Cellulase Activity and Gene Expression in Citrus Fruit Abscission Zones during and after Ethylene Treatment

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ABSTRACT. Mature and immature 'Valencia' orange [*Citrus sinensis* (L.) Osbeck] and immature 'Valencia' orange and 'Tahiti' lime (*Citrus latifolia* Tan.) fruit with attached pedicels were treated with 8 $\mu\text{L}\cdot\text{L}^{-1}$ ethylene for periods up to 24 hours. Endo- β -1,4-glucanase (cellulase) activity and gene expression were determined in fruit abscission zones during and after ethylene exposure. Cellulase activities were not detected in mature 'Valencia' orange and immature 'Tahiti' lime fruit abscission zones immediately following harvest and after 6 hours of ethylene treatment. After 12 hours of ethylene treatment, cellulase activity increased and was highest after 24 hours. Cellulase gene expression preceded the rise in cellulase activity and was detectable after 6 hours of ethylene treatment, but then declined after 12 hours. Following transfer to air storage, abscission zone cellulase activity in mature 'Valencia' fruit remained high, whereas activity in immature 'Tahiti' fruit declined. After 168 hours air storage, activity in abscission zones of mature 'Valencia' fruit decreased slightly, but activity in abscission zones of immature 'Tahiti' lime fruit increased to the highest level. Expression of abscission zone cellulase gene *Cel-a1* in abscission zones of mature 'Valencia' fruit markedly increased after transfer to air and was highest after 48 hours air storage. *Cel-a1* expression returned to low levels after 168 hours of air storage, but expression of cellulase gene *Cel-b1* remained at low levels throughout the air storage period. Expression of *Cel-a1* and *Cel-b1* declined in fruit abscission zones of immature 'Valencia' and 'Tahiti' lime fruit upon transfer to air. After 168 hours of air storage, expression of *Cel-a1* again rose to high levels but *Cel-b1* remained low. The results suggest that differences in cellulase activity and gene expression measured in mature and immature fruit abscission zones during ethylene treatment and subsequent air storage may, in part, explain the differential response of mature and immature fruit to abscission agents.

Most citrus fruit are still harvested by hand, and this has contributed to the recent trend in reduced returns for citrus growers. The cost of harvesting Florida citrus now exceeds the cost of production (Muraro, 1996). Tree trunk shakers in combination with abscission agents show promise for improving automation and economics of harvesting (Whitney, 1995). However, the force needed to remove mature fruit from the tree continues to be a major impediment to efficient and economical harvesting. A better understanding of the physiology and regulation of citrus fruit abscission is needed to develop effective automated harvesting management strategies based on abscission agents and mechanical harvesting units.

Abscission is a process that culminates in the shedding of plant organs. Ethylene is involved in abscission, and its presence hastens appearance and action of endo- β -1,4-glucanase (cellulase) and other cell wall hydrolases (Brummell et al., 1994; Sexton and Roberts, 1982). Although the *in planta* substrate for cellulase remains unknown, the likely substrate may be xyloglucan polymers of hemicellulose (Brummell et al., 1994). Polygalacturonase (PG) activity has also been correlated with breakdown of pectin polymers found throughout the middle lamella of abscission zones (Sexton and Roberts, 1982). The action of cell wall hydrolases directly influence fracture at the abscission zone and eventual detachment of the organ from the parent plant.

In abscising bean leaves, ethylene treatment induced accumulation of a cellulase-specific mRNA followed by *de novo* synthesis of pI 9.5 cellulase (Tucker et al., 1988). Continuous ethylene exposure was required to maintain expression of cellulase mRNA and cellulase activity. Increased activities of cellulase and PG were associated with abscission in citrus fruit, and ethylene accelerated the process (Burns et al., 1995; Burns, et al., 1998; Goren and Huberman, 1976; Greenberg et al., 1975; Huberman and Goren, 1979). Two abscission-related cellulase cDNAs encoding cellulase proteins of pI 5.5 (*Cel-a1*) and 8.0 (*Cel-b1*) were isolated from an ethylene-induced 'Valencia' fruit abscission zone cDNA library (Burns et al., 1998). Expression of both cellulase genes was detected 4 h after continuous ethylene treatment, suggesting that only short ethylene durations are necessary to reduce auxin to noninhibitory levels.

