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Cell wall metabolism in gibberellin-treated persimmon fruits

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Abstract

The application of gibberellin [GA_3] to persimmon fruits as an orchard spray, at least 2 weeks prior to harvest, has been shown to delay ripening of the fruit on the tree and its rate of softening after harvest. This effect persisted during and after cold storage. The delay in softening has been attributed to the effect of the phytohormone on cell wall metabolism. To examine this hypothesis, cell walls of GA_3-treated fruit were compared to those of non-treated fruit. Comparison between fruit was from harvest till the termination of post-storage softening. The study included TEM examinations, assay of certain hydrolase activities and determination of compositional changes occurring in the various cell-wall carbohydrate polymers. Our findings indicate that GA_3 either delays or inhibits all of the cell wall changes that were found to accompany fruit softening, including dissolution of the middle lamella, separation of the plasmalemma from the cell-wall, mitigation of the structural coherence and density of the primary cell wall, increased solubilization of pectic polymers, loss of neutral sugars, predominantly arabinose and galactose, and increased activities of exo-polygalacturonase [PG] and endo-1,4-β-glucanase [EGase]. The principal discernible compositional difference between GA_3-treated fruit and control fruit at harvest was a higher total carbohydrate content in the cell wall material extracted from GA_3-treated fruit, which was due chiefly to an increased amount of cellulose.

1. Introduction

Gibberellin has been shown to delay maturation and ripening of a number of fruits. With each species, however, the type of response is different, though there are some responses common to a number of species. Thus, for example, inhibition of chlorophyll breakdown and carotenoid or anthocyanin synthesis occur in tomato [13], apricot [1], citrus [25], strawberry [28] and persimmon[17]. Delayed maturation accompanied by increased growth is found in grapes [18] and cherries [26]. Increased firmness has been demonstrated in cherry [14] and in persimmon fruits [6, 23]. A delay in the onset of the climacteric respiration has been reported for banana [33], apricot [1], tomato [5] and persimmon [7]. As far as is known, there is not one fruit, which demonstrates all of these responses and the action of GA_3 on ripening processes is therefore pleiotropic.

The contribution of cell wall metabolism in ripening fruits to processes other than textural changes is a relatively new concept, based on the findings that the oligomeric products of cell wall hydrolysis have a number of physiological and regulatory effects [8]. Such oligomers can induce ethylene production [32], function as elicitors for fungal resistance [30], and may be involved in the release of proteins from the cell wall during ripening [20]. As there is some evidence that the cell wall is one of the sites responsive to GA_3 in some ripening fruits [25], and in vegetative tissues [22], it might be the site for initiation of ripening processes other than fruit softening. This study describes the changes incurred by GA_3 in the cell wall metabolism of ripening persimmon fruits.

2. Materials and Methods

2.1 *Plant material*

Persimmon (*Diospyros kaki* L., "Triumph") trees were sprayed at two locations with 50 mg l^{-1} GA_3, plus 0.025% Triton X-100, approximately 2 weeks prior to the peak of the estimated commercial harvest. Both orchards are in the southern coastal plain of Israel, about 5 km apart, planted in heavy loam on *D. virginiana* rootstock. Orchard A was planted in 1977, at a density of 330 trees/ha. Orchard B was planted in 1980 at a density of 400 trees/ha. Both orchards are irrigated and fertigated in a similar manner, orchard B receiving 20% more water than orchard A. The main difference between the two orchards is that the trees in orchard A are not pruned and reach a height of 6–7 m, whereas those in orchard B are pruned annually and maintained at a height of ca. 3 m. Sprays were applied in each orchard when the ground color of the fruit had turned from green to yellow-orange. Fruits from 5 sprayed and 5 untreated trees in each orchard were harvested 2 weeks after treatment. Sixty fruits of uniform size and color from each tree were sampled and divided into 3 subsamples for the assays described below, at harvest , after 3 months of storage at −1 °C and after a week at 20 °C, following cold storage. All fruit had the astringency removed prior to assaying, by exposure to 80% CO_2 for 24 h at 25 °C. Fruit firmness was measured with a mounted Hunter-Spring penetrometer using an 11 mm tip, on two pared cheeks of each of 20 fruits, at each examination. Five of these fruits, of approximately the average firmness value, were selected for preparation of the cell wall material, as described below.

2.2 *Cell wall carbohydrates*

A composite sample of 50 g pulp tissue was blended with 200 ml cold acetone (−20 °C) for 2 min. The slurry was centrifuged and the pellet was washed once in acetone and twice in 70% ethanol. The final pellet was rinsed in acetone and air-dried overnight before weighing. These acetone/ethanol insoluble solids (AEIS) included, apart from the cell wall material, the insoluble tannin, part of which had been precipitated by the CO_2 treatment to remove astringency. The insoluble tannin constituted approximately 50% of the AEIS, which ranged from 3.7% to 4.5% of the fresh weight. It was found necessary to insolubilize all the soluble tannin prior to cell wall extraction and thus include it in the AEIS, otherwise it would have been an unknown quantity, varying with the gradual softening of the fruit.

