# Ethylene Effects on In Vitro and In Vivo Growth of Certain Postharvest Fruit-Infecting Fungi

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## ABSTRACT

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Exposure of certain postharvest fruit-infecting fungi (*Alternaria alternata, Botrytis cinerea, Colletotrichum gloeosporioides, Monilinia fructicola, Penicillium digitatum, P. expansum, P. italicum, Rhizopus stolonifer,* and *Thielaviopsis paradoxa*) to ethylene ($C_2H_4$) at 1, 10, 100, and $10^3$ $\mu$l/L of air stimulated germination of *P. digitatum, P. italicum,* and *T. paradoxa* and germ tube elongation of most of the tested fungi, but had little influence on their final growth rates at 20 C. Treatment with $C_2H_4$ up to $10^3$ $\mu$l/L of air increased the total dry weight of *B. cinerea* grown both in vitro and in vivo (the latter on strawberries, as determined by glucosamine content) after 4 days at 20 C. Glucosamine content of *P. italicum* grown in vitro and in vivo (the latter on oranges) also increased in response to similar exposure of fruits to $C_2H_4$. $C_2H_4$ did not affect lesion diameters on navel oranges inoculated with *P. italicum* before $C_2H_4$ exposure was initiated. However, similar oranges treated with $C_2H_4$ for 3 days at 20 C before inoculation with *P. italicum* became more resistant and developed smaller lesions.

*Additional key words*: *Citrus sinensis, Fragaria chiloensis* var. *ananassa*, orange, postharvest pathology, strawberry.

Ethylene ($C_2H_4$), a plant hormone, is produced by almost all plants and plant organs as well as by a large number of microorganisms. Enhancement of $C_2H_4$ evolution generally occurs in most diseased plant organs (9,14,17,19). The possible involvement of $C_2H_4$ in plant pathogenesis has attracted the attention of many investigators (1,2,5,9,14,17). Stahmann et al (18) reported that sweet potato slices exposed to 8 $\mu$l of $C_2H_4$ per liter of air for 2 days became resistant to infection by *Ceratocystis fimbriata*. Also, $C_2H_4$-treated tangerines became more resistant to *Colletotrichum gloeosporioides* than untreated fruits (4). On the other hand, $C_2H_4$ stimulated disease development on some fruits (4,7,8,13).

The direct effect of $C_2H_4$ on plant pathogenic microorganisms has not been extensively investigated. Therefore, the objective of this research was to determine the effects of various concentrations of $C_2H_4$ on the in vitro and in vivo growth of some fungal postharvest fruit pathogens.

## MATERIALS AND METHODS

**Fungi.** Ten postharvest fruit-infecting fungi (*Alternaria alternata* (Fr.) Keissler; *Botryodiplodia theobromae* Pat.; *Botrytis cinerea* Pers. ex Fr.; *Colletotrichum gloeosporioides* (Penz.) Arx; *Monilinia fructicola* (Wint.) Honey; *Penicillium digitatum* Sacc.; *P. expansum* Lk. ex Thom; *P. italicum* Wehmer; *Rhizopus stolonifer* (Fr.) Lind.; and *Thielaviopsis paradoxa* (de Seynes) Höehn.) were provided by the second author. Cultures were stored at 4 C on potato-dextrose agar (PDA).

**Fruits.** The fresh navel and Valencia oranges (*Citrus sinensis* Osbeck) used in these studies were obtained from a commercial grove near Fresno, CA, transported to our laboratory at Davis, CA, within 3 hr, and held overnight at 7 C before the experiments were initiated. Fruits were sorted for uniformity of color, size, and freedom from defects and matched lots were selected for use in each experiment.

Strawberries (*Fragaria chiloensis* Duch. var. *ananassa* Bailey 'Aiko') were obtained from Watsonville, CA. The fruits were cooled to near 0 C within a few hours of harvest, transported to Davis within 3 hr, and held overnight at 0 C before being used for the experiment.

