Physiological and Compositional Changes in Orange Fruit in Relation to Modification of their Susceptibility to *Penicillium italicum* by Ethylene Treatments

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Abstract. Exposure of orange fruit to 1000 μl/liter C₂H₄ at 20°C for 2 to 6 days before inoculation with *Penicillium italicum* Wehmer reduced fungal growth as indicated by lesion diameter and glucosamine content. Respiration rates were stimulated by C₂H₄ treatments and fungal inoculation, which also increased C₂H₄ production rate. Ethylene treatments with or without inoculation did not influence soluble solids, pH, titratable acidity, or total phenolics content of fruit juice. Total phenolics of the rind increased in un inoculated fruit, but not in inoculated fruit, in response to C₂H₄ treatment. No polyphenol oxidase activity was detected. Phenylalanine ammonia-lyase activity exhibited a significant increase in rind of un inoculated fruit, but not in inoculated fruit which were subjected to C₂H₄ for 6 days followed by 6 days in air at 20°C.

An important mechanism for disease resistance in plants is their ability to accumulate rapidly relatively simple chemical substances (phytoalexins) to levels inhibitory to the growth and development of pathogens. Many abiotic and biotic factors cause the accumulation of phytoalexins (10). The possible involvement of C₂H₄ in plant–pathogen interactions is not fully understood (1). Ethylene was found to stimulate rot development by some fungi on citrus fruit (8, 12) and strawberries (5). On the other hand, C₂H₄ has been shown to induce resistance to certain pathogens in some harvested plant organs. Sweet potato slices exposed to 8 μl/liter C₂H₄ for 2 days became resistant to infection by *Ceratocystis fimbriata* (17). Lockhart et al. (11) found that C₂H₄ treatment inhibited apple rot development caused by *Gloeosporium album*.

Robinson tangerines exhibited resistance to *Colletotrichum gloeosporioides* when treated with C₂H₄ before inoculation, then exposed to additional C₂H₄ to complete removal of green color (4). Ethylene-treated tangerines accumulated more phenolic compounds and were more intensely lignified than untreated fruit (3). El-Kazzaz et al. (6) reported that exposure of orange fruit to C₂H₄ following inoculation with *Penicillium italicum* did not influence the rate of rot development during holding at 20°C. However, induced resistance to this fungus was noted in both ‘Valencia’ and ‘Navel’ oranges if they were treated with C₂H₄ for 3 days prior to inoculation and holding at 20°. The effectiveness of C₂H₄ increased with concentration up to 1000 μl/liter (6). These findings prompted us to investigate the physiological basis of this C₂H₄-induced resistance to pathogens.

We report here on some physiological and compositional changes in ‘Valencia’ and ‘Navel’ oranges associated with modification of their susceptibility to *P. italicum* by C₂H₄ treatments for various durations.

**Materials and Methods**

‘Navel’ and ‘Valencia’ oranges were obtained from commercial orchards near Fresno, Calif., and transported to Davis where
they were held at 7°C until the following morning when experiments were initiated. Fruit were sorted for uniformity of orange color, size, and absence of defects and matched lots were selected for use in each experiment. Fruit to be inoculated were surface-sterilized by immersion in sodium hypochlorite solution (1000 μg/ml) for 3 min, then air-dried. These fruit were inoculated with Penicillium italicum by inserting dissecting needles into fruit tissue (1 cm deep) and injecting 0.05 μl of the conidial suspension (1 × 10⁶ spores/ml) through the wound using a 1-μl syringe. All test fruit were held at 20°C in glass jars under a continuous flow of humidified air or air + C₃H₄. Respiration and C₃H₄ production rates were determined on 3 replicates of 5 fruit each per treatment using gas chromatography. Glucosamine content of the rind was determined as described previously (6) on infected fruit as a quantitative indicator of fungal growth on ' Valencia' oranges, which were subjected to 1000 μl/liter C₃H₄ for various durations at 20°C before inoculation with P. italicum. Fruit of each cultivar were sampled initially, after the incubation period, and either before or after C₃H₄ treatments for composition analysis. Soluble-solids content (SSC) in juice samples was determined with an Abbé refractometer. Titratable acidity, calculated as percent citric acid, was determined by titrating 6 g of juice with 0.1 N NaOH and pH was measured with a Corning Model 130 digital pH meter. Total phenolic compounds were determined by the method of Singleton and Rossi (16), using the Folin-Ciocalteau reagent and gallic acid as standard.

