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Carbon Dioxide Effects on Ethanol Production, Pyruvate Decarboxylase, and Alcohol Dehydrogenase Activities in Anaerobic Sweet Potato Roots[1]

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LING A. CHANG[2], LARRY K. HAMMETT, AND DAVID M. PHARR
Department of Horticultural Science, North Carolina State University, Raleigh, North Carolina 27650

ABSTRACT

The effect of varied anaerobic atmospheres on the metabolism of sweet potato (*Ipomoea batatas* [L.] Lam.) roots was studied. The internal gas atmospheres of storage roots changed rapidly when the roots were submerged under water. O_2 and N_2 gases disappeared quickly and were replaced by CO_2. There were no appreciable differences in gas composition among the four cultivars that were studied. Under different anaerobic conditions, ethanol concentration in the roots was highest in a CO_2 environment, followed by submergence and a N_2 environment in all the cultivars except one. A positive relationship was found between ethanol production and pyruvate decarboxylase activity from both 100% CO_2-treated and 100% N_2-treated roots. CO_2 atmospheres also resulted in higher pyruvate decarboxylase activity than did N_2 atmospheres. Concentrations of CO_2 were higher within anaerobic roots than those in the ambient anaerobic atmosphere. The level of pyruvate decarboxylase and ethanol in anaerobic roots was proportional to the ambient CO_2 concentration. The measurable activity of pyruvate decarboxylase that was present in the roots was about 100 times less than that of alcohol dehydrogenase. Considering these observations, it is suggested that the rate-limiting enzyme for ethanol biosynthesis in sweet potato storage roots under anoxia is likely to be pyruvate decarboxylase rather than alcohol dehydrogenase.

If excessive soil moisture occurs prior to harvest of sweet potato storage roots, it will cause roots to decay in the field as well as adversely affect the quality and storage life of the roots (10, 15). In flooded soil, O_2 exchange between the soil and the air is reduced about 10,000 times compared to diffusion in gas-filled pores (6). O_2, within flooded soils, may be depleted within 24 h (14). N_2 gas concentrations remain fairly constant in the soil with some fluctuation throughout the flooded period (14). CO_2, the byproduct of respiration, accumulates in flooded soils. The partial pressure of CO_2 in flooded soils ranges from 0.2 to 0.8 atm within 1 to 3 weeks of flooding depending on soil properties and temperatures (3). Likewise, the internal gas atmospheres of sweet potato roots displayed a similar pattern, except that N_2 was replaced gradually by CO_2 during a 72-h submergence period (1).

In many higher plants, pyruvate is converted to ethanol via PDC[3] and ADH under anoxia. High concentrations of ethanol and high activities of ADH have been found under anoxic conditions in species and/or genera known to be susceptible to wet soil (4). However, other lines of evidence support a reverse relationship (16). Higher levels of PDC and ADH were found in anaerobically treated plants than in aerobic controls (8, 16). A recent study indicated the apparent activities of PDC, ADH, and ethanol concentration in sweet potato roots were higher in submerged roots than in aerobically held roots (2).

This paper reports on the effect of different anaerobic environments, particularly that of CO_2, on the apparent activities of PDC and ADH and the production of ethanol in sweet potato storage roots.

MATERIALS AND METHODS

Plant Material. Transplants of two flood-tolerant sweet potato (*Ipomoea batatas* [L.] Lam.) cultivars, Centennial and Jasper, and two flood-susceptible cultivars, Caromex and Jewel, were planted in Orangeburg loamy sand soil at the Horticultural Crops Research Station, Clinton, NC.[4] The roots were harvested 126 d after planting. All the cultural and postharvest handling practices followed recommended procedures (17).

Changes in Internal Gas Atmosphere of Submerged Roots. Ten freshly harvested roots of each of four cultivars were submerged in water-filled buckets at 22 ± 1°C to simulate anaerobic conditions encountered in flooded fields. Control (aerobic) roots were placed in open paper bags alongside the buckets. CO_2, N_2, and O_2 concentrations were measured on samples of two roots (no replication) at 0, 5, 11, 24 and 48 h after submergence following the methods described by Ahn *et al.* (1).

Effect of Different Anaerobic Environments on Ethanol Production. Four freshly harvested roots were placed in a 4-L glass jar and filled either with 100% CO_2 or 100% N_2 in a closed system or with water or air in an open system. There were four replications per cultivar and four cultivars for each treatment. For the closed system, each jar was exchanged with fresh CO_2 or N_2 after 24-h incubation in order to maintain the stated environment. At the end of 48 h, ethanol concentration in the roots was determined.

