

## The Effect of Ethylene on Susceptibility of Robinson Tangerines to Anthracnose

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### ABSTRACT

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The effect of ethylene on the development of anthracnose in mature, green-colored Robinson tangerines was twofold. Disease occurred when inoculated fruit were treated immediately with ethylene, but the incidence was reduced when fruit were exposed to ethylene for 3 days prior to inoculation. Ethylene stimulated disease, apparently by inducing the pathogen to develop infection hyphae that

penetrated the peel tissue. Ethylene also induced resistance in green fruits, but this resistance did not develop rapidly enough to inhibit infection except when the ethylene treatment preceded inoculation. Tangerines with good natural orange color were resistant to anthracnose, but this was partially overcome by exposing fruit to 100  $\mu$ liters ethylene per liter of air but not by exposure to 10  $\mu$ liters.

*Additional key words:* lignin, postharvest decay, *Colletotrichum gloeosporioides*.

Anthracnose, which is caused by *Colletotrichum gloeosporioides* Penz., is a serious postharvest decay of mature Robinson tangerines, but only when the fruit must be treated (degreened) with ethylene to improve peel color (5). Severity of the disease is dependent upon duration of degreening (1, 5) and ethylene concentration. More anthracnose developed in tangerines degreened with 50  $\mu$ liters ethylene per liter of air than in fruit treated with only 5  $\mu$ liters ethylene per liter of air (1). Observations of Robinson tangerines commercially treated with ethylene indicate that the disease is most prevalent on ethylene-treated fruit that degreens to a pale orange or yellow color instead of to the preferred deep orange.

Ethylene stimulates appressoria of *C. gloeosporioides* to form infection hyphae (1). The thin threadlike infection hyphae penetrate the cuticle and epidermal cells and subsequently form larger primary hyphae that are responsible for most of the fruit decay.

This study describes in greater detail the effect of ethylene on development of anthracnose and induced resistance which is associated with fruit color in Robinson tangerines.

### MATERIALS AND METHODS

Samples of mature fruit were collected at intervals from two commercial groves from early September through November. Fruit picked in September exhibited very little natural orange color break. During October until

the first of November, both green and orange-colored fruit were available because of natural variation that exists between groves and in individual fruit. In November, the only green-colored fruit available were those that originated from a bloom that developed 6-8 weeks after the major bloom.

Fruit color was measured with a Hunterlab D-25 Color Difference Meter described previously (8) which measures and numerically expresses reflected light as the a/b ratio (7). This value correlates well with apparent visual color and the U. S. Department of Agriculture Color Standards for citrus (7). Green color was represented by a negative value and a value of about zero indicated a yellow color. The magnitude of a positive value represented the intensity of a red-orange color in the fruit peel. Color of the fruit used in each experiment before ethylene treatment was determined and is indicated by the a/b ratio.

Freshly harvested tangerines were dipped in 1.0% sodium hypochlorite and immediately washed in running tap water, and then dried with cheesecloth. This procedure effectively killed and removed appressoria present on fruit surfaces from natural grove infection and prevented development of anthracnose from this inoculum during the ethylene experiments. Buttons (calyx + disk) of washed fruit were dipped in 600  $\mu$ g/ml of benomyl to prevent development of stem-end rot (which is caused by *Diplodia natalensis* P. Evans). Fruit were inoculated, either prior to or after ethylene treatments, with spore suspension droplets at the fruit equator as previously described (1), and held at near 100% relative humidity (RH) overnight to induce appressorium formation. Fruit then were placed in Plexiglas chambers that contained various ethylene concentrations obtained

by using previously described procedures (2, 8). During ethylene treatment, fruit were maintained at 28-30 C and 95% RH. Ethylene concentrations were maintained at  $\pm 10\%$  of the intended treatment concentration and verified with a gas chromatograph equipped with a flame ionization detector. Following treatment, fruit were stored in dishpans (1) at near 100% RH at 25-27 C.

Isolations of *C. gloeosporioides* were made as previously described (1) to determine if appressoria had formed infection hyphae.

Fresh sections, 24-36  $\mu\text{m}$  in thickness, were prepared with a Hooker Plant Microtome and stained for lignin with phloroglucinol-HCl.

TABLE 1. Influence of ethylene concentration on the incidence of anthracnose (caused by *Colletotrichum gloeosporioides*) in Robinson tangerines

Ethylene concentration ( $\mu\text{liters per liter of air}$ )	Fruit with anthracnose <sup>a</sup> (%)
0	0.0
10	16.7
20	56.7
30	65.5
40	86.7
50	85.9

<sup>a</sup>Average for three replications, each containing 10 inoculated green-colored fruit ( $a/b = -0.25$ , as determined with a Hunterlab D-25 Color Difference Meter, which reads  $a/b$  directly). Fruit were exposed to ethylene for 2 days, and stored for 1 week at 26 C at near 100% RH.

