Ethylene Production by *Bipolaris sorokiniana* and *Curvularia geniculata* on Methionine and Inorganic Salts

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ABSTRACT

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Chlorosis of *Poa pratensis* leaves infected by *Bipolaris sorokiniana* is due in part to elevated levels of endogenous ethylene in response to infection. The severity of the disease also is often enhanced by fertilization. Chlorosis does not occur in response to leaf infection by *Curvularia geniculata*, and the lesions produced are not affected by fertilization. *B. sorokiniana* requires methionine (MET) for the production of ethylene, but the effect of fertilizer salts on ethylene production by the pathogen is unknown. The ability of *C. geniculata* to produce ethylene on MET or fertilizer salts is unknown. Research was initiated to evaluate the ability of *B. sorokiniana* and *C. geniculata* to produce ethylene on a complex medium (leaf blade infusion of *P. pratensis*) and on a synthetic medium amended with 1 and 10 mM MET, $(NH_4)_2SO_4$, NH_4NO_3, or K_2SO_4. The two organisms produced ethylene in similar quantities on the unamended complex medium; no detectable level of ethylene was produced by either organism on the unamended synthetic medium. The addition of 1 or 10 mM MET to both media resulted in increased ethylene production by both organisms. Production was greatest (2,600–3,000 pmol) on the complex medium, with the two organisms producing similar amounts, except that production by *C. geniculata* declined after 5 days of growth on the complex medium amended with 10 mM MET. The two organisms produced similar amounts of ethylene on the MET-amended synthetic medium, but not as much as on the complex medium. The inorganic salts at 1 or 10 mM had no effect on ethylene production by either organism when added to the complex medium, and at 1 mM they had no effect in the synthetic medium. The addition of 10 mM $(NH_4)_2SO_4$ or NH_4NO_3 to the synthetic medium stimulated low-level ethylene production by *B. sorokiniana* but had little effect on *C. geniculata*.

Bipolaris sorokiniana (Sacc.) Shoem. and *Curvularia geniculata* (Tracy & Earle) Boedijn cause leaf spot diseases of *Poa pratensis* L. (3,8,14). *B. sorokiniana* is a strong pathogen that produces leaf lesions surrounded by chlorotic halos and eventually chlorosis of tissue beyond the lesions (13). *C. geniculata* is a weak leaf pathogen that produces small lesions without halos (14). Some infected leaves show chlorosis at infected cut ends, but general chlorosis of leaves does not occur (12). Increases in endogenous ethylene in leaves infected by *B. sorokiniana* is responsible for much of the leaf chlorosis beyond the immediate area of the lesion (13).

Methionine (MET) enhances or is required for the production of ethylene by several fungal organisms (1,2,4,9, 20–22). The utilization of MET by *B. sorokiniana* to produce ethylene and other intermediates of the higher-plant biosynthetic pathway of ethylene suggests that this pathogen may contribute directly or indirectly to the increase in endogenous ethylene during pathogenesis (6,7). The ability of *C. geniculata* to produce ethylene in the presence of MET is unknown.

Fertilizer salts increase the severity of infection of *P. pratensis* by *B. sorokiniana* (5,10,11,18) and infection of germinating seeds of *Festuca rubra* L. by *C. geniculata* (17). The direct effect of inorganic salts on the production of ethylene by fungal organisms is essentially unknown. Phosphate concentration can affect ethylene production by *Penicillium digitatum* (15), but nothing is known of the effects of nitrogen and potassium salts. The purpose of the research presented is to determine the ethylene-producing capabilities of two closely related fungal pathogens, with different pathogenic characteristics, in the presence of MET and several fertilizer salts.