Samples of the AEIS were analyzed to determine the carbohydrate components of the cell wall. Water-soluble pectin (WSP) was extracted twice from 100 mg AEIS with 30 ml H_2O, by stirring each time for 1 h. The supernatants were combined and the pellet was extracted in a similar manner with 50 *mM* trans-1,2-diaminocyclohexane-N,N,N′,N′ tetraacetic acid (CDTA), pH 6.0, to collect the calcium pectate fraction (CSP). The pellet was resuspended in CDTA, brought to pH 11.0 and stirred for 30 min. to de-esterify the insoluble pectic fraction (ISP). The de-esterified pectin was solubilized with 10 mg fungal pectinase (Sigma), after reducing the pH to 5.5 with glacial acetic acid. The resulting uronic acids were measured colorimetrically [2].

Non-cellulosic neutral sugars (NS) were derivatized to alditol acetates by hydrolysis with 2*N* trifluoroacetic acid (TFA), reduction and acetylation [3]. The derivatives were separated and identified by gas chromatography on a Carlo-Erba chromatograph fitted with a 30 m fused silicon capillary column (DB-225, J & W Scientific). The oven temperature was 210 °C and H_2 was used as a carrier gas. Quantitation was based on integration of the peak area from the flame ionization detector, using myo-inositol as an internal standard. Cellulose was measured in the TFA-insoluble fraction by the anthrone colorimetric assay [12]. Sugar component analysis of this fraction by gas chromatography, as described above, showed that 98% was glucose.

2.3 *Enzyme assays*

Polygalacturonase (PG). Ten g of peeled fruit tissue were diced and blended with 4% polyethylene glycol 6000 (PEG) in an Ultra-Turrax homogenizer. The slurry was centrifuged for 10 min. at 10,000 *g* at 0 °C and the supernatant was discarded. The pellet was washed with distilled H_2O, re-sedimented as above and resuspended [1:1 (w/v)] in 0.1 *M* citrate-phosphate buffer, pH 6.0, containing 1 *M* NaCl, and stirred in the cold for 90 min. The supernatant was collected after centrifugation at 10,000 *g* and dialyzed overnight against 100 volumes of distilled water at 4 °C. The final supernatant was the crude extract used for PG assay, based on the procedure developed by Honda *et al.* [21] and adapted by Gross [16]. The reaction mixture contained 0.2% polygalacturonic acid in 0.05 *N* acetate buffer, pH 5.2, and the incubation time was 20 h. A

unit of enzyme activity is defined as nmole reducing sugar released per hour.

Endo-1,4-β-glucanase (EGase, EC 3.2.1.4). Ten g of peeled fruit tissue were diced and homogenized in 25 ml 0.1 *M* citrate-phosphate buffer, pH 6.0, containing 1 *M* NaCl and 2% PEG 6000. The slurry was stirred for 1 h at 5 °C, centrifuged for 10 min. at 10,000 *g* and the supernatant served as the crude extract for the assay of EGase. Activity was determined by measuring the decrease in the relative viscosity of a 1% carboxymethyl cellulose (CMC) solution at 30 °C in an Ostwald-Fenske viscosimeter 150. The CMC substrate was prepared in 50 m*M* acetate buffer, pH 5.2, containing 0.01% cycloheximide and 0.01% chloramphenicol. The change in viscosity was measured during 1 h and activity was calculated as percent reduction in relative viscosity per g fresh weight in 1 h.

Pectin Esterase (PE). Ten g of peeled fruit were diced and homogenized in 50 ml 2 *M* NaCl plus 2% PEG 6000. The slurry was brought to pH 6.0 with 1 *N* NaOH and stirred for 1 h at room temperature. The supernatant, after 10 min. centrifugation at 10,000 *g*, was the crude extract used for PE assay. Activity was assayed by measuring the release of carboxyl groups from 1% citrus pectin in 0.2 *M* NaCl at pH 7.5 and 30 °C, by titration with 0.05 *M* NaOH. A unit of PE activity was defined as that which causes the release of 1 microequivalent of carboxyl groups per minute [4].

The softening and hydrolytic activity of PG, PE and EGase in GA_3-treated and control fruit was followed during shelf-life at 20 °C, after 3 months' storage at −1 °C. A composite sample of 40 fruits from each treatment were held in air at 20 °C after 24 h in 80% CO_2 to remove astringency. At each examination, 5 unblemished fruits were chosen randomly for determination of firmness and enzymatic activity.

