungal and bacterial growth. Inline filters from the root chamber were also plated and cultured for 2 weeks, then examined. All contaminated replications were discarded.

Statistical Analysis

Analysis of variance was used to determine the statistical differences among the treatments in this investigation. The hierarchal or nested design was used for all experiments involving the root chamber. The average of each root chamber was used to calculate the sums of squares in the analysis of variance. A randomized block design was used for the bioassay experiments. The average value of each replication was used to calculate sums of squares in the analysis of variance. Significant differences among the data were determined by the least significant difference (LSD) method.

Gas Chromatography and Mass Spectroscopy

Ethanol was identified and measured by injecting the samples, taken from the reservoir, directly into the gas chromatograph. One microliter samples were injected into a Beckman GC-2A gas chromatograph equipped with a hydrogen flame detector and a 2 m x 3.2 mm stainless steel column packed with Porapak QS (100/120 mesh). Oven temperature was isothermal at 175 C with the flow rate of the helium carrier gas at 80 ml min^{-1}.

The nutrient solution and exudates from three chambers were lypholized to dryness. Exudates were silylated in the

presence of the remaining salts before analysis by gas chromatography and mass spectroscopy.

The silylated derivatives (TMSi) of the root exudates were formed by substituting a silyl group for an active hydrogen and/or replacing the metal component of a salt; Pierce (1970) and personal communication with the Biochemistry Department, M.S.U. A sixty molar excess of N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA)* containing 1% of the catalyst trimethylchlorosilane (TMCS) was added to the dried sample and sonicated for five hours at 70 C in an ultrasonic cleaning vessel filled with water. The ultrasonic treatment physically dismantled the salt-exudate complex enhancing silylation. During silylation the solvent properties of BSTFA and its reaction products were used to dissolve the amino acids and carbohydrates in the exudates. The dissolved TMSi samples were centrifuged and concentrated by evaporation. The sample was redissolved in 50μl of BSTFA containing 1% TMCS and 50μl dimethylformamide. The above reactions were carried out in a Kimax vial (100 x 13 mm) covered with a teflon-lined cap.

The TMSi derivatives were quantitatively and qualitatively analyzed by using a Perkin-Elmer 900 gas chromatograph and an LKB 9000 gas chromatograph-mass spectrometer, respectively. Quantitative analysis of the TMSi derivatives was performed by injecting 5μl samples into the gas chromatograph equipped with a hydrogen flame detector. The column used was a 3.3 m x 3.2 mm stainless steel tube packed with 3% SE-30

---

*Obtained from the Regis Chemical Co., Chicago, Ill.

ultraphase on chromosorb W (HP)* (80/100 mesh). The oven temperature was programed from 90-200 C at 2° min^{-1}. The injector and manifold temperatures were 250 C. Flow rate of the helium carrier gas was 40 ml min^{-1}. Standards of glucose, fructose, ribose, and sucrose were weighed, then silylated by the same procedure used for the exudates.

Mass spectrometric analysis was performed by eluting the samples through the SE-30 column used above. The separated compounds flowed from the column, whose temperature was programed from 90-200 C at 2 C min^{-1}, into the ionization chamber of the LKB single-focusing mass spectrometer. The ionization voltage was 70 ev while the temperature of the ion source was 290 C. TMSi derivatives were volatilized at 250 C in the flash heater at the head of the column. Helium carrier gas flowed through the system at 40 ml min^{-1}.

Conditions for the gas chromatography-mass spectrometric analysis of the exhaust gases from the rhizosphere were similar to those listed above. The changes for this analysis included a 3.3 m x 3.2 mm stainless steel column packed with 3% OV 101 on chromosorb W (HP) (80/100 mesh). A 15.2 cm x 6.35 mm stainless steel pre-column was packed with the 0.50 g Porapak Q. The unknown compounds in the pre-column were volatilized at 275 C and eluted into the main column which was at room temperature. Then the main column was programed at 6° min^{-1} from 50-150 C and 2° min^{-1} from 150-250 C. All compounds were identified by studying the important fragmented ions recorded on a bar graph spectrogram.

---

*Obtained from the Pierce Chemical Co., Rockford, Ill.

RESULTS AND DISCUSSION

Mist Chamber

The mist chamber proved to be an excellent system for studying the effects of gas composition on plant roots. By simply altering the flow rate of gases entering the mist chamber, the gaseous composition could be changed very rapidly for determining the short-term effects on plant roots or very slowly simulating natural field conditions, Figure 3. The system also provided a means for manipulating the gas environment, extracting root exudates, observing morphological changes, and inoculating roots without contaminating the root zone. Sterile conditions were maintained for 88% of the experiments. The present design should also maintain sterile conditions for prolonged periods when aseptic plant roots are transferred to the system.

Additional uses of this system might include measurements of water and nutrient uptake, evaluations of disease resistance, the energy requirements of roots in the presence and absence of soil microflora and the effects of rhizosphere gases on these reactions.

Content of Exudates

Ethanol: Roots of intact pea seedlings were tested for loss of ethanol into the rhizosphere during anaerobiosis.

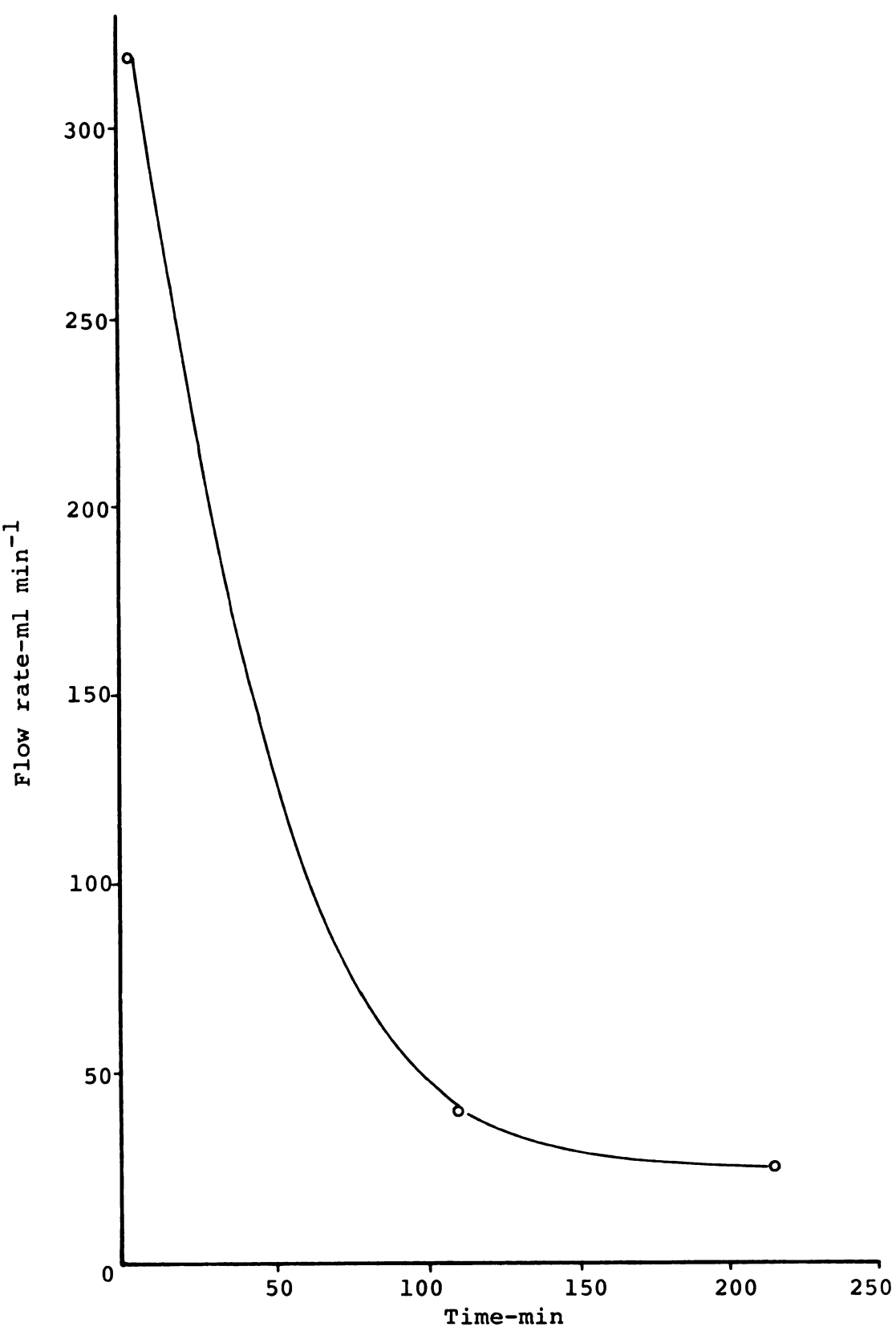


Figure 3. Time required to remove oxygen from mist chamber by flushing the system with N_2 gas at various flow rates.

Data obtained from the treatments with different gases under high and low intensities of light are presented in Figures 4-7 and Tables 2-7. The accumulation of ethanol in root exudates was quite different depending upon the gas composition of the rhizosphere and the light intensity.

Ethanol was absent from the root exudates of peas grown in a sterile aerobic rhizosphere. In contrast, large quantities of ethanol accumulated in root exudates under anaerobic conditions, Table 2. Similar results have been found by others; Kenefick (1962) and Bolton and Erickson (1970). However, the accumulation of ethanol in the root exudates of their experiments was greatly reduced as the roots were not under aseptic conditions.

Table 2. The effects of gas composition on the net accumulation of ethanol in the root exudates of peas treated for 6 days.

Treatment	Ethanol mg/g dry root
Air	0.0[a]
Air containing 30% CO_2	134.3
N_2 without CO_2	146.2
N_2 containing 30% CO_2	269.5

[a]Each value represents the average of three replications.

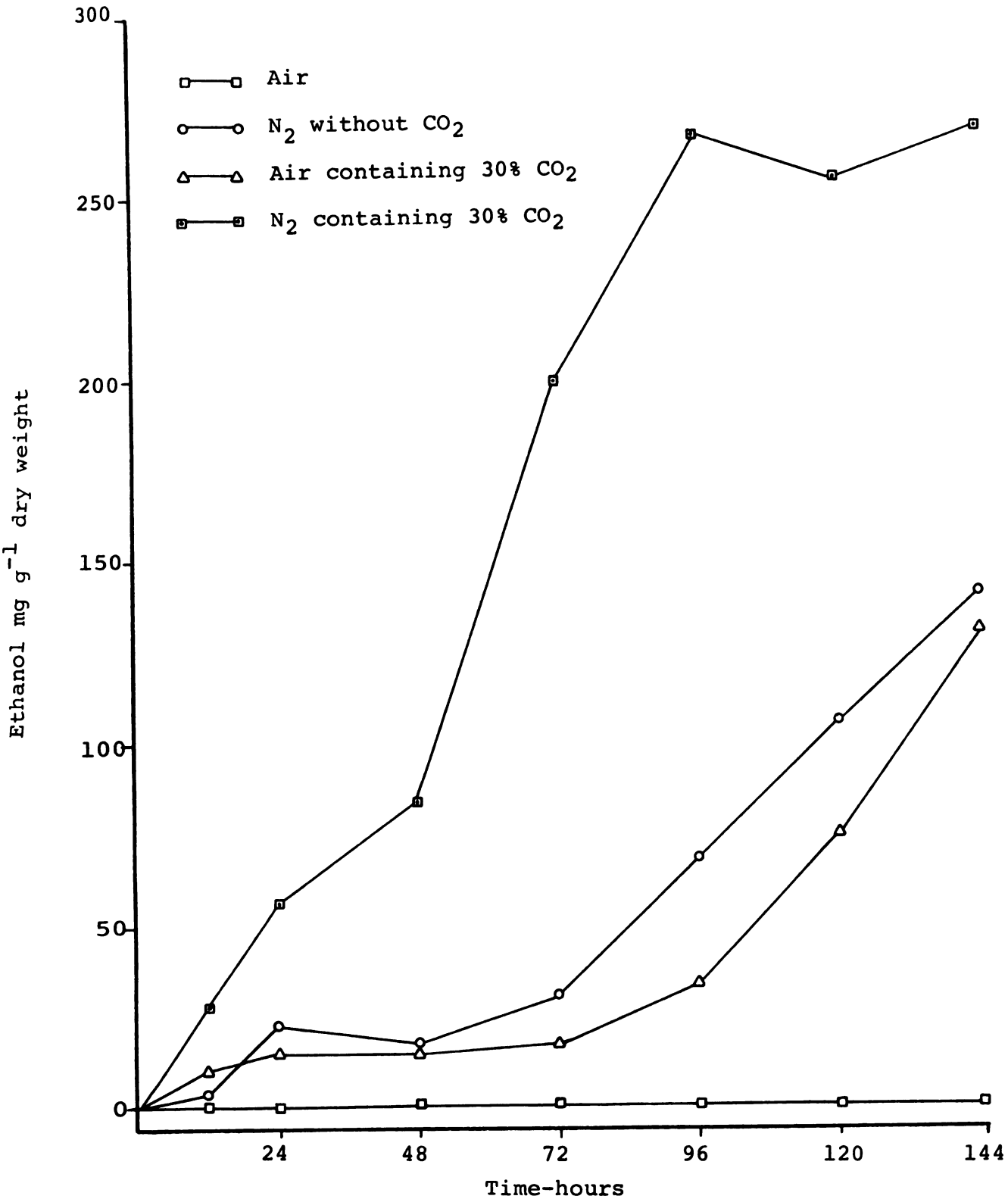


Figure 4. Effect of gas composition on ethanol production by the roots of aseptic peas grown under low light conditions. (Each point represents the average value of three replications.)

