Effects of Virulent and Hypovirulent *Endothia parasitica* and Their Metabolites on Ethylene Production by Bark of American and Chinese Chestnut and Scarlet Oak

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ABSTRACT

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Ethylene production was stimulated in bark of American chestnut and scarlet oak up to 8 cm from wounds or expanding initial lesions caused by virulent and hypovirulent strains of *Endothia parasitica*, before lignification. Stimulation of ethylene production ceased when, or soon after, a lignified zone formed and initial lesions stopped expanding. The stimulation of ethylene production was repressed 0.5–2 cm from the expanding initial lesion in American chestnut inoculated with virulent *E. parasitica* but not in the other host-treatment combinations. On American chestnut, stimulation of ethylene production with repression close to the canker also was observed for naturally occurring cankers that were expanding because of growth of mycelial fans. Stimulation of ethylene production was not observed for naturally occurring cankers on scarlet oak or Chinese chestnut, possibly because the cankers were not expanding. Mycelium-agar disks of virulent and hypovirulent *E. parasitica* stimulated ethylene production in bark plugs of American and Chinese chestnut and scarlet oak as compared with controls. American chestnut was stimulated more than scarlet oak and Chinese chestnut. Oxalate, a known metabolite of *E. parasitica*, inhibited ethylene production in bark plugs of chestnut and oak. Sterile filtrates of virulent and hypovirulent *E. parasitica* cultures stimulated ethylene production in American chestnut when the fungi were grown on chestnut or oak bark-extract broth but not when the fungi were grown on potato-dextrose broth. Thus both the stimulatory factor(s) and the repressive factor(s) may be present in culture filtrates.

The mechanism for the extraordinary susceptibility of American chestnut (*Castanea dentata* (Marsh.) Borkh.) to blight, caused by *Endothia parasitica* (Murr.) P. J. & H. W. And., is unknown. This is true also for the mechanism by which the agents of cytoplasmic hypovirulence render *E. parasitica* hypovirulent. McCarroll and Thor (12) found a pectinase inhibitor in blight-resistant Chinese chestnut (*C. mollissima* Blume) that was more active than a similar fraction from American chestnut. They also exposed inner bark of Chinese and American chestnut to oxalate buffers and reported that Chinese chestnut bark turned brown at a lower concentration or a higher pH (11). Samman et al (15) found a lipid-phase growth inhibitor of *E. parasitica* in Chinese chestnut bark that was more active than a similar fraction in American chestnut bark. Finally, Hebard and Kaufman (8), building on previous work, found a strong correlation between hydrolysable tannin concentration and blight resistance in callus cultures. The significance of the last finding is obscured by the ability of *E. parasitica* to degrade chestnut tannins (1,4). However, at physiological concentrations, chestnut tannins do inhibit the growth of *E. parasitica*, and tannins from Chinese chestnut are reported to be more inhibitory than tannins from American chestnut (13).

Hebard et al (7) found that the rapidity of formation and growth of mycelial fans, rather than the rapidity of formation and rate of extension of wound periderm, distinguished blight-resistant from blight-susceptible chestnut and virulent from hypovirulent *E. parasitica*. This finding suggested to them that the ability of the fungus to obtain nutrition from colonized chestnut bark, and to not be inhibited by it, was crucial for canker development. It also suggests that events that occur between artificial inoculation and mycelial fan formation 20–40 days later can serve as a model system for the critical aspects of pathogenesis.

Shain and Wheeler (17) found that oats resistant to victoria blight produced much less ethylene than susceptible oats when exposed to victorin. This probably reflects the greater damage that victorin inflicts on susceptible oats. Ethylene production in plants generally is enhanced during stress and has been associated with events leading to resistance to plant disease (3). The speed and ease with which ethylene can be measured make it a useful assay of plant damage. The objectives of the current study were to measure ethylene production by hosts differing in resistance to *E. parasitica* after artificial inoculation by virulent and hypovirulent strains and to compare that to known events during canker development, to explore the utility of ethylene measurements in determining the blight-resistance of hosts of *E. parasitica*, and to develop an ethylene-based assay for toxic metabolites of *E. parasitica*. Preliminary reports have been published (9,10).

