Plant Physiol. (1991) 95, 921–927
0032-0889/91/95/0921/07/$01.00/0

Received for publication June 26, 1990
Accepted September 6, 1990

The Effect of Ethylene and Propylene Pulses on Respiration, Ripening Advancement, Ethylene-Forming Enzyme, and 1-Aminocyclopropane-1-carboxylic Acid Synthase Activity in Avocado Fruit[1,2]

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ABSTRACT

When early-season avocado fruit (*Persea americana* Mill. cv Hass) were treated with ethylene or propylene for 24 hours immediately on picking, the time to the onset of the respiratory climacteric, *i.e.* the lag period, remained unchanged compared with that in untreated fruit. When fruit were pulsed 24 hours after picking, on the other hand, the lag period was shortened. In both cases, however, a 24 hour ethylene or propylene pulse induced a transient increase in respiration, called the pulse-peak, unaccompanied by ethylene production (IL Eaks [1980] Am Soc Hortic Sci 105: 744–747). The pulse also caused a sharp rise in ethylene-forming enzyme activity in both cases, without any increase in the low level of 1-aminocyclopropane-1-carboxylic acid synthase activity. Thus, the shortening of the lag period by an ethylene pulse is not due to an effect of ethylene on either of the two key enzymes in ethylene biosynthesis. A comparison of two-dimensional polyacrylamide gel electrophoresis polypeptide profiles of *in vitro* translation products of poly(A$^+$) mRNA from control and ethylene-pulsed fruit showed both up- and down-regulation in response to ethylene pulsing of a number of genes expressed during the ripening syndrome. It is proposed that the pulse-peak or its underlying events reflect an intrinsic element in the ripening process that in late-season or continuously ethylene-treated fruit may be subsumed in the overall climacteric response. A computerized system that allows continuous readout of multiple samples has established that the continued presentation of exogenous ethylene or propylene to preclimacteric fruit elicits a dual respiration response comprising the merged pulse-peak and climacteric peak in series. The sequential removal of cores from a single fruit has proven an unsatisfactory sampling procedure inasmuch as coring induces wound ethylene, evokes a positive respiration response, and advances ripening.

The avocado is a climacteric fruit, wherein ethylene plays a key role in ripening (7). In several varieties of avocado (*e.g.* cv Hass and Fuerte), fruit fail to ripen on the tree after the fruit are mature and picking initiates the ripening process (24). Failure to ripen on the tree has been attributed to a putative inhibitory tree factor (2, 24). The endogenous ripening syndrome comprises an extended period of low, steady respiration with insignificant ethylene production, the so-called lag phase, followed by a sharp increase in respiration, the climacteric, accompanied by extensive ethylene production and other ripening phenomena including color change, aroma and flavor development, and softening. Whereas the interval from the onset of the climacteric rise to its peak is essentially constant (about 3 d), the lag period varies among individual fruit (12), as well as with season (13) and external ethylene concentration (5). The lag period plays an ethylene-dependent role in the onset of the climacteric even though ethylene production during the lag period may be low (3, 8). Thus, on the one hand the initiation of ripening is prevented when internal ethylene levels are lowered by holding fruit under hypobaric conditions at an oxygen concentration equal to that in air (10), while on the other, ripening is advanced when fruit, *viz.* bananas, are treated with ethylene at concentrations too low to evoke an overt response (20).

After recognition of the crucial role of endogenous ethylene in ripening (1, 8) there followed studies on the influence of exogenous ethylene on ripening initiation. As noted by Biale (5), ethylene administered continuously at high concentrations (*e.g.* 10 μL/L) evokes an immediate climacteric response in avocado, while lower concentrations shorten the lag period to a lesser extent. By contrast, ethylene pulses of 6 to 24 h promptly elicit a transitory respiration peak (which we have termed the pulse-peak) with respiration rates that approach those of the true climacteric peak (12, 13), before returning to low lag-phase rates. Nevertheless, pulses advance the time to the true climacteric (12). In a later study Eaks (13), by pulsing with propylene in place of ethylene (see ref. 18), was able to determine the effect of pulsing on endogenous ethylene formation. In contrast to the respiratory climacteric, which is accompanied by a spate of ethylene production, no ethylene is produced in conjunction with the pulse-peak (13). Whereas it has been reported that the lag period is not shortened by an ethylene pulse in freshly picked fruit (14), a contrary view has been presented (2, 28, 29) that stresses fruit maturity as a factor in the response of freshly harvested fruit (2). The triggering of the climacteric presumably follows upon a developmental increase in tissue sensitivity, or responsiveness, to ethylene that occurs during the lag period (25) (*cf.* ref. 27).

