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Heat Shock Response in Field-grown, Ripening Papaya Fruit

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Abstract. **Mesocarp softening during papaya *(Carica papaya* L.) ripening was impaired by heating at 42C for 30 min followed by 49C for 70 min, with areas of the flesh failing to soften. Disruption of the softening process varied with stage of ripeness and harvest date. The respiratory climacteric and ethylene production were higher and occurred 2 days sooner in the injured fruit than in the noninjured fruit that had been exposed to 49C for only 30 min. Skin degreening and internal carotenoid synthesis were unaffected by the heat treatments. Exposure of ripening fruit to either 42C for 4 hr or 38 to 42C for 1 hr followed by 3 hr at 22C resulted in the development of thermotolerance to exposure to the otherwise injurious heat treatment of 49C for 70 min. Four stainable polypeptide bands increased and seven declined in single-dimensional acrylamide gels following incubation of fruit at the nondamaging temperature of 38C for 2 hr. Three polypeptides showed marked increases when polysomal RNA was translated. These polypeptides had apparent molecular weights of 17, 18, and 70 kDa. Proteins with molecular weights of 46, 54, and 63 kDa had slight increases after heat treatment. The levels of these polypeptides peaked 2 hr after heat treatment and declined within 24 hr. The amount of these polypeptides in the unheated control varied with the batch of fruit. The concentration of three translated polypeptides, with apparent molecular weights of 26, 37, and 46 kDa, declined. Other polypeptides continued to be translated during and after holding papayas for 2 hr at 38C.**

Many plants briefly exposed to high temperatures acquire transient thermotolerance (Yarwood, 1961; Chen et al., 1982; Key et al., 1985). As this thermotolerance response develops, a unique group of proteins, known as heat shock proteins (HSP), are synthesized. HSP and associated thermotolerance have been reported for field-grown cotton leaves (Burke et al., 1985), soybean leaves (Kimpel and Key, 1985), and sorghum (Ougham and Stoddart, 1986). HSP are rapidly synthesized in response to increases of 5 to 10C above ambient, especially above 37C (Kimpel and Key, 1985). The decay of HSP occurs with the loss in thermotolerance. These HSP may protect cellular function during high-temperature stress, but their role is still unclear.

Mature papaya fruit exposed to a hot water treatment of 30 min at 42C, followed immediately by 20 min at 49C, as well as to other brief heat treatments for fruit fly disinfestation, sometimes fail to soften (Jones, 1939; Akamine, 1966). Heat treatments are also used for postharvest disease control (Couey, 1989). Papaya fruit softening is unusual in that polygalacturonase and xylanase are only produced briefly, i.e., when the fruit is 40% to 60% yellow (Paull and Chen, 1983). This pattern contrasts with the continual production of polygalacturonase in tomatoes (Grierson et al., 1985) and cellulase in avocado (Christoffersen et al., 1982).

Disruption of tomato (Yoshida et al., 1984; Picton and Grierson, 1988), apples (Liu, 1978; Porritt and Lidster, 1978), and pear (Maxie et al., 1974) fruit ripening occurs when fruit are exposed to temperatures > 30C for 48 hr or more. This disruption is characterized by a failure to develop normal pigmentation, little softening, and marked decline in ethylene production (Maxie et al., 1974; Yoshida et al., 1984; Picton and Grierson, 1988). Elevated temperature treatments may lead to inhibition and or reduced expression of softening and ripening-related mRNAs (Picton and Grierson, 1988).

We studied the response of ripening papaya fruit to short-term heat treatments. The variation in fruit sensitivity to heat treatment during the year and conditions necessary to produce maximum induced thermotolerance also were determined.

Materials and Methods

Fruit. The papaya ('Sunset') fruits used were harvested between 1985 and 1988 at the Poamoho Experimental Station, Oahu, Hawaii, were used. Fruit were picked at the first sign of skin yellowing, and only fruit having < 10% yellow skin, unless otherwise indicated, were used in these tests. The fruit was brought into the laboratory within 4 hr of harvest and sorted according to ripeness on the basis of skin color. Ten to 15 fruit were used for each treatment, and all tests were repeated at least twice.

Heat treatment. The standard noninjurious hot water treatment involved immersing fruit in water at 42C for 30 min, followed immediately by transfer to 49C for 30 min (Couey and Hayes, 1986). Thermocouples, inserted into the center of the fruit, indicated that the temperature at the center of the fruit had reached 36C. To test thermotolerance, the second heat treatment time was increased to 70 min, when the center of the fruit reached 49C. Heating for 70 min at 49C was designated as the injurious heat treatment. Immediately following heat treatments, the fruit were cooled to < 30C by immersion in water at 23.5 ± 1.5C for 30 min. Fruit were then dipped into 650 µl thiabendazole/liter (Merck, Rahway, N.J.) fungicide for 1 min.

Fruit evaluation. Fruit were allowed to ripen at 25C for 8 to 10 days after heat treatment. Unless otherwise indicated, fruit were then evaluated according to the following criteria: Hunter calorimeter "b" value (Hunter Associates, Reston, Va.) or CIE

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Table 1. Effect of heat treatments on various fruit characteristics after ripening for 10 days at 25C. All fruit, except for those evaluated at harvest, were treated for 30 min at 42C followed within 3 min with various lengths of time at 49C.

Time at 49C (rein)	Final color (% yellow)	Internal red color[z] (%)	Internal injury[y] (scale)	Deformation force (N)
0 (at harvest)	53	54	0	200
0	94	108	0	29.4
10	90	109	0	43.2
20	93	110	0	42.2
30	88	101	0	59.8
40	89	96	0.2	84.3
50	89	96	3.9	79.4
60	93	100	4.5	84.3
70	91	100	5.0	120
Significance[x]	L,Q	L,Q	L,Q	L
r^2	0.325*	0.447***	0.865***	0.625***

[z]Percen~ages of internal redness > 100% indicate overripeness with water-soaked areas in the flesh.

