

0 time storage and the indicators determined after the various storage times. A possible explanation of this result could be due to the relatively small differences found and to variations within samples.

In summary, a total of five harvests of freeze-damaged Valencia oranges were made during the 1983-84 and 1984-85 seasons. Storage of statistically equivalent samples for periods of up to three days produced no statistically significant differences in 12 analytical indicators of juice quality and yield. However, in several cases, trends were observed indicating a deterioration in juice quality. The most notable were an 11.2% increase in total glycoside content and a 9.1% decrease in flavor score over the 3 days storage. Small but important increases and decreases were found in Brix/% acid ratio and juice yield, respectively.

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DIPLODIA STEM-END ROT, A DECAY OF CITRUS FRUIT INCREASED BY ETHYLENE DEGREENING TREATMENT AND ITS CONTROL

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Abstract. *Diplodia* stem-end rot is a major decay of ethylene-degreened fruit. Concentrations of ethylene in excess of the 5-10 $\mu\text{l/l}$ required for optimum degreening significantly enhanced the decay. The increase in disease at high ethylene (50 $\mu\text{l/l}$) was not associated with more rapid abscission of the button or to stimulated activity of quiescent infections in button tissue. However, *D. natalensis* P. Evans did penetrate the base of the fruit more rapidly at an ethylene concentration of 50 $\mu\text{l/l}$ than at 10 $\mu\text{l/l}$. Ethylene may have enhanced the growth rate of the fungus or predisposed cells of the abscission zone to hyphal penetration. Fungicide treatments were more effective in controlling *Diplodia* stem-end rot when applied before degreening than after degreening.

Stem-end rot (SER) caused by *Diplodia natalensis* P. Evans is a serious postharvest decay of degreened citrus fruit in Florida (9). *Diplodia natalensis* is usually present in mature, harvested fruit in necrotic tissue on the surface of the floral calyx and disc (button) (4). The fungus grows from the necrotic tissue into the rind through natural openings that occur when the button abscises (5). Degreening encourages development of SER because ethylene initiates abscission. The temperature of 30°C required for optimal chlorophyll removal is also optimal for growth of the fungus. Concentrations of ethylene in excess of the 5-10 $\mu\text{l/l}$ required for degreening cause an even higher incidence of SER (6,9). In this study, much of which has been previously reported (1), some factors are identified

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that are responsible for enhanced disease development on fruit exposed to high ethylene levels. Timing of fungicide treatments to combat the increased risk of SER caused by degreening was investigated.

Materials and Methods

Valencia and Pineapple oranges (*Citrus sinensis* L. Osbeck) were harvested by clipping and leaving the button attached. Experiments were initiated within 4 hr of harvest. Ethylene treatments were conducted with a continuous flow-through system (2) at 30°C and 94-96% relative humidity (RH) with ethylene maintained at $\pm 10\%$ of the desired concentration.

Abscission vs. ethylene concentration. Pineapple oranges with stems about 5 cm long were treated with ethylene at 0, 1, 10 or 50 $\mu\text{l/l}$. The bonding force of the button to each of 20 fruits was determined initially and at 12 or 24 hr intervals with a chatillon pull tester (8) on separate lots of fruit at each time of testing.

Stem-end rot vs. ethylene concentration. The effect of ethylene on the incidence of SER was evaluated by treating graded and randomized lots each of 75 Valencia oranges with ethylene at 0, 1, 10 or 50 $\mu\text{l/l}$ for 96 hr. Rate of fungal penetration was evaluated by treating fruit with ethylene at 0, 10, or 50 $\mu\text{l/l}$ for 48, 72 or 96 hr and then dipping the fruit in thiabendazole (1000 $\mu\text{g/ml}$) for 15 sec. Each treatment, consisting of three replicates of 55 fruit, was stored at 29-30°, 94-96% RH for 4 wk. Ethylene vs. fungal penetration was also studied by treating 100 fruit with ethylene at 1, 10 or 50 $\mu\text{l/l}$ for 48 hr. Buttons were removed from half of the fruit in each treatment and all fruit were stored for disease development. An additional series of 75 fruit were treated at each of these ethylene concentrations for 48 hr. Susceptibility of tissue beneath the button to hyphal penetration was studied by removing

the button and placing 1 ml of an aqueous mycelial suspension (70% transmittance at 600 μm) of *D. natalensis* in the stem cavity. Disease was evaluated after holding the fruit for 2 wk at 29-30°C and 94-96% RH.

