EFFECTS OF SOIL FLOODING ON ETHANOL CONTENT OF TOMATO PLANTS RELATED TO CERTAIN ENVIRONMENTAL CONDITIONS

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A. E. Erickson
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ABSTRACT

EFFECTS OF SOIL FLOODING ON ETHANOL CONTENT OF TOMATO PLANTS RELATED TO CERTAIN ENVIRONMENTAL CONDITIONS

by Everett F. Bolton

Effects of certain environmental conditions associated with soil flooding were related to the metabolic status of tomato plants. Ethanol, an indicator of celluar oxygen deficiency, was used as a parameter of these effects. Concentration of ethanol responded markedly to environmental factors under anaerobic conditions resulting from flooding.

Simulated environments were provided with a growth chamber, a transpiration chamber and mist chambers. Ethanol concentration was determined in xylem exudate, transpired condensate and in plant tissue samples by gas chromatographic procedures. A method was developed for distilling samples from tissue.

Air temperature markedly influenced ethanol concentration in xylem exudate of flooded tomato plants. High air temperature resulted in the largest ethanol concentration compared with lower temperatures. At high temperature, concentration approached a maximum in xylem exudate at the 24-hour flooding period. At medium air

temperature an intermediate ethanol concentration resulted and reached a maximum at the 12-hour period of flooding. Low air temperature produced the lowest ethanol concentration.

Light intensity influenced plant ethanol concentration under certain conditions of flooding. At medium air temperature high light increased ethanol over low light for the 12- and 24-hour periods. Under high air temperature high light intensity increased ethanol at only the 24-hour flood period.

Under flooded conditions soil temperature showed little effect on ethanol concentration at low air temperature and low light. At medium air temperature and high light intensity a decrease in soil temperature delayed the rate of ethanol accumulation.

Transpiration losses of ethanol were small but were proportional to exudate concentration for each flooding period.

A significant concentration of ethanol occurred in root excretions after 12 hours of flooding.

Concentration of ethanol in anaerobic plants was highest in the bottom stem and top roots and decreased in the foliage and in the bottom roots.

Carbon dioxide at 20.1 percent, combined with oxygen at 20.1 percent, did not increase ethanol concentration over that of aerobic plants.

Ethanol concentration was higher in plants flooded in 1.9 liter soil volumes than for larger volumes. A reduced rate of ethanol exudation for larger soil volumes only occurred, however, during the early flooding period. Reduced ethanol exudation was attributed to entrapped air.

Field flooding of tomatoes resulted in ethanol concentrations that agreed closely with growth chamber data for equivalent environmental conditions.

The investigation indicated the usefulness of ethanol as a measure of environmental effects associated with soil flooding of tomato plants. Since tomato plant growth and yield previously have been related to soil oxygen supply during short flooding periods ethanol measurement provides a potential means of correlating flood damage intensity with environmental conditions.

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By

Everett Francis Bolton

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

LIST OF APPENDICES

INTRODUCTION

Soil flooding recurrently inflicts severe damage on many cultivated crops. Short periods of flooding often reduce growth and yield while extended flooding periods may result in death of plants and complete loss of crop (7). Soil physical characteristics predispose some soils particularly to flooding conditions and, in addition, plant species have been observed to differ in susceptibility to flood damage. It has frequently been observed, also, that concurrent climatic conditions affect the severity of flood damage. Specialization and intensity of cropping in certain soil regions focus increased attention on the flooding factor in agriculture. This problem demands a search for greater knowledge of the plant reaction to flooding conditions.

The soil physical system has been defined considerably with respect to flooding and water saturated soils are now considered to be synonymous with oxygen deficiency (11, 33). Early work showed that oxygen was essential for plant growth in nutrient solutions and extension of studies to field conditions supported this conclusion. In the field soil oxygen supply, within the available soil moisture range, is normally dependent upon the large pore spaces in the soil, which represent a capacity factor. In addition

to the capacity factor it is now known that a continuous supply of oxygen to the plant, representing a rate factor, is essential for normal growth. The platinum micro-electrode technique of Lemon and Erickson (24) has contributed greatly in defining the rate of oxygen supply for different soil pore space and moisture conditions. As soil moisture increases within the available moisture range the air capacity is decreased and the increasing thickness of moisture films reduces the rate of oxygen supply. In a completely saturated soil, constituting flooded conditions, the air pore space is eliminated and the oxygen diffusion rate is greatly reduced. Under these conditions oxygen supply is dependent upon solubility of oxygen in water and oxygen solubility in water under prevailing soil temperatures is low.

Rapid advances in biochemistry and related fields have extensively clarified the role of oxygen in the plant cell and the nature of oxygen deficiency. Oxygen is known to be a final electron acceptor in the electron transport chain within the cell. In this capacity oxygen enables degradation of energy rich products of photosynthesis to carbon dioxide and water with the concomitant release and transient storage of energy for carrying out cell functions. A deficiency of oxygen alters metabolic routes and, as a consequence, reduces energy production and results in the formation of reaction products that differ from those of aerobic cells (8).

Ethanol is one of the resultant cellular products of metabolism produced under oxygen deficient conditions (42). In the aerobic cell, hexose products of photosynthesis are degraded via the glycolytic sequence to pyruvic acid and thence through the tricarboxylic acid cycle to CO_2 and water. In anaerobic cells the tricarboxylic acid cycle is restricted since it is dependent upon oxygen and the electron transport chain to reoxidize the coenzymes essential in its enzyme systems. The glycolytic sequence can continue to function under anaerobic conditions since the coenzyme, nicotinamide adenine dinucleotide, which is reduced early in the sequence, is subsequently reoxidized when acetaldehyde, the decarboxylated product of pyruvic acid, is reduced to ethanol. Most plant cells have enzyme systems (16) exhibiting the ethanol, or fermentation, reaction.

Since ethanol production in the cell has been thus shown to be oxygen dependent, measurement of ethanol in the plant has been investigated as an index of anaerobic conditions. Studies by Kenefick (20), using an enzyme assay procedure, and investigations by Grineva (13), using an alcohol oxidant, showed an increase in alcohol under anaerobic conditions in sugar beets, corn and sunflowers. The alcohol was presumed to be predominantly ethanol. Fulton (12), using gas chromatographic analysis, showed that ethanol concentration increased markedly in root xylem exudate of tomatoes under flooded conditions. In addition

the ethanol concentration of tomato exudate was highly correlated with oxygen diffusion rate, at least during the early periods of flooding.

It is considered that the ethanol assay can be used to provide valuable and extensive information on the reaction of plants to the anaerobic environment of flooded soils. Soils research has advanced considerably on the basis of chemical analysis of plants in relation to the chemical and physical characteristics of soils. Chemical analyses of nitrogen in plants, for example, have been related to the many environmental factors of soils. These factors have included fertility level of this nutrient within the soil and soil physical factors influencing the amount of nitrogen released for plant use (3). In an analogous manner the measurement of ethanol in plants should provide a means of determining oxygen requirement and supply to the plant under given environmental conditions. In principle, the ethanol assay should provide a more sensitive measure with respect to oxygen supply than plant analyses provide for nutrient supply since the ethanol is part of a short term reaction while chemical analyses measure the integrated product of long term effects. Soil physicists, Baver and Russell, (5, 33), among others, have repeatedly pointed to the need for such measurements to indicate plant response to soil conditions.

The objective of this investigation was to measure effects of certain soil environmental conditions on ethanol

accumulation in plants under conditions of flooding. Tomato plants were used primarily throughout this study since this species is known to be damaged extensively by flooding. Tobacco plants, another sensitive species, were used in certain phases of the investigation.

The primary objectives include:

1. Determination of the effect of air temperatures and light intensities on the accumulation of ethanol in xylem exudate of tomato plants flooded for short periods of time.
2. Determination of the soil temperature effect on the accumulation of ethanol in xylem exudate of roots under certain air temperatures.
3. Measurement of transpired ethanol from tomato and tobacco plants to determine the effectiveness of transpiration as a means of eliminating ethanol under flooded conditions.
4. Evaluation of the magnitude of root excretion as a means of eliminating ethanol from flooded plants.
5. Determination of ethanol distribution in anaerobic plants.
6. Comparison of ethanol accumulation in tomato plants flooded in the field with results from plants flooded in the growth chamber to test the applicability of simulated conditions.

LITERATURE REVIEW

Flooding reduces plant growth principally due to reduced oxygen supply. This effect was originally demonstrated through aeration of plant culture solutions (33). Studies extended to soil systems also indicated low oxygen supply to be a causative factor in reduced growth under these conditions (7, 9).

Oxygen supply in soil has been attributed to the amount of large soil pores (44) which contain air at moisture contents within the available soil moisture range. Baver (5) and others (1, 6) have shown that the amount of large pores, referred to as air pore space, is important for plant growth and that reduction of this pore space beyond certain limits markedly reduces growth and yield depending on plant species.

The oxygen supply to the large pores and especially to plant roots has been shown to depend upon a diffusion process. A platinum microelectrode technique, developed by Erickson and Lemon (24, 25, 26, 27), enabled measurement of oxygen movement by diffusion in the liquid phase of soil. Since the platinum microelectrode must be in contact with soil moisture films to be operative it is considered to simulate the conditions under which plant roots contact oxygen.

The amount of space available for storage of air constitutes a capacity factor while the rate of diffusion represents the rate of oxygen supply (22, 26, 27). It has been shown (19) that the effect of each of these factors on oxygen supply is dependent upon moisture content within and above the available soil moisture range. As moisture content increases the moisture films around the soil particles increase in thickness with a resultant decrease of oxygen diffusion across the films. Concurrently, the amount of soil pore space available for air decreases with increasing moisture content.

Where the soil is completely saturated with water, as occurs during flooding, the supply of oxygen decreases rapidly (38) and the rate of oxygen diffusion has been shown (19) to diminish very quickly. Under these conditions the oxygen supply depends upon the amount of oxygen dissolved in water and the amount of oxygen dissolved is very small under field conditions. The effect of increasing soil temperature, within plant growth limits, increases oxygen diffusion rate slightly and decreases solubility (17) but the alterations are negligible with respect to plant oxygen demands.

Erickson and co-workers (11) have shown that short periods of flooding accompanied by decreased oxygen diffusion rate, reduced plant growth and yield. Oxygen deficient periods of twenty-four hours decreased growth of tomato plants in the field, especially when the oxygen deficient period occurred during the early growth periods.

A greater reduction in growth occurred when the oxygen deficient period was applied under high light intensities than under low light, an effect that the authors attributed to higher rates of photosynthesis and transpiration under these conditions.

The nature of reduced oxygen supply within the plant has been considerably clarified by rapid advances in biochemistry. Limitation of oxygen supply within the cell has been shown (8, 42) to alter the metabolic pathways available for degradation.

Energy within the cell is derived from enzymatic oxidation of reduced products of photosynthesis. It is used to effect synthesis and carry out cell functions. The energy release occurs as electrons are transferred from degraded photosynthetic products through a highly organized series of compounds arranged in order of decreasing electronegative potential. Ferredoxin is probably the initial electron acceptor and is followed by oxidized pyridine nucleotides and other components of a system collectively known as the electron transport chain. Oxygen is normally the final electron acceptor in this electron transfer system. This transfer process mediates the formation of a high energy compound, adenosine triphosphate.

Where an adequate supply of oxygen exists, glucose is degraded to pyruvic acid by the glycolytic reaction sequence and is subsequently oxidized to CO_2 and H_2O by way of the tricarboxylic acid cycle. Under anaerobic

conditions the tricarboxylic acid cycle is inhibited since inadequate oxygen supply limits operation of the electron transport chain and thus restricts reoxidation of pyridine nucleotides. The glycolytic sequence can continue anaerobically since the NAD, which is reduced early in the sequence, in turn reduces acetaldehyde, the decarboxylated product of pyruvic acid, with the formation of ethanol. As a consequence, a low oxygen supply to the plant cells results in reduced energy production and in both a qualitative and quantitative change in reaction products (34).