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MATERIALS AND METHODS

Plant materials and culture media. *B. sorokiniana* and *C. geniculata* were cultured on 20 ml of 3% (w/v) Bacto agar in 100- × 15-mm sterile plastic petri dishes at 20 C for at least 20 days. The organisms were transferred from the agar to liquid media to determine the effect of salt treatments on ethylene production. A leaf blade infusion (complex medium) of *P. pratensis* and a synthetic medium were used as control media. The complex medium was prepared from shredded *P. pratensis* leaves (150 g, fresh weight) boiled in 2 L of deionized distilled water for 1 hr. The leaves were briefly homogenized in a blender to disrupt the tissue. The homogenate was filtered through cheesecloth, and the grass broth filtrate diluted with deionized distilled water to 10 L. The synthetic medium consisted of 3 g of sucrose, 0.1 g of dipotassium phosphate, and 0.05 g of potassium chloride in 1 L of deionized distilled water.

Treatments and ethylene analysis. Treatments consisted of the complex and the synthetic media amended with 1 or 10 mM $(NH_4)_2SO_4$, NH_4NO_3, K_2SO_4, or L-MET. The unamended complex and synthetic media served as controls. Each treatment medium (100 ml) was placed in a 125-ml Erlenmeyer flask stoppered with a Styrofoam plug, autoclaved 30 min, and cooled. Two 4-mm plugs of *B. sorokiniana* or *C. geniculata* on Bacto agar were added to the media. The cultures were incubated statically at 21 C with a 12-hr photoperiod and an irradiance of 27 $\mu E \cdot m^{-2} \cdot sec^{-1}$.

For ethylene analysis, 1.0-ml gas samples were taken from the headspace of flasks every 24 hr for 7 days after 48 hr of incubation. The Styrofoam plugs in the flasks were replaced by serum caps during each 12-hr dark period of the 7 days, to trap the ethylene evolved after each dark period. The 1.0-ml gas sample removed from the headspace of a flask was injected into a gas chromatograph (GC) (Varian 3700) with an injector temperature of 150 C, a column temperature of 155 C, a flame ionization detector temperature of 250 C, and a flow rate of 30 $cm^3 \cdot min^{-1}$ for the carrier gas (He). The signal from the flame ionization detector was connected to a Cary (model 401) electrometer coupled to a Spectra-Physics 4100 computing integrator. After calibration, sample data were plotted and quantified by the computing integrator. Ethylene concentrations were expressed in picomoles.

The experiment was a split-plot design. The studies were repeated four times with each treatment medium. Means among fungal organisms and within salt treatments, and means among salt treatments and within fungal organisms were analyzed by the *F*-test and Fisher's least significant difference ($P = 0.05$).

RESULTS

Ethylene production on the complex

medium. Both *B. sorokiniana* and *C. geniculata* produced ethylene on the complex medium (Fig. 1). Ethylene production by *B. sorokiniana* on the complex medium was greater than that of the control blank on days 6 and 7, as was production by *C. geniculata* on days 5, 6, and 7, with no difference between the amounts produced by the two organisms. Production by either organism on the complex medium amended with 1 or 10 mM $(NH_4)_2SO_4$, NH_4NO_3, or K_2SO_4 did not differ from that of the amended or unamended complex medium blanks.

Ethylene production by both organisms grown 3 days or longer on the complex medium amended with 1 or 10 mM MET was substantially greater than that of MET-amended blanks (Fig. 2). The two organisms did not differ in ethylene production in response to 1.0 mM MET; production by both organisms increased to day 6 and then declined. On the complex medium amended with 10 mM MET, production by *B. sorokiniana* increased rapidly from day 3 to day 8, whereas production by *C. geniculata* increased from day 3 to day 5 and then decreased until it was significantly lower than that of *B. sorokiniana* by day 8 (Fig. 2).

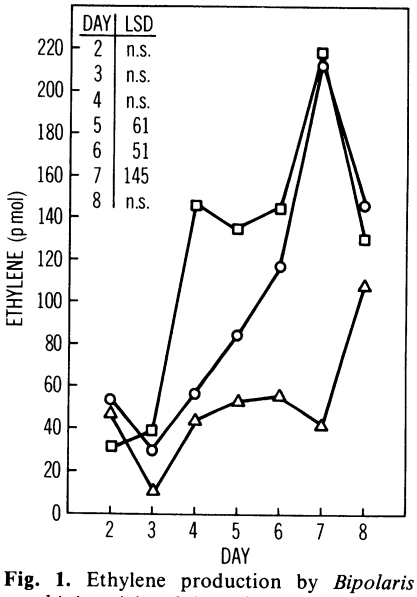

Fig. 1. Ethylene production by *Bipolaris sorokiniana* (o) and *Curvularia geniculata* (□) on the complex medium and by the control blank (Δ). LSD = least significant difference ($P = 0.05$); n.s. denotes that the *F*-test was not significant at that level.