[y]Internal injury was recorded using a scale based on the volume of mesocarp showing hard lumps. The scale was based upon one-fifth of the angular transformation from 0° to 90°; 0 = 0%; 1 = 0% to 10%; 2 = 10% to 35%; 3 = 35% to 65%; 4 = 65% to 90%; 5 = 90% to 100%; 6 = 100%.

[x]Coefficient of determination (r^2) for best-fit model. L and Q represent significance of linear (L) or quadratic (Q) components at $P < 0.01$ (n = 78), respectively. Values at harvest not included in analysis.

*,***Significant at $P = 0.05$ or 0.001, respectively.

"b" value (Minolta Chromameter CR-110, Minolta, Ramsey, N.J.) of the blossom end at harvest and at the equator after ripening; subjective estimation of extent of skin yellowing (0% to 100% yellow); deformation force (softness) measured by a penetrometer (AmTek LKG-14, Ametek, Largo, Fla.) fitted with a 1.5-cm disc (force required to depress the disc 2 mm into the fruit); internal color development, i.e., subjective estimation of degree of internal flesh showing carotenoid development (0% to 100% red-orange). External scalding and internal injury (failure to soften) were separately recorded using a similar scale based on area or volume affected. The subjective scale used was based on one-fifth of the angular transformation from 0° to 90°; 0 = 0%; 1 = 0% to 10%; 2 = 10% to 35%; 3 = 35% to 65%; 4 = 65% to 90%; 5 = 90% to 100%; 6 = 100%. This scale was designed for more comfortable visual discrimination and less error in estimation extent. The raw data were evaluated on the rating, not the percentage. External scald symptoms evaluated were dark olive to light brown spots, depending on stage of fruit ripeness. Internal areas of the mesocarp that failed to soften were slightly lighter in color than normal tissue. These areas occurred as a hard shell around the fruit seed cavity or hard lumps spread through the flesh.

Respiration and ethylene production. Ten treated fruit were enclosed in jars at 20C and flushed continuously with air previously scrubbed of ethylene by passing through a column made of potassium permanganate absorbed onto an aluminum silicate support. The CO_2 evolution was determined at 3-hr intervals by an automatic gas sampling manifold connected to an infrared gas analyzer (Model IR-703, Infrared Industries Inc., Santa Barbara, Calif.). The jars were sealed daily for 1 hr and a 1-ml gas sample was taken from the head space for ethylene determination. Ethylene was determined on a gas chromatography fitted with an alumina column and photoionization detector (Bassi and Spencer, 1985).

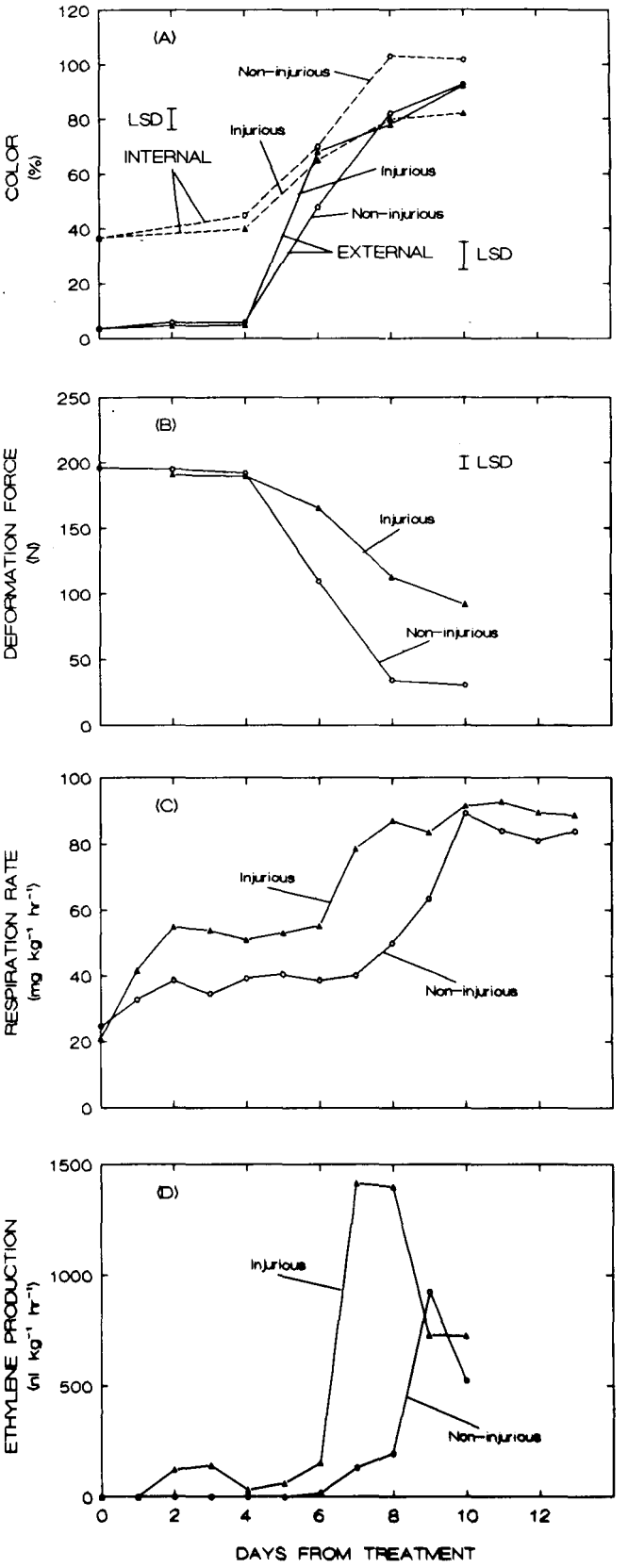

Fig. 1. Effect of an injurious heat treatment (70 min at 49C) (Δ) and a noninjurious treatment (20 min at 49C) (O) on papaya fruit ripened at 25C. All fruit were pretreated for 30 min at 42C. **(A)** External and internal color development. **(B)** Fruit softness. **(C)** Respiration. **(D)** Ethylene production. Vertical bars represent LSD ($P = 0.05$).

