Changes in the Pathway of Ethylene Production Following Citrus Exocortis Viroid Infection in Tomato Plants

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ABSTRACT. Ethylene production was stimulated during the systemic reaction of Rutgers tomato to citrus exocortis viroid (CEV) infection. The increase in ethylene production of CEV-infected leaf discs was parallel to the increase in 1-aminocyclopropane-1-carboxylic acid (ACC) production, and content. Moreover, the capacity to convert ACC into ethylene (ethylene forming enzyme activity) in CEV-infected leaves also increased. The blockage of ACC synthase with aminooxyacetic acid (AOA) completely prevented the viroid-induced production of ethylene, thus indicating that this enzyme is the primary controlling step of ethylene biosynthesis acted upon in viroid infection. The increased ethylene-forming enzyme (EFE) activity acts as a secondary contributor to the enhanced production of ethylene.

Index words. 1-aminocyclopropane-1-carboxylic acid production, ethylene-forming enzyme activity.

The idea that viroids induce in the host plant a nonspecific developmental response mediated by ethylene has been proposed (2, 3).

To further reinforce the hypothesis of the involvement of ethylene in the viroid-induced response, we studied the biosynthetic pathway of ethylene as altered by citrus exocortis viroid (CEV) infection.

It is generally admitted that the pathway of ethylene biosynthesis involves the following steps: methionine → S-adenosylmethionine (SAM) → 1-aminocyclopropane (ACC) → Ethylene (1).

We have concentrated our attention on the last two steps of the pathway, which were found to be critical in the stimulation of ethylene production associated with several plant diseases and stress (9).

MATERIALS AND METHODS

Plant material. Rutgers tomato plants were grown from seed in a greenhouse at temperatures ranging from 25 to 32 C. Plants were inoculated with CEV by stem puncture as described previously (7).

Measurement of ethylene production. Leaf discs (20 mm diameter) were incubated in 1 ml of water in 35-ml sealed serum flasks at 25 C in fluorescent light. At the end of the incubation period, a 1-ml gas sample was taken from the sealed flasks with a syringe and assayed for ethylene with a gas chromatograph equipped with a flame ionization detector. Ethylene production was calculated as the average of three independent incubations.

Extraction and determination of ACC. Leaf discs (20 mm diameter) were frozen in liquid nitrogen, ground with a mortar and pestle and 5% sulphosalicylic acid (3 ml/g fresh weight) added. The resulting extract was centrifuged at 30,000 x *g* for 20 min. One-half ml of the supernatant was used for determination of ACC (6).

Determination of *in vivo* ACC production. This activity was assayed following the procedure of De Laat and Van Loon (4) with slight modifications and constitutes a measure of ACC synthase activity *in vivo* (4). Production of ACC *in vivo* was calculated by determining ACC accumulation during incubation of tomato leaf discs under anaerobic conditions. The last step in ethylene biosynthesis is blocked by anaerobiosis and consequently ACC accumulates.

Discs from one-half leaf were extracted to determine the ACC content

at the start of incubation and discs from the other half were placed in an atmosphere of N_2. ACC accumulation was constant for at least 6 h. At the end of the incubation period, the leaf material was frozen in liquid N_2 for determination of ACC.

Assay of ethylene-forming enzyme (EFE) activity. *In vivo* activity of EFE was determined by measuring conversion of applied ACC to ethylene. Ten-mm diameter discs were floated for 30 min on a solution containing 1 mM ACC, blotted dry on a filter paper, and then enclosed in a 7-ml tube to measure ethylene production.

RESULTS

Figure 1 shows the pattern of ethylene production in CEV-infected tomato apical leaf discs as compared to healthy controls during the period of symptom appearance and development. The evolution of ethylene production in CEV-infected leaves occurs in two stages: 1) an early, steep rise lasting 2 days, and 2) steady level during the development of symptoms.

A parallel increase in ACC level (fig. 2), in the *in vivo* activity of ACC synthase (fig. 3) and in the capacity of converting ACC into ethylene (EFE) (fig. 4) of CEV-infected apical leaves also occurred.