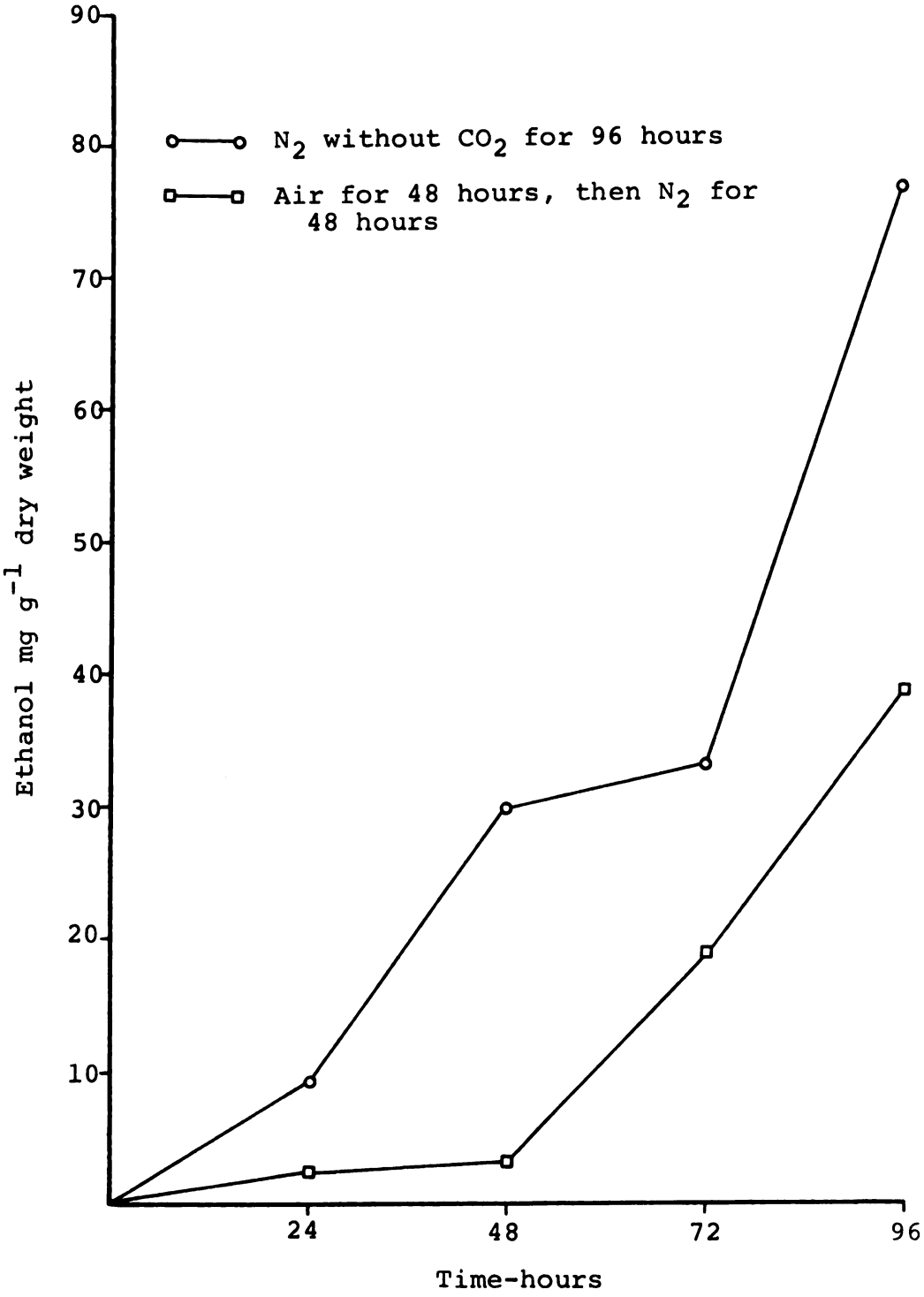


Figure 5. Effects of flooding period on ethanol production by aseptic roots under low light conditions. (Each point represents the average value of three replications.)

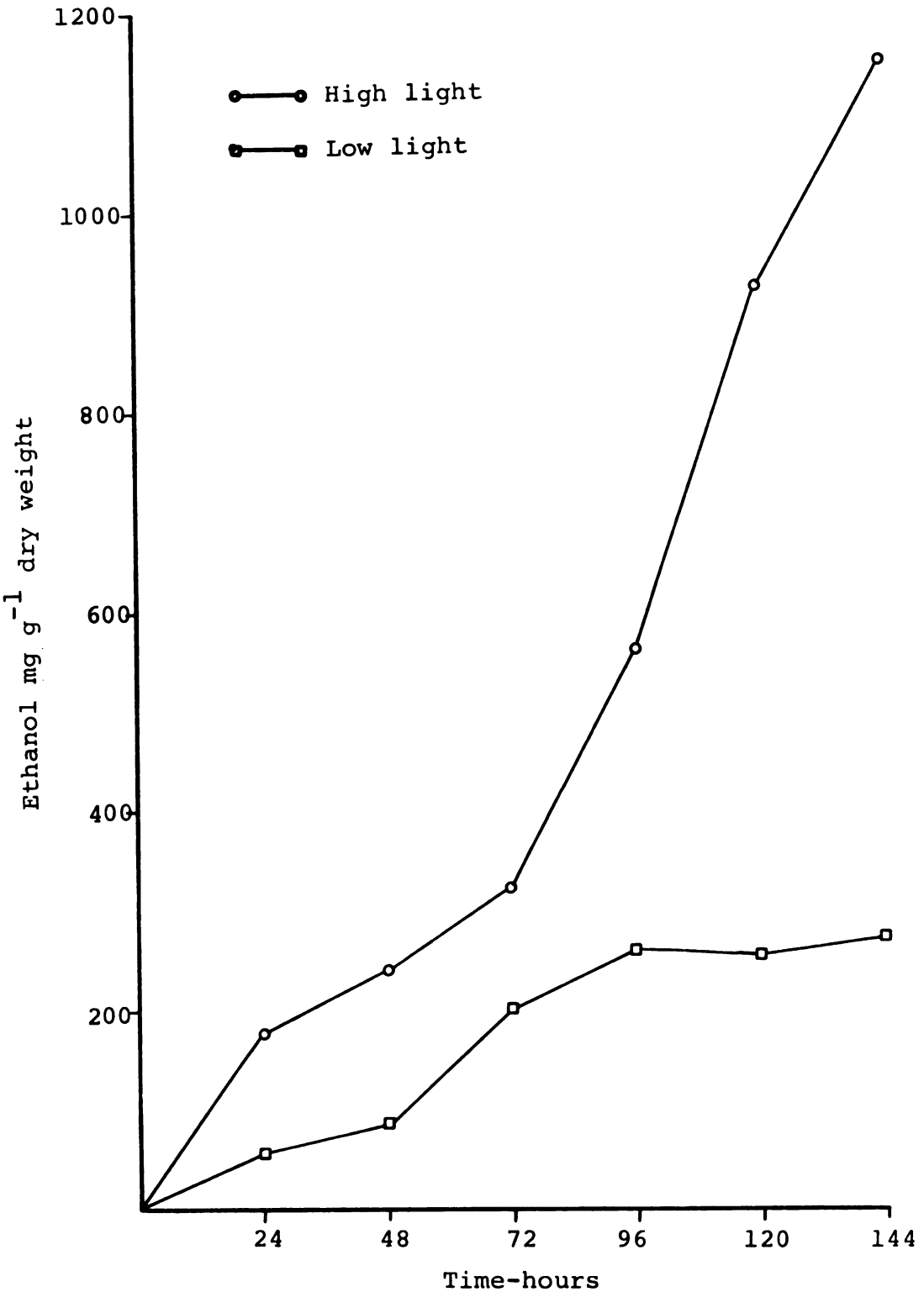


Figure 6. Effects of light intensity on ethanol production by aseptic pea roots subjected to N_2 containing 30% CO_2. (Each point represents the average value of three replications.)

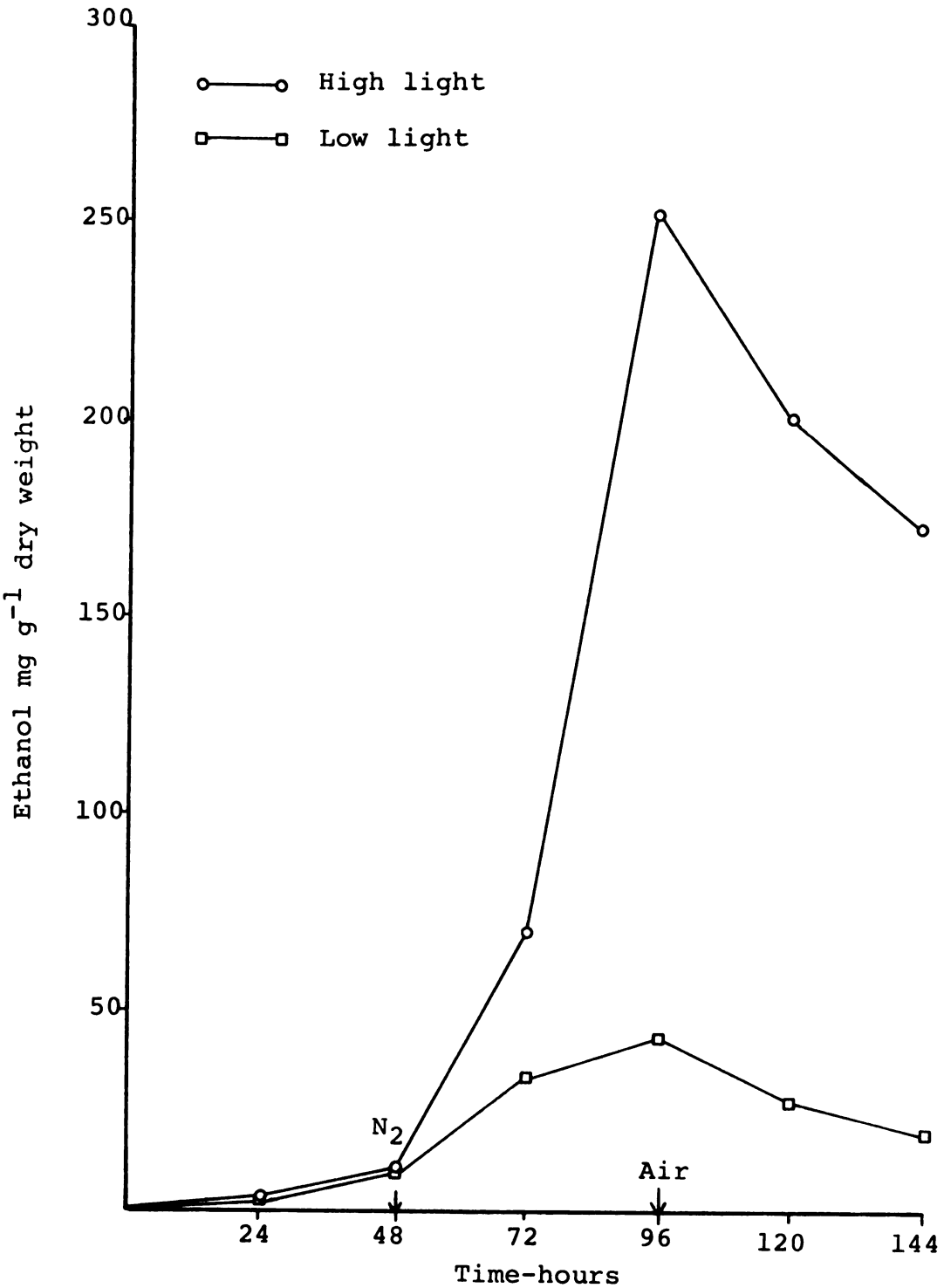


Figure 7. Effects of light intensity on the ethanol production by aseptic pea roots treated with air for 48 hours, then N_2 without CO_2 for 48 hours, then air for 48 hours. (Each value represents the average of three replications.)

Table 3. Ethanol production in the exudates from roots of four pea plants treated with N_2 containing 30% CO_2.

Duration of treatment hours	Ethanol concentration ppm	Remaining volume of nutrient solution ml
17	6[a]	174
24	9	171
35	14	168
43	11	164
48	14	160
60	33	156
66	37	152
72	36	148
87	43	144
96	51	140
108	51	136
113	53	132
120	53	128
132	56	124
140	65	120
144	62	116

[a]Each value represents the average of three replications.

Table 4. Ethanol production in the exudates from roots of four pea plants treated with 30% CO_2 and air.

Duration of treatment hours	Ethanol concentration ppm	Remaining volume of nutrient solution ml
6	1[a]	173
24	2	165
48	2	152
72	2	140
96	5	126
120	12	111
126	16	107

[a]Each value represents the average of three replications.

Table 5. Ethanol production in the exudates from roots of four pea plants treated with air for 48 hours, then N_2 without CO_2 for 48 hours, then air for 48 hours at high light intensity.

Duration of treatment hours	Ethanol concentration ppm	Remaining volume of nutrient solution ml
48	2[a]	173
56	3	166
58	8	162
70	15	158
74	30	154
80	36	150
86	50	146
96	62	142
120	57	127

[a]Each value represents the average of three replications.

Table 6. Ethanol production in the exudates from roots of four pea plants treated with N_2 without CO_2.

Duration of treatment hours	Ethanol concentration ppm	Remaining volume of nutrient solution ml
12	1[a]	172
24	2	168
48	6	156
72	7	140
132	37	104

[a]Each value represents the average of three replications.

Table 7. Ethanol production in the exudates from roots of four pea plants treated with air for 48 hours, then N_2 without CO_2 for 48 hours, then air for 48 hours.

Duration of treatment hours	Ethanol concentration ppm	Remaining volume of nutrient solution ml
12	1[a]	175
24	1	170
54	1	160
66	4	152
72	6	147
79	7	142
90	12	136
92	14	130
138	10	120
142	8	114
149	10	110

[a]Each value represents the average of three replications.

The possibility that CO_2 might affect root exudation of ethanol during soil flooding was also considered. The fact that gases taken from an anaerobic soil at a depth of two meters contained 15.5% CO_2 (Baver, 1956) suggests that very high concentrations of CO_2 may occur in the rhizosphere of respiring roots when flooded. Values of 20% CO_2 have also been reported to accumulate in very heavy soils containing decomposing organic matter; Kidd (1914). To study this possibility, air containing 30% CO_2 and a mixture of 70% N_2 and 30% CO_2 were obtained from the Matheson Gas Products Company. These gases were circulated through the mist chamber for six days and the ethanol content of the circulating mineral nutrient solution was monitored. Data is presented

in Figures 4 and 6. The addition of 30% CO_2 to 70% N_2 caused a 200% increase in ethanol accumulation. With 30% CO_2 and 70% air, ethanol accumulated at a rate similar to that of N_2. This is illustrated by the nearly parallel curves for these two treatments in Figure 4.