2.4 *Transmission electron microscopy (TEM)*

Control and GA_3-treated fruit were sampled for TEM at harvest, upon removal from storage, when still firm, and after shelf-life at 20 °C, when soft. Small elongated pieces of fruit pulp were sampled 5 mm beneath the peel and from the central region between the peel and the core. The samples were fixed in 2.5% glutaraldehyde in 0.02 *M* phosphate buffer, pH 7.2, for 1 h. They were then post-fixed for 1 h in 1% osmium tetra-oxide in the same buffer and dehydrated in a series of ethanol solutions. Finally, the fixed pieces were embedded in Spurr's medium [31]. The tissue blocks were sectioned transversely and the sections were stained with uranyl acetate and lead citrate and then examined with a JEOL-JEM 100 CXII electron microscope.

Table 1. The effect of preharvest GA_3 treatment on persimmon fruit firmness at harvest, after 3 months' storage at −1 °C and following 7 days at 20 °C

Treatment	Fruit firmness N	
	Orchard A	Orchard B
	At harvest	
Control	78.1 b*	64.7 a
GA_3	98.5 a	75.8 a
	After cold storage	
Control	81.6 ab	66.3 a
GA_3	98.4 a	71.4 a
	After cold storage + ripening	
Control	24.0 c	16.6 b
GA_3	70.8 b	32.5 b

* Mean separation within columns at $p < 0.05$

3. Results

3.1 *Fruit firmness*

Persimmon fruits, pre-treated with GA_3 were found to be of greater firmness compared to untreated fruit (control) at harvest, but the differences in firmness were statistically significant only in orchard A (Table 1). This difference was maintained after storage at −1 °C, as there was no significant softening even of the control fruit, during 3 months' storage. A marked effect of GA_3 in retarding the rate of fruit softening became apparent when the fruit was transferred from cold storage to 20 °C. Whereas the control fruit was entirely soft after 1 week, the treated fruit from orchard A still remained firm. Fruit from orchard B was just turning soft at this time, (firmness below 40 N indicates fruit responding to finger pressure). The softening of GA_3-treated fruit from orchard B indicate that the ability to eventually ripen was maintained.

3.2 *Cell wall structure*

TEM analysis for structural comparison of the cell walls from GA_3-treated fruit with those of untreated

Table 2. The effect of preharvest GA_2 treatment on pectic fractions (mg g^{-1} f.wt) in cell wall material from persimmon fruits at harvest, after 3 months' storage at −1 °C and following 7 days at 20 °C. (Each value is the mean of 5 replicates and for any value the error was less than 10%.)

Treatment	Orchard A				Orchard B			
	WSP[1]	CSP[2]	ISP[3]	Total	WSP[1]	CSP[2]	ISP[3]	Total
				At harvest				
Control	1.49	0.66	2.66	4.81	1.74	0.50	2.02	4.26
GA_3	1.38	0.79	3.20	5.32	1.43	0.58	2.92	4.93
				After cold storage				
Control	1.65	0.69	2.09	4.43	2.36	0.52	1.64	4.52
GA_3	1.46	0.74	2.56	4.76	2.88	0.60	1.61	5.09
				After cold storage + ripening				
Control	2.64	0.82	0.73	4.19	2.52	0.71	0.54	3.97
GA_3	2.10	0.76	1.67	4.53	2.42	1.07	0.64	4.23

[1] WSP = water-soluble pectin
[2] CSP = CDTA-soluble pectin
[3] ISP = Insoluble pectin

fruit at harvest, indicated a more structured middle lamella and compact primary wall in the former (Figure 1, A and B). A similar comparison was conducted after cold storage and subsequent holding at 20 °C, between treated and untreated fruit that had softened. Marked differences were observed between the two in the region of the middle lamella, the primary cell wall and the interface of the cell wall with the plasmalemma (Figure 1, C and D).The GA_3- treated fruit still maintained a more organized structure within both cell wall regions. In addition, the coherence between the plasmalemma and the cell wall, observed in the GA-treated fruit tissue, was absent from the control fruit tissue.

3.3 *Structural and compositional cell wall changes*

The most obvious structural change that occurred with fruit softening, as shown in the electron micrographs, was the disassembly of the middle lamella, albeit to a different extent in the GA_3-treated versus the untreated fruit. This region of the cell wall is considered to be rich in pectic substances, predominantly calcium pectate. In fruit sampled at harvest and after storage, no consistently significant differences in the soluble fractions were observed between the treated and non-treated fruit from both orchards, but the insoluble fraction tended to be greater in the GA-treated fruit so that the total pectin content was higher (Table 2). At harvest and especially during storage there was a noticeable difference in the WSP levels between the two orchards. In orchard B it was initially high and it increased even more so during storage, but especially in the GA_3-treated fruit. However, after shelf-life the total amount of uronides had decreased similarly in both treated and control fruit in both orchards, although there were significant differences in their solubility. In the soft fruit, whether treated or not, the insoluble pectic fraction had decreased markedly with an increase in both H_2O-soluble and CDTA-soluble fractions. These increases however, did not fully account for the decline in the insoluble fraction. In the GA_3-treated fruit from orchard A, which was flexible but not yet soft, the decrease in total uronide was less than in fruit from orchard B, with the insoluble pectin still forming 37% of the total pectin, more than twice of that in the soft fruit.

