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FLOODING-INDUCED SOIL AND PLANT ETHYLENE ACCUMULATION AND WATER STATUS RESPONSE OF FIELD-GROWN TOBACCO*

by P. G. HUNT, R. B. CAMPBELL, R. E. SOJKA and J. E. PARSONS**

KEY WORDS

C_2H_4 Eh Ethylene Flooding O_2 Redox potential Sandy soil

SUMMARY

Ethylene (C_2H_4) accumulation in flooded soil was related to oxygen (O_2), redox potential (Eh), and flooding rate. The water status response of tobacco (*Nicotiana, tabacum* L.) to these conditions was evaluated from stem diameter, relative water content, leaf water potential, and C_2H_4 content of leaf tissue. Treatments were: flooded with either 0, 5, or 15 cm of water per day for 6 days. By the third day, O_2 in the soil decreased to less than 9% in treatments flooded with 5 or 15 cm of water. When O_2 in the soil air was less than 9% and redox potential (Eh) was less than +150 mv, most of the soil air samples contained some C_2H_4 and 16% contained more than 6 ppm. Very little C_2H_4 was present in soil air when O_2 exceeded 9%. Tobacco leaf C_2H_4 peaked 3 days after flooding and then declined to the preflooding level a day later, one day ahead of the rapid increase in soil C_2H_4. Wilting developed progressively beginning with the rise of C_2H_4 in the soil; leaf water potential, stem diameter, and relative leaf water content all were decreased. Soil-and plant-produced C_2H_4 are suggested as factors in reducing root permeability and increasing resistance to water uptake by tobacco.

INTRODUCTION

In the Southeastern Coastal Plains of the United States, crops are exposed to both excessive soil water and drought due to a combination of erratic rainfall and low water retention of the predominant soil types[33]. Physical properties of soils from this region have been described by Campbell, Reicosky, and Doty[9]. In a study on a Varina loamy sand, Campbell and Phene[7] found that matric potentials of less than −85 mb were associated with low O_2 concentration in soil-air and reduction of millet (*Pennisetum glaucum* (L.) R. Br.) yield. Tobacco (*Nicotiana tabacum* L.), a crop more sensitive to low O_2 and high soil water than millet[24,25,26], is economically important in this physiographic region. Wilting has been shown to take place in the presence of optimal soil water when oxygen

* Contribution of the USDA-SEA/AR, in cooperation with the South Carolina Experiment Station.
** Soil Scientists and Mathematician, USDA-SEA/AR, Coastal Plains Soil and Water Conservation Research Center, Florence, SC 29502.

has been excluded, or under flooding per se. Campbell[5] reported that flue-cured tobacco yields were reduced 40% when tobacco was flooded for longer than 48 hours. Kramer and Jackson[26] reported that reduced soil O_2 levels and reduced permeability of root systems under flooding could not totally explain the injury of tobacco in flooded soils, and suggested the involvement of microorganisms in the damage. Other scientists have more recently suggested nutritional involvement[12,15,37]. They have shown sufficiently rapid nutritional concentration changes to account for some physiological responses. They indicated partial relief of stress symptoms with nitrate addition under partially flooded conditions. However, under totally flooded conditions nitrate addition to the soil did not prevent flooding damage.

Ethylene, a plant hormone, has been found in numerous soils around the world[10,35,36]. Ethylene production in Norfolk loamy sand was cited as the possible cause of damage to potato plants and tubers[6]. Laboratory studies showed C_2H_4 accumulations were associated with low O_2 and high soil water content in Norfolk loamy sand[16]. Soil C_2H_4 has also been reported to be translocated through the roots to the shoots of tomato plants and to cause epinastic growth when the soil concentration of C_2H_4 was greater than 2 ppm[17]. Their results suggest that flooding damage to sensitive plants can be caused by soil-produced C_2H_4. However, tomato plants can also produce C_2H_4 in shoots in response to stress, specifically when roots are exposed to low O_2 concentrations[19]. Bradford and Dilley[2] have substantiated that C_2H_4 is produced by tomato plants under flooded conditions and that this C_2H_4 caused epinastic growth. They also showed that silver nitrate, a C_2H_4 inhibitor, blocks the epinastic effect. These results suggest that both plant-produced and soil-produced C_2H_4 may be related to the devastating flooding damage to tobacco. We conducted a field study to more clearly define *in situ* (1) the extent of C_2H_4 production in relation to soil oxygen, redox potential (Eh), and flooding and (2) to evaluate leaf water potential, stem diameter and leaf relative water content of tobacco in relation to C_2H_4 and O_2 concentrations produced in time by flooding of soil.