These results indicate that high concentrations of CO_2 affect not only the permeability of the root membranes but also the metabolism of plant roots.

The low pH (5.5) created by saturating the mineral nutrient solution with 30% CO_2 may have increased the permeability of membranes in stressed roots. This phenomenon explains in part the greater accumulation of ethanol in the exudates of roots subjected to N_2 with 30% CO_2. However, the formation of ethanol in roots treated with air containing 30% CO_2 suggests the high partial pressures of CO_2, occurring in cells subjected to high concentrations of CO_2, inhibit the normal cycling of the tricarboxylic acid (TCA) cycle, White et al. (1968). Just as in the case of oxygen stress, when reactions in the TCA cycle are inhibited by their accumulated products (i.e., reduced pyridine nucleotides) high concentrations of CO_2 could inhibit the decarboxylation reactions, reducing the rate of the cycle. Assuming this occurs, pyruvate is then reduced to ethanol forming an alternate electron sink even in the presence of oxygen. In any case, whether or not the forementioned alternate pathways occur, the results present concrete evidence that metabolic pathways of aerated roots are altered by high concentrations of CO_2.

Ethanol production during anaerobiosis was also affected by light intensity. Figures 6 and 7 show more ethanol accumulated in the root exudates of plants subjected to high light regardless of the gas used in the rhizosphere. Bolton and Erickson (1970) reported similar results by showing higher ethanol concentrations accumulated in xylem exudates of tomato plants grown under high light conditions. It is believed this phenomenon occurs as a result of higher photosynthetic rates during the higher light and temperature conditions. Ethanol was absent from the exudates of aerobic roots under high light.

The rate at which ethanol accumulates during anaerobiosis appeared to be unaffected by the concentration of ethanol in the rhizosphere; Figure 5. However, when the atmosphere of the root zone is made aerobic, the ethanol content of anaerobic root exudates declined as shown in Figure 7. These results indicate that exuded ethanol was reabsorbed and/or oxidized by the roots. Similar results were reported by Cossins and Turner (1962). They reported that an active alcohol dehydrogenase in the cotyledons is active in peas during germination but the activity declines soon after germination. In this study, ethanol disappeared from the exudate of 11-day-old pea roots indicating that an active alcohol dehydrogenase enzyme may be present in the roots. These data provide evidence that the reaction in equation (1) occurs in the roots of peas:

$$\underset{\text{acetaldehyde}}{CH_3\text{-}COH} \xleftrightarrow[DPN^+ \quad DPNH]{\text{alcohol dehydrogenase}} \underset{\text{ethanol}}{CH_3CH_2OH} \qquad (1)$$

Diphosphopyridine nucleotide (DPNH) is the reduced pyridine

nucleotide and DPN^+ is the oxidized pyridine nucleotide. Since the direction of equation (1) in peas is controlled by the presence or absence of oxygen it appears that initially the reaction is driven to the right during anaerobiosis by the abundant supply of substrate and DPNH.

TMSi derivatives: Root exudates of intact pea seedlings were analyzed for amino acids and carbohydrates. Data obtained from chromatographing their TMSi derivatives are presented in Figures 8-15 and Table 8. The exudation patterns were quite different depending upon the length and degree of anaerobiosis and the presence or absence of Fusarium.

The column used in this study gave excellent separation of the TMSi derivatives of root exudates as long as they were in small quantities. Alanine, valine, leucine, glycine, proline, serine, threonine, and glutamic acid were eluted before the pentoses and hexoses. Good separation of exudate carbohydrates also occurred on the SE-30 column.

At the onset of this investigation attempts were made to silylate root exudates using BSA in pyridine. This procedure was soon abandoned as most of the amino acids were masked by the tailing pyridine peak. Attempts were made to remove the pyridine by partitioning the TMSi derivatives in hexane and water as described by Wood et al. (1965). This procedure is excellent for TMSi sugars as hexane is insoluble in water yet retains the carbohydrates, but only compounds the TMSi amino acid-pyridine problem.

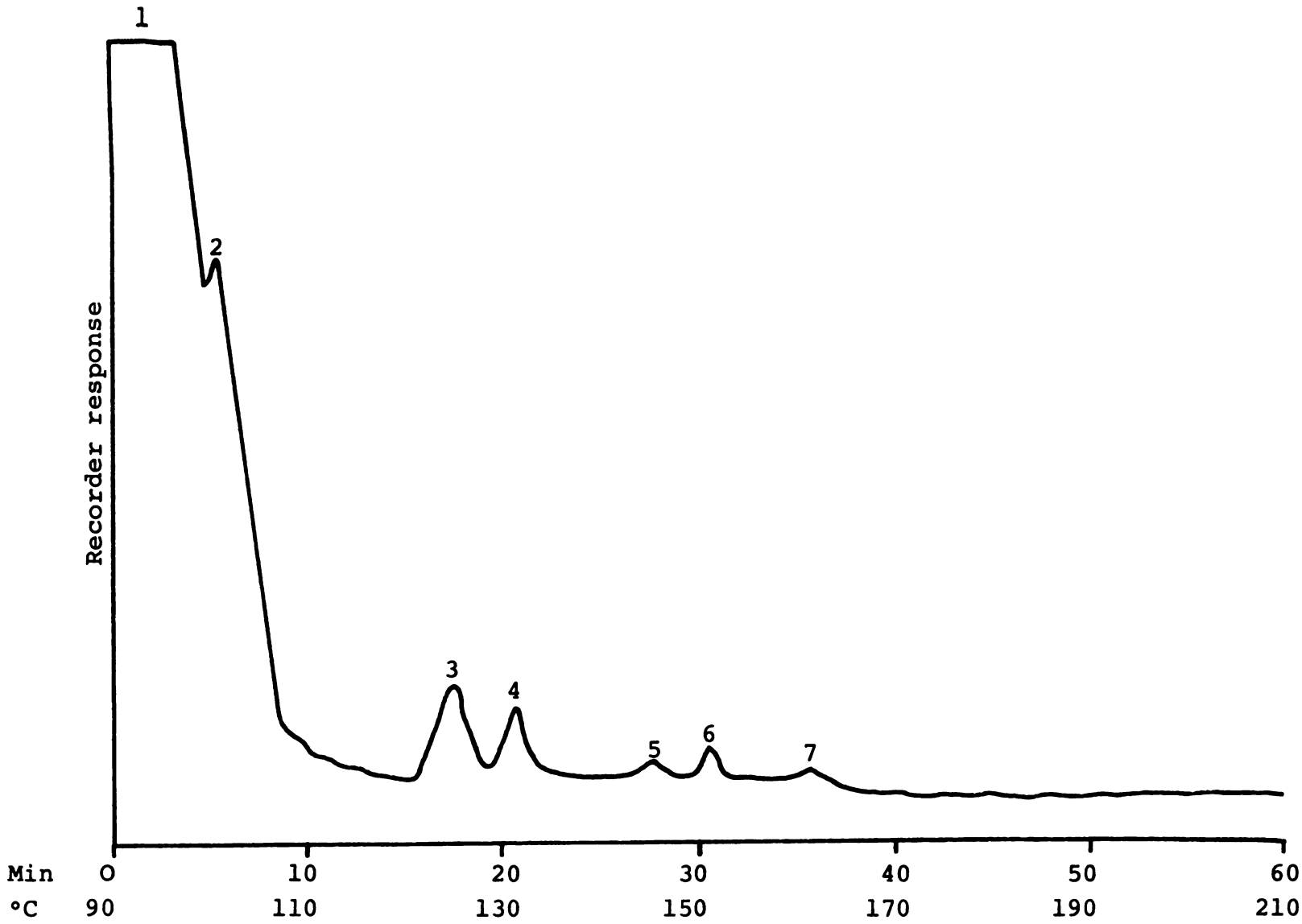


Figure 8. Gas chromatogram of TMSi derivatives of exudates from roots of 12 pea plants treated with air for 6 days. Peaks are: 1, alanine; 4, ribose; 5, fructose; 6 and 7, glucose; 2 and 3, unknowns.

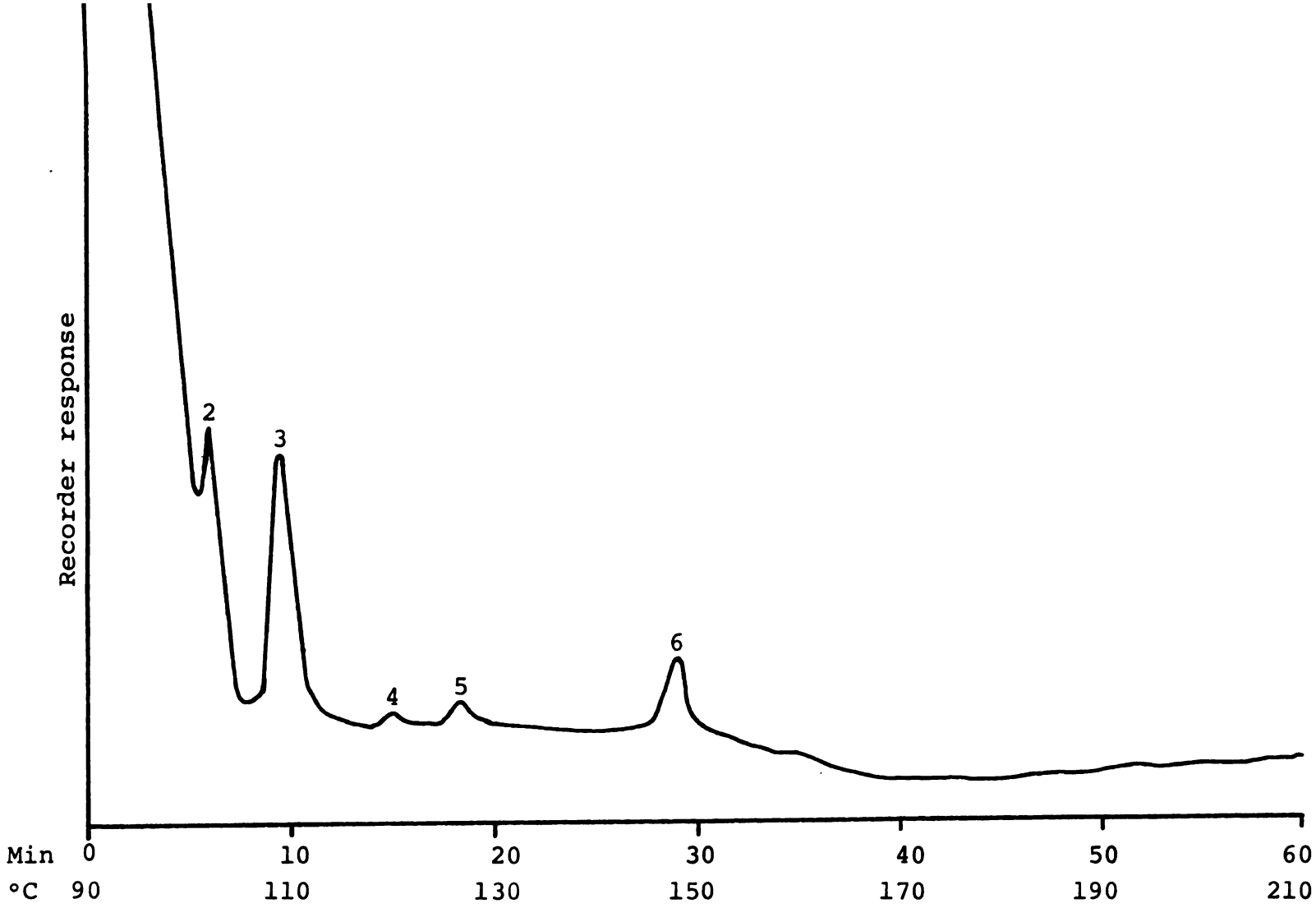


Figure 9. Gas chromatogram of TMSi derivatives of exudates from roots of 12 pea plants treated with N_2 for 6 days. Peaks are: 1, alanine; 3, threonine; 5, ribose; 6, glucose; 2 and 4, unknowns.