Abscission agents act by releasing ethylene either directly by breakdown of the agent, through metabolism of the agent or by tissue injury (Davies et al., 1976). Abscission agents used in the past for citrus fruit removal caused a burst of ethylene evolution from fruit followed by variable fruit drop within 2 to 4 d (Holm and Wilson, 1977). Internal ethylene typically rose to 1 to 8 $\mu\text{L}\cdot\text{L}^{-1}$ for 24 h or more before adequate fruit loosening was achieved. Various abscission agents considered for use in citrus harvesting must loosen only mature fruit. Specificity of abscission materials for mature fruit is critical especially for 'Valencia', since mature and immature fruit are usually found on trees during the harvest period. We hypothesized that variable fruit drop could be attributed to a reduced abscission response due to the loss of ethylene, such as would occur with metabolism and/or breakdown of an abscission agent in the field (Holm and Wilson, 1977). In addition, the reported specificity of some abscission agents for mature fruit might be related to difference in response of mature and immature fruit abscission zones to ethylene. To test this, we exposed mature

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and immature 'Valencia' and immature 'Tahiti' lime fruit with attached pedicels to ethylene for 24 h followed by storage in air. 'Tahiti' lime was chosen because fruit are typically harvested when immature. We monitored abscission by measuring cellulase activity, by following cellulase gene expression, and in mature 'Valencia' by following anatomical changes in the fruit/pedicel abscission zone, located at the juncture of the pedicel and peel immediately beneath the calyx (Goren, 1993), with light microscopy.

Materials and Methods

PLANT MATERIAL. Mature 'Valencia' oranges $\approx$ 12 months after full bloom (MAFB) with 2 to 4 cm attached pedicels were harvested at the Citrus Research and Education Center in Lake Alfred, Fla., at weekly intervals beginning 25 Mar. 1996 and ending 8 Apr. 1996. An additional harvest of mature fruit (12 MAFB) was made on 12 Apr. 1997 (12 MAFB) and immature fruit ($\approx$ 6 MAFB) on 10 July 1997. 'Tahiti' limes ($\approx$ 6 MAFB) were harvested at the Tropical Research and Education Center in Homestead, Fla., at weekly intervals beginning 8 July 1996 and ending 22 July 1996. For 'Valencia' orange and 'Tahiti' lime, three harvests were made 1 week apart in 1996, and each harvest comprised one of three replicate samples. Pull force was measured on 20 fruit from six representative trees at each harvest by measuring the force necessary to detach the pedicel from the fruit using a modified Magness-Taylor firmness meter. Harvested fruit from both citrus species were measured for horticultural maturity ($^{\circ}$ Brix and acid for 'Valencia' orange and fruit length and diameter for 'Tahiti' limes) using current industry maturity standards (Florida Department of Agriculture and Consumer Services, 1994).

Fruit with attached pedicels used for enzyme and RNA studies were harvested and transported to the CREC packinghouse, selected for uniform size and randomized prior to separation into seven treatment groups of 100 fruit each. The control group was sampled directly before ethylene treatment. The remaining 6 groups were placed in an ethylene chamber set at 27 $^{\circ}$ C, 95% relative humidity (RH), and 8 μ L-L $^{-1}$ ethylene. Ethylene concentration was monitored by gas chromatography during treatment. Periodic checks of temperature and humidity in the ethylene chamber and storage room (see below) indicated that both remained constant during treatment.