MATERIALS AND METHODS

The field experiment consisted of two blocks that contained: nonflooded (F_0), intermittently flooded (F_1), and continuously flooded (F_2) treatments. During a 6-day flooding period, 5 cm of water was applied to F_1 each day at 0800. For the F_2 treatment, approximately 15 cm of water was applied each day to keep the surface continuously flooded. Each block contained four replicates each of F_1 and F_2, but only one F_0 treatment (extra F_1 and F_2 replicates resulted from pooling nonsignificant organic matter and nitrate treatments).

Tobacco, 'Coker 319', was transplanted on 28 June 1977 into 4.7 × 5.1 m plots in a Norfolk loamy sand soil (*Typic paleudults*). The plants were arranged in four rows spaced 1 m apart with 0.46 m between each of the 9 plants within a row.

Ethoprop and trifluralin as Mocab and Treflan* and a balanced fertilizer (5–10–15) were all applied at recommended rates and incorporated prior to planting. Plantings were later side dressed with 225 kg/ha of sodium nitrate. Carbaryl and methomyl as Sevin and Lannate were applied at recommended rates for insect control. Plants were sprinkler irrigated to avoid drought stress before flooding. Soil temperature was measured at the 10-cm depth with thermocouples and mercury thermometers.

On 14 September, plots were enclosed with small earthen dikes and flooded for 6 days with water applications of 30 and 90 cm for F_1 and F_2, respectively. Prior to flooding, gas exchange chambers[8] were placed at the 15-cm depth in four locations within each plot. Tubes connected to the chambers extended above the soil surface and were sealed with rubber septa. Gas samples were taken during the flooding period with gas-tight syringes. The O_2 concentration of gas samples taken from the gas exchange chambers was determined with a Cd–Au electrode. Ethylene was measured by flame ionization gas chromatography using an activated alumina column maintained at a temperature of 110°C. Bright platinum electrodes were placed at 15 cm depths in four locations near each of the four gas sampling chambers in each of the eight plots. Redox potentials (Eh) were then measured using the millivolt scale of an Orion pH meter and a standard calomel electrode.

The stems of two tobacco plants in two replications of each flooding treatment and in the control were instrumented with linear variable displacement transducers[32] to monitor stem diameter change. Incoming radiation was monitored with an Eppely pyranometer. Stem diameter and incoming radiation were logged at 20-min intervals on an automated data acquisition system. Soil matric potential was monitored with tensiometers. During flooding the most recently matured, fully expanded leaves were sampled two or three times per day from 1000 h to 1500 h to determine relative water content and leaf water potential. Relative water content was calculated as follows:

$$\%\,\mathrm{RWC} = \frac{\text{turgid wt} - \text{fresh wt}}{\text{turgid wt} - \text{dry wt}} \times 100$$

Leaf water potential was determined using a hydraulic leaf press[15,34] (estimates water potentials equivalent to pressure bomb derived leaf water potential value). Ethylene in plant tissue was determined by cutting ten, 8-mm diameter disks from the newest fully mature leaves and incubating them in sealed 37-ml bottles. The bottles were then sampled for C_2H_4 three times during incubation: immediately, at 24 hours, and at 48 hours.