To ascertain whether ACC synthesis was the key step of the CEV-induced ethylene production, as has been described for the tobacco-mosaic virus (TMV) system (5), we studied the effect of amiooxyacetic acid (AOA), a recognized inhibitor of the ACC synthase (1), on the ethylene production of CEV-infected and healthy leaf discs.

AOA inhibited ethylene production almost completely (ca. 81% and 73% in CEV-infected and noninfected discs, respectively) and AOA did not influence the ethylene production by leaf discs from healthy or CEV-infected plants incubated in ACC (table 1).

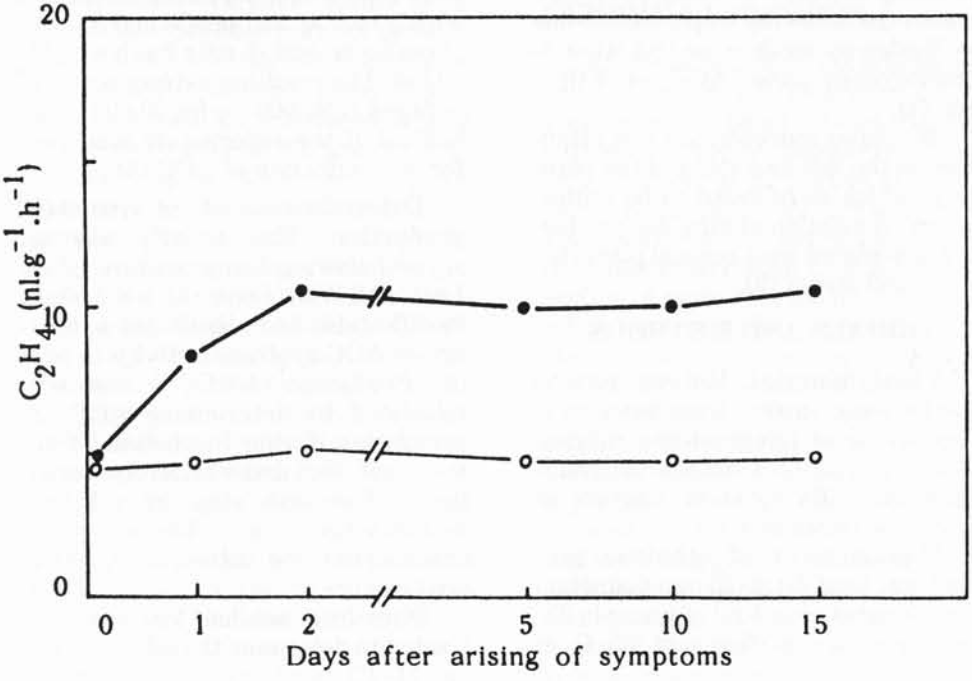

Fig. 1. Time course of ethylene production by leaf discs from: healthy plants ○, CEV-infected plants ●.