**Ethylene effects on in vitro growth.** Four plates per fungus, each containing 15 ml of PDA, were centrally inoculated with 6-mm-diameter mycelial plugs obtained from the edge of 4- to 9-day-old cultures. The plates were vented by positioning a bent sterilized wire to raise the lid slightly and were placed in 8.5-L cylindrical glass containers with rubber stoppers fitted with inlet and outlet openings. These containers were continuous-flow ventilated with either humidified air or air plus the desired $C_2H_4$ concentration flowing at 100 ml/min governed by capillary tubes employed as flow meters as described by Claypool and Keefer (6). All tested fungi were subjected to 0, 1, 10, 100, and $10^3$ $\mu$l of $C_2H_4$ per liter of air at 20 C. Two additional $C_2H_4$ concentrations ($16 \times 10^3$ and $28 \times 10^3$ $\mu$l/L of air) were used with *B. cinerea*. Ethylene concentrations were verified periodically by flame ionization gas chromatography. Colony diameters were measured daily for 2–9 days depending on the sensitivity of the fungal species being treated.

To determine $C_2H_4$ effects on spore germination and germ tube elongation of the tested fungi, 6-mm-diameter PDA disks were placed on sterile glass slides (three disks each) and one drop (approximately 0.05 ml) of conidial suspension was placed on each disk. The conidia were harvested by adding Tween-80 solution. A concentration of $10^6$ conidia per milliliter was attained by relating absorption at 490 nm in a Bausch & Lomb Spectronic 20 colorimeter to a standard curve previously established with a hemacytometer for a similar spore suspension. The glass slides were placed under the same atmospheres indicated above. Spore germination was checked with a light microscope after 6, 14, and 24 hr, and the rate of spore germination was determined by counting 400 spores after a suitable incubation period for each species. The length (micrometers) of 10 germ tubes in each of four replicates per treatment was measured.

**Ethylene effects on in vivo growth.** Orange fruits were washed, surface sterilized by immersion in sodium hypochlorite solution ($10^3$ $\mu$g/ml) for 3 min, then air-dried. Fruits were inoculated with *P. italicum* by inserting dissecting needles 1 cm into fruit tissue and injecting 0.05 ml of the conidial suspension ($10^6$ spores per millilter) into the wound with a 1-ml syringe. Strawberry fruits were wound-inoculated with *B. cinerea* by puncturing them with a needle (2-mm long) previously dipped in a spore suspension containing $10^6$ spores per milliliter. Three replicates of 10 strawberry or five orange fruits each were used per treatment and were placed into 11.5-L glass jars that were ventilated with air or air plus the desired $C_2H_4$

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concentration at a flow rate of 160 ml/min for strawberries or 550 ml/min for oranges at 20 C.

In one test, groups of orange fruits were inoculated with *P. italicum*, then subjected to $C_2H_4$ treatments for 6 days. In the other test, fruits were exposed to $C_2H_4$ for 3 days before inoculation, then held for an additional 6 days either in air or the same $C_2H_4$ atmospheres. Following the incubation periods indicated above, fruits were examined for rot development and the diameter of each lesion was measured.

**Glucosamine content of fungi grown in vitro.** Cultures of *B. cinerea* and *P. italicum* were grown in 250-ml Erlenmeyer flasks containing 50 ml each of either synthetic Botrytis medium (20 g sucrose, 5 g peptone, 2 g ammonium phosphate, 2 g potassium nitrate, 0.5 g magnesium sulfate, and 0.1 g calcium chloride in 1.0 L of distilled water) or Czapek Dox broth medium (30 g saccharose, Difco, 3 g sodium nitrate, 1 g dipotassium phosphate, 0.5 g magnesium sulphate, 0.5 g potassium chloride, and 0.01 g ferrous sulfate in 1.0 L of distilled water). These cultures were incubated at 21–24 C on a rotary shaker (140 rpm). Two flasks from each culture were removed daily for up to 6 and 10 days for *B. cinerea* and *P. italicum*, respectively. The mycelium was collected on a 40-$\mu$m-mesh sieve, washed with distilled water, and homogenized for 1 min in 20 ml of distilled water in a Brinkmann homogenizer. The mycelial suspensions were assayed for glucosamine according to the method of Ride and Drysdale (15,16) as modified by Jarvis (10) and Bishop et al (3). Three 3-ml samples from each of the mycelial suspensions were taken in tared aluminum weighing cups and dried to constant weight at 70 C in a vacuum oven for dry weight determination. The glucosamine data were expressed as micrograms of glucosamine per milligram (dry weight) of the fungus.