Fruit were treated with ethylene for 6, 12, and 24 h. One hundred fruit were removed from the chamber at each of the ethylene treatment periods. Following 24 h of ethylene treatment, 300 fruit were placed in a storage room set at 27 $^{\circ}$ C and 95% RH and held for 24, 48, or 168 h. One hundred fruit were removed from the storage room at each storage time. Gas chromatography verified that ethylene was below detectable levels in the storage room.

A sharpened 4 mm diameter cork borer was used to remove the abscission zone. The borer was slipped over the pedicel, and pushed through the calyx and the fruit peel. The location of the abscission zone was visually determined in this cylinder of tissue, and trimmed to $\approx$ 3 mm above and below the abscission plane using a razor blade. The abscission zone tissue taken for subsequent determinations was $\approx$ 6 mm in length by 4 mm in width. Abscission zone tissue from 50 fruit was immediately frozen in liquid nitrogen and placed in -80° C storage for future RNA extraction.

DETERMINATION OF CELLULASE ACTIVITY. For mature 'Valencia' orange and immature 'Tahiti' lime harvested in 1996, tissue from the remaining 50 abscission zones was weighed and then pulverized using an Astramixer rotary homogenizer in a 20 $\times$ volume of ice-cold acetone (Burns et al., 1998). The pulverized tissue/

acetone mixture was filtered by aspiration through a Whatman no. 41 filter. The insoluble material was removed from the filter and reextracted in a 20 $\times$ volume of ice-cold acetone. The remaining insoluble material (acetone powders) was recovered, dried at room temperature, weighed, and then stored under desiccant at -20° C.

Acetone powders were suspended in 10 volumes of solubilization buffer (1 M NaCl and 40 mM K₂PO₄, pH 7.0) and stirred at 4 $^{\circ}$ C. After 1 h, the suspension was centrifuged at 12,000 g_n . The supernatant was removed and placed on ice. This supernatant was used immediately for enzyme activity assays and total protein estimates. Protein concentration was estimated by using Coomassie Plus Protein Assay Reagent (Pierce, Rockford, Ill.) following manufacturer's protocol.

Cellulase activity was assayed by measuring the release of reducing groups (Nelson, 1944). An aliquot of the extract was incubated in 0.1 M sodium acetate pH 4.7 with 1% carboxymethyl cellulose (sodium salt, medium viscosity) as the substrate at 37 $^{\circ}$ C for 24 h.

NUCLEIC ACIDS. Mature and immature 'Valencia' orange and immature 'Tahiti' lime fruit abscission zones were used for extraction of RNA. Plant total RNA was extracted by following a modified phenol-SDS method outlined in Current Protocols Unit 4.3 (Ausubel et al., 1989). mRNA was isolated by selecting on 200 μ L of BioMag Oligo (dT)₂₀ nuclease-free magnetic beads (PerSeptive BioResearch Products, Cambridge, Mass.) as outlined by the manufacturer. Yield of total RNA and mRNA was estimated by measuring absorbance at A260.

DNA probes were developed from two distinct citrus abscission-specific cellulase cDNA clones (*Cel-a1* and *Cel-b1*) previously isolated from an ethylene-induced fruit abscission zone cDNA library (Burns et al., 1998). *Cel-a1* and *Cel-b1* hybridization probes were generated using the NEBlot Phototope Random Primer Biotin Labeling Kit (New England Biolabs, Beverly, Mass.).

The method used for electrophoresis of mRNA was adapted from McMaster and Carmichael (1977) and Ausubel et al. (1989). One microgram of mRNA/lane was loaded on a 1.2% agarose gel for visualization, or 250 ng/lane for quantification (see below). RNA was transferred from the gel to the membrane in the downward direction. Gels were blotted overnight onto Immobilon-S nylon membranes (Millipore, Bedford, Mass.). Following transfer, membranes were dried and UV cross-linked (50 mJ). Membranes were then hybridized overnight with biotin-labeled *Cel-a1* or *Cel-b1* under conditions of high stringency (68 $^{\circ}$ C). Membranes were developed using the Phototope Chemiluminescence Detection Kit (New England Biolabs).