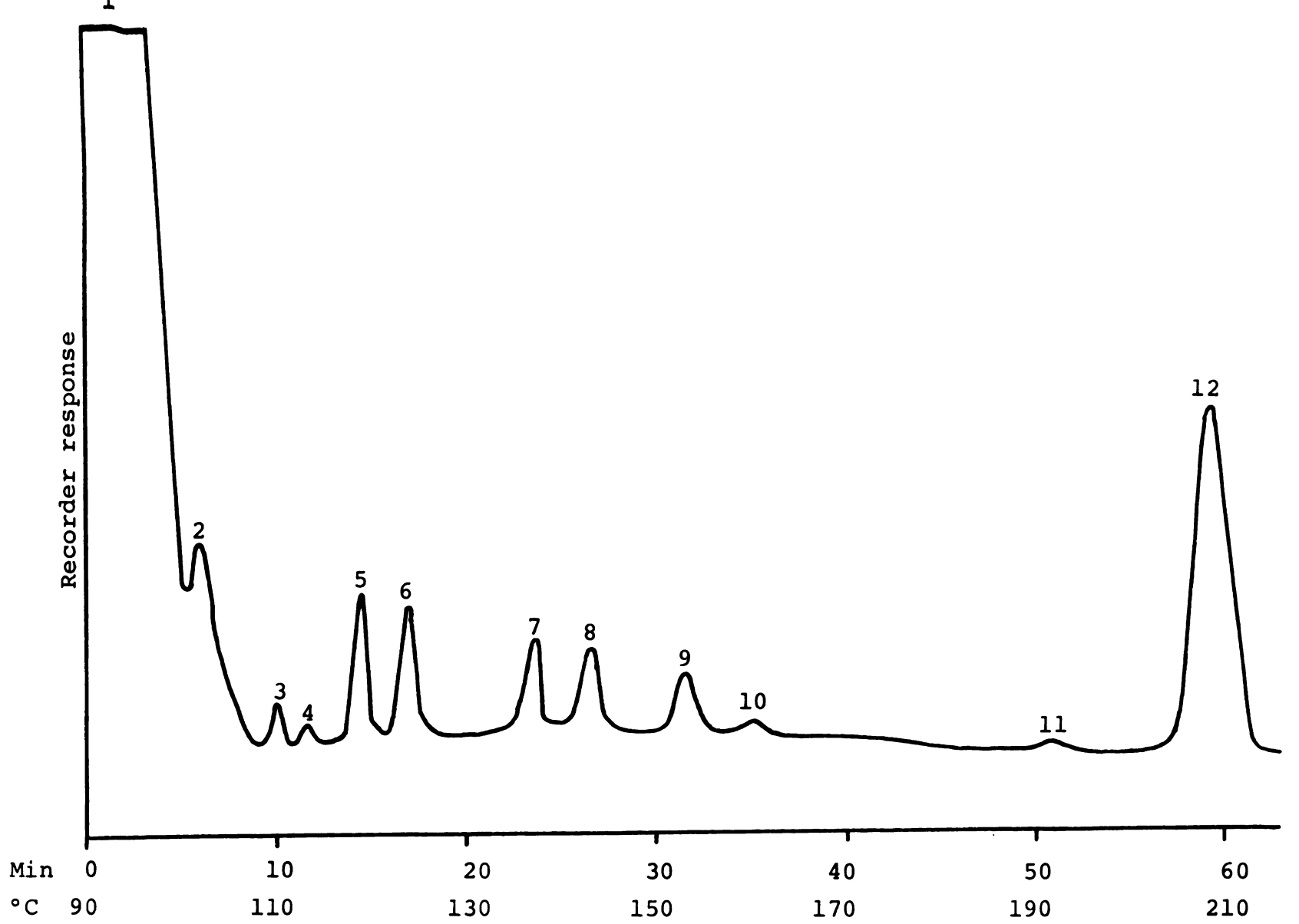


Figure 10. Gas chromatogram of TMSi derivatives of exudates from the roots of 12 pea plants treated with air for 48 hours, then N_2 without CO_2 for 48 hours, then air for 48 hours under low light. Peaks are: 1, alanine; 2, leucine; 4, aspartic acid; 5, glutamic acid; 8, fructose; 9 and 10, glucose; 12, sucrose; 3, 6, 7, and 11, unknowns.

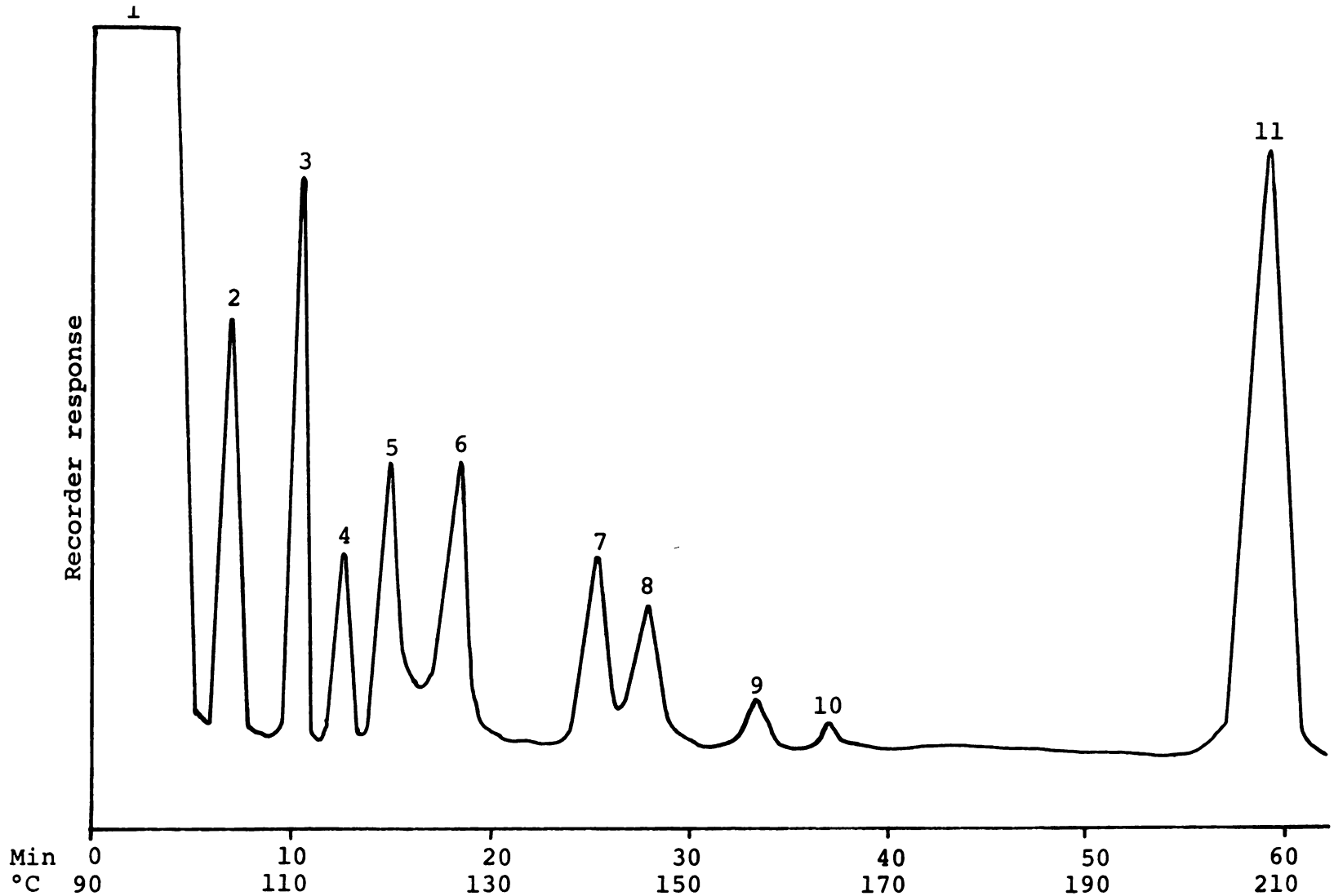


Figure 11. Gas chromatogram of TMSi derivatives of exudates from roots of 12 plants treated with N_2 containing 30% CO_2 for 6 days. Peaks are: 1, alanine; 2, leucine; 4, aspartic acid; 6, ribose; 7 and 8, fructose; 9, glucose; 11, sucrose; 5 and 10, unknowns.

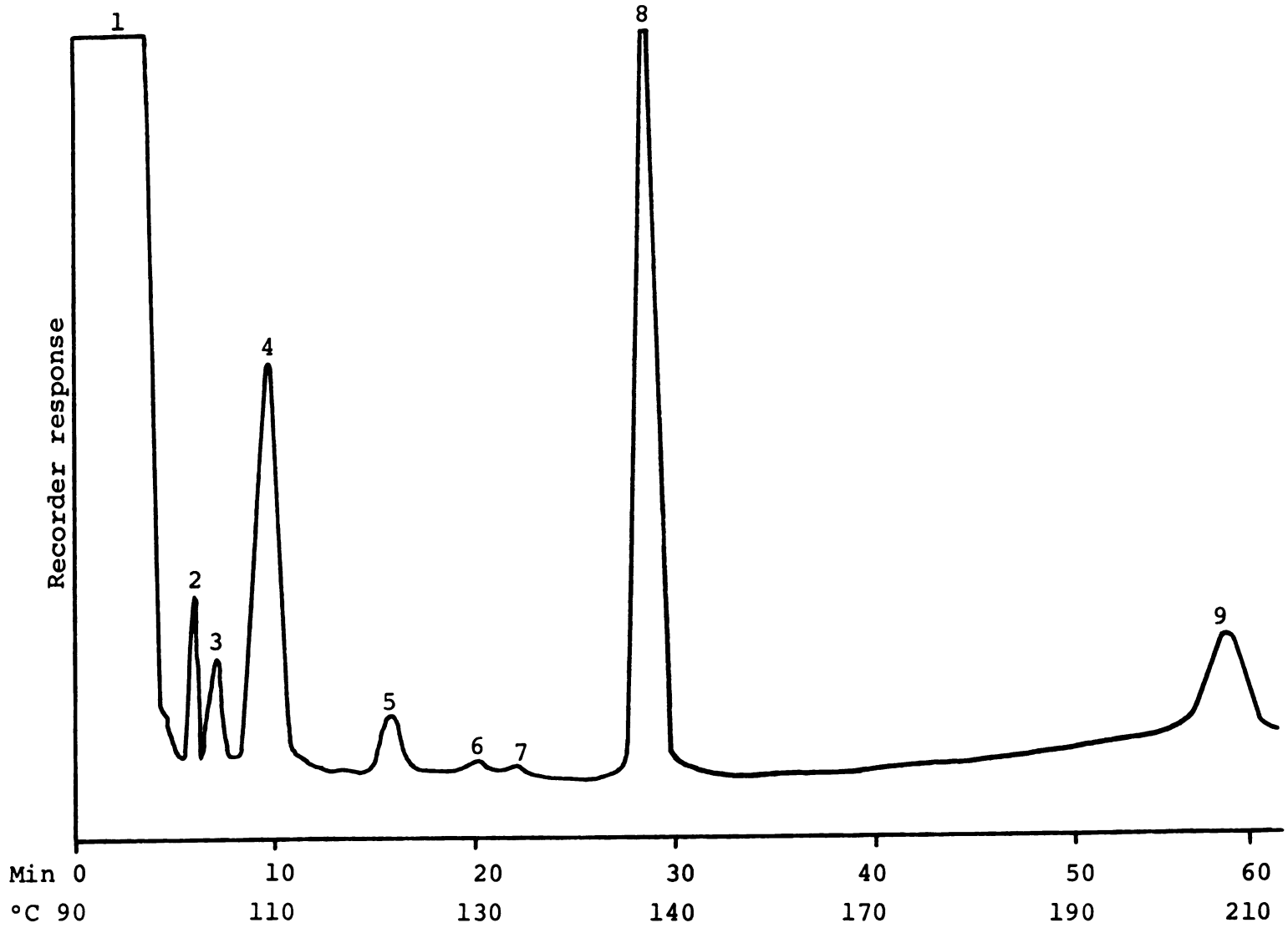


Figure 12. Gas chromatogram of TMSi derivatives of exudates from the roots of 12 pea plants treated with air containing 30% CO_2 for 6 days. Peaks are: 1, alanine; 5, glutamic acid; 8, glucose; 9, sucrose; 2, 3, 4, 6, and 7, unknowns.

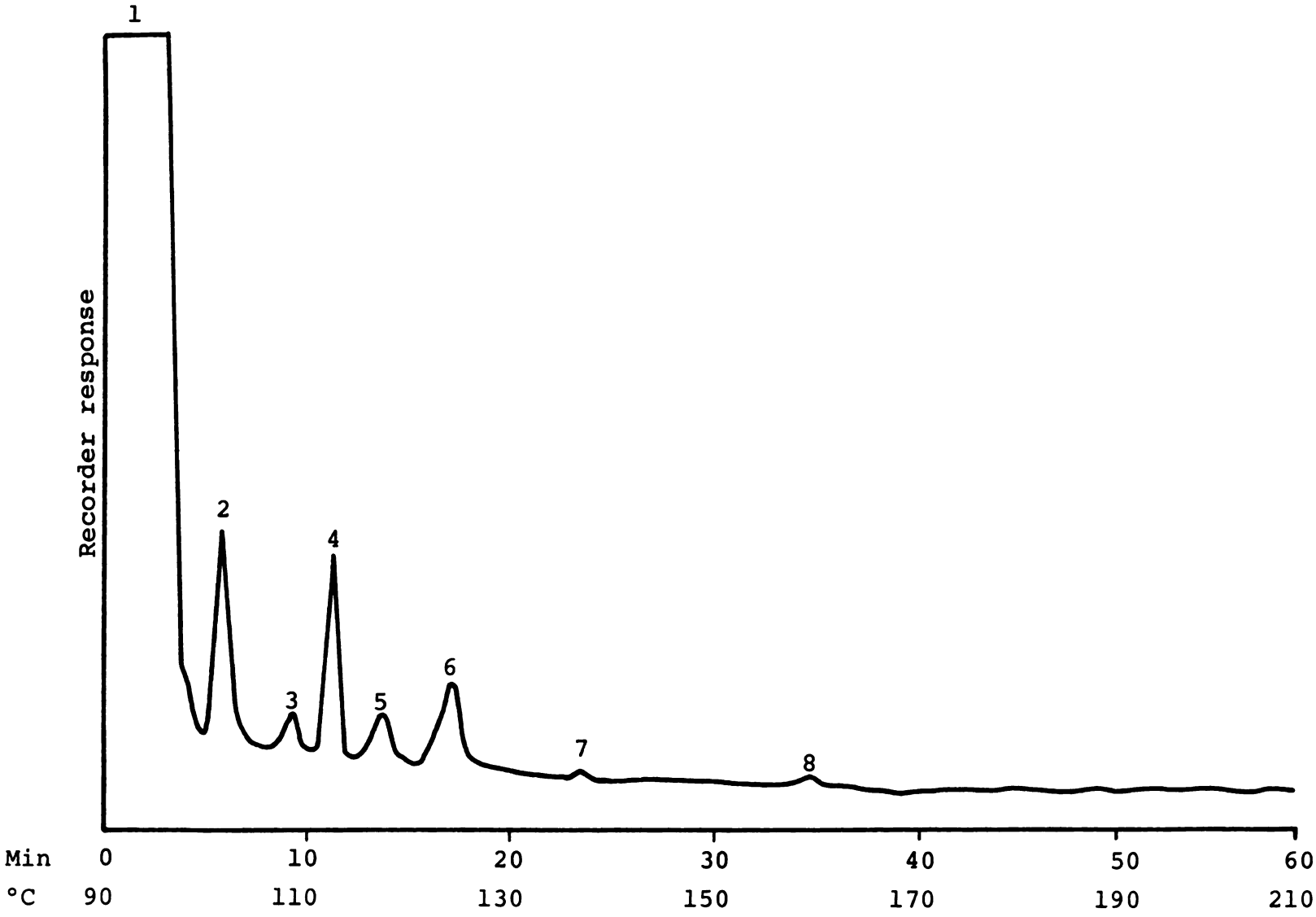


Figure 13. Gas chromatogram of TMSi derivatives of exudates from roots of 12 pea plants treated with air for 48 hours, then N_2 without CO_2 for 48 hours, then air for 48 hours under high light. Peaks are: 1, alanine; 2, leucine; 4, aspartic acid, 5, glutamic acid; 8, glucose; 3, 6, and 7, unknowns.