Ethanol accumulation in plants has been determined as an index of anaerobic conditions. Kenefick (20), using an enzyme redox reaction, showed that alcohols present in sugar beets increased under anaerobic conditions. Transpired samples also indicated that alcohol, considered to be ethanol, was transpired from the leaves. Grineva (13) using potassium dichromate as an oxidant showed the presence of alcohol in samples from corn and sunflower plants subjected to anaerobic conditions.

Fulton (12) found a very high correlation between ethanol content of root exudate from tomato plants and oxygen supplying power of the medium during the early stages of flooding. In this investigation ethanol was identified and quantitatively measured by gas chromatographic techniques. The results indicated that for short term flooding periods, at least, measurement of ethanol provided a satisfactory means of measuring the extent to which

anaerobic soil conditions could influence the fermentation reaction. Ethanol occurred at a higher concentration in plants flooded in light than in the dark. Morphological age of tomato plants also affected ethanol accumulation to some extent and plants in flower contained more ethanol than younger plants.

Alcohols, including ethanol, have been shown to have a stimulating effect on plant growth at very small concentrations (14). On the other hand ethanol has proven toxic to plants (12, 14) when supplied from external sources in concentrations equivalent to those occuring in anaerobic plants.

It is well known that plants, edaphically adapted and grown under favorable moisture conditions within the available soil moisture range, are markedly influenced by environmental factors (29). Rate of metabolite production by means of photosynthesis is dependent on light intensity and temperature while rate of degradation through respiration depends upon air and soil temperature.

The metabolic behavior of upland plants has received considerably less attention at soil moisture conditions in excess of the available range. Work by Erickson cited above has indicated that deleterious effects of soil anaerobic conditions on plants are associated with light intensity. In addition, Fulton showed a difference for ethanol content of plants flooded in the light compared with those flooded in the dark. The above considerations

would suggest the feasibility of using the ethanol assay to characterize certain environmental effects with respect to plants subjected to flooded conditions for short periods of time.

MATERIALS AND METHODS

Description of Growth Chamber

Where other factors permitted, a large Sherer-Gillett walk-in type growth chamber was used for control of environmental conditions in these experiments. The inside dimensions of the chamber were 3.35 meters square by 2.80 meters high. The unit was equipped to enable simulation of field conditions with respect to temperature, light intensity and humidity.

Temperature was controlled by a cam-shaped template which programmed temperature in a daily cycle. With this equipment temperature cycles could be chosen within a temperature range from near freezing to slightly more than 40° C. Air was continuously recirculated through the chamber from bottom to top.

The unit was designed to provide a range of quality and intensity of light (30) through the use of separately controlled banks of fluorescent and incandescent bulbs. As a result it was possible to select combinations of fluorescent and incandescent light intensities. A further control of light intensity was achieved by means of the plant platform, within the chamber, which could be raised or lowered. The fluorescent banks consisted of 40, 20, and 12 bulb units while the incandescent lights consisted of 40, 25, and 16

bulb units, where each unit was controlled by a separate time clock. With this equipment it was possible to obtain a programmed cycle of light intensities for periods of the day that approached light conditions during the main summer growing season.

Light intensity was measured within the growth chamber for combinations of light sources referred to as "low" and "high" light intensities. The low light intensity consisted of a 16 bulb bank of incandescent lights combined with a 12 bulb bank of fluorescent lights. The high intensity light source contained 16, 25, and 40 bulb banks of incandescent lights combined with 12, 20, and 40 bulb banks of fluorescent lights.

The light energy emitted for each intensity was measured with a Beckman and Whitley thermal radiometer attached to a Sargent recorder. Measurement was made at 3 distances from the light sources by placing the black surface sensing element of the radiometer at 25, 56, and 127 cm from the incandescent light bulbs. The distances corresponded, respectively, to the top and mid points of the tomato plants under treatment and to the surface of the table upon which the containers were placed.

At the low light intensity, energies measured at the top and mid point of the plants were .512 and .480 gm-cal cm^{-2} min^{-1}, respectively. Corresponding energy values at the high light intensity were 1.417 and 1.286 gm-cal

cm^{-2} min^{-1}. The energy of direct sunlight, measured in July, was 1.472 gm-cal cm^{-2} min^{-1}.

Humidity could also be controlled within the chamber by means of moisture nozzles which were activated by a wet bulb control. It was possible to obtain relative humidities from approximately 50 percent to those near saturation although only higher relative humidities were maintained for these experiments.

Description of Plant Growth Media and Growth of Plants for Air and Soil Temperature Studies

Growth Medium

A coarse sand material was used as the plant growth medium in all experiments where air temperatures, light intensities and soil temperatures were varied. Total pore space of this material, calculated from bulk density measurements (5), approximated 40 percent of the volume and about 36 percent of the total pore space represented air pore space. This medium was used to insure aerobic conditions for plants prior to flooding.

The sand was placed in 15 cm diameter pipe containers, of galvanized metal, which were inserted within 20 cm diameter pipes constructed of similar material. Both pipes were 61 cm in height. The outside container was constructed with a bottom while the inside pipe containing the sand was open at both ends. This arrangement permitted rapid entry and exit of water to and from the sand. Water

was added to the sand as required and was pumped daily from the outside container. This technique was used to ensure adequate aeration for the plants during the entire growth period.

Care of Plants

Fireball variety tomatoes, Lycopersicum esculentum Mill., were used in all experiments involving tomatoes. The plants were grown from seeds planted in greenhouse flats containing a potting soil mixture and were transplanted when the first true leaves appeared.

The plants were grown in the greenhouse until flower buds were initiated and were then placed in the growth chamber for two days under medium temperature and high light conditions. The plants were watered twice daily. Major and minor nutrients were supplied to the soil at planting and subsequently at weekly intervals.

Description of Plant Growth Media and Growth of Plants for Transpiration Studies

Growth Medium

A greenhouse potting soil composed of 2 parts loam soil: 1 part sand: 1 part sphagnum peat moss was prepared for growing tomato and tobacco plants to be used in the transpiration studies. When this soil mixture was placed in 13 cm clay pots it reached a bulk density of approximately 1.20 gm cc^{-1}, equivalent to 55.0 percent total pore space.

Care of Plants

Tomato and tobacco seedlings were transplanted to the 13 cm clay pots at the first true leaf stage. Subsequently these plants were grown in the greenhouse and received applications of water and nutrients as required for normal growth.

Description of Soil Temperature Control Water Baths

Greenhouse Water Baths

Insulated water baths were used to control soil temperature in the greenhouse. These tanks were 132-cm long, 86-cm wide and 69-cm deep, inside dimensions. They were sufficiently large to accommodate six tomato plants in galvanized pipe containers. Temperatures of 10.0, 18.2 and 26.7°C were maintained in the three tanks, respectively, by means of thermostatically controlled heating and cooling equipment. The water baths provided a satisfactory degree of temperature control within the plant root medium and temperature varied only within one degree of the bath temperature.

Growth Chamber Water Baths

Cylindrical galvanized containers were adapted for use with the soil containers used in the growth chamber, since the growth chamber could not accommodate the large temperature control baths. The small baths were 61 cm in height, the same as the soil containers, and were 30 cm in diameter so that one soil container could be

placed inside each bath. Water pipe attachments, 13 mm I. D., were attached at the top and bottom of each bath and the baths were connected in series with garden hose so that a complete replicate could be run simultaneously.

Description of the Transpiration Chamber

A chamber was constructed of 6-mm plexiglass for obtaining a transpiration sample from tomato and tobacco plants. The chamber was 33-cm square and 38-cm in height with a 13-cm diameter opening on the bottom to enable placement over the plant. Solid plastic tubing, 6-mm in diameter, was installed in the side, near the bottom, for entry and, near the top on the opposite side, for removal of air which was fed through the chamber from a compressed air line to sweep out the transpired sample.

A separate container of 15-cm cubic dimensions was constructed of plexiglass, also, and was used to contain the 13-cm clay pot with the plant. This chamber had an inlet and outlet tube for water. The pot containing the plant and one inch of the stem was sealed off with a plastic lid and secured with tape. The chamber enclosing the top of the plant was sealed onto the bottom container with stopcock grease and held in place by the weight of the light cooling bath.

Light was supplied to the plant by five 300-watt bulbs that were immersed in water which removed some far infrared light and was recirculated to reduce radiant heat.

Fluorescent lights were placed to the side to extend the range of light quality. The light source supplied 1.21 gm-cal cm^{-2} min^{-1} at the mid-point of the chamber.

Description of Mist Chambers

Mist chambers, designed and constructed by Erickson, were used for growth and treatment of plants in experiments where root secreted ethanol and ethanol concentration within the plant were measured. These chambers were constructed of 6-mm plexiglass and were 122-cm in length, 32-cm wide and 61-cm deep at the mid-section. The bottom sloped from each end in order that the condensed mist would return rapidly to the liquid chamber for recirculation. A commercial room humidifier, equipped with fan, was installed in a central compartment in the base of the chamber. This provided a continuous mist of Hoagland's solution to the plant roots that was adequate for growth.

Openings were spaced at intervals in the top of the chamber for the young plants. Tomato plants were transplanted to the mist chamber at the first leaf stage by wrapping each plant in cotton and attaching it to the opening with masking tape.

An opening was left in the top for addition of nutrient solution and for aeration of the plants during growth. Other openings were available for admitting nitrogen gas that was used to effect anaerobic conditions in the chamber and for extraction of gas and vapor samples used for ethanol

measurement. A pump also was attached to one opening to permit oxygen measurement with a Beckman oxygen analyzer, in order to ensure that anaerobic conditions were maintained during treatment. Openings were also placed in the bottom of the chamber, directly beneath the plants, so that plastic cups with tubes attached could be used to collect mist condensate samples from the roots.

Measurement of Ethanol

The ethanol assay procedure, developed by Fulton (12), was used to measure ethanol in samples obtained from xylem exudation, transpiration and root excretion. The sampling techniques are described below. Ethanol was measured with the same Beckman GC2A gas chromatograph and hydrogen flame attachment described by Fulton.

The method involved injection of a 20 ul sample into the chromatograph and elution of the sample through a 1.83 meter stainless steel column to the flame. Column temperature was maintained at 100° C and helium was used as the carrier gas with a flow rate of 80 cc min^{-1}. Chromosorb W* was used as solid support material in the column. Diethylene glycol succinate* comprised the liquid partition phase and phosphoric acid was used as activator in earlier experiments. Carbowax 400 was used as the partition phase in later experiments. Elution time and peak characteristics

*Columns with these materials were prepared by Beckman Instruments Inc., Fullerton, Calif., U.S.A.

were similar for both materials but more sensitive quantitative detection was provided by the carbowax. Elution peaks were integrated with a Sargent model SR recorder. Standard curves were prepared from a series of ethanol standards ranging in concentration from 5 to 300 parts per million on a weight basis.

Determination of ethanol in condensed tissue samples necessitated a different column for separation of ethanol and methanol. A 1.83-meter x 6-mm column was prepared for this purpose using a commercial material designated as Porapak type Q.*

Entrapment of Transpiration Samples

Transpiration samples from tobacco and tomato plants were entrapped in a U-tube and Erlenmeyer flask assembly which was submersed in a salt-ice bath at -15° C. The condensing apparatus was connected by tygon tubing to a solid plastic inlet tube in the wall near the top of the plexiglass transpiration chamber described above. Compressed air from an air line was fed through the chamber continuously at a flow rate of 4-5 liters min^{-1} for the 24-hour period. The condensing trap was connected to the chamber only for two-hour periods when samples were obtained.

Distillation of Ethanol from Plant Tissue

A distillation method was developed to extract tissue-free samples from plant material. Such a method was

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*This material was obtained from Waters Associates, Inc., Framingham, Mass., U.S.A.

chosen in preference to the use of expressed tissue samples since methods to express tissue result in a release of large molecular weight components in addition to cellular structures. Contamination by these high molecular weight and particulate materials was found to alter column characteristics.

The present method was adapted to extract plant sap, containing ethanol, from stems, leaves and roots of tomato plants. Fresh tissue samples ranging in weight from 2 to 5 gm were placed within a glass tube annealed to the inside of a 125-ml Erlenmeyer flask. The flask was sealed with a foil-covered rubber stopper and the sample was distilled for two hours under a bank of 300-watt flood lamps.