[1] Supported in part by a grant from the National Science Foundation and in part by the University of California Los Angeles Academic Senate Committee on Research. D. A. S. was supported in part by a U.S. Department of Agriculture National Needs Fellowship.

[2] In memory of Jacob Biale: dear friend, esteemed colleague, devoted plant physiologist.

In endogenous ripening, responsiveness increases with fruit maturity, with a corresponding shortening of the lag period (6). By the same token the climacteric peak draws closer to the pulse-peak the later the season (13).

The integral role played by endogenous ethylene in the lag period and climacteric, as well as the effects of exogenous ethylene, suggest that ethylene biosynthesis itself may be important in determining the lag period length. ACC[3] synthase and EFE are the key enzymes in the ethylene biosynthetic pathway (27), with ACC synthase activity the apparent rate limiting step (22). Whereas EFE activity has been shown to be induced by an ethylene pulse (22), in normal ripening the activity of both enzymes remains at relatively low levels until the onset of climacteric ethylene production (15). Because both pulsing and wounding (*i.e.* slicing [4, 23]) accelerate the ripening process, those preclimacteric events that advance ripening in response to a pulse or wounding are of interest. Accordingly, we have investigated the effect of ethylene pulsing on shortening the lag period both with respect to the time of pulse application and the consequence of pulsing on the expression of the two key enzymes of ethylene biosynthesis, *viz.* ACC synthase and EFE. Further, we have examined the influence of an ethylene pulse on the polypeptide profile of poly(A^+) mRNA *in vitro* translation products during the course of ripening, compared with profile characteristics attending endogenous ripening.

MATERIALS AND METHODS

Plant Material

Avocado fruits (*Persea americana* Mill. cv Hass) were harvested from the University of California South Coast Field Station between 10 and 12 AM, or from an orchard in Carpenteria, CA, between 11 AM and 1 PM. Within 2 to 3 h, single fruit were placed in 500 mL leakproof plastic canisters (Tupperware), fitted with input and output ports, or three to six fruit in 4 L jars kept at 20°C in a constant temperature incubator. The fruit were treated with a continuous 30 mL/min flow of water-saturated air, ethylene at 10 μL/L in air, or propylene at 500 μL/L in air. A system of control valves allowed for easy switching of gas mixtures during the course of an experiment.

Respiration and Ethylene Determinations

Respiration was measured by monitoring CO_2 production. Outlet ports from canisters or jars were connected to an IR gas analyzer (Anarad, model AR500) through a system of solenoid-activated switches controlled by an IBM PC equipped with a customized software program (Sable Systems, Los Angeles, CA) designed to control sampling and record the rate of CO_2 evolution. Each fruit container was sampled once every hour by switching its out-flow to the gas analyzer, purging the gas analyzer with out-flow gas for 3.5 min, and then monitoring CO_2 levels for 10 s. During each 10 s, thousands of individual readings of total CO_2 concentration are recorded and automatically computed as an average value. The latter is converted to respiratory CO_2 by subtracting a constantly calculated baseline value derived from a gas stream passing through a container without fruit. The computer expresses the final value in μL CO_2 per gram fresh weight per hour and depicts on the screen the course of respiration for each fruit throughout the entire ripening period.

Ethylene production was measured by manually withdrawing a 10 mL sample of gas from each outlet port and injecting it into a back-flush fitted GC (Hach Carle 04254-C) with a column at 70°C packed with specially modified packing material for ethylene determination (Hach Carle application 254-C). Sampling periodicity depended on the experiment. To minimize the effect of fruit variability, many fruit were used for each treatment, and experiments were repeated.

Coring

A 14 mm diameter cork borer was used to punch cores from the skin down to the seed at points around the equatorial region of the fruit. One to four cores per fruit were removed at specific time points. Cores were sliced into smaller pieces and immediately frozen in liquid N_2. The holes were filled with lanolin and the fruit returned immediately to respiration monitoring chambers.

