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Effect of Ethanol, Acetaldehyde, Acetic Acid, and Ethylene on Changes in Respiration and Respiratory Metabolites in Potato Tubers

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ABSTRACT

Ethanol, acetaldehyde, and acetic acid, when applied in a volatile state in air to potato tubers, led to a climacteric-like upsurge in respiration. The respiratory upsurge was markedly enhanced when the volatiles were applied in 100% O_2.

Ethanol induced a decline in the level of 2-phosphoglyceric acid and phosphoenolpyruvate while leading to the accumulation of tricarboxylic acid cycle intermediates including isocitrate and α-ketoglutarate. The action of these compounds was similar to, but independent of, the action of ethylene.

The application of ethylene to potato tubers can induce a climacteric-like upsurge in respiration (7). Solomos and Laties (9) found that the ethylene-induced increase in respiration is accompanied by changes in respiratory intermediates and suggested that ethylene enhances aerobic glycolysis.

Other naturally occurring volatiles may act similarly to ethylene. In fruit the application of acetaldehyde resulted in the stimulation of respiration (4, 6) and enhancement of ripening processes (5). Acetaldehyde and ethanol were also shown to increase dramatically during the onset of fruit ripening (5) and may act, together with ethylene, in the stimulation of respiration and possibly other ripening processes. In ripening fruit, ethanol and acetaldehyde increase concomitantly with ethylene and for that reason it is difficult to discern the interdependency of the two classes of volatiles.

In the present work we used potato tubers to examine the effect of ethanol and other volatile compounds including acetaldehyde and acetic acid, on the respiratory upsurge independently of ethylene action. We also studied the effect of applied ethanol on the changes in respiratory metabolites as they compare to the changes induced by ethylene.

MATERIALS AND METHODS

Locally grown potatoes (*Solanum tuberosum* L., var. Norchip) were preconditioned at room temperature for 2 weeks following harvest. Six whole tubers, each weighing approximately 100 g, were placed in 2-liter jars, and were ventilated with different gas mixtures at a rate of 400 ml/min. The gas mixtures consisted of air (air control), or 100% O_2 (O_2 control) either alone or in combination with 10 μl/l) ethylene and different concentrations of ethanol, acetaldehyde, or acetic acid. The potatoes held in the different gas mixtures were used for measuring respiration, and for the preparation of tissue extracts for the measurement of respiratory intermediates.

The various concentrations of volatiles were prepared by diluting a gaseous source mixture of these compounds with air or 100% O_2. The ethylene source mixture consisted of a commercial gas cylinder containing a known concentration of ethylene. The source mixture of ethanol, acetaldehyde, or acetic acid was prepared by placing a storage vessel of one of these compounds (in the liquid phase) in a large chamber which was ventilated with air or 100% O_2. The size of the storage vessel and the rate of gas flow through the chamber determined the evaporation rate of the compounds which could be maintained at a fairly constant value. The concentration of the volatiles leaving the source chamber was determined by gas chromatography and was adjusted to the desired concentrations by mixing the source mixture with air or 100% O_2.

Measurement of Respiration. The output of CO_2 by the tubers was measured periodically following the application of ethylene, ethanol, acetaldehyde, or acetic acid mixtures as outlined before (2).

Extraction and Assay of Respiratory Intermediates. Respiratory intermediates were extracted based on the method of Barker *et al.* (1). One hundred g of potato tissue, representing a composite sample of two potatoes, were homogenized in 100 ml of 10% trichloroacetic acid. The homogenate was vacuum-filtered and the residue reblended with 50 ml of 5% trichloroacetic acid. The combined filtrates were centrifuged for 15 min at 17,000*g* and the resulting supernatants lyophylized to dryness and dissolved in about 50 ml of water. The solutions were centrifuged at 17,000*g*. Additional purification steps in the extraction were described by Barker *et al.* (1). The final sample was lyophilized, dissolved in 6 ml of water, filtered under vacuum through a charcoal column (0.5 × 3 cm), and made up with water to a volume of 10 ml. All intermediates including 2-PGA,[2] 3-PGA, PEP, lactate, α-ketoglutarate, and isocitrate, were determined in the extracts using enzymic reactions coupled to changes in NADH levels (1, 10).

Acetaldehyde and Ethanol Determinations. Changes in acetaldehyde and ethanol in the potato tubers were measured according to the method of Davis and Chace (3). Twenty-five g tissue increments from each of two potatoes were combined and homogenized in 50 ml of cold trichloroacetic acid. A 40-ml aliquot of the homogenate was placed in a 125-ml serum bottle and the latter capped. The serum bottles were incubated in a water bath at 37 C for 1 h. After incubation, 1-ml samples were drawn from the head space and used for the determination of acetaldehyde and ethanol by gas chromatography, using a Porapak Q column. The concen-

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[2] Abbreviations: 2-PGA: 2-phosphoglyceric acid; 3-PGA: 3-phosphoglyceric acid; PEP: phosphoenolpyruvate.

