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Effects of Cyanide and Ethylene on the Respiration of Cyanide-sensitive and Cyanide-resistant Plant Tissues[1]

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ABSTRACT

The effects of cyanide and ethylene, respectively, were studied on the respiration of a fully cyanide-sensitive tissue—the fresh pea, a slightly cyanide-sensitive tissue—the germinating pea seedling, and a cyanide-insensitive tissue—the cherimoya fruit. Cyanide inhibition of both fresh pea and pea seedling respiration was attended by a conventional Pasteur effect where fermentation was enhanced with an accumulation of lactate and ethanol and a change in the level of glycolytic intermediates indicative of the activation of phosphofructokinase and pyruvate kinase accompanied by a sharp decline in ATP level. In these tissues, ethylene had little or no effect on the respiration rate, or on the level of glycolytic intermediates or ATP. By contrast, ethylene as well as cyanide enhanced both respiration and aerobic glycolysis in cherimoya fruits with no build-up of lactate and ethanol and with an *increase* in the level of ATP. The data support the proposition that for ethylene to stimulate respiration the capacity for cyanide-resistant respiration must be present.

We have shown that cyanide as well as ethylene induces a respiratory climacteric and ripening in avocados (18) as well as an increase in respiration in potato tubers (19). In both cases, glycolysis is enhanced and the gases produce seemingly identical physiological and biochemical changes. It has been suggested that for ethylene to enhance plant respiration, the cyanide-insensitive, or alternate, path (4) must be present and potentially operative (17). Below we compare the effects of cyanide and ethylene, respectively, on green peas and pea seedlings, where respiration is inhibited by cyanide, and on cherimoyas, where respiration is enhanced by cyanide.

MATERIALS AND METHODS

Fresh green peas were removed from the pods aseptically and samples of 10 g were placed in jars through which a stream of ethylene-free air was passed at a rate of 60 ml/min at 20 C. Germinating peas were prepared as follows: pea seeds, *Pisum sativum*, var. Alaska, were surface-sterilized by soaking for 15 min in 1.5% sodium hypochlorite solution. The seeds were then washed several times, and soaked overnight in distilled H_2O. Uniformly soaked seeds (100 g) were put between wet filter papers in 4-liter jars. The papers were kept wet by the addition of 4- to 10-ml portions of sterile H_2O daily. All materials were sterilized. A stream of sterile ethylene-free air was passed through the jars at a rate of 60 ml/min at 20 C. Cherimoyas, *Annona cherimola*, of unknown variety were collected from a private orchard in San Diego county. Fruits were placed singly in respiratory jars. In all cases, the rates of O_2 uptake and CO_2 output were measured with a Beckman oxygen and infrared CO_2 gas analyzer, respectively. Ethylene and cyanide were applied as described previously (18). Sugar phosphates were extracted and estimated according to previously published methods (5, 18). In the case of pea seedlings, the seed coat was removed and the whole seedlings were dropped into liquid nitrogen and powdered therein. ATP was first isolated by use of an ion exchange column (10) and subsequently estimated enzymically (5). Ethanol and lactate were extracted by homogenizing the tissue in cold 10% perchloric acid. The homogenate was centrifuged, and the pellet homogenized twice with cold 5% perchloric acid. All supernatants were combined and neutralized with KOH. Potassium perchlorate was removed by centrifugation. Lactate and ethanol were determined enzymically (5). The enzymes used were from Sigma.

RESULTS

Effect of Cyanide and Ethylene on Respiration of Fresh Peas. The respiration of fresh pea seeds declines with time from shelling (Fig. 1c). Ethylene (60 μl/l) does not affect the respiration rate despite the high concentrations used (Fig. 1b). Cyanide, at 100 μl/l, which is in equilibrium with 1 mM cyanide in solution (13), lowers the rate of O_2 uptake by about 75%, while CO_2 evolution is decreased some 40%, with the result that the RQ value increases from 1 to about 2, an indication of enhanced fermentation. It is known from previous studies that anaerobiosis induces a noticeable Pasteur effect in peas (3, 21).