Peel samples were analyzed for total phenolic compounds and polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PALL) activity. Five grams of peel tissue were homogenized in 100 ml 0.1 N HCl in methanol using a Brinkmann homogenizer. Model PT 10-35. The homogenate was filtered through Whatman filter paper No. 1 under vacuum. The filtrate was washed with n-hexane several times until all lipids were removed and then assayed for total phenolics (16). PPO was extracted according to the method described by Flurkey and Jen (7) using 0.2 g of acetone powder in 100 ml citric acid-phosphate buffer, pH 6.2. The mixture was stirred for 10 min and filtered through Whatman filter paper No. 42. To the reaction mixture containing 10 ml enzyme preparation, 5 ml of 0.1 M catechol were added and mixed for 30 sec. Deionized water was used in the blank. After 1 min, absorbance was read at 420 nm. Results were expressed in units of PPO activity, where each unit of PPO activity was equivalent to an increase of 0.1 absorbance unit per min. PALL was assayed in acetone powders prepared from peel tissues according to the method described by Zucker (18) using 0.75 g acetone powder suspended in sodium borate buffer, pH 8.8. The reaction mixture contained 0.5 ml enzyme preparation, 1.5 ml borate buffer (0.2 M, pH 8.8), 1 ml of 1% phenylalanine, and 2.5 ml deionized water. One ml deionized water was added instead of phenylalanine as a blank. The mixture was incubated at 40°C for 1 hr. The reaction was stopped by adding 0.5 ml of 5% HCl to each tube. The enzyme was assayed at 290 nm and the activity was expressed as μ moles of trans-cinnamic acid per gram fresh weight of the rind.

Results

Exposure of ' Valencia' oranges to 1000 μl/liter C₃H₄ for 2 to 6 days before inoculation with P. italicum reduced blue mold disease development (Table 1). The one-day C₃H₄ treatment had no effect. No significant differences were observed between fruit exposed to C₂H₄ for 2, 3, and 4 days before inoculation. Fruit subjected to C₂H₄ for 5 and 6 days showed the most resistance to the disease. Brown discoloration around the site of inoculation was observed on fruit exposed to C₂H₄ for 2 to 6 days prior to inoculation.

The effects of pre-inoculation C₂H₄ treatments on limiting fungal growth and disease development were also shown by glucosamine content of the orange rind (Table 1), while the one-day C₂H₄ treatment resulted in higher glucosamine content reflecting greater fungal growth. Treatments for longer durations reduced glucosamine content, with the 5- and 6-day C₂H₄ exposures being the most effective (Table 1).

Since changes in CO₂ production by 'Navel' and ' Valencia' oranges in response to C₂H₄ and inoculation treatments were similar, the data for only ' Valencia' oranges are included. Respiration rates of ' Valencia' fruit held in air at 20°C decreased with time (Fig. 1). Inoculation with P. italicum stimulated respiration rates. Fruit treated with C₂H₄ for 3 days and then moved into ambient air showed an increase in their respiration rate during exposure to C₂H₄. The magnitude of increase in respiration rates was proportional to C₂H₄ concentration up to 100 μl/liter, which did not differ significantly from the 1000 μl/liter treatment. Ethylene production by control fruit changed little and remained between 0.05 and 0.1 μl/kg/hr during holding at 20°C for 9 days (Fig. 2). Inoculation stimulated C₂H₄ production about 10-fold by the 6th day (3 days after inoculation).

Initial juice composition of ' Navel' and ' Valencia' oranges included SSC of 12.7% and 11.8%, pH of 3.9 and 3.3, titratable acidity of 0.7%, and 1.2% citric acid, and total phenolics of 84 and 47 mg/100 g fresh weight, respectively. Differences in these constituents between treatments following exposure to C₂H₄ and holding at 20°C for various durations before and/or after inoculation were not significant (data not included).

Total phenolics in the rind of uninoculated fruit increased in response to C₂H₄ treatment (Table 2). Infected fruit that were treated with C₂H₄ after inoculation showed no change in their total phenolic content. Fruit that were exposed to C₂H₄ for 3 days before inoculation, then held in air for 6 days, had higher final total phenolics than those which were inoculated before exposure to C₂H₄. Total phenolic content of the adjacent non-

| Table 1. Effect of duration of exposure to 1000 μl/liter C₃H₄ before inoculation with Penicillium italicum on rot development as indicated by lesion diameter and glucosamine content in the rind of ' Valencia' oranges held at 20°C for 6 days after inoculation. |
|-----------------|-----------------|-----------------|-----------------|
| Treatment       | Mean Diameter of lesions (mm) | mg glucosamine/g dry wt of the rind |
| air control     | 53 c            | 7.44 d          |
| Exposed to C₂H₄ for the following durations before inoculation: | | |
| 1 day           | 55 c            | 10.30 c         |
| 2 days          | 42 b            | 3.20 b          |
| 3 days          | 42 b            | 2.49 b          |
| 4 days          | 44 b            | 2.55 b          |
| 5 days          | 36 a            | 1.92 a          |
| 6 days          | 34 a            | 1.97 a          |

*Glucosamine content in the rind of uninoculated fruit = 0.10 mg/g dry wt.
*Mean separation in columns by Duncan's new multiple range test, 5% level.
infected rind tissues of inoculated fruit was not significantly different from that found in the infected rind tissues (data not shown).

There was no PPO activity in both inoculated and noninoculated fruit regardless of the \( \text{C}_2\text{H}_4 \) treatments. PAL activity increased significantly only in the rind of control fruit exposed to 1000 \( \mu \text{liter} \) \( \text{C}_2\text{H}_4 \) for 6 days and held in air for an additional 6 days (Table 2). Inoculated oranges exhibited a much lower PAL activity than did noninoculated fruit.