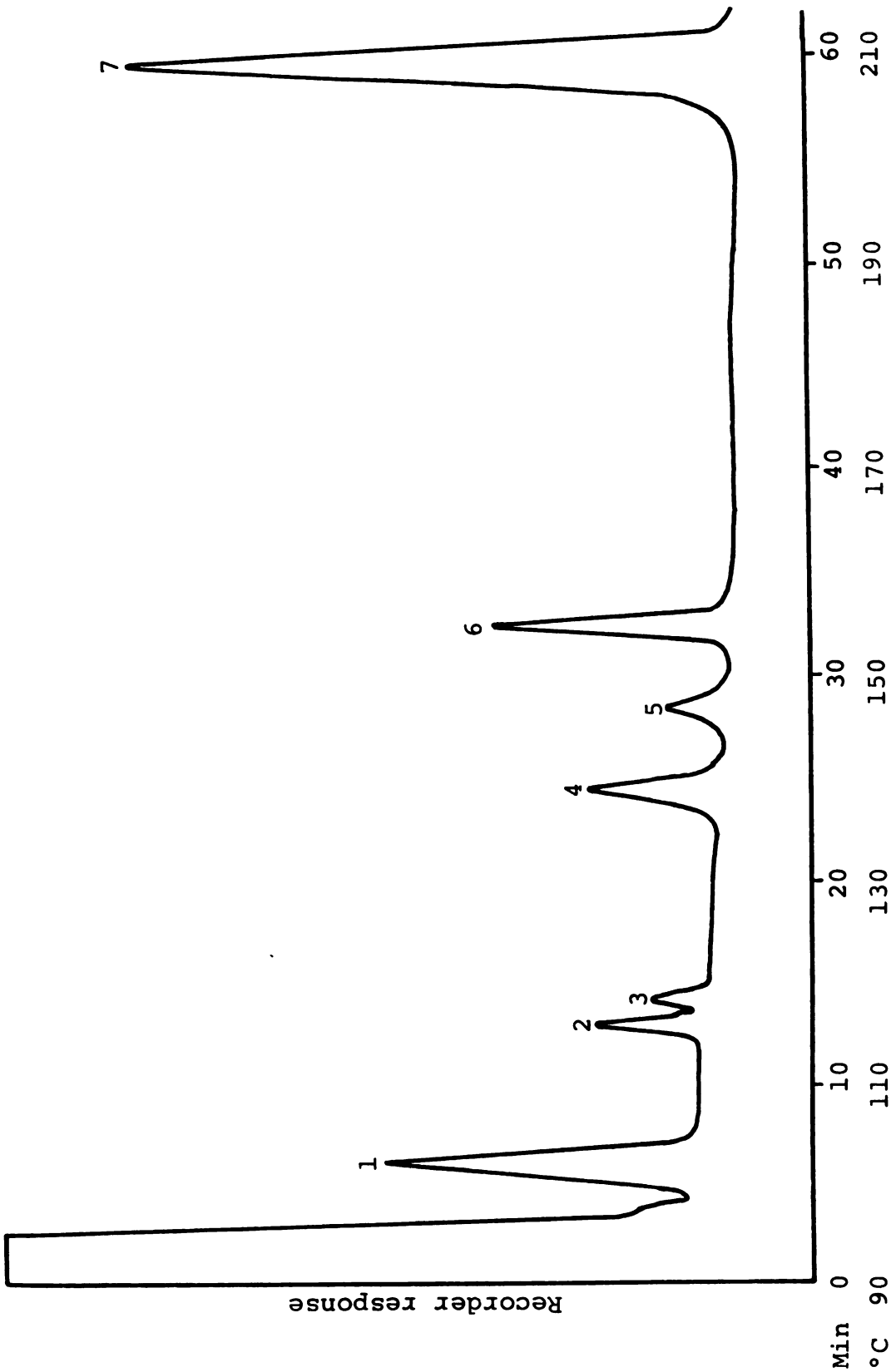


Figure 14. Gas chromatogram of TMSi derivatives of exudates from roots of 8 pea plants treated with air for 17 days. Fusarium was added on day 5 and experiment was terminated on day 17. Peaks are: 1, leucine; 2, glutamic acid; 5, fructose; 6, glucose; 7, sucrose; 3 and 4, unknowns.

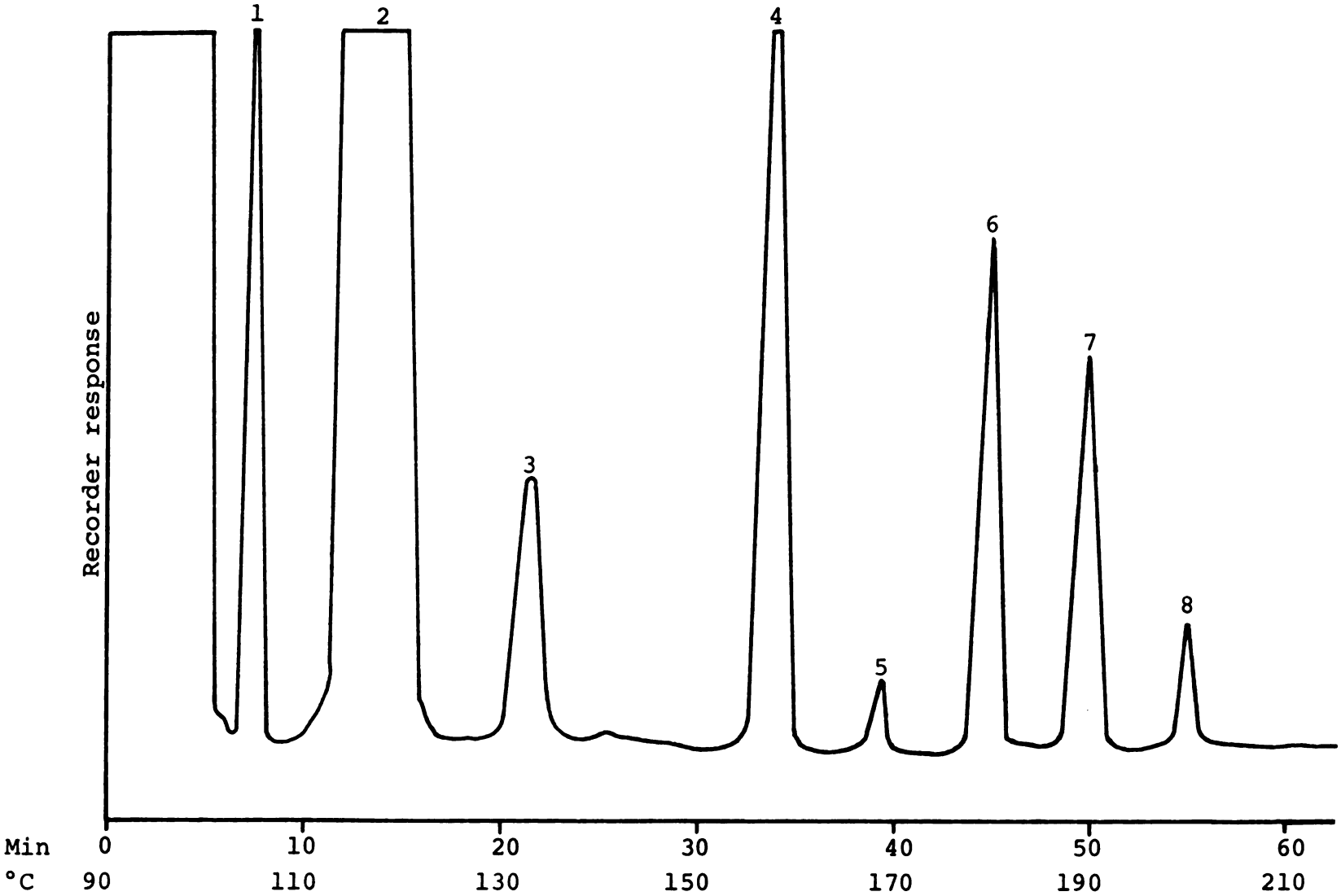


Figure 15, Gas chromatogram of TMSi derivatives of exudates from roots of 8 pea plants treated with N_2 containing 30% CO_2 for 10.3 days, then air for 6.7 days. Fusarium was added on day 5 and experiment was terminated on day 17. Peaks are: 2, glutamic acid; 4, glucose; 1, 3, 5, 6, 7, 8, unknowns.

Table 8. Effects of the gas composition of the rhizosphere on the amino acid and carbohydrate contents in root exudates of peas grown in an aseptic mist chamber. (Each value represents the net accumulation of exudates from 12 plants during a six-day treatment.)

Treatment	Amino acids - μg/g dry root					Carbohydrates - μg/g dry root				
	Ala	Leu	Asp	Glu	Total	Rib	Fru	Glu	Suc	Total
Low light										
Air	+	0	0	0	+	+	+	+	0	+
Air with 30% CO_2	7051	11	0	52	7114	0	0	271	59	330
N_2 without CO_2	271	+	0	0	271	0	0	9	0	9
N_2 with 30% CO_2	8403	949	345	0	9697	1380	2527	13	690	4610
Air--N_2--Air	2936	+	+	66	3002	0	153	5	212	370
High light										
Air--N_2--Air	2252	80	66	58	2456	0	0	69	0	69

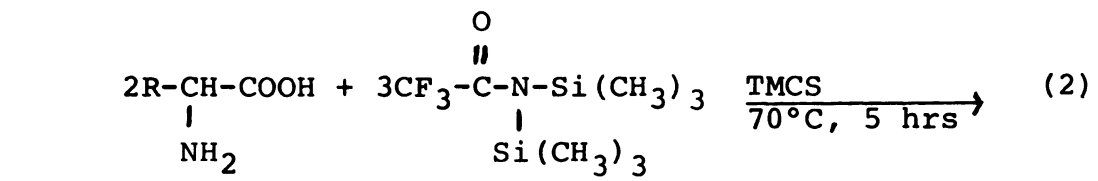
+Present but no quantitative evaluation possible.

As reported by Stalling et al. (1968) TMSi alanine and TMSi glycine cannot be separated from mono (trimethylsilyl) acetamide, a reaction product of BSA. BSTFA and its reaction product mono (trimethylsilyl) trifluoroacetamide are more volatile than BSA and do not coincide with the elution peaks of alanine or glycine. The success in silylating amino acids and sugars in the presence of high salt concentrations with BSTFA holds considerable promise in identifying additional root exudates of plants as the derivatives can be prepared in a single step and in a relatively short time.

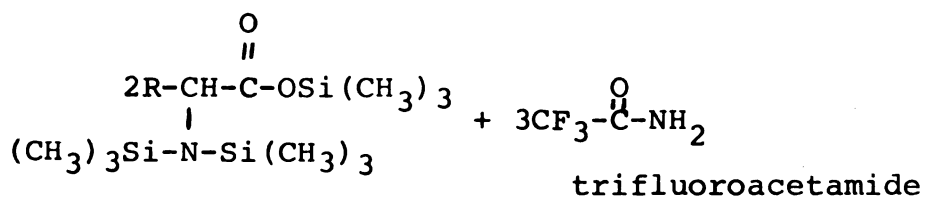
Attempts were also made to separate the mineral salts in the nutrient solution from root exudates using an ion exchange resin, Rollins et al. (1962). This, as well as organic methods of separating salts and amino acids, did not efficiently separate the small quantities of amino acids from the mineral salts.

Exudates were quantitatively analyzed by the methods outlined by Sawardeker and Sloneker (1965). The minimum quantity of TMSi sugars detected by the hydrogen flame was 0.25 μg and 1.0 μg for the amino acids.

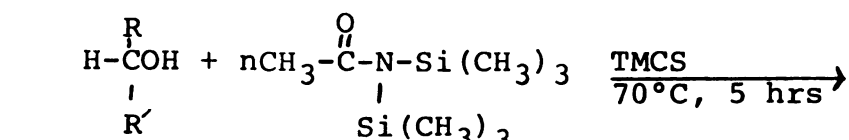
Mass spectrometric analyses showed the silylation procedure used in this study formed the silylamine or silylester of essentially all the amino or carboxyl and/or hydroxy groups, respectively. The general reaction is recorded in equations (2) and (3). Fully silylated amino acids were previously reported by Ruhlman (1961) and others cited there. The above conditions for derivatization



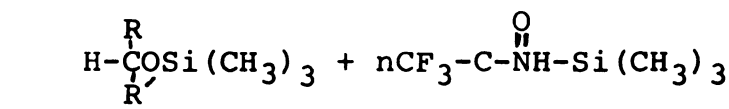
amino acid BSTFA



TMSi amino acid trifluoroacetamide



polyhydroxy sugar



poly TMSi sugar mono (trimethylsilyl) trifluoroacetamide

were selected on the basis of the dissolution and silylation of the amino acid and carbohydrate standards. One milligram of each dried standard was silylated in 450 µl BSTFA and 50 µl DMF. Samples, 5 µl, were injected into the gas chromatograph. Anomerization of some carbohydrates may have occurred during this treatment; Sweeley et al. (1963). Consequently, the anomeric peaks, if they were identified, were combined during the quantitative measurements of the carbohydrates.

Considering the amino acid content of pea root exudates, Figures 8-15 show that 4 or possibly 7 amino acids were exuded by the roots of 6-day-old pea seedlings. This is

fewer than the 21 identified by Rovira (1956) in the root exudates of 14-day-old peas grown in sand. Apparently additional amino acids leaked from the roots during the removal of sand by washing. Their methods of removing the mineral salts from the exudates and forming the hydrochloride salts of the amino acids may have hydrolized the enzymes (Chang and Bandurski, 1964) and other proteins in the root exudates.

