There may be—no doubt there are —variations in the establishment of new vascular tissues between stock and scion. There may be differences among cultivars in capacity to support reproduction of TmRSV. There may be differences in capacity to destroy TmRSV particles or components. There may be variation related to differences in physical status of the host plants within a given clone.

One further possibility cannot be ruled out by our data or by others' reports we have seen: the possibility exists that at least some plants may respond to relatively slow virus influx with some sort of defense mechanism that either temporarily or permanently inhibits access of the virus.

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Ethylene, Fungi, and Summer Fruit Drop of Navel Orange[1]

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Abstract. **Species of *Alternaria* and *Gloeosporium* were most often isolated from fruit with blossom-end yellowing (BEY), a disorder associated with summer fruit drop of navel orange [*Citrus sinensis* (L.) Osbeck]. Fruit inoculated with pure cultures of these fungi did not develop BEY; however, wounded fruit which were inoculated with fungi produced higher levels of ethylene and more extensive BEY than wounded, noninoculated fruit. Fruit with BEY produced higher amounts of ethylene than symptomless fruit. The methoxy analog of rhizobitoxine (methoxyvinylglycine) did not reduce ethylene levels, and silver nitrate increased ethylene production from fruit with BEY. Ethylene and fungi are associated with BEY of navel orange but do not appear to be causal factors.**

The yield of navel orange is generally lower than that of other citrus cultivars (10, 17, 26, 27). Several periods of heavy fruit drop contribute to this reduced yield, including a recently characterized summer fruit drop (19). Fruit that drop during this period usually first develop blossom-end yellowing (BEY), which is then followed by abscission.

BEY begins in the secondary fruit (navel), advances to surrounding tissue and intensifies within the navel area prior to abscission. Insects also invade the navel and have been associated with fruit severely affected by BEY (10, 19). *Alternaria citri* Ellis & Pierce has been associated with "June drop" of 'Washington' navel orange in California (10) and BEY of navel oranges in Florida (13). However, BEY symptoms observed in the field are unlike those symptoms characteristic of *Alternaria* black rot of orange caused by *Alternaria citri*. Ethylene is known to cause yellowing of diseased tissue (7, 28), but has not previously been measured in BEY fruit. Objectives of this research were to investigate the roles of fungi and ethylene in navel orange BEY and summer fruit drop. Species of *Alternaria* and *Gloeosporium* were selected for use in this study because these fungi have been frequently isolated from citrus fruit (25). Moreover, they are known to cause disease symptoms similar to BEY.

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Materials and Methods

Plant material. Fruit were collected from 15-year-old 'Washington'-type navel orange trees on sour orange (*Citrus aurantium* L.) rootstock near Eustis, Fla.

Field pretreatments and inoculations. Fruit at stage II were selected from the periphery of trees about 1.0 to 2.0 m above soil surface on May 30, 1980. Two ethylene-producing materials were applied to fruit as treatments prior to fungal inoculation: (2-chloroethyl)phosphonic acid (ethephon), an ethylene-releas-

ing compound, and 5-chloro-3-methyl-4-nitro-1H-pyrazole (Release), a compound used to induce wound ethylene production, were applied to fruit in order to determine whether or not ethylene was associated with development of BEY and fruit abscission, and whether or not it altered fungal penetration and pathogenicity. A total of 12 fruit, 20 to 40 mm in diameter, 4 from each of 3 trees, were treated prior to fungal inoculation by 1 of the following procedures: 1) wounding at the edge of the fruit stylar abscission zone with a 20 gauge needle, which punctured both flavedo and albedo tissue, in order to generate wound ethylene and provide entryway or stimulate fungal infection; 2) dipping in 200 ppm Release plus 0.1% X-77 adjuvant for 10 sec; 3) dipping in 300 ppm ethephon plus 0.1% X-77 adjuvant for 10 sec; 4) or dipping in 0.1% X-77 for 10 sec. Fruit were then inoculated with PDA fungal discs (5 mm in diameter) of either *Alternaria* or *Gloeosporium* taken from the periphery of actively growing colonies (about 7 to 14 days old) isolated from 1-to 2-month-old fruit. Fungal isolates were obtained from young fruit because BEY had not yet developed under field conditions. However, microscopic investigation showed that fungal isolates from 1980 fruit were like those obtained from BEY fruit from 1979. This was later confirmed upon examination of fungal isolates from 1980 BEY fruit. Each disc containing mycelia and conidia was placed on the cotton pad of a sterile bandage previously saturated with distilled water. Bandages bearing PDA fungal discs were placed securely over the wound or directly over the stylar-end of treated fruit.