Initial attempts to measure ethanol in the distillate using the carbowax and diethylene glycol succinate partitioning materials proved unsuccessful. In addition to the ethanol peak, which eluted at 1.8 minutes, another compound eluted at 1.7 minutes. It was not possible to resolve the ethanol peak under these conditions.

Preliminary work with suspected compounds showed that methanol was the interfering agent. In view of the temperature (80° C) used to distill the samples from plant tissue it was concluded that methanol was formed non-enzymatically during the distillation process.

It was necessary to use a partitioning material that could separate the two components. The commercial material, Porapak Q, described above was used for ethanol analysis.

With this material methanol formed a peak at 2.2 minutes while ethanol formed a peak at 5.6 minutes with column temperature at 160° C and a carrier gas flow rate of 100 cc min^{-1}.

To insure that the measured ethanol was a metabolic product rather than a product of autolysis recovery of known standards was measured. Dried tomato stem tissue was placed in aliquots of 15, 25 and 50 ppm ethanol standards. The saturated stem tissue was distilled by the same procedure used for fresh tissue.

A greater amount of methanol was released from the dried tissue than from fresh tissue and this resulted in some interference from the methanol peak even with this column. However, duplicate ethanol standard samples were quite reproducible and the peaks aggreed closely with standards. It was readily concluded that the ethanol measured by this procedure was the metabolic product.

Statistical Analysis

Analysis of variance was used to determine statistical differences for treatments in all experiments except the transpiration and mist-chamber experiments. Where analysis of variance was conducted F values were calculated and are included in the appendices along with the data for each experiment.

A randomized block design was used for all experiments to which statistical analyses were applied. Treatments were

replicated 4 to 6 times, the number of replications depending primarily on preliminary results.

Considerable variation due to error occurred in these experiments and this was attributed to biological variability. Although this variability was present the treatments significantly influenced ethanol content as indicated below.

Transpiration and mist-chamber data were not treated statistically in this investigation. Only two replicates were used in the transpiration study and consequently the average values were used. In the mist-chamber experiment also, the design to give the desired information was not predisposed to statistical analysis and average values were used.

EXPERIMENTAL RESULTS

Air Temperature and Light Intensity Effects on Ethanol Accumulation in Flooded Tomato Plants

Description of Experiment

The effects of air temperature and light intensity on ethanol accumulation in xylem exudate of flooded tomato plants was investigated by treatment of plants in the growth chamber described above. A set of flooding treatments, each replicated in quintuplicate, was established within each of six simulated environments, with each set of plants being subjected to only one environment during flooding. Three air temperatures, each combined with a low and high light intensity comprised the six environments. The growth chamber was maintained at approximately 75 percent relative humidity within each temperature and light intensity.

The three air temperatures were cyclic and were selected to represent a range of temperatures under which plants may be subjected to field flooding conditions in Michigan (4). A temperature gradient was present in the growth chamber but thermometer readings indicated that temperature was maintained at the desired level within the immediate plant zone. The high air temperature was programmed from a minimum of 16.7 to 35.6° C for the 24-hour

flooding period and during the 12-hour day period ranged from 31.7 to 35.6° C. Minimum and maximum temperatures for the medium temperature range were 9.1 and 30.0° C respectively, for the 24-hour period. Air temperature for the medium cycle ranged from 22.2 to 30.0°C for the 12-hour day period at full light intensity. The low temperature cycle increased from 16.0 to 20.6° C for the 12-hour day period and ranged from 10.0 to 20.6° C for the full day.

The plants used in the experiment were transplanted as seedlings into the sand containers and were grown in the greenhouse until they approached treatment stage where blossom clusters appeared. Transplantings were carried out in sequence in order that the plants for each experiment would be at a similar morphological age at the time of treatment. As plants reached the early bloom stage the containers with plants were placed in the growth chamber for a two-day period at the medium temperature cycle and high light intensity in order that the condition of the plants would be constant at the time when that group of plants was subjected to a specific temperature and light regime during treatment.

Flooding treatments within each combination of air temperature and light intensity consisted of an unflooded control and flooding with water to the sand surface for 4- and 12-hour periods. Additional 24-hour flood periods were subsequently applied to plants at the high temperature cycle combined with low and high light intensities and at the

medium temperature cycle combined with the high light intensity.

Xylem exudate samples were obtained by decapitating plants at the end of the flooding period and collecting samples in tygon tubing attached to the decapitated stem. The flood water was pumped from the outside container at the end of the designated flood period.

Results

Table 1 shows that the oxygen diffusion rates of the flooded soils were sufficiently low for all environments to harm tomato plant growth (11, 24). The slightly higher rate for the shorter flooding period is attributed to entrapped air.

TABLE 1.--Oxygen diffusion rates gm x 10^{-8} cm^{-2} min^{-1} in soils flooded at three cyclic air temperatures and two light intensities.

Flooding Period Hours	High Light	Low Light
	High Temperature	
4	17.1	15.2
12	10.7	8.8
	Medium Temperature	
4	18.3	14.3
12	11.6	10.7
	Low Temperature	
4	13.3	14.3
12	11.8	7.7

Results in Fig. 1 indicate that air temperature had a pronounced effect on ethanol accumulation in xylem exudate of flooded tomato plants, especially beyond the 4-hour flooding period. At the high temperature cycle, where a maximum of 35.6° C was reached, ethanol concentration continued to increase at 12 hours and subsequent measurements at 24 hours showed a considerably greater ethanol concentration. The shape of the curve, however, indicated that, at the 24-hour flood period, ethanol concentration was approaching a maximum at the 35.6 ° C. At the 30.0° C range, ethanol concentration reached a maximum at the 12-hour flooding period. This is in agreement with results obtained by Fulton. At the low temperature, where a maximum of 20.6° C was reached, ethanol concentration was considerably lower at the 12-hour flooding period than for the medium temperature at high light intensity.

Light intensity had a less consistent effect on ethanol concentration in xylem exudate than air temperature, in these experiments. In the high temperature range ethanol concentrations were nearly identical for both levels of light intensity up to and including the 12-hour flooding period. At the 24-hour flood period, however, ethanol concentration decreased from the 12-hour period where light intensity was low while concentration continued to rise under high light. The level of light intensity had its greatest effect on ethanol concentration within the medium temperature range. In the medium temperature experiments,

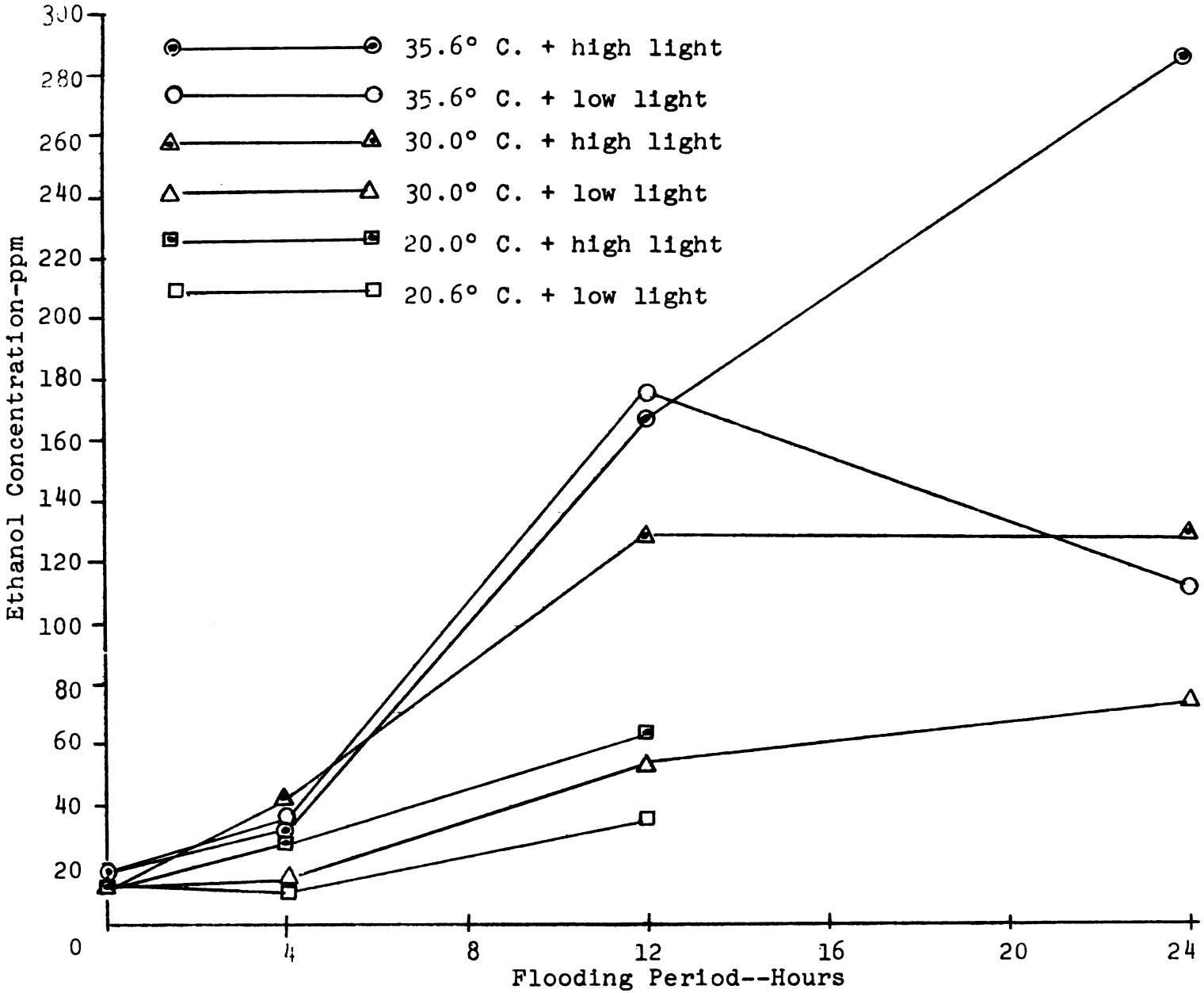

Fig. 1.--Ethanol concentration in xylem exudate of tomato plants flooded under three ranges of air temperature cycled to indicated maxima and at two light intensities. (Each value is the average of five replicates.)

the ethanol concentrations in plants flooded for 12- and 24-hour periods under low light were less than for high light. Under the low light and medium air temperature regime ethanol concentrations approximated values for low air temperature.

Light is reported (29) to be limiting in photosynthesis for tomato plants when light is less than 1/3 to 1/4 full light intensity. It is probable that the low light intensity used here only approached the limiting value. Nevertheless, the low light level used in this experiment, is less than values generally obtained in cloudy conditions during the growing season for this latitude(44).

Soil Temperature Effect on Ethanol Accumulation in Flooded Tomato Plants

Description of Experiment in Greenhouse

The review of Richards et al. (31) has indicated the importance of soil temperature on plant growth and physiological processes. Plant roots, like plant tops, have been shown to have optimum temperature ranges for growth (10) and for accumulation or depletion of constituents (31). In addition, increased soil temperature has been calculated to impose greater oxygen demands on respiring roots (28). Consequently, measurement of ethanol should indicate the intensity of anaerobiosis at different soil temperatures.

The present experiment was established to measure the effect of ethanol accumulation in xylem exudate of tomato

plants flooded at three soil temperatures. Water bath temperatures employed were 10.0, 18.2 and 26.7° C and soil temperatures within the pipe containers were maintained within one degree of these values. Air temperatures in the greenhouse during treatment varied from 18.2 to 21.1° C, and a low light intensity was established with fluorescent light. The plants used in the study were grown in the greenhouse and placed in the growth chamber for a conditioning period before being placed in the water baths.

Results of Soil Temperature Experiment-Greenhouse

Fig. 2 shows a slight increase in average ethanol concentration at the 12-hour flood period with increasing soil temperature. All values, however, were low and there was high variability within treatments. On the basis of these results it is concluded that soil temperature was a factor influencing ethanol accumulation in roots but that the effect was not great compared with the effect of high air temperatures.

