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FACTORS AFFECTING ETHYLENE ACCUMULATION IN A NORFOLK SANDY LOAM SOIL[1]

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ABSTRACT

Laboratory incubation studies of a Norfolk sandy loam (Typic Paleudult), a soil typical of the southeastern Coastal Plains, were conducted to assess factors affecting ethylene accumulation. Soil from the Ap horizon accumulated more ethylene than soil from either the A2 or B horizon. In the presence of initially high oxygen and methionine, the Ap soil accumulated 35 nanograms of ethylene per gram of soil in 14 days. The same soil with methionine incubated under initial oxygen levels less than 10 percent, however, accumulated less than 5 nanograms per gram of ethylene in 14 days. When incubated under low (< 1 percent) initial oxygen concentrations, ethylene accumulation from soils from all three horizons was unaffected by the addition of methionine. Since methionine did not increase the ethylene concentration in soil with low oxygen concentrations, significant methionine-induced ethylene accumulation in the fields of Norfolk sandy loam does not appear probable. Unamended Ap soil incubated under high oxygen levels did not accumulate significant ethylene unless the water content was near saturation (> 28 percent water by weight). Adding finely ground Bermudagrass residue (0.5 to 2.0 percent) induced an increase in ethylene accumulation. The time required for peak ethylene accumulation decreased with increasing amounts of added plant residue. Nitrate, particularly at concentrations greater than 75 milligrams per kilogram, suppressed the accumulation of ethylene. The research suggests that ethylene accumulation in Norfolk sandy loam is enhanced by high moisture, low oxygen, and added organic matter, and is suppressed by high nitrate concentrations.

INTRODUCTION

Ethylene (C_2H_4) can have rather dramatic effects on such plant processes as maturation, and it has been used commercially for ripening fruit. Ethylene has also been used in the United States to control witchweed (Abeles 1973; Eplee 1975). When C_2H_4 is injected into the soil, it causes the premature germination of the witchweed seed, an event that normally occurs only in the presence of certain hosts, such as corn. Freytag, Wendt, and Lira (1972) injected C_2H_4 into soils on which sorghum and cotton were grown to hasten maturity and increase productivity; the approach was not uniformly successful.

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The natural occurrence of C_2H_4 in soil has been shown in several parts of the world: in England by Smith and Russell (1969), in Australia by Smith (1973), in the northwest United States by Cook and Smith (1977), and in the eastern Coastal Plains of the United States by Campbell and Moreau (1979). Generally, the presence of C_2H_4 in soil has been associated with low soil oxygen (O_2) levels, warm temperatures, and organic substrates (Smith and Russell 1969; Smith and Dowdell 1974; Cornforth 1975; Cook and Smith 1977). Inorganic substances, such as nitrate, and physicochemical conditions, such as redox potential (Eh) and pH, may affect ethylene production. Smith (1976) discussed the effect of these and other factors on soil C_2H_4 in a review on C_2H_4 in soil biology.

Campbell and Moreau (1979) have indicated possible ethylene damage to potatoes in Coastal Plains soils. The concentration of ethylene they found in the Norfolk sandy loam soil (Typic Paleudult) was greater than that which would cause epinastic growth in tomatoes (> 2 ppm). Because C_2H_4 produced in the soil could be culturally significant to tomatoes and many other sensitive plants (Smith and Robertson 1971), the factors governing its production in soils need to be better understood.

Unfortunately, the mode of C_2H_4 production in the soil is not well understood, and available explanations of the processes are not agreed upon. Some authors believe that production in the soil is an anaerobic, spore-forming bacterial process (Smith 1973; Smith and Cook 1974; Cook and Smith 1977). Others have indicated that ethylene can be produced aerobically by soil fungi and bacteria with the addition of methionine (Lynch and Harper 1974; Lynch 1975; Primrose 1976 and 1977). Lynch (1975) suggests that the substrate for C_2H_4 production may be produced in anaerobic microsites, but the C_2H_4 production may actually occur in aerobic microsites. We conducted this study to determine more precisely factors affecting ethylene accumulation in a Norfolk sandy loam as affected by aerobic and anaerobic conditions in the presence and absence of certain C_2H_4 stimulating and inhibiting substances.

