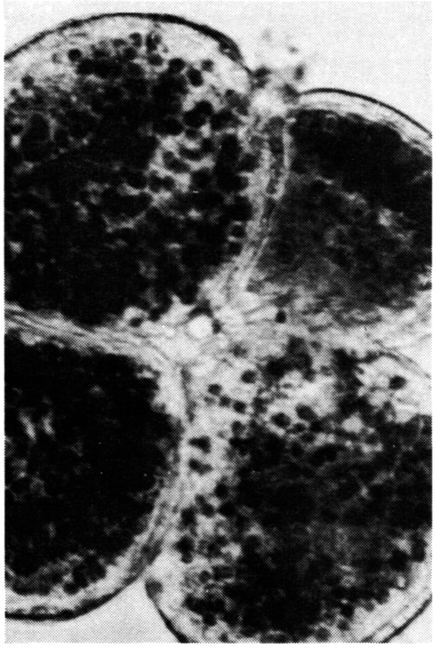

Fig. 1. Pollen grains at anthesis (Jan. 2) showing tetrad stage with thick walls and full of starch. Stained with KI•I. (×940).

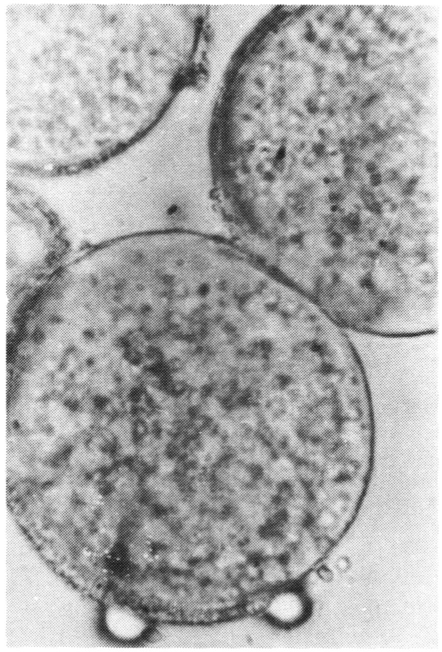

Fig. 2. Pollen grains at anthesis on Feb. 1, showing thin walls and no starch grains. Stained with KI•I. (×940).

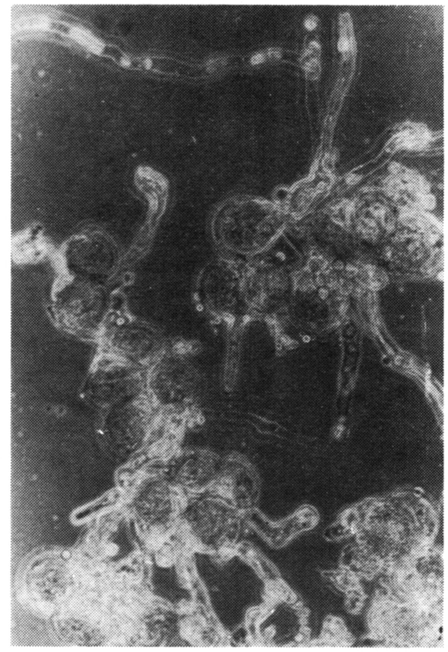

Fig. 3. Germinated pollen grains 2 hr after incubation on Kwack's medium. (Phase contrast. ×128).

pollinated and immediately sprayed with water on Jan. 19, 25 and Feb. 1, and fruit sets of 43.7%, 57.0% and 94.4%, respectively, were obtained. The fruit remained in the tree until maturity, and produced, in general, well-conformed sizable fruits.

Pollen used on Feb. 1 had a greater proportion of individual grains with thin walls, no starch grains (Fig. 2) and greater cytoplasmic streaming than those of the first date. Many grains germinated after a few hours (Fig. 3).

Even though the first flowers to open gave a poor fruit set with hand pollination, their setting was actually very good when treated with growth regulators (over 90%). Furthermore, later fruit growth was also found to be excellent (unpublished results).

Therefore it is proposed that the vegetative condition of the tree may influence ripening and viability of the pollen rather than development of the ovaries and the availability of organic and inorganic nutrients necessary to maintain the development of the fruit.