Soil temperature influenced rate of root exudate in accordance with results reported elsewhere (18, 35). Rates of ethanol exudate were expressed as ugm hr^{-1} and are presented in Fig. 3. Results expressed in this form appear more meaningful with regard to ethanol accumulation under these conditions. They do not appear to alter the earlier conclusion, however that soil temperature effect was small under low air temperature and low light intensity.

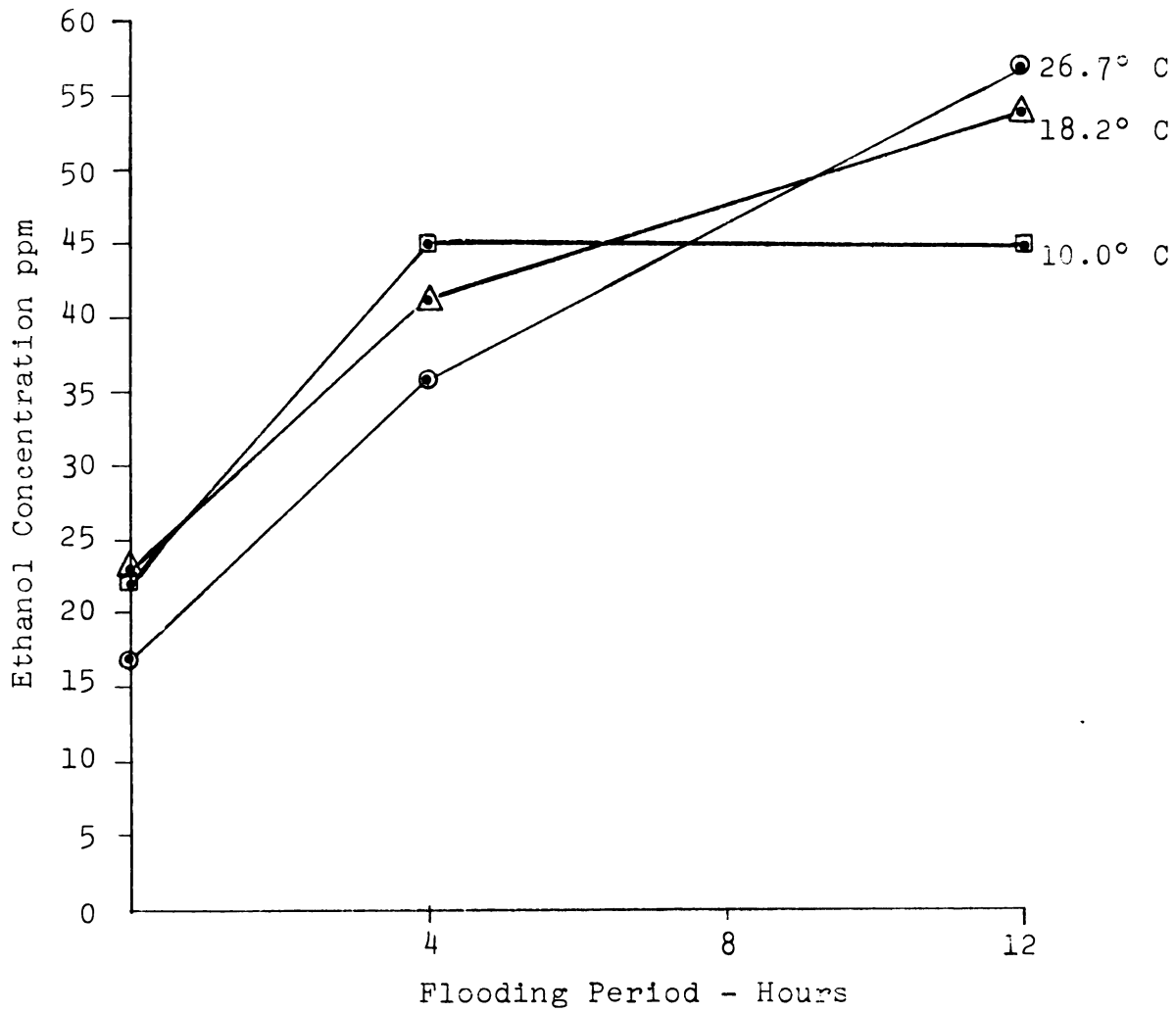

Fig. 2.--Ethanol concentration in xylem exudates of tomato plants flooded at three soil temperatures in the greenhouse at an air temperature of 18.3-21.1° C and low light intensity. (Each value is the average of six replicates.)

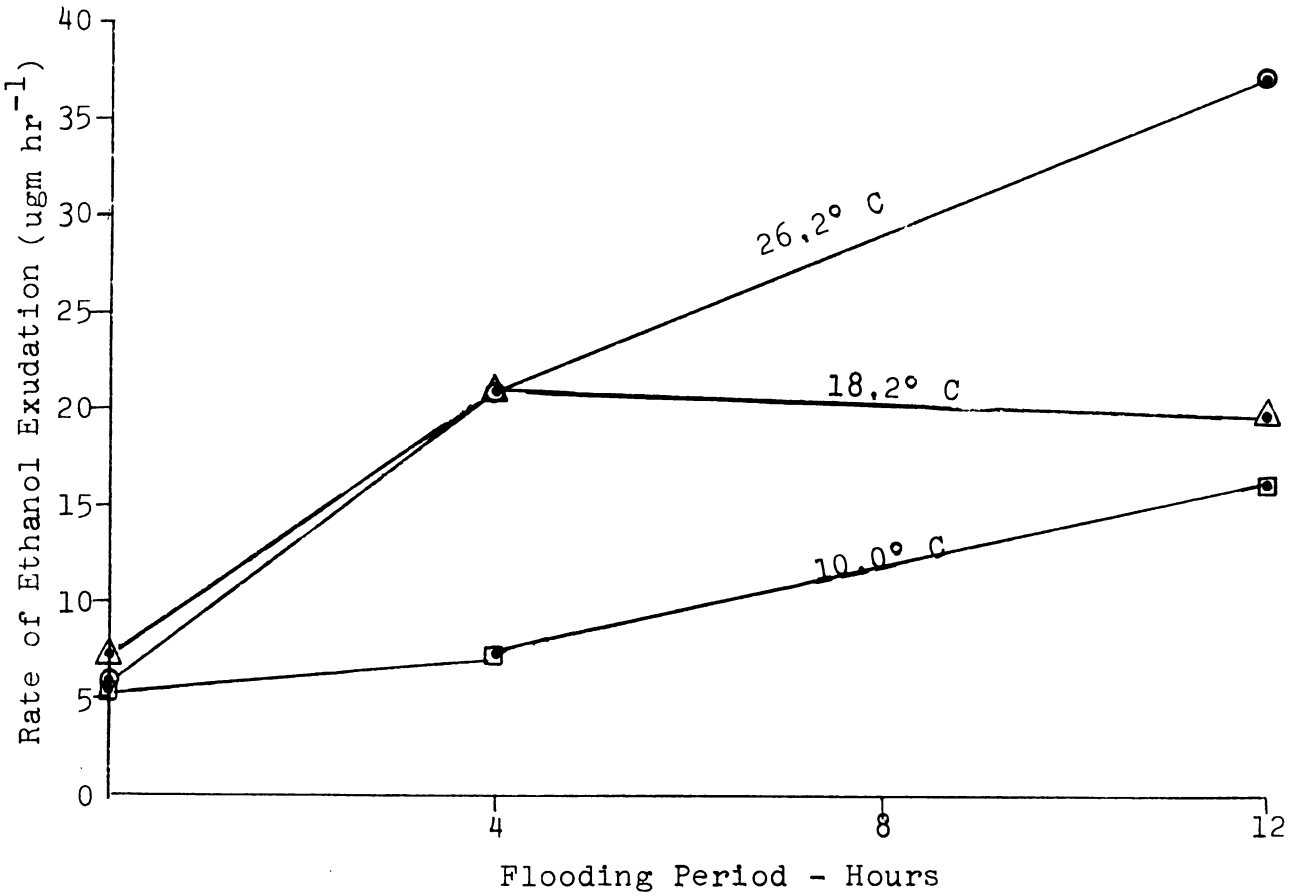

Fig. 3.--Rate of ethanol exudation from tomatoes flooded at three soil temperatures in the greenhouse at an air temperature of 18.2-21.1° C and low light intensity. (Each value is the average of six replicates.)

Description of Soil Temperature Experiment in Growth Chamber

Results of the greenhouse soil temperature experiment suggested that effect of soil temperature may be more important at a higher air temperature and greater light intensity. In the air and light intensity experiments, reported above, soil temperature in the containers followed air temperature closely in a similar cycle at the 7- and 15-cm depths.

An experiment was established at the medium temperature in the growth chamber to determine the effect of soil temperature controlled at 18.2° C while air temperature followed its regular cycle. The cylindrical water baths, described above, were used for this experiment and water was circulated through the pipes to maintain soil temperature within one degree of 18.2° C. The baths were insulated with commercial insulating material during treatment.

Results of Soil Temperature Experiment in Growth Chamber

Ethanol concentration Fig. 4 was numerically lower up to 12 hours flooding where soil temperature was maintained at 18.2° C than where soil temperature followed the medium temperature cycle (30.0° C maximum). Rates of exudation were similar for the two soil temperatures and it is concluded that soil temperature had some influence under these air environmental conditions. In the field, soil temperatures tend to follow air temperatures closely in a similar

but delayed diurnal cycle within surface depths (31). Consequently, it is considered that the growth chamber data obtained from the 9.1-30.0°C soil temperature range would apply more readily to field situations than would data for the constant soil temperature of 18.2 centigrade.

Transpiration Losses of Ethanol From Tobacco and Tomato Plants

Description of Experiment

The small molecular size and relatively high volatility of ethanol would suggest that this compound could be readily removed by the transpiration stream. Investigations by Kenefick (20) showed that transpiration served as a means of eliminating alcohol from sugar beet leaves under anaerobic conditions. Fulton (12) showed that intact tomato plants had less ethanol than decapitated plants flooded for identical periods of time. This effect was attributed in part to elimination capacity of the foliage.

The following experiment was conducted to determine the effectiveness of transpiration for removal of ethanol from tomato and tobacco plants. Tobacco plants were included in this experiment since these plants have large leaves and have also been shown to accumulate large ethanol concentrations when flooded.*

*Erickson, A. E., J. M. Fulton and G. H. Brandt. New Techniques for Relating soil Aeration and Plant Response. Trans. 8th International Cong. Soil Sci. Bucharest (in press).