MATERIALS AND METHODS

Host. American chestnut served as the susceptible host while Chinese chestnut and scarlet oak (*Quercus coccinea* Muenchh.) served as resistant hosts. Trees were located in Powell and Harlan counties, Kentucky. Naturally occurring, basal cankers associated with *E. parasitica* are particularly prevalent on scarlet oak in Powell County. In general, these species were growing on different sites, so effects of environment and genetics were confounded. Differences in innate blight-susceptibility between the resistant and susceptible hosts, however, are sufficiently great to outweigh this relatively minor environmental component.

Pathogen. A virulent strain of *E. parasitica*, Ep155, has been infected separately with the hypovirulence agents H_{I1}, H_{I2}, H_{M2}, or H_{T2} to form hypovirulent strains designated Ep779, 780, 868, and 905, respectively. Hypovirulence agents I_1 and I_2 originated in Italy, while M_2 and T_2 originated in Michigan and Tennessee, respectively (5). Fungi were grown in the laboratory on Difco potato-dextrose agar amended with 100 mg of methionine per liter and 1 mg of biotin per liter at ambient temperature (23–27 C) and light (16 hr/day, about 1.4 μE m^{-2} s^{-1} from cool-white fluorescent tubes). Subcultures were checked to ensure they retained appropriate morphology. At intervals of approximately 3 mo, fresh cultures were started from stock cultures maintained on agar slants at 4 C.

Ethylene measurement. Bark plugs (0.4 cm diameter, 0.2–0.8 cm thick) were collected with an increment hammer. The plugs consisted of all living bark tissue systems, i.e., functioning and nonfunctioning secondary phloem, cortex, and periderm. Dead outer bark (rhytidome) and xylem, if present, were removed from plugs before incubation. When bark plugs were collected from trees with cankers, each was sealed in a separate 6-ml Vacutainer tube and the tubes cooled on ice until all plugs were collected. Sealed Vacutainer tubes then were incubated in the dark for about 20 hr at 25 C. Ethylene was determined by withdrawing 1 ml of gas and injecting it into a gas chromatograph equipped with a column of activated alumina and a flame ionization detector (16). Ethylene production is expressed as nl hr^{-1} g^{-1} dry weight. Preliminary tests indicated that ethylene production was linearly proportional to weight, within treatments, over the range encountered during this study. Within a species, the weight of a plug was directly proportional to its thickness. The log of ethylene evolution was used to equalize variances for statistical analysis.

Ethylene production near naturally occurring cankers. Three trees were sampled from each of two sites per species. One set of sites was sampled 6 May 1986 and the other on 13 May. The American chestnuts were 5–8-yr-old sprouts that were 2–6 cm in diameter at breast height (dbh, 1.4 m above ground). They were growing in clearcuts. Each tree had only one canker, which was located within 1 m of the ground. The cankers did not encircle the trees. The Chinese chestnuts were of seedling origin, about 12 cm dbh, and about 20 yr old. Each tree had multiple cankers. We selected trees with only one canker on a 4-m length of the bole. The cankers were within 3 m of the ground. The scarlet oak were about 25 cm dbh growing in wooded areas last cut about 50 yr previously. The scarlet oak also had multiple cankers. These cankers were particularly prevalent at the base of the trees, where they were associated with a swollen butt. Samples were obtained from uncankered tissue 1,2,4,8,16,32,64, and 128 cm up or down the stem from cankered tissue. In scarlet oak, sampling was done upward from the swollen butt.

Ethylene production near cankers incited by virulent and hypovirulent *E. parasitica*. Cankers were initiated using the cork-borer, agar-disk method (#2 cork borer) (6). Agar disks were obtained about 0.5 cm behind the growing edge of a colony 7–10 days old. The hosts were scarlet oak and American chestnut. The American chestnuts were in the understory of a site that had been selectively cut about 10 yr before. Thus, they were partially released from competition. Trees ranged from 3.5 to 7.7 cm dbh. The scarlet oak were growing at a site that had been clearcut about 8 yr before. They ranged from 2 to 6 cm dbh. Unblighted Chinese chestnut trees were not found in sufficient number for this experiment, but Chinese chestnut performed similarly to scarlet oak in preliminary trials.