Effect of Cyanide and Ethylene on Respiration of 5-Day-Old Pea Seedlings. The rate of seedling respiration increases for about 90 hr, following which there is no appreciable change up to 170 hr (Fig. 2c). The RQ values before the application of either ethylene or cyanide change with time from higher to lower than unity before approaching 1 (Fig. 3, a, b, and c). In germinating peas, ethanol and lactate accumulate initially, subsequently to be metabolized (8, 9). The noted shift in RQ may reflect these events. Ethylene induces a limited increase in respiration rate, which, after 47 hr, is 30% higher than that of the control (Fig. 2b). Cyanide (180 μl/l), on the other hand, decreases the rate of O_2 uptake by about 40% in the 1st 7 hr, while CO_2 output increases by about 20% in the same period. The RQ rises from 1 to 1.8, indicative of an increase in the rate of ethanol formation (Table I). The respiration continues to decline and drops to about 35% of the control 47 hr after cyanide addition. Ethanol and lactate levels rise rapidly within this period and remain high.

Effect of Cyanide and Ethylene, Respectively, on Glycolytic Intermediates and ATP in Germinating Pea Seedlings. Table I

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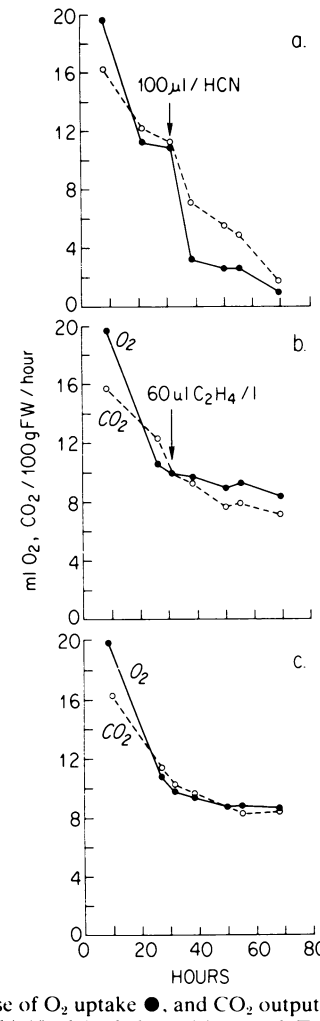

FIG. 1. Course of O_2 uptake ●, and CO_2 output ○, of fresh peas: (a) 100 µl/l HCN; (b) 60 µl/l ethylene; (c) control. Temperature, 20 C.

indicates a 50-fold increase in ethanol and lactate content of germinating pea seedlings in response to cyanide. This increase in aerobic glycolysis is associated with a decrease in ATP, G6P,[3] and PEP levles. The decrease of the latter is much more pronounced than that of the other two metabolites. FDP, on the other hand, increases by more than 10-fold. This pattern of change, evoked by cyanide in this case, is typical of that observed in various tissues and organisms when a Pasteur effect is induced by anaerobiosis (3, 11, 20). By contrast with cyanide, ethylene elicits no appreciable changes in the levels of glycolytic intermediates, glycolytic end-products, or ATP in germinating pea seedlings.

Effect of Cyanide and Ethylene, Respectively, on Respiration and Levels of G6P, FDP, and ATP in Cherimoya Fruits. Cherimoyas, as a climacteric fruit,respond to ethylene with a rise in respiration and eventual ripening (6). Figure 3, a and b depicts the response of cherimoya fruits to 17 µl/l C_2H_4 and 400 µl/l HCN, respectively. Cyanide induces a marked rise in respiration as readily as does ethylene. Cyanide treatment eventually leads to an increase in ethylene evolution (Fig. 3b). Table II shows that the rise in respiration in response to both cyanide and ethylene results in about a 3-fold increase in ATP level. Similar increases in ATP content during the climacteric have been observed with other fruits (14, 22). While G6P increases slightly, the most pronounced changes are shown by FDP, whose level increases severalfold. This pattern of changes in G6P and FDP

