

Chlorophyll Fluorescence Imaging Allows Early Detection and Localization of Lemon Rind Injury following Hot Water Treatment

David Obenland¹ and Paul Neipp

U.S. Department of Agriculture, Agricultural Research Service, Crop Protection and Quality, San Joaquin Valley Agricultural Sciences Center, 9611 South Riverbend Avenue, Parlier, CA 93648

Additional index words. *Citrus limon*, essential oil

Abstract. Green lemons (*Citrus limon* (L.) Burm.) were imaged for chlorophyll fluorescence (CF) 30 minutes after immersion of the fruit into 55 °C water for 5 minutes to determine if CF could be used to identify areas of hot water-induced rind injury before the appearance of visible symptoms. Fluorescence was variable in intensity over the surface of the rind with defined areas of enhanced fluorescence being present that corresponded in shape and location with visible injury that later developed during 24 hours of storage. Images showing minimum fluorescence (F_0) and maximal fluorescence (F_m) provided the best image contrast between injured and noninjured areas of the rind. Total F_0 present in the image was closely correlated ($r^2 = 0.87$) with the area of rind injury present following storage. Holding the fruit under conditions of low humidity for 24 h before hot water treatment prevented both the formation of areas of enhanced fluorescence and the corresponding rind injury. Imaging of CF has potential as a means to identify areas of incipient rind injury in citrus to facilitate study of the causal mechanisms of postharvest rind disorders.

Measurement of chlorophyll fluorescence (CF) has been widely used as a tool for detecting and monitoring stress in horticultural commodities. A variety of stresses have been studied using this technique, some examples being changes in atmospheric composition (DeEll et al., 1995; DeLong et al., 2004; Prange et al., 2002) and exposure to high (Joyce and Shorter, 1994; Smillie, 1992; Smillie and Gibbons, 1981; Tian et al., 1996) or low temperatures (DeEll et al., 2000; Forney et al., 2000; Lurie et al., 1994). Primarily, measurements of CF have been performed using single point measurements, but instrumentation has recently become commercially available that allows CF to be imaged over a large surface area, thus enabling visualization of heterogeneity in CF on the surface of the fruit and a more realistic overall determination of CF of the whole commodity. Ciscato et al. (2001) used CF imaging to predict the storage potential of apples during long-term storage. The authors noted that storage caused a loss in CF and the appearance of fluorescing spots that were related to the loss of photosynthetic capability in these areas. Nedbal et al. (2000b) imaged ripening lemons and observed differences in the photosynthetic parameters F_0 (minimum fluorescence), F_m (maximal fluorescence) and F_v (variable fluorescence, $F_m - F_0$) over the fruit surface that were related to differing levels of

chlorophyll content. The F_v/F_m ratio, an estimate of the maximum quantum yield of photosystem 2 (Genty et al., 1989), however, was uniform over the fruit surface. These results were taken to illustrate the need to monitor more than a single fluorescence parameter when attempting to identify areas of surface damage. A ratio of F_0/F_v was found to be the most effective in identifying areas of decay in the peel of whole lemons and could predict decay before the appearance of visual symptoms (Nedbal et al., 2000b).

Nonpathological rind disorders of citrus fruit are a common occurrence that can greatly reduce marketability. These disorders can be initiated by a variety of both pre and postharvest factors (Grierson, 1981). One type of disorder is characterized by the development of darkened sunken areas, or pits, on the rind and can be induced with exposure to either chilling (Obenland et al., 1997; Underhill et al., 1995) or high temperatures (Obenland et al., 1996). Postharvest pitting may also occur in citrus fruit held at warm temperatures following waxing (Petracek et al., 1995). The water status of the peel is believed to be key in the development of this type of pitting as the occurrence of the disorder is enhanced by transfer of fruit from low to high humidity (Alferez and Burns, 2004). The physiological basis underlying these various types of postharvest pitting is incompletely understood. One of the greatest impediments to understanding pitting disorders is the nonuniform distribution of the injured regions on the surface of the rind. Waiting for injury to become visible before initiating biochemical studies allows the injury to become very advanced and the tissue senescent, making it difficult to discern initial causes. A means of visualizing early on in the development of

a pitting disorder where injury will eventually occur would be extremely useful.