Cankers were initiated on the same side of each tree in August 1986, with virulent Ep155 and hypovirulent Ep905. Inoculation points were 128 cm apart on American chestnut and 64 cm apart on the shorter scarlet oak. Rhytidome precluded use of larger scarlet oak. An uninoculated wound was made on the opposite side of each tree, midway between the two inoculation points, or at an equal distance above or below them. A nonwounded, control area was located 128 or 64 cm above or below the uninoculated wound on American chestnut or scarlet oak, respectively. This design ensured that the virulent and hypovirulent cankers were as far apart as possible, minimizing chances of cross-contamination. Between trees, the orientation and height of the two pairs of treatments on a tree and the relative height of the elements of each pair were randomized. The basal-most treatment point was 10 cm above ground on American chestnut and 30 cm above ground or the top of the rhytidome on scarlet oat. These four treatments comprised the first split in the split-split plot design employed. The second split was distance from each canker or wound. Two bark plugs were removed for ethylene determination at 0.5 cm and one plug at 2, 8, and 32 cm from each canker or wound. Two plugs were removed at 0.5 cm to increase the precision of measurement at that critical distance. Bark plugs were not obtained at distances other than 0.5 cm from the healthy, untreated region, so this treatment was excluded for statistical analysis. Trees were grouped in five blocks of four individuals of each species. A previously unsampled tree was selected randomly from each block 5, 12, 19, 26, and 47 days after inoculation (dai). Tissue samples also were obtained from one tree of each species at each collection date and processed for histopathological examination (7).

Rain destroyed some data for two replicates of scarlet oak at 5 dai and two replicates of American chestnut at 12 dai, so a Fuller-Batese transformation was used on the ethylene data and on the model before analysis by ordinary least squares regression (2). The unbalance also precluded treating distance as a repeated measures factor. Means were obtained as least squares estimates after the transformation. The significance of coefficients of a regression of log ethylene production on log distance for each species-treatment-date combination was assessed using the error term for the whole experiment. Because there were four replicated distances, the significance of linear, quadratic, and cubic trends could be assessed in the regression. Coefficients also were compared between combinations by orthogonal comparisons.

Interaction of bark with mycelium, culture filtrates, and oxalate. Bark plugs were obtained from one uncankered, previously unsampled sprout or branch of each species. The portion sampled was free of rhytidome. The scarlet oak and American chestnut were 3–8-yr-old sprouts growing in clearcuts. The Chinese chestnut were about 20-yr-old seedlings growing in wildlife plantings or gardens; plugs were obtained from branches about 5 yr old.

Bark plugs to be exposed to mycelium or agar were placed cambium-side down on 0.9-cm-diameter agar-mycelium or agar disks in Vacutainer tubes. Plugs to be exposed to culture filtrates or potassium oxalate were placed cambium-side down on 0.9-cm disks of No. 4 Whatman filter paper soaked with 20 μl of the treatment solution. Ethylene production was measured after incubation for about 20 hr. The tubes then were flushed with air, and ethylene production was determined after a second 20-hr incubation. These experiments were arrayed as randomized complete blocks, replicated five times. Some early experiments with oxalate were replicated fewer times; however, each experiment was repeated at least twice. Repetitions of major experiments were performed during both the growing and dormant seasons. The experiments described in detail in the Results were performed during the growing season. The treatments included host species, *Endothia* strain, and its growth medium, age of fungus culture, and location on the culture, and concentration and pH of test solutions. Means were separated by preplanned, orthogonal, single degree-of-freedom comparisons.

The fungal growth media included potato-dextrose broth amended with 1 mg of biotin per liter and 100 mg of methionine per liter (PDBmb), Puhalla and Anagnostakis (14) minimal medium (broth), and bark extract broth. Bark media were prepared by grinding fresh-frozen bark in a Wiley mill with dry ice. Within 1 hr of grinding, frozen bark meal was placed into hot (90–100 C) water at a concentration of 108 g/L and autoclaved.

RESULTS

Ethylene production near naturally occurring cankers. Ethylene was not evolved from agar-mycelium disks of *E. parasitica*, nor from colonized bark, which is necrotic. Thus, the ethylene evolved in these experiments was produced by the host.

Ethylene produced by bark plugs obtained at increasing distances from naturally induced cankers on Chinese and American chestnut and on scarlet oak is shown in Figure 1. Ethylene production was stimulated in plugs obtained near cankers on the American chestnut trees, as indicated by a significant ($p < 0.0001$) negative linear trend in a regression of the log of ethylene production versus the log of distance from the canker. The stimulation of ethylene production was repressed 0 and 1 cm as compared with 2 cm from cankers on American chestnut, as indicated by a significant ($p < 0.05$) cubic trend in the regression. The cubic trend also indicated a second bend in the curve, near 32 cm, where stimulation ceased.