A second set of observations based on EM was the compactness and organization of the primary cell wall in the GA_3-treated fruit as compared to the control fruit. The principal cell wall component responsible for its structured appearance is cellulose, which forms the microfibrillar backbone of the cell wall [9]. At harvest, the cellulose fraction of the cell wall in GA_3-treated fruit was significantly higher than in the control fruit (Table 3). In orchard A, where GA_3 was more effective, cellulose was 44% more abundant, as compared to a 28% higher abundance in orchard B. The size of this fraction decreased by about 10% during storage and

Table 3. The effect of preharvest GA_3 treatment on cellulose content of persimmon fruits at harvest, after 3 months storage at −1 °C and following 7 days ripening at 20 °C. (Each value is the mean of 5 replicates followed by the standard error in brackets.)

Treatment	Cellulose (mg g^{-1} f wt.)	
	Orchard A	Orchard B
	At harvest	
Control	3.51 (0.22)	3.19 (0.06)
GA_3	5.05 (0.38)	4.08 (0.31)
	After cold storage	
Control	3.15 (0.19)	3.04 (0.23)
GA_3	4.70 (0.42)	3.83 (0.24)
	After cold storage + ripening	
Control	3.25 (0.02)	2.87 (0.18)
GA_3	4.57 (0.45)	3.68 (0.05)

shelf-life, in fruit from both treatments, so that the relative amounts in fruits from each orchard remained unchanged and the differences between the treatments were maintained.

The changes that occurred in the neutral sugar (NS) component of the cell wall polysaccharides during and after storage, do not point to a direct correlation with fruit softening, although some involvement is indicated during its latter stages, after removal from cold storage (Table 4). During cold storage, there was a total loss of between 15 to 35% of cell wall NS, which was predominantly glucose, and the greater decrease was in GA_3-treated fruit. This change was apparently unrelated to fruit softening, as there was no loss of firmness at this time (Table 1). However the changes during post-storage ripening at 20 °C, appeared to be related to fruit firmness in that the smallest decline in NS content occurred in the GA_3-treated fruit from orchard A, to a greater extent in the GA_3-treated fruit from orchard B and in the control fruit from orchard A, and to the greatest degree in control fruit from orchard B. This trend parallels the firmness measurements after shelf-life (Table 1). The 44% decline in the NS content of the cell wall from control fruits was due principally to a reduction in the amounts of arabinose, xylose and galactose. In the GA_3-treated fruit from orchard A these sugars decreased less with a total NS decline of 20%. The decrease in arabinose and galactose, which constituted equal proportions of the cell wall at harvest, was about 65% in soft fruit, irrespective of treatment, and only ca. 40% in firm GA_3-treated fruits. Xylose, which at harvest constituted a smaller proportion of the neutral sugars (14% and 16% in control and GA-treatment respectively) was also reduced in quantity during ripening, but to a smaller degree (50% and 25% in soft and firm fruit, respectively).

The sum of the three fractions – pectins, neutral sugar and cellulose, renders the carbohydrate content of the cell wall (Figure 2). At harvest, the fruit in both orchards had similar cell wall carbohydrate contents which were, in both cases, greater in GA_3-treated than in control fruit. There was a gradual overall decrease in carbohydrate content during storage, which was not affected by pre-harvest treatment and therefore the levels in GA_3-treated fruit still remained higher than in the control fruit. During holding at 20 °C the decrease in the total cell wall carbohydrate was accelerated, coinciding with fruit softening. The factor which was principally responsible for this decline was the 50% reduction in neutral sugars.

3.4 *Hydrolytic enzyme activity*

During post-storage holding at 20 °C control fruit softened rapidly and became totally disintegrated within 5 days (Figure 3A). The softening of the GA_3-treated fruit can be divided into three 5-day stages: during the first stage softening was almost linear with a 50% reduction in firmness values; softening almost ceased during the second stage and was resumed at the initial rate of softening during the third stage. GA-treated fruit eventually softened fully but did not disintegrate to the same degree as the control fruit.