MATERIALS AND METHODS

Five incubation experiments were conducted to evaluate the effects of glucose, methionine, nitrate, and grass residues under anaerobic and aerobic conditions. The soil used in the study was a Norfolk sandy loam composed of 75 percent sand, 22 percent silt, and 3 percent clay. This is a moderately well-drained soil common to the southeastern Coastal Plains. The soil samples were air-dried and sieved through a 2-mm space sieve. The organic matter content was approximately 1.3 percent, and the soil pH was 5.2. Soil water contents corresponding to varying water potential levels were determined on duplicate samples subjected to differential pressures across a permeable ceramic membrane in pressure chambers (Richards 1948). Samples for incubation were placed in 37-ml bottles and capped with rubber septa. We sampled the atmosphere of the bottles with a syringe and analyzed the gas for oxygen (O_2) by passing gas samples through a cuvette supporting an O_2 sensor with a Cd-Au electrode. Ethylene was measured on a Hewlett Packard Model 5075 gas chromatograph, GC, with flame ionization detector.[3] The GC column was packed with activated alumina 70/80 mesh (Analabs, Inc.), and the oven temperature was maintained at 110°C.

In experiment 1, 10-g soil samples from the Ap and A2 soils were amended with 2.5 ml of 0.5 percent glucose and methionine solution, and the B soil received 3.5 ml of the same solution. This brought the two soils to the same matric potential due to differences in soil textures. The soils were incubated in gaseous atmospheres containing about 0, 1, 5, 10, and 20 percent O_2. To assess the influence of glucose and methionine, separate Ap soil samples were amended with 3.5 ml of 0.5 percent glucose, 0.5 percent methionine, 0.5 percent glucose plus 0.5 percent methionine, or water. They were compared for C_2H_4 accumulation after being incubated under gaseous atmospheres initially containing 0 or 20 percent O_2. To assess the influence of biological components on C_2H_4 accumulation, separate samples of Ap soils similarly amended with glucose or methionine or water were incubated under atmospheres of 0 or 20 percent initial O_2 atmospheres after autoclaving at 105°C. The O_2 contents were obtained by flushing the air space in the incubation bottles with nitrogen (N_2) and then replacing part of the N_2 with air to obtain the desired O_2 percentages. Then the sample bottles were incubated 14 days at 24°C before gas samples were analyzed for O_2 and C_2H_4.

In experiment 2, 10-g soil samples taken from the same horizons were incubated at saturation water content under the same O_2 contents as in experiment 1, but without methionine and glucose amendments. Again, gas samples from the bottles were analyzed for O_2 and C_2H_4 after a 14-day incubation period at 24°C.

Soil water content and initial O_2 were selected as the treatment variables in experiment 3. Water was added in small increments to adjust the soil water content to 35, 30, 25, 20, 15, and 3 percent (air-dry) in triplicate. The samples were incubated at 1 and 20 percent initial O_2 for 14 days before O_2 and C_2H_4 of the atmosphere were determined.

In experiment 4, 20-g samples of Ap soil were amended with finely ground residue of Bermudagrass (*Cynodon dactylon* L.) at rates of 0.0,

[3] Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment by the USDA.

0.5, 1.0, 1.5, and 2.0 percent organic matter by weight. The soil-grass mixtures were adjusted to 28 percent water and incubated at 24°C, and the atmosphere was analyzed for C_2H_4 and O_2 after 1, 4, 11, 19, and 24 days of incubation.

In experiment 5, 20-g samples of Ap horizon soil were amended with 1 percent finely ground Bermudagrass as an organic matter supplement, and 0, 25, 50, 75, and 100 ppm nitrate-N ($NaNO_3$) additions were the treatment variables. The incubation water content was 28 percent, and the incubation temperature was 24°C. The gas space was sampled and analyzed for O_2 and C_2H_4 over a 15-day period.