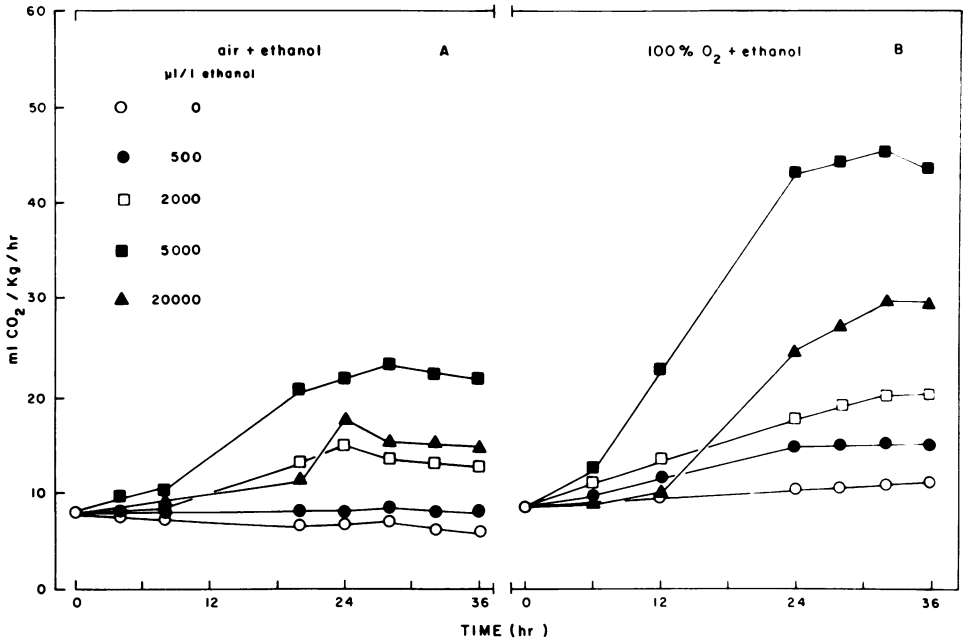

FIG. 1. Effect of different ethanol concentrations in air (A) and in 100% O_2 (B) on CO_2 production of whole potato tubers.

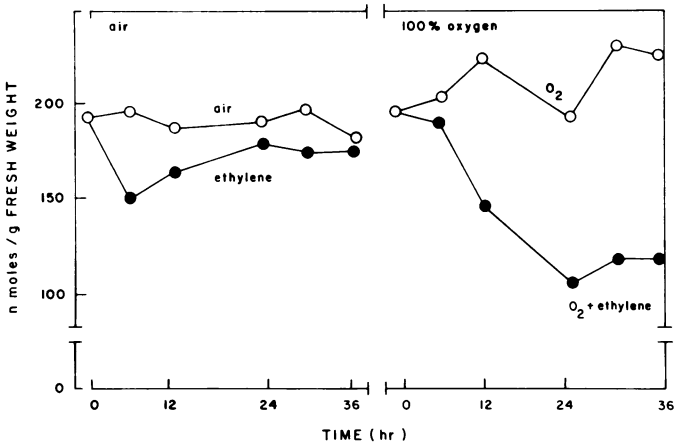

FIG. 2. Effect of ethylene (10 µl/l) in air or 100% O_2 on the changes in the level of 3-PGA in whole potato tubers. Each value in this and subsequent figures represents the average of two to three replications, and each experiment was generally repeated two to three times.

trations of the tested gases were compared against known concentrations of the volatiles incubated in serum bottles under the same conditions.

RESULTS AND DISCUSSION

Figure 1 shows the effect of ethanol on the stimulation of respiration in whole potato tubers. The increase in the concentration of ethanol from 500 to 5,000 µl/l in the ventilating air led to a progressive increase in respiration (Fig. 1A). Higher concentrations (20,000 µl/l) resulted in a diminished respiratory upsurge. When the ethanol was applied in 100% O_2 (Fig. 1B), the respiratory upsurge was more pronounced, as compared with the effect of equivalent concentrations of ethanol in air. As in air, the highest ethanol concentration led to a decrease in the respiratory upsurge.