There were no significant ($p < 0.05$) trends in the overall

regression of ethylene production on distance for Chinese chestnut and scarlet oak. However, some individual cankers did have a significant regression. These cankers may have been expanding, whereas cankers lacking stimulation of ethylene production may not have been expanding. These trees were sampled in May, but similar results were obtained for trees sampled in March and October 1986.

Ethylene production and canker development. Initial lesion growth was evident by 5 dai with Ep155 (virulent) and Ep905 (hypovirulent) in American chestnut and scarlet oak. Lignified zones were observed microscopically in radial sections of American chestnut at 12 dai and in scarlet oak at 19 dai. At 19 dai in American chestnut, the lignified zone was bypassed near the vascular cambium by individual hyphae or aggregates of one to five hyphae of Ep155 (virulent) but not Ep905 (hypovirulent). However, by 26 dai, the initial lesion of Ep155 was contained by a lignified zone in American chestnut. Wound periderm formation began 19 dai in wounded scarlet oak and 26 dai in scarlet oak inoculated with *E. parasitica*. Wound periderm was complete in all treatments on both scarlet oak and American chestnut 47 dai.

Ethylene production by bark plugs removed from American chestnut was stimulated at 5, 12, and 19 dai with Ep155 (virulent), as indicated by significant ($p < 0.05$) quadratic (Fig. 2A and C) or cubic (Fig. 2B) trends in the log ethylene versus log distance regression. As with natural cankers, the significant quadratic and cubic trends also indicated that stimulation was repressed adjacent to the canker margin, but peaked at 2–8 cm. Stimulation ceased by 26 dai (Fig. 2C and D). Stimulation resumed at 47 dai (Fig. 2E) when cankers began linear expansion due to growth of mycelial fans. There was no repression of stimulated ethylene production near these cankers at 47 dai, but repression was observed in the following growing season.

With Ep905 (hypovirulent) on American chestnut, initial lesion expansion ceased by 12 dai, and there was no statistically significant stimulation of ethylene production by 19 dai (Fig. 2A vs. B–E). With Ep905 and Ep155 on scarlet oak, initial lesion expansion ceased by 26 dai and there was no elevated production of ethylene beyond 26 dai (Fig. 2F–I vs. J). Thus, ethylene evolution was stimulated before the onset of lignification and persisted for about 1 wk after lesion expansion ceased. The lesion around uninoculated wounds did not expand; there was no stimulation of ethylene production near uninoculated wounds by 12 dai in American chestnut (Fig. 2A vs. B–E) and by 26 dai in scarlet oak (Fig. 2F–H vs. I and J).

There was no direct evidence for a repression in the stimulation of ethylene production with treatments other than Ep155 on American chestnut. Of those other treatments, there was only one instance of a quadratic or cubic trend, which occurred in the wounds on scarlet oak at 19 dai. It indicated that stimulation was occurring 0.5 cm from the wound but not at other distances (Fig. 2H). However, the lack of significant stimulation near Ep905 cankers on American chestnut at 5 dai while it was occurring near Ep155 or noninoculated wounds (Fig. 2A) suggests repression may have been occurring. The delay until 12 dai in stimulation of ethylene production with all treatments on scarlet oak (Fig. 2F and G), and the longer persistence of stimulation on scarlet oak than American chestnut (Fig. 2I vs. D), may reflect differing patterns of lesion growth and wound closure: lignified zones did not begin to form in scarlet oak until 19 dai, whereas they began to form in American chestnut at 12 dai; lignified zones were complete and initial lesion growth ceased around 26 dai in scarlet oak compared with 19 dai in American chestnut.