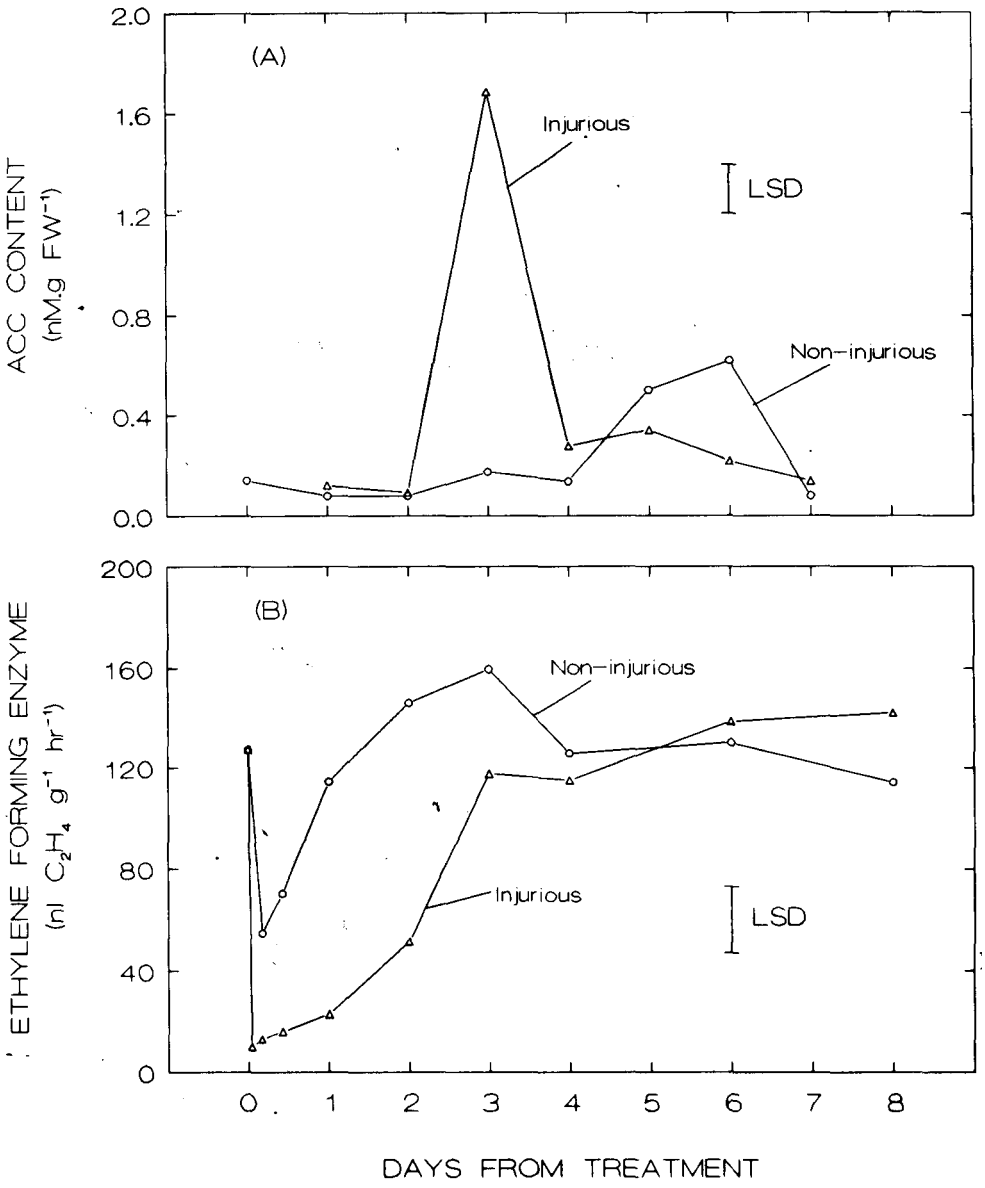

Fig. 2. Effect of an injurious heat treatment (70 min at 49 C) (Δ) and a noninjurious treatment (20 min at 49C) (O) on papaya fruit. **(A)** ACC content. **(B)** Net ethylene-forming enzyme activity during ripening at 25C, the difference in activity with and without additional ACC. Zero-time samples taken just before treatment. Vertical lines represent LSD (P = 0.05).

ACC and ethylene-forming enzyme activity. Content of 1-amino-cyclopropane-l-carboxylic acid (ACC) was determined in equatorial mesocarp tissue that was frozen and ground into powder in liquid N_2. Powdered tissue was extracted with 4 vol of 95% (v/v) ethanol for 20 min at 75C. After centrifugation (12,000 × *g*, 10 min), the pellet was re-extracted as before and the two supernatants combined. The combined extracts were evaporated to dryness in vacuo and redissolved in a known aliquot of water, then washed twice with one-half the volume of chloroform. The ACC content was assayed according to the method of Lizada and Yang (1979).