Chlorophyll fluorescence is highly sensitive to stress and injury and it seemed likely that cellular processes involved in the induction of rind injury in citrus peel may also cause changes in CF early in the development of the injury. The purpose of this study was to evaluate the use of CF imaging to scan the fruit surface and identify areas of rind injury in lemons before the occurrence of visual injury. Hot water immersion was chosen as a means to induce rind injury for the study as injury could be rapidly and reproducibly induced. In addition, experimentation was conducted to provide an initial look at potential causes of the observed hot-water-induced changes in CF and associated rind injury.

Methods and Materials

Hot water treatment and CF imaging. Thirty green 'Lisbon' lemons (*Citrus limon* (L.) Burm.) were picked from a field at the Parlier USDA facility. Prior experimentation had shown that although fully mature yellow lemons did not contain an adequate amount of chlorophyll to provide a fluorescent image, lemons with only a small visible amount of green rind coloration (silver to light-yellow color stage) could be imaged (data not shown). Lemons of dark-green to light-green coloration were used throughout this study. The fruit were washed in distilled water before imaging. To allow a comparison of CF images and subsequent rind injury the lemons were marked by drawing a line around the circumference of the fruit across both stem and blossom ends and each half was given a designation to identify it. The lemons were then placed into a wire basket and immersed into a 55 °C water bath for 5 min. Following treatment the fruit were removed from the hot water bath and dried using paper towels.

After hot water treatment and before CF imaging the lemons were dark adapted for 30 min by placing them in a lightproof box. Imaging of CF was then conducted using a FluorCam system (Photon Systems Instruments, Brno, Czech Republic) that was described in Nedbal et al. (2000a). The instrument utilized a CCD camera to capture fluorescence images in response to exposure to different irradiation. Lemons were placed into the FluorCam system and oriented with one of the marked halves facing the camera and both halves individually imaged. The automated measurement protocol was initiated by exposure of the fruit to 3 s of nonactinic measuring flashes to provide an image of F_0 . This was followed by a 1-s pulse of saturating light ($2000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to allow imaging of F_m . Digital photographs were taken of each lemon after 24 h of storage at 22 °C to document the resulting rind injury.

Quantification of rind injury. Twenty-two additional 'Lisbon' lemons were hot water-treated as described above except that immersion times of 1, 2, 3, and 4 min were used to help provide a wider range of rind injuries. Five fruit were treated using the 1, 2, and 3 min times and seven fruit using the 4 min time. Following hot water treatment the fruit were imaged for CF and then after 24 h of storage at 22 °C were photographed

Received for publication 38 Mar. 2005. Accepted for publication 16 Apr. 2005. Mention of trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

¹To whom reprint requests should be addressed; e-mail dobenland@fresno.ars.usda.gov.

using a digital camera. Each half of the fruit was carefully oriented so that the photographed portion corresponded closely with the part of the fruit that had been earlier imaged for CF. Photographs from one side of each of the 22 fruit were selected for further image processing based upon the need to provide an array of injury severities. Software (Adobe Photoshop, San Jose, Calif.) was used to convert the images to grey scale and to change the color of all of the injured areas to white and noninjured areas to black. The percentage of each color was then estimated from the converted image using Scion Image (Scion Corporation, Frederick, Md.). The percent white was used as an estimate of the percentage of rind injury. Regression analysis of the data was performed using StatView (Abacus Concepts, Berkeley, Calif.).

Pretreatment storage experiment. Eighteen green 'Lisbon' lemons were harvested from a field at the Parlier USDA facility and washed with distilled water. The lemons were warmed to room temperature (22 °C), divided into three groups of six and immersed in hot water (55 °C for 5 min) after the following treatments: 1) None, immediate treatment; 2) Storage on a laboratory bench (22 °C and 40% RH) for 24 h; 3) Storage inside sealed plastic bags containing a moistened paper towel for 24 h. Fruit were imaged for CF after hot water treatment and photographed 24 h later to document the development of rind injury. To compare measurements of overall CF an analysis of variance procedure was conducted using StatView to determine significant differences at $P \leq 0.05$.