EFE and ACC Synthase Assays

For every EFE time point one fruit was removed from its container after measuring ethylene production, and cut longitudinally into slices 4 mm thick with a slicer comprising an adjustable microtome knife fixed into an appropriate bed. Slices were punched with a No. 9 cork borer yielding discs 14 mm in diameter. Discs were randomized, quickly rinsed five times with distilled water, and blotted dry. Fifteen discs (*ca.* 7.25 g fresh weight) were placed on each of two circular stainless steel screens, each positioned on a 3 inch square piece of 3MM filter paper wet with 4 mL of water in 25 mm × 140 mm Petri dishes. A serum cap was inserted into a hole drilled in the lid. A 25 μL droplet of 100 mM ACC in water was applied to each disc in the experimental dish; a 25 μL droplet of deionized H_2O was applied to each of the control discs. Dishes were sealed with Parafilm and incubated at 20°C. After a period of time, usually 2 h, 5 mL samples of gas were withdrawn through the serum cap with a syringe and injected into the GC. An amount of 50 mM ACC in a 25 μL droplet was previously shown to yield maximal rates of EFE for tissue with the greatest activity.

EFE activity was calculated from the rate of ethylene evolution by discs treated with ACC. Ethylene produced in the absence of exogenous ACC was considered to reflect ACC synthase activity, because the addition of ACC sharply increased the rate of ethylene production. Furthermore, inhibition of ethylene production by untreated (*i.e.* water-treated) discs from ripe fruit by aminoethoxyvinyl glycine, an inhibitor of ACC synthase, confirmed this assumption. The possibility that ACC synthase activity might be concealed by sequestration of ACC into 1-(malonylamino)cyclopropane-1-carboxylic acid was considered negligible because 1-(malonylamino)cyclopropane-1-carboxylic acid levels remained low and fairly constant throughout the lag period (data not shown).

[3] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; EFE, ethylene-forming enzyme.

These experiments were repeated two to three times, multiple fruits were assayed per time point, and many time points were assayed per time course to minimize the consequences of fruit variability.

RNA Isolation and Two-Dimensional Gel Analysis

Total RNA was isolated by the guanidinium isothiocyanate method (11). Fifteen grams of tissue were mixed with dry ice and pulverized in an electric coffee grinder (Krups), then placed at −70°C for 48 h to allow the CO_2 to sublime. The tissue powder was homogenized in guanidinium buffer (11) in a mortar and pestle, filtered, and the homogenate centrifuged 10 min at 12,000*g* in an SS-34 rotor (Beckman) to separate out cellular debris and the oils commonly found in avocados. The oil-free supernatant was layered over a 2.5 mL pad of 5.7 M CsCl and centrifuged 20 h in an SW-41 rotor (Beckman) at 35,000 rpm (average, 150,000*g*). The resulting pellet (total RNA) was resuspended in water and precipitated with 0.1 volume 3 M sodium acetate and 2.5 volumes ethanol at −20°C.

Poly(A^+) mRNA purification was accomplished essentially as described by Tucker and Laties (26). The total RNA ethanol precipitate was resuspended in elution buffer (10 mM Tris-HCl, pH 7.6; 1 mM EDTA; 0.1% SDS), heated to 70°C for 3 min, rapidly cooled to room temperature, and 0.1 volume of 5 M NaCl added. As an additional step, the suspension was then passed through a cellulose column equilibrated with binding buffer (0.5 M NaCl in elution buffer) to remove contaminating polysaccharides. The effluent was passed twice by gravity flow through an oligo-dT column equilibrated with binding buffer. Poly(A^+) mRNA was eluted from the column with elution buffer, and precipitated in 0.1 volume 3 M sodium acetate and 2.5 volumes ethanol at −20°C. The poly(A^+) mRNA pellet was resuspended in water, aliquoted into 0.5 mL Eppendorf tubes, and stored at −70°C.

Poly(A^+) mRNA was translated in a wheat germ extract in the presence of [^{35}S]methionine (21). Two-dimensional gel analysis was done essentially according to O'Farrell (19) except that 10 mM iminodiacetic acid was used as the anode chamber buffer and 10 mM ethylene diamine as the cathode chamber buffer. Gels were infiltrated with 2,5-diphenyloxazole, dried, and exposed to Kodak XAR-5 x-ray film at −70°C. Relative intensities of spots were compared from gels representing the different time points of the time course. Four spots of different intensities which remained constant throughout the time course were used as standards for comparison. The spots of interest were compared with the standards at each time point, and the relative intensities estimated.

Materials

High purity ACC was purchased from Sigma; 99.9% pure ethylene and propylene used to make gas mixtures were purchased from Scott Specialty Gases; compressed air was purchased from Liquid Air Corporation.