Activity of the ethylene-forming enzyme (EFE) was determined in vivo by measuring the conversion of exogenously applied ACC to ethylene. Mesocarp discs (5 mm in diameter) were excised using a cork borer. Discs (1 g) were incubated in 2 ml of solution containing 50 mM 2-(N-morpholino)ethanesulfonic acid (Mes) buffer (pH 6.1), 2% sucrose, and 0.1 mg chloramphenicol in the presence and absence of 2 mM ACC in a closed 40-ml vial for 1 hr at 20C with constant shaking. Ethylene production was assayed by injection of 1 ml of head space gas into a gas chromatography (Varian Model 1440, Varian Industries, Downey, Calif.). Net EFE activity was calculated by the difference between assays with and without added ACC.

Extraction of fruit proteins. Problems were experienced in extraction of proteins from papaya mesocarp tissue, as indicated by protein degradation on the gels that are associated with the high level of proteinase and soluble pectins present during ripening (Paull and Chen, 1983). Variously heat-treated papaya fruit with 2% to 10% skin yellowing were used. Extraction of protein was performed according to the method of Schuster and Davies (1983), with the following modification. Mesocarp (5 g) was ground to a powder in liquid N_2 and extracted with 20 ml of extraction buffer (0.7 M sucrose; 0.5 M Tris, pH 7.4; 50 mM EDTA; 0.1 M KCl; lmM phenylmethylsulfonyl fluoride (PMSF); and 1.5 mM dithiopyridine). After 5 min incubation at 2C, an equal volume of water-saturated phenol was added. The phases were separated by centrifugation after 15 min of shaking. The phenol phase was recovered and re-extracted with an equal volume of extraction buffer. The proteins were precipitated from the phenol phase by the addition of 5 vol of methanol with 0.1 M ammonium acetate and held at –20C overnight. The precipitate was washed twice with 0.1 M ammonium in

Table 2. Seasonal changes in fruit response to heat treatments as indicated by internal injury. All fruit had < 10% skin yellowing initially and were treated for 30 min at 42C, and then, within 3 min, were exposed for various durations to 49C. Fruit were then allowed to ripen for 10 days at 25C before evaluation.

Time at 49C (min)	Date of harvest						
	9 July[z] 1985	10 Aug. 1985	20 Sept. 1985	26 Nov. 1985	21 Jan. 1986	11 Mar. 1986	8 April 1986
	Internal injury (scale)						
0	0	0	0	0	0.4	0	0
10	0	---	---	0	0	0	0
20	0	0	0	0	0	0	0
30	0	0	0.1	0	0.2	0	0
40	0	0	0	0.2	1.8	0.1	0
50	1.9	1.1	3.7	3.9	4.0	2.1	2.3
60	3.6	4.1	4.9	4.5	5.0	4.5	5.0
70	3.9	4.9	---	5.0	5.0	5.0	5.0
Significance[y]	L,Q	L,Q	L,Q	L,Q	L,Q	L,Q	L,Q
r^2	0.678***	0.866***	0.799***	0.864***	0.867***	0.908***	0.835***
n	132	96	90	79	80	120	96
Harvest date				***			
Treatment				***			
Harvest date × treatment				***			
n				680			

[z]Internal injury (failure to soften) hard lumps scale described in Table 1.
[y]Coefficient of determination (r^2) for best-fit model. L and Q represent significance of linear (L) or quadratic (Q) components at $P < 0.01$, respectively.
***Significant at $P = 0.001$.

methanol, then twice with acetone. The pellet was resuspended in 1 ml 50 mM Tris buffer, 0.6% $NaDodSO_4$, 5% 2-mercaptoethanol, and 0.1 M PMSF (pH 7.5), dialyzed overnight with three changes of 20 mM ammonium bicarbonate. After centrifugation (12,000 × *g*, 20 min), the supernatant was lyophilized to dryness.

Polysomal RNA isolation and in vitro translation. Mesocarp frozen in liquid N_2 was ground into powder with a mortar and pestle. Polysomal associated RNA was isolated by centrifugation through a sucrose cushion following the method of Spiers et al. (1984). The RNA was translated using ^{35}S-methionine in a nuclease-treated rabbit reticulocyte lysate translation system (New England Nuclear, Boston). A mixture of protease inhibitors (Sigma, St. Louis, Mo.) made of leupeptin (50 µg·ml^{-1}), antipain (20 µg·ml^{-1}), and chymostatin (25 µg·ml^{-1}) was added to the reaction. The translated proteins were precipitated on filter paper with 10% (w/v) trichloroacetic acid (TCA) and washed in boiling 5% (w/v) TCA, followed by cold 5% (w/v) TCA before an ethanol wash and freeze-drying. The proteins were redissolved for electrophoresis as described below.

Electrophoresis and fluorography. The proteins were dissolved in a minimum amount of sample buffer and separated by $NaDodSO_4$-PAGE 1.5-mm-thick 12.5% polyacrylamide slab gels (Laemmli, 1970). The following molecular weight standards were run alongside the extracted proteins: phosphorylase-B, 94 kDa; albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20.1 kDa; and α-lactalbumin, 14.4 kDa. The same amount of protein (25 µg) or counts were loaded in each sample well. Protein content was estimated by the dye-binding method (Bradford, 1976) using the Bio-Rad Coomassie blue reagent (Bio-Rad, Richmond, Calif.). Gels were then stained in a solution containing 0.025% (v/v) Coomassie Blue, 10% acetic acid, and 50% methanol. The gels were destained with 10 acetic acid : 50 methanol : 40 water (by volume). Coomassie-stained and radioactive gels were fixed in 30 methanol : 14 acetic acid : 56 water (by volume) and prepared for fluorography and exposed to preflashed X-ray film at –80C (Spiers et al., 1984). Gels were scanned with a video densitometer (Bio-Rad).

