

# Role of Ethylene in Avocado Fruit Development and Ripening

I. FRUIT DROP

I. ADATO

*Department of Subtropical Horticulture, Agricultural Research Organization,  
The Volcani Centre, Bet Dagan, Israel*

AND S. GAZIT

*The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel*

Received 25 August 1976

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## ABSTRACT

A survey of avocado (*Persea americana* Mill.) fruit drop and ethylene production was carried out during fruit development. Natural drop of avocado fruitlets started soon after set (May) and continued at a gradually decreasing rate until September, except for a temporarily increasing rate in late July. Fruitlets weighing up to 0.2 g dropped at a rate of over 30 per cent per week. With larger fruits, the rate was under 1 per cent per week. Fruit drop ceased after September, when fruit growth declined and the seed coat began to shrivel.

A positive correlation was found between the rate of fruitlet and fruit drop and ethylene production. Fruitlets with defective seeds produced ethylene at a very high rate of 7-10 times more than apparently normal fruits. The high incidence of defective seeds might be the cause of the very high levels of ethylene production by young avocado fruitlets.

The seed was found to be the main site of ethylene production in fruitlets.

Abscisic acid (ABA) levels in young abscising fruits were 7 times as high as those in non-abscising fruits.

## INTRODUCTION

In the course of fruit development the abscission rate frequently determines the eventual yield and thus abscission is considered as a severe problem in many crops (Blanpied, 1972; Edgerton, 1971; Leopold, 1964; Lipe and Morgan, 1972, 1973; Martin and Nishijima, 1972; Nitsch, 1965). Since it was demonstrated that ethylene is a natural abscising hormone (Abeles, Craker, and Leather, 1971; Jackson and Osborn, 1970), attempts have been made to use it to cause abscission of plant organs in general (Daniell and Wilkinson, 1972; Edgerton and Greenhalgh, 1969; Stenbridge and Gambrell, 1971), and of young and mature fruits in particular (Bukovac, Zucconi, Larsen, and Kesner, 1969; Edgerton and Blanpied, 1968; Hall Truchelot, Leinweber, and Herreo, 1957; Lipe and Morgan, 1972, 1973). However,

Contribution from the Agricultural Research Organization, The Volcani Centre, Bet Dagan, Israel. 1976 Series, No. 260-E.

only recently were studies made of the relationship between the abscission of fruitlets and developing fruits, and their ethylene production rate. In one report such a connection was established (Blanpied, 1972) and in the case of young cotton balls it was shown that ethylene was indeed the hormone responsible for abscission (Lipe and Morgan, 1972, 1973).

Young avocado, mango, and lychee fruits were found to produce ethylene at a very high rate, which decreases to a very low rate at progressive stages of development (Akamine and Goo, 1973).

In this work we studied the relation between ethylene production and natural fruit drop in avocado during fruit development.

## MATERIALS AND METHODS

### *Parameters for the characterization of the developing fruits*

About a month after set, avocado fruits (*Persea americana* Mill., cv. Hass) of uniform size (diameter 1–1.5 cm) were tagged on a large number of trees in one orchard. Representative fruit samples were taken (10 tagged fruits at each date) for the determinations carried out during the long period of fruit development and maturation. The parameters determined were the weights of fruit, embryo, and seed coat, and the oil content in the mesocarp, which can serve as an index for fruit maturity (Table 1).

On five trees, 40 fruits per tree were kept for the survey of fruit drop during the year. The remaining fruits on the same trees were used for the determinations of endogenous ethylene concentration.

### *Determination of ethylene production and respiration*

Ambient temperature was kept at 21 °C. Plant material was sealed for 15 min in glass containers with a volume of 3, 8, 30, 60, or 750 ml in accordance with the volume of the tissue being examined. A single fruit, as well as different tissues derived from one fruit, were sealed per container. The ethylene or CO<sub>2</sub> level was determined in a 1–2 ml sample by means of gas chromatography (Adato and Gazit, 1974). Since the extraction of 1 ml from a container having only 3 or 8 ml volume would create a partial vacuum, water was introduced into the test tube as the air sample was withdrawn.