Mycelia of *B. cinerea* and *P. italicum* grown on PDA in petri dishes under various $C_2H_4$ treatments for 4 and 8 days, respectively, at 20 C were collected as previously described with broth media by melting the media in a water bath at 90 C. Two 0.6-ml portions of the mycelial suspension were centrifuged ($1.5 \times 10^3$ *g*, 10 min). The supernatant was removed, and the residue was mixed with 3 ml of concentrated KOH solution (120 g KOH/100 ml) and placed in an oven at 130 C for 1 hr or autoclaved for 15 min at 121 C. After being cooled, the alkaline solution was mixed with 8 ml of 75% ethanol and allowed to stand in ice water for 15 min. Nine-tenths milliliter of Celite (Johns-Manville, New York, NY 10016) suspension (1 g in 20 ml of 75% ethanol) was layered on top, and the tubes were centrifuged ($1.5 \times 10^3$ *g*, 10 min). The supernatant was removed, and the residue was washed with 40% ethanol and recentrifuged for 10 min. The pellet was washed twice with cold distilled water. The residue was adjusted to 1.5 ml with distilled water and a 1.5-ml aliquot of $NaNO_2$ (5% w/v) and a similar volume of 5% $KHSO_4$ was added to the centrifuge tube. The mixture was stirred for 15 min and centrifuged at $1.5 \times 10^3$ *g* for 2 min. A 1.5-ml portion of supernatant was mixed with 0.5 ml of 12.5% $NH_4SO_4$-$NH_2$, and the mixture was shaken for 5 min, then 0.5 ml of 0.5% 3-methyl-2-benzothiozolone hydrazone (MBTH) was added and mixed. The mixture was heated in a boiling-water bath for 3 min, cooled, and 0.5 ml of 0.5% FeCl was added. After standing at room temperature for 30 min, the absorbancy was read at 650 nm, and glucosamine concentration was calculated from a standard curve and expressed as micrograms of glucosamine per sample.

**Determination of fungal glucosamine in infected fruits.** Three replicates of healthy or inoculated strawberries (10 berries each) per treatment were weighed and homogenized in an omnimixer. For oranges, three 5-g samples from uninoculated or inoculated fruit rind (15-mm-diameter disk around the cite of inoculation) were homogenized in 25 ml of distilled water in a Brinkmann homogenizer. The fresh weight of the homogenate was determined in each case. Portions (2 g) of strawberries and orange rind suspensions were transferred to 15-ml graduated glass centrifuge tubes and diluted with 9 ml of acetone. The samples were centrifuged at $1.5 \times 10^3$ *g* for 2 min, the supernatant was removed, and the solids were resuspended in 9 ml of acetone and recentrifuged. The dried pellet was suspended in 4 ml of concentrated KOH and the above-mentioned procedure for glucosamine determination was followed.

## RESULTS

No significant differences in percent germination were noted among spores exposed to air (control) or various $C_2H_4$ concentrations up to $10^3$ $\mu$L in *A. alternata, C. gloeosporioides, P. expansum*, and *R. stolonifer* (Table 1). Exposure to 100 or $10^3$ $\mu$l of $C_2H_4$ per liter of air slightly reduced percent spore germination in *B. theobromae* and *B. cinerea*, but other $C_2H_4$ concentrations had no effect. Spore germination of *M. fructicola* was stimulated by exposure to $C_2H_4$ concentrations of 1 and 10 $\mu$l/L of air, but higher concentrations than these were comparable to air controls. Spores of *P. digitatum, P. italicum,* and *T. paradoxa* showed an increase in percent germination in response to $C_2H_4$ treatments, but their spore germination was less than 70% in all treatments (Table 1).