Results

Fruit ripening. Exposure of fruit to 42C for 30 min followed by 49C for up to 70 min permitted development of a normal yellow skin (Table 1); however, fruit heated for 70 min at 49C was slower to develop internal carotenoid color, and internal color development was always less than those for the other treatments (Fig. 1A). Exposure for 40 min or longer at 49C led to the development of hard lumps in the flesh. This manifestation of injury increased in extent as the length of exposure time was increased (Table 1). Softening of fruits treated in this manner was delayed, as indicated by higher deformation force 10 days after treatment (Table 1). Some postharvest softening occurred, as indicated by a decline in deformation force in fruit that was held more than 40 min at 49C (Fig. 1B). However, softening in response to more than 40 min at 49C occurred in both the outer shell and inner zone of the mesocarp near the seed cavity. The inner zone of the mesocarp was found to be the one most frequently and severely affected with hard lumps due to heat-treatment-induced failure of softening. The respiratory climacteric (Fig. 1C) and ethylene production (Fig. 1D) were accelerated by heating for 70 min at 49C.

ACC content and EFE activity. The ACC content of the fruit increased during ripening (Fig. 2A). The level of ACC in the mesocarp in three experiments in which fruit received the injurious heat treatment (70 min at 49C) had a consistently higher peak than the milder treatment (20 min at 49C), and the rise occurred 2 to 3 days earlier. The net EFE activity was rapidly depressed by both heat treatments (Fig. 2B). The activity in the

Table 3. Response of papaya fruit at different stages of ripeness to a potentially injurious heat treatment. All fruit treated for 30 min at 42C, then treated for varying durations held at 49C. Fruit allowed to ripen for 10 days at 25C then evaluated

Time at 49C (min)	Deformation force (N)			Internal injury (scale)		
	Color-break	One-tenth ripe	Quarter-ripe	Color-break	One-tenth ripe	Quarter-ripe
0 (at harvest)	220	217	209	0	0	0
20	33.3	24.5	21.6	0	0	0
40	66.7	64.7	50.9	0	0	0
50	73.6	58.8	78.5	0	0.9	3.7
60	82.4	91.2	151	4.0	4.3	5.0
70	117	149	145	4.8	5.0	5.0
Significance[y]	L,C	L,Q,C	L,C	L,Q,C	L,Q	L,Q,C
r^2	0.677***	0.710***	0.767***	0.873***	0.861***	0.861***
n	75	75	75	75	75	75
Heat treatment		***			***	
Stage ripeness		***			***	
Stage ripeness × treatment		***			NS	
n		225				

[z]Internal injury (failure to soften) hard lumps scale described in Table 1.
[y]Coefficient of determination (r^2) for best-fit model. L, Q, and C represent significance of linear (L), quadratic (Q), or cubic (C) components at $P < 0.01$, respectively. Values at harvest not included in analysis.
[NS, ***]Nonsignificant or significant at $P=0.001$, respectively.

injured fruit was ≈ 10% of the original activity 4 hr after treatment, whereas that of the noninjured fuit had declined to 40% of the original. Net EFE activity returned to original levels within 2 days following the noninjurious heat treatment and within 3 days for fruit subjected to the injurious heat treatment. The nonheated control showed no change in net EFE activity (data not shown).

Seasonal variation and stage of fruit ripeness. The sensitivity of fruit to the injurious treatment was greatest during the winter (Table 2). During January, exposure to >27 min at 49C was sufficient to induce areas in the mesocarp that failed to soften, whereas up to 45 min was required during April, July, August, and September.

The stage of fruit ripeness at the time of heat treatment, as judged by the degree of skin yellowing, had a significant influence on the extent of the flesh that failed to soften, as shown by higher deformation force and the extent of hard lumps (Table 3). Quarter-ripe fruit at the time of treatment developed extensive hard lumps in the inner mesocarp after >40 min at 49C. Color-break fruit (<2% yellow skin) developed areas in the mesocarp that failed to soften after >50 min exposure to 49C.

Pretreatment and sensitivity. Increasing the exposure of the fruit to the noninjurious 42C from 30 min to 4 hr before holding it 20 min (control) or an injurious 70 min at 49C decreased the occurrence of hard lumps in the mesocarp and lowered deformation force (Table 4). There were no hard areas in the fruit mesocarp treated for >2 hr at 42C. In other experiments, hard lumps occurred after 8-hr pretreatment at 42C (data not shown).

Pretreating fruit for 1 hr at 42C before holding it 3 hr at 22C reduced the proportion of the mesocarp that failed to soften and resulted in softer fruit following the injurious heat treatment of 42C for 30 min plus 70 min at 49C relative to shorter or longer exposures (Table 5). Pretreating the fruit for 1 hr at 35C, then allowing the fruit to stand for 3 hr at 22C, was sufficient to induce some heat tolerance (Fig. 3). Exposure to 40C almost completely prevented internal injury. The best interval of incubation at 22C was 3 to 4 hr (data not presented). With pretreatment at 38C, a 1- to 2-hr interval at 22C was also required for maximum reduction in damage. However, increasing the pretreatment time at 42C to 2 hr or longer followed by 3 hr at 22C resulted in more hard lumps and higher deformation forces in fruit whether heat-treated at the injurious of noninjurious level.