Ethylene production on the synthetic medium. Ethylene evolution from the unamended synthetic medium was 0–20 pmol, and ethylene production by *B. sorokiniana* and *C. geniculata* on this medium was not significantly different from that of the medium blank. The addition of 1.0 mM $(NH_4)_2SO_4$, 1.0 mM NH_4NO_3, or 1.0 or 10 mM K_2SO_4 to the synthetic medium did not affect ethylene production by either organism. Production by *B. sorokiniana* on the synthetic medium amended with 10 mM $(NH_4)_2SO_4$ was significantly greater than that of the respective control blank on days 3, 4, 5, and 7, and on the synthetic medium amended with 10 mM NH_4NO_3 it was significantly greater on days 6, 7, and 8 (Fig. 3). Production by *C. geniculata* on the synthetic medium amended with 10 mM $(NH_4)_2SO_4$ was greater than that of the control blank only on day 4; the 10 mM NH_4NO_3 amendment did not increase production by *C. geniculata* (Fig. 3). Ethylene was not detected in the control blanks of the synthetic medium amended with $(NH_4)_2SO_4$ or NH_4NO_3.

The increased production of ethylene by *B. sorokiniana* and *C. geniculata* on the synthetic medium amended with 1.0 mM MET was significant on days 7 and 8 and on days 5, 7, and 8, respectively (Fig. 4); on the medium amended with 10 mM MET, production by both organisms was significantly greater than that of the control from day 4 to day 8 (Fig. 4).

DISCUSSION

B. sorokiniana and *C. geniculata* were capable of producing ethylene on the complex medium derived from host plant leaves, but they produced very little on the synthetic medium. The addition of MET to either medium resulted in substantial increases in ethylene production by both organisms (Figs. 2 and 4).

