Ethylene Biosynthesis in *Poa pratensis* Leaves in Response to Injury or Infection by *Bipolaris sorokiniana*

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ABSTRACT

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Research was initiated to evaluate 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity, ACC content, and endogenous ethylene of *Poa pratensis* leaf blades subjected to wounding or infection by *Bipolaris sorokiniana*. Wounding stimulated ACC synthase activity, endogenous ethylene, and ACC content of leaf blades. ACC synthase activity peaked 1 hr after wounding followed by a peak in ethylene production 2 hr after wounding that slowly declined. ACC content increased rapidly and peaked 3 hr after wounding. Infection of leaf blades by *B. sorokiniana* resulted in peak ethylene production 36 hr after inoculation followed by peak ACC synthase activity at 72 hr. Endogenous ethylene produced in response to infection was somewhat greater than that produced in response to wounding and peak ACC synthase activity was more than five times greater in response to infection than to wounding. Infection resulted in an accumulation of ACC in leaf blades 96 hr after inoculation that did not occur in response to wounding. The results are discussed relative to the source of ethylene and ACC in infected leaf blades.

Ethylene production by stressed (15,18,27), mechanically wounded (5,13,14,17,24,30), and diseased plants (9,10,12,16,19, 21,25) has been studied extensively. Biosynthesis of ethylene in host-pathogen interactions is believed to differ from that of wounded plants or plants undergoing changes in development (16,19,25). Biosynthesis of ethylene by higher plants and by microbes also is thought to occur by different pathways (7,20,26,29). Ethylene biosynthesis in higher plants seems to occur primarily by the following pathway: Methionine (Met)→S-adenosylmethionine (SAM)→1-aminocyclopropane-1-carboxylic acid (ACC)→ethylene (29). This pathway seems to function in higher plants in response to stress or mechanical injury; i.e., ACC increases along with ethylene. The increase in ethylene in host-pathogen interactions involving fungal pathogens may be accompanied by an accumulation of ACC in infected tissue (1,2). The accumulation of ACC in infected tissue is believed to be of host origin.

Endogenous ethylene increases in leaves of *Poa pratensis* L. infected by *Bipolaris sorokiniana* (Sacc. ex Sorok.) Shoem. (12). Ethylene produced in host leaves is associated with a general chlorosis of infected leaves, which can be substantially decreased when intact diseased plants are grown under reduced atmospheric pressure. The origin of the ethylene in this host-pathogen interaction is unknown. Recent studies, however, show *B. sorokiniana* to produce ethylene from Met, to possess ACC synthase activity, and to secrete ACC into a synthetic culture media (8). These observations suggest that *B. sorokiniana* might contribute ethylene and/or ACC to the host-pathogen interaction during pathogenesis. This study was initiated to evaluate endogenous ethylene, ACC synthase activity, and ACC production in leaves of *P. pratensis* subjected to wounding (abiotic stress) or to infection (biotic stress) by *B. sorokiniana* and to determine any potential contribution by *B. sorokiniana* to ethylene biosynthesis during pathogenesis.

MATERIALS AND METHODS

Mechanical wounding of *P. pratensis* leaf blades. Four leaf blades of intact *P. pratensis* shoots grown in 7.5-cm^2 plastic pots were rinsed with distilled water, and alternate 1-cm sections of the blades were crushed in a rolling action with a glass rod. Enough pressure was applied to the leaf to cause internal liquid to be expressed and for a water-soaked area to appear. Wounded plants were incubated in plastic refrigerator crispers containing 100 ml of distilled water to prevent desiccation of injured tissues. The leaf blades (about 0.5 g fresh weight) of each shoot were sampled at various intervals between 0 and 24 hr after wounding and were analyzed for endogenous ethylene, ACC content, and ACC synthase activity. The wounding treatment and analysis at specific times were replicated three times with two analytical samples per treatment. Controls consisted of uninjured, healthy leaf blades assessed at time 0 before wounding for endogenous ethylene, ACC synthase activity, and ACC content.

Infection of *P. pratensis* by *B. sorokiniana*. Inoculation and subsequent infection of four intact leaf blades of *P. pratensis* shoots with *B. sorokiniana* were accomplished by a previously described method (9). Strips (2 mm × 2 cm) of Bacto-agar (3%, v/v) infested with *B. sorokiniana* were placed on leaf blades, and distilled water was applied to the leaf surface with a syringe to provide a water interface between the pathogen and leaf surface. Inoculated plants were incubated in refrigerator crispers at 23 C for up to 4 days with a 9-hr photoperiod. Four infected leaves from an individual shoot were sampled at 18, 24, 36, 48, 60, 72, and 96 hr and analyzed for endogenous ethylene, ACC content, and ACC synthase activity. The inoculation treatment and analysis at specific time periods were replicated three times with two analytical samples per treatment. Controls consisted of uninjured, healthy plants.