Induction of unique proteins. A 2-hr incubation at 38C resulted in quantitative changes in at least 13 polypeptides separated on one-dimensional NaDodSO$_4$–PAGE slab gels (Fig. 4). The apparent molecular weights of the seven polypeptides that declined were 117, 38, 25, 24, 22, 16, and 14 kDa after a further 2 hr at 22C, following the end of the induction. Four polypeptides, with molecular weights of 70, 30, 20, and 19 kDa, increased in density of Coomassie staining. These polypeptides did not accumulate in the control. Twenty-four hours after heat treatment, small increases occurred in two polypeptides at 30 and 27 kDa.

Translated polysomal RNA extract from fruit showing < 10% skin yellowing showed an increase in at least three RNAs following exposure to 38C for 2 hr (Fig. 5). The proteins had apparent molecular weights of 17, 18, and 70 kDa. These proteins were present at low levels in fruit harvested in July of the non-heat-treated controls (data not shown). The increase in these proteins occurred by the end of the 2-hr heat treatments at 38C, reaching a peak 2 hr after treatment. The 17- and 18-kDa proteins declined more rapidly than the 70-kDa protein. These two low-molecular-weight proteins disappeared 24 hr after the treatments, while the large molecular weight protein was still visible. Three additional translated RNAs decreased in translation ability within 4 hr of treatment, but recovered within 24 hr. Other polypeptides continued to be translated during and after the 2-hr heat treatment at 38C.

Discussion

The synthesis of ripening-specific mRNAs and new enzymes is induced during ripening (Tucker and Grierson, 1982; Grierson et al., 1985; Christoffersen et al., 1984). Exposure of pears

Table 4. Effect of pretreating color-break papaya at 42C on fruit failure to soften after an injurious heat treatment. The fruit was treated for varying times at 42C, followed by either 20 min (control) or 70 min (injurious) at 49C. Fruit allowed to ripen at 25C.

Time at 42C (hr)	Deformation force (N)		Internal injury (scale)[z]	
	Control	Injurious heat	Control	Injurious heat
0.5	31.4	99.0	0	4.6
1.0	23.5	84.3	0	4.8
2.0	49.0	63.7	0.3	3.2
4.0	54.9	55.9	0	0
6.0	54.9	47.1	0	0
Significance[y]	L	L	NS	L
r^2	0.363***	0.479***	---	0.664**
n	75	76	75	76
Heat treatment	***		***	
Length of treatment	NS		***	
Heat × length	***		***	
n	151		151	

[z]Internal injury (failure to soften) hard lumps scale described in Table 1.
[y]Coefficient of *determination*(r^2) for best-fit model. L represents significance of linear components at $P < 0.01$.
NS,**,*** Nonsignificant or significant at $P = 0.01$, or 0.001, respectively.

Table 5. Effect of pretreatment duration at 42C on failure of fruit to soften after heat treatment for 20 or 70 min. Pretreatments at 42C were followed by 3 hr at 22C. Following exposure to 22C, fruit were held for 30 min at 42C, followed within 3 min by either 20 min (control) or 70 min (injurious) at 49C.

Length of pretreatment (hr)	Heat treatment			
	20 min	70 min	20 min	70 min
	Deformation force (n)		Internal injury (scale)[z]	
0	46.0	168	0	5.0
0.5	25.5	69.6	0	4.9
1.0	30.4	51.9	0.3	4.0
2.0	41.2	78.5	0.5	4.8
3.0	121	162	5.0	5.0
Significance[y]	L,Q	Q	L,Q,C	Q
r^2	0.728***	0.844***	0.891***	0.157***
n	75	73	74	72
Heat treatment	***		***	
Length of pretreatment	***		***	
Heat × length	*		***	

[z]Internal injury (failure to soften) hard lumps scale described in Table 1.
[y]Coefficient of determination (r^2) for best-fit model. L, Q, and C represent significance of linear (L), quadratic (Q), or cubic (C) components at $P<0.01$, respectively.
*,**,***Significant at $P=0.05$, 0.01, or 0.001, respectively.

(Maxie et al., 1974) and tomato fruit (Ogura et al., 1976; Yoshida et al., 1984; Picton and Grierson, 1988; Biggs et al., 1988) to temperatures > 30C for 24 hr or more resulted in disruption of various aspects of ripening. Respiration rate and ethylene synthesis are also affected by exposure to high temperartures (Maxie et al., 1974; Ogura et al., 1976). Twelve days or more at 33C resulted in the suppression of respiration, ethylene synthesis, and fruit softening, and this action was not completely reversed after returning the fruit to ambient temperatures (Yoshida et al., 1984; Ogura et al., 1976). The inhibition of ripening above 30C has been ascribed to disruption of protein-related to ethylene synthesis (Ogura et al., 1975), of the polygalacturonase regulatory mechanism that controls the generation of activation of the enzyme (Chan et al., 1981), and to suppression of ripening-related mRNA synthesis (Picton and Grierson, 1988).

The exposure of papaya fruit to high temperatures for <90 min resulted in the disruption of softening (Fig. 1B, Table 1). The pattern of other ripening-related events, such as the change in skin color (Fig. 1A), climacteric respiration (Fig. 1C), ethylene production (Fig. 1D), ACC content (Fig. 2A), net EFE activity (Fig. 2B), and internal carotenoid synthesis (Fig. 1A) were also altered by the high-temperature treatment. These data contrast with those reported for the effects of extended periods of exposure to high temperatures on the suppression of the ripening respiratory climacteric, ethylene production, and softening of tomatoes and pears (Maxie et al., 1974; Ogura et al., 1976; Yoshida et al., 1984).