A duplicate experiment had been performed April 29, 1980, where 5 fruit, 15 to 20 mm in equatorial diameter, on each of 3 trees had been treated prior to fungal inoculation by 1 of the 4 above procedures. A total of 180 fruit was used. Results of these duplicate experiments were pooled because no significant differences existed between experiments.

Isolation of fungi. Fungi were isolated from 12 navel fruit showing early BEY symptoms and from those showing symptoms as a result of the above treatments. A transverse cut was made 2 to 4 mm deep across the navel and 2 mm^3 V-shaped tissue pieces were excised from the center and edge of the yellowed tissue. Excised tissue was surface sterilized in approximately 0.3% sodium hypochlorite for 3 min, blotted dry on sterile filter paper, cut in half, and cultured on acidified potato dextrose agar (APDA). The APDA was prepared as described by El-Gholl et al. (11). Twenty drops of 50% lactic acid were added to 300 ml of PDA to inhibit bacterial growth. Tissue pieces on APDA were incubated under 25 μ E m^{-2} sec^{-1} fluorescent light daily under an 8.5 hr photoperiod and 25 ± 2°C regime. When a dense mycelial mat had formed, APDA fungal plugs (taken from 7-day-old cultures) were transferred to sterile APDA in order to obtain pure fungal cultures. Fungal species were identified by viewing spores under a light microscope.

Ethylene production from treated and inoculated detached fruit. Five stage II (the cell expansion phase) fruit per treatment, each including a peduncle and 3 to 4 cm of attached shoot, were clipped from each of 3 trees in duplicate experiments on April 29 and May 30, 1980. Fruit were surface-sterilized in about 0.3% sodium hypochlorite for 3 min, rinsed with distilled water, air-dried, and subjected to the same treatments and inoculum sources that were used in field studies. Fruit-shoot combinations were removed from water-filled vials and sealed in 480 ml clear glass jars for 1 hr at 25 ± 2°C under 25 μ E m^{-2} sec^{-1} fluorescent light. A 1-ml gas sample was drawn from each jar and analyzed for ethylene by gas chromatography. Jars were purged with air after sampling. Fruit-shoot combinations were removed and shoots were reclipped under water, inserted into a water-filled vial, and reincubated in the dark at 25° until the next sampling time. CO_2 does not accumulate significantly during a 1 hr incubation period (G. K. Rasmussen, personal communication); consequently, no precautions were taken to control CO_2 levels within the container.

Ethylene inhibition and BEY. Blossom-end-yellowed and apparently healthy green navel orange fruit were collected on Aug. 8, 1979. Fruit were dipped for 15 sec in solutions of either 100 or 250 ppm $AgNO_3$ or 20 ppm methoxy analog of rhizobitoxine, or the broad spectrum fungicide Difolatan [cis-N-(1,1,2,2-tetrachloroethyl) thio)-4-cyclohexene-1,2-dicarboximide]. Each solution contained 0.1% X-77 adjuvant. Each of 3 replicates consisted of 3 fruit (9 fruit/treatment) placed in a 1400-ml plastic beaker. Ethylene levels were determined at regular intervals for 24 hr after treatment.