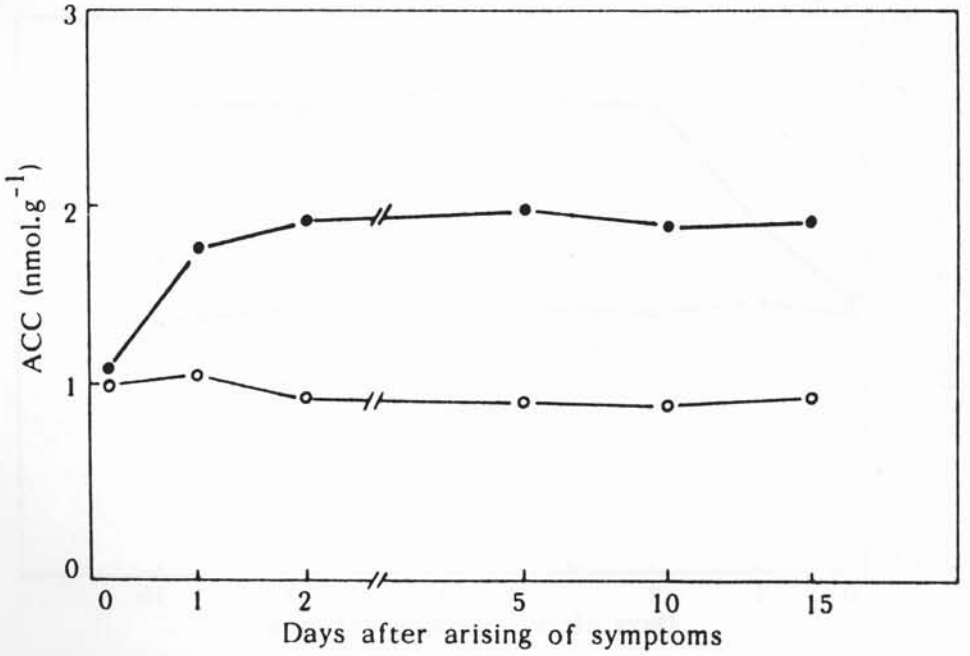

Fig. 2. Time course of 1-aminocyclopropane-1-carboxylic acid content in tomato leaf discs from: healthy plants ○, CEV-infected plants ●.

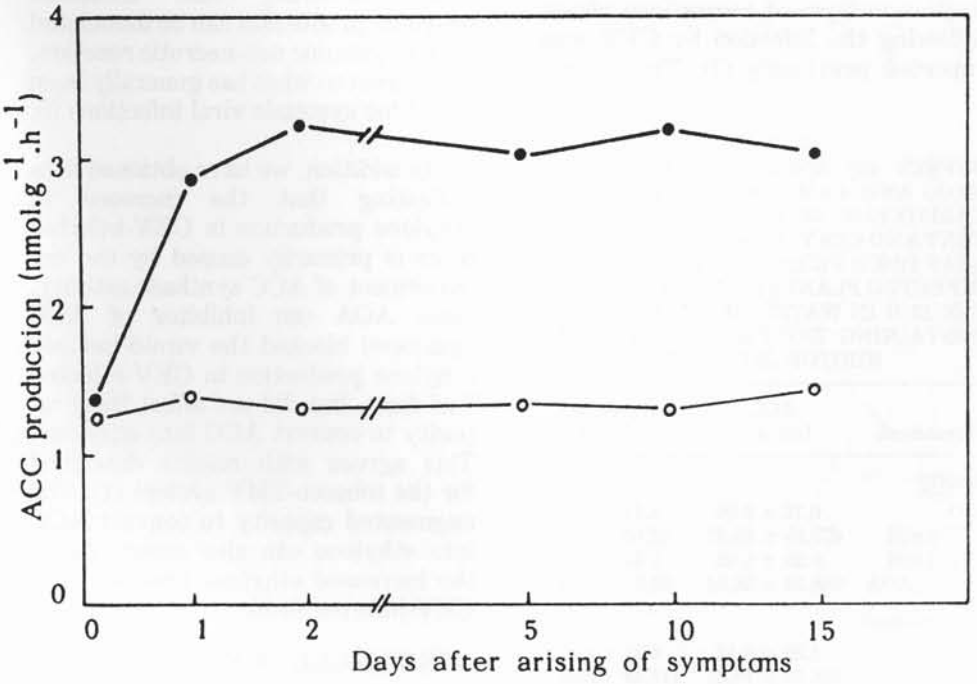

Fig. 3. Changes in the rate of production of 1-aminocyclopropane-1-carboxylic acid in Rutgers tomato leaves from: healthy plants ○, CEV-infected plants ●.