The pattern of increasing PG activity followed that of fruit softening (Figure 3B). The initial activity in the control and the GA_3-treated fruit was the same for 2 days after transfer from −1 °C to 20 °C, even though the control fruit was already significantly softer than the GA_3-treated fruit on the second day. On the third day, PG activity began to increase rapidly in the control fruit, whereas in the GA_3-treated fruit the increase in activity was much slower and more gradual, following a similar pattern to that of softening, with a more rapid rate of increase in the latter stages of softening. Eventually, the level of PG activity attained after 20 days in the GA_3-treated fruit was similar to that reached in the control fruit after 5 days at 20 °C.

The increase in EGase activity in fruit paralleled the decline of firmness in control fruit (Figure 3C). In GA_3-treated fruit a low level of activity was maintained throughout the 3 stages of softening. PE activity,

Table 4. The effect of preharvest GA_3 treatment on neutral sugar composition of persimmon cell walls from fruit sampled at harvest, after 3 month's storage at −1 °C and following 7 days at 20 °C. (Each value is the mean of 5 replicates and for any value the error was less than 10%.)

Treatment	Orchard	Neutral sugar content (mg g^{-1} f wt.)							
		Rha	Fuc	Ara	Xyl	Man	Gal	Glu	Total
					At harvest				
Control	A	0.20	0.25	1.87	1.26	0.32	1.97	2.16	8.03
	B	0.16	0.30	1.76	0.94	0.44	1.80	2.94	8.32
GA_3	A	0.25	0.34	2.27	1.69	0.27	2.57	1.73	9.14
	B	0.19	0.37	2.28	1.34	0.46	2.57	2.48	9.70
					After cold storage				
Control	A	0.23	0.31	1.55	1.45	0.19	1.95	1.16	6.84
	B	0.19	0.29	1.63	1.14	0.22	1.56	1.17	6.22
GA_3	A	0.12	0.29	1.70	1.27	0.20	2.23	1.17	7.09
	B	0.18	0.32	1.70	1.05	0.25	1.58	1.22	6.30
					After cold storage + ripening				
Control	A	0.13	0.13	0.70	0.70	0.19	0.78	1.83	4.47
	B	0.11	0.14	0.61	0.44	0.32	0.55	1.76	3.91
GA_3	A	0.21	0.24	1.50	1.27	0.29	1.40	2.37	7.27
	B	0.13	0.13	0.77	0.63	0.28	0.68	2.82	5.45

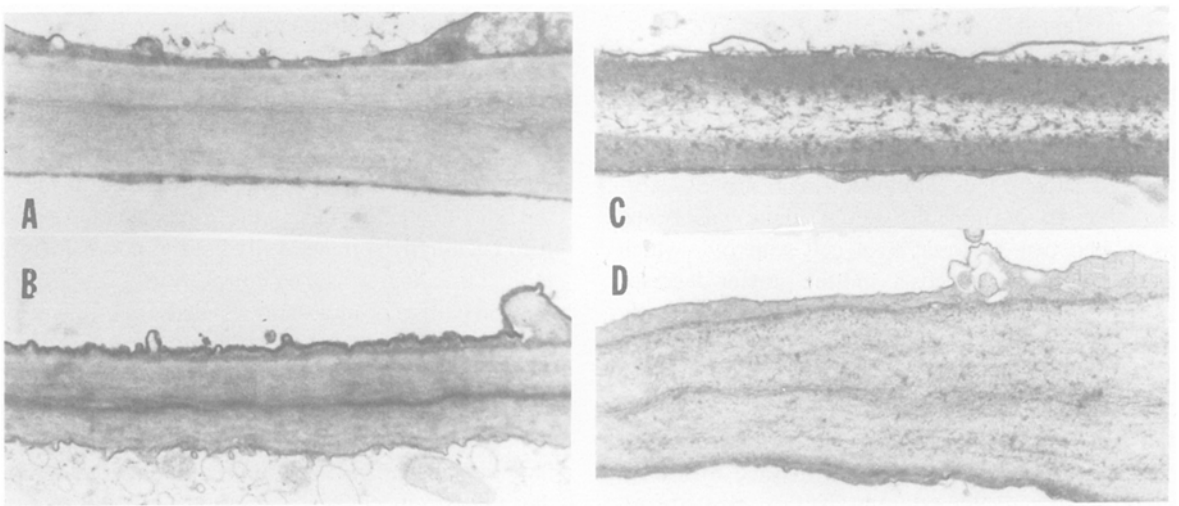

Figure 1. Cell walls from persimmon fruit at harvest (A, B) and soft fruit after post-storage ripening (C, D): A, C – control; B, D – GA_3 treated.

which has been proposed as a component of persimmon softening [4], increased slightly , but erratically with time, whether the fruit softened or not. The measured values of 20–40 units·g^{-1} fresh weight, were similar in control and GA_3-treated fruits (data not shown).