QUANTIFICATION OF HYBRIDIZATION SIGNALS. A comparative analysis of hybridization signal strength between treatments was performed. Exposed X-ray films from northern blots loaded with 250 ng/lane mRNA were digitized by scanner and optical density analyzed using OS-Scan Image Analysis Software (Oberlin Scientific Corporation, Oberlin, Ohio).

TISSUE PREPARATION FOR MICROSCOPY. Abscission zone plugs were removed as above but were also sliced longitudinally into equal halves. Tissue was prepared for microscopy as previously described (Burns et al., 1994).

Results

PLANT MATERIAL. Pull force, horticultural maturity measurements (acid, $^{\circ}$ Brix, ratio in 'Valencia' and length and diameter in 'Tahiti') and abscission zone total protein did not change significantly in the 3 weekly harvests in 1996 so data were pooled. Pull

Fig. 1. Cellulase activity in fruit abscission zones of mature 'Valencia' orange (top panel) and immature 'Tahiti' lime (bottom panel) fruit during ethylene treatment and subsequent air storage. Fruit were removed from ethylene treatment (indicated by ↓) and transferred to air storage. Bars indicate standard error of the mean.

force at harvest was higher in 'Valencia' orange (8.35 ± 0.24 kg) than 'Tahiti' lime (4.94 ± 0.10 kg). Mature 'Valencia' juice acid was $0.66 \pm 0.06\%$ and °Brix was 11.23 ± 0.18 , giving a brix/acid ratio of 17.10 ± 1.68 . Immature 'Tahiti' limes were 5.35 ± 0.21 cm long and 5.01 ± 0.23 cm in diameter. Abscission zone fresh mass of 'Valencia' orange or 'Tahiti' lime also did not change significantly between replicates (data not shown). Fresh mass of excised fruit abscission zones averaged 61 ± 12 and 82 ± 15 mg/abscission zone for 'Valencia' orange and 'Tahiti' lime, respectively. Total protein did not significantly change during the ethylene treatment and air storage period in 'Valencia' orange and 'Tahiti' lime. Total protein ranged from 2.85 ± 0.26 to 3.64 ± 1.40 mg protein/g fresh tissue mass in mature 'Valencia' orange to 2.14 ± 0.35 to 3.00 ± 0.53 mg protein/g fresh tissue mass in immature 'Tahiti' lime.

CELLULASE ACTIVITY. There were no differences between replicates in total cellulase activities at any ethylene treatment or air storage time, so data were pooled. Differences in cellulase activity were measured in 'Valencia' orange and 'Tahiti' lime abscission zones during ethylene treatment and subsequent air storage (Fig. 1). In both citrus fruit, cellulase activity was not detected until 12 h of continuous ethylene treatment. Cellulase activity in 'Valencia' orange rose to its highest level after 24 h of continuous ethylene treatment. After transfer to air storage, cellulase activity remained high, even after 168 h (1 week) of storage. In contrast, mean cellulase activity in 'Tahiti' lime decreased after 24 h of air storage and remained at a similar level after an additional 24 h of

storage. Increased cellulase activity was measured in abscission zones of 'Tahiti' lime following 168 h of air storage.

TOTAL AND mRNA YIELD AND mRNA ACCUMULATION. No differences were seen in total RNA or mRNA yields between replicates, so yield data were pooled. Mean yield of total RNA varied between 168.7 ± 7.3 and 214.0 ± 33.3 μg·g⁻¹ fresh tissue mass and 146.8 ± 46.4 and 204.2 ± 60.7 μg·g⁻¹ fresh tissue mass in 'Valencia' orange and 'Tahiti' lime, respectively. Yields of mRNA ranged between 7.3 ± 0.9 and 14.9 ± 3.2 ng·μg⁻¹ total RNA in 'Valencia' and 4.8 ± 0.7 and 13.6 ± 0.8 ng·μg⁻¹ total RNA in 'Tahiti' lime. Continuous ethylene exposure resulted in a slight increase in total RNA in 'Valencia' and mRNA in 'Valencia' orange and 'Tahiti' lime. Yields of total RNA and mRNA remained constant throughout the air storage period.