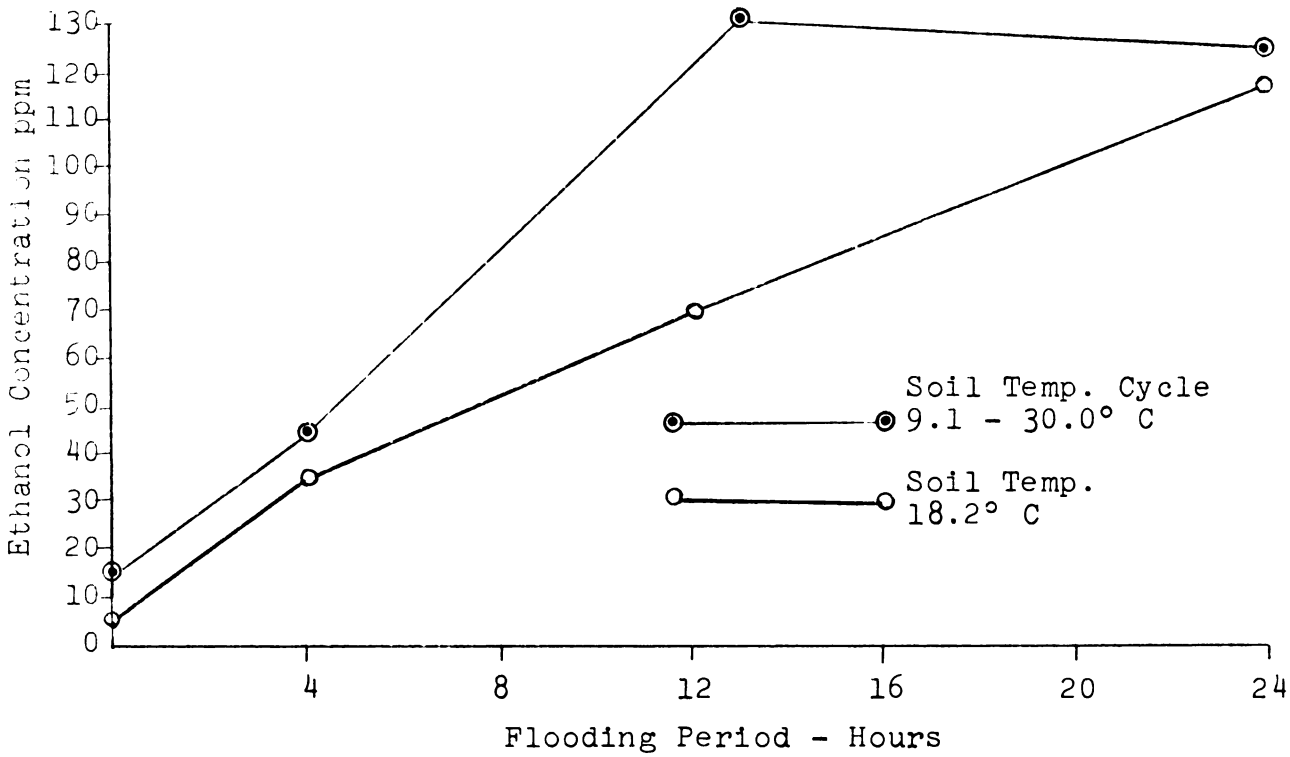

Fig. 4.--Ethanol concentration in xylem exudate of tomato plants flooded at two soil temperatures under medium air temperature and high light intensity. (Each value is the average of five replicates.)

Tomato and tobacco plants were grown in 13-cm clay pots in a greenhouse potting soil and were treated when the plants yet could be accommodated in the plastic chamber described above. At this time the tomato plants were in the early bloom stage with one or two blossom clusters. The tobacco plants were in the foliar stage.

Two replicates of each plant species were subjected to flooding in the laboratory while another set was flooded in the growth chamber. Light intensities were 1.20-1.30 gm-cal cm^{-2} min^{-1} at the mid-plant zone in both experiments. Air temperature in the lab experiment was constant at 26.7° C within the chamber while chamber temperature within the Sherer-Gillett unit reached 35.6° C in a cyclic manner. Transpiration samples were trapped by the method described previously.

Results

Ethanol concentration in transpiration condensates of both tobacco and tomato plants, Fig. 5 and Fig. 7 increased with duration of flooding period. Maximum transpiration concentration was reached at the 12-hour flooding period for tomato plants in both the laboratory and growth chamber experiments. The maximum concentration for tobacco plants was also reached at 12 hours in the constant temperature experiment but continued to increase at 24 hours in the growth chamber. Concentrations for tomato plants followed the pattern established in exudate accumulation, although concentrations were much

lower in transpired condensates. Tomato plants had a slightly higher ethanol concentration than tobacco plants at both the constant and cyclic temperatures.

Since concentrations were small, rates of ethanol loss in the transpired samples were calculated and presented in Fig. 6 and Fig. 8 for tobacco and tomatoes. Results expressed in this manner disclosed a cyclic pattern for ethanol loss in transpired samples under the cyclic temperature and light environment. Loss rate was high at the 4-hour flood period when transpiration was high and was low at 12 hours when transpiration was negligible. Transpiration loss increased again at the 24-hour period.

Effect of Flooding Tomato Plants in Different Soil Volumes

Description of Experiment

Flooding of tomato plants in the greenhouse and growth chamber involved the use of relatively small soil volumes. It is known that saturation of soil is associated with entrapment of air in the soil (5). The amount of air entrapped would depend on volume. In addition the work of Williamson (43) has indicated that oxygen diffusion rates are dependent to some extent on soil volume. Such an effect could presumably result from decreased oxygen concentration due to increased plant requirement per unit soil volume.

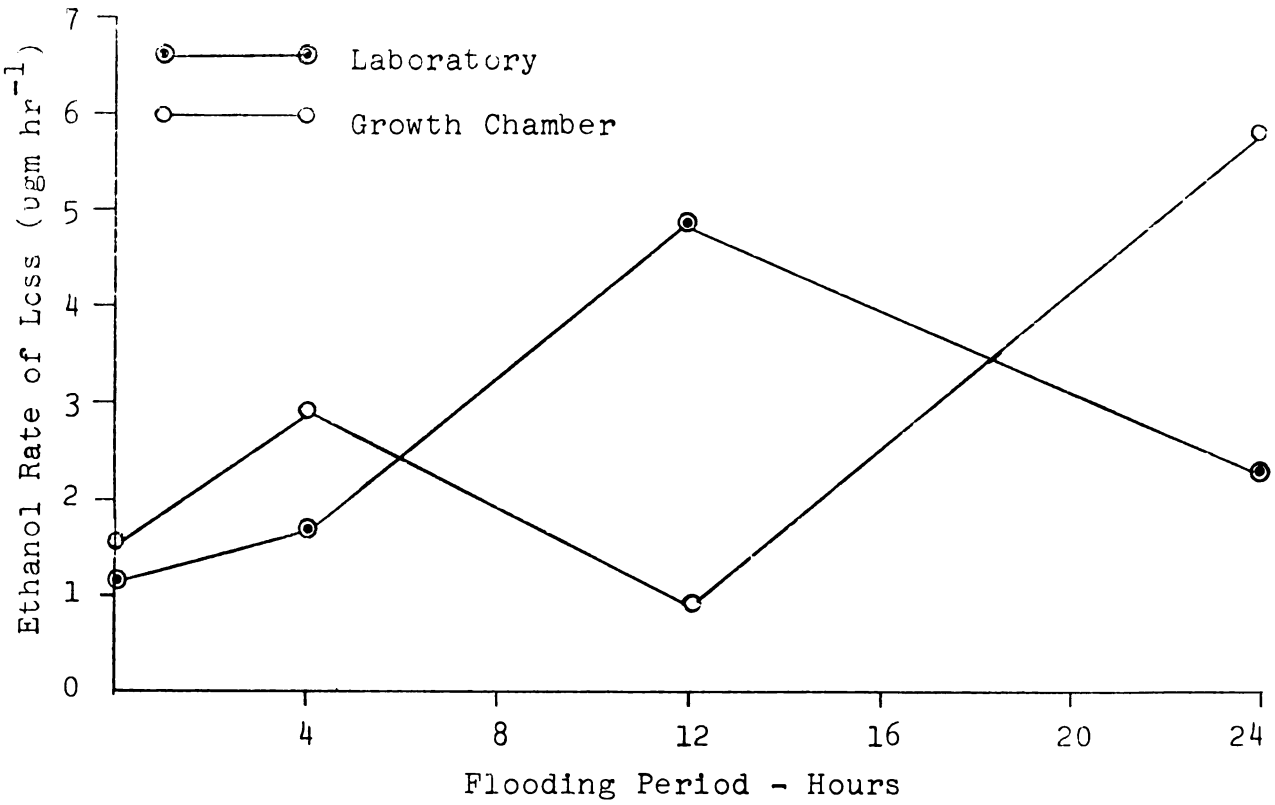

Fig. 6.--Rate of ethanol loss by transpiration from tobacco plants flooded at a constant temperature in the laboratory and at a cyclic temperature in the growth chamber. (Each value is the average of two replicates.)

The present experiment was established to measure ethanol concentration in xylem exudate of tomato plants flooded in three different soil volumes. Tomato plants were grown in a potting soil mixture in 1.9, 3.8 and 7.6 liter glazed crocks. Flooding treatments were applied when the plants were in the bloom stage and each flooding treatment was replicated in quadruplicate within each soil volume. The treatments consisting of a control and 3-, 6- and 9-hour flooding periods were carried out in the growth chamber at the medium air temperature combined with high light intensity.

Experimental Results

Ethanol concentration, Fig. 9, increased during the 9-hour period within each soil volume and appeared to reach a maximum concentration at 6-hours in the 7.6 liter containers, while ethanol continued to increase in the smaller containers. Plants in the 1.9 liter containers had considerably more ethanol than plants in larger soil volumes at the 6- and 9-hour periods. Ethanol concentration in 3.8 liter containers was slightly greater than in 7.6 liter volumes at 9-hours.

Plants in the three soil volumes differed in exudation rate and results in Fig. 10 show this effect. On this basis, rate of ethanol exudation was slightly greater at 3- and 6-hour periods from plants in 1.9 liter containers than from plants in larger volumes. At the

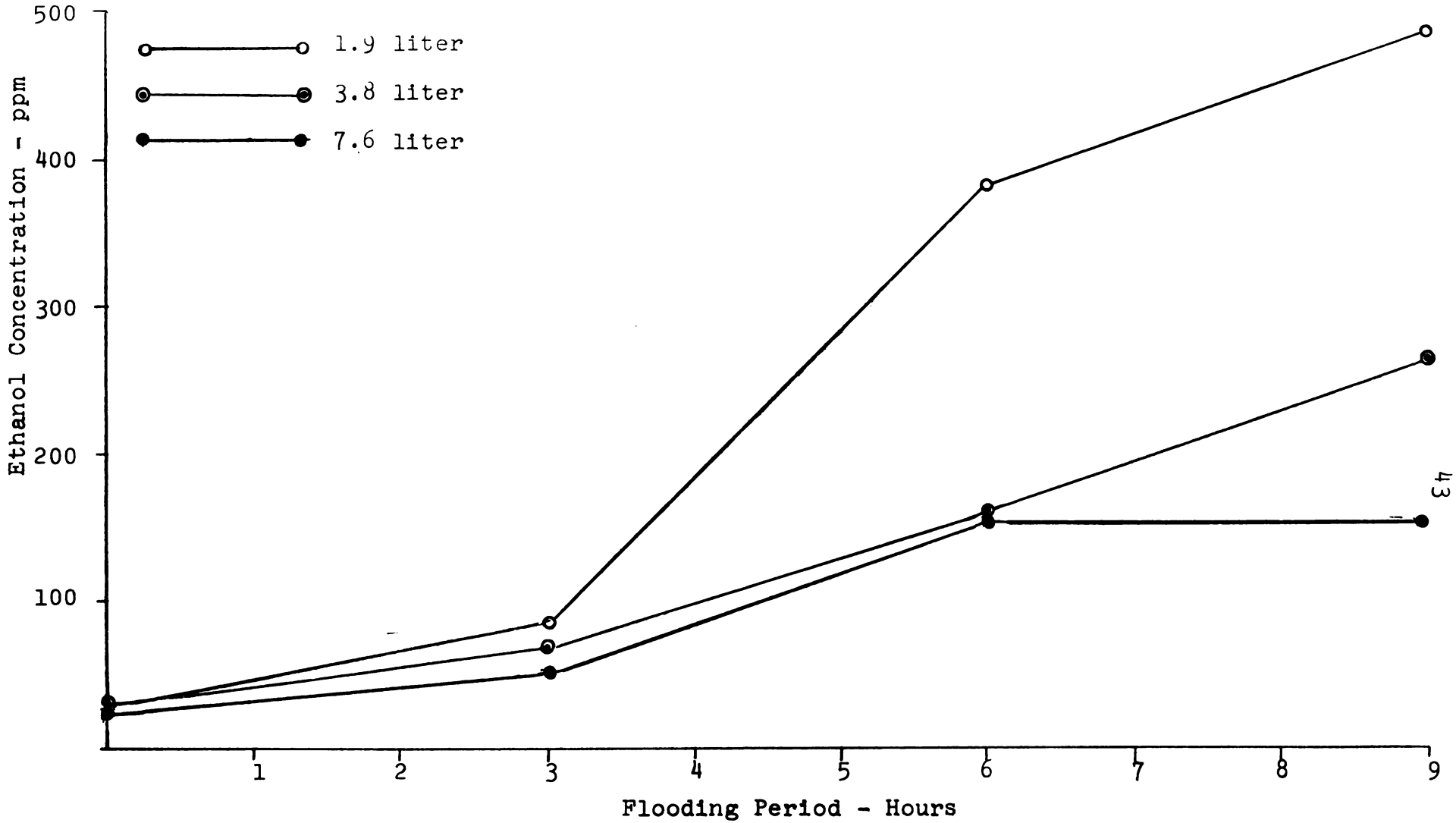

Fig. 9.--Ethanol concentration in xylem exudate of tomato plants flooded in three different soil volumes at medium air temperature and high light intensity. (Each value is the average of four replicates.)