4. Discussion

The most noticeable effect of GA_3 on the cell wall composition of the fruit at harvest was the high cellulose content which was on average 37% more than in the control fruit. This may have been responsible for the greater firmness of GA_3-treated fruits at harvest, but not necessarily for the retarded rate of softening

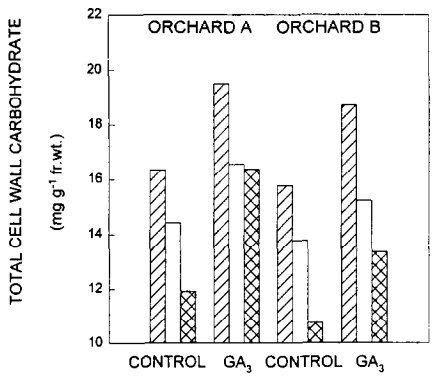

Figure 2. Total cell wall carbohydrates from control and GA_3-treated persimmon fruits at harvest (▨), after 3 months' storage at −1 °C (□) and following 7 days at 20 °C (▩).

after storage and shelf-life, as the fruit from orchard B softened quite rapidly in spite of a 28% higher cellulose content at harvest. The pectin and hemicellulose components of the cell wall were also somewhat higher in the GA_3-treated fruit than in the control (11–17%). The result was a 19% higher carbohydrate content of the cell walls. This may be due in part to reduced cell expansion following GA_3 treatment [6]. The cell wall would then represent a larger proportion of the fruit tissue, but this would not account for the total increase in cell wall material. As the cell wall composition reflects the balance between synthesis and hydrolysis, both of which occur throughout development and ripening [15], the particularly high cellulose level could result from an enhancement of cellulose synthase, an inhibition of EGase, or both. EGase activity in fruit at harvest was very low in control fruit and was undetectable in GA_3-treated fruit (data not shown). Evidence for the possible stimulation of cellulose synthesis by GA_3 was found when it was applied to pericarp discs from mature green tomatoes that ripened thereafter (Greve and Labavitch, personal communication). Moreover, the minimum of 7 days required following GA_3 treatment till harvest, for the treatment to become effective [6], suggests that a synthetic process might be involved.

GA_3-induced retardation of softening in persimmon fruit, which has been previously demonstrated [6, 23], did not appear to be simply the result of an overall deferral of fruit ripening, in that the effects on cell wall metabolism were not purely a temporal delay in the sequence of events occurring during the natural course of fruit softening. Certain aspects , such as pectin solubilization, which is probably not the initial step occur-

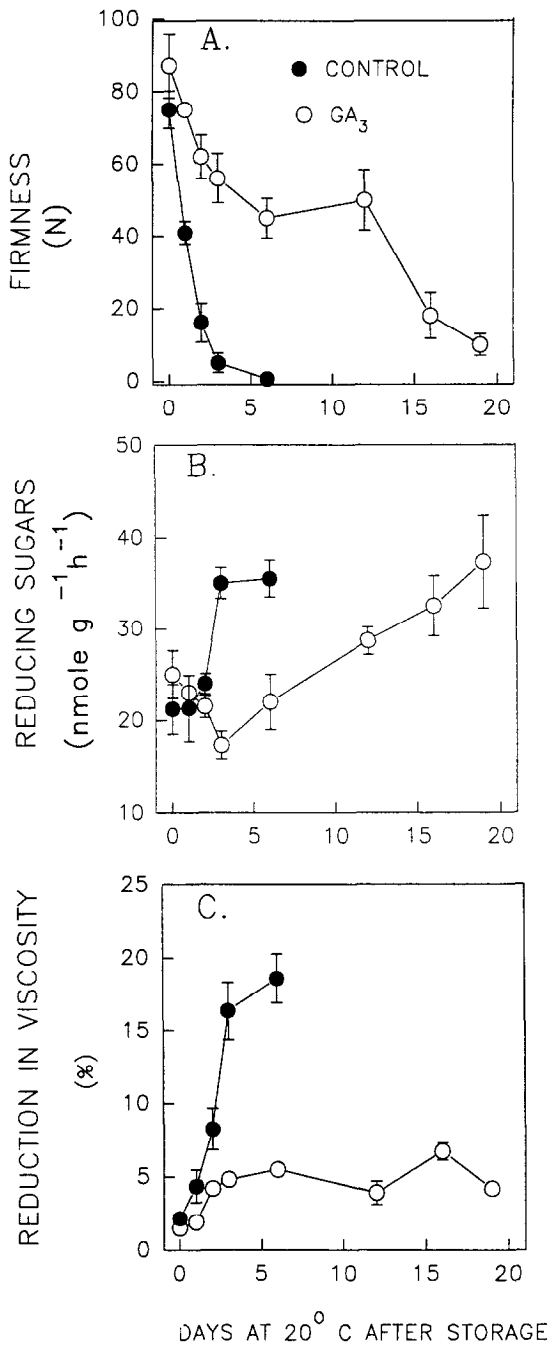

Figure 3. The effect of preharvest GA_3 treatment on softening of persimmon fruit at 20 °C (A), and the activities of PG (B) and EGase (C), in comparison with control fruit after 3 months' at −1 °C (Orchard A).