Cellulase cDNA clones *Cel-a1* and *Cel-b1* were used as probes to demonstrate the expression of cellulase genes during ethylene-induced abscission. The cDNA clones exhibited low sequence identity and could be distinguished in hybridization experiments with high stringency conditions (Burns et al., 1998). *Cel-a1* and *Cel-b1* probes hybridized to a 1.8 kb mRNA from ethylene-treated abscission zones in 'Valencia' orange (Burns et al., 1998) and 'Tahiti' lime. Very little cellulase mRNA hybridization was detected by northern blot analysis in untreated abscission zones from 'Valencia' orange and 'Tahiti' lime (Fig. 2). In both citrus species, hybridization of the *Cel-a1* and *Cel-b1* probes increased following 6 h of continuous ethylene treatment. Mature 'Valencia' orange cellulase mRNA levels declined after 12 h of continuous ethylene treatment, and continued to decline after 24 h of continuous ethylene treatment. Hybridization of *Cel-a1* and *Cel-b1* probes differed upon transfer to air storage. Levels of *Cel-b1*-hybridizing mRNA slightly declined after 24 and 48 h of air storage but increased ~2-fold after 168 h of air storage (Fig. 3). Accumulation of *Cel-a1*-hybridizing mRNA markedly increased upon transfer to

Fig. 2. Expression of cellulase genes in fruit abscission zones of 'Valencia' orange (A) and 'Tahiti' lime (B). Single representative northern blots of 'Valencia' orange and 'Tahiti' lime are shown. Northern blots probed with *Cel-a1* (top) or *Cel-b1* (bottom). Lane 1, time 0; lane 2, 6 h ethylene; lane 3, 12 h ethylene; lane 4, 24 h ethylene; lane 5, 24 h ethylene + 24 h air storage; lane 6, 24 h ethylene + 48 h air storage; lane 7, 24 h ethylene + 168 h air storage.

Fig. 3. Cellulase mRNA levels in fruit abscission zones of mature 'Valencia' orange (top panel) and immature 'Tahiti' lime and 'Valencia' orange (bottom panel) fruit during ethylene treatment and subsequent air storage. Data were taken from single representative northern blots and expressed as the magnitude of increase in optical density (OD) compared to that of fresh harvested abscission zones before ethylene treatment. OD of fresh-harvested abscission zone lane on the northern blot was adjusted to zero for comparison. Fruit were removed from ethylene treatment (indicated by ↓) and transferred to air storage. ■ *Cel-a1* expression in mature 'Valencia' orange and immature 'Tahiti' lime; □ *Cel-b1* expression in mature 'Valencia' orange and immature 'Tahiti' lime; ● *Cel-a1* expression in immature 'Valencia' orange; ○ *Cel-b1* expression in immature 'Valencia' orange.

air storage and was highest after 48 h of air storage (72 h after harvest), but declined >4-fold after 168 h of air storage.

In contrast to mature 'Valencia' orange, cellulase mRNA accumulated to maximum levels in 'Tahiti' lime and immature 'Valencia' orange after 12 h of continuous ethylene treatment. An additional 12 h of ethylene treatment in 'Tahiti' lime either did not change (*Cel-b1*) or reduced (*Cel-a1*) the amount of cellulase-hybridizing mRNA detected. When immature 'Tahiti' lime and 'Valencia' orange fruit were transferred to air storage, the amount of cellulase mRNA declined after 24 and 48 h to levels approaching those of freshly harvested abscission zones. However, following 168 h of ethylene-free storage, a marked accumulation of *Cel-a1*-related mRNA was measured.