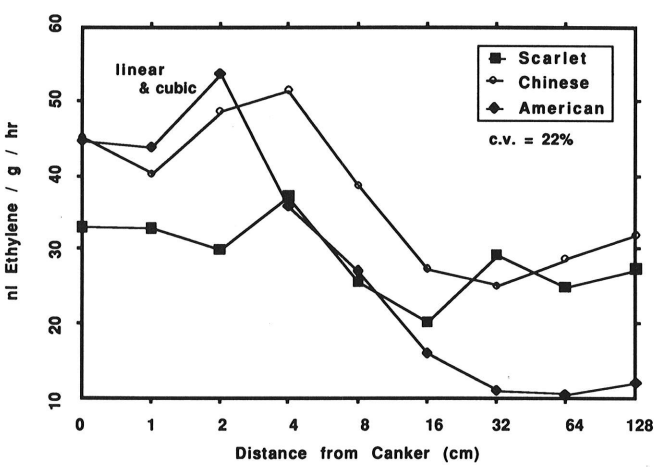

Fig. 1. Rates of ethylene production by bark plugs of Chinese or American chestnut or scarlet oak during the first day after their collection at increasing distances from naturally occurring *Endothia parasitica* cankers. Curves with significant ($p < 0.05$) linear, quadratic, and cubic trends in an ethylene-distance regression are so indicated. Error is expressed as the coefficient of variation (c.v.).

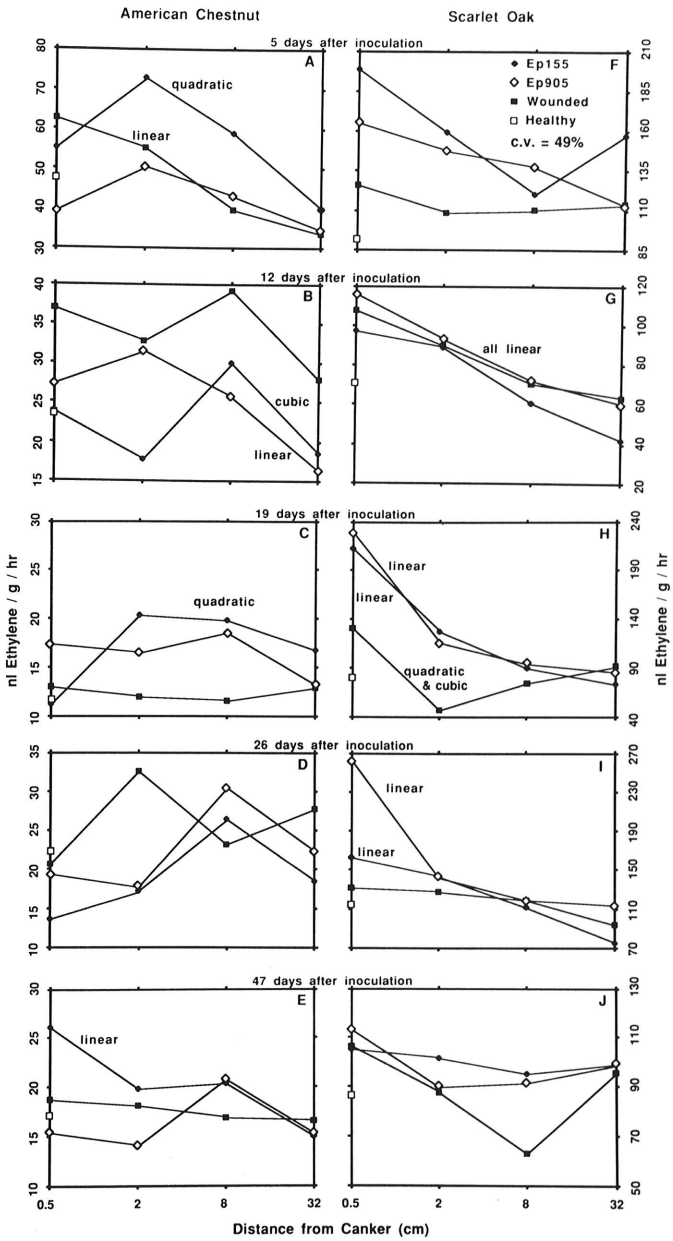

Fig. 2. Rates of ethylene production by bark plugs of American chestnut (A–E) or scarlet oak (F–J) during the first day after their collection at increasing distances from wounds or cankers induced by virulent (Ep155) or hypovirulent (Ep905) strains of *Endothia parasitica* at 5–47 days after inoculation, with nonwounded, healthy controls (on y axis only). Curves with significant ($p < 0.05$) linear, quadratic, and cubic trends in an ethylene-distance regression are so indicated. Error is expressed as the coefficient of variation (c.v.).