RESULTS

Detailed Respiration Profiles

The respiration profiles shown in Figure 1, A–C, represent control, propylene-pulsed, and continuously propylene-treated mid-season fruit (April). The curves described by hourly measurements confirm earlier observations made by Eaks (13) with twice daily measurements, and Zauberman and Fuchs (28) with daily, discontinuous measurements, while exhibiting finer detail. The climacteric ethylene production profiles of Figure 1, A and B, confirm earlier results (3, 7, 13, 16). An exogenous propylene pulse elicits a typical respiratory pulse-peak (Fig. 1B)—without concomitant ethylene production—which falls to air control levels after the pulse is discontinued (an ethylene pulse elicits a similar response). All fruit in the following experiments exhibited a pulse-peak when pulsed with ethylene or propylene. Fruit treated continuously with propylene exhibit two respiration peaks: the pulse and climacteric peaks, respectively, with an elevated level of respiration in between (Fig. 1C). The transient pulse-peak is induced by the exogenous propylene treatment, while the climacteric rise reflects a heightened response to propylene (or ethylene) due to a change in tissue sensitivity, or responsiveness (25, 27).

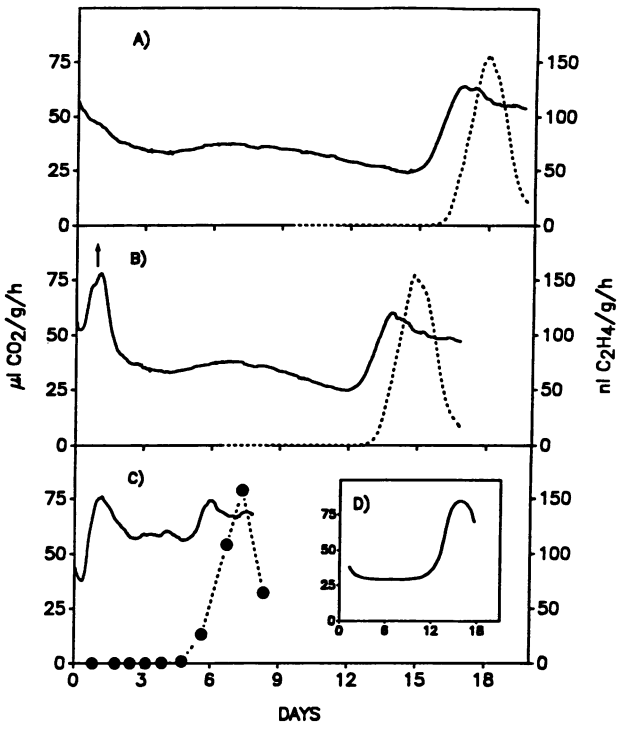

Figure 1. Respiration and ethylene production of propylene-treated Hass avocado fruit. CO_2 production (——) and ethylene production (- - - - -, ●-----●) of (A) control (*i.e.* endogenous ripening); (B) propylene pulsed; and (C) continuously propylene treated mid-season avocado fruit. The 24 h pulse was applied at $T = 0$ and discontinued at $T = 24$ h (arrow). CO_2 production was monitored every hour. Ethylene production was monitored daily until it became measurable, at which time it was measured every hour, except in (C) where it was measured every 18 to 24 h. Ethylene values which were zero or unmeasureable are not shown. (D) An idealized respiration profile of endogenous ripening (from ref. 7) is shown for comparison.

Pulse Effects on Ripening Time

Early-season fruit (Oct.–Jan., 6–9 months from anthesis) respond to a 24 h ethylene pulse applied immediately after

harvest ($T = 0$) with a typical pulse-peak with no change in the length of the lag period (Table I). By contrast, a 24 h pulse given 1 d after picking ($T = 24$) shortens the lag by an average of 4.5 d out of the normal 15 d lag period, while a 48 h pulse given at $T = 24$ shortens the lag even more (Table I). On the other hand, a 48 h pulse applied at $T = 0$ has the same effect as a 24 h pulse given at $T = 24$.

With mid-season fruit (Feb.–May, 10–13 months from anthesis), a 24 h pulse applied at $T = 0$ shortens the lag period by 1 out of 13 d, well less than the 4.5 d shortening effected by a 24 h pulse applied at $T = 24$. A 24 h pulse given to late-season fruit (June–Sept., 14–17 months from anthesis) at either $T = 0$ or $T = 24$ shortens the lag period by roughly the same time, *viz.* 4 to 5.5 out of 11 d. Ethylene applied continuously reduces the lag to an estimated 3 d and keeps the respiratory pulse-peak from dropping back to control levels (Fig. 1C). Eventually climacteric ethylene production is induced, together with the development of a respiratory climacteric.