Isolation of *D. natalensis* from buttons. Valencia oranges were treated with ethylene at 1 or 50 $\mu\text{l/l}$ for 48 hr. Buttons were removed, surface-sterilized with 1% sodium hypochlorite for 1 min, and plated on Difco potato-dextrose agar containing 50 mg of rose bengal per milliliter. After incubation at 30°C for 5 days, buttons from 40 fruit of each treatment were examined for mycelia of *D. natalensis*. The experiment was repeated twice.

Fungicidal control of stem-end rot. Fungicides were applied before degreening by dipping unwashed fruit in an aqueous suspension for 15 sec. Similar applications were made after degreening by spraying additional washed fruit with a non-recovery fungicide treatment applied on fungicide-saturated, rotating horsehair brushes before waxing with a solvent wax. Fruit were packed in fiberboard cartons and stored for 3 wk at 21°C and 85% RH.

Results and Discussion

Incidence of SER was 4, 24, 47 or 65% in fruit after 4 wk storage when treated with ethylene at 0, 1, 10 or 50 $\mu\text{l/l}$ of air, respectively (Fig. 1). The incidence of SER in fruit treated with ethylene at 10 or 50 $\mu\text{l/l}$ increased rapidly during the second wk of storage, with as much as 70% of the total SER developing during this period. Relatively little SER (4%) developed in fruit treated with air. These results illustrate why diploдия-induced SER is com-

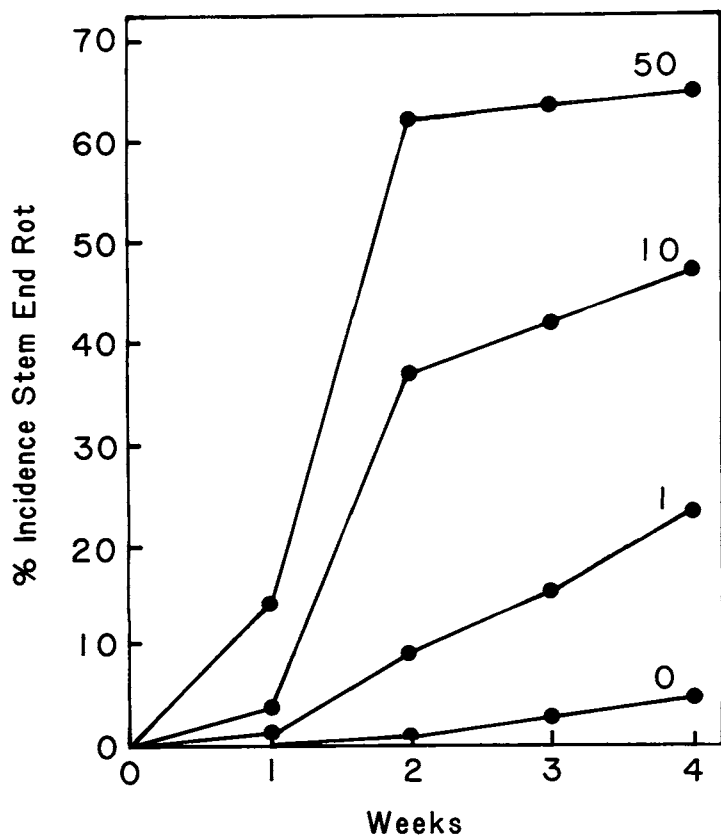


Fig. 1. Incidence of stem-end rot caused by *Diplodia natalensis* in Valencia oranges treated with ethylene at concentrations of 0, 1, 10 or 50 $\mu\text{l/l}$.

mon after using ethylene, but becomes minor later in the season when fruit does not have to be subjected to ethylene degreening.

A more rapid rate of abscission at higher ethylene concentrations might encourage more rapid disease development since entry of *D. natalensis* depends on natural openings that occur at the button (5). However, no difference in abscission rate could be detected at 10 vs. 50 μl ethylene/l of air (Fig. 2), and the cells of the abscission zone were so weakened at both these concentrations that no pull force was required after 48 hr exposure to remove the button. At a rate of 1 $\mu\text{l/l}$, the fruit bonding force did not approach 0 until after 72 hr. Development of SER in fruit treated at this low ethylene concentration could have been delayed because of the slower abscission rate. Even after 4 wk of storage, however, incidence of SER was much less in fruit treated with ethylene at 1 than at 10 or 50 $\mu\text{l/l}$ (Fig. 1). Thus, abscission of the button did not ensure that more decay would develop.