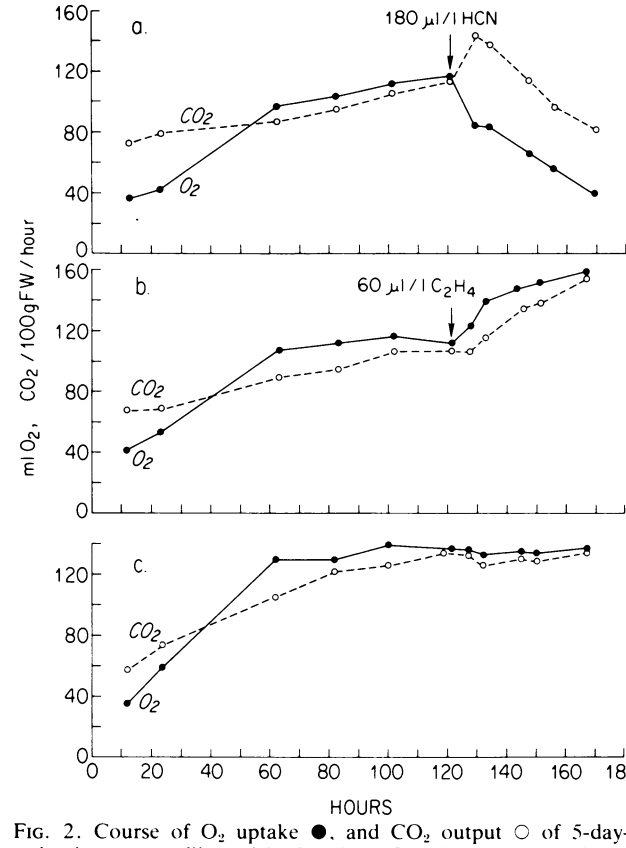

FIG. 2. Course of O_2 uptake ●, and CO_2 output ○ of 5-day-old germinating pea seedlings: (a) 180 µl/l HCN; (b) 60µl/l ethylene; (c) control. Temperature, 20 C.

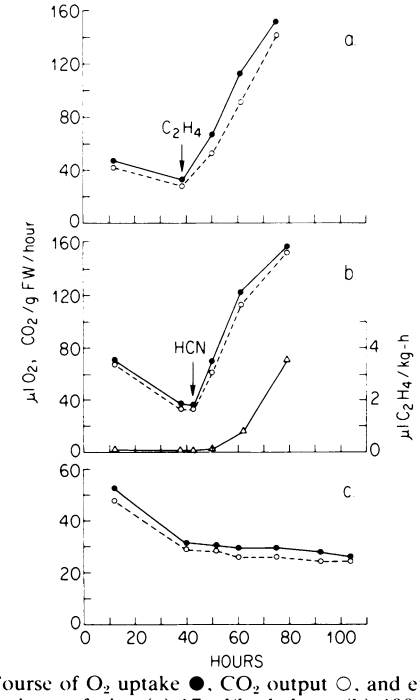

FIG. 3. Course of O_2 uptake ●, CO_2 output ○, and ethylene evolution △ of cherimoya fruits: (a) 17 µl/l ethylene; (b) 400 µl/l HCN; (c) control. Temperature, 20 C.

[3] Abbreviations: G6P: glucose 6-phosphate; FDP: fructose 1,6-diphosphate; PEP: phosphoenolpyruvate; PK: pyruvate kinase; PFK: phosphofructokinase.

Table I. *Effect of Cyanide and Ethylene, Respectively, on Level of Glycolytic Intermediates and End Products in Germinating Pea Seedlings*

Seeds were germinated in the dark for 5 days in respiration jars, following which they were subjected to a stream of air, or a mixture of air and HCN or C_2H_4, as indicated. Chemical analyses were carried out after 47 hr.

Sample	Treatment	G6P	FDP	PEP	ATP	Lactate	Ethanol
		nmol/g fresh wt					*μmol/g fresh wt*
1		264	2.2	75	140	18	2.1
2	Control	318	3.1	105	167	23	0.9
3		339	3.8	88		33	1.7
4	HCN	93	41	9	83	1090	91
5	180 μl/l	60	40	7.5	65	1250	92
6		64	36	8.2	82	1320	77
7		100	60	12	71	2260	109
8	C_2H_4	380	2.7	95	170	91	2.6
9	60 μl/l	390	2.5	106	175	75	1.8
10		320	2.1	100	143	36	4.2

Table II. *Effect of Cyanide and Ethylene, Respectively, on Respiration and Level of Glycolytic Intermediates in Cherimoya Fruits*