RESULTS AND DISCUSSION

Prior to day 1, all plots were maintained under uniformly favorable soil water regimes. Solar radiation (Fig. 1) dropped from day 1 to day 3, then jumped significantly on days 4 and 5. Following day 5, radiation dropped again but remained above the level recorded on day 3 throughout the remainder of the study. Soil temperature at the 10-cm depth averaged 24.8°C.

By the fourth day of flooding, the soil-O_2 concentration declined to less than 9% in both the F_1 and F_2 flooding treatments (Fig. 2). The F_2 treatment had the

* Mention of product names is for description only, and not an endorsement by USDA-SEA-AR.

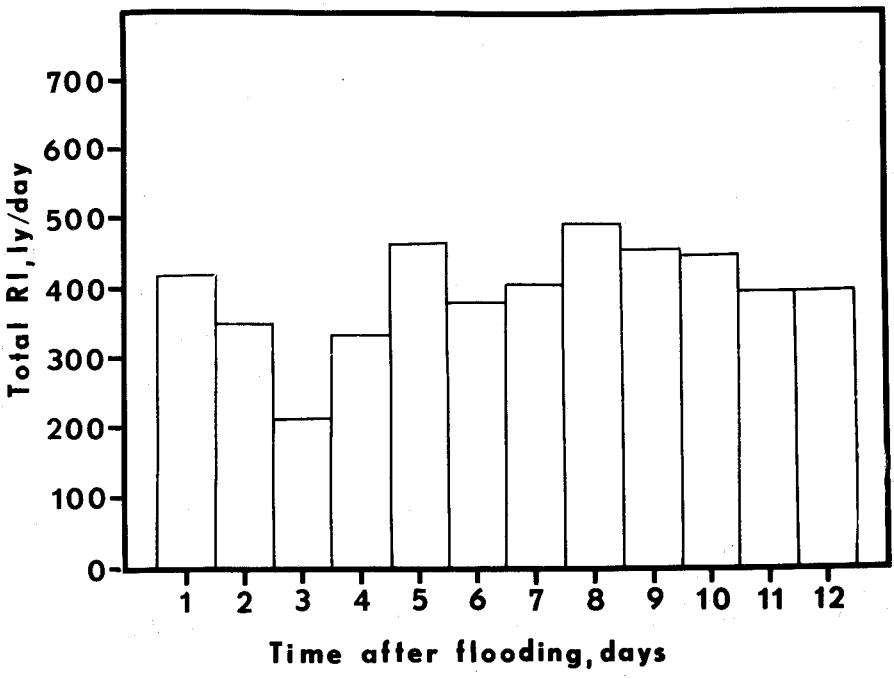

Fig. 1. Total solar radiation during the study.

lowest O_2 concentration and the highest C_2H_4 concentration. It should be noted that prior to the third day when soil O_2 was above 9%, C_2H_4 concentration was generally below 1 ppm.

When soil-air contained greater than 9% O_2, about 87% of the gas samples contained less than 1 ppm C_2H_4 (Fig. 3). When soil-air contained less than 9% O_2 and the Eh was greater than +150 mv, only 4% of the samples had less than 1 ppm C_2H_4; however, less than 5% of the samples had C_2H_4 concentrations greater than 6 ppm. With the most reduced boundary conditions (<9% O_2 and <150 mv Eh), about 16% of the samples had greater than 6 ppm C_2H_4. This is consistent with soil-air data of Campbell and Moreau[6], obtained from a potato experiment. It is also consistent with results of incubation studies using a similar soil[16].

