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Increased Production of Ethylene by Plant Tissues Treated with 1-Aminocyclopropane-1-carboxylic Acid[1]

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Abstract. **Ethylene production of tissues excised from root, stem, leaf, inflorescence, and fruit of 16 plant species greatly increased following the application of 1-aminocyclopropane-1 carboxylic acid (ACC), an intermediate in the conversion of methionine to ethylene. Treatment with 1 mM ACC invariably increased the rate of ethylene production 10 to 1000 times over controls, whereas methionine at the same concentration was ineffective. Treatment with 0.1mM ACC consistently increased ethylene production in all of the tissues tested, although only a few tissues responded to 0.01 mM.**

Ethylene is a plant hormone which regulates many aspects of plant growth and development, and many of these effects are of high commercial value in agriculture (1). Since it is active in trace amounts, a small increase in ethylene production can often elicit spectacular physiological responses. Adams and Yang (2) recently studied ethylene biosynthesis in apple tissue and established the following biosynthetic sequence: methionine → S-adenosylmethionine → ACC → ethylene. In mung bean hypocotyls the conversion of S-adenosylmethionine to ACC is the limiting step in the synthesis of ethylene. (4). ACC was first isolated from perry pears in 1957 by Burroughs (3), but no physiological role could be ascribed to it at that time.

The present study was designed to test various plant tissues for their ability to convert exogenously applied ACC to ethylene. Excised tissue weighing 1 to 5 g was placed in a 50-ml Erlenmeyer flask which contained 5 ml water. Where indicated, 1 mM methionine and ACC at 0.01, 0.1, or 1 mM were included. The flasks were sealed with serum caps and the tissue was incubated at 27°C with constant shaking. At the end of every 3-hr period, gas samples were withdrawn from the flasks for ethylene determination. The flasks were then flushed with air, sealed and returned to the water bath. Each experiment was run in duplicate, and the data represent mean values. Fruits and vegetables were obtained from a local market. Seedlings were grown in moistened vermiculite at 25° in either light or dark.

Stem tissues were represented by potato and celery, root tissues by sweet potato and carrot, and inflorescence tissues by broccoli and cauliflower. Although the ethylene production rates of these various tissues differed markedly, all exhibited a generally similar response to ACC (Fig 1) In most cases, excision itself increased ethylene production many fold over intact tissue, thus accounting for some high control rates. In all cases, 1 mM methionine had no significant effect. Conversely, tissue incubated in 1 mM ACC produced ethylene at 10 to 30 times the rate of the water controls, regardless of the