Sample	Treatment	Respiration Rate	G6P	FDP	ATP	Lactate
		μl O_2/g FW/hr	nmoles/g FW			
1	Control	42	122	0.5	48	100
2		39	110	0.5	40	75
3	HCN	110	98	2.1	156	90
4	400 μl/l	100	141	3.9	102	106
5		152	154	21	110	121
6	C_2H_4	144	232	2.9	115	85
7	17 μl/l	146	163	3.1	134	101
8		150	180	4.2	121	78

has been observed previously in climacteric bananas (2, 15), and a sharp increase in FDP has been shown to occur during the climacteric in other fruits (7, 18). Lactate is present in small amounts, and shows no appreciable change in either cyanide- or ethylene-treated fruits (Table II).

DISCUSSION

The present data support our previous findings that the cyanide-resistant respiratory electron path must be present for ethylene to stimulate respiration (17). Thus, in fresh green peas, where respiration is strongly inhibited by cyanide, ethylene has no effect despite the high concentrations tested. In fresh green peas, cyanide inhibition raises the RQ from 1 to about 2, which indicates enhancement of ethanol formation. Fresh green peas are known to show an appreciable Pasteur effect (3, 22). While germinating pea seedlings are somewhat more resistant to cyanide than fresh peas, oxygen uptake is decreased 40% by cyanide in the first 7 hr, while CO_2 evolution is increased by about 20% in the same period. The consquent rise in RQ is indicative of enhanced fermentation, which is further substantiated by the accumulation of ethanol and lactate (Table I). On the basis of the rate of ethanol and lactate production in response to cyanide, as well as the rate of respiration in its presence, it is estimated that the rate of glucose utilization is at least doubled in the first 7 hr. The enhancement of glycolysis is associated with a characteristic pattern of changes in glycolytic intermediates indicative of the activation of PFK and PK, two glycolytic enzymes which are considered central in the regulation of glycolysis in plant and animal tissues and microorganisms (1, 20). In germinating peas, the partial inhibition of respiration by cyanide simulates anaerobiosis in that it leads to the accumulation of lactate and ethanol and a decrease in ATP. In this tissue, the capacity of the alternate path is inadequate to cope with the enhanced rates of pyruvate production and to maintain the level of ATP. Ethylene, which marginally affects aerobic respiration in germinating peas, neither stimulates glycolysis noticeably nor appreciably affects the levels of glycolytic intermediates and end-products or ATP (Table I).

In cherimoyas, both ethylene and cyanide induce a rapid increase in respiration (Fig. 3, a and b) which is associated with a disproportionate relative increase in FDP (Table II) and presumably with a sharp enhancement of glycolysis (1, 20). In this case, however, the enhancement of glycolysis is associated neither with an increase in lactate and ethanol nor with a decrease in ATP (Table II). ATP, in fact, increases (Table II). Thus, enhancement of glycolysis in cherimoyas cannot be thought to reflect the evocation of a classical Pasteur effect (20). In this tissue, the capacity of the alternate path is adequate to accommodate the enhanced rates of pyruvate production. Furthermore, since site I phosphorylation is part of the over-all cyanide-resistant path, the net rate of ATP formation in the presence of cyanide or ethylene increases because of the 3- to 5-fold increase in electron flow through site I, together with the increase in substrate level phosphorylation.

Ethylene fails to affect glycolytic activity in peas while it enhances it in cherimoyas. These tissues differ in a way which indicates that the capacity for cyanide-resistant respiration is necessary for ethylene to enhance glycolysis.

In summary, the data herein suggest that for ethylene to enhance plant respiration, the presence of a functional cyanide-resistant electron path is necessary. The "activation" by ethylene (or cyanide) of electron transport through the alternate path in cherimoyas and certain other plant tissues (17–19) decontrols respiration, as evinced by the increase in respiration rate, and concomitantly stimulates glycolysis despite the increase in ATP (14, 19, 22). This activation may be the primary event which leads to respiratory stimulation, although it is unclear whether ethylene acts directly or indirectly in inducing cyanide-resistant respiration. Neither is the mechanism of activation of PFK and PK understood under conditions where ATP and possibly energy charge increase (14, 19, 22).

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