The lack of C_2H_4 accumulation at greater than 9% soil O_2 is not likely due to diffusion loss to the atmosphere. Hunt *et al.*[16] found that when this soil was

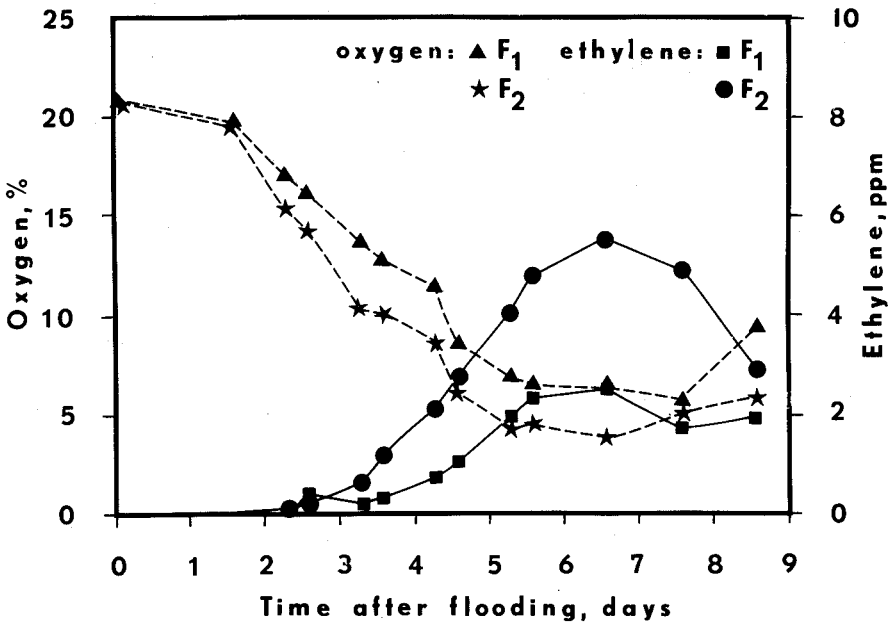

Fig. 2. Soil C_2H_4 and O_2 in a Norfolk loamy sand planted to tobacco.

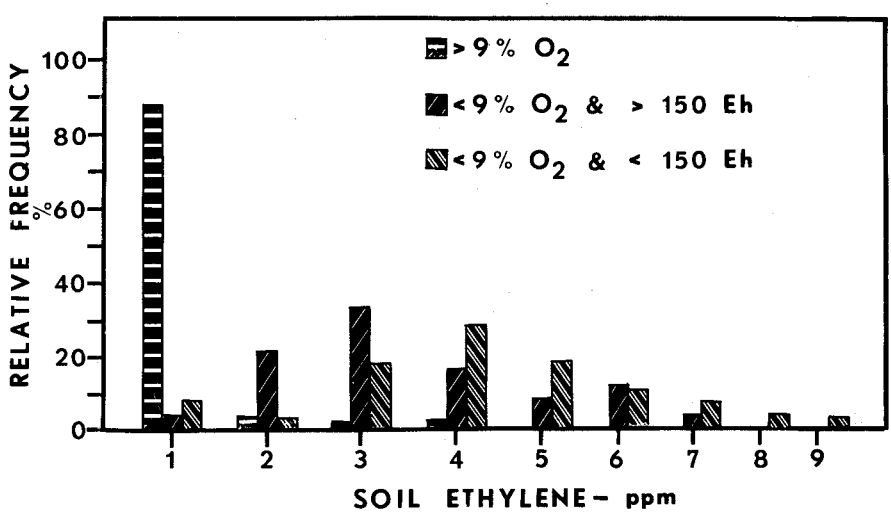

Fig. 3. Relative frequency of C_2H_4 concentrations with soil Eh and O_2 boundaries in a Norfolk loamy sand.

incubated in sealed containers C_2H_4 accumulated only when the O_2 concentrations in the containers were less than 1%. Cornforth[11] reported that in laboratory studies C_2H_4 decomposed much faster in aerobic than in anaerobic conditions. He also inferred that the anaerobic C_2H_4 production was not sufficient to allow significant accumulation of C_2H_4 in an aerobic atmosphere, because the rate of aerobic C_2H_4 decomposition in macropores was much more rapid.