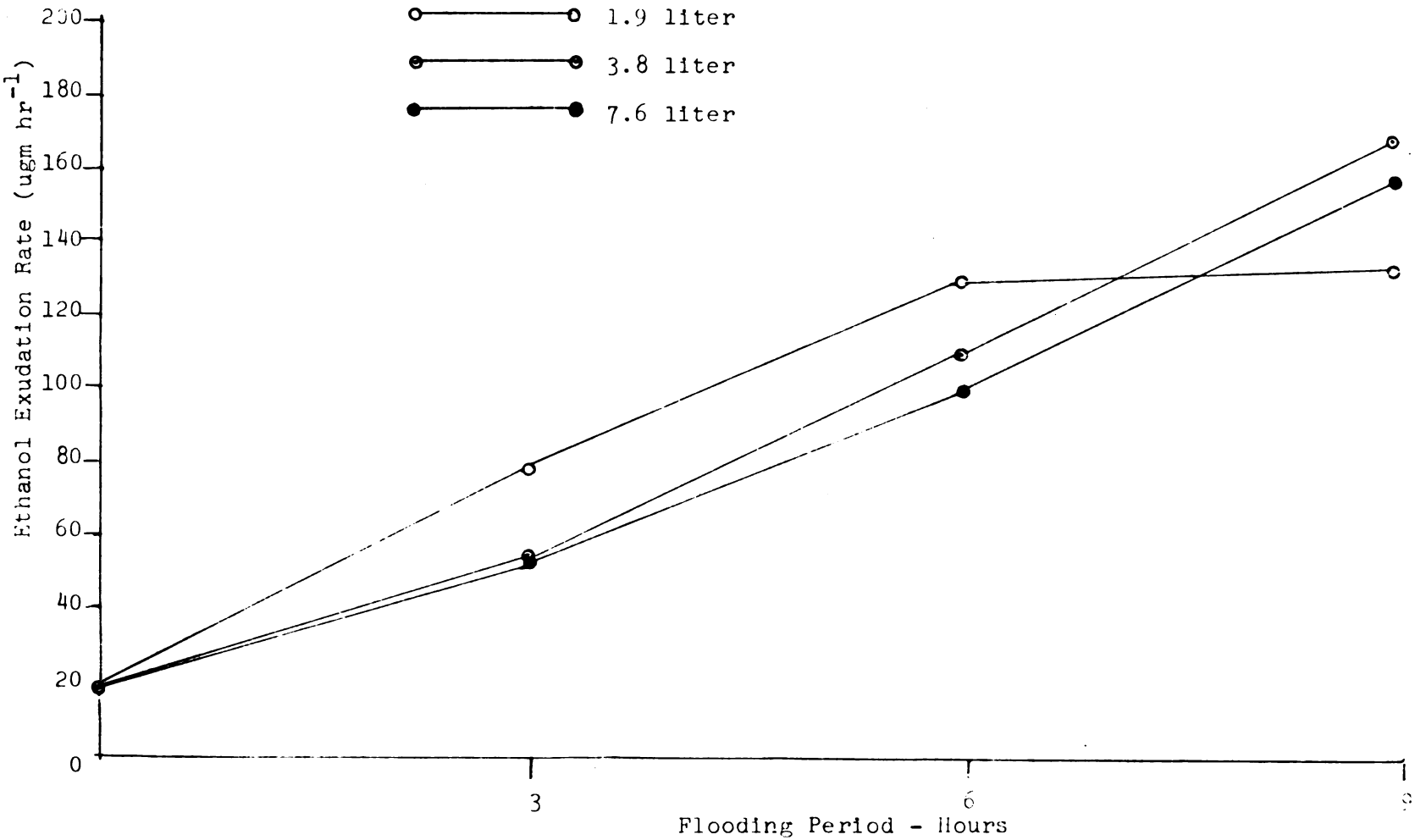

Fig. 10.--Rate of ethanol exudation from tomato plants flooded in three different soil volumes at medium air temperature and high light intensity. (Each value is the average of four replicates.)

9-hour period ethanol exudation rate was increasing more rapidly on the plants in larger soil volumes.

The results would suggest that soil volume altered the pattern of ethanol concentration and exudation through the amounts of entrapped air in the soil. Such an effect would be feasible during short term flooding periods. Entrapped air is commonly referred to (5, 33), as a factor effecting the wetting characteristics in soils. Oxygen diffusion rate was slightly higher in the 7.6 liter pots during flooding than on smaller soil volumes although all oxygen diffusion rates for flooding periods were low.

It is to be noted that plants in the 1.9 liter containers were smaller than in the larger soil volumes. The plants were at the same morphological stages, however, on the basis of flowering.

Distribution of Ethanol in Anaerobic Tomato Plants

Description of Experiment

The present experiment was established to determine the distribution of ethanol in anaerobic tomato plants and to measure ethanol secretion by the roots. The work of Grineva (13) showed that pyruvic acid, an ethanol precursor, was in higher concentration in sunflower tops than in the roots when the plants were subjected to anaerobic conditions. Measurements of oxygen in plants (23), (39) have indicated an increasing concentration from bottom

to top during conditions of photosynthesis. In addition, it has been shown that plants excrete many organic compounds through the root (32). Grineva showed that alcohols were excreted by anaerobic plants.

The plants in this experiment were grown and treated in a mist chamber described before. This culture technique was chosen to enable measurement of root excreted ethanol and to obtain clean roots for determination of ethanol content. Nutrients were supplied in the mist by Hoagland's solution and the plants were grown to the early bloom stage before anaerobic treatment was applied.

One plant was selected to serve as a control while four plants were subjected to anaerobic treatment. The anaerobic condition was established in the mist chamber, which contained the roots, by supplying a stream of nitrogen gas to the chamber for a 12-hour period. After treatment, the anaerobic plants, along with the control plant, were cut into sections designated as top leaves, bottom leaves, top stem, bottom stem, top roots and bottom roots. In addition to plant tissue samples, effluent samples were obtained from the root environment and condensed mist samples were collected from under each plant root.

Results and Discussion

Anaerobic conditions, established in the mist chamber, increased ethanol concentration, (Table 2), throughout the plant in comparison with the aerobic

TABLE 2.--Effect of flooding with nitrogen gas on ethanol distribution in tomato plants.

Part of Plant	No Flood Control	Treatment: 12-Hour Flood with Nitrogen Gas				
		Plant 1	2	3	4	Av.
		Ethanol ppm				
Top Leaves	3	19	24	26	17	22
Bottom Leaves	4	25	20	29	22	24
Top Stem	5	12	38	36	40	32
Bottom Stem	7	23	46	78	41	47
Top Root	7	28	38	25	28	30
Bottom Root	6	13	17	30	15	19

TABLE 3.--Effect of flooding with nitrogen gas on root excretion of ethanol.

Sample	Treatment: No Flood	Treatment: 12-Hour Flood
	Ethanol ppm	
Nitrogen Effluent	8	24
Plant 1	5	9
2	4	24
3	1	14
4	2	20
Average Root	3	17

control. In nitrogen treated plants, ethanol was in highest concentration in the bottom portion of the stem and decreased toward the top of the plant and decreased also in the bottom roots. Ethanol concentration was considerably lower than in the previous experiments where sand or soil constituted the root medium.

Ethanol concentration, (Table 3), determined on condensed vapor samples from the root atmosphere, was increased three-fold by the 12-hour flood period. In addition, ethanol was increased in condensed mist samples collected under the roots at the 12-hour flood period. Under the anaerobic conditions of this experiment it is indicated that ethanol is excreted to the external root environment in considerable concentrations.

Effect of Carbon Dioxide on Ethanol Concentration in Tomato Plants Under Aerobic Conditions

Description of Experiment

Carbon dioxide has been investigated (33) as a deleterious factor affecting plants on saturated soils. Carbon dioxide concentration in soils has often been associated reciprocally with low oxygen content.

An experiment was established, using the mist chamber technique, to determine whether high carbon dioxide concentration influences the fermentation reaction in tomato plants under aerobic conditions. Tomato plants were grown in the mist chamber by means of identical

culture techniques used in the nitrogen flooding experiment. Treatment of the plants in this experiment consisted of enclosing the roots in a gaseous mixture composed of 20.1 percent carbon dioxide, 20.1 percent oxygen and 59.8 percent nitrogen. Plant tissue, chamber effluent and mist condensate samples from below the plants were collected as in the previous experiment.

Experimental Results

Ethanol concentrations throughout the plants were low (Table 4) and showed no effect due to carbon dioxide concentration under the aerobic conditions provided. The concentrations of ethanol in the atmosphere surrounding the roots and in the condensed mist samples (Table 5), collected below the roots, were also low. Difficulty was experienced in obtaining accurate measurements of ethanol at concentrations below 5 parts per million.

The data indicate that carbon dioxide concentration did not influence the fermentation reaction under aerobic conditions. The effect of carbon dioxide concentration under anaerobic conditions was not investigated.

Quantities of Ethanol Accumulated and Eliminated from Tomato Plants

Total quantities of ethanol in tomato plants and the amounts eliminated from plants by transpiration and root excretion were determined for a 12-hour anaerobic period. The amounts of ethanol in plants and quantities excreted by roots were calculated from mist chamber data.

TABLE 4.--Effect of carbon dioxide on ethanol distribution in tomato plants under aerobic conditions.

Part of Plant	No Flood Control	Treatment: 12-Hour Treatment with Gas Mixture				
		Plant 1	2	3	4	Av.
		Ethanol-ppm				
Top leaves	2	4	2	4	5	5
Bottom leaves	2	2	4	2	7	4
Top stem	1	4	2	2	5	3
Bottom stem	3	4	2	2	5	3
Top root	3	2	2	2	4	3
Bottom root	2	2	4	2	3	3

TABLE 5.--Effect of carbon dioxide on root excretion of ethanol under aerobic conditions.

Sample		Treatment: No Flood	Treatment: 12-Hour Flood
		Ethanol-ppm	
Gas Effluent		2	2
Plant	1	3	0
	2	0	0
	3	0	5
	4	0	0

The quantities of ethanol eliminated by transpiration were calculated from the tomato transpiration results. Plants used in both experiments were similar in size and morphological stage of growth.

Table 6 shows that the rate at which ethanol was eliminated by plants was considerably less than the rate at which it accumulated. This result is expected since otherwise the compound would not accumulate in such readily measurable concentrations. The data does show that root excretion served more effectively than transpiration in eliminating some of this anaerobic product. In addition the amount of ethanol in aerobic plants is sufficiently high to suggest that this compound is normally metabolized, at least to a small extent, in the plant.

TABLE 6.--Ethanol accumulation and elimination from anaerobic tomato plants in ugm per plant.

Amount of Ethanol	Flooding Period - Hours	
	None	12
	ugm	
Total Plant	380	1829
Transpiration	2	127
Root Excretion	4	453

Flooding of Tomato Plants in 3.8 Liter Containers in the Field

Description of Experiment

Experiments in the growth chamber under simulated environments had shown specific responses to air temperature and light intensity using ethanol measurements as an indicator of anaerobic conditions. Soil temperature was also shown to influence the anaerobic effect to a lesser extent.

To further investigate the usefulness of ethanol measurements as indicators of flooding damage it was considered important to obtain measurements under field environmental conditions. Initially an experiment was established for this purpose by growing plants in a field location on a loam textured soil. In this earlier experiment the tomato seedlings were transplanted into the field at the first true leaf stage as was done in the greenhouse experiments. The plants were flooded by enclosing each plant in a 91-cm perimeter of aluminum sheeting inserted to a 10-cm depth in the soil. The entire soil area was extremely dry at the time the plants reached flooding stage and difficulty was experienced in attaining flooded conditions.

