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A BRIEF HISTORY OF FLORIDA CHILLING INJURY RESEARCH

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Chilling injury (CI) is a physiological disorder induced by low, but not freezing, temperatures. It can take forms varying from local surface pitting to complete physiological breakdown. Such CI is apparently distinct from the "low temperature disorders" of apples in long-term storage (Smock and Neubert, 1950). Very basic studies in California involving mitochondrial respiration reported a sharply defined disruption of cell membrane integrity at the onset of CI (Raison et al., 1971).

Species susceptibility. A very wide range of species is involved in CI susceptibility, usually but not always associated with plants of tropical origin. The USDA transportation manual lists 70 CI susceptible products (McGregor, 1987).

There is a tendency to expect CI only with (in the botanical sense) fruits and certain root crops such as sweet potatoes. However, whole plants, particularly seedlings, can be susceptible. Tomato and cotton seedlings are well known examples, but even some tropical grasses are susceptible to CI.

History. Long before the advent of refrigeration, alert growers were aware of CI susceptibility of certain seedlings, as when too early plantings of cotton or tomatoes were observed to be stunted by chilly, but not freezing, night temperatures.

With the coming of refrigerated storage and shipment, it soon became apparent that temperatures suitable for most temperate zone products severely injured such tropicals as bananas, avocados, and mangos; and some, but not all citrus fruits.

One of the most baffling aspects of CI is the unexplained variation in susceptibility among identical varieties (cultivars) grown in different areas and among different varieties of the same species grown in the same area. Valencia oranges grown in Florida are not CI susceptible, but the same cultivar grown in California is. This is why CI research in California has been largely concerned with oranges (Eaks, 1960).

Varietal differences. Varietal differences can involve horrid pitfalls. Foreseeing their use by our gift fruit industry, we at Lake Alfred did some physiological studies on carambolas using the Golden Star variety from TREC Homestead. We reported, surprisingly, that carambolas were not CI susceptible (Grierson and Vines, 1965). When sweeter, more popular varieties were released, a major shipper believed us and put a considerable quantity into cold storage. A very humbling memory! The new varieties proved to be highly CI susceptible.

Early grapefruit research. Our initial research was purely empirical, involving only temperature and time studies—how long could a grapefruit survive at what temperatures before developing CI?

Much research effort has been wasted by various research workers on studies analyzing healthy vs. CI injured tissues. This is basically a fruitless endeavor since necrotic tissues are inevitably anomalous. This included some of our early research at Lake Alfred. Finding that acetaldehyde evolution preceded visible CI lesions, we devoted much ultimately non-productive research exploring the hypothesis that CI was initiated by failure of the respiratory terminal oxidative system. My files are cluttered with similar wild goose chases by many research workers on many CI sensitive products.

Florida grapefruit research 1966-1982. Most of our CREC Lake Alfred research on CI of grapefruit was carried out in parallel with the USDA, ARS postharvest group at Orlando. Each group had its advantages and disadvantages. The USDA had far better funding, much better physical facilities, and also extensive worldwide capability. However, at times they were hampered by federal orthodoxy. Their Washington editors demanded experimental protocols providing for traditional statistical evaluation. To provide this, they used a very few very large experimental pickings. Such enforced orthodoxy in postharvest research can be a very real handicap. In my very early days at Lake Alfred, I was privileged to cooperate with John Winston, the USDA's grand old man of postharvest research (I used to say that I was Paul at the feet of Gamaliel!). Hydrocooling of peaches having proved a great success, there was considerable commercial pressure to also hydrocool citrus. I built a little experimental hydrocooler and John Winston provided samples of a wide range of citrus varieties. We soon found that, though in general, hydrocooling did no harm; it made some samples extremely susceptible to decay. This we wrote up in a manuscript that was vetoed by someone in Washington because it was then considered a tenet of faith that "a fruit or vegetable could not be cooled too soon or too fast." This report had to be published with no evidence of USDA participation (Grierson, 1957). This was unfortunate as these hydrocooling problems proved to be very real.

At Lake Alfred we had far less sophisticated facilities, absolutely minimal funding, and were limited to working in-state. However, we had a very great advantage in the involvement of a series of very intelligent, very hard-working, graduate students. Each graduate student's committee enlisted staff members who contributed expertise otherwise unavailable in our small postharvest group.

Thus, we settled into a fruitful cooperation in which the USDA handled the increasing demand for practical applications borne of the developing export trade. Meanwhile, we at Lake Alfred, were free to concentrate on more basic CI research. At least once a year, our two postharvest groups conferred to coordinate progress as much as possible. This was particularly important after it became clear that CI could be mitigated by various forms of preconditioning to low temperature storage or shipping. Both U.S. and Japanese regulations being involved, very specific instructions had to be given to exporters. These, and overseas shipping tests, were clearly the responsibility of the USDA.

Much time and effort was expended by both teams on various conditioning treatments (hot water dips, controlled atmospheres, vacuum holding, etc.) most of which proved to be little more than confirmation of a 1938 South African report that delayed cooling mitigated CI (Van der Plank, 1938). Both teams missed a very useful observation reported by the Israelis. This was that the fungicide TBZ had a useful effect in mitigating CI (Schiffmann-Nadel et al., 1975).