RESULTS AND DISCUSSION

In experiment 1, considerably more C_2H_4 accumulated in the bottles containing the Ap horizon soil than in either the A2 or B horizon soil (Table 1). This was particularly true for the bottles with an initial O_2 level of 20 percent. As all the soils were amended with both glucose and methionine, the difference was probably not directly attributable to organic substrate. It might be related, however, to the level of initial microbial population, which would have been higher in the Ap horizon soil because of its initially higher organic content. The difference in the A2 and B horizon soils under 20 percent initial O_2 can probably be explained by similar reasoning; the difference was much smaller, however. Lynch (1975) and Primrose (1976) reported that ethylene production was stimulated greatly by the presence of methionine under aerobic conditions. Increasing the O_2 content from 10 to 20 percent increased the ethylene accumulation more than ninefold in the Ap horizon soil. There were only very slight differences among the 0, 1, 5, and 10 percent initial O_2 level treatments, and the same relationship held for the Ap, A2, and B horizon soils. The magnitude of ethylene production under the 20 percent O_2 treatment, however, was lower for the A2 and B horizon soils. In a subexperiment (Table 2) with aerobic conditions, the addition of glucose alone only slightly increased ethylene production, but it caused a synergistic response with methionine. Methionine was responsible for the majority of the increases in ethylene production under aerobic conditions. Under anaerobic conditions, however, methionine was no more effective than the glucose in stimulating C_2H_4 production. In addition to being known as a precursor for C_2H_4, methionine could be stimulatory through microbial selection, as it is toxic to certain pure cultures of microorganisms at levels above 0.1 percent.[4] The biological nature of the C_2H_4 production was supported by data from another subexperiment where only 0.2 and 7.0 ng C_2H_4 per gram of soil were produced from autoclaved soil under anaerobic and aerobic conditions, respectively, while 2.2 and 37.5 ng C_2H_4 per gram of soil were produced from nonautoclaved soil under anaerobic and aerobic conditions, respectively.

Results of experiment 2 (Table 3) show that the unamended Ap horizon soil accumulated about 2.2 ng C_2H_4 per gram of soil when initial O_2 was 1 percent. This C_2H_4 level was essentially equal to that which the Ap soil accumulated under 0, 1, and 5 percent O_2 treatments amended with glucose and methionine. Ethylene accumulation differences in the 0 and 1 percent initial O_2 treatments were not significant. In contrast to the methionine-amended soil, however, the unamended soil accumulated less than 0.5 ng C_2H_4 per gram of soil when more than 1 percent O_2 was initially added. Since the final O_2 levels in the 5, 10, and 20 percent initial O_2 treatments were above 1 percent, it is likely that they simply did not become anaerobic enough for significant accumulation of C_2H_4. Thus, experiment 2 showed that C_2H_4 accumulated only when O_2 levels in the soil air space were very low in the absence of methionine. These findings agree with those of Campbell and Hunt,[5] who found

TABLE 1

Ethylene from 0.5% glucose and methionine amended soil of different horizons of a Norfolk sandy loam soil after 14 days incubation at 24°C (Exp. 1)

Initial O_2[a] %	Horizon		
	Ap	A_2	B
	C_2H_4, ng/g		
0	2.20	0.35	0.48
1	2.38	0.40	0.48
5	2.60	0.66	0.92
10	3.92	2.33	2.60
20	35.55	12.36	7.96
$LSD_{(0.05)}$	0.57	0.38	0.54
$LSD_{(0.01)}$	0.77	0.53	0.74

[a] Final O_2 values were all < 2.0 percent.

[4] J. W. Doran, USDA-SEA, University of Nebraska, Lincoln, Nebraska, personal communications.