TABLE 2. Effect of low ethylene concentrations on the susceptibility of green- vs. orange-colored Robinson tangerines to infection by *Colletotrichum gloeosporioides*

Fruit color <sup>a</sup> ( $a/b$ )	Ethylene exposure (days)	Fruit with anthracnose <sup>b</sup> (%)
-0.60 (green)	1	72.7
	2	97.0
1.13 (orange)	1	00.0
	2	00.0

<sup>a</sup>Fruit color was determined with a Hunterlab D-25 Color Difference Meter, which reads  $a/b$  directly.

<sup>b</sup>In each treatment, 50 fruit were inoculated and treated with 10  $\mu\text{liters}$  of ethylene/liter of air and stored at near 100% RH for 1 week at 26 C.

TABLE 3. Effect of a high ethylene concentration on the resistance of orange-colored Robinson tangerines to infection by *Colletotrichum gloeosporioides*

Harvest date (1975)	Fruit color <sup>a</sup> ( $a/b$ )	Ethylene concentration ( $\mu\text{liters per liter of air}$ )	Fruit with anthracnose <sup>b</sup> (%)
20 October	1.10 (orange)	10	00.0
		100	96.1
29 October	0.91 (orange)	10	00.0
		100	38.5

<sup>a</sup>Fruit color was determined with a Hunterlab D-25 Color Difference Meter, which reads  $a/b$  directly.

<sup>b</sup>Disease that developed in each treatment of about 50 fruit after ethylene treatment for 76 hours and storage at near 100% RH for 1 week at 26 C.

## RESULTS

**Ethylene concentration.**—No disease developed in inoculated green-colored tangerines that had not been treated with ethylene (Table 1). However, increasing ethylene concentrations in increments of 10  $\mu\text{liters}$  per liter of air increased the incidence of anthracnose until a maximum level of disease was reached in the 40  $\mu\text{liters}$  of ethylene per liter of air treatment. Subsequent experiments were conducted at 10  $\mu\text{liters}$  of ethylene per liter of air, the lowest level evaluated, because of the moderate amount of disease increase at that concentration.

**Association of fruit color with resistance to anthracnose.**—The extreme susceptibility of ethylene-treated green-colored Robinson tangerines to anthracnose as compared with the resistance of fruit with a good natural orange color is shown in Table 2. The inoculated green fruit ( $a/b = -0.60$ ) developed 72.7% anthracnose after only 1 day of treatment with 10  $\mu\text{liters}$  of ethylene per liter of air and 1 week of storage. None of the inoculated orange-colored fruit ( $a/b = 1.13$ ) with similar ethylene treatments developed anthracnose. The difference in anthracnose development in response to 10  $\mu\text{liters}$  ethylene per liter of air (Table 1 vs. Table 2) was attributed to the difference in degree of fruit greenness ( $a/b = -0.25$  vs.  $a/b = -0.60$ ). The resistance of naturally orange-colored tangerines ( $a/b = 1.10$  and  $a/b = 0.91$ ) to infection by *C. gloeosporioides* was partially overcome when fruit were treated with a high concentration of ethylene (100  $\mu\text{liters}$  per liter of air), for 76 hr (Table 3). No disease developed on orange-colored fruit treated with only 10  $\mu\text{liters}$  of ethylene per liter of air.

**Induced resistance with preinoculation ethylene treatments.**—Partial resistance could be induced by exposing green-colored fruit to ethylene for more than 1 or 2 days immediately prior to inoculation with *C. gloeosporioides* (Table 4). An ethylene treatment of 3 days, that resulted in a yellow fruit with some orange pigmentation ( $a/b = 0.43$ ) was as effective for inducing resistance as the 4-day exposure.

**Mode of resistance of orange-colored fruit to infection.**—Resistance of natural or ethylene-induced colored fruit to infection by *C. gloeosporioides* was manifested in two ways. In some inoculated orange-colored fruit, there were no external symptoms of infection following treatment with 10  $\mu\text{liters}$  of ethylene per liter of air for 2 days and 1 week of storage. *Colletotrichum gloeosporioides* was not isolated any

more frequently from surface-sterilized peel of these fruit than from comparable inoculated nonethylene-treated fruit. The ethylene treatment, therefore, did not cause the appressoria to form infection hyphae in the peel of several of the orange-colored fruit.

In other orange-colored fruit, external symptoms were present in the form of silver-gray halos around the individual appressoria. This was due to partial removal of the red-orange pigments following extensive development of the infection hyphae. Symptomatology, at this stage, was similar to that which resulted from infection of susceptible green-colored tangerines [Fig. 1-F in (1)]. However, there was no further progression of the infection in the orange-colored fruit. Fresh sections through the infected peel showed extensive browning due to the accumulation of lignin (Fig. 1) in a three- to four-cell deep layer of the flavedo. This response was not observed in orange-colored fruit where the resistance was overcome by treating with 100  $\mu$ liters of ethylene per liter of air.