ring in the cell wall of softening fruit [8], appeared to be deferred by GA_3 treatment. PG activity seemingly accompanied softening rather than induced it, and was especially high as the fruit pulp turned extremely soft. Other aspects, such as the inhibition of EGase activity, appeared to be a specific response to GA_3 application. However, the involvement of EGase in the softening process is evidently not essential, as the GA_3-treated fruit did eventually soften, in spite of the fact that the activity of the enzyme remained much lower than in softening control fruit.

The *in vivo* substrate of EGase in fruits is still an open question. The CMC substrate used for *in vitro* assays doesn't truly represent any of the cell wall polymers *in vivo*. There has been no evidence for cellulose depolymerization associated with ripening [8], and the suggestion that xyloglucan could be cleaved by this enzyme, often termed CMCase [19], has not been supported in recent studies due to its inability to hydrolyse xyloglucan *in vitro*, when extracted from avocado [29] or tomato [27]. The analysis of neutral sugars did, however, indicate marked changes in the cell wall hemicellulose polymers during persimmon fruit softening, though it is not readily apparent that xyloglucan was the major polymer to be altered, since the marked loss of xylose during softening was not matched by a similar loss of glucose from the cell wall material. The three predominant sugars which had been released from the cell walls of soft fruit – xylose (48%), arabinose (65%) and galactose (68%) – were maintained at higher levels within the cell wall material of firm GA_3-treated fruit (75%, 66% and 63% respectively of the initial harvest values).

Cutillas-Iturralde *et al.* [10] found no PG activity (neither endo- nor exo-) in persimmons during ripening despite a large decrease in pectin fractions as well as pectin depolymerisation, but they did not examine fruit in the final stages of fruit softening and disintegration, which were examined herein. The rise in PG reported above, occurred after considerable softening had occurred and it is suggested that this enzyme has a role chiefly in the final stages of fruit softening, when it becomes mushy. More recently, changes have been reported in the hemicellulosic fractions of the cell wall, which were also depolymerised and reduced in quantity with fruit softening [11]. However, the marked increase in the activity of xyloglucan endotransglycolysate (XET), which was considered responsible, began during the second stage of fruit development, reaching its maximum prior to cessation of fruit growth and initiation of softening, and therefore, was unlikely to be solely responsible for the hemicellulose changes which occurred during fruit softening. A more likely candidate for involvement in cell wall disassembly during fruit softening is β-galactosidase, which has recently been isolated from persimmons [23]. The loss of galactosyl residues form the cell wall, as well as arabinosyl, indicate that this and other enzymes probably work in synchrony to depolymerize the cell wall components and they may be affected by GA_3 in a similar way to EGase.

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References

1. Abdel-Gawad H and Romani RJ (1974) Hormone-induced reversal of color change and related respiratory effects in ripening apricot fruits. Physiol Plant 32: 161–165
2. Ahmed A and Labavitch JM (1977) A simplified method for accurate determination of cell wall uronide content. J Food Biochem 1: 361–365
3. Albersheim P, Nevins DJ, English PD and Karr A (1967) A method for the analysis of sugars in plant cell wall polysaccharides by gas-liquid chromatography. Carbohydr Res 5: 340–345
4. Awad M (1985) Persimmon pectin methylesterase: extraction and variation during ripening. J Food Sci 50: 1643–1645
5. Babbitt JK, Powers MJ and Patterson ME (1973) Effects of growth regulators on cellulose, polygalacturonase, respiration, color and texture of ripening tomatoes. J Amer Soc Hort Sci 98: 77–81
6. Ben-Arie R, Bazak H and Blumenfeld A (1986) Gibberllin delays harvest and prolongs life of persimmon fruits. Acta Hort 179: 807–813
7. Ben-Arie R, Roizman Y, Zutkhi Y and Blumenfeld A (1989) Gibberellic acid reduces sensitivity of persimmon fruits to ethylene. In: Clijsters H (ed) Biochemical and Physiological Aspects of Ethylene Production in Lower and Higher Plants, pp 165–171. Kluwer Academic Press
8. Brady CJ (1987) Fruit ripening. Ann Rev Plant Physiol 38: 155–178
9. Carpita N and Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J 3: 1–30
10. Cutillas-Iturralde A, Zarra I and Lorences EP (1993) Metabolism of cell wall polysaccharides from persimmon fruit. Pectin solubilization occurs in apparent absence of polygalacturonase activity. Physiol Plant 89: 369–375