Results and Discussion

Hot water treatment can easily and reliably induce rind injury in lemons and so was chosen as a means to test the ability of CF to identify areas of rind injury before the occurrence of visible symptoms. Although damage to the rind was not immediately visible following hot water treatment, injury in the form of brown sunken lesions on the flavedo developed within 24 h of storage at room temperature. A typical injury response of the lemon rind to hot water treatment is shown in the photograph in Fig. 1. The lesions were often irregularly distributed on the surface of the rind and varied greatly in number and severity from fruit to fruit. Immediately after hot water treatment and 30 min dark adaptation strongly fluorescing areas were identified by imaging the fruit for both F_0 and F_m with the FluorCam system (Fig. 1). These areas were clearly visible despite the presence of a gradient in fluorescence (higher values in center) across the surface of the fruit caused by unequal illumination due to the surface curvature. A comparison between the photo taken following 24 h of storage and the F_0 and F_m images taken 30 min after heat treatment showed a close correspondence in shape and position between the strongly fluorescing areas and areas of the rind that developed lesions following storage. These fluorescing areas occurred on all of the 30 heat-treated fruit that were imaged and were always predictive of rind injury. Injured and noninjured areas could also be identified by the F_v , F_v/F_m , and F_0/F_v images, although the

contrast between the areas was inferior to that obtained with F_0 and F_m (Fig. 1).

To obtain a numerical estimate of the correspondence between injury and total CF a further 22 lemons were hot water treated using a treatment duration ranging from 1 to 4 min, imaged for CF and photographed following 24 h of storage to provide an image from which to calculate the amount of rind injury present. An array of rind injury was obtained, ranging from 1% to 62% of the total rind area visible in the photograph. In agreement with visual observations from the previous experiment, the total F_0 from each of the fruit images (one side only per fruit) was positively correlated ($r^2 = 0.87$) with the total area (expressed as pixels) of the fruit that was injured (Fig. 2).

Allowing lemons to stand at room temperature for 24 h after harvest and before hot water treatment was effective in eliminating both the formation of regions of enhanced F_0 and the associated rind injury that develops following storage (Fig. 3A, B, D, and E). It seems likely that a loss of rind moisture was responsible for the decline in sensitivity to hot water injury since lemons stored for the same period of time under high humidity conditions were injured by hot water treatment Fig. 3C and F. All of the fruit within a storage treatment were found to respond in a similar manner. The treatment differences were also reflected in overall measurements of CF from the fruit surface where the CF parameters of F_0 , F_m , and F_v were significantly lower in fruit stored under low humidity (Table 1). High rind turgor in lemons caused by a high rind moisture content has long been associated with the development of rind injury following handling and storage (Sinclair, 1984). The rind injury, known as oleocellosis,

is associated with the release of phytotoxic oil onto the rind surface and subsequent collapse of tissue surrounding the oil glands in the affected area (Fawcett, 1916). Oil release also occurs from lemons during and following hot water treatment and may be at least partially responsible for the induced injury (Obenland et al., 1996). In this study rind oil was abundantly present on the fruit surface of lemons following hot water treatment, but only on those lemons that would later develop injury. Rind injury in citrus due to exposure to rind oil has been associated with plastid degradation (Knight et al., 2002; Shomer and Erner, 1989). In addition, large increases in F_0 as were observed in this study in the injured areas of the rind, are characteristic of damage to photosystem 2 (Schreiber and Berry, 1977). It is possible that oil release from the turgid lemons directly damaged the chloroplasts present in the rind and was responsible for the elevation in F_0 seen in the areas of the rind that would later develop visible injury. Although heat by itself is also known to have a detrimental effect on photosystem 2 activity (Schreiber and Armond, 1978), fruit that were allowed to stand for 24 h at low humidity before hot water treatment did not develop fluorescing areas or injury (Fig. 3B and E), indicating that some additional factor must be involved.

In summary, these results indicate that CF imaging can identify areas of the lemon rind that are damaged before the appearance of visual symptoms. Although this study exclusively looked at hot water-induced injury, it is hoped that the technique will also be useful in visualizing other forms of postharvest rind disorders in citrus and facilitate the study of the biochemical basis of the disorders.