Excepting *R. stolonifer* ($C_2H_4$ did not influence its germ tube length) and *P. expansum* (impossible to evaluate owing to excessive growth after 24 hr), $C_2H_4$ treatments enhanced germ tube elongation of the tested fungi (Table 2). There was no clear or consistent relationship between $C_2H_4$ concentration and the extent of germ tube elongation. In most cases, 1 $\mu$l of $C_2H_4$ per liter of air was adequate for attaining near maximum response. Spore germination and germ tube elongation of *B. cinerea* in response to $C_2H_4$ at $16 \times 10^3$ and $28 \times 10^3$ $\mu$l/L (*unpublished*) were comparable to those observed at $10^3$ $\mu$l/L. Differences in growth rates of the tested fungi were small and not significant, in most cases, among control and $C_2H_4$ treatments (Table 3). Certain $C_2H_4$ concentrations slightly increased growth rates of *C. gloeosporioides, M. fructicola, P. italicum, R. stolonifer,* and *T. paradoxa*. Again, there was no consistent $C_2H_4$ concentration effect on growth rates.

By using fungal chitin determination as an index of fungal growth, we found mean values of 21.3 and 19.3 $\mu$g of fungal glucosamine per milligram dry weight for *B. cinerea* and *P. italicum*, respectively. We also found that healthy (uninoculated) fruit tissues contained small quantities of glucosamine (about 8 and

TABLE 1. Spore germination of fungi on potato-dextrose agar as influenced by exposure to air with various concentrations of $C_2H_4$ at 20 C

| Fungus | Incubation (hr) | Mean percent spore germination of fungi exposed[y] to $C_2H_4$ at: | | | | |
|---|---|---|---|---|---|---|
| | | Air | 1 $\mu$l/L | 10 $\mu$l/L | 100 $\mu$l/L | 1,000 $\mu$l/L |
| *Alternaria alternata* | 6 | 99.7 a[z] | 98.0 a | 99.3 a | 95.0 a | 99.5 a |
| *Botryodiplodia theobromae* | 6 | 98.3 a | 99.7 a | 95.3 ab | 92.3 b | 91.0 b |
| *Botrytis cinerea* | 6 | 98.3 a | 99.7 a | 95.3 ab | 94.0 b | 94.0 b |
| *Colletotrichum gloeosporioides* | 6 | 95.7 ab | 96.7 a | 98.0 a | 97.3 a | 92.0 b |
| *Monilinia fructicola* | 6 | 82.0 b | 95.0 a | 91.7 a | 82.3 b | 83.0 b |
| *Penicillium digitatum* | 24 | 44.7 c | 52.7 b | 51.0 b | 64.0 a | 64.0 a |
| *P. expansum* | 24 | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a |
| *P. italicum* | 24 | 40.7 c | 67.7 a | 65.7 a | 56.7 b | 63.3 a |
| *Rhizopus stolonifer* | 14 | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a |
| *Thielaviopsis paradoxa* | 14 | 38.3 b | 45.0 b | 49.3 b | 47.0 b | 64.7 a |

[y] In vented PDA culture plates centrally inoculated with 6-mm-diameter mycelial plugs from 4- to 9-day-old PDA cultures.
[z] Mean separation in rows according to Duncan's new multiple range test, $P = 0.05$.