11. Cutillas-Iturralde A, Zarra I, Fry SC and Lorences EP (1994) Implication of persimmon fruit hemicellulose metabolism in the softening process. Importance of xyloglucan endotransglycosylase. Physiol Plant 91: 169–176
12. Dische Z (1962) Color reactions of carbohydrates. *In* RL Whistler, ML Wolfrom (Eds) Methods in Carbohydrate Chemistry, Academic Press, New York, pp. 475–514
13. Dostal HC and Leopold AC (1967) Gibberellin delays ripening of tomatoes. Science 158: 1579–1580
14. Facteau TJ (1982) Levels of pectic substances and calcium in gibberellic acid-treated sweet cherry fruit. J Amer Soc Hort Sci 107: 148–151
15. Greve LC and Labavitch JM (1991) Cell wall metabolism in ripening fruit V. Analysis of cell wall synthesis in ripening tomato pericarp tissue using a D-(U- ^{13}C) glucose tracer and gass chromatography-mass spectrometry. Plant Physiol 97: 1455–1461
16. Gross J, Bazak H, Blumenfeld A and Ben-Arie R (1984) Changes in chlorophyll and carotenoid pigments in the peel of Triumph persimmon (*Diospyros kaki* L.) induced by preharvest gibberellin [GA_3] treatment. Sci Hortic 24: 305–314
17. Gross KC (1982) A rapid and sensitive spectrophotometric method for assaying polygalacturonase using 2-cyanoacetamide. HortScience 17: 933–934
18. Guelfat-Reich S and Safran B (1973) Maturity responses of Sultanina grapes to gibberellic acid treatments. Vitis 12: 33–37
19. Hayashi T (1989) Xyloglucans in the primary cell wall. Ann Rev Plant Mol Biol 40: 139–168
20. Hobson GE, Richardson C and Gillham DJ (1983) Release of protein from normal and mutant cell walls. Plant Physiol 71: 635–638
21. Honda S, Matsuda Y, Takahashi M, Kakehi K and Ganno S (1980) Fluorimetric determination of reducing carbohydrates with 2-cyanoacetamide and application to automated analysis of carbohydrate as borate complexes. Anal Chem 52: 1079–1082
22. Jones RL (1982) The role of gibberellins in plant cell elongation. CRC Crit Rev Plant Sci 1: 23–47
23. Kang IK, Suh SG, Gross KC and Byun JK (1994) N-terminal amino acid sequence of persimmon fruit β-galactosidase. Plant Physiol. 105: 975–979
24. Kitagawa H, Sugiura A and Sugiyama M (1966) Effect of gibberellin spray on storage quality of kaki. HortScience 1: 59
25. Lewis LN, Coggins CW, Labanauskas CK and Dugger WM (1967) Biochemical changes associated with natural and gibberellin A_3 delayed senescence in the Navel orange rind. Plant Cell Physiol 8: 151–160
26. Looney NE and Lidster PD (1980) Some growth regulator effects on fruit quality, mesocarp composition and susceptibility to postharvest surface marking of sweet cherries. J Amer Soc Hort Sci 105: 130–134
27. Maclachlan G and Brady C (1994) Endo-1,4-β-glucanase, xyloglucanase, and xyloglucan endo-transglycosylase activities versus potential substrates in ripening tomatoes. Plant Physiol 105: 965–974
28. Martinez GA, Chaves AR and Anon MC (1994) Effect of gibberellic acid on ripening of strawberry fruits (*Fragaria annanassa* Duch.) J Plant Growth Regul 13: 87–91
29. O'Donoghue E and Huber DJ (1992) Modification of matrix polysaccharides during avocado *(Persea americana)* fruit ripening: an assessment of the role of Cx-cellulase. Physiol Plant 86: 33–42
30. Ryan CA, Bishop P, Pearce G, Darvill AG, McNeil M and Albersheim P (1981) A sycamore cell wall polysaccharide and a chemically-related tomato leaf polysaccharide possess similar proteinase inhibitor-inducing activities. Plant Physiol 68: 616–618
31. Spurr AR (1969) A low viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruc Res 26: 31–43
32. Tong C, Labavitch JL and Yang SF (1986) The induction of ethylene production from pear cell culture by cell wall fragments. Plant Physiol 81: 929–930
33. Vendrell M (1970) Acceleration and delay of ripening in banana fruit tissue by gibberellic acid. Aust J Biol Sci 23: 553–559
34. Wallner SJ and Bloom HL (1977) Characteristics of tomato cell wall degradation *in vitro*. Implications for the study of fruit softening enzymes. Plant Physiol 60: 207–210