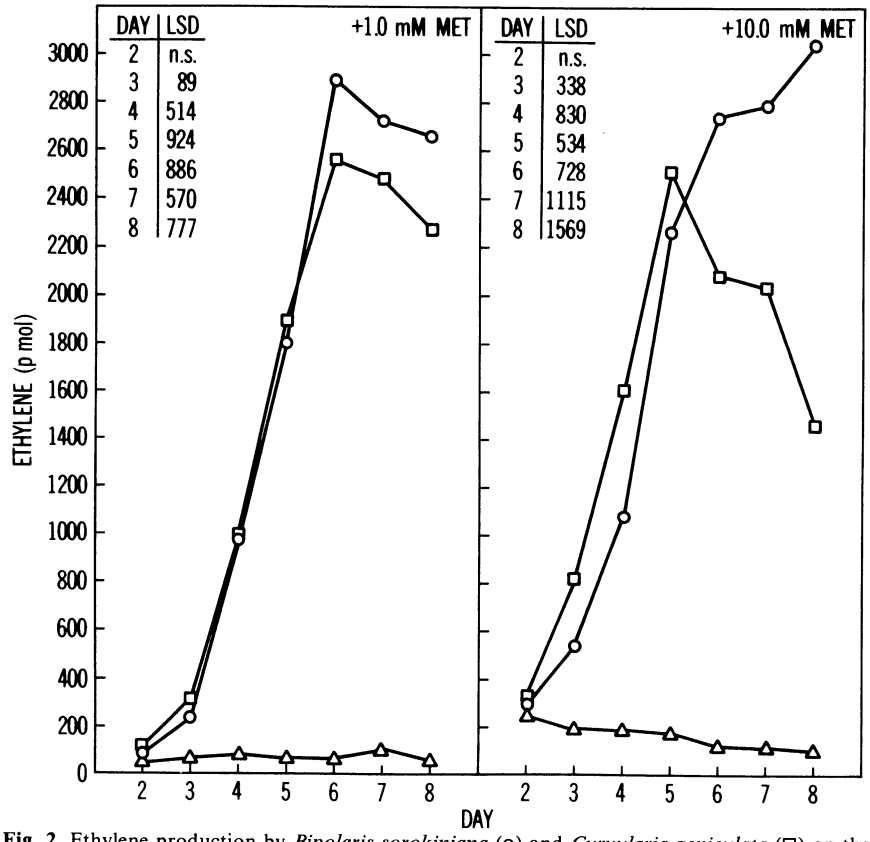

Fig. 2. Ethylene production by *Bipolaris sorokiniana* (o) and *Curvularia geniculata* (□) on the complex medium amended with 1 and 10 mM methionine (MET) and by the amended blanks (Δ). LSD = least significant difference ($P = 0.05$); n.s. denotes that the *F*-test was not significant at that level.

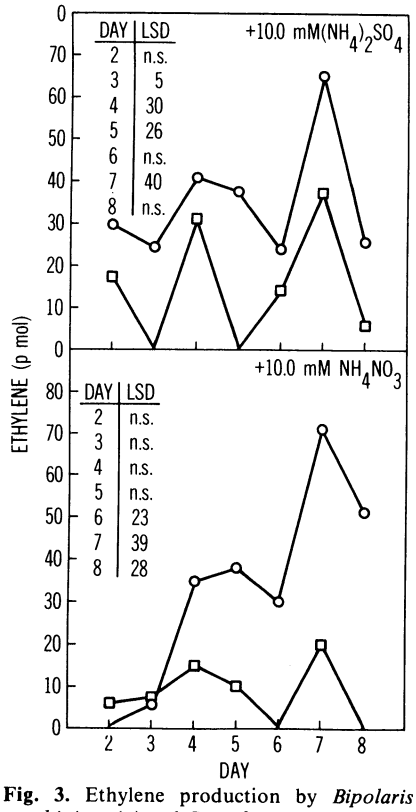

Fig. 3. Ethylene production by *Bipolaris sorokiniana* (o) and *Curvularia geniculata* (□) on the synthetic medium amended with 10 mM $(NH_4)_2SO_4$ and NH_4NO_3. LSD = least significant difference ($P = 0.05$); n.s. denotes that the *F*-test was not significant at that level. No line is shown for the amended blanks, which failed to produce detectable levels of ethylene.

MET is a common constituent of grasses (16), and the complex medium probably provides MET or other metabolites that both organisms can utilize for ethylene production.

The production of ethylene by *B. sorokiniana* and *C. geniculata* varied on the complex and synthetic media amended with 10 mM MET. The decline in production by both organisms after 6 days on the complex medium amended with 1.0 mM MET (Fig. 2) suggests that available MET may have been exhausted by both organisms. The decline in production by *C. geniculata* after 5 days on the complex medium amended with 10 mM MET (Fig. 2) is suggestive of autoinhibition of ethylene biosynthesis, like that observed in some higher plants (19,23). The continuing increase in production by *B. sorokiniana* on the complex medium amended with 10 mM MET (Fig. 2) suggests that MET availability is not the limiting factor in the decrease in production by *C. geniculata*.

Ethylene production by both organisms on the synthetic medium amended with 1.0 mM MET was relatively low (Fig. 4), compared to that on the complex medium amended with 1.0 mM MET (Fig. 2). Although there was no decrease in production by *C. geniculata* on the synthetic medium amended with 10 mM MET (Fig. 4), maximum production (about 2,100 pmol) required 8 days of growth and was below the maximum (about 2,500 pmol) produced on days 6 and 5 on the complex medium amended with 1 and 10 mM MET, respectively (Fig. 2). The difference between the quantity of ethylene produced by both organisms on the MET-amended complex medium and the quantity produced on the synthetic medium is probably related to metabolites in the complex medium.