Effect of CO_2 and N_2 Environments on PDC, ADH Activities, and Ethanol Production. Cured roots of 'Jewel' were gassed in a

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[3] Abbreviations: PDC, pyruvate decarboxylase; ADH, alcohol dehydrogenase.

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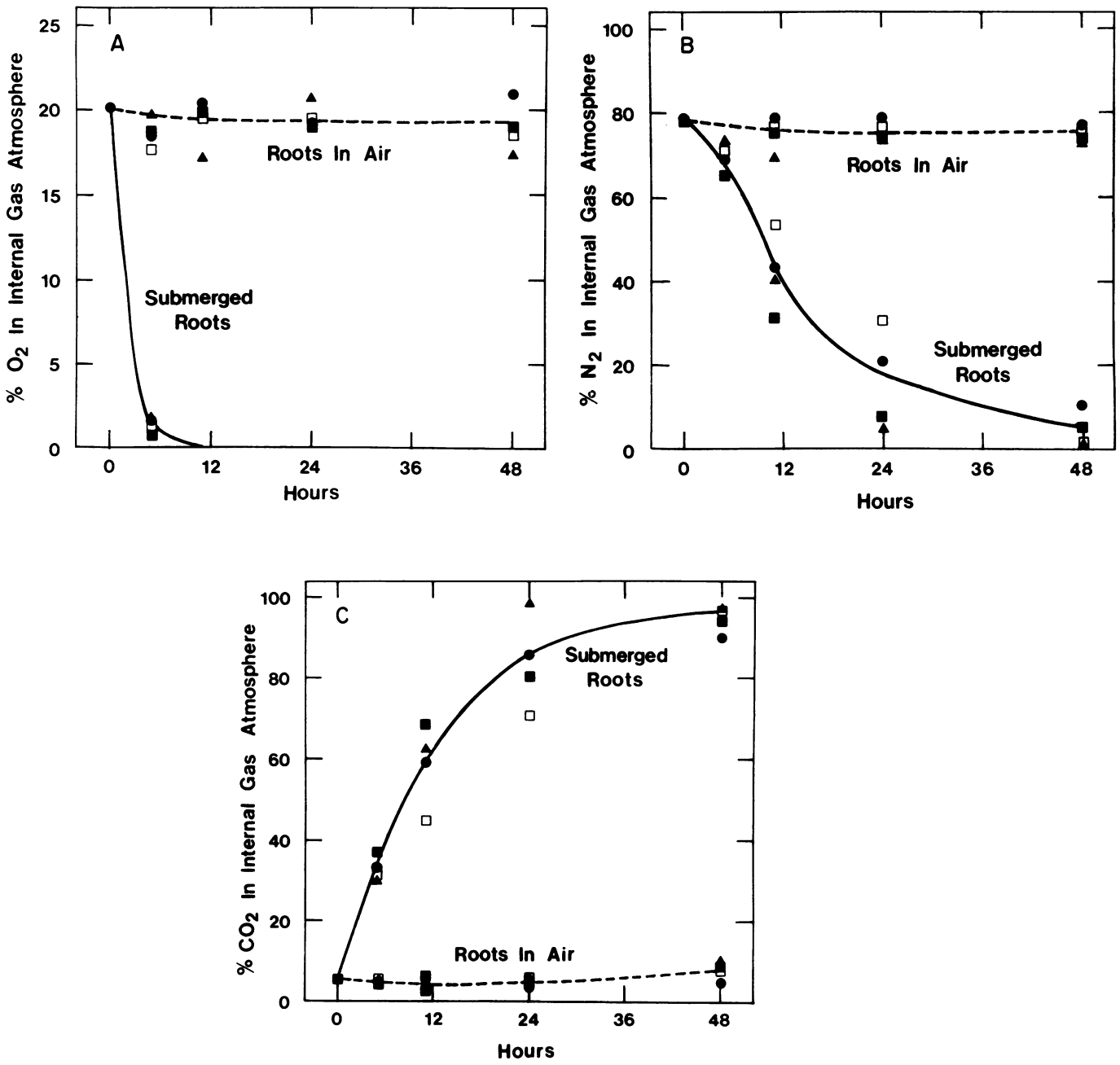

FIG. 1. Changes in internal O_2 (A), N_2 (B), and CO_2 (C) gas concentrations in storage roots of four sweet potato cultivars when held submerged under water for 48 h at 22°C. The following cultivars were used: (▲), Caromex; (□), Jewel; (●), Centennial; (■), Jasper.

4-L glass jar with 100% CO_2, 100% N_2, or air at a flow rate of 50 ml/min. At time 0, 24, and 48 h during the gassing period, three roots were taken from each treatment to determine the levels of ethanol, PDC, and ADH. The experiment was done three times.