Ethylene production from fungi. PDA discs, 5 mm in diameter, of actively growing cultures of *Alternaria* or *Gloeosporium* species (same age as those discs used for field inoculations), bearing mycelia, conidia and 5 PDA discs serving as controls, were set on 8-ml slants in 25-ml test tubes with 5 replications per treatment. Each tube was plugged with a cotton swab and incubated under 25 μ E m^{-2} sec^{-1} light for 8 hr at 25 ± 2°C. Tubes were sealed with tight-fitting serum rubber stoppers 1 hr before each sampling. Gas samples for ethylene analysis were drawn at 24 hr intervals for 5 days, by which time a dense mycelial mat had formed. Sterile conditions were maintained throughout.

Results and Discussion

Fruit inoculations, isolations, and ethylene. No statistical differences existed in percentage abscission owing to treatment (i.e., ethephon, Release, and wounding) prior to inoculation with fungi; therefore, data for the inoculum sources were combined (Table 1). Ethephon treatment with or without fungi induced abscission after 14 days, with very little abscission in the following week. Wounding alone did not induce fruit abscission; however, wounding plus fungi did induce abscission. Most fruit treated with ethephon, ethephon plus fungi, or wounding plus fungi showed BEY symptoms prior to abscission.

Wounds can provide entryways for fungal invasion of citrus or can alter host-pathogen interactions, making fungal invasion more likely (14, 21, 24). Fruit wounding appeared to help establish a fruit-wound-fungal interaction, which accelerated or induced chemical processes needed for fruit abscission under conditions studied.

Table 1. Effects of wounding, Release, ethephon, and inoculation with species of *Alternaria* and *Gloeosporium* on navel fruit abscission.

Treatment (ppm)	*Gloeosporium* and *Alternaria*	Abscission (%) Days after Treatment 7	14	21
Control	−	0	0	0
	+	0	0	0
Wound	−	0	0	0
	+	0	42	42
Release (200)	−	0	0	0
	+	0	8	8
Ethephon (300)	−	0	25	33
	+	0	42	42

Isolations of fungi were made from navel fruit showing early BEY symptoms prior to fruit abscission. Fungi were not recovered in 4 of 11 BEY fruit. Species of *Alternaria* were recovered in 7 of 11 and species of *Gloeosporium* were recovered in 2 of 11 fruit. Fruit with BEY, BEY induced by ethephon, ethephon plus fungi, or wounding plus fungi did not always harbor fungi, even though BEY symptoms were present prior to abscission. Furthermore, *Alternaria, Gloeosporium,* and other fungi have been isolated from healthy navel orange fruit (25). Fungi were often associated with BEY fruit but apparently were not the primary cause of the disorder.

Ethylene production from navel fruit treated with ethephon was significantly greater than from wounded fruit at 12 and 24 hr (Fig. 1) and from non-wounded or Release-treated fruit at all intervals except 72 hr. Ethylene production was significantly greater in wounded fruit with fungi than in wounded fruit alone at 24 hr and non-wounded fruit at 12, 24, and 48 hr. Ethylene production by fruit decreased at 24 and 48 hr, but generally increased at 72 hr.

The time-dependent ethylene production from wounded, wounded plus fungi, or ethephon-treated fruit is analogous to the "M-shaped" curve reported by Kossuth and Biggs (16) for detached citrus fruit treated with Release. They described the first peak of ethylene production as a "chemical peak" which occurred 30 hr after treatment with 300 ppm Release. This peak was associated with fruit abscission. The second ethylene peak occurred 3 to 4 days after detachment and may have resulted from endogenous ethylene production due to aging. The ethylene peaks observed at 12 hr in wounded, wounded plus fungi, or ethephon-treated fruit may be analogous to the chemical peak, and the 72-hr peak may represent ethylene production in aged, detached citrus fruit as reported previously (16). In the experiments reported here, Release did not stimulate ethylene production (Fig. 1). Similarly, low ethylene production was also found in immature 'Hamlin' orange treated with Release (12).