Both teams (and all published reports) agreed that early fall pickings were extremely susceptible to CI, gradually becoming less susceptible as the season progressed. A major area of disagreement was that we, but not the USDA, always found a marked increase in CI susceptibility in late spring harvests. Later, an alert graduate student, Kaz Kawada, found a 1936 USDA publication reporting fall and spring susceptibility in California grapefruit (Harvey and Rygg, 1936). This had been overlooked by our USDA colleagues due to a most obscure title and the lack of modern "keyword" indexing.

At Lake Alfred, really systematic progress became possible with two breakthroughs in methodology. Both USDA and CREC teams had until then compiled data from each picking in terms of degree of CI and amounts and kinds of decay from multiple treatments at three or more weekly examinations. This generates a great clutter of data, much of it not necessarily pertinent to CI susceptibility. Instead, we reduced our observations for each treatment at each picking to a single value, days at 40 °F (4.5 °C) before appearance of CI. As soon as that value was obtained, the sample was discarded (Grierson, 1979).

The second breakthrough was getting our own trees. It being Holy Writ in Florida citrus that production research is *never* concerned with postharvest problems, we needed trees of our own. These came when our Director, Dr. Herman Reitz, assigned us an 18-tree grapefruit buffer row for our exclusive use.

Now we had the opportunity to really explore seasonal changes in susceptibility of grapefruit to CI. These 18 trees were sampled every 2 weeks for 5 yr, regardless of fruit maturity. Each picking included fruit from all areas of each tree, north and south, east and west inside and outside, high and low. Fruit from each picking was consolidated, then divided into four samples one of which went into 40 °F (4.5 °C) storage immediately. Fruit were examined at weekly intervals for CI and the results graphed. As soon as "days to incipient CI" was known, the sample was discarded. Thus, a single value was recorded for each treatment at each biweekly picking.

This paid off handsomely. It soon became apparent that every year there were sharp peaks in resistance to CI, but when they occurred was not predictable. Then the grapefruit was again highly susceptible to CI. The USDA's single big spring harvests had never happened to occur after this return to CI susceptibility. This finding we presented in a combined paper at the 1977 Orlando meeting of the International Society of Citriculture (Grierson and Hatton, 1977).

Use of a single value for "days to incipient CI" proved to be a bonanza in the hands of a superb statistician. Our graduate students had to divide their time between Lake Alfred and Gainesville. Lake Alfred is a weather reporting station. For his thesis work at Gainesville I assigned Kaz Kawada the task of correlating 5 yr of weather data with 100 biweekly "days to incipient CI." The result was a memorable memo starting "I send you a great holy angel." This was a 0.963 correlation between "days to incipient CI" and "grove temperature deviation from the long term mean" (Any 0.9+ correlation in agricultural research is phenomenal). The more the trees were stressed, the more resistant grapefruit were to CI (Kawada et al., 1978).

Back to South Africa, where the very cooperative research workers at Nelspruit sent us library copies of their Annual Reports. In short, at Nelspruit on the High Veldt, their grapefruit were as CI susceptible as ours. But after being shipped for 3 d across the Karroo Desert in *unrefrigerated steel freight cars* to the port at Capetown they were immune to CI. We did not have a Karroo Desert, nor a steel freight car, but there was a disused metal structure on the roof of our building and I had a strong young assistant. At each biweekly picking, Jim Rushing (now Professor at Clemson) climbed a ladder with a carton of grapefruit to that very hot metal shed on the roof. Until the weather cooled, 3 d in that very hot rooftop shed and grapefruit were virtually immune to CI.

But why? This "heat stress effect" worked equally well on or off the tree. The tree communicates with its fruit by means of growth regulators (GRs), (hormones). So we would study GRs. These tend to work as opposed pairs, a "promoter" and an "inhibitor." On the advice of Dr. Adair Wheaton, Kaz set to work analyzing gibberellins, the operative GRs in growth flushes, and abscisic acid (ABA), the inhibitor of growth and promoter of dormancy. The results are summarized in a chapter in a recent textbook (Grierson, 1999).

To condense a cauldron of research into a teaspoon: in times of growth flush or bloom grapefruit are very susceptible to CI. Grapefruit from drought-stressed trees are very resistant to CI. This explains the mid-winter resistant period, which coincides with Florida's dry season. Similar stress to harvested grapefruit has the same effect.

These findings neatly correlate basic and applied research. Gibberellins are always associated with juvenility, and juvenility with CI susceptibility. This review started with very basic California research showing that CI injury is initiated by temperature-induced cell membrane disruption. A British report identifies ABA as a specific protector of cell membrane integrity (Lea and Collins, 1979).

Obviously, packers planning to export or store grapefruit should work in close cooperation with their production managers. This being totally contrary to Florida citrus theology, the production manager is probably happily irrigating the grapefruit grove to be picked for export or storage next week.

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