Data from laboratory studies show that many Coastal Plain soils also accumulated C_2H_4 under water saturated conditions (unpublished data Hunt, *et al.*), and Cook and Smith[10] report significant C_2H_4 accumulations from soils at 0.5 bar metric potential as well as at saturation. While the highest concentrations of C_2H_4 in this experiment occurred under the most highly reduced conditions and little C_2H_4 accumulated when O_2 levels were above 9%, there was, nonetheless, significant accumulation of C_2H_4 in mildly reduced soil with O_2 concentrations between 5 and 10%. This experiment and earlier data by Campbell and Moreau[6] leave no doubt that C_2H_4 accumulates in Norfolk loamy sand '*in situ*' with only moderate suppression of soil macropore O_2 concentration. Campbell and

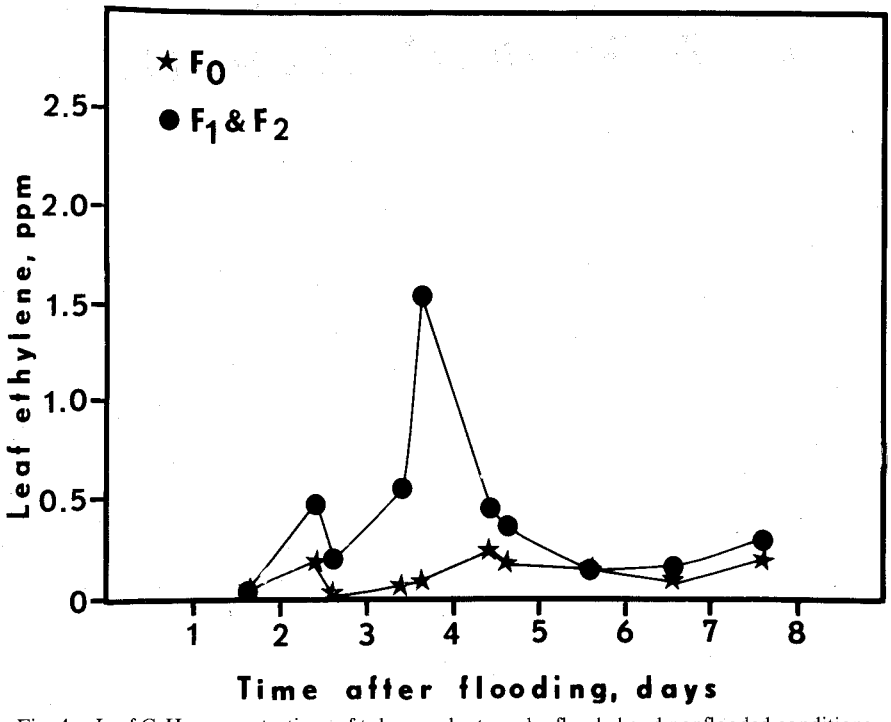

Fig. 4. Leaf C_2H_4 concentrations of tobacco plants under flooded and nonflooded conditions.

Phene[7] found that 10% soil O_2 concentration is associated with a soil water potential of less than −85 mb in the summer. Millet yields were reduced under these conditions. Since elevated C_2H_4 is also associated with this O_2 boundary, −85 mb soil water matric potential could probably be used as a management criterium for soils of this type in the summer.

While treatments F_1 and F_2 produced significantly different C_2H_4 concentrations in the soil, plant responses were not significantly different comparing the two flooded treatments. Both flooding (F_1 and F_2) treatments, however, did produce plant responses which differed significantly from the nonflooded (F_0) treatment (Fig. 4).

Soil and plant C_2H_4 levels are related to one another[17,18,19]. Sufficiently higher C_2H_4 concentrations may have existed in soil micropores at proximity to roots to explain the rise in leaf-C_2H_4 on day three by diffusive processes.

Regardless of whether C_2H_4 originated in the soil or in the plant itself, the plant water status was not immediately affected by the elevated leaf-C_2H_4.

