plants with less than 8% polyembryony are considered to be strictly sexual.

Until critical populations can be scored for the presence or absence of nucellar seedlings, the exact genetic basis of nucellar embryony cannot be determined. However, it is clear that monoembryonic tetraploids can be obtained from crosses involving polyembroyonic tetraploids, and that sexual plants may be recovered in proportions greater than would be expected on the basis of a single dominant gene.

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# Influence of Canopy Depth on Susceptibility of 'Marsh' Grapefruit to Chilling Injury[1]


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*Additional index words. Citrus paradisi, Phyllocoptruta oleivora,* citrus rust mite, postharvest disorders, soluble carbohydrates



*Abstract.* **'Marsh' grapefruit (*Citrus paradisi* Macf.) harvested from the exterior canopy of the tree were more susceptible to chilling injury (CI) at 4.4°C than grapefruit harvested from the interior canopy of the same tree. No differences were found in the levels of total soluble carbohydrates, reducing sugars, and sucrose in the peels of the fruit from the 2 canopy depths. An unusual pattern of CI, remarkably similar to the pattern of citrus rust mite [*Phyllocoptruta oleivora* (Ashm.)] damage, was observed in several of the exterior canopy fruit. Although the fruit had no visible rust mite damage at harvest, fruit remaining on the tree developed the characteristic bronzing associated with rust mite injury within 2 to 3 weeks after the test fruit were harvested. It is suggested that environmental and biotic factors predispose grapefruit to CI.**


Susceptibility of grapefruit to CI at 4.4°C is seasonal with fruit harvested early (October-December) and late (April-June) generally being more susceptible to CI than fruit harvested at midseason (4, 5, 10). The mechanism of the midseason resistance is not known, but may be related to soluble metabolites or metabolic activity of the peel (5, 10).

Fruit in various canopy positions are exposed to widely differing microclimates during growth and maturation (3, 11). Differences were observed in the internal quality of fruit from the sunlit exterior canopy positions and the shaded interior canopy positions, especially early in the season (11). For this reason, it was of interest to determine if canopy depth influenced the susceptibility of grapefruit to CI.

'Marsh' grapefruit were harvested separately from the interior and exterior canopies of trees on 2 different dates during the 1979-80 season. Fruit were harvested in mid-November 1979 from 4 trees and combined into one interior and one exterior canopy sample. In mid-January, fruit were harvested from 3 trees and maintained as separate samples instead of being combined as was done for the November harvest. The fruit were washed with detergent, rinsed, and air dried. Forty fruit of each lot were stored at 4.4°C and CI (peel pitting) was rated at weekly intervals as previously described (4, 10).

For carbohydrate analyses 5 fruit were randomly selected from each lot, the flavedo (colored) portion of the peel was removed, and sugars were extracted in 80% ethanol as previously described (10). Total soluble carbohydrates were determined by the anthrone method (6) and reducing sugars and sucrose were determined by gas-liquid chromatographic procedures (7) modified as follows: 1 ml of the resin treated ethanol extract was dried and 1 ml of Tri-Sil (Pierce Chemical Co., P. O. Box 117, Rockford, IL 61105) added. The sample was sonicated for 20 min followed by centrifugation. Two and one-half µl of the sample were injected into the gas chromatograph.

Fruit harvested from the exterior canopy were more susceptible to CI than fruit harvested from the interior canopy (Fig. 1). The fruit harvested in November from both the exterior and interior canopies were more susceptible to CI than fruit harvested in January. All of the exterior fruit and 60% of the interior fruit harvested in November had CI lesions after only 4 weeks of storage at 4.4°C (Fig. 1).

Although seasonal resistance of grapefruit to CI was previously related to high reducing sugar levels in the peel (5, 10), no differences were found in the levels of either total soluble carbohydrates, reducing sugars, or sucrose in the peel of exterior and interior canopy fruit (data not shown). These results do not necessarily rule out the possibility that reducing sugars play a role in the resistance of grapefruit to CI. While the interior fruit developed greater resistance to CI, the exterior canopy fruit may have been predisposed to CI by an environmental or biotic factor absent in the interior canopy.