Enhanced recovery of *D. natalensis* from buttons treated with high ethylene would suggest some role of ethylene in the stimulation of quiescent infections. However, treatment of fruit with ethylene at 50 $\mu\text{l/l}$ did not increase the frequency of isolation of *D. natalensis* (Table 1). In fact, the fungus was recovered less often from buttons of fruit treated with 50 $\mu\text{l/l}$ than from buttons of fruit receiving 1 $\mu\text{l/l}$ (72 vs. 88%).

Invasion of cells at the base of the fruit by hyphae growing from the button occurred more rapidly after treatment with a high ethylene level. This was proven by the results obtained in two separate experiments. Stem-end rot can be prevented by removing the infected buttons from the fruit before the fungus grows into cells at the abscission zone. Removing the buttons from fruit after treatment with ethylene for 48 hr at 1 or 10 $\mu\text{l/l}$ reduced SER by 90 and 91%, respectively (Table 2). However, SER was reduced by only 65% in fruit receiving ethylene for 48 hr at 50 $\mu\text{l/l}$. More rapid penetration of the fruit by hyphae at high ethylene rates was also demonstrated by treating fruit with thiabendazole immediately after the ethylene treatment (Fig. 3). Thiabendazole, being relatively nonsystemic on

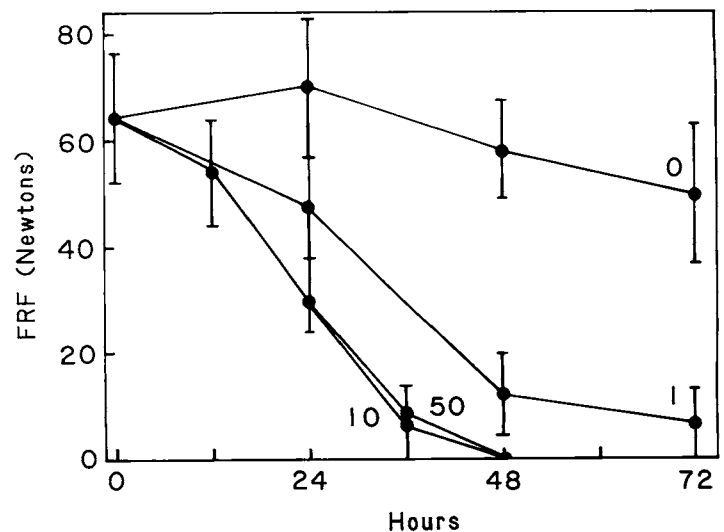


Fig. 2. Influence of ethylene (0, 1, 10 or 50 $\mu\text{l/l}$) on abscission (force in Newtons to remove stem) of Pineapple oranges during 72 hr at 30°C. Bars represent standard errors of the mean of 20 observations.

Table 1. Recovery of *Diplodia natalensis* from buttons removed from Valencia oranges receiving two concentrations of ethylene.

Ethylene $\mu\text{l/l}$	Recovery of <i>Diplodia natalensis</i> (%) ^z	
	Trial 1	Trial 2
1	80	97
50	63	78

^zButtons were removed from clipped Valencia oranges treated with ethylene for 48 hr and plated on selective media for the isolation of *Diplodia natalensis*.

Table 2. Stem-end rot caused by *Diplodia natalensis* of Valencia oranges treated with three concentrations of ethylene as affected by removal of the button before four weeks of storage.

Ethylene $\mu\text{l/l}$	Incidence of stem-end rot (%) ^z	
	Button intact	Button removed
1	20	2
10	46	4
50	74	26

^zFruit were treated with ethylene for 48 hr at 30°C, then stored at 29-30°C and 94-96% relative humidity.

citrus fruit (7), controls surface mycelia but not those that have penetrated more deeply into the tissue. Incidence of SER in fruit treated with ethylene at 50 $\mu\text{l/l}$ was consistently greater than that in fruit treated with 10 $\mu\text{l/l}$ after either 48, 72, or 96 hr of ethylene exposure. The disease was of minor importance in the absence of ethylene, but became significant with increases in degreening time or ethylene concentration.