Endogenous ethylene, ACC content, and ACC synthase activity. Endogenous ethylene of leaf blades was determined from the vacuum extracted internal atmosphere of leaves in accordance with a previously described method (11). ACC was extracted by grinding about 0.5 g fresh weight wounded or infected leaf tissue in 30 ml of distilled water followed by centrifugation at 10,000 *g* for 10 min. The supernatant was concentrated to 3 ml under reduced pressure at 40 C, treated with 0.1 ml of 10% trichloroacetic acid,

and centrifuged at 30,000 *g* for 1 hr. The pellet was discarded and the ACC content of the supernatant assayed using 0.1 ml of 50 mM $HgCl_2$ and 0.1 ml of NaOCl:NaOH (2:1, v/v). Ethylene evolved by this alkaline decomposition was determined by gas chromatography (8). The activity of ACC synthase was determined in accordance with a previously described method (29).

RESULTS

Endogenous ethylene, ACC synthase activity, and ACC content of mechanically injured leaf blades. Mechanical injury of leaf blades increased endogenous ethylene, ACC synthase activity, and ACC content. ACC synthase activity peaked 1 hr after injury and preceded the rise in endogenous ethylene (Fig. 1). Maximum increase in endogenous ethylene occurred 2 hr after injury and decreased slowly for up to 6 hr after wounding. ACC content of mechanically wounded leaf blades peaked 4 hr after wounding and then slowly declined (Fig. 2). Leaf blades of uninjured control plants at time 0 averaged 0.6 pmol of endogenous ethylene, ACC synthase activity of 4.4 pmol ACC 4 hr^{-1} mg^{-1} of protein and 0.06 nmol of ACC per gram fresh weight.

Endogenous ethylene, ACC synthase activity, and ACC content of leaf blades infected by *B. sorokiniana*. Infection of leaf blades increased endogenous ethylene, ACC synthase activity, and ACC content. Endogenous ethylene peaked 36 hr after inoculation and remained relatively high through 72 hr, before declining (Fig. 3). Maximum ACC synthase activity occurred late in disease development at 72 hr after inoculation (Fig. 3) and preceded the substantial accumulation of ACC in diseased tissue at 96 hr (Fig. 4). ACC peaked and declined twice in diseased leaves before the accumulation at 96 hr.

DISCUSSION

The increase in endogenous ethylene and the intermediates (ACC synthase, ACC) required for its biosynthesis in mechanically wounded or *B. sorokiniana*-infected leaf blades of *P. pratensis* suggest that the ethylene produced from wounding or infection

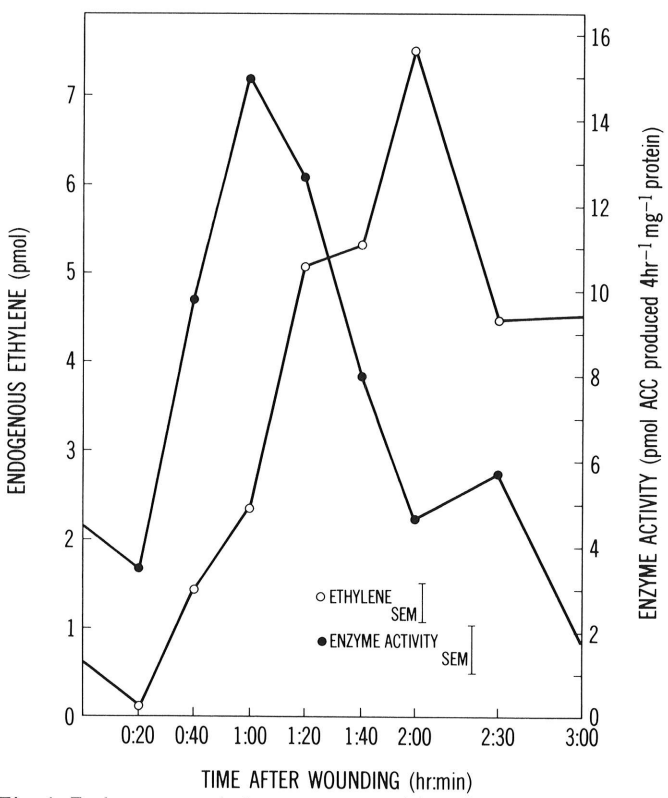

Fig. 1. Endogenous ethylene content and 1-aminocyclopropane-1-carboxylic acid synthase activity of mechanically wounded leaf blades of *Poa pratensis*.