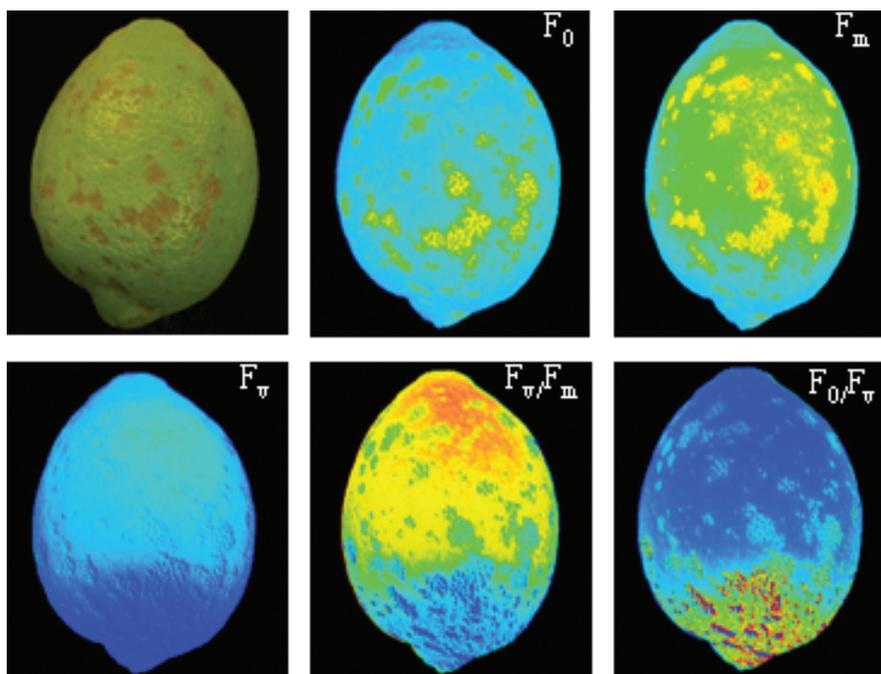


Fig. 1. A green lemon with rind injury induced by hot water treatment and 24 h of storage is shown in the photograph. Fluorescence images of F_0 , F_m , F_v , F_v/F_m and F_0/F_v taken from the same fruit 30 min after treatment are shown in the remaining panels. Blue color indicates low fluorescence and green, yellow and red signify increasingly higher intensities of fluorescence and measuring ratios. In relative numerical units the overall chlorophyll fluorescence (CF) readings from the associated images were: F_0 (426.8), F_m (679.8), F_v (252.9), F_v/F_m (0.36), and F_0/F_v (1.75). This result was typical of that obtained from treating and imaging 30 lemons.

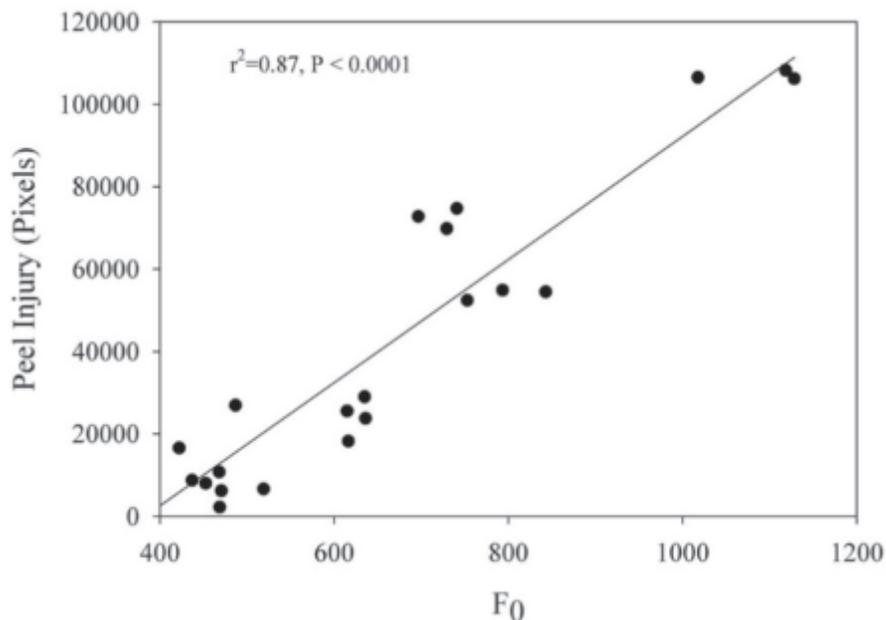


Fig. 2. The relationship of rind injury, expressed as the number of pixels in the injured area, with total F_0 in the entire area of the rind imaged. Each data point represents an image taken from a separate lemon.