Similar effects on respiration were obtained by acetaldehyde and by acetic acid, when applied in air or 100% O_2, except that acetaldehyde appears to be the most effective in inducing the stimulation in respiration compared to the other volatiles (data

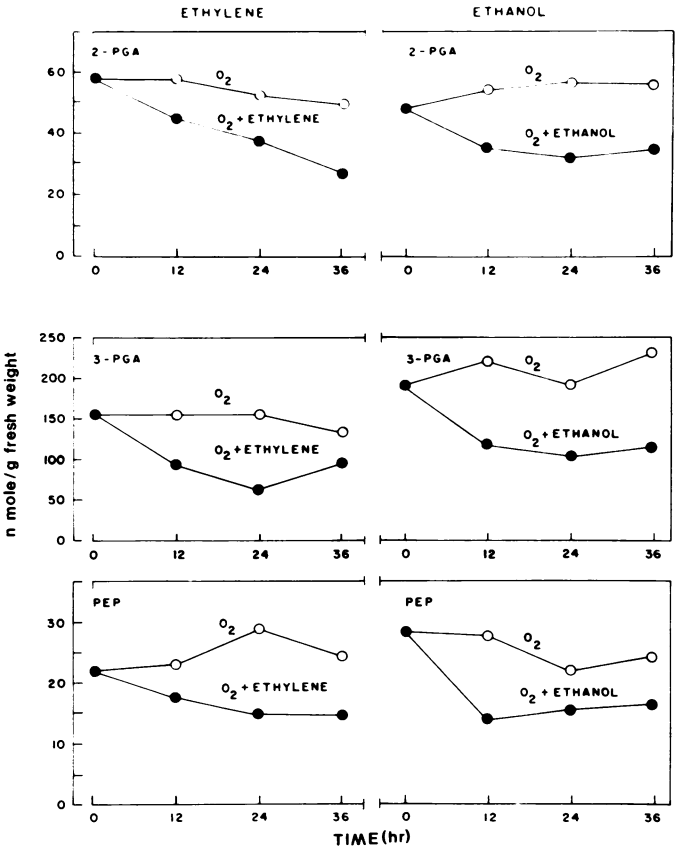

FIG. 3. Effect of ethylene (10 µl/l) and ethanol (5,000 µl/l) in air or 100% O_2 on the changes in glycolytic intermediates in whole potato tubers.

not shown). The responses obtained resemble the effect of ethylene on stimulating respiration in potatoes (7). Also as with ethylene the effect of the volatiles on potato respiration was augmented when applied in high O_2 concentrations (2).

In the present study we also examined the changes in some

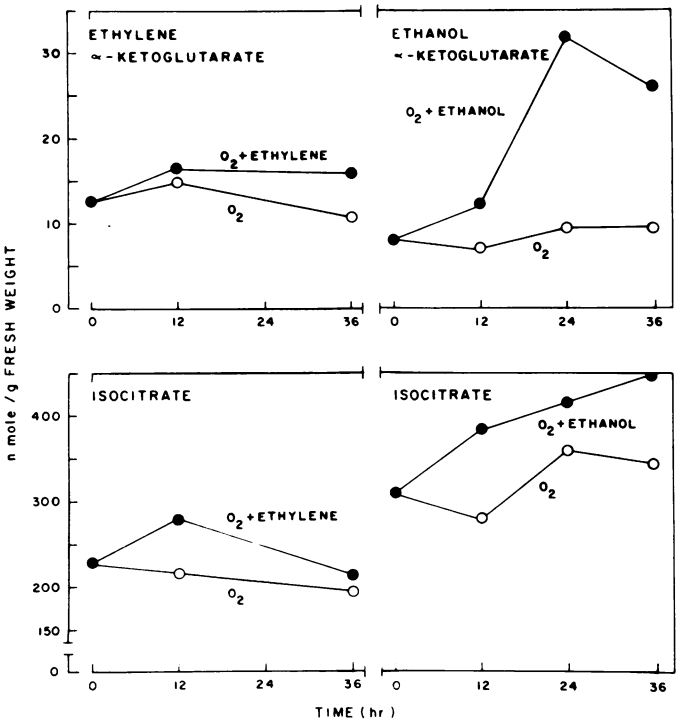

FIG. 4. Effect of ethylene (10 μl/l) and ethanol (5,000 μl/l) on the changes in tricarboxylic acid intermediates in whole potato tubers.

Table I. Time course changes in the formation of ethylene by whole potato tubers as influenced by a continuous ventilation with air, 100% O_2, and 100% O_2 and 3000 ul/l acetaldehyde.