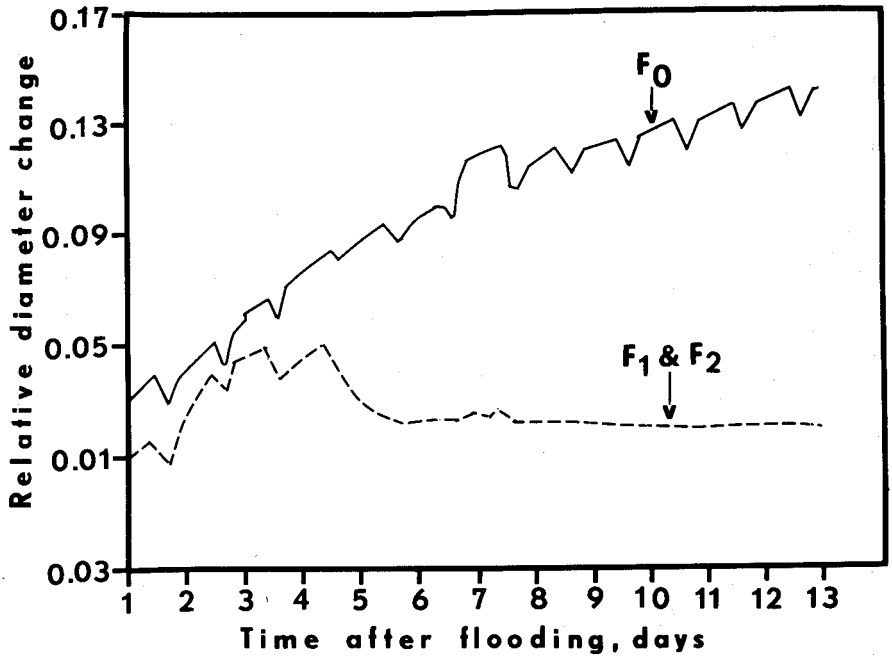

Fig. 5. Relative stem diameter change of flooded and nonflooded plants on several days following flooding.

Changes in plant water status were first observed on day 4 (1 day after C_2H_4 increased in the leaves) when soil-O_2 dropped below 9%, soil-C_2H_4 rose above 1.5 ppm, and leaf-C_2H_4 dropped to less than 0.5 ppm. This suggests that the major effects of low soil-O_2 and high soil-C_2H_4 on plant water status were associated with the root system and its membranes. All plants in flooded plots wilted significantly, whereas no plants wilted in the control plots.

Stem diameter (Fig. 5) increased uniformly throughout the study in nonflooded plots. In flooded plots, however, stem diameter peaked by day 4 and then declined rapidly, reaching an equilibrium diameter by day 6 that was insensitive to further diurnal environmental shifts in the flooded plots. This plateau in stem diameter occurred at a higher concentration of O_2 than reported by Willey[38] for tobacco grown in a mist-chamber. The difference could be due to the fact that lower O_2 and higher C_2H_4 occurs in soil micropores than is measured via the soil sampling tubes, which predominently reflect concentrations in the soil macropores. Trought and Drew[37] have also suggested that lower root surface O_2 concen-