LIGHT MICROSCOPY. Light micrographs showed a distinct separation layer in the abscission zone tissue of untreated 'Valencia' fruit (Fig. 4a). The separation zone was three to seven cell layers wide, composed of parenchyma cells and traversed the cortex, pith, and vascular bundles. Starch grains were associated with the region. Cell walls in the separation layer appeared relatively thin, indicating that no abscission-related cell wall degradation had occurred in untreated samples. Little change in the abscission zone layer was observed after 6 h of ethylene exposure (Fig. 4b). After

12 h of continuous ethylene treatment, however, the cytoplasm of abscission layer cells became dense and starch grains became prominent (Fig. 4c). After 24 h of continuous ethylene treatment, no further changes in abscission layer cells were evident (Fig. 4d). Following 48 h of air storage, cell wall dissolution had begun as indicated by the thickened appearance of cell walls in the separation layer (Fig. 4e). Following 168 h of air storage, no further significant changes in abscission layer cells were seen, and cells remained intact with abundant starch grains (Fig. 4f).

Discussion

The time of harvest of mature 'Valencia' oranges during this study coincided with normal commercial harvest for late-season fruit in central Florida. Pull-force measurements taken on 'Valencia' fruit at harvest fell within ranges described by Cooper and Wilson (1971) and Wheaton et al. (1977). Maturity measurements of 'Valencia' oranges were within acceptable maturity standard limits as outlined by the Florida Department of Agriculture and Consumer Services (1994). 'Tahiti' limes were harvested for this study during the heaviest production period of June, July and August in South Florida (Campbell, 1978). 'Tahiti' lime maturity standards, based largely on fruit size and color (Campbell, 1978), fell within the acceptable range. Fruit-removal forces, fruit size measurements and maturity standards for fruit used in this study established that fruit were in good horticultural condition and not undergoing abscission at harvest.

Our results demonstrate that cellulase activity and gene expression are correlated with events in fruit abscission zones triggered by ethylene treatment in 'Valencia' orange and 'Tahiti' lime. Accumulation of cellulase mRNAs was followed by an increase in total cellulase activity. Cellulase mRNA was expressed after 6 h of ethylene treatment in 'Valencia' orange and 'Tahiti' lime. Total cellulase activity rose from nondetectable levels in freshly harvested abscission zones to relatively high levels after 12 h of continuous ethylene treatment. Light micrographs verified that abscission-related cell wall degradation had begun after 12 h of continuous ethylene treatment of mature 'Valencia' orange fruit. Measurable increases in cellulase activity following ethylene treatment generally occur from 12 to >30 h, depending on the tissue (Bonghi et al., 1992; Ferrarese et al., 1995; Greenberg et al., 1975; Pollard and Biggs, 1970; Ratner et al., 1969; Tucker et al., 1988). The relatively short lag period between ethylene exposure and accumulation of cellulase mRNA and increase in cellulase activity in citrus suggests that fruit harvested during this time of year may contain low amounts of auxin or auxin-type compounds. Changes in abscission zone auxin or auxin-type compounds may in part explain why pull forces fluctuate during the harvest season, especially when new flushing shoots and developing fruit are present on the tree (Goren and Goldschmidt, 1970; Pollard and Biggs, 1970; Wheaton et al., 1977).

Continuous ethylene was thought to be essential for accumulation of cellulase-related RNA (Tucker et al., 1988) and for maintenance of cellulase activity (Pollard and Biggs, 1970) during abscission. However, Greenberg et al. (1975) demonstrated that cell wall hydrolase activity continued in air storage following ethylene treatments for >24 h in Shamouti orange abscission zones. We also demonstrated that cellulase activity continued in abscission zones of mature 'Valencia' fruit under similar conditions. In addition, cellulase gene expression continued in fruit abscission zones of mature 'Valencia' orange 48 h after transfer to air storage. Continued cellulase mRNA accumulation and cellulase activity was much less in immature fruit abscission zones of

Fig. 4. Light micrographs of mature 'Valencia' fruit abscission zones during ethylene treatment and subsequent air storage. Fruit abscission zones (a) at harvest; (b) after 6 h ethylene treatment; (c) after 12 h ethylene treatment; (d) after 24 h ethylene treatment; (e) after 24 h ethylene treatment and 48 h air storage; (f) after 24 h ethylene treatment and 168 h air storage. Micrographs are 230 $\times$ magnification. Arrowhead indicates general abscission layer area.