Stage of development alters responsiveness of fruit to abscission-inducing chemicals (18); therefore, it was necessary to investigate several treatments by which ethylene could be produced in stage II fruit. Those treatments which induced ethylene production after 12 and 24 hr also showed more BEY and had the greatest percentage of abscission (Table 1). Apparently, ethylene has a role in the abscission of these stage II fruit if present at threshold levels.

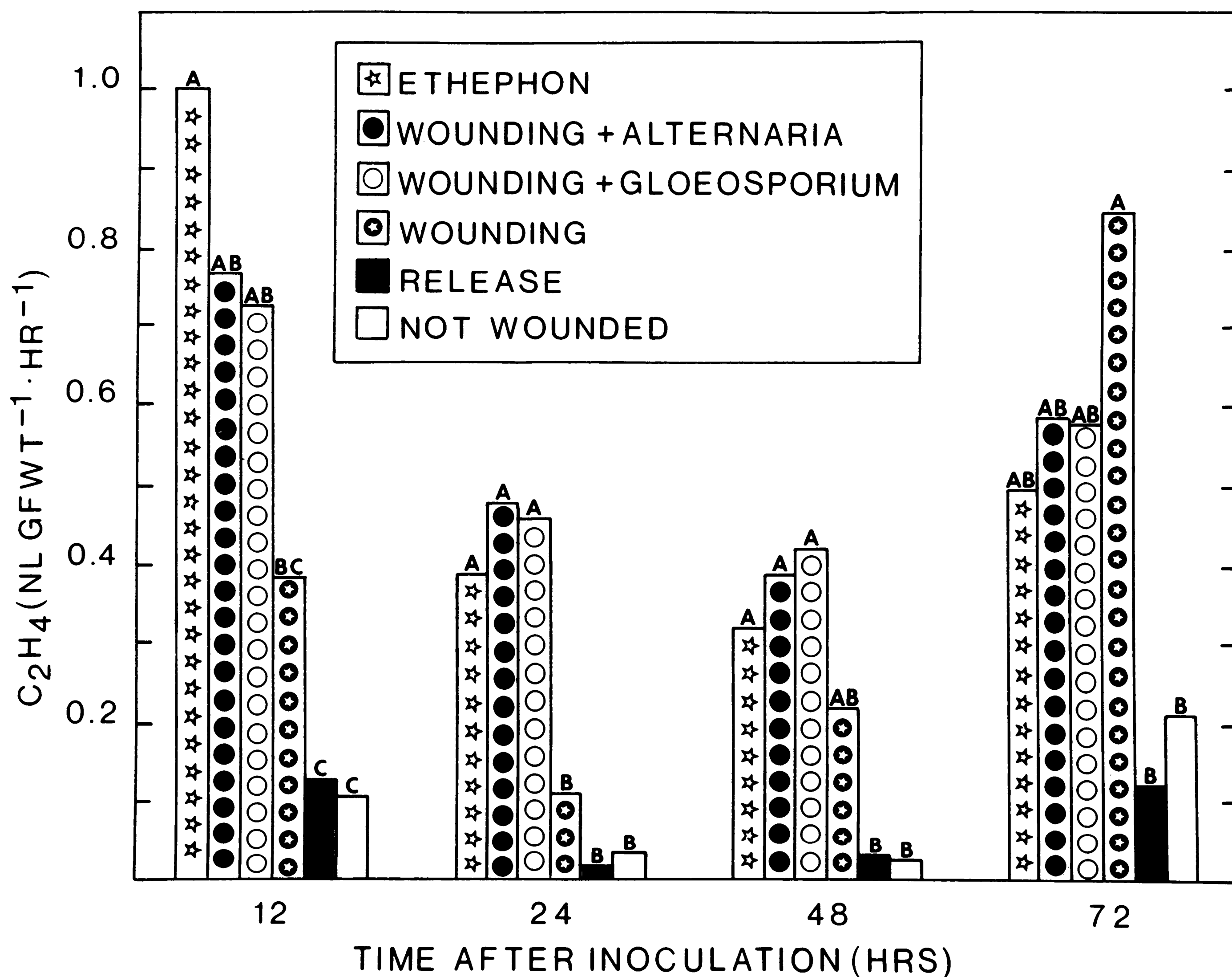

Fig. 1. Ethylene production from detached navel orange fruit at various intervals after treatment with ethlene-releasing compounds and inoculation with fungi. Mean separation among treatments within each time interval and among incubation periods by Duncan's multiple range test, 5% level. Bars are means of 5 replicates.

Some pathogens are capable of synthesizing ethylene independently of the host (7, 15, 22) and such synthesis could have augmented ethylene production found in wounded plus fungi treatments. However, no measureable ethylene was produced *in vitro* by actively growing cultures of species of *Alternaria* or *Gloeosporium*. Moreover, 1, 10, and 100 ppm ethylene did not affect growth of 2 morphologically different strains of species of *Gloeosporium* grown at room temperature. *Gloeosporium* was selected for these experiments because it was commonly found in both BEY and apparently healthy fruit (25), and because ethylene can alter growth (20) or stimulate induction of infectious hyphae from latent appresoria that previously had penetrated tangerine peel tissue (8, 9).

Ethylene production and inhibition from BEY fruit. Ethylene production from BEY fruit was not inhibited by chemical treatments known to inhibit ethylene production or action in citrus and other plants (Table 2). The methoxy analog of rhizobitoxine inhibited ethylene production by citrus peel discs in less than 6 hr (12), but had no inhibitory effect on ethylene production