TABLE 2. Germ tube length of fungi on potato-dextrose agar as influenced by exposure to various $C_2H_4$ concentrations at 20 C

| Fungus | Incubation (hr) | Mean germ tube length ($\mu$m) of fungi exposed to $C_2H_4$ in air at: | | | | |
|---|---|---|---|---|---|---|
| | | Air | 1 $\mu$l/L | 10 $\mu$l/L | 100 $\mu$l/L | 1,000 $\mu$l/L |
| *Alternaria alternata* | 6 | 298 b[y] | 405 a | 403 a | 309 b | 366 a |
| *Botryodiplodia theobromae* | 14 | 125 c | 144 bc | 154 ab | 130 c | 164 a |
| *Botrytis cinerea* | 6 | 124 c | 284 a | 212 b | 220 b | 232 b |
| *Colletotrichum gloeosporioides* | 24 | 306 c | 682 b | 707 b | 874 a | 689 b |
| *Monilinia fructicola* | 24 | 126 c | 516 a | 617 a | 337 b | 310 b |
| *Penicillium digitatum* | 24 | 28 b | 63 a | 58 a | 67 a | 50 a |
| *P. expansum*[z] | 24 | ... | ... | ... | ... | ... |
| *P. italicum* | 24 | 18 b | 69 a | 53 a | 74 a | 65 a |
| *Rhizopus stolonifer* | 14 | 207 a | 259 a | 286 a | 231 a | 220 a |
| *Thielaviopsis paradoxa* | 14 | 108 b | 115 b | 156 ab | 121 b | 164 a |

[y] Mean separation within rows according to Duncan's new multiple range test, $P = 0.05$.
[z] Densely packed mycelium made measurement of germ tube length impossible.

TABLE 3. Growth rates of fungi on potato-dextrose agar as influenced by exposure to various $C_2H_4$ concentrations at 20 C

| Fungus | Incubation (days) | Mean increase in colony diameter (mm/day) of fungi exposed to $C_2H_4$ in air at: | | | | |
|---|---|---|---|---|---|---|
| | | Air | 1 $\mu$l/L | 10 $\mu$l/L | 100 $\mu$l/L | 1,000 $\mu$l/L |
| *Alternaria alternata* | 10 | 6.5 a[z] | 6.8 a | 7.0 a | 7.1 a | 6.9 a |
| *Botryodiplodia theobromae* | 4 | 17.7 ab | 17.9 ab | 17.8 ab | 17.4 b | 18.1 a |
| *Botrytis cinerea* | 4 | 16.0 abc | 15.7 bc | 16.2 ab | 16.7 a | 15.2 c |
| *Colletotrichum gloeosporioides* | 9 | 6.3 b | 6.7 a | 6.5 ab | 6.6 a | 6.5 ab |
| *Monilinia fructicola* | 7 | 8.5 b | 8.6 ab | 8.5 b | 9.4 a | 9.3 ab |
| *Penicillium digitatum* | 8 | 5.9 a | 6.0 a | 6.1 a | 5.9 a | 5.9 a |
| *P. expansum* | 8 | 5.5 a | 5.8 a | 5.7 a | 5.8 a | 5.8 a |
| *P. italicum* | 8 | 6.4 b | 6.6 ab | 6.6 ab | 6.5 ab | 6.7 a |
| *Rhizopus stolonifer* | 2 | 29.8 c | 32.4 a | 31.0 b | 32.0 a | 30.6 b |
| *Thielaviopsis paradoxa* | 4 | 18.3 b | 18.6 a | 18.4 b | 18.4 b | 18.4 b |

[z] Mean separation within rows according to Duncan's new multiple range test, $P = 0.05$.

TABLE 4. Glucosamine content of *Botrytis cinerea* and *Penicillium italicum* grown on potato-dextrose agar and exposed to a range of $C_2H_4$ concentrations for 4 or 9 days, respectively, at 20 C

| Treatment ($\mu$l $C_2H_4$ per liter of air) | Glucosamine ($\mu$g) | |
|---|---|---|
| | *B. cinerea* | *P. italicum* |
| 0 (air control) | 285 d[z] | 597 e[z] |
| 1 | 410 b | 615 d |
| 10 | 405 b | 706 c |
| 100 | 341 c | 843 b |
| 1,000 | 408 b | 875 a |
| 16,000 | 387 b | Not tested |
| 28,000 | 455 a | Not tested |

[z] Mean separation in columns according to Duncan's new multiple range test, $P = 0.05$.