The effects of abscission, inoculum concentration and rate of hyphal growth on SER was minimized by removing the button before inoculating with *D. natalensis*. This was accomplished by degreening fruit for 48 hr at ethylene concentrations of 1, 10, or 50 $\mu\text{l/l}$, removing the button, and placing an aqueous suspension of *D. natalensis* in the stem cavity. After 2 wk in air at 29-30°C, fruit treated with ethylene at 1, 10 or 50 $\mu\text{l/l}$ developed 27, 44 or 89% SER, respectively. Regardless of the ethylene concentration, fruit that resisted infection developed red pigment in the surface cells of the abscission zone within 2 days of inoculation. Surface mycelial growth of *D. natalensis* was less extensive on fruit that developed pigment than on infected fruit. No pigment developed within the cavity of uninoculated fruit following any of the ethylene treatments. The chemical nature of the red pigment and its role, if any, in resistance is not known.

Table 3. Efficacy of fungicides applied before or after degreening for control of stem-end rot caused by *Diplodia natalensis*.

Fungicide	Rate ($\mu\text{g/ml}$)	Percentage decay control ^z	
		Before	After
Benomyl	600	100	91
Thiabendazole	1000	91	59
		97	52
Imazalil	1000	84	63
		78	44
Guazatine	1000	86	41

^zDecay control in experiments where fungicides were applied as drench treatments to unwashed fruit before degreening for 72 hr or as non-recovery sprays to washed fruit after degreening before waxing.

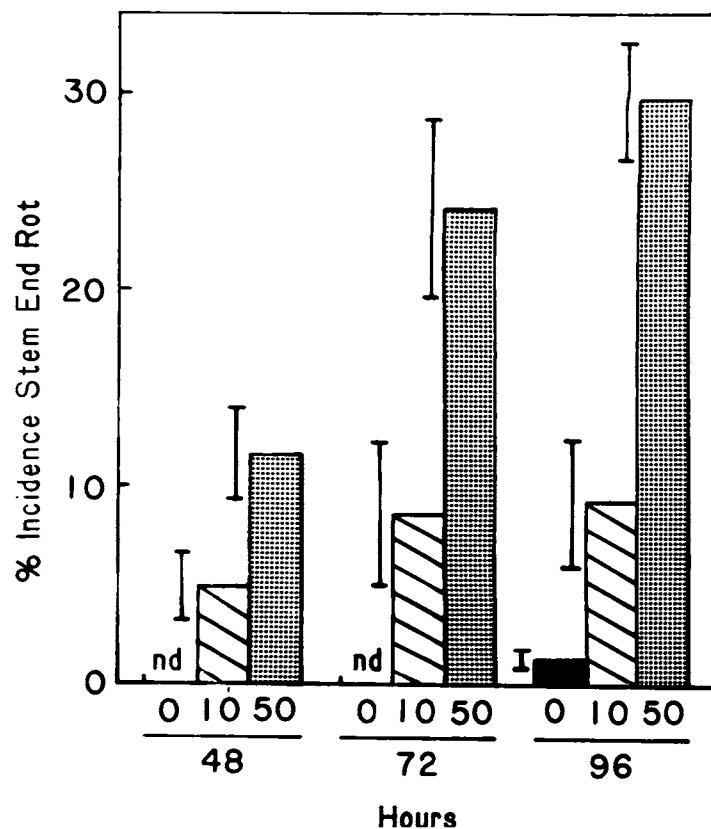


Fig. 3. Incidence of stem-end rot caused by *Diplodia natalensis* in Valencia oranges treated with thiabendazole (1000 $\mu\text{g/ml}$) after exposure to ethylene (0, 10 or 50 $\mu\text{l/l}$) for 48, 72, or 96 hr and stored for 4 wk at 29-30°C and 94-96% relative humidity. Bars represent standard errors of the means of 3 replications.

Enhanced decay at high ethylene rates was associated with more rapid establishment of the fungus in cells of the fruit beneath the button. This may have been due to more rapid growth of the fungus, but probably the penetration was more rapid because high ethylene interfered in some manner with the resistance of the host cells to the invading hyphae.