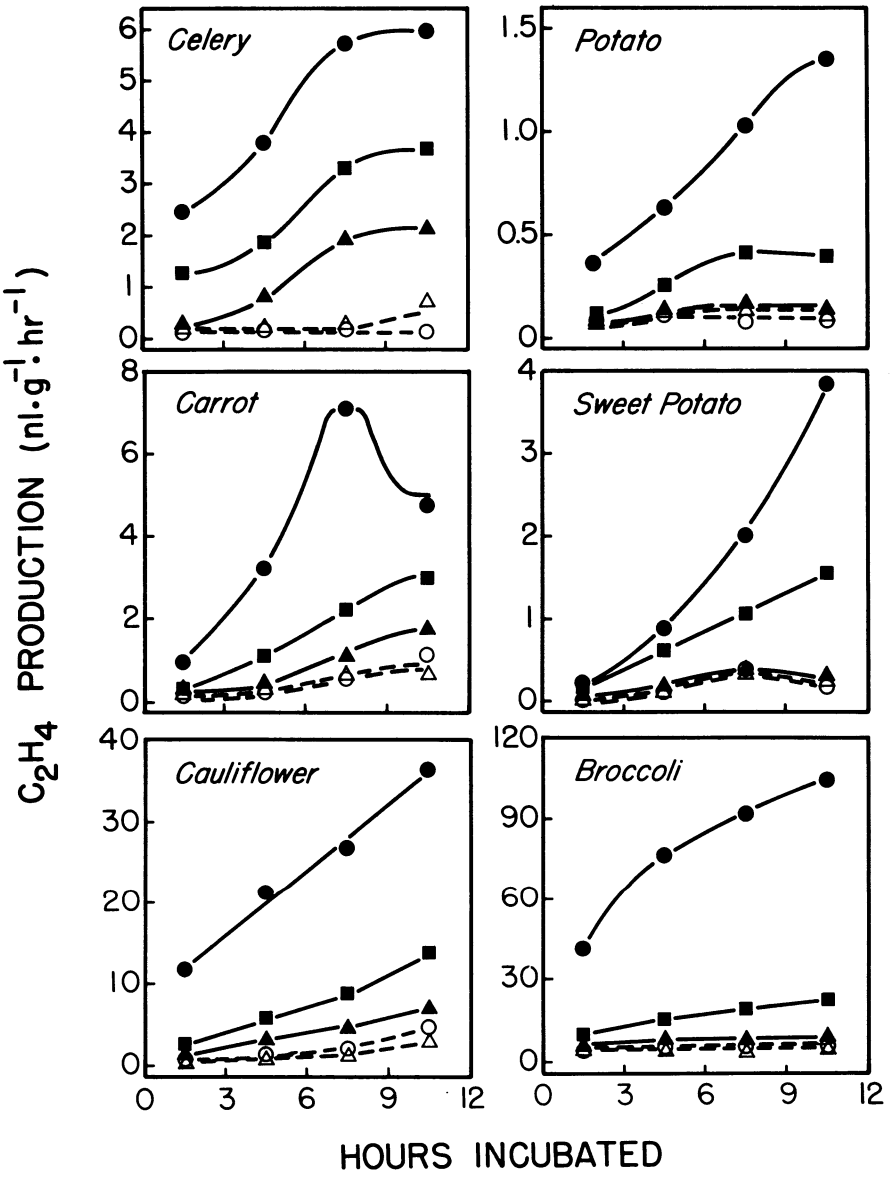

Fig 1. Influence of ACC on ethylene production rates in vegetative and reproductive tissues. Rates were measured at 3-hr intervals, ○, control;△, 1 mM methionine;▲, 0.01 mM ACC; ■, 0.1 mM ACC;●,1 mM ACC.

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control rate. At lower concentrations there was a considerable variation in the ability of different tissue to convert ACC, although there was a consistent increase in the rate of ethylene production at 0.1 mM. A lag phase in the conversion of 1 mM was generally observed, and the rate increased with time of incubation. This lag phase might result from slow penetration of ACC to the active sites, or might represent the time required for induction of new enzymes by ACC.

Tissues excised from tomato fruits at 3 stages of ripening were evaluated for their ability to convert ACC to ethylene during the ripening process. ACC at a concentration of 1 mM evoked a significant increase in ethylene production at each stage of ripening, although the absolute values were much higher during the breaker stage than in the mature green and red stages. (Fig 2) This suggests that the ability to convert ACC to ethylene is highest at the time normal ethylene production is highest. However, mature green tomatoes obviously possess the enzyme(s) necessary to convert ACC to ethylene, and the rate of production of ACC probably limits the rate of ethylene production. This postulate is further supported by the observation that a marked evolution of ethylene followed sub-epidermal injection of ACC in mature green tomatoes, while controls injected with water maintained a very low rate (A. C. Cameron and S. F. Yang, unpublished observation). The reason for the steady decline which followed the initial increase in the rate of ethylene production by breaker tomatoes treated with 1 mM ACC is not known.

The response of cucumber, bell pepper, and squash, which are non-climacteric fruits, was similar to that of red tomato, although there was a great divergence in the efficiency with which the tissues converted ACC to ethylene (Fig 2). All 3 fruits displayed a 10- to 20-fold increase in rate of ethylene production in the presence of 1 mM ACC, as compared to controls, while none responded significantly to 1 mM methionine.

Seedling stem tissue was also tested for its response to ACC. Neither 1 mM methionine nor 0.01 mM ACC had an appreciable effect on ethylene production rates in corn stem tissue, whether light- or dark- grown (Fig 3). There was a large response to 0.1 mM or 1 mM ACC, however, and the response of tissue of dark-grown seedlings was far greater than that of those grown in light. Sorghum seedling stem tissue also responded to the higher concentration of ACC. The difference in response between light- and dark-grown seedlings was apparent in sorghum tissue treated with 0.01 or 0.1 mM ACC, but no dif-