Ethylene production by bark plugs exposed to mycelium. On exposure to mycelium from 12-day-old cultures of *E. parasitica*, ethylene production by American chestnut bark plugs was stimulated ($p < 0.001$) about fourfold over plugs exposed only to agar, or untreated plugs exposed only to air (Fig. 3). Inoculated Chinese chestnut bark plugs were stimulated ($p < 0.001$) about twofold over similar controls, a significant ($p < 0.001$) difference from the American chestnut. Scarlet oak was stimulated significantly ($p < 0.001$) less than Chinese chestnut. In other repetitions of this experiment, mycelium of *E. parasitica* did not stimulate scarlet oak bark plugs significantly ($p > 0.05$) more than the agar control. While mycelium from the margin of a 12-day-old culture stimulated ethylene production more in American chestnut than in Chinese chestnut and scarlet oak, similar mycelium from 5-day-old cultures did not. In some experiments, there were differences in ethylene production by bark plugs exposed to virulent and hypovirulent fungal strains, but, while these differences were consistent between host species, they were inconsistent between experiments. The inconsistencies were due to variation in the response to the hypovirulent strain.

Ethylene production by bark plugs exposed to fungal metabolites. Compared with fungus-free controls, ethylene production in American chestnut bark plugs was stimulated ($p < 0.001$) by filtrates from cultures grown on bark-extract broths for 5, 7, 11, 14, and 21 days. Figure 4 shows representative data for 11-day-old cultures grown on bark broths and PDBmb. Filtrates from bark broths that had supported either Ep155 or Ep905 stimulated ethylene production similarly ($p > 0.05$). With PDBmb, significant ($p < 0.05$) stimulation occurred for filtrates from 5-day-old cultures, but not for older cultures. In subsequent trials, filtrates from Puhalla and Anagnostakis (14) minimal medium also did not stimulate ethylene production.

Ethylene production by bark exposed to oxalate. Ethylene production was not stimulated at any concentration of oxalic acid-potassium oxalate solutions, from 0.01 to 0.5 M, at pH 3 to 7. Ethylene production was not detected at oxalate concentrations above about 0.1 M. Below about 0.1 M, ethylene production was similar to the water control. There was no significant ($p > 0.05$) effect of the pH of oxalate solutions on ethylene production. An oxalate concentration was not found at any pH where the three hosts differed in ethylene production or browning of inner bark, despite extensive tests with up to 12 replicates.

DISCUSSION

Ethylene production in the bark of all hosts tested was stimulated up to 8 cm from *E. parasitica* cankers when they were expanding. This result, and those of our other assays, are consistent with the hypothesis that the degree of ethylene stimulation reflects the degree of damage inflicted on the host by *E. parasitica*. However, the hypothesis needs to be modified with the caveat that host cells are stimulated as long as they are not damaged too much. The stimulation near virulent cankers on American chestnut was repressed adjacent to the canker margin. This may reflect the impending death of host cells in that region, which occurs at least 350 μm in front of the mycelial fan (7); ethylene synthesis is an active process requiring the metabolism of live cells. The stimulation of ethylene production was not repressed close to the canker in scarlet oak inoculated with virulent *E. parasitica* or in scarlet oak or American chestnut inoculated with a highly debilitated, hypovirulent *E. parasitica*. This lack of stimulation was associated with smaller initial lesions; the damage to host tissues occurring around those smaller lesions may not have been sufficient to repress the stimulation of ethylene evolution. An interesting question for further research is what role, if any, this repression of stimulation has in the extraordinary pathogenicity of *E. parasitica* to American chestnut.

Mycelium of *E. parasitica* stimulated ethylene production by bark plugs, and the degree of stimulation was inversely correlated with blight resistance. This system appears promising as a rapid assay of blight resistance. A comparable assay for pathogenicity did not emerge from the present work. It is possible that hypovirulence agents do not reduce fungal pathogenicity after only 1 or 2 days' growth of hyphae into bark plugs, especially if replication of the agents lags behind initial growth of the hyphae. Hebard et al (7) saw no differences between a virulent and a moderately aggressive, hypovirulent *E. parasitica* until mycelial fans formed. In the current work, ethylene production by bark plugs exposed to mycelium varied with the age of fungal cultures and varied between experiments for some hypovirulent strains. This may have reflected the ability of the fungi to colonize the bark plugs rapidly and extensively.