[5] R. B. Campbell and P. G. Hunt, unpublished data, USDA-SEA, Florence, South Carolina.

that ethylene was not formed in field sites of Norfolk sandy loam unless the O_2 concentration in the soil atmosphere was less than 10 percent. Campbell and Phene (1977) have also shown that soil O_2 levels below 10 percent were associated with soil matrix potentials greater than 80 millibars and reduced yield of millet on a similar soil.

Experimental data of experiment 3 (Table 4) summarize the effect of soil water content (0 to 15,000 millibars) and atmospheric composition on C_2H_4 accumulation. When air containing 20 percent O_2 was initially used, ethylene was very low in the pores of the soils containing less than 30 percent moisture by weight. When the O_2 concentration was initially very low, however, there was not a significant difference in ethylene production among soils ranging from 15 to 35 percent moisture content. These responses are in agreement with our observation of O_2-related C_2H_4 accumulation under soil conditions mentioned earlier and with those reported by Cook and Smith (1977). Thus, the behavior of Norfolk sandy loam of the southeastern Coastal Plains is similar to soils in other parts of the world in C_2H_4 accumulation as related to O_2 and water content of soil.

TABLE 2

Ethylene from an amended Norfolk sandy loam after 14 days incubation at 24°C

Amendment	Initial O_2, % 0	20
	C_2H_4, ng/g	
Control	1.98	0.09
Glucose	2.77	3.04
Methionine	2.07	18.70
Glucose & methionine	2.33	33.18
$LSD_{(0.05)}$ = 0.56		
$LSD_{(0.01)}$ = 0.92		

TABLE 4

Ethylene accumulated after 14 days at 24°C from a Norfolk sandy loam at varying water content and initial O_2 levels (Exp. 3)

Soil water, %	Matric potential, mb[a]	Initial O_2 1%	20%
		C_2H_4, ng/g	
Air-dry	15000	0.18	0.09
15	110	2.16	0.00
20	50	2.07	0.00
25	20	2.21	0.13
30	0	1.94	1.58
35	0	2.25	1.50
$LSD_{(0.05)}$ = 0.17			
$LSD_{(0.01)}$ = 0.23			

[a] Estimated from a soil water-matric potential curve for disturbed samples.

The data from experiments 1, 2, and 3 suggest that two processes may be involved in C_2H_4 accumulation: an aerobic process stimulated greatly by methionine and glucose, and an anaerobic process stimulated very little by methionine and glucose. Thus, these findings support both the opinions of Cook and Smith (1977), and those of Lynch and Harper (1974) and Primrose (1976). Simply stated, when the soils are amended with methionine and kept aerobic, they produce C_2H_4. When they are incubated under low O_2 conditions, either with or without methionine, they produce ethylene. In terms of agricultural significance, most field production of C_2H_4 in soils is related to low levels of soil atmospheric O_2 and high soil water content. It is, therefore, our conclusion from these data that ethylene production in soils, mediated by aerobic conditions and methionine, is not likely to be an agriculturally important process. As Lynch (1975) and Lynch and Harper (1974) have pointed out, however, there is the possibility

TABLE 3

Ethylene accumulated from different horizons of a nonamended Norfolk sandy loam soil after 14 days incubation at 24°C (Exp. 2)

Initial O_2, %	Ap	A_2	B	Ap	A_2	B
		Horizons				
		Final O_2, %			C_2H_4, ng/g	
0	2	2	2	2.16	0.31	0.20
1	1	2	2	2.20	0.26	0.35
5	3	7	6	0.13	0.13	0.26
10	7	11	12	0.40	0.13	0.18
20	15	20	20	0.18	0.13	0.13
$LSD_{(0.05)}$				0.57	0.38	0.54
$LSD_{(0.01)}$				0.77	0.53	0.74

that the methionine or its precursor is provided in some way from the anaerobic microsites to the aerobic microsites where it is converted to ethylene.