An unusual pattern of pitting was observed in several of the exterior canopy fruit harvested in January which contributed to both the speed of development and severity of CI. Instead of being randomly located and widely scattered over the surface of the fruit, a typical characteristic of CI in grapefruit (Fig. 2A), the pits were restricted to and generally covered an entire hemisphere of the fruit (Fig. 2B, C, D). CI of these fruit was more rapid (pitting occurring in less than 2 weeks) and


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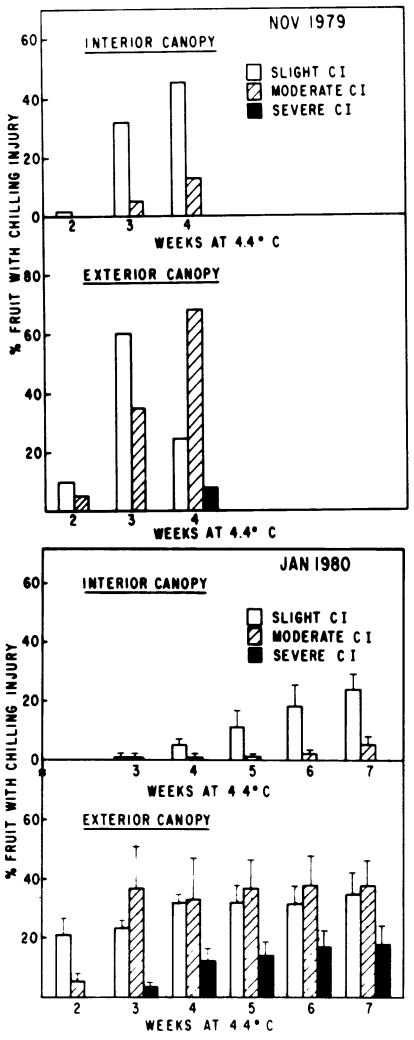


Fig. 1. Comparison of chilling injury in 'Marsh' grapefruit harvested from the interior and exterior tree canopies during November 1979 and January 1980. Bars represent SE.