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Seasonal Susceptibility of Grapefruit to Chilling Injury as Modified by Certain Growth Regulators[1]

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Additonal index words. 2,4-D, gibberellic acid, benzyladenine, *Citrus paradisi*, cold storage

Abstract. **Changes in susceptibility to chilling injury of grapefruit (*Citrus paradisi* MacF.) were found to vary directly with the growth activity of the trees. Exogenous growth regulators were applied to test the hypothesis that they may be involved in seasonal variations in susceptibility to chilling injury. Benzyladenine, gibberellic acid and 2,4-dichlorophenoxyacetic acid applied postharvest and benzyladenine and 2,4-D applied preharvest significantly altered susceptibility to chilling injury although the direction and extent of the changes were neither consistent nor predictable.**

Grapefruit and lime (*C. aurantifolia* Swing.) are the most susceptible among citrus species to the low temperature disorder known as chilling injury (CI). Affected fruit develop discolored rind pitting between 12.8°C (55°F) and 0°C (32°F), which becomes necrotic (Fig. 1) and results in subsequent fruit decay. Lyons (2) hypothesized a pathway of events leading to CI in which mitochondrial membranes are the initial site at which this degenerative process begins. Grierson (1) postulated that susceptibility of grapefruit to CI throughout the season is related to the general growth condition of the tree, probably via its growth regulator (GR) levels at time of harvest; i.e., fruit from actively growing trees are more susceptible to CI than those from inactive ones. Schiffmann-Nadel et al. (3) have drawn attention to the analogy between reduction of CI by thiabendazole (TBZ) and the inhi-

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[2]Florida Department of Citrus.

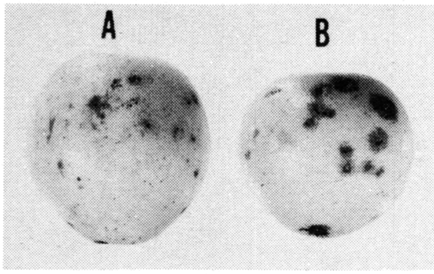

Fig. 1. typical symptoms of 2 types of chilling injury encountered on 'Ruby Red' grapefruit held for 7 weeks at 4.4°C: A. Distinct sunken pitting, B. Pitting surrounded by halo of discolored injured tissue.

Table 1. Effect of postharvest application of a mixture of 3 growth regulators (GR)[z] on chilling injury (CI) and decay of 'Marsh' grapefruit during and after storage at 4.4°C.

Variable	Control	GR's[y]	Significance
Avg CI[x] score after 28 days at 4.4°C	6	5	NS
Avg CI[x] score after 56 days at 4.4°C	25	12	1%
Days at 4.4°C until CI = 10[w]	35	52	5%
Avg decay after 56 days at 4.4°C (%)	56	13	1%
Avg decay after further 14 days at 21°C (%)	82	35	1%

[z]500 ppm GA_3, 100 ppm BA, and 44 ppm 2,4-D.

[y]Each treatment included 8 cartons of grapefruit.

[w]Chilling injury scored on a 0 to 100 point scale (see text).

[w]A CI score of 10 indicates incipient chilling injury and thus termination of successful cold storage.

Table 2. Effect of 3 postharvest growth regulator treatments on chilling injury (CI) of 'Marsh' grapefruit; (Averages of monthly harvests from Sept. 10, 1973 to May 27, 1974).

	Chilling injury score[z]			
	Time at 4.4°C			Days to reach
Treatment[y]	16 days	30 days	44 days	CI = 10
Control (water)	3a[z]	13a	19a	35a
500 ppm GA_3	7b	17a	23a	28ab
100 ppm BA	4a	15a	21a	27b
44 ppm 2,4-D	4a	15a	22a	28ab

[z]CI score on a 0-100 points scale (see text).

[y]Aqueous sprays applied over horsehair bruses.

[x]Mean separation within columns by Duncan's multiple range test, 5% level.

bition of senescence by certain growth regulators. No firm data are available, however, despite such circumstantial evidence, as to any role of GR's nor the possible effect of applied GR's on susceptibility or resistance to CI. This study, therefore, was to investigate effects of pre- and postharvest applications of an auxin, a kinin, and a gibberellin on CI of grapefruit.

Postharvest applications. Initial trials were made in mid-March, 1973, just as the 1972-73 crop season was drawing to a close. A survey-type experiment was set up to investigate whether application of exogenous growth regulators might have any effect on CI using a single application combining 500 ppm gibberellic acid (GA_3), 100 ppm benzyladenine (BA) and 44 ppm 2,4-D. This was applied postharvest as a nonrecovery spray to 8 cartons (251 fruit), with another 8 cartons (257 fruit) used as a control. The fruit were then stored 56 days at 4.4°C followed by 2 wks at 21°. Peel injury was scored on a previously used 0-100 scale on which an average value of 10 indicates the onset of visible CI but a level that would not normally be noticed in marketing (1). Differences were not significant for the first 4 weeks, but both chilling injury and subsequent decay were very much reduced in the samples receiving the growth regulator treatment by the end of the 8-wk storage period (Table 1).