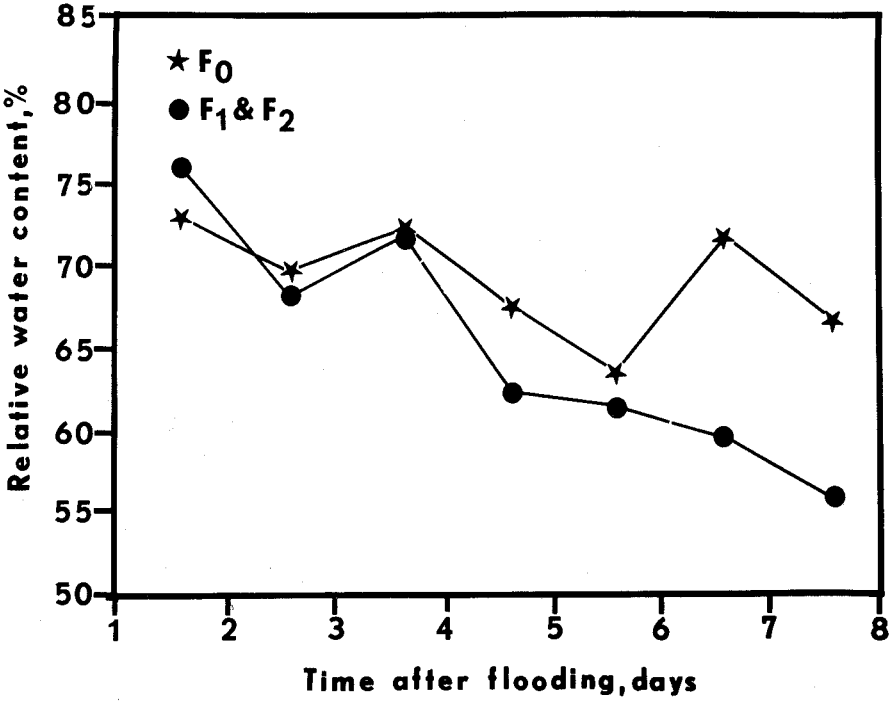

Fig. 6. Relative water content of flooded and nonflooded plants several days following flooding.

trations may be related to flooding symptoms prior to low bulk soil-O_2 concentrations as shown in their time course studies. Soil-O_2 concentrations measured in the soil gas sampling cups is likely higher than the actual soil-O_2 concentration near active root surfaces[27].

Decrease in stem diameter is associated under droughthy conditions with lowered plant water potentials[32], loss of turgidity, and wilting. Flooding caused a similar set of responses in this study. Both relative water content and pressure potentials of leaves dropped significantly in the flooded treatments (Fig. 6 and 7). Stem diameter change, relative water content, and leaf water potential declined even though the flooded plants had high root zone water potentials and low soil resistance to water movement.

Although rapid pathogenic infection could not be entirely ruled out, the observed reversal of normally expected plant water status in the presence of high root-zone water availability is probably best explained by an increase in the radial resistance to water flux in the roots of the flooded plants[25]. As explained by Elfving *et al.*[13], such an increase in root resistance would lower plant water

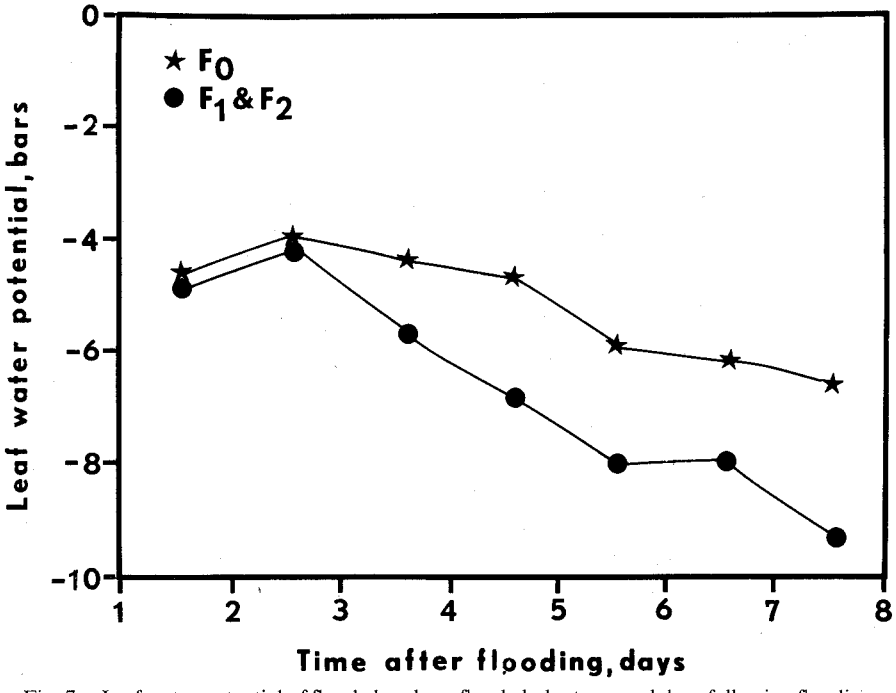

Fig. 7. Leaf water potential of flooded and nonflooded plants several days following flooding.

potentials and cause stomatal closure. Water potentials did drop in our experiment, and although we did not measure stomatal response, the dampening of stem diameter change in response to diurnal shifts in the aerial environment (See Fig. 5) suggests stomatal closure had probably occurred.