Effect of CO_2 Concentration on the Internal Gas Atmosphere, Ethanol Production, and PDC Activity. Eleven roots of cured 'Jewel' were placed in a 20-L glass jar and gassed either with 0% CO_2, 100% N_2; 50% CO_2, 50% N_2; or 100% CO_2, 0% N_2 at a flow rate of 100 ml/min. At the end of 48 h, two roots were removed from each jar for the determination of internal CO_2 and N_2 concentrations. The remaining nine roots (three roots per sample) were used to estimate tissue ethanol concentration and PDC activity.

Extraction and Assay for Ethanol, PDC, and ADH. The procedures for ethanol, PDC, and ADH determinations were the same as described in an earlier paper (2). The enzyme activities were expressed as units per g fresh weight where a unit is defined as the amount of enzyme necessary to produce 1 μmol product per min.

RESULTS

Internal gas concentrations of freshly harvested sweet potato roots submerged in water exhibited a similar pattern as previously observed for cured roots (1). O_2 gas concentration decreased rapidly within the first 5 h and became undetectable after 11-h submergence (Fig. 1A). N_2 gas concentration dropped at a slower rate and was nominal by the end of 48 h (Fig. 1B). Within 11 h, streams of bubbles were noted from the surface of the submerged roots. The internal gas atmosphere was replaced by CO_2 and the submerged roots were nearly saturated with CO_2 by the end of 48 h (Fig. 1C). Roots held in air changed little in internal gas

Table I. *Ethanol Accumulation in Storage Roots of Four Sweet Potato Cultivars*

Ethanol was determined following exposure of the roots to an atmosphere of 100% CO_2 or 100% N_2, or following submergence under water for 48 h at 22°C.

Cultivar	Ethanol Content			
	Control	Submergence	CO_2 Treatment	N_2 Treatment
		μmol/g fresh wt		
Centennial	0.22	41.92	70.52	33.38
Jasper	0.24	61.39	72.86	35.90
Jewel	0.27	61.86	73.96	23.53
Caromex	0.22	75.71	72.90	30.83

$LSD_{(0.05)} = 3.33$

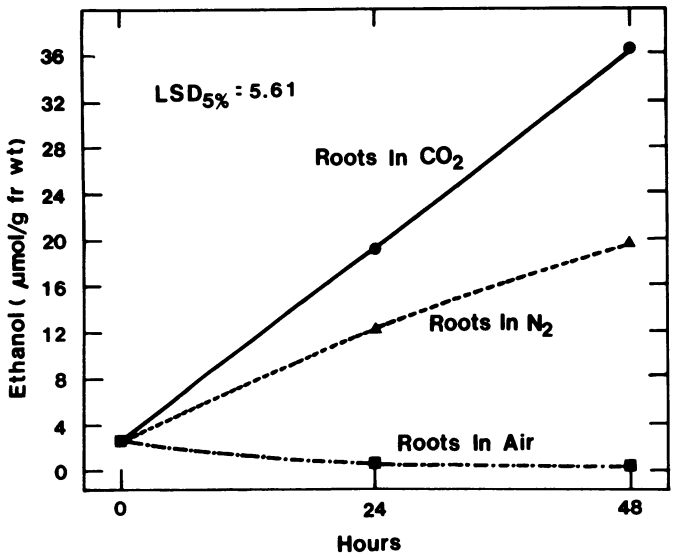

FIG. 2. Effect of 100% CO_2 or 100% N_2 on ethanol production during a 48-h exposure at 22°C by sweet potato roots of 'Jewel'.

Table II. *Effect of CO_2 or N_2 on Sweet Potato Roots of 'Jewel'*

PDC and ADH activities were determined during a 48-h exposure to 100% CO_2 or 100% N_2 at 22°C.

Exposure Time and Condition	PDC	ADH
	units/g fresh wt	
0 h	0.020	5.14
24-h Air	0.023	6.29
24-h N_2	0.033	7.57
24-h CO_2	0.040	6.50
48-h Air	0.027	7.02
48-h N_2	0.050	10.68
48-h CO_2	0.093	9.07
$LSD_{(0.05)}$	0.017	2.22

composition.

Ethanol concentration was highest in roots held in 100% CO_2 gas in all the cultivars except in Caromex (Table I). Submerged roots contained the second highest concentration of ethanol, followed by 100% N_2-treated roots. Very low concentrations of ethanol were found in air-control roots. Differences in ethanol content were greater among cultivars in roots which were submerged than in roots from the CO_2 or N_2 treatments. In roots which had been submerged, there was twice the concentration of ethanol present in the most susceptible cultivar Caromex as in the tolerant cultivar Centennial. These differences among cultivars were less obvious in CO_2- or N_2-treated roots.