Coring Effects

Avocado fruit to some degree display an inherent variability in their ripening behavior. Time-course studies of enzymatic activities or changes in mRNA populations would best be done on tissue taken repeatedly from the same fruit. To this end we have investigated the feasibility of obtaining tissue repeatedly from the same fruit by coring. The respiration of cored fruit exhibits a double respiratory peak, following which respiration returns to control levels after 72 h. This phenomenon is repeated with each subsequent coring event (Fig. 2B, *cf.* 2A). A small wound-ethylene response underlies the modest coring-induced respiration rise. Early-season cored fruit reach a climacteric peak an average of 2 d sooner than control fruit (Table I) and in some cases the coring response leads directly to a climacteric rise (Fig. 2C). Fruit that were simultaneously pulsed (24 h) and cored at $T = 24$ show the bimodal respiration response (data not shown), with a lag period the same as in pulsed controls, and shorter than in control fruit. Because coring does affect ripening behavior—specifically respiration, ethylene production, and length of the lag period—it does not provide an accurate representation of normal ripening, and accordingly is an unacceptable technique.

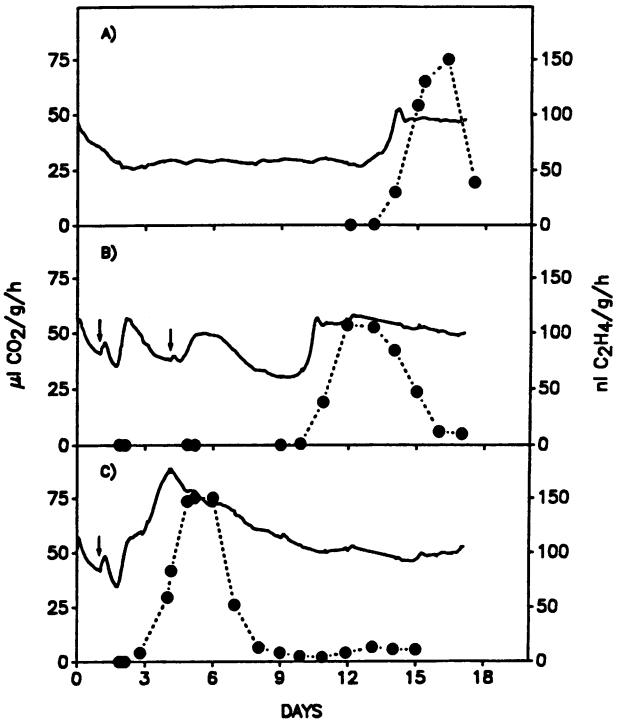

Figure 2. Respiration and ethylene production of cored Hass avocado fruit. CO_2 production (——) and ethylene production (●----●) of (A) control and (B, C) cored mid-season avocado fruit. CO_2 production was measured every hour. Ethylene production was monitored at daily intervals, except following coring, where it was measured every 6 h for 48 h. Ethylene values which were zero or unmeasureable are not shown. Two cores were taken at d 1, and 1 core at d 4 (arrows) as described in the text.

Table I. *Effect of Ethylene Pulses and Coring on Length of Lag Period in Hass Avocado Fruit*

$T = 0$ and $T = 24$ represent hours from harvest and indicate the point of application of the pulse. All values are averages of four to six fruit.

Treatment	Time to Climacteric Peak	Lag Shortening
	d	
November		
$T = 0$, 24 h pulse	15	0
$T = 24$, 24 h pulse	11.5	4.5
$T = 0$, 48 h pulse	11.5	4.5
$T = 24$, 48 h pulse	9	6
Cored	13	2
Control	15	
March		
$T = 0$, 24 h pulse	12	1
$T = 24$, 24 h pulse	8.5	4.5
Control	13	
July		
$T = 0$, 24 h pulse	7	4
$T = 24$, 24 h pulse	5.5	5.5
Control	11	

Pulse Effect on EFE and ACC Synthase Activity

In response to a 24 h propylene pulse EFE activity rises to 90 nL C_2H_4 g^{-1} h^{-1} compared with the 2 nL C_2H_4 g^{-1} h^{-1} of control fruit (Fig. 3, A–B). EFE activity rises to roughly the same levels whether the pulse is applied at $T = 0$ or $T = 24$ (Table II). After the pulse is removed EFE activity falls off and continues to decrease for 6 to 7 d, reaching a level slightly higher than that in control fruit before burgeoning in conjunction with the climacteric. EFE activity is induced to the same level with the same kinetics with pulses as short as 8 h (data not shown). ACC synthase activity is little affected by the pulse, and remains essentially nil, increasing only at the onset of autocatalytic ethylene production, as in control fruit. EFE activity in control fruit increases gradually through the course of the lag period (from 1–2 to 3–8 nL C_2H_4 g^{-1} h^{-1}), and then sharply at the onset of the ethylene climacteric.