Gas Treatment Applied to Whole Tubers	Time of Treatment (hr) 0	12	24	30	36	48
	Ethylene Evolution (ul/kg/hr)					
air	1.97	.92	1.36	.62	.59	1.97
100%	1.97		1.29	.60	.58	1.92
100% 0 + acetaldehyde	1.97	1.25	1.48	.64	.85	2.29

Table II. Time course changes in the formation of ethanol or acetaldehyde, in whole potato tubers as influenced by a continuous ventilation with 100% O_2, and 100% O_2 and 10 ul/l ethylene

Gas Treatment Applied to Whole Tubers	Time of Treatment (hr) 0	12	24	36	48
	Acetaldehyde (mg/100 g tissue)				
O_2	.5	.7	.65	.2	.5
O_2 + C_2H_4	.5	.65	.45	.5	.65
	Ethanol (mg/100 g tissue)				
O_2	6.5	6.5	9.0	6.5	9.0
O_2 + C_2H_4	6.5	6.5	6.5	6.5	9.0

intermediates of glycolysis, including 2-PGA, 3-PGA, PEP, and lactate, and some tricarboxylic acid cycle acids, including α-ketoglutarate and isocitrate, to determine whether the increase in respiration induced by ethanol is accompanied by metabolic changes similar to those induced by ethylene. We first compared the effect of ethylene in air with that in 100% O_2 on the changes in 3-PGA. Figure 2 shows that the application of ethylene in O_2 caused a more pronounced difference in the level of 3-PGA than it did in air. Henceforth, further determinations of the respiratory intermediates were performed on potatoes treated in 100% O_2, in combination with ethylene or ethanol.

Figure 3 shows the changes in glycolytic intermediates in potatoes held in 100% O_2 in combination with ethylene or ethanol. The level of the tested glycolytic intermediates, namely, 2-PGA, 3-PGA, and PEP, declined significantly in both the ethylene- and the ethanol-treated tubers by comparison with the O_2 control. The decrease in these glycolytic intermediates was observed typically when glycolysis was speeded up as for example during anaerobiosis, and was also observed by Solomos and Laties (9) in potatoes treated with ethylene in air. We found that the lactate level did not change (result not shown).

Figure 4 shows the changes in the level of some tricarboxylic acid intermediates in tubers held in 100% O_2 in combination with ethylene or ethanol. In tubers treated with ethylene the levels of isocitrate and α-ketoglutarate increased as compared to the O_2 control. A similar increase was also observed in potatoes treated with ethanol. According to Solomos and Laties (9), the rise in the level of these tricarboxylic acid cycle intermediates, concomitant with the increase in respiration, suggests an enhanced flux of metabolites through the tricarboxylic acid cycle.

The present data show that the pattern of change in the intermediates level was virtually the same in ethylene- and ethanol-treated tubers, although the magnitude of the changes induced by ethanol was greater than those induced by ethylene, especially in 3-PGA and α-ketoglutarate. These results suggest that the effect of ethanol on respiratory metabolism resembles that of ethylene, and thus may also involve the acceleration of glycolysis with the enhanced flux of metabolites to the tricarboxylic acid cycle.

We examined the possibility that the effect of ethanol or acetaldehyde may reflect an indirect action of ethylene. Table I shows the changes in ethylene in potato tubers kept in air, in 100% O_2, and O_2 plus acetaldehyde. Although ethylene synthesis was slightly higher in potatoes treated with acetaldehyde, it is doubtful that this increase can account for the marked stimulation in respiration. Reid and Pratt (7) showed that a linear increase in respiration in potatoes is a function of an exponential increase in ethylene. In the acetaldehyde-treated potatoes the ethylene production is only slightly higher than the control and therefore not likely to induce a marked stimulation in respiration. Table II shows that ethylene does not stimulate the formation of either ethanol or acetaldehyde in potatoes, indicating that the action of ethylene may not be attributed to the action of glycolytic volatiles. These data suggest that the stimulation of respiration by ethylene and by the other volatiles are independent of each other.

It is not clear just how ethanol, acetaldehyde, or acetic acid may influence respiratory metabolism. It does not appear that these compounds are acting as substrates for the liberation of CO_2. For example, it is calculated that slightly more than 10% of the acetaldehyde supplied must be absorbed and metabolized to yield the amounts of CO_2 liberated. In our study less than 1% was actually absorbed, which could account for less than 10% of the CO_2 evolved. However, their action appears to resemble that of ethylene. Similar to ethylene, these compounds trigger a respiratory upsurge in potatoes, lead to changes in the level of respiratory intermediates, and as with ethylene their action is augmented in high O_2 concentrations. Solomos and Laties (9) suggested that ethylene may stimulate the activity of an alternate (cyanide-insensitive) respiratory pathway. In other studies (8) we support this hypothesis and also suggest that ethanol, acetaldehyde, or acetic acid can lead to the development of the cyanide-insensitive respiration. Thus, the outlined respiratory changes in potatoes are not induced exclusively, or specifically by ethylene, and may represent a more general response in storage tissues.

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