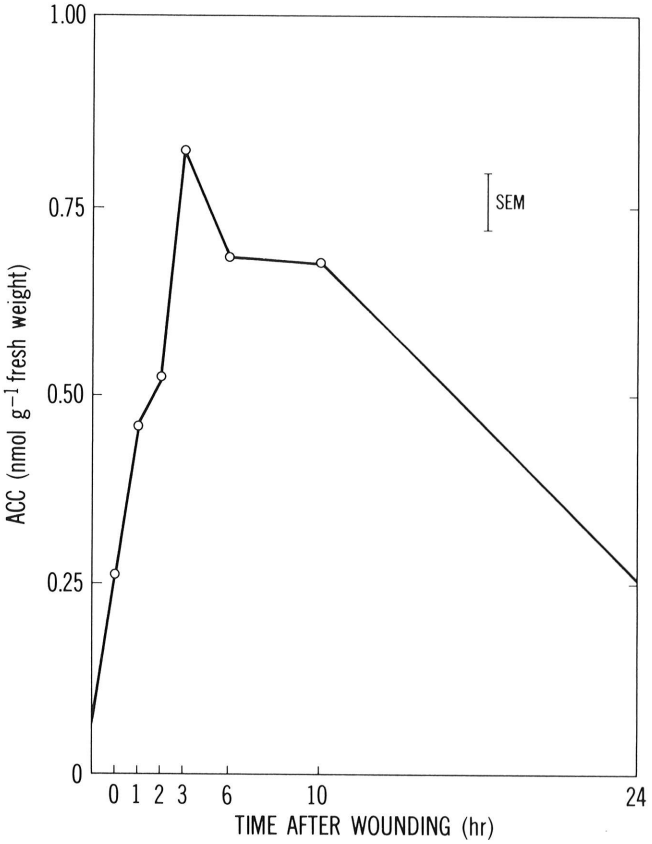

Fig. 2. 1-Aminocyclopropane-1-carboxylic acid content of mechanically wounded leaf blades of *Poa pratensis*.

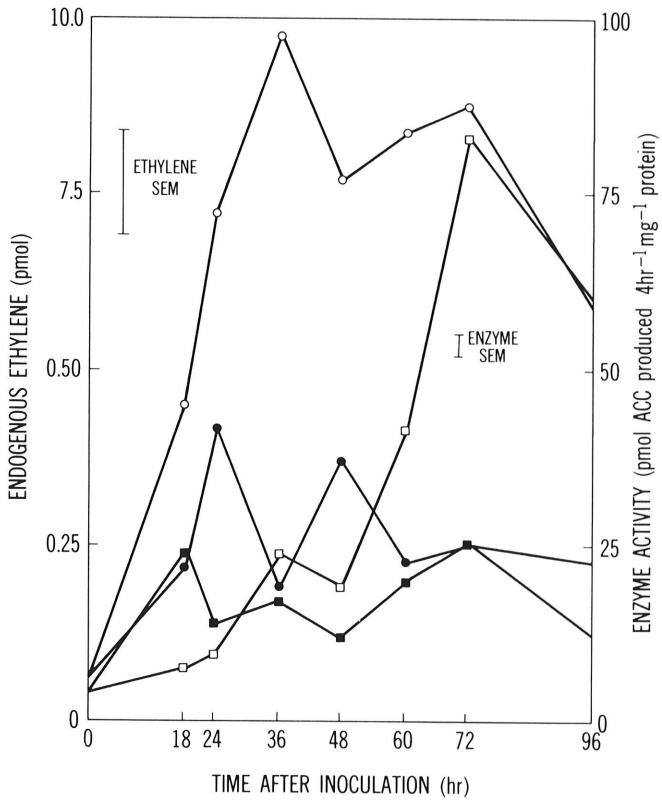

Fig. 3. Endogenous ethylene content and 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity of leaf blades of *Poa pratensis* infected with *Bipolaris sorokiniana*. Endogenous ethylene of control (•) and inoculated (o) plants. ACC synthase activity of control (■) and inoculated (□) plants.