Ten harvests of 'Marsh' grapefruit were made at about monthly intervals from Sept.-May during the 1973-74 season. Four treatments, control, 500 ppm GA_3, 100 ppm BA, and 44 ppm 2,4-D, were applied at each picking. All samples were stored at 4.4°C. An overall analysis of CI data (Table 2) gave some indication of significant differences. Curves showing tolerance to cold storage as related to growth status of the trees throughout the 10-month harvesting season are of much greater interest. Resistance to CI increased as the trees became progressively less active (Fig. 2). Then resistance to CI decreased as the trees resumed growth, bloomed and put out progressively more vigorous flushes until grapefruit picked at the end of May were more CI-susceptible than the first harvest the previous Sept. BA and 2,4-D had an almost identical effect in depressing resistance to CI. The effect of GA was more obscure, the entire curve being skewed toward earlier harvests.

Preharvest applications. A preliminary experiment was conducted during the 1973-74 season with 25 year-old 'Ruby Red' grapefruit trees. GA_3, BA, and 2,4-D were applied singly and in combinations to 2 single-tree replications. This test indicated that preharvest GR applications might also modify susceptibility to CI.

Forty-four ppm 2,4-D and 50 ppm BA were applied separately on Nov. 26, 1974 and Feb. 18, 1975 to 3 single-tree plots of 25 year-old 'Ruby Red' grapefruit. Samples were harvested 7 days and 7 weeks after spraying. Fruit were washed, surface-dried and stored in 4/5-bu cartons at 4.4°C for 7 weeks.

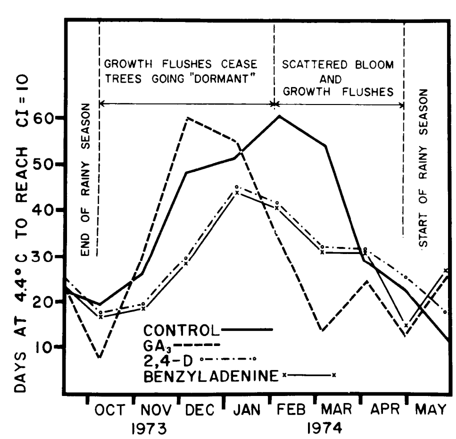

Fig. 2. Changes in susceptibility to chilling injury of 'Marsh' grapefruit picked monthly from mid-Sept. to the end of May, together with the effect of 3 postharvest growth regulator treatments.

Side effects were numerous, ranging from a stabilization of the scattered bloom prevailing the 1973-74 and 1974-75 seasons to an apparently significant increase in yield. Data on CI only are given here for BA and 2,4-D sprays applied in Nov. and picked 1 week later. Data on subsequent CI from the 2-tree plots in 1973 and the 3-tree plots in 1974 have been combined (Table 3). Differences in CI among treatments were not significant after 3 weeks at 4.4°C. CI in grapefruit from the 2,4-D sprayed trees was significantly less than in the control after 5 weeks cold storage, however CI was significantly less in both the BA and 2,4-D treatments after 7 weeks at 4.4°. Postharvest BA and 2,4-D tended to *decrease* resistance to CI; whereas, both these GR's applied preharvest tended to *increase* resistance to CI.

Table 3. Chilling injury during cold storage of 'Marsh' grapefruit from trees sprayed with 2 growth regulators 1 week prior to picking. (combined data for 1973-74 and 1974-75 seasons)[y].

	Chilling injury score[z]		
Preharvest	Time at 4.4°C		
spray	3 wks	5 wks	7 wks
Control	7a[z]	12a	20a
50 ppm BA	2a	6ab	10b
44 ppm 2,4-D	1a	4b	8b

[z]CI score on a 0-100 points scale (see text).

[y]Mean separation within examination dates by Duncan's multiple range test, 5% level.

It is apparent that both pre- and postharvest GR applications modified susceptibility of grapefruit to CI, although the type and magnitude of the response was not predictable. Effects of GR application applied pre- and postharvest were often opposite. Evidence presented here supports the hypothesis that growth regulator activity does affect the susceptibility of grapefruit to CI.