The increase in ethylene production by *B. sorokiniana* on the synthetic medium amended with 10 mM $(NH_4)_2SO_4$ or NH_4NO_3 was not shared by *C. geniculata* (Fig. 3). Maximum production by *B. sorokiniana* was between 60 and 70 pmol; this very low level suggests that the metabolism of these salts by *B. sorokiniana* may contribute in some manner to ethylene biosynthesis. It is of interest to note that the synthetic medium amended with 1 and 10 mM $(NH_4)_2SO_4$ contained 0.5–1.27 mM MET per milliliter after supporting the growth of *B. sorokiniana* for 8 days,, and it contained 0–0.22 mM MET per milliliter after 8 days of growth of *C. geniculata*. These were not controlled or replicated observations, but they may suggest that *B. sorokiniana* is capable of greater MET biosynthesis than *C. geniculata*. This could account for the inability of *C. geniculata* to produce significant amounts of ethylene on the synthetic medium. Future research will explore this hypothesis.

P. pratensis infected by *B. sorokiniana* is often more severely diseased under conditions of high fertility than under low or normal fertility (18). Ethylene is partly responsible for the general chlorosis of infected leaves of *P. pratensis* (13), and perhaps the fertilizer salts stimulate the pathogen to contribute additional ethylene during pathogenesis. Ethylene production by *C. geniculata* is not influenced by fertilizer salts, and there is no known ethylene-chlorosis relationship associated with the small lesions produced by this organism.

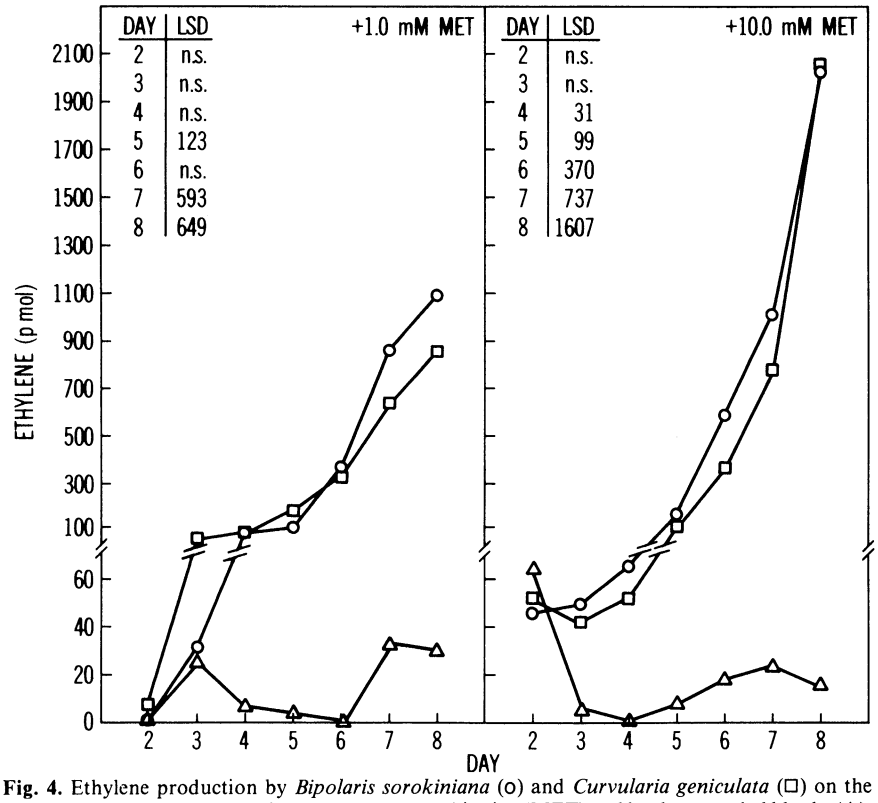

Fig. 4. Ethylene production by *Bipolaris sorokiniana* (o) and *Curvularia geniculata* (□) on the synthetic medium amended with 1 and 10 mM methionine (MET) and by the amended blanks (Δ). LSD = least significant difference ($P = 0.05$); n.s. denotes that the F-test was not significant at that level.

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