TABLE 5. Glucosamine content of strawberries infected with *Botrytis cinerea* and Valencia oranges infected with *Penicillium italicum* exposed to different concentrations of $C_2H_4$ (per liter of air) for 4 or 6 days, respectively, at 20 C

| Treatment ($\mu$l $C_2H_4$ per liter of air) | Glucosamine ($\mu$g/g dry wt) | |
|---|---|---|
| | Strawberry/*B. cinerea* | Orange/*P. italicum* |
| Preinoculation | | |
| 0 Initial | 7.8 d[z] | 100 c[z] |
| Postinoculation | | |
| 0 (air control) | 41.1 c | 7,440 b |
| 1 | 59.6 a | ... |
| 10 | 64.4 a | ... |
| 100 | 50.0 b | ... |
| 1,000 | 62.2 a | 9,470 a |

[z] Mean separation within columns according to Duncan's new multiple range test, $P = 0.05$.

100 $\mu$g/g dry weight of strawberries and oranges, respectively). These concentrations did not change when the fruits were analyzed after exposure to air or air + $10^3$ $\mu$l of $C_2H_4$ per liter of air for 6 days at 20 C.

Glucosamine content of *B. cinerea* and *P. italicum*, in vitro, significantly increased in response to $C_2H_4$ treatments up to $10^3$ $\mu$l/L for *P. italicum* and up to $28 \times 10^3$ $\mu$l/L for *B. cinerea* (Table 4). Glucosamine content increased with the increase in $C_2H_4$ concentration in the case of *P. italicum*. No clear relationship between glucosamine content and $C_2H_4$ concentration was observed in *B. cinerea*, except that the highest glucosamine value coincided with the highest $C_2H_4$ concentration. The effects of $C_2H_4$ on stimulating fungal growth as indicated by glucosamine determinations were also observed in the in vivo studies. Glucosamine content of strawberries infected with *B. cinerea* increased in response to $C_2H_4$ treatments, and Valencia orange rind infected with *P. italicum* and exposed to $C_2H_4$ for 6 days at 20 C contained more glucosamine than that held in air (Table 5).

Exposure of navel oranges to various $C_2H_4$ concentrations for 6 days at 20 C following their inoculation with *P. italicum* did not influence lesion development (Table 6). However, lesions on fruits subjected to $C_2H_4$ treatments for 3 days before inoculation developed more slowly when fruits were held in air for 6 days at 20 C. The $10^3$ $\mu$l of $C_2H_4$ per liter of air treatment was the most effective in limiting lesion development (Table 6).

The effectiveness of preinoculation exposure to $C_2H_4$ in decreasing lesion development was also found in Valencia oranges inoculated with *P. italicum* (Table 7). The retarding effect of $C_2H_4$ on lesion development was noted in fruits exposed to 1, 10, 100, or $10^3$ $\mu$l of $C_2H_4$ per liter of air for 3 days before inoculation, then held in air for 6 days at 20 C. However, the $C_2H_4$ effect was limited to those fruits that were exposed to 100 or $10^3$ $\mu$l of $C_2H_4$ per liter of air before inoculation when they were subsequently held in air plus $C_2H_4$ for 6 more days at 20 C.

## DISCUSSION

Ethylene significantly stimulated spore germination of *P. digitatum, P. italicum,* and *T. paradoxa,* but it had little or no effect on the other tested fungi. These results differ from those reported

TABLE 6. Effect of $C_2H_4$ treatments on lesion development due to *Penicillium italicum* on navel oranges at 20 C

| Treatment ($\mu$l $C_2H_4$ per liter of air) | Mean diameter of lesions (mm) | |
|---|---|---|
| | A[y] | B[y] |
| 0 (air control) | 51 a[z] | 60 a |
| 1 | 53 a | 62 a |
| 10 | 46 a | 56 b |
| 100 | 53 a | 55 b |
| 1,000 | 51 a | 49 c |

[y] A = fruits incubated for 6 days following inoculation, and B = fruits subjected to $C_2H_4$ treatments for 3 days before inoculation, then incubated in air for 6 days.
[z] Mean separation in columns according to Duncan's new multiple range test, $P = 0.05$.