Table 8 shows oxygen stress and/or high partial pressures of CO_2 definitely promoted root exudation of alanine. Alanine was the predominant amino acid in the exudate of all gas treatments. This finding coincides with that of Cossins (1964) who reported large amounts of alanine metabolized via the transamination of pyruvate in young pea seedlings. In this study the largest concentrations of alanine accumulated in the exudates of plants treated with N_2 and/or CO_2. This phenomenon is evidence that only a portion of the pyruvate is reduced to ethanol during anaerobiosis. More alanine accumulated in the exudates of plants subjected to air, then N_2, then back to air at intervals of 48 hours than when subjected to N_2 for 6 days. There was also more alanine in the exudate of plants treated with the same gases but under low light. The tremendous quantity of alanine produced in the air treatment containing 30% CO_2 is evidence for a carbon dioxide stimulated process in the metabolism of alanine under aerobic conditions. It should be mentioned that the attenuation of the alanine peaks in Figures 8-13 was too high (160-320) to be included in these chromatograms.

The accumulation of leucine and aspartate appeared to be a function of high CO_2 and high light, as 8.4 and 0.9 mg/g dry roots were excreted into the rhizospheres treated with no oxygen and high CO_2. Additional light also increased the accumulation of these two amino acids in the rhizosphere treated with an intermittent aerobic-anaerobic atmosphere. Glutamate accumulated only in the rhizospheres treated with gases containing oxygen, except the control.

Dubinina (1961) reported oxygen deficiencies of pumpkin, tomato, and willow roots led to increases in endogenous free amino acids. The fact that more amino acids were exuded by stressed pea roots in the present study is evidence that greater quantities of free amino acids are produced in anaerobic pea roots.

The greatest loss of identified carbohydrates by plant roots occurred during periods of oxygen stress. Low oxygen compounded by high concentrations of CO_2 caused up to 4.6 mg of carbohydrates to be exuded per gram of root, Table 8. Pea roots treated with air exuded trace amounts of carbohydrates. With the addition of 30% CO_2, carbohydrate exudation increased to at least 0.3 mg per gram of dry root. Treatment with N_2 caused very little leakage of carbohydrates. The addition of 30% CO_2 to N_2 caused 4.6 mg of the measured carbohydrates to leak into the rhizosphere. Roots treated with an intermittent aerobic-anaerobic atmosphere also showed an increase in the carbohydrate content when compared to the control.

Among the carbohydrates listed in Table 8, glucose is the most consistent exudate. For the air treatment root exudates contained a trace of glucose while 0.3 mg/g was exuded when 30% CO_2 was added. A nitrogen environment caused 9μg of glucose to accumulate in the rhizosphere. By adding 30% CO_2 to the N_2, the glucose content increased to 13μg. The treatments with 48-hour intervals of aerobic-anaerobic atmospheres contained 5 and 69μg/g of root for low and high light, respectively.

Except for the air control, ribose was found only in the N_2 treatment containing 30% CO_2. Besides the control, fructose occurred in the exudates from the air-N_2-air (low light) and N_2 with 30% CO_2 treatments. Sucrose was identified in the air-N_2-air (low light), and the air with 30% CO_2 and N_2 with 30% CO_2 treatments. They contained 212, 59, and 690μg/g dry root, respectively.

These results demonstrate the adverse effects anaerobic rhizosphere conditions have upon root exudation. That anaerobic conditions simulated by an N_2 atmosphere are intensified by adding 30% CO_2 is indicated by the larger quantity of exudates. These results are similar to those of Hiatt and Lowe (1967) and others. These authors suggest that anaerobiosis causes the cytoplasm to derange and become less dense than that of aerobic roots. Similar changes of the cytoplasm result from treatment of roots at a pH of 4.4. This phenomenon may have contributed to the increased exudation of roots subjected to 30% CO_2 as the pH of the circulating nutrient solution dropped to 5.5.

Several peaks were eluted from the column at temperatures greater than 165 C. Of these only sucrose was positively identified. Many of these peaks had mass spectrograms very similar to those characteristic of TMSi carbohydrates. For example, the spectrogram of the peak at 168 C in Figure 11 contained fragmented ions whose masses were 204 and 217 which were 10 and 40% of the parent peak and are characteristic of carbohydrate fragments. It also contained fragments with a mass of 305 which was 30% the height of the parent peak. The peak is often found in the mass spectrograms of closed ring carbohydrates.

Glycerol phosphate was also identified in the exudates of all treatments by mass spectroscopy. No quantitative measurements of this compound were made during this investigation.

The possibility that volatile organic compounds evolved from the roots of stressed plants was also considered. To study this possibility a porapak organic compound trap was attached to the gas outlet of the mist chamber for 48 hours. Porapak containing the trapped gases was emptied into the precolumn of the gas chromatograph-mass spectrometer. Qualitative measurements of the unknowns was possible by loading the column at room temperature and eluting these compounds into the mass spectrometer by programing the temperature of the column. Background components of this procedure were determined by applying the above procedures to Porapak which filtered the gases prior to flushing the

rhizosphere. The ethylene and/or acetylene content of the rhizosphere was determined by the direct injection of 10 ml gas samples into the gas chromatograph.

Ethanol, acetaldehyde, and water were trapped and identified from the exhaust gases of stressed plants. No organics were detected in the exhaust gases of the rhizosphere treated with air. Quantitative evaluation of these organics was impossible by these methods as the samples were expended during mass spectroscopy. However, the relative height of the parent peaks of acetaldehyde to ethanol was 4:5 in the bar graph spectrogram.

Volatilization of ethanol, accumulated during anaerobic germination of peas, was also reported by Cossins and Turner (1962). They showed 5% of the ethanol produced was lost by evaporation. The above results are interpreted as evidence that the alcohol dehydrogenase complex of peas was operative during aerobic and anaerobic conditions when the ethanol substrate was present. Since no acetaldehyde was detected in samples chromatographed for ethanol, it is suggested that most of the acetaldehyde produced by metabolism in the rhizosphere is lost into the gaseous atmosphere of the soil. Microbial contamination as a possible explanation for the observed production of acetaldehyde has been excluded by tests which indicated strict asepsis was maintained.

Carbon dioxide evolution by the treated roots was determined by measuring the differential CO_2 content of the inlet and exhaust gases. Data from these measurements are

presented in Tables 9-13. Generally, less CO_2 was produced by roots treated with air than those treated with N_2 containing no CO_2. Evolution of CO_2 by plant roots subjected to air-N_2-air at 48-hour intervals was less during the N_2 treatment under low light conditions but increased when treated with high light, Tables 11 and 12. As expected, respiration also fluctuated with the time of day.

The increase in root respiration immediately after the rhizosphere was converted from aerobic to anaerobic conditions without CO_2, as shown in Table 11, provides additional evidence of the over-response mechanisms characteristic of plants. Infected roots pretreated with a mixture of 70% N_2 and 30% CO_2, then changed to air, produced more CO_2 than infected roots grown in an aerobic atmosphere, Table 13.

Plant Morphology

After six days of treatment, plants were removed from the mist chamber and separated into roots, shoot, and seed. The component parts were measured, dried, and weighed. The data are presented in Tables 14 and 15.

Growth of the primary root was reduced very little by the gas treatments. Secondary root development was greatly reduced by oxygen stress and/or high CO_2 concentration. As shown in Table 14, more secondary roots developed and grew in the treatment containing 21% oxygen. When the O_2 content decreased to 14.7% and the CO_2 content increased to 30%, essentially no secondary roots formed. N_2 gas resulted

Table 9. Carbon dioxide evolution by the roots of four pea plants treated with air.

Date - Time	Gas	[CO_2] ppm	[CO_2] mg/g/hr
3/5 - 830	Air	88[a]	2.20[a]
3/5 - 1015	Air	105	2.62
3/5 - 1100	Air	76	1.90
3/5 - 1400	Air	88	2.20
3/5 - 1500	Air	97	2.42
3/5 - 1600	Air	94	2.35
3/6 - 1430	Air	101	2.52
3/6 - 1600	Air	110	2.75
3/6 - 1750	Air	103	2.57
3/6 - 1900	Air	95	2.73
3/7 - 600	Air	93	2.32
3/7 - 1230	Air	104	2.60

[a]Each value represents the average of three replications.

Table 10. Carbon dioxide evolution by the roots of four pea plants treated with N_2 without CO_2.

Date - Time	Gas	[CO_2] ppm	[CO_2] mg/g/hr
3/11 - 2230	N_2	161[a]	15.45[a]
3/12 - 830	N_2	245	23.51
3/13 - 1600	N_2	112	10.75
3/14 - 1030	N_2	107	10.27
3/14 - 1230	N_2	108	10.37
3/14 - 1630	N_2	102	9.79
3/14 - 2200	N_2	156	14.97
3/15 - 900	N_2	112	10.75
3/15 - 1030	N_2	113	10.84

[a]Each value represents the average of three replications.

Table 11. Carbon dioxide evolution by the roots of four pea plants treated with air for 48 hours, then N_2 without CO_2 for 48 hours, then air for 48 hours under low light.

Date - Time	Gas	$[CO_2]$ ppm	$[CO_2]$ mg/g/hr
3/16 - 1830	Air	122[a]	7.90[a]
3/16 - 2000	Air	135	8.74
3/17 - 1000	Air	117	7.58
3/17 - 1730	Air	101	6.54
3/17 - 2015	Air	102	6.60
3/17 - 2200	Air	101	6.54
3/18 - 2230	Air	91	5.89
3/18 - 2300	N_2 added	--	--
3/18 - 2315	N_2	339	21.95
3/19 - 900	N_2	63	4.08
3/19 - 2130	N_2	52	3.37
3/21 - 2100	Air	74	4.79
3/22 - 915	Air	108	6.99
3/22 - 1400	Air	102	6.60
3/22 - 2115	Air	95	6.15

[a]Each value represents the average of three replications.

Table 12. Carbon dioxide evolution by the roots of four pea plants treated with air for 48 hours, then N_2 without CO_2 for 48 hours, then air for 48 hours under high light.

Date - Time	Gas	$[CO_2]$ ppm	$[CO_2]$ mg/g/hr
4/10 - 1130	Air	88[a]	7.39[a]
4/10 - 2000	Air	95	7.98
4/11 - 900	Air	105	8.82
4/11 - 1700	N_2	134	11.26
4/12 - 830	N_2	133	11.17
4/12 - 1530	N_2	133	11.17
4/14 - 1430	Air	105	8.82

[a]Each value represents the average of three replications.

Table 13. Carbon dioxide evolution by the roots of four pea plants treated with air and N_2 containing 30% CO_2 (anaerobic) and infected by Fusarium.

	$[CO_2]$			
	Aerobic		Anaerobic	
Date - Time	ppm	mg/g/hr	ppm	mg/g/hr
4/19 - 1130	107[b]	5.14[b]	--[a]	--[a]
4/20 - 910	118	5.67	--	--
4/20 - 1900	133	6.39	--	--
4/21 - 1600	113	5.43	--	--
	---------- Addition of pathogen ---------			
4/21 - 1845	120	5.77	--	--
4/21 - 2230	84	4.04	--	--
4/21 - 6380	64	3.08	--	--
4/22 - 6324	63	3.03	--	--
4/22 - 1030	102	4.90	--	--
4/22 - 1440	112	5.38	--	--
4/22 - 2045	105	5.05	--	--
4/24 - 2315	93	4.47	--	--
4/26 - 1015	103	4.95	--	--
			- Changed to Air -	
4/27 - 1400	139	6.68	341[b]	59.48[b]
4/27 - 1545	133	6.39	218	38.03
4/27 - 1915	114	4.81	181	31.57
4/27 - 2130	115	5.53	218	38.03
4/28 - 2000	114	4.81	213	37.16
4/29 - 1100	97	4.66	141	24.42
4/29 - 2130	169	8.12	187	32.62

[a]Dashed lines indicate CO_2 content beyond range of instrument.

[b]Each value represents the average of three replications.

Table 14. Effects of gaseous composition of root environment upon the morphological characteristics of pea plants treated for 6 days.

Treatments	Length of primary roots--cm	Secondary root initials	Length of secondary roots--cm	Length of shoot cm
Before treatment	3.5[a]	0[a]	0[a]	2.4[a]
Low light				
Air	5.0	19	0.2	4.7
Air containing 30% CO_2	4.2	1	0	3.3
N_2 without CO_2	4.8	11	0.1	4.1
N_2 containing 30% CO_2	4.4	5	0.4	4.0
Air--48 hours, then N_2 without CO_2--48 hours, then Air--48 hours	6.8	18	2.5	4.3
High light				
Air--48 hours, then N_2 without CO_2--48 hours, then Air--48 hours	4.3	12	0.6	5.0
LSD .05	1.10	3	2.4	0.47
LSD .01	1.51	4	3.3	0.68

[a]Each value represents the average of 12 plants.

Table 15. Effects of gaseous composition of root environment upon the dry weight of pea plants treated for 6 days.

Treatment	Root mg	Shoot mg	Seed mg	Total mg
Low light				
Air	118.7[a]	128.0[a]	324.4[a]	571.1[a]
Air containing 30% CO_2	16.4	68.8	381.0	466.2
N_2 without CO_2	30.9	68.2	379.5	478.6
N_2 containing 30% CO_2	26.4	98.6	379.2	504.1
Air--48 hours, then N_2 without CO_2--48 hours, then air--48 hours	45.8	122.3	337.7	505.8
High light				
Air--48 hours, then N_2 without CO_2--48 hours, then air--48 hours	35.3	125.9	320.2	481.4
LSD .05	83.6	36.6	43.8	--

[a]Each value is the average of three replications.

in the reduction of the number of secondary root initials nearly 50%. When 30% CO_2 was added to the N_2 the number was reduced an additional 50%. Air, then N_2, then air at 48-hour intervals did not reduce secondary root development nor growth unless plants were grown under high light. Shoot and root growth was best when the roots were treated with air. In contrast, significant reductions in the length of shoots occurred among plants whose roots were treated with an atmosphere low in O_2 and high in CO_2.

Roots subjected to anaerobic conditions for 2 or 6 days were darker than aerated roots. However, new but thinner secondary root tips, which were white, continued to grow or elongate after the 2-day stress. Roots subjected to high concentrations of CO_2 were considerably thicker than those grown in the presence or absence of O_2.