Delays in fungicide application because of degreening favor SER development (Table 3). Applications of fungicide before degreening, when the fungus is more accessible to the chemical, are more effective than applications of the same material following degreening. Preharvest sprays of benomyl (4) or drench treatments of either benomyl or thiabendazole (3) to pallets of fruit before degreening are effective control measures.

Any practices that reduce the degreening time will subsequently aid in the reduction of diplodia SER. Delaying harvest until development of more natural color or spot picking for color are effective methods. Even though *D. natalensis* is ubiquitous, inoculum levels are generally less in trees with the least deadwood, such as in young plantings or in blocks of vigorous trees which are usually those receiving the best nutrition, pesticide & irrigation programs. Such blocks should be selected, if at all possible, for the early harvests that require extensive degreening. Finally, use of precooling or cold storage after packing will delay decay development and complement the fungicide treatments. Diplodia SER is significantly delayed at 15°C and essentially curtailed at 10°C.

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USE OF POSTHARVEST TREATMENTS FOR REDUCING SHIPPING DECAY IN KUMQUATS

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Abstract. Mature kumquats are subject to some of the same postharvest diseases as citrus. Since the kumquat is a near relative (genus *Fortunella*) of the citrus (genus *Citrus*) the similarities in the fruit suggest that it would react to postharvest treatments in a similar manner. Treatments tested were chlorine, sodium o-phenylphenate (SOPP), thiabendazole (TBZ), 2,4-dichlorophenoxyacetic acid (2,4-D), and waxes. All treatments except 2,4-D alone improved resistance to decay, while a combination of SOPP and TBZ with a wax gave the best results. At the level tested 2,4-D had little apparent effect.

The kumquat (genus *Fortunella*) is subject to losses from postharvest decay during shipment. Due to its popularity with some ethnic groups it commands a high price on the market and is usually shipped in small packages. Kumquat production in Florida is a small volume operation, amounting to only about 10,000 bushels before the 1984 & 1985 freezes greatly reduced the amount of fruit available (F. Gude, Kumquat Growers, Inc., personal communication). A true citrus, the kumquat fruit is small in size, typically 3/4 to 1 1/4 inches diameter (26). Depending upon variety, the fruit will be round to elongated in shape (8,26,27). The fruit are used for decoration in gift packs (26) and for use in various jams and preserves (22,23). They are also eaten fresh, peel included (8,19,26).

Relatively large quantities of the fresh fruit were shipped to markets in Los Angeles and San Francisco, California, Chicago, Illinois, and New York (F. Gude, personal

communication). The large population of orientals in these cities probably accounts for this market. The kumquat probably originated in China and is still popular in most of the orient as a fresh fruit (8,25,26,27).

Eaten fresh, the kumquat has a peculiar sweetness not like that of other citrus. The peel has been found to contain dihydrochalcone flavonoids (10,13,14) similar to those recently developed as artificial sweetening agents (11,12,15). Differences in the oil of *Fortunella* (kumquat) as compared to *Citrus* have also been noted (17). Another notable difference between the kumquat and other members of the citrus family is its resistance to sour orange scab (20).

Before restrictions were placed upon fruit handling due to the outbreak of citrus canker in Florida (16), the fruit was clipped from the tree and packed loose into cartons or berry baskets for shipment to out of state markets. In addition, a considerable amount of fruit was harvested by clipping a small branch with several leaves and 3 or 4 fruit attached. These were used for decoration in gift boxes. Since canker quarantine restrictions require that all leaves and stems longer than 1 inch be removed from the fruit before shipping, this latter use has been discontinued.

Before canker quarantine restrictions were put into effect, whole fruit were clipped from the tree and packed into shipping containers with a minimum of handling. The restrictions require a chemical treatment (either chlorine or sodium o-phenylphenate (SOPP)) be given the fruit before packing (16). One Florida packer noted that since they began using a chlorine treatment in order to meet the quarantine requirements they have experienced increased decay in shipments (F. Gude, personal communication).

Their method of treatment was to dump the harvested fruit into a large wire basket (approximately 3 bushel capacity) which was lowered into a chlorine solution for 2 minutes. This basket of fruit was then transferred to another tank containing fresh water to rinse, then the fruit was dumped upon a perforated metal table to drain before packing. This extra handling apparently was causing injury to the fruit making it susceptible to decay (2). Also contributing to injury was the requirement that all leaves and excess stem be removed. Picking thus, pickers tended to include more pulled or plugged fruit.

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