Arnold[1] pointed to the root endodermis as the most significant barrier to radial water flux into roots, due to the Casparian strip forcing all absorbed water to pass through plant protoplasts. Root resistance could increase if the membrane structure or function of the protoplasts were rendered less permeable to water.

Increase in root resistance to water flow have been observed or implied in various flooding and aeration studies and in experiments involving introduction of respiration inhibitors[1,3,4,23,24,25,28,29,31]. In our study, there was a marked coincidence between the onset of C_2H_4 production and the deterioration of plant water status in the flooded plots. Kramer and Jackson[26] found that tobacco wilting following flooding was reversed if soil was removed from near the roots to improve aeration.

Leaf yellowing, epinasty, and adventitious root development in numerous crops when flooded, have suggested the induction of bio-active chemicals in plants under flooded conditions[18,25]. This was early associated with similar responses produced by exposure to C_2H_4 gas[14,39], although Jackson[20] ascribed the direct cause of injury to aerial parts to decreased root water absorption. However, Kawase[21] concluded that, for sunflower, C_2H_4 played a significant direct role as well.

More recently, Jackson and Campbell[17] and Jackson *et al.*[19] established the source of C_2H_4 production as being both diffusion from soil to roots and self production within tomato plant roots. They further showed that substantial amounts of C_2H_4 moved from plant roots to shoots and that C_2H_4 accumulation was greatest in a partly anaerobic environment but dropped when a system becomes totally anaerobic.

One study of a synthetic lipid-protein system in which an egg lecithin-cholesterol bilayer membrane was used to separate electrolytes suggested C_2H_4 activity cannot be explained by its simple physical effects on membranes[30]. The results from the artificial system do not seem borne out, however, by observations of C_2H_4 effects on plasma membrane densities in kidney bean abscission zones[22]. In the latter study, a significant density increase could be attributed to C_2H_4 exposure. While the mode of action in the root endodermis is likely to be of a different nature than that observed for an abscission zone, their study identifies the distinct ability of C_2H_4 to alter membrane constitution in living plant tissues.

CONCLUSION

The wilting or 'flopping' of field-grown tobacco in response to flooding may be related to physiological changes in the root. Ethylene was strongly implicated as the cause of this physiological response. Flooding produces an environment favorable to C_2H_4 production in the soil and/or in roots by reducing the soil-O_2 concentration and Eh. Ethylene accumulated in soil gas samples when soil-O_2 was less than 9% even at greater than +150 mv Eh. At less than 9% soil-O_2 and Eh below +150 mv, soil-C_2H_4 accumulation is even greater.

Because the soil-gas sampling techniques used sample soil-air predominantly from the macropores of soil at some distance from active roots, it is not possible to exclude soil-produced C_2H_4 as the source of at least some of the observed leaf-C_2H_4 accumulation. Actively respiring roots may create a zone of decreased soil O_2 concentration near the root surface[27]. In this zone C_2H_4 might be produced and be available for diffusion into the plant. This would still not exclude the possibility of production of C_2H_4 in the plant root itself.

In this experiment, wilting in plant tops was associated with decreased stem diameter, lower plant water potentials, and lower leaf relative water contents. These plant responses, like wilting, are generally associated with soil water stress. Rapid pathogenic infection induced by soil-C_2H_4 could have been involved. However, our results are probably best explained by an increase in root resistance to water entry from the surrounding soil. Changes in cell membrane structure that could relate to such rapid increases in root resistance to water flux have been associated with plant response to C_2H_4.

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