Leaves of plants treated with N_2 and N_2 with 30% CO_2 became chlorotic after 24-48 hours. Treatments containing 30% CO_2 brought on chlorosis more rapidly than N_2 alone. Plants treated with N_2 for 48 hours became chlorotic 1-2 days after treatment. The leaves of all plants subjected to treatments containing 30% CO_2 appeared to have thicker cuticles than those treated with air or N_2 alone.

The above results compare favorably with those reported by Williamson and Splinter (1968) and others. The results indicate that either a lack of O_2 or excessive CO_2 causes injury to the root and with time causes physiological changes in the leaves.

The dry weight of plants treated in this investigation are reported in Table 15. These values are closely related to the morphological data presented above. Root weights were significantly less in those plants treated with mixtures of gases low in O_2 and/or high in CO_2. These gases also increased the sloughing off of cell debris. The quantity of debris accumulated on the nutrient solution filter was: 3.6, 2.3, 1.1 and 0.4 mg for 8.5, 7.0, 0.3, and 0.9% of the total root weight of treatments; N_2 containing 30% CO_2, air containing 30% CO_2, N_2 without CO_2 and intermittent air-N_2-air at 48-hour intervals, respectively. The hydrolytic products of this debris provide substantial quantities of soluble material in the soil environment. However, in the sterile mist chamber any hydrolytic enzymes secreted into the circulating mist media by the root would be diluted, hence inactive. Consequently, it is believed the exudates reported in this study are truly the products of excretion and not autolysis.

Plant Disease

Roots of intact pea seedlings were tested for their increased susceptibility to Fusarium root rot during severe anaerobiosis. The gas treatment containing N_2 and 30% CO_2 was chosen for its adverse effects upon root exudation and morphology and its suggested stimulative effects at this CO_2 concentration upon Fusarium growth; Papavizas and Davey (1962). Macroconidia of F. solani f. pisi, cultured and prepared by the methods described earlier, were injected into the sterile nutrient solution 5 days after the onset of the

experiment. Samples were removed from the reservoir via the access port and analyzed for spore germination and ethanol content.

Table 16 shows a definite increase in the germination and growth of Fusarium in the rhizosphere of anaerobic plants. Two hours after inoculation, spore germination was nearly 300% greater in the root zone of stressed plants. At 18 hours the percent germination was the same but growth (i.e., germ tubes) of the pathogen was greater in the stressed treatment. After 29 hours germination had doubled and germ tube growth was 27-fold greater in the anaerobic than in the aerobic rhizosphere. By 94 hours germination had peaked at 91% in the anaerobic rhizosphere with concurrent germ tube growth of 400μ including the branches. Many of the macroconidia in the rhizosphere containing air had constricted forming chlamydospores whose contents were granular having the same appearance as those cultured in distilled water. Six days after inoculation the atmosphere of the stressed plants was changed to air until the experiment was terminated.

The presence of Fusarium in the anaerobic rhizosphere decreased the rate of ethanol accumulation as shown in Figure 16. The point of inflection in the ethanol curve 12 hours after inoculation was concurrent to the rapid growth of the inoculum, Table 16. The fact that the decrease in the rate of ethanol accumulation was simultaneous to pathogen growth provides additional evidence that ethanol was metabolized by the pathogen. The ethanol

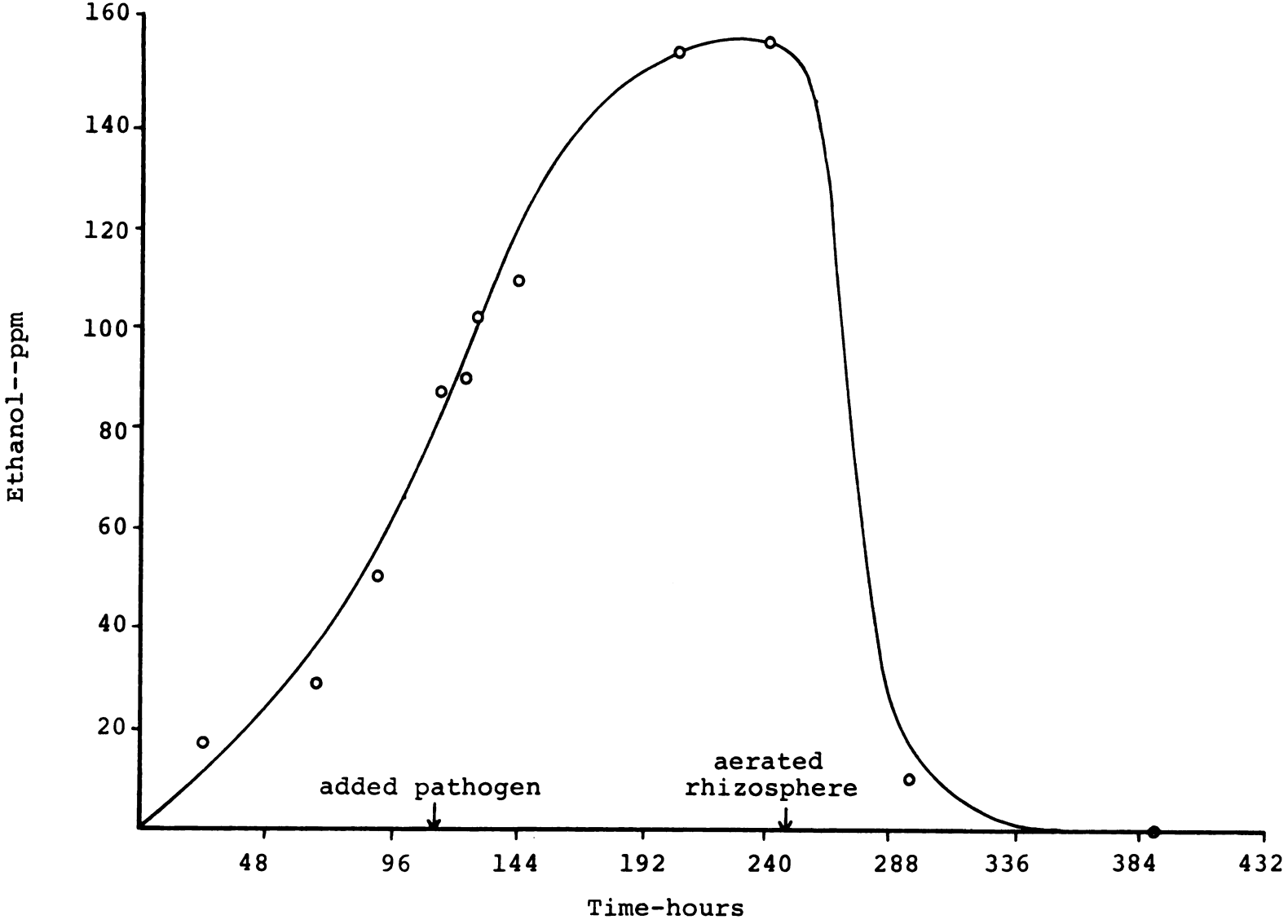


Figure 16. The effects of anaerobiosis and the presence of Fusarium upon ethanol accumulation in the rhizosphere of four plants. Each point represents the average value of two replications.)

Table 16. The effects of time and gaseous atmosphere upon the germination and growth of F. solani f. pisi macroconidia in the rhizosphere of pea roots.

Time hrs	Treatments			
	Aerobic		Anaerobic	
	Germination %	Length of germ tube μ	Germination %	Length of germ tube μ
0	0[a]	0[a]	0[a]	0[a]
3	13	0	36	12
8	16	0	41	12
18	39	0	39	56
30	43	4	82	108
42	--	--	92	300
96	26	16	91	400

[a]Each value represents the average of two replications.

content declined very rapidly soon after the rhizosphere was aerated, Figure 16. This rate far exceeded that of auto-oxidation in a sterile rhizosphere, Figure 7, suggesting that ethanol was oxidized by the pathogen. These results support those of Cochrane et al. (1963) who showed the exogenous carbon requirements of germinating macroconidia could be fully replaced by ethanol.

Anaerobic conditions caused a 5-fold increase in the growth of Fusarium with a concomitant 4-fold increase in infection and disease, Table 17. Roots grown in the mist chamber having an atmosphere of air were infected at the broken walls of the primary root where the secondary roots had emerged. Roots grown in a mixture of 70% N_2 and 30% CO_2 showed black lesions on both the roots and epicotyl.

Table 17. Effects of severe anaerobiosis on the intensity of root rot disease and growth of Fusarium in the rhizosphere of peas.

Treatment	Disease index[a]	Dry weight[b] - mg
Air	1.9[c]	8.1[c]
70% N_2, 30% CO_2	7.6	42.7

[a]Disease index is rated 0-12, with 12 assigned to dead plants.

[b]Dry weight of pathogen growth in rhizosphere for 12 days. Fusarium mycelia were removed from nutrient solution by filtration. This weight includes the cell debris.

[c]Values are the average of two replications.

The tips of roots treated with N_2 containing 30% CO_2 were completely black while the aerobic root tips were not infected. This phenomenon suggests that more nutrients were exuded at the root tips or the integrity of this area of the root was affected more by anaerobiosis or a combination of both resulted in greater disease. This data agrees with that of Lockwood (1962) who showed 24% more disease occurred in pea roots grown in saturated conditions. Since the plants in his study were not grown under aseptic conditions, the smaller increase in the disease of saturated plants may have resulted from the competitive absorption of root exudates.

Pea roots became necrotic 8 days after inoculation. A brown exudate was noted from the anaerobic treatment 4 days after inoculation while it was not observed in the exudate of the aerobic treatment until 8 days after inoculation. A similar exudate containing 10 to 20 phenolic compounds was reported by Sherrod and Domsch (1970). They showed the release of phenols was an integral part of the pathogenicity mechanism of pea root disease.

As in previous treatments, the length of the primary root was unaffected by anaerobiosis. However, secondary root growth and development was severely restricted by the anaerobic conditions as is shown in Table 18. Total dry weight of roots grown in the air treatment was 15.4 mg while 4.2 mg of dry weight was produced by each plant treated with N_2 containing 30% CO_2. Shoot growth was also reduced by anaerobiosis. The length of stems and leaves was reduced 40% while the dry weight of the shoots was reduced by nearly 80%. These results are interpreted as evidence supporting the work of Kende (1964) and others. They reported that an auxin or kinetin-like substance synthesized in the root is essential for proper growth and development of the shoot. The author contends the synthesis and transport of these substances is inhibited by anaerobiosis.

As reported earlier in Table 13, greater quantities of CO_2 were produced by infected roots in the anaerobic treatment when it was changed to air than by infected plants treated aerobically. The 4-9 fold increase in CO_2, expressed on the basis of root weight, may be explained by the greater growth of the pathogen in this treatment as shown in Table 17.

Fewer carbohydrates accumulated in the rhizosphere of anaerobically treated plants after they were inoculated by Fusarium. Table 19 shows a 70% decrease in the measured carbohydrate content of exudates in the treatment involving N_2 which contained 30% CO_2 as compared to the aerobic

Table 18. The effects of anaerobiosis upon the morphology of plants infected with Fusarium root rot.

Treatment	Length of primary root cm	Secondary root initials	Length of secondary roots cm	Total dry root weight per plant mg	Length of shoot cm	Total shoot weight per plant mg
Aerobic[a]	4.4	26	4.0	15.4	8.8	110.6
Anaerobic[a]	4.3	6	0.3	4.2	5.4	35.5

[a]Each value represents the average of 8 plants.

treatment. Both fructose and sucrose were absent from the exudates of diseased and anaerobic plants. Ribose was absent from the exudates of both treatments. In contrast, glucose was more abundant in the exudates of the anaerobic than in the aerobic treatment.

Fewer amino acids were identified in the anaerobic exudates of diseased plants, Table 19 and Figure 15. There was a 60-fold increase in glutamate production in the exudates of the anaerobic as compared to the aerobic treatment. The conspicuous absence of alanine, present in the exudates of sterile plants of both treatments (Table 8) and the absence of aspartate and leucine identified in the sterile exudate of the mixture of 70% N_2 and 30% CO_2 suggest these three amino acids were metabolized by Fusarium.

By comparing the total carbohydrate and amino acid contents of the sterile roots, Table 8, and inoculated roots, Table 19, it is apparent that the presence of Fusarium may have increased root exudation. This phenomenon may in part be the result of plant age. But, as Rovira (1956) points out, there is just a 20% increase in root exudates of peas from day 10-21. Since the increase in exudates exceeds this value, even when a growing pathogen is present, it is believed this increase results from the presence of the pathogen.

Bioassay

From the results obtained in the mist chamber several factors were operative, causing the increased disease in

Table 19. Effects of gas composition on the accumulation of carbohydrates and amino acids in the rhizosphere of peas grown in an aseptic mist chamber and inoculated with Fusarium. (Each value represents the accumulation of exudates from eight plants during a 17-day treatment.)

Treatment	Amino acids - μg/g dry root					Carbohydrates - μg/g dry root				
	Ala	Leu	Asp	Glu	Total	Rib	Fru	Glu	Suc	Total
Air	0	66	0	22	88	0	34	74	769	877
N_2 containing 30% CO_2	0	0	0	1340	1340	0	0	255	0	255

the anaerobic rhizosphere. These factors could possibly be separated by isolating the microorganism and subjecting it to various concentrations of sterile gases and ethanol.

Gases sterilized by filtration were bubbled through 100 ml of sterile redistilled water containing 0, 10, 100, and 1000 ppm of ethanol. Macroconidia of F. solani f. pisi were added to the solution and incubated at 25 C for 24 hours. Data are presented in Table 20.

Germination was low in all the treatments. Ethanol concentration alone had essentially no effect upon germination regardless of the treatment gas. Those gases containing very low concentrations (less than 0.41 ppm) of oxygen inhibited germination and development of the germ tube. The air treatment containing 30% CO_2 showed some stimulation of germination by ethanol. After 24 hours 33% of the spores had germinated in the solution containing 1000 ppm ethanol with 34, 16, and 3% germinating in 100, 10 and 0 ppm of ethanol, respectively. Approximately 50% germination occurred in those solutions diffused with air. Again there was no effect of ethanol concentration on germination.