The first flooding attempts consisted of applying water within each aluminum enclosure until about 8 cm of water remained on the soil surface around the plant. Drainage characteristics of the soil were rapid and it

was necessary to provide a continuous supply of water to the plant area. Oxygen supply in the soil surface decreased to levels known to limit plant growth as indicated by oxygen diffusion rates. Ethanol concentration, however, was very low in root exudates from the plants and it was concluded that soil flooding was not achieved throughout the root zone, and the oxygen supply did not become limiting.

An experiment was designed to measure the field environmental effect under conditions where soil flooding could be assured. To do this plants were grown in 3.8 liter glazed pots in a potting soil mixture similar to that used in the soil volume experiments. The plants were grown outside until they reached the early bloom stage. Two days before treatment the plants were transferred to the field and enclosed in the soil in order that temperature in the plant root zone could equilibrate with that of the soil environment.

Results

In Figure 11 data from this field experiment are compared with data obtained at two temperatures and at high light intensity in the growth chamber (cf. Figure 1). Ethanol concentrations in the field flooding experiment followed a pattern for flooding duration similar to that obtained in the growth chamber. In addition the average value at the 24-hour flood period was between the high temperature and low temperature concentrations.

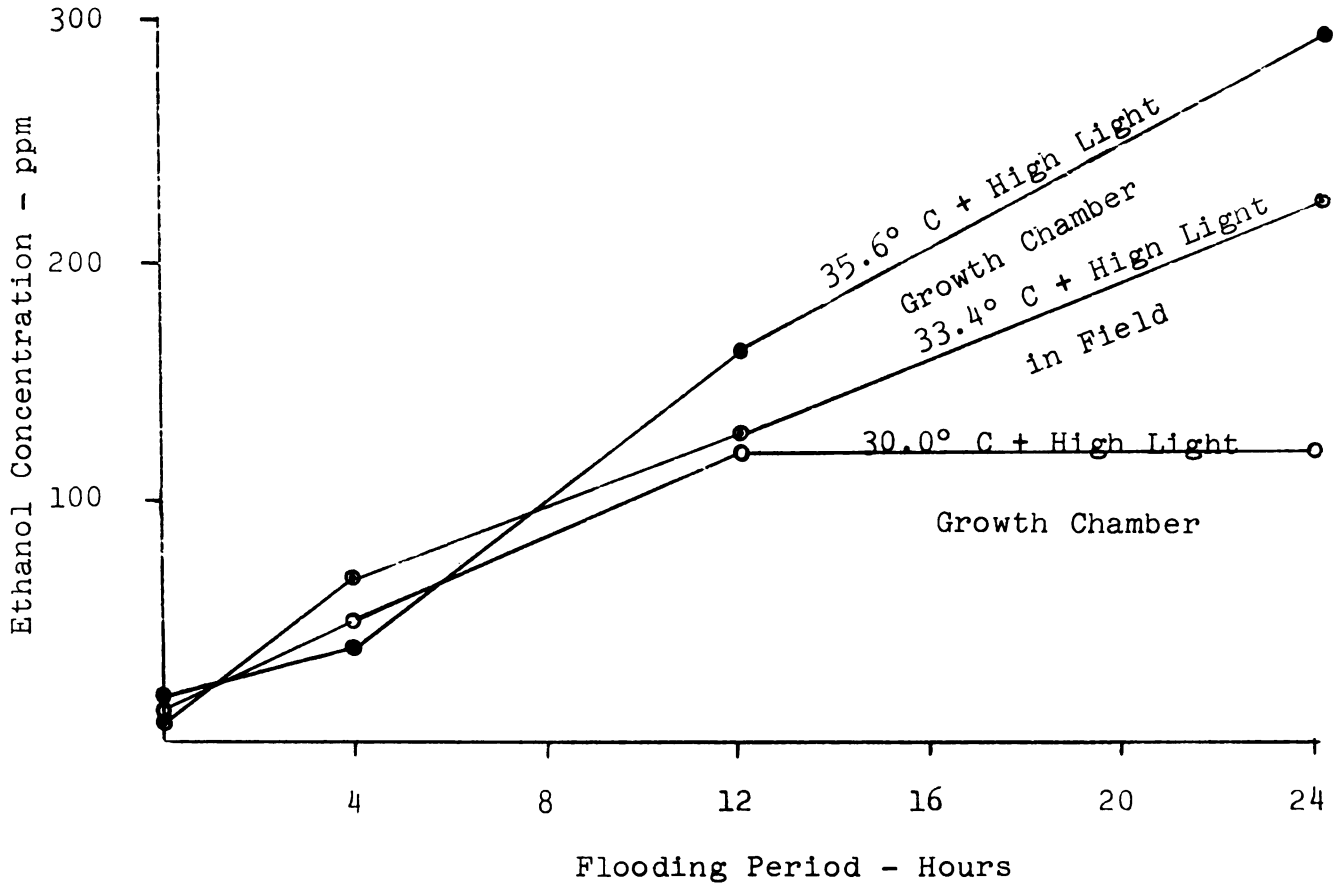

Fig. 11.--Ethanol concentration in xylem exudate of tomato plants flooded in the field in comparison with data obtained in growth chamber experiments.

Air temperature in the field reached a maximum of 33.4° C which was close to the high temperature cycle maximum of 35.6° C in the chamber. Light intensity was high in both locations except that a gradient would not exist in the field as occurred in the simulated conditions. Soil temperature in the field did not reach the maximum temperature recorded in the chamber but was 3 to 5° C lower. It is evident from the data that the simulated conditions in the growth chamber were quite similar to the field conditions and it is concluded that results obtained in the growth chamber are applicable for field situations.

DISCUSSION OF RESULTS

Air temperature had a pronounced influence on ethanol concentration in xylem exudate of flooded tomato plants. Within each air temperature environment, ethanol concentration increased with flooding period during the first 12 hours, in agreement with results reported elsewhere (12). At the high air temperature level, in the presence of high light intensity, ethanol concentration continued to increase at 12 hours but appeared to reach a maximum at the 24-hour period. For all other air and light environments maximum ethanol concentration was reached at 12 hours.

Light intensity, within the range supplied in these experiments, had a smaller effect than air temperature on ethanol accumulation. High light intensity, under medium air temperature, increased ethanol concentration at 12- and 24-hour periods of flooding. At the high air temperature ethanol concentration was lower at the 24-hour period under low light than under high light intensity. Since the low light intensity provided in these experiments was lower than light intensity values for cloudy daytime periods in the field it is concluded that light intensity would be of less practical significance than air temperature with regard to the fermentation reaction.

Soil temperature, in these experiments, had a smaller effect on ethanol concentration in tomato root exudate than air temperature. Where light intensity and air temperature were low, in the greenhouse experiment, ethanol concentration was also low, although the amount exuded was dependent on soil temperature. At medium air temperature and high light intensity in the growth chamber, where a maximum air temperature of 30.0° C was reached, soil temperature had a greater effect on the concentration of ethanol in xylem exudate than in the greenhouse experiment. This effect was especially evident during the early periods of flooding. Where soil temperature followed the air temperature cycle ethanol concentration reached a maximum at 12 hours. Where soil temperature was maintained constant at a lower level of 18.2° C ethanol concentration approached the same maximum but the maximum was not reached until the 24-hour period. Also, ethanol concentration at the 18.2° C soil temperature reflected a linear relationship with flooding period.

The amount of ethanol transpired from tomato and tobacco plant foliage was small in relation to the amount of this compound exuded by the roots. Concentration of transpired ethanol, however, was proportional to exudate accumulation with respect to duration of flooding conditions. In addition, a maximum transpiration concentration appeared to be reached that coincided with the maximum of root exudate for similar conditions (21).

Results of the above experiments indicated that the fermentation reaction as measured by ethanol concentration was dependent upon a continuous supply of photosynthetic products (41) rather than upon degradation of stored compounds. At high air temperature and high light intensity ethanol concentration continued to increase up to the 24-hour period while at low light intensity and high air temperature ethanol concentration decreased from the 12- to the 24-hour period. The soil temperature experiment, conducted in the greenhouse, indicated that ethanol concentration was increased with soil temperature but the amounts of ethanol involved were small. The effect of lower soil temperature in the growth chamber was to produce a linear increase in ethanol concentration that approached the maximum obtained earlier where soil temperature followed the air temperature cycle. The cyclic nature of amounts of ethanol transpired would also indicate the dependency of the reaction upon the photosynthetic supply rather than upon stored metabolites.

Ethanol was excreted in small amounts by aerobic tomato plants and in considerably larger quantities by anaerobic plants. This compound was present in the root atmosphere and in condensed mist samples from the plant roots. Anaerobic conditions for this phase of the study were established by treatment with nitrogen gas in the mist chamber and ethanol concentrations were lower in the plants than where soil was used as the plant medium. Nevertheless,

the results show that root excretion provides a potentially important means of ethanol elimination by flooded tomato plants. Comparison of excretion data with transpiration results also points to root excretion as being a more effective means of ethanol elimination than transpiration. Although the excretion of ethanol by plant roots may provide a means of alleviating toxicity within the plant the process may also induce pathogenicity. Weinhold (40) has shown that ethanol concentrations within the range established by this experiment are effective in initiating invasion of plant roots by certain soil pathogens.

Ethanol in tomato plants subjected to 12 hours of flooding was observed to vary with the portion of the plant in which it was measured. The highest concentration of ethanol was observed in the lower stem and in the top roots. Ethanol concentration decreased in the upper leaves and in the bottom roots. Lower ethanol concentration in the upper leaves is attributed to transpiration of the compound from the leaves and to higher oxygen concentration in the upper portions of the plant. Since ethanol removal was small the effect of oxygen due to photosynthesis and gaseous exchange is an important factor. Root excretion data indicate that ethanol concentration in the lower root system results from excretion of the compound and point to the lower root region as the most active zone of ethanol excretion. It is apparent that since ethanol increases in the plant with flood period that the means normally available for disposal or utilization of this compound are insufficient to offset the amounts

produced. The distribution study indicates, however, that root environment provides a significant sink for elimination of this constituent.

Volume of soil, in which tomato plants were flooded, was shown to influence the time pattern of ethanol accumulation. Ethanol concentration was considerably higher on plants flooded in the smallest soil volume compared with concentration in plants flooded in volumes of double or quadruple magnitude. The rate of ethanol exudation was also higher in the smallest soil volume than in the larger volumes during early periods of flooding but this result was reversed at the longest flooding period. Potential oxygen availability in flooded treatments, measured as oxygen diffusion rate, was slightly higher on the largest soil volume than in the two smaller soil volumes, although all volumes provided values shown to be associated with the fermentation reaction. It is concluded that a greater supply of oxygen relative to plant size was available in the form of entrapped air in the larger soil volumes. It is further concluded that the volume effect continued only until this supply of oxygen was consumed, which in this experiment was approximately 6 hours.

Field flooding of tomatoes showed that field environmental conditions resulted in ethanol concentrations similar to those obtained in the growth chamber for identical flooding periods. It was necessary to use pot containers for the plants tc ensure accurate flooded conditions in the

field experiment and the size of container, 3.8 liters in this experiment, could have influenced the actual concentration of ethanol produced. Nevertheless the results showed that similar effects for flooding occurred for both environments and it is concluded that measurements in the growth chamber have valid application to field conditions.

Considerable variation occurred between ethanol values within each treatment throughout the experiments in this investigation. It is believed that these differences were due to variability inherent in the plant materials. Regardless of this variability sufficient consistency was obtained to establish statistical differences described between treatments and it is concluded that the averages fairly represent treatment effects.

The investigations reported here show that measurement of ethanol concentration in tomato plants is a useful means of determining the degree of damage resulting from environmental conditions during short periods of flooding. The studies conducted by Kenefick and also by Grineva indicated that alcohol measurement served as an index of insufficient oxygen supply. Fulton determined ethanol qualitatively and showed a quantitative relationship between this compound in xylem exudate of tomato plants and soil oxygen diffusion rate. The present study is in agreement with these results and further shows that environmental conditions during a short term flooding period can be assessed on the basis of ethanol measurement.