Table III. *Effect of Ambient CO_2 Concentrations on Sweet Potato Storage Roots of 'Jewel'*

Internal CO_2 concentration, ethanol production, and PDC activity were determined following a 48-h exposure of the roots to a 0%, 50%, or 100% CO_2 environment with N_2 as the balancing gas at 22°C.

Ambient CO_2 Concn.	Internal CO_2 Concn.	Ethanol	PDC
%		*μmol/g fresh wt*	*units/g fresh wt*
0	12.0	19.03	0.021
50	73.2	31.76	0.092
100	95.8	52.96	0.140
$LSD_{(0.05)}$	1.0	5.66	0.033

Accumulation of ethanol in roots increased as anaerobiosis continued (Fig. 2), and the accumulation was about twice as high in CO_2-treated roots as in N_2-treated roots at the end of a 48-h gassing period. PDC activity followed a similar pattern under the different gas environments (Table II). ADH activity was found to be approximately two orders of magnitude higher than PDC activity in sweet potato roots (Table II). The activity of ADH did increase under anaerobic conditions as has been reported for other species (8, 16). Roots held in CO_2 gas tended to have lower levels of ADH than those held in N_2 gas, although these apparent differences were not statistically significant.

The internal CO_2 concentration in roots was about 12% and 23% higher than the imposed 0% and 50% ambient CO_2 environments and 5% lower in 100% CO_2 ambient (which may have been due to some contamination during the extraction) (Table III). Other than CO_2, the balancing internal gas atmosphere was N_2 (data not shown). A positive relationship was found between the amount of ethanol formed and PDC activity as both increased when the ambient CO_2 concentration increased (Table III).

DISCUSSION

The internal gas atmosphere of submerged sweet potato roots was replaced rapidly by CO_2. The internal CO_2 concentration of the roots was found to be higher than the ambient CO_2 atmosphere under anaerobiosis. This is also true for the less aerated parts of many plants when in a normal aerobic environment (11). These observations are important as CO_2-enriched anaerobic environments were found to cause more ethanol to accumulate in sweet potato roots than other imposed anaerobic conditions, and the effect was proportional to the ambient CO_2 concentration (Tables I and III; Fig. 2). Therefore, high concentrations of CO_2 found in the internal atmosphere of submerged sweet potato roots and in the gas atmosphere of flooded soils (3, 14) could be of considerable physiological importance in flood damage to sweet potatoes.

In parallel with ethanol levels, PDC activity was also found at higher activity in CO_2-treated sweet potato roots. PDC was present at rate-limiting levels relative to ADH, and thus changes in PDC activity might be expected to modulate the flux of carbon toward ethanol under anaerobic conditions. PDC exhibits several characteristics which suggest that it may be under fine control *in situ*. The saturation curve for pyruvate is sigmoidal (7). The sweet potato enzyme is optimally active between pH 6.1 and 6.6, with activity falling rapidly above pH 7 (9, 12). Additionally, high ratios of NADH to NAD are known to stimulate the enzyme (8). Transitions from an aerobic to an anaerobic environment may favor a higher concentration of pyruvate, increased ratio of

NADH to NAD, and lowered pH of the cytoplasm due to an initial accumulation of organic acids (5). High CO_2 concentration may also shift the pH of cytoplasm to a more acid range (13). These factors may contribute to a high activity of PDC *in situ* under anaerobiosis.

In a previous study, it was observed that PDC levels were higher in roots which were submerged for 48 h after harvest than in roots held in air (2). The higher activity of PDC in the submerged roots could have been due to elevated levels of CO_2 in the submerged roots as compared to the air control roots as was observed in the present work. However, PDC activities among the sweet potato cultivars in the previous work (2) were not well correlated with ethanol formation by the cultivars during submergence. Thus, cultivars differing in the capacity for ethanol formation during flooding may differ in factors other than PDC which also influence ethanol formation. Nevertheless, the apparent induction of PDC by CO_2 in submerged roots may be one factor which accelerates ethanol formation.

Finally, it should be mentioned that the higher activity of PDC in anaerobic sweet potato roots (Table II) is apparently due to either more of the enzyme in the tissue or a relatively permanent modification of existing enzyme molecules to a more active form, as the enzyme was assayed at a saturating concentration of pyruvate (5 mM) (2), and desalting of the extracts on Sephadex G-25 in a preliminary trial did not appreciably alter the measured activity.

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