The strong stimulation of ethylene production in American chestnut bark plugs by culture filtrates of *E. parasitica* parallels the response we observed in the whole plant and in bark plugs exposed to mycelium. Thus, it appears to be a good bioassay for toxic metabolites of that fungus. Oxalate, which has been proposed as an important toxin in *E. parasitica* (11), only inhibited ethylene production in bark plugs. Thus, some factor other than oxalate

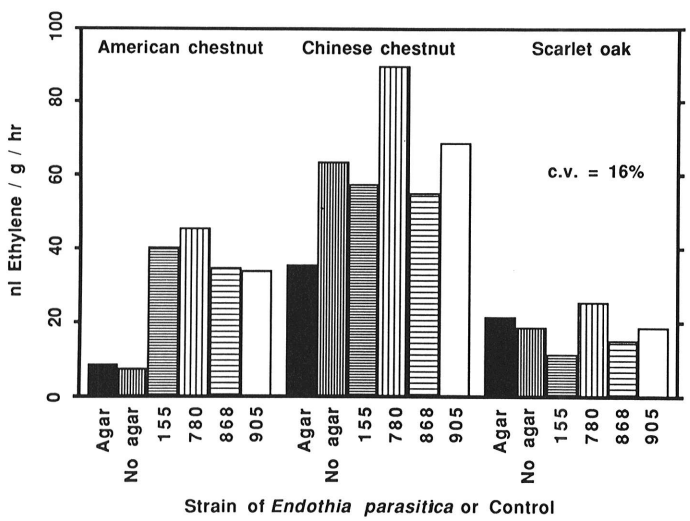

Fig. 3. Rates of ethylene production by bark plugs of American or Chinese chestnut or scarlet oak between about 20 and 40 hr after exposure to agar-mycelium disks of virulent (155) or hypovirulent (780, 868, 905) strains of *Endothia parasitica*, or to agar or no agar controls. Error is expressed as the coefficient of variation (c.v.).

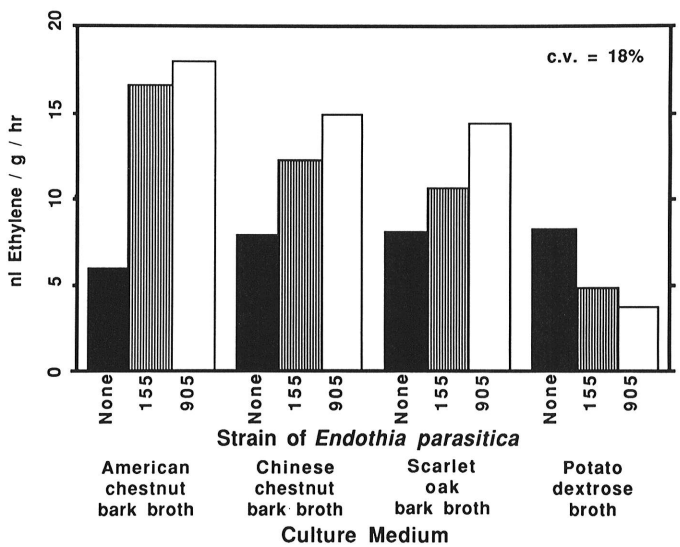

Fig. 4. Rates of ethylene production by American chestnut bark plugs between about 20 and 40 hr after exposure to filter-sterilized broth from 11-day-old cultures of virulent (155) or hypovirulent (905) strains of *Endothia parasitica*, or to fungus-free media (None); cultures were grown in potato-dextrose broth amended with methionine and biotin or in bark extract broths of American or Chinese chestnut or scarlet oak. Error is expressed as the coefficient of variation (c.v.).

must be stimulating ethylene production. Oxalate and other organic acids may be responsible for the repression of stimulation immediately next to expanding cankers on American chestnut. Such acids also may be responsible for the lack of stimulation of American chestnut bark plugs by PDBmb, which had supported *E. parasitica*. We found no differences in ethylene production between Chinese and American chestnut bark plugs exposed to oxalate, nor were we able to repeat McCarroll's results with an inner bark browning assay (11).

Stimulation of ethylene production began before lignification. Both stimulation of ethylene production and the onset of lignification were delayed in scarlet oak compared with American chestnut. Similarly, initial lesion expansion, lignification, and stimulated ethylene production persisted longer when American chestnut was inoculated with a virulent than with a highly debilitated hypovirulent strain of *E. parasitica*. Thus, an elevated ethylene level in bark may trigger formation of a lignified zone, which is associated in turn with the subsequent formation of wound periderm.