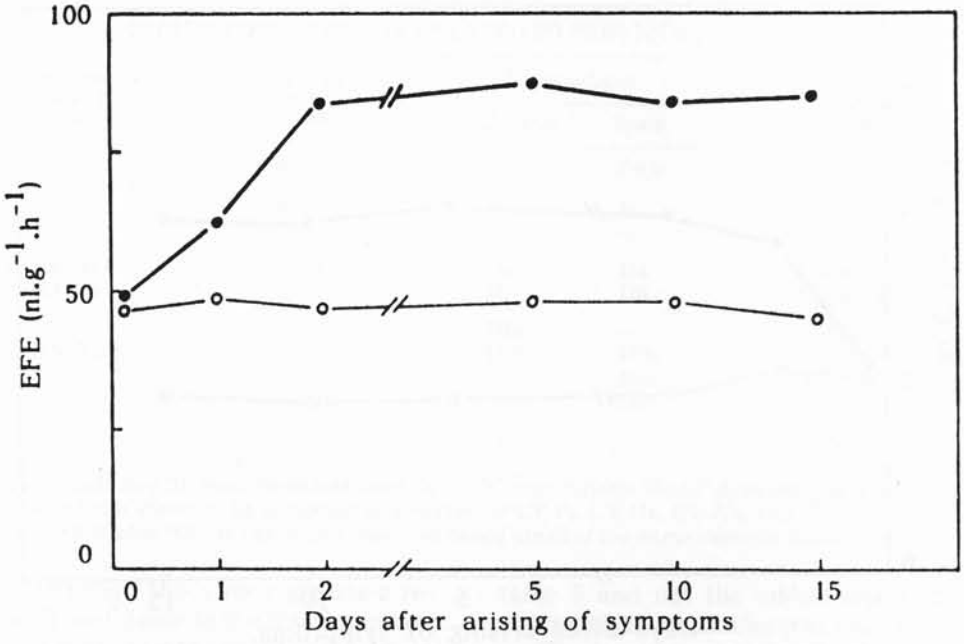

Fig. 4. Ethylene production of leaf discs cut from healthy ○ or CEV-infected ● plants after incubation in 1 mM solutions of 1-aminocyclopropane-1-carboxylic acid at 25 C in light for a 4-h period. EFE = ethylene forming enzyme.

DISCUSSION

The enhancement of ethylene production in *Gynura aurantiaca* plants following the infection by CEV was reported previously (3). The results presented in this paper extend this finding to the tomato-CEV system thus confirming that enhanced ethylene production can be associated with a systemic non-necrotic reaction, in contrast to what has generally been found for systemic viral infections (5, 8).

TABLE 1
EFFECT OF AMINOOXYACETIC ACID (AOA) AND 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) ON ACC CONTENT AND ETHYLENE PRODUCTION OF LEAF DISCS FROM HEALTHY AND CEV-INFECTED PLANTS INCUBATED AT 25 C FOR 12 H IN WATER OR IN SOLUTIONS CONTAINING THE PRECURSOR OR INHIBITOR INDICATED

Treatment	ACC (nmol/g)	Ethylene (nl/g/h)
Healthy		
H_2O	0.72 ± 0.06	4.71 ± 0.57
ACC 1 mM	673.55 ± 55.87	52.66 ± 6.12
AOA 1 mM	0.21 ± 0.02	1.34 ± 0.10
ACC + AOA	668.32 ± 58.54	50.91 ± 6.31
CEV-infected		
H_2O	1.39 ± 0.12	8.33 ± 0.15
ACC 1 mM	686.70 ± 70.65	117.42 ± 13.24
AOA 1 mM	0.42 ± 0.03	1.71 ± 0.19
ACC + AOA	694.93 ± 68.79	112.79 ± 13.45

In addition, we have obtained data indicating that the increase in ethylene production in CEV-infected discs is primarily caused by the enhancement of ACC synthase activity, since AOA (an inhibitor of ACC synthase) blocked the viroid-induced ethylene production in CEV-infected leaf discs, but did not affect their capacity to convert ACC into ethylene. This agrees with results described for the tobacco-TMV system (4). The augmented capacity to convert ACC into ethylene can also contribute to the increased ethylene production in CEV-infected discs.

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