One worker, Letey (2), was unable to obtain a relationship between ethanol concentration, in tomato plants, and different oxygen concentrations in nutrient solutions. However, in Letey's investigation oxygen levels were established by bubbling oxygen through the solution medium at 21, 10, 3.5 and 1.5 percent concentrations. Russell (33) has questioned the applicability of dynamic conditions of this nature in studying oxygen requirements for plants. In addition the treatments were continued for periods extended to three weeks. The question is also raised concerning adaptability of the plants to these low oxygen conditions through development of aerenchyma tissue as has been shown (36, 37) for certain plants. The data shown by Letey for tomato plants are in close agreement with those reported by Fulton and Erickson where similar conditions were provided during treatment.

It is considered that ethanol determinations constitute a very suitable tissue test method for evaluating the conditions involved in soil anaerobic stress for plants. Undoubtedly other plant metabolites have potential usefulness in this area. In addition the metabolic processes may only be an initial indicator of anaerobic effects since structural changes could also follow, especially with prolonged periods of oxygen deficiency. It is concluded that the plant fermentation reaction, as evaluated by ethanol measurements, can serve a useful purpose in answering

questions regarding flooding effects on plants and in seeking means to alleviate the disastrous results that can occur where plants are subjected to flooding.

APPENDICES

TABLE 1.--Ethanol concentration in xylem exudate of tomato plants flooded under three ranges of air temperature and at two light intensities.

Flooding Period Hours	Rep 1	2	3	4	5	Av.	Flooding Period Hours	Rep 1	2	3	4	5	Av.
							Ethanol Concentration ppm						
	High Temperature and High Light							High Temperature and Low Light					
None	23	18	26	17	10	19	None	3	10	14	41	25	18
4	19	16	55	48	24	32	4	34	26	27	58	35	36
12	240	162	106	177	148	167	12	183	184	109	183	222	176
24	400	396	242	162	235	287	24	95	76	109	143	138	112
	Medium Temperature and High Light							Medium Temperature and Low Light					
None	0	21	33	7	18	15	None	34	14	4	20	10	16
4	26	54	57	47	23	41	4	13	15	19	16	13	15
12	170	175	110	23	165	129	12	31	57	73	58	56	56
24	220	60	70	120	165	127	24	135	51	44	20	122	74
	Low Temperature and High Light							Low Temperature and Low Light					
None	33	8	9	21	7	16	None	19	30	15	23	11	20
4	48	29	27	20	15	28	4	9	10	15	12	17	13
12	48	95	53	80	40	63	12	38	49	31	28	26	34

Summary of Table 1.

Source of Variance	F	Nec. F	
		0.05	0.01
Flood Period	94.72	3.13	4.92
Air Temperature	24.14	3.13	4.92
Light Intensity	8.47	3.98	7.01
Flood Period x Air Temperature	15.62	2.50	3.60
Flood Period x Light Intensity	1.38	3.13	4.92
Air Temperature x Light Intensity	2.27	3.13	4.92

TABLE 2.--Ethanol concentration in xylem exudate of tomato plants flooded at three soil temperatures in greenhouse.

Flooding Period Hours	Soil Temp. ° C	Rep 1	2	3	4	5	6	Av.
		Ethanol Concentration ppm						
None	10.0	22	16	19	26	22	24	22
	18.2	30	36	18	17	16	18	23
	26.7	17	7	21	23	16	17	17
4	10.0	68	67	28	45	36	27	45
	18.2	51	81	24	27	25	37	41
	26.7	48	79	26	22	23	15	36
12	10.0	16	74	29	28	69	53	45
	18.2	49	73	33	34	78	57	54
	26.7	61	72	27	18	63	100	57

Summary of Table 2.

Source of Variance	F	Nec. F 0.05	Nec. F 0.01
Flood Period	16.43	3.23	5.18
Soil Temperature	.12	3.23	5.18
Flood Period x Soil Temperature	.71	2.61	3.83

TABLE 3.--Rate of ethanol exudation by tomato plants at three soil temperatures in the greenhouse.

Flooding Period Hours	Soil Temp. °C	Rep 1	2	3	4	5	6	Av.
		Ethanol, ugm hr^{-1}						
None	10.0	.7	2.2	6.5	18.9	.7	4.3	5.6
	18.2	5.4	8.3	18.0	5.6	1.4	4.0	7.1
	26.7	3.9	5.1	9.9	9.7	2.4	2.6	5.6
4	10.0	6.1	6.7	20.4	6.0	3.6	1.1	7.3
	18.2	12.8	11.3	21.6	46.7	1.0	30.3	20.6
	26.7	13.4	20.5	24.1	24.9	32.4	9.0	20.7
12	10.0	2.0	2.0	29.9	28.0	21.4	13.3	16.1
	18.2	16.3	20.3	17.5	17.5	27.3	20.0	19.8
	26.7	13.4	16.6	25.1	20.3	65.5	82.0	37.2

Summary of Table 3.

Source of Variance	F	Nec. F	
		0.05	0.01
Flood Period	9.54	3.23	5.18
Soil Temperature	3.78	3.23	5.18
Flood Period x Soil Temperature	1.66	2.61	3.83

TABLE 4.--Ethanol concentration in xylem exudate of tomato plants flooded at two soil temperatures under medium air temperature and high light intensity.

Flooding Period Hours	Rep 1	2	3	4	5	Av.
	Ethanol Concentration ppm Soil Temperature 9.1 - 30° C					
None	0	21	33	7	18	16
4	26	54	57	47	23	41
12	170	175	110	23	165	129
24	220	60	70	120	165	127
	Soil Temperature 18.2° C					
None	4	10	9	4	10	7
4	52	10	48	14	40	32
12	140	24	55	49	79	69
24	100	132	130	144	94	120

Summary of Table 4.

Source of Variance	F	Nec. F 0.05	Nec. F 0.01
Flood Period	18.70	2.95	4.57
Soil Temperature	2.97	4.20	7.64
Flood Period x Soil Temperature	1.13	2.95	4.57

TABLE 5.--Transpiration loss of ethanol from flooded tobacco plants.

Plant	Flooding Period - Hours				24-Hour Xylem Exudate
	None	4	12	24	
Ethanol Concentration, ppm in Transpiration Condensate at 26.7° C in Laboratory					
1	1	2	4	6	362
2	3	4	10	4	120
Average	2.0	3.0	7.0	5.0	241
Ethanol Concentration, ppm in Transpiration Condensate at 14.4 - 35.6° C in Growth Chamber					
1	5	4	2	20	212
2	2	2	7	7	25
Average	3.5	3.0	4.5	13.5	119

TABLE 6.--Effect of flooding on rate of ethanol transpiration from tobacco plants.

Plant	Flooding Period - Hours			
	None	4	12	24
	Ethanol ugm hr^{-1}			
Rate of Transpiration of Ethanol at 26.7° C in Laboratory				
1	1.6	2.1	1.6	3.7
2	.7	1.3	8.3	.8
Average	1.2	1.7	5.0	2.3
Rate of Transpiration of Ethanol at 14.4 - 35.6° C in Growth Chamber				
1	1.6	3.8	.3	6.4
2	1.5	2.1	1.4	5.1
Average	1.6	3.0	.9	5.8

TABLE 7.--Transpiration loss of ethanol from flooded tomato plants.

Plant	Flooding Period - Hours				24-Hour Xylem Exudate
	None	4	12	24	
			Ethanol ppm		
			Ethanol Concentration in Transpiration Condensate at 26.7° C in Laboratory		
1	2	4	11	7	200
2	3	6	9	8	138
Average	2.5	5.0	10.0	7.5	179
			Ethanol Concentration in Transpiration Condensate at 14.4 - 35.6° C in Growth Chamber		
1	5	7	12	10	212
2	4	2	4	7	105
Average	4.5	4.5	8.0	8.5	159

TABLE 8.--Effect of flooding on rate of ethanol transpiration from tomato plants.

Plant	Flooding Period - Hours			
	None	4	12	24
		Ethanol, ugm hr^{-1}		
		Rate of Transpiration of Ethanol at 26.7° C in Laboratory		
1	3.4	7.0	13.5	2.9
2	2.6	8.3	8.5	5.6
Average	3.0	7.7	11.0	4.3
		Rate of Transpiration of Ethanol at 14.4 - 35.6° C in Growth Chamber		
1	3.6	26.3	16.0	16.8
2	.6	2.3	.9	3.7
Average	2.1	14.3	8.5	10.3

TABLE 9.--Ethanol concentration in xylem exudate of tomato plants flooded in three soil volumes at medium temperature and high light intensity.

Soil Volume (Liters)	Rep. No.	Flooding Period - Hours			
		None	3	6	9
			Ethanol, ppm		
	1	12	60	305	330
	2	40	105	420	500
1.9	3	42	135	378	525
	4	16	38	425	600
	Av.	28	85	382	489
	1	22	45	140	245
	2	32	33	160	250
3.8	3	50	70	150	300
	4	10	125	195	260
	Av.	29	68	161	264
	1	8	27	75	185
	2	37	39	142	212
7.6	3	20	65	320	145
	4	23	70	80	70
	Av.	22	50	154	153

Summary of Table 9.

Source of Variance	F	Nec. F	
		0.05	0.01
Flood Period	74.08	2.90	4.46
Soil Volume	35.54	3.30	5.34
Flood Period x Soil Volume	10.24	2.40	3.42

TABLE 10.--Rate of ethanol exudation from tomato plants flooded in three soil volumes at medium temperature and high light intensity.

Soil Volume (Liters)	Rep. No.	Flooding Period - Hours			
		None	3	6	9
		Ethanol ugm hr^{-1}			
	1	24	33	154	78
	2	5	63	33	229
1.9	3	6	96	97	79
	4	28	119	235	142
	Av.	16	78	130	132
	1	19	10	44	149
	2	10	28	54	237
3.8	3	22	75	189	118
	4	16	94	148	166
	Av.	17	52	109	168
	1	21	32	76	161
	2	24	42	113	267
7.6	3	17	64	86	195
	4	6	66	122	9
	Av.	17	51	99	158

Summary of Table 10.

Source of Variance	F	Nec. F	
		0.05	0.01
Flood Period	15.15	2.90	4.46
Soil Volume	.08	3.30	5.34
Flood Period x Soil Volume	.54	2.40	3.42

TABLE 11.--Oxygen diffusion rates in three soil volumes flooded at medium temperature and high light intensity.

Soil Volume (Liters)	Flooding Period Hours	Rep. 1	2	3	4	Average
		Oxygen, gm x 10^{-8} cm^{-2} min^{-1}				
1.9	None	43.8	38.2	32.6	28.8	35.9
	3	17.0	15.1	11.3	19.8	15.8
	6	13.2	14.3	16.5	10.7	13.7
	9	13.5	9.0	11.0	11.2	11.2
3.8	None	38.4	45.5	40.1	32.6	39.2
	3	16.5	15.0	13.2	13.5	14.6
	6	14.7	13.3	16.2	13.2	14.4
	9	12.5	11.0	13.6	12.4	12.4
7.2	None	41.4	28.4	27.0	24.1	30.2
	3	13.8	17.6	17.3	17.0	16.4
	6	18.2	16.9	13.8	16.2	16.3
	9	20.1	15.9	11.6	15.9	15.9

Summary of Table 11.

Source of Variance	F	Nec. F	
		0.05	0.01
Flood Period	101.22	2.90	4.46
Soil Volume	.30	3.30	5.34
Flood Period x Soil Volume	2.96	2.40	3.42

TABLE 12.--Ethanol concentration in xylem exudate of tomato plants flooded in the field at a medium-high air temperature and high light intensity.

Flooding Period Hours	Rep. 1	2	3	4	5	Average
			Ethanol, ppm			
None	14	0	5	32	7	12
4	39	70	120	47	33	62
12	65	125	145	200	150	137
24	85	200	245	285	345	232

Summary of Table 12.

	F	Nec. F	
		0.05	0.01
Flood Period	17.53	3.49	5.95

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