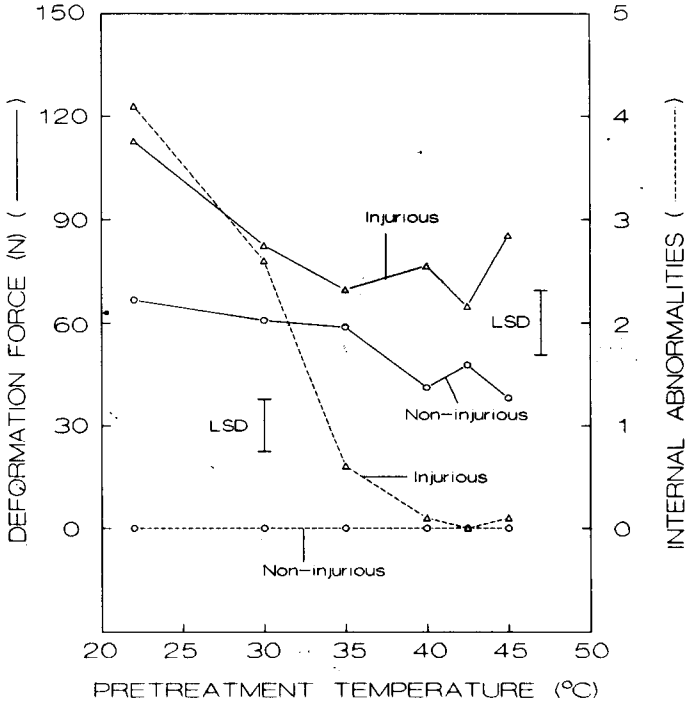

Fig.3. Effect of different pretreatment temperatures for l hr followed by 3 hr at 22C on fruit failure to soften following a noninjurious 20 min at 49C (O) or an injurious 70 min at 49C (Δ) expressed as deformation force (fruit softness) (—) and internal injury (----) after 10 days of ripening at 25C. Vertical bars represent LSD (P = 0.05).

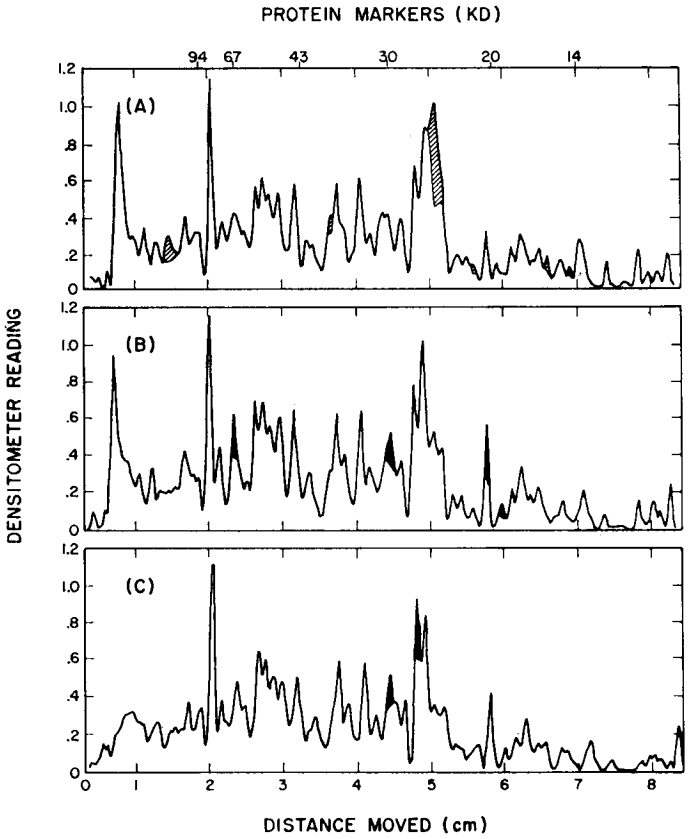

Fig. 4. Densitometer scans of proteins extracted from papaya fruit mesocarp and separated by NaDod SO_4–PAGE. **(A)** Control, no heat treatment. **(B)** Two hours after exposure to 38C for 2 hr. **(C)** Twenty-four hours at 22C after exposure to 38C for 2 hr. Hatched areas indicate protein bands that decline following exposure to 38C for 2 hr. Proteins that appear or accumulate are shown in black. Numbers at top show molecular weight standard protein position.

An ACC peak occurred sooner and was higher in fruit subjected to the injurious heat treatment (Fig. 2A). This peak consistently occurred in all experiments and coincides with a small peak in ethylene production 3 days after an injurious heat treatment (Fig. 1D). The rapid loss of EFE activity (Fig. 2B) in papaya fruit subjected to similar heat treatments has been previously reported (Chan, 1986); however, full recovery of EFE occurred within 3 days from the injurious heat treatment (Fig. 2B). Thus, it is unlikely that the disruption of ethylene synthesis was the cause of the failure to soften. This assumption is supported by the ability of tomato fruit to recover ethylene synthesis following long exposures to high temperature (Ogura et al., 1976; Biggs et al., 1988). The recovery of ethylene production ability possibly requires protein synthesis (Biggs et al., 1988), suggesting low-level production of ethylene-related mRNAs (Picton and Grierson, 1988) or reactivation of pre-existing proteins. Wound-induced ethylene synthesis is less sensitive than climacteric-related ethylene synthesis to heat stress inactivation (Biggs et al., 1988).

The disruption of papaya softening following heat stress (Figs. 1B and 3) was not recovered following return to ambient conditions. Chan et al. (1981) observed that papaya fruit with 25% or 50% of the skin yellow had less polygalacturonase than fruit at the color-break stage after 65 min of exposure to 46C. This result could be due to either denaturation or disruption of polygalacturonase synthesis. Because polygalacturonase shows first-order heat denaturation kinetics and is 60% denatured by 20 min at 70C (Chan and Tam, 1982), exposure of fruit to 49C would induce minimal denaturation. This failure to soften possibly is attributable to suppression of the mRNA for wall-softening enzymes, as found for tomatoes (Picton and Grierson, 1988). The loss of polygalacturonase mRNA in tomatoes is not overcome by exogenous ethylene (Picton and Grierson, 1988), but polygalacturonase activity does return following after a 6-day lag when returned to 25C (Yoshida et al., 1984).