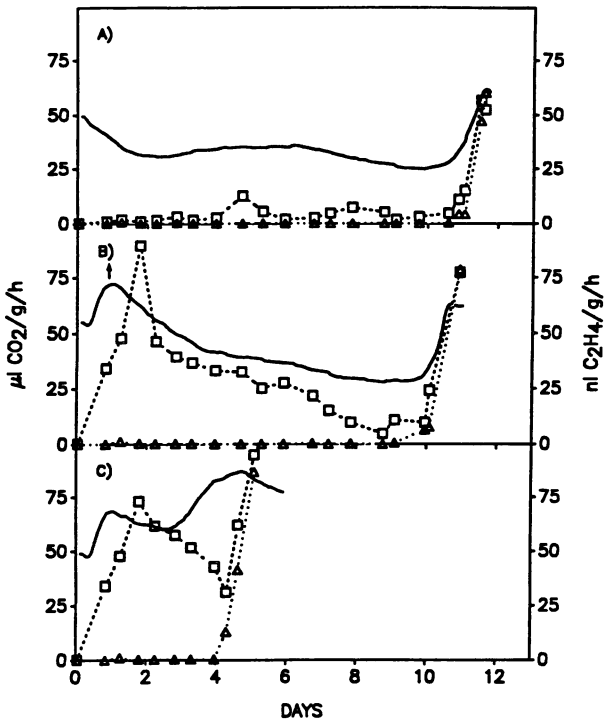

Figure 3. Respiration and EFE activity of propylene-treated Hass avocado fruit. CO_2 production (——), EFE activity (□——□), and ACC synthase activity (△· · · · · △) of (A) control; (B) propylene pulsed; and (C) continuously propylene treated mid-season avocado fruit. The 24 h propylene pulse was applied at $T = 0$ and discontinued at $T = 24$ hours (arrow). CO_2 production was measured every hour. EFE and ACC synthase activities were assayed twice daily. Ethylene production by intact fruit (immediately prior to harvesting for assays) roughly matched ACC synthase activity as measured in slices (see "Materials and Methods"); for the sake of clarity ethylene values are not shown.

EFE activity of continuously propylene-treated fruit first rises sharply as with a pulse, then subsequently decreases gradually, while remaining higher than the corresponding pulse-induced levels until the onset of the climacteric. Upon initiation of the climacteric, EFE once again rises, together with a burst of endogenous ethylene. ACC synthase activity remains at essentially control levels throughout the treatment, rising only at the onset of the ethylene climacteric.

Pulse Effect on mRNA Translation Profiles

Poly(A^+) mRNA extracted from pulsed and control fruit at different times over the ripening time course reveals numerous changes in the prevalence of specific translatable poly(A^+) mRNA species. Two-dimensional PAGE gel analysis of *in vitro* translation products made from poly(A^+) mRNA populations (data not shown) shows various patterns of expression for different polypeptides. Figure 4 shows the patterns of five representative signals that were strong and showed noticeable changes.

One species of mRNA (●) that is present at relatively high levels immediately after picking, and drifts down by the time of the climacteric, is reduced dramatically by a 24 h propylene pulse. Another species (○), which rises at the climacteric in control fruit, is induced to climacteric levels within 24 h by the pulse. A third species (■) in the control rises moderately by 24 h and shows a slight further increase at the climacteric, whereas a pulse increases the intensity above the control by 24 h only to have the level drop below the control at the climacteric. The remaining two species of mRNA (□ and △) are little affected by the pulse; both increase during the lag period and rise to high levels by the climacteric.

DISCUSSION

The ripening syndrome in climacteric fruit, in particular, avocado, comprises two distinct phases: the lag phase, wherein endogenous ethylene at low concentrations, so-called system I ethylene (17, 27), mediates a developmental augmentation

Table II. *EFE and ACC Synthase Activity of T = 0 versus T = 24 h Propylene-Pulsed Fruit*

EFE and ACC synthase activities are expressed as nanoliters C_2H_4 produced per gram fresh weight per hour. $T = 0$ and $T = 24$ represent hours from harvest and indicate the point of application of the pulse. All fruit data are average values of four fruit ± SE except March pulsed fruit which are average values of six fruit ± SE.