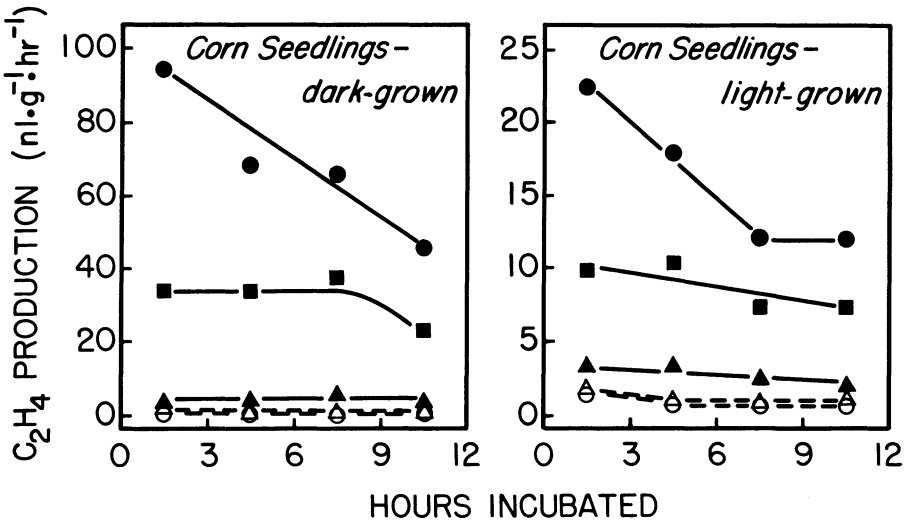

Fig 2. Influence of ACC on ethylene production rates in fruit tissues. See legends in Fig 1.

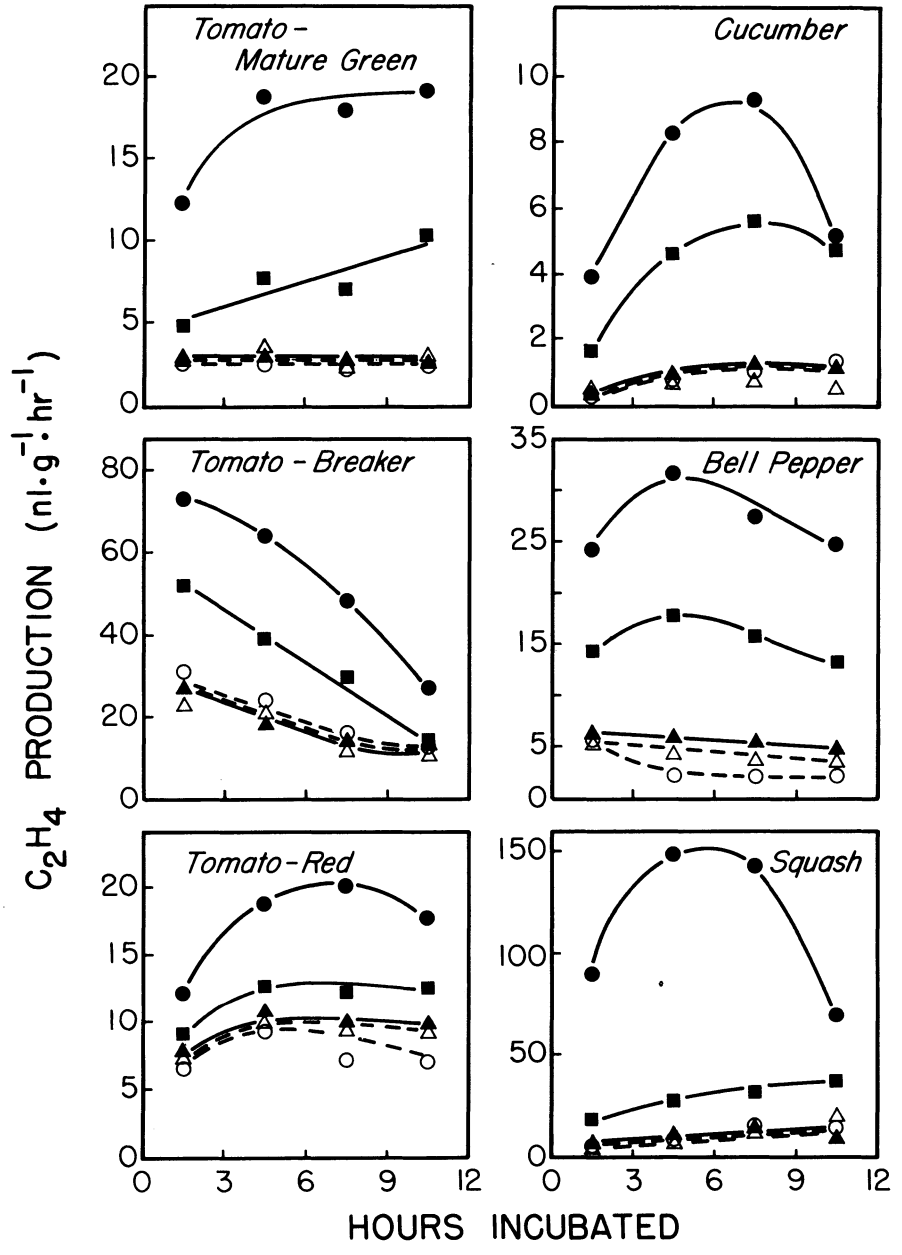

Fig 3. Influence of ACC on ethylene production rates in corn seedling stem tissues. See legends in Fig 1.

Table 1. Influence of ACC on ethylene production rates in various plant tissue. Rates were measured at 3-hr intervals.