from BEY fruit over a 24-hr incubation period. Some plant tissues are rhizobitoxine-sensitive, while others are rhizobitoxine-resistant (2). Ethylene biosynthesis in BEY navel fruit may be similar to the rhizobitoxine-resistant system found in avocado and ripe tomato tissue (2). Vacuum infiltration of rhizobitoxine or aminoethoxyvinylglycine (AVG) at higher concentrations would help to clarify these findings.

Ethylene production from BEY navel fruit was significantly increased by 100 and 250 ppm $AgNO_3$ 24 hr after treatment (Table 2). Similary, Evenson (12) found that $AgNO_3$ increased ethylene production in citrus peel discs. This increase may be due to wounding, increased methionine conversion to ethylene (1), or to accumulation of ethylene due to inadequate incorporation into tissues because $Ag+$ may bind to a site specific for ethylene and, thus, block ethylene action (1, 5, 6).

Alternatively, Aharoni et al. (1) suggested that $Ag+$ stimulated ethylene production in tobacco leaf discs by preserving indole-3-acetic acid (IAA) in the tissue. Typically, $AgNO_3$ reduced ethylene levels from post-climacteric banana and apple tissue (23), ethylene-induced epinasty in tomato and cucumber, and ethylene-induced fruit abscission in cotton, cucumber, and tomato (3, 4). Difolatan did not significantly decrease ethylene levels, further indicating that fungi alone are probably not involved in BEY.

We conclude that while *Alternaria* and *Gloeosporium* were frequently isolated from young navel orange fruit (25) and from BEY fruit, they did not appear to play a causal role in summer drop of navel orange. Ethylene was produced by BEY navel orange fruit prior to abscission and a fruit-wound-fungal interaction was responsible for increased ethylene production and a greater amount of abscission. Thus, we believe that ethylene is associated with BEY navel fruit and their premature abscission, but it is not the primary cause of BEY of navel orange.