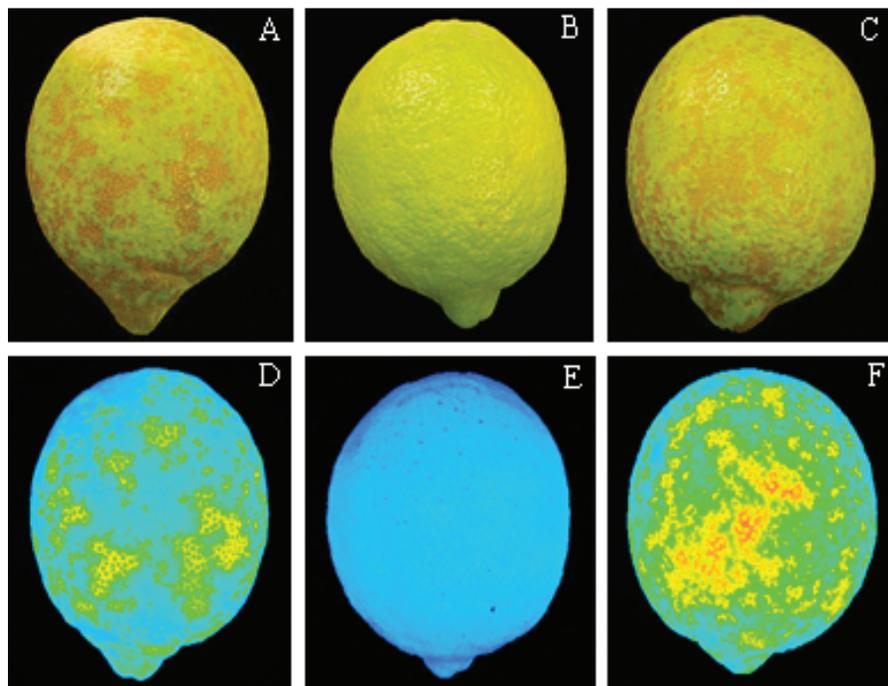


Fig. 3. Photographs of green lemons treated with hot water immediately after harvest (A) or following 24 h of storage in either low (B) or high (C) humidity. The photographs were taken after 24 h of storage following treatment. Corresponding fluorescence images (F_0) of the same fruit taken 30 min after hot water treatment are in the lower panels (D, E, and F). Blue color indicates low fluorescence and green, yellow and red signify increasingly higher intensities of fluorescence. The photos and images are representative of the results obtained.

Table 1. Parameters of chlorophyll fluorescence (CF) following various pretreatment storage regimes and hot water treatment at 55 °C for 5 min.

Pretreatment storage ^z	CF parameter (relative units)		
	F_0	F_m	F_v
None	731.2	943.0	211.8
24 h, Low humidity	324.0	474.0	150.0
24 h, High humidity	868.6	1091.0	222.4
LSD ^y	153.5	188.4	40.8

^zbefore hot water treatment.

^yLSD at $P \leq 0.05$ ($n = 6$).