'Tahiti' lime (cellulase gene expression and cellulase activity) or 'Valencia' orange (cellulase gene expression) 48 h after transfer into air storage. This suggests that mature fruit continue abscission-associated events at the fruit abscission zone after ethylene is removed, or when an applied abscission agent is degraded or metabolized. Cell wall degradation was apparent in light micrographs of 'Valencia' orange fruit abscission zones 48 h after transfer into air storage. Immature fruit may be less likely to loosen in the absence of continued accumulation of cellulase mRNA and resulting enzyme activity. The differences between mature and immature fruit cellulase gene expression measured after ethylene treatment and subsequent air storage were likely attributable to

differences in stage of fruit maturation (Biale and Young, 1981) and not necessarily related to inherent differences between 'Valencia' orange and 'Tahiti' lime. This fact may explain why mature fruit preferentially respond to abscission agents that cause rapid but transient ethylene production, while immature fruit remain attached to the tree.

Although our results support the involvement of cellulase in citrus fruit abscission, genes for other cell wall-modifying enzymes such as pectinesterase are also expressed in ethylene-induced citrus abscission zones (Nairn et al., 1998). Polygalacturonase activity also contributes to the abscission process in citrus fruit and leaf abscission as well (Burns et al., 1995, 1998; Greenberg et al., 1975; Huberman and Goren, 1979; Riov, 1974).

In citrus, cellulase is encoded by a multigene family that contains at least four genes (Burns et al., 1998). Expression of two of those genes, *Cel-a1* and *Cel-b1*, are abscission specific. Our data show that expression patterns of both cellulase mRNAs in abscission zones differed during air storage of immature and mature 'Valencia' orange

fruit. Cellulase activities generally followed trends similar to cellulase gene expression. Expression of *Cel-b1* was much greater in mature fruit during ethylene treatment. This suggests that each gene may encode a cellulase protein with a distinct biochemical function and/or compartmentation, or that is active at distinct developmental stages. Once *Cel-a1* and *Cel-b1* and their encoded enzyme products are fully characterized, both genes could be manipulated to enhance expression and reduce fruit pull force for more efficient and economical mechanical harvesting. Additional cellulase genes may be involved in citrus fruit abscission, and their expression may be developmentally regulated as in the case of tomato flower abscission (del Campillo and Bennett, 1996).

After 168 h of air storage, cellulase mRNA accumulation and cellulase activity increased in immature limes and 'Valencia' fruit abscission zones or remained high in abscission zones of mature 'Valencia' orange fruit. This rise in cellulase activity after 168 h of storage has been reported before (Greenberg et al., 1975; Huberman and Goren, 1979) and may be specific to detached fruit. Periodic checks for the presence of ethylene in the storage room indicated

the absence of detectable ethylene or fruit decay. It is possible, however, that the high levels of cellulase activity and gene expression were brought about by a wound-healing process at the abscission zone, or induced by internal ethylene generated from natural senescence processes, or possibly by enhanced senescence brought about by superficial wounds or pathogens, and not necessarily abscission related (Greenberg et al., 1975; Huberman and Goren, 1979).

In summary, fruit abscission zone cellulase activity and gene expression differed during abscission of mature 'Valencia' orange and immature 'Valencia' orange and 'Tahiti' lime fruit. Persistent cellulase activity in mature fruit following ethylene treatment suggested that mature fruit continued the process of abscission after external ethylene was removed. The expression of two abscission-specific cellulase genes were shown to differ during ethylene treatment and subsequent air storage, suggesting that each has a specific role during the abscission process.

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