Table 2. Ethylene production from BEY-affected fruit after treatment with $AgNO_3$, rhizobitoxine, or the fungicide Difolatan.[z]

Treatment	Ethylene production[y] in 24 hr (nl g fresh wt^{-1})
Symptomless navel	0.06 ± 0.03
BEY-affected navel	0.28 ± 0.11
$AgNO_3$ (250 ppm)	1.96 ± 0.56
$AgNO_3$ (100 ppm)	0.64 ± 0.03
Rhizobitoxine[x] (20 ppm)	0.57 ± 0.18
Difolatan (200 ppm)	0.43 ± 0.15

[z]Experiment performed Aug. 8, 1979. Representative of 2 experiments.
[y]Mean of 3 replicates ± SE.
[x]Methoxy analog (Ro-7-7957) of rhizobitoxine was dissolved in Na-phosphate buffer at pH 4.9.

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Cuticular Retention *in Vitro* and Localization of Zn after a Foliar Application of Zinc-containing Fungicides[1]

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Abstract **The cuticular retention of zinc contained in 4 fungicides (Antracol, Calyram, M-Special, and Polyram Combi) and zinc chloride was studied using isolated pear leaf cuticles (*Pyrus communis* L. cv. Passe Crassane).The retention of Zn by the cuticular discs was significant after a 24-hour immersion in each fungicide (0.2% w/v). The distribution coefficient, calculated as the ratio of the zinc retained by the cuticles (in μg/g) to the zinc content of the immersion liquid (in μg/ml), varied from 25.1 to 88.2 for the fungicides, but was only 5.7 in the case of $ZnCl_2$. Laser probe mass spectrography indicated that Zn supplied with the fungicides remained superficially localized 24 hours after a foliar application on maize (*Zea mays* L. cv. Dekalb 202); Zn was detectable only in the surface zone corresponding to a maximum depth of 30 μm from the adaxial leaf surface.**

Many Zn-containing fungicides are currently sprayed on leaves for plant protection from pathogens. Some observations suggest that the Zn in these fungicides remains localized at the leaf surface level (6) but further work is needed to specify its exact localization. This study was undertaken with 2 objectives: a) to determine cuticular retention of the Zn provided by the fungicides by using isolated pear leaf cuticles; and b) to determine the localization of Zn in maize leaves treated with Zn-containing fungicides by laser probe mass spectrography.

Materials and Methods

Chemicals. The commercial and common names of the Zn-containing fungicides are listed in Table 1.

Determination of the cuticular retention. Cuticular discs (10-mm diameter) from the upper surface of fully expanded 'Passe Crassane' pear leaves, harvested in August in an orchard near Grenoble, France, were enzymatically isolated from the underlying tissues using 2% pectinase and 0.2% cellulase buffered at pH 3.8 as previously described (3). Samples of 21 dry cuticular discs were immersed for 24 hr in 100 ml 0.2% (w/v) of one of the different fungicides or $ZnCl_2$; each solution contained Tween 20 at 0.1% (v/v) as a surfactant. The pH values were 5.5, 4.9, 8.9, 6.5, and 4.4 with Antracol, Calyram, M-Special, Polyram Combi, and $ZnCl_2$, respectively. The period of immersion was long enough to be sure that equilibrium was attained, as was previously shown with Zn as $ZnCl_2$ (4). The experiments were carried out in a gyrotory water bath shaker (New Brunswick Model G 76- speed 5.25) at 25° C. At the end of the immersion period, the cuticles were washed 5 min in 100 ml of deionised water by continous agitation. The cuticular discs were recovered in a nylon net and dried on filter paper. Each batch of 21 discs was divided into 3 samples of 7 discs each, which were separately mineralized and analysed for Zn by atomic absorption spectrometry. Thus, 3 determinations were obtained for each chemical (fungicide or zinc chloride). The results were subjected to a one-way analysis of variance.

Localization studies. The localization experiments were carried out on 15-day-old plants of 'Dekalb 202' maize hydroponically grown in a climate chamber (1). Three or five 2-μl droplets

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