Treatment	T = 0		T = 24	
	EFE	ACC synthase	EFE	ACC synthase
	nL C_2H_4/g fresh wt/h ± SE			
November				
24 h pulse	63.6 ± 4.3	1.8 ± 1.2	42.0 ± 8.5	1.7 ± 0.9
Control	0.6 ± 0	0.1 ± 0	0.6 ± 0.13	0.3 ± 0.04
January				
24 h pulse	90.9 ± 15.6	1.8 ± 0.3	61.9 ± 4.2	3.2 ± 1.5
Control	3.1 ± 0.73	0.8 ± 0	4.8 ± 0.6	1.7 ± 0.4
March				
24 h pulse	26.3 ± 1.1	0.6 ± 0.03	35.8 ± 5.5	1.0 ± 0.34
Control	1.0 ± 0.15	0.2 ± 0	1.3 ± 0.37	0.3 ± 0

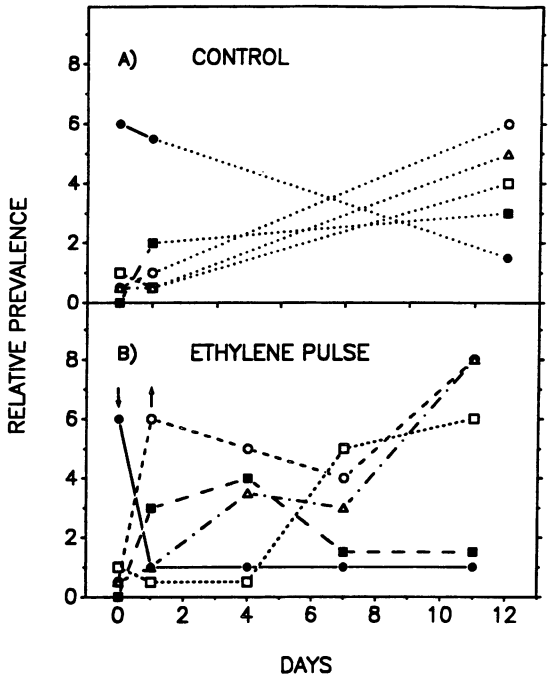

Figure 4. Relative abundance of [^{35}S]methionine-labeled *in vitro* translation products of avocado fruit poly(A$^+$) mRNA from different time points. (A) Endogenous ripening control and (B) ethylene pulsed mid-season avocado fruit. Five prominent spots with varying intensities were compared with four selected spots whose intensities remained constant through all gels of the time course. The four control spots were of four different intensities and were used as standards for estimation of relative spot intensities of the five spots of interest. The ethylene pulse was applied from $T = 0$ to $T = 24$ h (arrows).

of tissue sensitivity, or responsiveness to ethylene (20, 25), and the second phase, or climacteric, wherein a burst of autocatalytically produced ethylene, so-called system II ethylene, causes and accompanies a respiratory climax attended by a cohort of ripening phenomena. Inasmuch as ethylene causes a respiration response in citrus divorced from ripening (5), and a brief ethylene pulse does the same in both banana (9) and avocado (12, 13), there has been reason to suspect that the pulse response in preclimacteric fruit is an independent event extraneous to ripening, mediated by an ethylene receptor distinct from that which initiates ripening. However, Eaks (12, 13) has noted that a propylene pulse (as effective as ethylene [see ref. 18]; throughout this discussion we use ethylene pulse generically to refer to a pulse of ethylene or propylene) that elicits a transient respiration response while failing to cause ethylene synthesis in preclimacteric avocado, nevertheless advances the true climacteric (*cf.* ref 2), much as low levels of ethylene that cause no overt response hasten ripening of preclimacteric bananas (20). We have verified Eaks's observations and sought to determine whether any demonstrable pulse-induced changes in enzymatic activity or gene expression, particularly with respect to ethylene biosynthesis, underlie the respiratory pulse response. Whereas EFE is sharply augmented in the course of a 24 h ethylene pulse, there is no increase in ACC synthase activity (Fig. 3) (*cf.* ref. 22), which by our methods remains essentially unchanged throughout the pulse. The induction of EFE by an ethylene pulse is the same when fruit are pulsed immediately after picking as when pulsed 24 h after picking (Table II). Because, by contrast, pulsing freshly harvested early-season fruit has no effect on the time of onset of the climacteric, whereas pulsing fruit 24 h after picking advances the onset of the climacteric (Table I), it follows that EFE augmentation in response to pulsing is not responsible for advancing the climacteric. Furthermore, EFE declines after a pulse, only to rise again when ACC synthase too is triggered, in conjunction with the initiation of the climacteric (Fig. 3). Thus, the pulse must influence developmental phenomena other than ethylene synthesis per se (*cf.* ref. 29), a proposition supported by Figure 4, which suggests up- and down-regulation of a number of genes in response to an ethylene pulse. The results in Figure 4 provide us with hope for finding prominent genes which are influenced by a pulse and may be involved in shortening the lag period. For example, the expression of at least two genes, as judged by the prevalence of their translatable poly(A$^+$) mRNAs, is markedly affected by a 24 h pulse, and the effect persists through the ripening period (Fig. 4). In fact, many translation products change with pulsing, encoded by genes that may be isolated by differential screening of pulsed and control-derived cDNA libraries, a project we are currently undertaking. Whereas EFE rises in response to a pulse and subsequently drops off, reaching its post-pulse minimum at the onset of the climacteric, EFE activity in endogenously ripened fruit rises gradually throughout the lag period increasing substantially only at the onset of autocatalytic ethylene production (Fig. 3A of refs. 22 and 27). Continuously treated fruit exhibit patterns similar to pulsed fruit with EFE activity falling off after 24 h and drifting down, though less than in pulsed fruit (Fig. 3, B and C). ACC synthase, however, remains at low control levels throughout ethylene treatment, rising only when the fruit manifests autocatalytic ethylene production at the climacteric rise. Thus the induction of ACC synthase is correlated with climacteric ethylene production, confirming earlier observations that the conversion of *S*-adenosylmethionine to ACC is the rate limiting step in ethylene biosynthesis in ripening avocado fruit (15, 22).