LITERATURE CITED

1. Bazzigher, G. 1955. Uber tannin—und phenol spaltrende Fermente von *Endothia parasitica*. Phytopathol. Z. 24:265-282.
2. Cornelius, P. L. 1986. A computational method for unbalanced two-fold nested-error linear models. Pages 166-177 in: Am. Stat. Assoc., 1986 Proc. Statistical Computing Sect.
3. Ecker, J. R., and Davis, R. W. 1987. Plant defense genes are regulated by ethylene. Proc. Natl. Acad. Sci. USA 84:5202-5206.
4. Elkins, J. R., Lawhorn, Z., and Weyand, E. 1982. Utilization of chestnut tannins by *Endothia parasitica*. Pages 141-144 in: Proc. USDA Cooperators' Meetings. H. C. Smith and W. L. MacDonald, eds. West Virginia University Books, Morgantown.
5. Elliston, J. E. 1982. Effects of selected North American and Italian cytoplasmic hypovirulence agents on North American and Italian strains of *Endothia parasitica*. Pages 134-140 in: Proc. USDA For. Serv. Am. Chestnut Cooperators' Meeting. H. C. Smith and W. L. MacDonald, eds. West Virginia University Books, Morgantown.
6. Griffin, G. J., Hebard, F. V., Wendt, R. W., and Elkins, J. R. 1983. Survival of American chestnut trees: Evaluation of blight resistance and virulence in *Endothia parasitica*. Phytopathology 73:1084-1092.
7. Hebard, F. V., Griffin, G. J., and Elkins, J. R. 1984. Developmental histopathology of cankers incited by virulent and hypovirulent *Endothia parasitica* on susceptible and resistant chestnut trees. Phytopathology 74:140-149.
8. Hebard, F. V., and Kaufman, P. B. 1978. Chestnut callus cultures: Tannin content and colonization by *Endothia parasitica*. Pages 63-69 in: Proc. Am. Chestnut Symposium. W. L. MacDonald, F. C. Cech, J. Luchok, and C. Smith, eds. West Virginia University Books, Morgantown.
9. Hebard, F. V., and Shain, L. 1986. Ethylene production by resistant and susceptible hosts near cankers caused by virulent and hypovirulent *Endothia parasitica*. (Abstr.) Phytopathology 76:1113.
10. Hebard, F. V., and Shain, L. 1987. Early stimulation of ethylene production in hosts challenged by *Endothia parasitica* or its culture filtrates. (Abstr.) Phytopathology 77:1716.
11. McCarroll, D. R., and Thor, E. 1978. The role of oxalic acid in the pathogenesis of *Endothia parasitica*. Pages 60-62 in: Proc. Am. Chestnut Symposium. W. L. MacDonald, F. C. Cech, J. Luchok, and C. Smith, eds. West Virginia University Books, Morgantown.
12. McCarroll, D. R., and Thor, E. 1985. Pectolytic, cellulytic and proteolylic activities expressed by cultures of *Endothia parasitica*, and inhibition of these activities by components extracted from Chinese and American chestnut inner bark. Physiol. Plant Pathol. 26:367-378.
13. Nienstaedt, H. 1953. Tannin as a factor in the resistance of chestnut, *Castanea* spp., to the chestnut blight fungus, *Endothia parasitica*. Phytopathology 43:32-38.
14. Puhalla, J. E., and Anagnostakis, S. L. 1971. Genetics and nutritional requirements of *Endothia parasitica*. Phytopathology 61:169-173.
15. Samman, S., Schell, F. M., and Thor, E. 1978. Effect of some compounds of American and Chinese chestnut inner bark on the growth of *Endothia parasitica*. Pages 70-72 in: Proc. Am. Chestnut Symposium. W. L. MacDonald, F. C. Cech, J. Luchok, and C. Smith, eds. West Virginia University Books, Morgantown.
16. Shain, L., and Hillis, W. E. 1972. Ethylene production in *Pinnus radiata* in response to Sirex-Amylosterum attack. Phytopathology 62:1407-1409.
17. Shain, L., and Wheeler, H. 1975. Production of ethylene by oats resistant and susceptible to victorin. Phytopathology 65:88-89.