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Effects of Prestorage Carbon Dioxide Treatments and Delayed Storage on Stem-end Rind Breakdown of 'Marsh' Grapefruit[1]

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Additional index words. fruit physiology, *Citrus paradisi,* storage, chilling injury

Abstract. **Prestorage treatments of 10, 20, and 40% CO_2 for 3 and 7 days at 21°C significantly reduced stem-end rind breakdown in grapefruit (*Citrus paradisi* Macf.) held at 4.5° for 8 and 12 weeks. Three days' exposure to CO_2 was as effective as 7 days' exposure; however, 20 and 40% concentrations of CO_2 were significantly more effective in reducing stem-end rind breakdown than was 10% CO_2. Fruit stored continuously at 4.5° in air or that exposed to 21° in an air prior to storage at 4.5° had significantly more stem-end rind breakdown than that exposed to CO_2.**

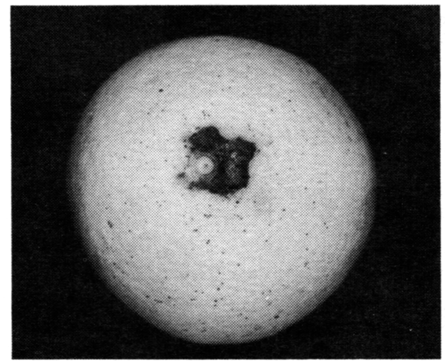

Fig. 1. Stem-end rind breakdown of 'Marsh' grapefruit.

Grapefruit, when stored fully mature, is subject to the physiological disorder generally referred to as stem-end rind breakdown (SERB) and sometimes as aging, brown or burnt stem (5). The affected rind around the stem button wilts and shrivels and is usually accompanied by a collapse of outer rind tissues; the area may turn brown and the oil glands may collapse (Fig. 1). The areas of breakdown gradually increase and tend to coalesce, followed by stem-end decay, especially at high temp. SERB in grapefruit was reported to increase when the fruit was exposed to 10 to 30% CO_2 at 4.5° for 7 days and then stored at the same temp (4).

The purpose of this study was to determine the effects of prestorage CO_2 concn and exposure times at 21° on development of SERB in grapefruit during storage at 4.5° and for the 14 days at 21° after storage.

'Marsh' grapefruit were harvested from 3 commercial groves in Indian River County. Fruit from individual groves was randomized but kept separate throughout, and transported to the USDA Horticultural Research Laboratory in Orlando. Four harvests were made from the same trees on Dec. 13, 1972, May 9, 1973, Jan. 23, 1974, and May 15, 1974. The day after each harvest the fruit was washed, treated with 1,000 ppm thiabendazole (TBZ), graded, waxed with a solvent-type wax, and packed.

The total no. of grapefruit included in the test was 4,800. Treated grapefruit were exposed to the desired CO_2 concn at 21° for 3 and 7 days, then placed in conventional air storage at 4.5° for 8 and 12 weeks. Three control groups were used. Two were stored at 21° in air for 3 and 7 days prior to conventional air storage at 4.5° and the other was held continuously at 4.5°. Relative humidity during storage ranged from 88 to 92%.

Grapefruit were placed in gastight containers designed for CA studies for exposure to CO_2 with concn monitored automatically by equipment described previously (1, 2). Sequential analysis of separate atmospheres was controlled by time programming of solenoid valves to a gas chromatograph with a thermal conductivity detector. Atmospheres were monitored continuously after an initial flushing with CO_2 to establish the desired concn and air, oxygen, or CO_2 was added to maintain proper CO_2 levels.

Samples were inspected immediately after storage and again after 1 and 2 weeks at 21°. Sound fruit and fruit affected with SERB were retained until the next inspection; individual pieces of fruit were discarded at the time actual decay was detected.

Stem-end rind breakdown was present in all instances, but no significant differences were noted in fruit of any specific treatment group from the 4 harvests, from the 3 groves, or between the 3- and 7-day periods of prestorage exposure to CO_2; consequently, data were combined. No significant differences were found in SERB of fruit from any specific treatment group for the 8- and 12-week storage periods, so these data were also combined.

Table 1. Stem-end rind breakdown of 'Marsh' grapefruit on removal from 4.5°C storage, and after 14 days at 21° as affected by prestorage treatments of CO_2.[z]

Prestorage treatment (3 and 7 days)	After storage (8 and 12 wk) (%)[y]	After storage + 7 days at 21° (%)[y]	After storage + 14 days at 21° (%)[y]
Air, 21°, 4.5°	8.9c	10.3c	9.2cd
Air, 4.5° only	8.6bc	9.2bc	9.8d
40% CO_2, 21°	2.5a	4.8a	4.2a
20% CO_2, 21°	3.4a	4.5a	5.1ab
10% CO_2, 21°	5.9b	7.6b	7.4bc

[z]Each prestorage treatment represents 960 grapefruit obtained from 4 harvests of 3 groves each and 20 fruit per sample; data from fruit stored 8 and 12 weeks and treated for 3 and 7 days were combined.

[y]Means with unlike superscripts (a-d) in any columns differ at 5% level.

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