TABLE 7. Effect of $C_2H_4$ treatments for 3 days before inoculation with *Penicillium italicum* on lesion development on Valencia oranges at 20 C

| Treatment ($\mu$l $C_2H_4$ per liter of air) | Mean diameter of lesions (mm) | |
|---|---|---|
| | A[y] | B[y] |
| 0 (air control) | 40 a[z] | 40 a |
| 1 | 36 b | 40 a |
| 10 | 35 b | 38 a |
| 100 | 34 b | 32 b |
| 1,000 | 33 b | 30 b |

[y] A = fruits incubated in air for 6 days following inoculation, and B = fruits incubated in air + $C_2H_4$ for 6 days following inoculation.
[z] Mean separation in columns according to Duncan's new multiple range test, $P = 0.05$.

by Kepczynski and Kepczynska (11), who found stimulation of spore germination of *B. cinerea, P. expansum,* and *R. stolonifer* in response to 100 $\mu$l $C_2H_4$ per liter of air at room temperature (not specified). They also reported that exposure to 10,000 $\mu$l of $C_2H_4$ per liter of air inhibited germination of these three fungi. Our results show that $C_2H_4$ concentrations up to 28,000 $\mu$l/L did not affect spore germination of *B. cinerea.* These differences may have been due to incubation temperature and the timing of observations. The temperature used in our experiments, ie, 20 C, is slightly below optimum for most of the tested fungi. It is possible that $C_2H_4$ effects on spore germination and other growth parameters are temperature dependent. Additional studies are needed to clarify this point as well as the observed inconsistent $C_2H_4$ concentration effects.

Stimulation of germ tube elongation occurred with all tested fungi, with two exceptions, at all $C_2H_4$ concentrations. These stimulatory effects of $C_2H_4$ on germ tube elongation may have been partially due to early spore germination in some fungi, since it was not consistently reflected in terms of rate of increase in colony diameter.

Glucosamine determination is an accurate indicator of fungal growth, since chitin is a major constituent of fungal cell walls, but little or no chitinlike materials occur in higher plants (3). Our data show that fungal growth is more accurately measured by glucosamine content than by radial growth since the former method represents colony mass. The stimulatory effect of $C_2H_4$ on fungal growth was observed for both *B. cinerea* and *P. italicum*, in vitro and in vivo, as indicated by the increase in glucosamine. This increase in the in vivo studies was mainly due to fungal glucosamine since we found no change in the glucosamine content of uninoculated fruit tissues.

Lesion development on inoculated oranges or strawberries was accelerated by $C_2H_4$ treatment immediately following inoculation. Similar results indicating the stimulatory effect of $C_2H_4$ on disease development have been reported (4,7,8,13). On the other hand, some inhibitory action of $C_2H_4$ on rot development on infected fruits or other plant organs have also been described (4,12,18). These apparently contradictory results may be due to differences in timing of $C_2H_4$ treatment, temperature, and the method used for measuring fungal growth.

The partial resistance induced by treating orange fruits with $C_2H_4$ for 3 days before inoculation may be due to alteration of the host metabolism, eg, increased activity of certain enzymes or formation of a toxin that interacted with the pathogen's metabolites. Induced resistance in tangerines to anthracnose has been reported following their exposure to $C_2H_4$ for 3 days before inoculation (4). Similarly, sweet potato slices exposed to low $C_2H_4$ concentrations for 2 days became resistant to infection by *Ceratocystis fimbriata* (18); these findings were not confirmed in another study (5).

Both the direct stimulatory effects of $C_2H_4$ on postharvest fruit fungi and the indirect inhibitory effects via possible modifications of the host metabolism have practical implications in the postharvest biology of fresh horticultural crops. Additional research should evaluate the potential benefits of avoiding exposure to $C_2H_4$ and its removal from around fruits on rate of rot development. On the other hand, the possible use of $C_2H_4$ treatment to induce disease resistance and reduce susceptibility of fruits to decay merits further investigation.

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