Literature Cited

- Alferez, F. and J.K. Burns. 2004. Postharvest peel pitting at nonchilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. *Postharvest Biol. Technol.* 32:79–87.
- Ciscato, M., M. Sowinska, M. vandeVen, F. Heisel, T. Deckers, J. Bonany, and R. Valcke. 2001. Fluorescence imaging as a diagnostic tool to detect physiological disorders during storage of apples. *Acta Hort.* 553:507–512.
- DeEll, J., C. Vigneault, and S. Lemerre. 2000. Water temperature for hydrocooling field cucumbers in relation to chilling injury during storage. *Postharvest Biol. Technol.* 18:27–32.
- DeEll, J.R., R.K. Prange, and D.P. Murr. 1995. Chlorophyll fluorescence as a potential indicator of controlled-atmosphere disorders in 'Marshall' McIntosh apples. *HortScience* 30:1084–1085.
- DeLong, J.M., R.K. Prange, J.C. Leyte, and P.A. Harrison. 2004. A new technology that determines low- O_2 thresholds in controlled-atmosphere-stored apples. *HortTechnology* 14:262–266.
- Fawcett, H.S. 1916. A Spotting of Citrus Fruits due to the Action of Oil Liberated From the Rind. *California Ag. Expt. Sta. Bul.* 266:259–270.
- Forney, C.F., M.A. Jordan, K.U.K.G. Nicolas, and J.R. DeEll. 2000. Volatile emissions and chlorophyll fluorescence as indicators of freezing injury in apple fruit. *HortScience* 35:1283–1287.
- Genty, B., J.-M. Briantais, and N. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys. Acta.* 990:87–92.
- Grierson, W. 1981. Physiological disorders of citrus fruits. *Proc. Intl. Soc. Citricult.* 2:764–767.
- Joyce, D.C. and A.J. Shorter. 1994. High-temperature conditioning reduces hot water treatment injury of 'Kensington Pride' mango fruit. *HortScience* 29:1047–1059.
- Knight, T.G., A. Klieber, and M. Sedgley. 2002. Structural basis of the rind disorder oleocellosis in Washington navel orange (*Citrus sinensis* L. Osbeck). *Ann Bot.* 90:765–773.
- Lurie, S., R. Ronen, and S. Meier. 1994. Determining chilling injury induction in green peppers using nondestructive pulse amplitude modulated (PAM) fluorometry. *J. Amer. Soc. Hort. Sci.* 119:59–62.
- Nedbal, L., J. Soukupová, D. Kaftan, J. Whitmarsh, and M. Trtílek. 2000a. Kinetic imaging of chlorophyll fluorescence using modulated light. *Photosynthesis Res.* 66:3–12.
- Nedbal, L., J. Soukupová, J. Whitmarsh, and M. Trtílek. 2000b. Postharvest imaging of chlorophyll fluorescence from lemons can be used to predict fruit quality. *Photosynthetica* 38:571–579.
- Obenland, D.M., D.C. Fouse, L.H. Aung, and L.G. Houck. 1996. Release of d-limonene from noninjured and injured lemons treated with hot water and low temperature. *J. Hort. Sci.* 71:389–394.
- Obenland, D.M., D.A. Margosan, L.G. Houck, and L.H. Aung. 1997. Essential oils and chilling injury in lemon. *HortScience* 32:108–111.
- Petracek, P.D., W.F. Wardowski, and G.E. Brown. 1995. Pitting of grapefruit that resembles chilling injury. *HortScience* 30:1422–1426.
- Prange, R.K., J.M. DeLong, J.C. Leyte, and P.A. Harrison. 2002. Oxygen concentration affects chlorophyll fluorescence in chlorophyll-containing fruit. *Postharvest Biol. Technol.* 24:201–205.
- Schreiber, U. and P. Armond. 1978. Heat-induced changes of chlorophyll fluorescence in isolated chloroplasts and heat damage at the pigment level. *Biochim Biophys. Acta.* 502:138–151.
- Schreiber, U. and J. Berry. 1977. Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of the photosynthetic apparatus. *Planta* 136:233–238.
- Shomer, I. and Y. Erner. 1989. The nature of oleocellosis in citrus fruits. *Bot. Gaz.* 150:281–288.
- Sinclair, W. 1984. *The Biochemistry and Physiology of the Lemon.* Univ. Calif., Oakland.
- Smillie, R.M. 1992. Calvin cycle activity in fruit and the effect of heat stress. *Scientia Hort.* 51:83–95.
- Smillie, R.M. and G.C. Gibbons. 1981. Heat tolerance and heat hardening in crop plants measured by chlorophyll fluorescence. *Carlsberg Res. Commun.* 46:395–403.
- Tian, M., A. Woolf, J. Bowen, and I. Ferguson. 1996. Changes in color and chlorophyll fluorescence of broccoli florets following hot water treatment. *J. Amer. Soc. Hort. Sci.* 121:310–313.
- Underhill, S.J.R., R.L. McLauchlan, and I.L. Eaks. 1995. 'Eureka' lemon chilling injury. *HortScience* 30:309–312.