The failure of avocado fruit to ripen on the tree has been attributed to an inhibitory tree factor (24, 27). Whether this tree factor is the same factor which renders an early mature fruit recalcitrant to a pulse applied immediately after harvest, with respect to the lag period, or whether there are separate factors for inhibiting ripening of attached fruit and for the early recalcitrance of picked fruit, remains an open question. In any event, Biale (6) early established that response to ethylene varies with fruit maturity (*cf.* ref 2), and Zauberman and Fuchs (29) and Adato and Gazit (2) demonstrated that ethylene given late mature fruit directly after harvest hastens softening, an earlier assertion by Gazit and Blumenfeld (14) to the contrary notwithstanding. Consistent with the foregoing we find that pulse effectiveness in advancing ripening varies with season, inasmuch as late harvest fruit respond to a pulse immediately after picking, whereas early-season fruit do not (Table I) (*cf.* ref 2). Parenthetically, although it is ethylene at low internal concentrations (hence system I ethylene, see Yang [27], McGlasson [17]) that mediates the increase in

tissue sensitivity that leads to triggering of the climacteric, continuous exposure to exogenous ethylene shortens the lag phase in proportion to ethylene concentration (5) in a manner that bespeaks a product law, *i.e.* where response is a function of concentration × time. In this view, ironically, the system I receptor must have a high K_m for ethylene.

While the pulse-induced respiration peak *per se* may be incidental to the responses that influence the length of the lag period, it is nevertheless symptomatic. Why then does the classical depiction of the respiratory time course of avocado fruit given continuous ethylene (5) resemble the idealized respiratory profile of endogenously ripened fruit in showing no evidence of a pulse-like response (Fig. 1D, *cf.* 1C)? Two reasons may be offered: (a) with high concentrations of exogenous ethylene and mid- to late-season fruit, the pulse response is merged with the climacteric (see refs. 13 and 26); and (b) the poor kinetic definition of the respiration time course with measurements made at extended intervals fails to perceive its inherently dual nature. Parenthetically, with continuous respiration measurements of ethylene or propylene treated avocado fruit of three different varieties (*viz.* Hass, Fuerte, and Scott), Eaks invariably observed a pronounced bimodal respiration response (private communication).

As fruit are pulsed later and later in the season, the pulse-peak and climacteric peak draw closer together, until finally the pulse-peak is just a blip (13) or shoulder (26) on the climacteric peak. We propose that in endogenous ripening, ripening-related events that can be accelerated by an ethylene pulse normally occur over prolonged time in the absence of exogenous ethylene. In this view the pulse response is a bona fide component of the ripening syndrome. Whether pulse-induced respiration per se has anything to do with ripening, or is simply an independent, extraneous phenomenon, remains an open question.

ACKNOWLEDGMENTS

The authors wish to thank Professor Howard Ohr at the University of California, Riverside, for his kindness in providing us with avocado fruit from the South Coast Station orchard, and Bill Catlin for graciously providing us access to his orchard in Carpenteria, CA. We are indebted to Dr. John Lighton for the custom computer software programs developed for our use.

LITERATURE CITED

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