Experiment 4 shows how additions of plant residue affect C_2H_4 accumulation in this soil. During the first 12 h, C_2H_4 accumulations increased with each increment of added organic matter (Table 5). By day 4, C_2H_4 accumulation in the 2 percent organic matter treatment had peaked, and the 1.5 percent treatment had the highest C_2H_4 level. In 11 days C_2H_4 accumulation in the 1.0, 1.5, and 2.0 percent residue treatments were decreasing, and the 0.5 percent residue treatment had the highest concentration of C_2H_4. Soil without added residue did not exceed any residue-treated soil in ethylene concentration at any time. On days 19 and 24, all residue treatments still contained substantial amounts of C_2H_4. This result indicates that ethylene could accumulate in soils at higher levels when high levels of crop residue were produced, and it could be an important aspect of crop residue management on soils that were exposed to excess water.

Data in Table 6 indicate that the C_2H_4 accumulation processes in the Norfolk sandy loam soil are depressed by nitrate, and Smith (1976) has referred to observation of a similar response to nitrate in soils from other parts of the world. Through day 2, no nitrate-N treatment significantly suppressed C_2H_4 accumulation. By day 7, however, when C_2H_4 accumulation was nearly 10 times as great as on day 2, all nitrate treatments suppressed C_2H_4 accumulation as compared with the control. As would be expected, as time passed and denitrification proceeded, the effect of nitrate diminished. By day 12, there was no significant difference among the nitrate treatments and control. These findings differ from those of Goodlass and Smith (1978), who reported that nitrate at levels below 500 mg/kg did not suppress C_2H_4 accumulation.

We know that ethylene production is, in some way, related to reduced O_2 levels and reduced Eh conditions in the soil, and that nitrate poises

TABLE 5

Ethylene accumulation from a Norfolk sandy loam soil amended with Bermudagrass residue and incubated at 24°C (Exp. 4)

Residue, %	Time, days				
	0.5	4	11	19	24
			C_2H_4, ng/g		
0.0	0.27	0.31	0.84	1.45	0.77
0.5	0.61	2.70	3.56	2.49	2.90
1.0	1.38	5.80	2.15	2.18	1.56
1.5	1.76	8.44	2.15	2.36	1.72
2.0	4.71	7.52	2.06	2.33	1.08
$LSD_{(0.05)}$	0.50	1.22	0.34	0.65	0.35
$LSD_{(0.01)}$	0.68	1.65	0.46	0.84	0.47

TABLE 6

Ethylene accumulated from a Norfolk sandy loam amended with 1% Bermudagrass and various amounts of nitrate and incubated at 24°C (Exp. 5)

Nitrate-N, mg/kg	Time, days[a]				
	1	2	7	9	12
			C_2H_4, ng/g		
0	0.75	1.58	11.62	13.20	5.85
25	0.88	1.36	4.60	8.80	8.58
50	0.37	1.21	3.81	5.94	5.48
75	1.28	1.12	4.14	3.08	4.14
100	0.92	0.73	3.23	4.18	3.23
$LSD_{(0.05)}$	NS[b]	1.00	2.41	NS	NS
$LSD_{(0.01)}$		1.45	3.51		

[a] = O_2 levels were below 2 percent after day 1.

[b] = not significantly different at the 0.05 level by the F-test.

soil Eh at a relatively mild level of reduction. It would, therefore, be reasonable to postulate that nitrate affects C_2H_4 production through an effect on Eh level. Soils differ in their rate of Eh change due to many reasons, such as NO_3, Mn, Fe, and organic contents. The difference in ethylene production caused by nitrate could be related to these differences in redox potential.

In summary, the Norfolk sandy loam used in this study accumulates C_2H_4 aerobically with methionine amendments. It accumulated C_2H_4 without methionine additions under low O_2 conditions. Ethylene accumulation under normal atmospheric O_2 content is restricted to soils near water saturation content. Ethylene accumulation is enhanced by the addition of Bermudagrass residue and suppressed by addition of nitrate-nitrogen. Accordingly, this soil has the potential of accumulating levels of ethylene that can be deleterious to sensitive crops. More research is needed, however, before a definitive statement can be made on the actual extent to which C_2H_4 is involved in moisture damage to crops grown on this soil.

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