### DISCUSSION

Ethylene increased or reduced the incidence of disease, depending upon the times and concentration of ethylene treatment and inoculation. Upon exposure of inoculated green-colored tangerines to ethylene, the infection process and resistance mechanism(s) are apparently initiated simultaneously. However, the infection process developed more rapidly.

Infection of green-colored fruit only occurred after exposure to ethylene (1). The lack of infection of nonethylene-treated fruit does not appear to be due totally to any inherent resistance of these fruit. Although appressoria were formed, relatively few produced infection hyphae until after the fruit were exposed to ethylene (1). Therefore, it appears that ethylene is required for penetration from appressoria of *C. gloeosporioides*, thereby resulting in numerous penetrations within a given area of the fruit surface. Lesion development on Robinson fruit is related to the inoculum concentration expressed as appressorial density

(1). Thus, penetration of the fruit surface by more of the appressoria in response to ethylene would increase the effectiveness of the inoculum. Ethylene may induce germination of the appressoria by causing nutrients to be released or made more available at the fruit surface. Ethylene could possibly alter the quantity or quality of cellulolytic or pectolytic enzymes produced by *C. gloeosporioides*, which subsequently could affect its ability to colonize host tissue. A similar suggestion was made by Hislop et al. (3) in studies of brown rot of apples caused by *Sclerotinia fructigena*. However, they were not able to demonstrate that ethylene affected the secretion of extracellular pectolytic enzymes by *S. fructigena* growing in liquid culture (3).

Resistance induced by the use of ethylene has been reported for other host-pathogen combinations. Ethylene concentrations required to induce resistance of sweet potato roots to *Ceratocystis fimbriata* (6) and to retard lesion development in apples by *S. fructigena* (3) were similar to those that induced resistance in green-colored fruit in our study. Lockhart et al. (4), however, used ethylene levels considerably higher than ours to retard pathogenesis of *Gloeosporium album* in McIntosh apples. Resistance in green-colored tangerines was induced after 3 days of exposure to ethylene. However, *C. gloeosporioides* can penetrate similar green-colored fruit within about 2 days (1). Thus, infection in such green-colored tangerines can develop before resistance induced by ethylene becomes effective. As the fruit develops more natural orange color associated with peel maturity, the resistance process often is already initiated; thus, the ethylene treatment does not induce extensive anthracnose development. Therefore, initiation of the resistance mechanism(s) in relation to development of fungal penetration is very important. As this resistance in orange-colored fruit can be broken by the use of high ethylene concentrations, a critical balance between susceptibility and resistance apparently exists. Since ethylene hastens maturation, the factor or factors responsible for resistance may be similar, whether induced by ethylene or developed naturally.

Resistance was evoked either at the appressorium cuticle interface when infection hyphae were not formed or at the time of penetration of infection hyphae into the

TABLE 4. Ethylene-induced resistance of green-colored Robinson tangerines to infection by *Colletotrichum gloeosporioides*

Ethylene exposure <sup>a</sup> (days prior to inoculation)	Fruit color (a/b ratio) <sup>b</sup>	Fruit with anthracnose <sup>c</sup> (%)
0	-0.60	84.9
1		94.0
2		64.1
3	0.43	18.4
4		18.5

<sup>a</sup>In each treatment, 50 fruit were exposed to 10  $\mu$ liters of ethylene/liter of air prior to inoculation.

<sup>b</sup>Fruit were initially green-colored but became orange with continued exposure to ethylene. The a/b ratios were determined only for 0 and 3 days, and were measured with a Hunterlab D-25 Color Difference Meter, which reads a/b directly.

<sup>c</sup>Following inoculation, all fruit were treated again with 10  $\mu$ liters of ethylene/liter of air for 3 days and stored for 1 week at 26 C at near 100% RH.

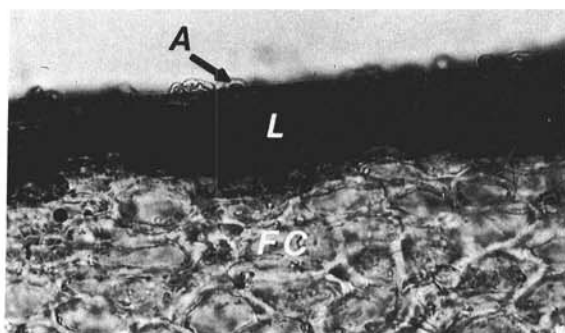


Fig. 1. Browning of tissues resulting from lignin accumulation in response to penetration by *Colletotrichum gloeosporioides* into flavedo cells of an orange-colored Robinson tangerine ( $\times 320$ ) (A = appressorium; L = lignin; and FC = flavedo cells).

flavedo cells. Penetration into these cells resulted in the accumulation of lignin and possibly other inhibiting compounds produced in response to infection. Resistance could be due to the presence of these compounds rather than, or in addition to, a physical barrier formed by the lignin.

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