The failure of papaya to recover the ability to soften could be related to its unique aspect, by which the wall-degrading enzymes polygalacturonase and xylanase have a peak activity when the fruit is 40% to 60% yellow (Paull and Chen, 1983). This relationship suggests that mRNA for polygalacturonase and xylanase in papaya are produced for only a short period during one stage of ripening. Softening of outer mesocarp tissue with failure of the inner mesocarp to soften supports the above conclusion because ripening proceeds from the inner mesocarp outward. The inner mesocarp exhibited noticeable carotenoid development when the skin was < 10% yellow, while the outer mesocarp showing no carotenoids (Paull and Chen, 1983). This suggests that the inner mesocarp was at a more-advanced stage of ripening; this stage may be more susceptible to heat stress than the less-mature outer region. This conclusion was supported by the greater damage to riper fruit when exposed to injurious temperature (Table 3). This interpretation would suggest that the inner mesocarp received an injurious temperature treatment that ultimately disrupted softening, while the outer mesocarp, even though it reached a higher temperature, was not injured because it was not at a sensitive stage.

The disruption of fruit softening by the injurious heat treatment (70 min at 49C) was reduced or prevented by a pretreat-

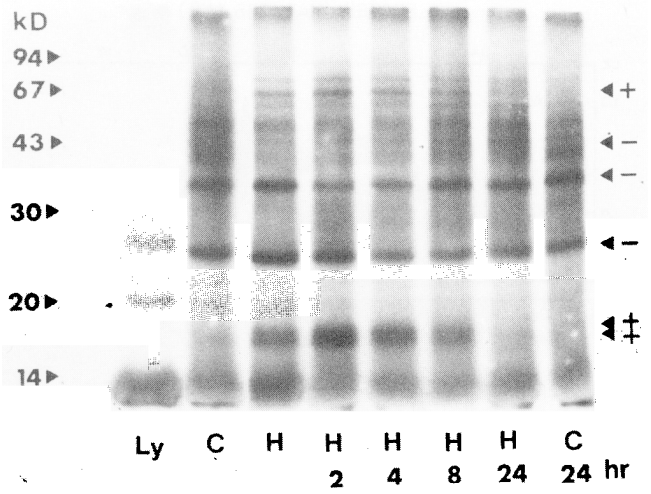

Fig. 5. Fluorogram of $NaDodSO_4$–PAGE slab gel of translated polysomal RNA proteins from fruit mesocarp at various times at room temperature after 2 hr at 38C. Lane 1, lysate; lane 2, control, no treatment; lane 3, immediately following 2 hr at 38C; lanes 4–7—2, 4, 8 and 24 hr after heat treatment; lane 8, no heat treatment 24 hr later. + Indicates an increase in staining; –, a decrease.

ment of 4 hr at 42C (Table 4) or a 1-hr pretreatment at temperatures > 35C (Fig. 3, Table 5), followed by 3 hr at 22C. The extent of protection of softening disruption was markedly dependent on season (Table 2), stage of fruit ripeness at exposure (Table 3), and pretreatment time (Tables 4 and 5) and temperature (Fig. 3). This seasonal variation (Table 2) probably explains the complete protection shown in respect to changes in deformation (Fig. 3), with limited protection found in the other experiment reported (Table 5). This seasonal variation could be due to presence of field-induced thermotolerance. Field induction of heat shock proteins has also been shown for cotton (Burke et al., 1985), soybean (Kimpel and Keys, 1985), and sorghum (Ougham and Stoddart, 1986).

The induced physiological thermotolerance has been associated with heat shock proteins (Key et al., 1985). Papaya fruit protein synthesis changed following exposure to 38C for 2 hr (Fig. 4). Comparison of polypeptides on $NaDodSO_4$-PAGE slab gels revealed at least four polypeptides with increased staining. The quantities of seven polypeptides decreased as the incubation time at 22C increased following 2 hr of exposure to 38C, whereas those of four others increased. No accumulation was seen in the control held at 22C for 10 hr. Translated polysomal RNA confirmed that new polypeptides were synthesized following heat shock induction. (Fig. 5). Continued increases in the apparent amount of stainable polypeptides occurred with increasing preincubation at 42C, while thermotolerance decreased (Table 5) under the same conditions. These results suggest that treatment at 42C was at the limit for induction of tolerance and that continued exposure, though still allowing HSP–polypeptide synthesis, was also causing damage. These results are in agreement with those for tomato fruit treated for long periods at 35C (Picton and Grierson, 1988). Translated mRNA from papaya fruit harvested in July showed that these new polypeptides were already present, indicating that summer field temperatures were adequate to induce this response.

The sensitivity of wall-degrading enzymes in papaya reported here was possible due to a suppression of mRNA synthesis for these enzymes, during and just after exposure to heat treatment, as suggested by Picton and Grierson (1988) for tomatoes. The selective failure of papaya to soften after this heat disruption suggests that, in papaya mRNA(s), for this or more enzymes was produced only during a short period of ripening. The suggestion that synthesis of secreted proteins are selectively disrupted by heat shock (Sachs and Ho, 1986) suggests an explanation for the heat disruption of only softening-related enzymes. Heat treatment could also be a useful probe for studying control of papaya softening.

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