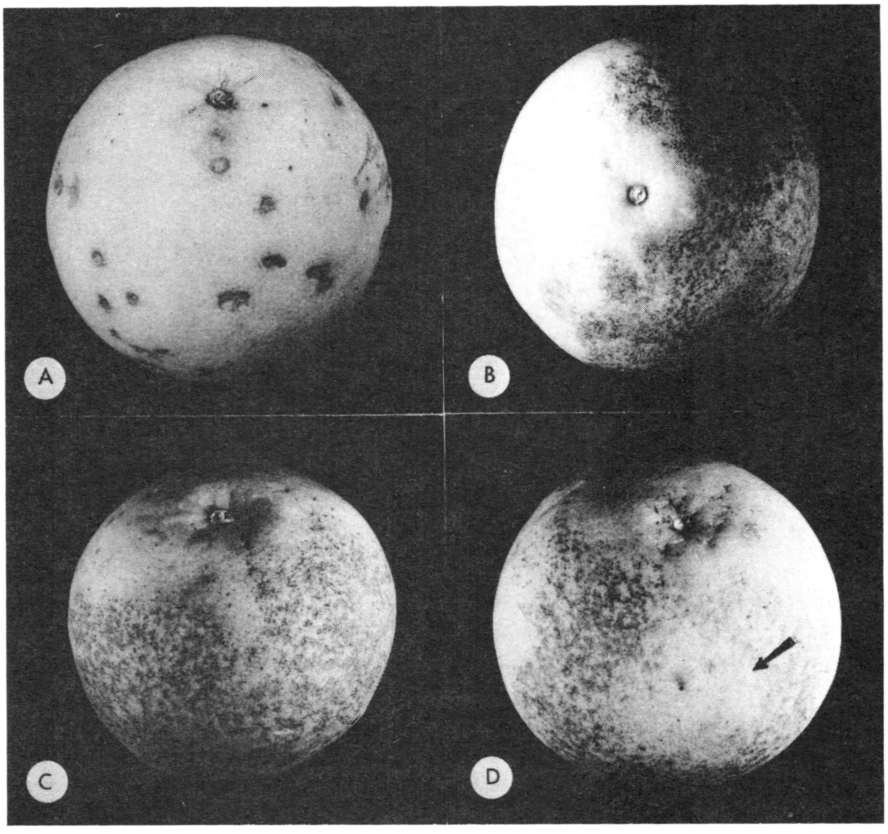


Fig. 2. A. Typical pitting on 'Marsh' grapefruit stored at 4.4°C for 7 weeks. B. C. D. Pitting on some exterior canopy fruit stored at 4.4°C for 3 weeks. Arrow indicates hot spot, an area generally avoided by rust mites.

more severe than other fruit in the same lot. The pattern of CI in these fruit was remarkably similar to the general pattern of rust mite damage on citrus fruit (1, 9). Mite densities are frequently greater on one hemisphere (usually the sunlit side) of the fruit (1, 3, 9). However, they tend to avoid bright sunlit or hot spots on the fruit (1, 3). In the present study, some of the exterior fruit had areas corresponding to the hot spots which did not chill injure (Fig. 2D).

At the time of harvest, no rust mite injury was apparent on any of the fruit. However, the characteristic bronzing associated with rust mite damage (1, 9) appeared on the exterior canopy fruit remaining on the trees within 2 to 3 weeks after the test fruit were harvested. High populations of rust mites must be maintained on fruit for 2 to 3 weeks before visible damage occurs (2), so it is likely that the test fruit had high rust mite populations prior to harvest. No rust mite damage was seen on the remaining interior canopy fruit. In addition, exterior canopy fruit from the trees with the most rust mite damage chill injured most severely.

If the rust mite predisposes grapefruit to CI as suggested by these observations, predisposition apparently occurred prior to the characteristic bronzing reactions. It is possible that metabolic reactions which lead to bronzing of the peel are also involved in the mechanism of CI and rust mite injury having induced these reactions would therefore increase the severity of CI. Although no quantitative estimates of citrus rust mite populations were made on the fruit which chill injured, the observations strongly suggest that the rust mite may predispose grapefruit to CI. Experiments have been designed to quantify the dynamics of rust mite populations with susceptibility of grapefruit to CI during the next harvest season.

Obviously, differences in the peel of the exterior canopy fruit other than injury caused by the rust mite contributed to the observed differences in susceptibility of the exterior and interior canopy fruit to CI. The temperature of the sun exposed surface of grapefruit can be 10 to 15°C higher than the temperature of the shaded surface of the same fruit (3, 11). Such differences in temperatures undoubtedly alter the metabolism of the peel, although no differences were observed in the levels of soluble sugars measured in this study. In addition, light quality and intensity are important regulators of many physiological and biochemical processes. It is interesting to note, however, that 'Honey Dew' melons exposed to sunlight during maturation, are significantly less susceptible to CI than those shaded by the vines (8).

In addition to season (4, 5, 10), canopy depth is also an important determinant of susceptibility of grapefruit to CI. The nature of the difference in exterior and interior canopy fruit responsible for the difference in susceptibility to CI is not known, but could be related to both environmental and biotic factors.

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# Somatic Embryogenesis in Cell Cultures of *Carica stipulata*[1]

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*Additional index words. in vitro* propagation, tissue culture

***Abstract.*** **Somatic embryogenesis was induced in cell suspension cultures of callus derived from peduncle explants of *Carica stipulata* Badillo. The presence of activated charcoal stimulated embryogenesis particularly when BA and NAA were both in the growth medium. Plantlets derived from embryos have been successfully transferred to potting mixture.**

*Carica stipulata*, although having no commercial importance, is related to the papaya (*C. papaya* L.). *C. stipulata* is resistant to infection by papaya ringspot virus (PRV) which causes a serious disease of papayas throughout the New World tropics (1). Virus resistance is conferred by a single dominant gene (5). The two *Carica* species are sexually incompatible, and efforts to achieve hybridization through the use of mediator pollen and embryo culture have not been successful.