Tissue	Chemical and concn (mM)	C_2H_4 production nl·g^{-1}·hr^{-1}) 0.3 hr	3-6 hr	6-9 hr	9-12 hr
Sorghum seedlings (dark-grown)	Water	1.5	6.3	4.6	2.9
	Methionine, (1)	3.0	5.4	3.5	2.4
	ACC (0.01)	3.0	15	16	12
	ACC (0.1)	14	30	34	33
	ACC (1)	32	32	35	33
Sorghum seedlings (light-grown)	Water	1.2	1.2	1.2	1.2
	Methionine, (1)	0.9	0.9	1.4	1.3
	ACC (0.01)	2.0	2.0	3.1	4.2
	ACC (0.1)	13	10	13	16
	ACC (1)	39	28	31	31
Mung bean seedlings (dark-grown)	Water	1.3	0.8	0.2	0.2
	ACC (0.01)	2.6	2.1	2.2	2.9
	ACC (0.1)	26	27	40	67
	ACC (1)	107	167	232	324
Radish root	Water	0.6	0.8	0.9	0.8
	Methionine (1)	0.5	0.9	1.1	1.4
	ACC (0.01)	1.0	1.4	1.7	1.7
	ACC (0.1)	2.6	5.5	7.4	5.3
	ACC (1)	6.5	10	14	12
Spinach leaf	Water	2.8	1.4	1.8	1.6
	Methionine (1)	3.1	1.2	1.5	1.9
	ACC (0.01)	5.3	4.9	5.4	3.9
	ACC (0.1)	14	12	12	8.0
	ACC (1)	40	30	21	14
Orange peel	Water	0.2	0.2	0.6	
	ACC (0.1)	0.3	0.6	1.5	
	ACC (1)	0.8	2.6	6.7	

ference was apparent when the concentration was raised to 1 mM (Table 1).

Orange peel, spinach leaf disks, dark-grown mung bean seedlings, and radish roots were also tested (Table 1). Each of these tissues was capable of converting ACC to ethylene, primarily at concentrations of 0.1 mM and 1 mM. In mung bean hypocotyls, ethylene production was increased more than 200-fold by 0.1 mM ACC and 1,000-fold by 1 mM ACC during the later part of the incubation periods.

To examine the possibility that some or all of the ACC-stimulated ethylene production might have been extracellular, incubated tissue and incubation media were separated and placed in separate flasks after ethylene production had begun. In each instance, essentially all of the ethylene production originated from the tissue and none from the solution.

This survey indicates that the ability to convert exogenously supplied ACC to ethylene is common among a wide variety of plant tissues. The large variation in ACC-stimulated ethylene production between different tissues may be due to the different quantity of the enzyme present in the tissues or to different rates of ACC uptake. Methionine, a known precursor of ethylene, is evidently distant from ethylene in the biosynthetic pathway, and its conversion to ethylene is normally restricted by a rate-limiting step. The discovery of ACC as an intermediate in the biosynthesis of ethylene (2) and its dramatic role in stimulating ethylene production rates in a wide variety of plant tissues, as shown in this report, provide new insight into the study of the mechanism and regulation of ethylene production. Considering the fact that ACC is a natural amino acid (3), and is readily converted to ethylene by plant tissues, it may prove to have important uses in agriculture.

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Low-cost Carbon Dioxide Analyzer for Greenhouses[1]

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Abstract. **A conductimetric CO_2 analyzer was designed, built, and tested. The principle of operation is based on measuring the increase in electrical conductivity of recirculated, deionized water when a sample air stream is bubbled through the water. An improvement over previous models was the addition of temperature compensation so the instrument could be used in greenhouses to reliably measure CO_2 concentration in the 0-3000 ppm range at 1/9th the cost of an infrared CO_2 analyzer.**

Enriching the air around plant leaves with CO_2 has generally increased rates of photosynthesis as well as marketable plant yield. Scientists who study the effects of CO_2 enrichment on plants have commonly used infrared gas analyzers to control the CO_2 concentration around their plants, but infrared analyzers are rather expensive. Therefore, commercial greenhouse operators generally choose simple time clocks to control their CO_2 generators, but the resulting CO_2 concentrations often are not optimum for plant growth. Therefore, we designed, built, and tested a CO_2 analyzer that is accurate enough for scientists and inexpensive enough for commercial growers.

Description. A conductimetric method of detecting CO_2 with recirculated, deionized water, was developed by James (2, 3) and used by Bowman (1) to control the CO_2 concentration of plant enclosures. Slack and Calvert (4) improved Bowman's device somewhat and used separate analyzers to control the CO_2 concentration of 9 different greenhouse compartments. However,

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[2]R. Allen helped provide temperature compensation for the analyzer.