The second bioassay was similar to the first except for the addition of a nitrogen source in the form of 20 μg of alanine. In this experiment ethanol content was determined before and after the treatment period and adjustments were made for volatilization. When the ethanol standards were autoclaved and cooled approximately 30% of the ethanol was lost by volatilization.

Table 20. The effects of ethanol concentration and gaseous composition upon the germination and initial growth of F. solani f. pisi macroconidia.

Treatments		Germination-%[a]			Length of germ tube-μ[a]		
Gas composition	Ethanol ppm	6	12	24	6	12	24
Air	0	52	55	58	24	22	38
Air	10	40	26	50	13	23	29
Air	100	0	3	8	0	12	6
Air	1000	30	44	31	27	23	18
N_2 containing 30% CO_2	0	0	0	0	0	0	0
N_2 containing 30% CO_2	10	1	0	0	12	0	0
N_2 containing 30% CO_2	100	0	0	0	0	0	0
N_2 containing 30% CO_2	1000	1	1	0	5	5	0
Air containing 30% CO_2	0	0	1	3	0	13	16
Air containing 30% CO_2	10	6	18	16	11	14	17
Air containing 30% CO_2	100	0	7	34	0	12	33
Air containing 30% CO_2	1000	12	8	33	6	8	16
N_2 without CO_2	0	0	0	0	0	0	0
N_2 without CO_2	10	2	1	0	4	4	0
N_2 without CO_2	100	0	0	0	0	0	0
N_2 without CO_2	1000	2	2	0	6	4	0
N_2 without O_2	0	2	0	0	7	0	0
N_2 without O_2	10	0	0	0	8	0	0
N_2 without O_2	100	0	0	0	0	0	0
N_2 without O_2	1000	11	0	1	12	0	3

[a]Each value represents the average of two replications.

Germination and growth of Fusarium occurred only in treatments diffused with air and air containing 30% CO_2. When an exogenous source of nitrogen was present greater growth resulted in those treatments containing ethanol. As before, germination and growth of the macroconidia appeared to be reduced by increasing the concentration of ethanol, Table 21. However, after 72 hours fungal growth showed a significant increase in the highest ethanol concentration used.

These results are interpreted to mean that ethanol alone has very little effect upon germination and growth of Fusarium. Conversely, when an exogenous source of nitrogen is added, ethanol does promote the growth of Fusarium. The greater germination in those treatments containing only alanine (i.e., 0 ppm ethanol) is evidence that nitrogen enhances spore germination. But with growth the endogenous reserves of carbon are expended and the requirements for exogenous carbon increase. These results agree with Cochrane (1963) and indicate the carbohydrate supply of the culture medium of these spores was more than adequate.

Table 21. Effects of ethanol concentration and gaseous composition upon the germination and growth of F. solani f. pisi in solution containing 20 μg alanine.

Treatments	Ethanol consumption[b] ppm	Percent germination[a]	Length of germ tube[a] μ	Dry weight of pathogen[b] mg
Air--ethanol ppm				
0	--[c]	91[c]	67[c]	2.4[c]
10	6	65	61	2.0
100	65	53	78	2.9
1000	196	39	20	3.3
Air containing 30% CO_2-- ethanol ppm				
0	--	86	28	2.7
10	2	31	40	2.8
100	55	67	52	2.2
1000	370	55	23	4.1
LSD .05	--	33.9	12.5	0.74
LSD .01	--	46.6	17.2	1.02

[a]Measurements made at 24 hours.

[b]Measurements made at 72 hours.

[c]Each value represents the average of two replications.

CONCLUSIONS

1) A mist chamber was designed to maintain sterile conditions in the rhizosphere. The gaseous atmosphere could be changed at will with continuous monitoring of the exudates.

2) Pea root exudation was increased by anaerobiosis. Exudation of ethanol, amino acids, and carbohydrates was increased by both oxygen stress and high CO_2 partial pressure. These effects were additive as more root metabolites were lost when the rhizosphere was treated with a mixture of 70% N_2 and 30% CO_2.

3) Both ethanol and acetaldehyde volatilized from the rhizosphere.

4) Greater respiration occurred in the rhizosphere of roots pretreated with N_2 and 30% CO_2 than from roots treated with air.

5) Oxygen stress and high concentrations of CO_2 reduced the growth and development of secondary roots. Shoot growth was also reduced by anaerobiosis.

6) Pathogen germination and growth as well as plant disease were increased by anaerobic conditions in the rhizosphere.

7) No germination of F. solani f. pisi occurred in absence of oxygen.

8) Ethanol content increased Fusarium growth only in the presence of an exogenous source of nitrogen.

These results provide evidence that anaerobiosis promotes the severity of Fusarium root rot of peas. Low concentrations of oxygen combined with 30% CO_2 reduced plant growth and development. Under these circumstances, the integrity of the root declines resulting in the loss of additional metabolites which in turn promote fungal germination and growth. Increased growth of the pathogen intensifies the inoculum potential of the fungus. The reduced integrity of the cells lowers the resistance of the root to the surrounding biota. The result of these combined factors was increased plant infection. Once the plant actively responds to the presence of the pathogen the ensuing metabolic responses are both complex and varied.

LITERATURE CITED

LITERATURE CITED

Ayers, W. A. and R. H. Thornton. 1968. Exudation of amino acids by intact and damaged roots of wheat and peas. Plant and Soil 28:193-207.

Baver, L. D. 1956. Soil Physics. John Wiley and Sons Inc. N.Y. p. 206.

Brown, G. E. and B. W. Kennedy. 1966. Effect of oxygen concentration on Pythium seed rot of soybeans. Phytopath. 54:407-411.

Bolton, E. F. and A. E. Erickson. 1970. Ethanol concentration in tomato plants during soil flooding. Agron. J. 62:220-224.

Boulter, D. et al. 1966. Amino acids liberated into the culture medium by pea seedling roots. Plant and Soil 24:121-127.

Burg, S. P. 1962. The physiology of ethylene formation. An. Rev. Plant Physiol. 13:265-302.

Chang, C. W. and R. S. Bandurski. 1964. Exocellular enzymes of corn roots. Plant Physiol. 39:60-64.

Cline, R. A. and A. E. Erickson. 1959. The effect of oxygen diffusion rate and applied fertilizer on the growth, yield and chemical composition of peas. Soil Sci. Soc. Amer. Proc. 23:333-335

Cook, R. J. and N. T. Flentje. 1967. Chlamydospore germination and germling survival of Fusarium solani f. pisi in soil as affected by soil water and pea seed exudation. Phytopath. 57:178-182.

Cook, R. J. and W. C. Snyder. 1965. Influence of host exudates on growth and survival of germlings of Fusarium solani f. phaseoli in soil. Phytopath. 55:1021-1025.

Cossins, E. A. 1964. Formation and metabolism of lactic acid during germination of pea seedlings. Nature 203:989-990.

Cossins, E. A. and E. R. Turner. 1959. Utilization of alcohol in germinating pea seedlings. Nature 183: 1559-1600.

_____ and _____. 1962. Losses of alcohol and alcohol dehydrogenase activity in germinating seeds. Annals of Bot. 26:591-597.

_____ and _____. 1963. The metabolism of ethanol in germinating pea seedlings. J. Exptl. Bot. 14:290-298.

Crawford, R. M. M. 1966. The control of anaerobic respiration as a determining factor in the distribution of the genus Senecio. J. Ecol. 54:403-413.

_____. 1967. Alcohol dehydrogenase activity in relation to flooding tolerance in roots. J. Exptl. Bot. 18: 458-464.

Dubinina, M. 1961. Metabolism of roots under various conditions of aeration. Fiziol. Rast. Akad. Nauk S.S.S.R. 8:395-406.

Durbin, R. D. 1955. Straight-line function of growth of microorganisms at toxic levels of carbon dioxide. Science 121:734-735.

Erickson, A. E. and D. M. Van Doren. 1960. The relation of plant growth and yield to oxygen availability. VII Trans. Int. Cong. Soil Sci. 3:428-434.

Fulton, J. M. and A. E. Erickson. 1964. Relation between soil aeration and ethanol accumulation in xylem exudates of tomatoes. Soil Sci. Soc. Amer. Proc. 29:610-616.

Garrett, S. D. 1970. Dormant survival by resting propagules of root-infecting fungi. In Pathogenic root-infecting fungi. University Press, Cambridge.

Geisler, G. 1965. The morphogenic effect of oxygen on roots. Plant Physiol. 40:85-88.

Goos, R. D. 1963. Further observations on soil fungi in Honduras. Mycologia 55:142-150.

Griffin, D. M. 1968. Observations on fungi growing in a translucent particulate matrix. Trans. Br. Mycol. Soc. 51:319-322.

Hiatt, A. J. and R. H. Lowe. 1967. Loss of organic acids, amino acids, K, and KCl from barley roots treated anaerobically and with metabolic inhibitors. Plant Physiol. 42:1731-1736.

Karkkainen, J. and R. Vihko. 1969. Characterisation of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-glactose, and related compounds as their trimethylsilyl derivatives by gas-liquid chromatography-mass spectroscopy. Carbohydrate Res. 10:113-120.

Kende, H. 1964. Preservation of chlorophyll in leaf sections by substances obtained from root exudate. Science 145:1066-1067.

Kenefick, D. G. 1962. Formation and elimination of ethanol in sugar beet roots. Plant Physiol. 37:434-439.

Kidd, F. 1941. The controlling influence of carbon dioxide in the maturation dormancy and germination of seeds. Part I. Proc. Royal Soc. London 87:408-421.

Lin, T. Y. et al. 1965. Ethyl alcohol metabolism in leguminous seedlings. Plant Physiol. 40:1261-1268.

Lockwood, J. L. 1962. A seedling test for evaluating resistance of pea to Fusarium root rot. Phytopath. 52:557-559.

McBride, W. J., Jr. and J. D. Klingman. 1968. In Lectures in gas chromatography. L. R. Mattick and H. A. Szymanski (ed.). Plenum Press, N.Y. Vol. 2. p. 1.

McDougall, Barbara M. and A. D. Rovira. 1969. Sites of exudation of C^{14} substances from wheat roots. New Phytologist (submitted).

Marchant, R. and Monica F. White. 1966. Spore swelling and germination in Fusarium culmorum. J. Gen. Microbiol. 42:237-244.

Newcombe, Margaret. 1960. Some effects of water and anaerobic conditions on Fusarium oxysporum f. cubense in soil. Trans. Br. Mycol. Soc. 43:51-59.

Papavizas, G. C. and C. B. Davey. 1962. Activity of Rhizoctonia in soil as affected by carbon dioxide. Phytopath. 52:759-766.

Payne, P. I., J. M. Milton, and D. Boulter. 1966. Studies on the amino acid composition of root exudates using a Technicon autoanalyzer. Automation in Anal. Chem. V Technicon Symposia, 1965, London. pp. 680-681.

Pearson, R. and D. Parkinson. 1961. The sites of excretion of ninhydrin positive substances by broad bean seedlings. Plant and Soil 13:391-396.

Pierce, A. E. 1970. Silylation of organic compounds. Pierce Chem. Co., Rockford, Ill.

Rollins, C., L. Jensen, and A. N. Schwartz. 1962. Desalting of amino acid solutions by an ion retardation resin. Anal. Chem. 34:711-712

Rovira, A. D. 1956. Plant root excretions in relation to the rhizosphere effect. I. The nature of root exudates from oats and peas. Plant and Soil 7:178-194.

_____. 1965. Interactions between plant roots and soil microorganisms. Ann. Rev. Microbiol. 19:241-266.

_____. 1969. Plant root exudates. The Bot. Rev. 35: 35-57.

Ruhlman, K. and W. Giesecke. 1961. Gas chromatography of silylated amino acids. Translated from Angewandte Chemie 73:113-117.

Sawardeker, J. S. and J. H. Sloneker. 1965. Quantitative determination of monosaccharides by gas liquid chromatography. Anal. Chem. 37(7):945-947.

Schroth, M. N. and D. C. Hildebrand. 1964. Influence of plant exudates on root infecting fungi. Ann. Rev. Phytopath. 2:101-132.

Schroth, M. N. and W. C. Snyder. 1961. Effect of host exudates on chlamydospore germination of the bean root rot fungus Fusarium solani f. phaseoli. Phytopath. 51:389-393.

Sherrod, L. L. and K. H. Domsch. 1970. The role of phenols and B-glycosidase in the pathogenicity mechanism of Gliocladium catenulatum to roots of peas (Pisum sativum L.). Soil Biol. Biochem. 2: 197-201.

Sisler, H. D. and C. E. Cox. 1954. Effects of tetramethylthiuram disulfide on metabolism of Fusarium roseum. Am. J. Bot. 41:338-345.

Stalling, D. L., C. W. Gehrke, and R. W. Zumwalt. 1968. A new silylation reagent for amino acids bis (trimethylsilyl) trifluroacetamide (BSTFA). Biochemical and Biophysical Communications 31:616-622.

Sweeley, C. C., R. Bentley, M. Makite, and W. W. Wells. 1963. Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. J. Amer. Chem. Soc. 85:2497-2507.

Toussoun, T. A., Shirley M. Nash, and W. C. Snyder. 1960. The effect of nitrogen sources and glucose on the pathogenesis of Fusarium solani f. phaseoli. Phytopath. 50:137-140.

White, A., P. Handler, and E. L. Smith. 1968. Principles of Biochemistry, 4th ed. McGraw-Hill Co., N.Y. pp. 326-327.

Williamson, R. E. and W. E. Splinter. 1968. Effect of gaseous composition of root environment upon root development and growth of Nicotiana tabacum L. Agron. J. 60:365-368.

Wood, R. D., P. K. Raju, and R. Reiser. 1965. Gas-liquid chromatographic analysis of monoglycerides as their trimethylsilyl ether derivatives. J. of Amer. Oil Chem. Soc. 42:161-165.

Zumwalt, R. W., K. Kuo, and C. W. Gehrke. 1971. Application of a gas-liquid chromatographic method for amino acid analysis. A system for analysis of nanogram amounts. J. Chrom. 55:267-280.