Previous studies have indicated that *Carica* species have a reasonably high morphogenetic potential. *In vitro* propagation of papaya is feasible not only from shoot tip cultures (7) but also by stimulation of adventitious shoot formation from callus (11). Haploid plants have been obtained from cultured anthers of *C. papaya, C. cauliflora* Jacq., and *C. stipulata* (8). Litz and Conover (9) have proposed an *in vitro* scheme for effecting interspecific hybridization between papaya and PRV-resistant *C. stipulata* by inducing protoplast fusion between the 2 species.

The exploitation of protoplast fusion in plant improvement depends on the ability to regenerate plants from cells efficiently. In this report we present a procedure for large-scale production of somatic embryos from cell cultures using *C. stipulata* as a model.

Peduncles were removed from mature, container-grown plants of *C. stipulata* and 1-cm pieces were disinfested by rinsing briefly in absolute alcohol followed by immersion in 1% (wt/vol) sodium hypochlorite for 10-12 min with continuous agitation. After transfer through 3 rinses in sterile, distilled water (10 min each), tissue was placed in culture on sterile plant growth medium composed of Murashige and Skoog medium (10) with 30 g/liter sucrose, 2 μM 6-benzylamino purine (BA), 1 μM naphthaleneacetic acid (NAA) and 8 g/liter Difco Bacto agar. The pH was adjusted to 5.8 with 1N KOH before sterilization at 120°C and 1 kg/cm$^2$ for 15 min. Cultures were maintained in a growth room at 25-27°C with a photoperiod of 16 hr light at 1.5 klux supplied by Agro Lite fluorescent tubes and 8 hr darkness.

Equal quantities of friable peduncle callus were transferred from solid medium into 125 ml Erlenmeyer flasks containing 30 ml Murashige and Skoog medium at pH 5.8. The growth regulator components were altered accordingly: 1) 2 μM BA and 1 μM NAA (6); 2) 2 μM BA; 3) no growth regulators; 4) 1 μM NAA. Activated charcoal (1% wt/vol) was added to half of the flasks in each treatment. There were 6 flasks in each treatment and the experiment was repeated once. The pH was adjusted to 5.8 prior to autoclaving. Cultures were agitated at 150 rpm on a rotary shaker for 1 month to facilitate the break up of cell clumps. Environmental conditions were 1.5 klux illumination with a 16 hr photoperiod at 25-27°C. The flasks then remained stationary for another 8 weeks before the cultures were decanted and examined.

Rapidly growing, friable callus was obtained on solid medium. After transfer of callus inoculum to liquid media, cell cultures continued to increase rapidly in flasks with NAA alone and in the control treatment

Table 1. Effect of growth regulators and activated charcoal on somatic embryogenesis of *Carica stipulata*.

| Treatment | 1% activated charcoal | BA (μM) | NAA (μM) | Avg no. embryos/ flask |
|---|---|---|---|---|
| 1 | – | 2 | 1 | 45 |
| | + | 2 | 1 | 1560 |
| 2 | – | 2 | 0 | 0 |
| | + | 2 | 0 | 10 |
| 3 | – | 0 | 0 | 0 |
| | + | 0 | 0 | 13 |
| 4 | – | 0 | 1 | 0 |
| | + | 0 | 1 | 10 |

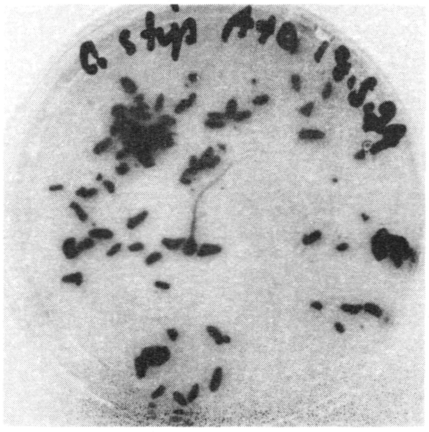

Fig. 1. Torpedo-shaped somatic embryos of *C. stipulata* prior to transfer to solid medium.

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