comes primarily from a common pathway. However, the rapid, lower, and less sustained responses of ethylene, ACC synthase, and ACC to wounding (most occurred within 24 hr after wounding) (Figs. 1 and 2) compared with the slower, higher, and more sustained responses to infection (most occurred between 18 and 96 hr after inoculation) (Figs. 3 and 4) suggest that the origin of the intermediates required for the biosynthesis of ethylene in response to infection might be different. It has been demonstrated that the biosynthetic pathway(s) for ethylene in microorganisms differs from that of higher plants (7,20,29). Recent observations, however, show *B. sorokiniana* to produce ethylene from Met, to possess ACC synthase activity, and to secrete ACC into a synthetic culture media supplemented with Met. Although *B. sorokiniana* produces ACC, the conversion of ACC to ethylene by the pathogen is inefficient compared with the conversion of Met to ethylene (8). These differences suggest that the pathogen probably produces ethylene by more than one pathway (7,20,26). Several fungal pathogens need or can use Met for production of ethylene, but none have been shown to produce ACC (3,4,6,20). The fact that *B. sorokiniana* can produce ACC suggests that it may contribute to the increase in ethylene via the higher plant pathway during pathogenesis.

The ability of *B. sorokiniana* to produce ACC (8) provides the basis for a working hypothesis relative to the early rise in ethylene and the late accumulation of ACC in the host-pathogen interaction (Figs. 3 and 4). The early rise in ethylene (18–24 hr) (Fig. 3) after inoculation precedes any significant rise in ACC synthase or ACC (Figs. 3 and 4). It is possible that this early increase in ethylene is of pathogen origin and from an alternate pathway (8). Endogenous ethylene peaked 36 hr after inoculation and remained relatively high through 72 hr. The period between 36 and 72 hr is accompanied by increasing ACC synthase activity (Fig. 3) and substantial (though erratic) increases in ACC (Fig. 4). The increase in ACC synthase activity and ACC between 36 and 72 hr after inoculation is probably of host origin. It is possible, however, that ACC also may be secreted into the host tissue by the pathogen (8) during this period and that the ACC may be metabolized to ethylene by the host tissue. During this period of infection, the host tissue has not yet succumbed to the pathogen; any potential metabolism of pathogen-supplied ACC by the host at this point in pathogenesis could, in part, account for the high levels of endogenous ethylene (compared with wounding) in infected tissue between 36 and 72 hr (Fig. 3) and may be responsible for chlorotic symptom expression (12).

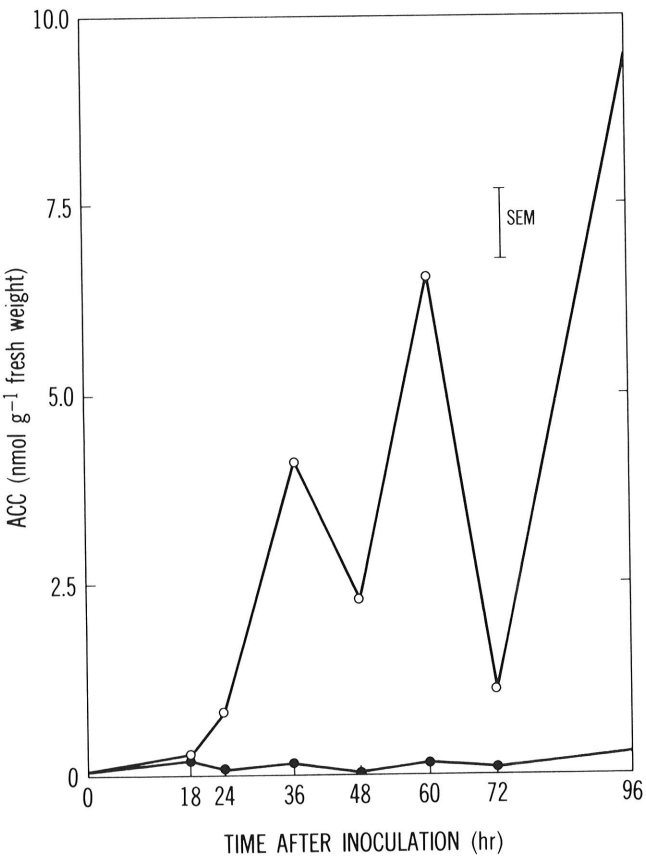

Fig. 4. 1-Aminocyclopropane-1-carboxylic acid content of leaf blades of *Poa pratensis* infected with *Bipolaris sorokiniana*. Control (•) and inoculated (o) plants.