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PAL Activity (( \mu \text{mole of trans-} \text{cinamic acid/g fresh wt} ))</th>
<th>% total phenolics (fresh-wt basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>1.7 b</td>
<td>1.30 bc</td>
</tr>
<tr>
<td>Held in air for 12 days</td>
<td>5.2 b</td>
<td>1.38 b</td>
</tr>
<tr>
<td>Exposed to ( \text{C}_2\text{H}_4 ) for 6 days followed by 6 days in air</td>
<td>14.3 a</td>
<td>1.54 a</td>
</tr>
<tr>
<td>Inoculated, then held in air for 6 days</td>
<td>2.1 b</td>
<td>1.23 c</td>
</tr>
<tr>
<td>Inoculated, then exposed to ( \text{C}_2\text{H}_4 ) for 6 days</td>
<td>1.9 b</td>
<td>1.24 c</td>
</tr>
<tr>
<td>Exposed to ( \text{C}_2\text{H}_4 ) for 3 days before inoculation, then held in air for 6 days</td>
<td>1.5 b b</td>
<td>1.45 a</td>
</tr>
</tbody>
</table>

*Mean separation in columns by Duncan’s new multiple range test, 5% level.*

**Discussion**

Although it is well known that \( \text{C}_2\text{H}_4 \) enhances senescence of fruit and consequently decreases their resistance to pathogens, we found that orange fruit exposed to \( \text{C}_2\text{H}_4 \) for 2 to 6 days increased resistance to rot development; exposure for 5 or 6 days was more effective than shorter exposures. This may be due to synthesis of antifungal compound(s), which restricted fungal growth as reflected in smaller lesion diameter and lower glucosamine content. Resistance to *Ceratocystis fimbriata* induced...
by \( \text{C}_2\text{H}_4 \) has been reported in sweet potato roots and was accompanied by an increase in activity of peroxidase and polyphenol oxidase (17). Lockhart et al. (11) indicated that \( \text{C}_2\text{H}_4 \) treatment at either high or low concentrations was effective in inhibiting apple rot development caused by \textit{Gloeosporium al-}

bum. Resistance to \textit{Colletotrichum gloeosporioides} was induced in green-colored tangerines after 3 days of exposure to \( \text{C}_2\text{H}_4 \) and was related to accumulation of lignin and other inhibiting compounds produced in response to infection (3, 4). However, Brown and Barnmore (4) concluded that resistance in orange-colored fruit can be broken by the use of 100 \( \mu \)l/liter \( \text{C}_2\text{H}_4 \) concentration for 76 hr. In contrast, we found that resistance to \textit{P. italicum} developed better at 1000 \( \mu \)l/liter for 5 and 6 days than for shorter treatment periods before inoculation.

We did not detect any PPO activity in healthy or infected rind tissues. The dark-brown discoloration development in lesions of \( \text{C}_2\text{H}_4 \)-treated fruit may have been due to oxidation and polymerization of phenolic compounds and formation of brown products by other oxidative agents. High PAL activity was detected only in uninoculated fruit exposed to 1000 \( \mu \)l/liter \( \text{C}_2\text{H}_4 \) for 6 days, followed by 6 more days in air at 20°C. PAL activity decreased dramatically in response to inoculation with \textit{P. italicum}, indicating that this enzyme may play a role in the synthesis of phytoalexins or other aromatic antifungal compounds, as proposed by other workers (13, 15). Our results agree with those of Rivi et al. (13) who obtained the highest PAL activity in citrus fruit peel with the highest \( \text{C}_2\text{H}_4 \) concentrations (100 and 1000 \( \mu \)l/liter). Our results also support the findings of Ismail and Brown (9) who found that PAL activity in inoculated 'Valencia' flaveddi infected with \textit{Penicillium digitatum} increased slightly after 24 hr, then declined as inoculated areas started to soften due to decay.

The effects of \( \text{C}_2\text{H}_4 \) treatments and inoculation on respiration rates of orange fruit were in line with previous studies of oranges and other nonclimacteric fruit (2). Ethylene production by orange fruit was also stimulated in response to disease stress. However, the amount of \( \text{C}_2\text{H}_4 \) produced was very small relative to the added \( \text{C}_2\text{H}_4 \) concentration (1000 \( \mu \)l/liter) which significantly influenced disease resistance.

Total phenolics increased in the rind of uninoculated fruit when treated with \( \text{C}_2\text{H}_4 \), but did not change in inoculated rind. The decrease of total phenolics in fruit exposed to \( \text{C}_2\text{H}_4 \) for 4 to 6 days before inoculation may have been a result of oxidation of these compounds and consequently the development of brown discoloration which was observed in infected tissues treated with high \( \text{C}_2\text{H}_4 \) concentrations. Accumulation of total phenols in carrot roots treated with \( \text{C}_2\text{H}_4 \) has been reported earlier (14). Further research is needed to elucidate \( \text{C}_2\text{H}_4 \) effects on specific phenolic compounds and possibly other constituents in orange fruit which may be responsible for increased resistance to pathogens.

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**Literature Cited**


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