The accumulation of ACC in infected host tissue late in pathogenesis (96 hr) (Fig. 4) suggests that *B. sorokiniana* may produce ACC in the host tissue. By 96 hr after inoculation, infected leaf blade sections are severely chlorotic with large necrotic areas. It is reasonable to assume that at this stage of disease development, normal physiological functions of the leaf tissues have for the most part succumbed to catabolism. The only vital entity in the host tissue is the heterotrophic pathogen. Under these circumstances, it seems plausible that the accumulation of ACC in the dead and dying leaf tissue may be of pathogen origin (8) due to the inability of dying host tissue to metabolize the ACC to ethylene and to the relatively inefficient conversion of ACC to ethylene by the pathogen (8). The fluctuations in ACC content of infected leaves between 24 and 72 hr (Fig. 4) may be related to variations in the rate of infection or in development of symptoms. Ethylene production also can be rhythmic (22,23), and fluctuations in ACC content of infected leaf blades from 24 to 72 hr (Fig. 4) may be related to diurnally regulated conversion of ACC to ethylene (11,28).

Accumulation of ACC in necrotic tissue has been observed in other host-pathogen interactions. ACC accumulation in grapefruit tissue infected by *Penicillium digitatum* is believed to be of host origin and due to impaired ability of the host tissue to convert ACC to ethylene (1,2). A similar accumulation of ACC in virus-infected tobacco leaves has been observed in the lesions and tissues directly adjacent to the lesions (9). The accumulation of ACC in *P. digitatum*-infected grapefruit is attributed to impairment of host-cell membranes to convert ACC to ethylene in cells still capable of ACC synthesis. Unlike *B. sorokiniana*, there is no evidence that *P. digitatum* produces ACC. The potential for ACC production by *B. sorokiniana* prevents an interpretation that would exclude any contribution of ACC by the pathogen to dead and dying tissue. There also exists the problem of how long cells with impaired membranes can actively synthesize ACC. Therefore, we must tentatively conclude from our observations of *B. sorokiniana*, and from its interactions with *P. pratensis* during pathogenesis, that some potential contribution of intermediates by the pathogen to the higher plant biosynthetic pathway of ethylene may occur. However, much additional research on the pathogen and on the host-pathogen interaction will be needed before conclusive evidence can be provided.

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Disease Control and Pest Management

Control of Metalaxyl-Resistant Causal Agents of Late Blight in Potato and Tomato and Downy Mildew in Cucumber by Cymoxanil

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ABSTRACT

Cohen, Y., and Grinberger, M. 1987. Control of metalaxyl-resistant causal agents of late blight in potato and tomato and downy mildew in cucumber by cymoxanil. Phytopathology 77:1283-1288.

A relatively high dosage of cymoxanil foliar spray was required to control late blight in potato and downy mildew in cucumber in growth chambers. ED_{90} values for control of metalaxyl-sensitive field isolates of *Phytophthora infestans* ranged between 164 and 459 μg/ml, and for metalaxyl-resistant isolates, between 112 and 525 μg/ml. ED_{90} values for control of metalaxyl-resistant isolates of *Pseudoperonospora cubensis* ranges between 201 and 878 μg/ml. Complete control of both pathogens was achieved at concentrations between 500 and >1,000 μg/ml. Preventive and curative efficacy of the fungicide lasted for 5 and 3 days, respectively. Cymoxanil was readily taken up by leaves, roots, and stems. When applied to roots and stems, it showed acropetal systemic translocation in cucumber and tomato but not in potato. No systemic translocation occurred when applied to leaf laminae. Translaminar translocation occurred in potato and tomato but not in cucumbers. The fungicide was toxic to roots of tomato plants.

Additional key words: Oomycetes, systemic fungicides.

Cymoxanil (Curzate), a systemic fungicide selectively active against fungi of the Peranosporales, has been commercially available since 1979 (8,14). In plants, it has a half-life of only a few days (11); therefore, it is more effective when combined with either a protectant (e.g., mancozeb), another systemic (e.g., oxadixyl or propramocarb), or with both a systemic and a protectant (1,3, 8–11,14,15). In Europe, cymoxanil used in a foliar spray at a relatively low concentration of 80–112 μg/ml demonstrated good preventive and curative activity against grape downy mildew and