Determinations were performed 2–3 h after harvest. Preliminary studies showed no significant changes in ethylene production and respiration between 0.5 and 5 h after harvest. Dissection did not cause significant changes in ethylene production within the first hour.

### *Abscisic acid determination*

The collection and storage of the plant material, and its extraction, methylation, and final determination have been described elsewhere (Adato, Gazit, and Blumenfeld, 1976; de Boer and Backer, 1954).

### *Methods used for determining endogenous ethylene concentrations in fruitlets and fruits*

*Fruitlets, size 0.05–0.5 g.* Fruitlets (6–22, depending on size) were placed in a 3 ml test tube. Six to ten groups (each one constituting a replication) were examined. The remaining space in the test tube was filled with tap water, which had been boiled the previous day, and then sealed. With the aid of a 50 ml syringe the water was drawn out of the test tube, creating a vacuum. This vacuum was maintained for 5 min, then the water was restored to the test tube, together with the gases which had been extracted from the fruitlets as a result of the vacuum. The gases formed an air bubble which was extracted by means of a 1 ml syringe and injected into a gas chromatograph for ethylene determination. All operations were performed while test tubes were immersed in water.

*Fruitlets, size 0.5–1.5 g.* Extraction was performed by the method described above, but three or four fruitlets were placed in an 8 ml test tube.

*Fruits, size 1.5–6.0 g.* Extraction of the gaseous phase was performed by two methods. (a) Three fruitlets were placed in a 30 ml test tube, and gas was extracted from them by the method as described above. (b) A 4 mm hole was pierced in each fruitlet by means of a cork borer. The hole was plugged with a rubber stopper, through which the needle of a 50 ml syringe was inserted; a vacuum was created by using the syringe under water. After 5 min 0.2–1.0 ml of endogenous air had accumulated. Eight to ten replications were examined by each method; both methods gave similar values of the endogenous ethylene.

*Fruits above 6 g.* Gas extraction was performed by the second method as described in the preceding paragraph.

## RESULTS

### *Fruitlets: ethylene production, and drop rate*

The survey of fruitlet abscission was begun on 10 May. Most of the fruitlets abscised within 18 d, leaving 0–5 fruitlets on each inflorescence (Table 2).

TABLE 1. *Weight of cv. Hass fruit, embryo, and seed coat and the oil content of the mesocarp during the season*

Results are averages of 10 fruits (replicates)  $\pm$  s.e.

Date	Whole fruit	Embryo		Seed coat		Oil content of mesocarp (%)
	Weight (g)	Weight (g)	% of whole fruit	Weight (g)	% of whole fruit	
25 May	2.2	—	—	0.1	4.5	0.7 $\pm$ 0.03
7 June	5.5	0.1	1.1	0.2	4.0	0.7 $\pm$ 0.04
26 June	27	0.5	1.8	0.6	2.2	0.9 $\pm$ 0.05
11 July	53	1.4	2.7	1.1	2.0	1.3 $\pm$ 0.1
24 July	70	2.5	3.5	1.2	1.7	1.6 $\pm$ 0.1
8 Aug.	109	4.9	4.5	1.7	1.6	2.3 $\pm$ 0.1
21 Aug.	122	7.7	6.3	1.8	1.5	3.0 $\pm$ 0.2
4 Sept.	140	11.2	8.0	2.1	1.5	4.8 $\pm$ 0.3
25 Sept.	162	15.2	9.4	2.4	1.5	5.7 $\pm$ 0.3
24 Oct.	185	20.4	11.0	2.2	1.2	8.4 $\pm$ 0.3
27 Nov.	205	28.5	13.9	1.8	0.9	10.5 $\pm$ 0.5
1 Jan.	210	27.3	13.0	0.9 <sup>a</sup>	0.4	13.0 $\pm$ 0.5
25 March	205	28.5	13.9	—	—	15.5 $\pm$ 0.5

<sup>a</sup> Seed coat brown and shrunken.

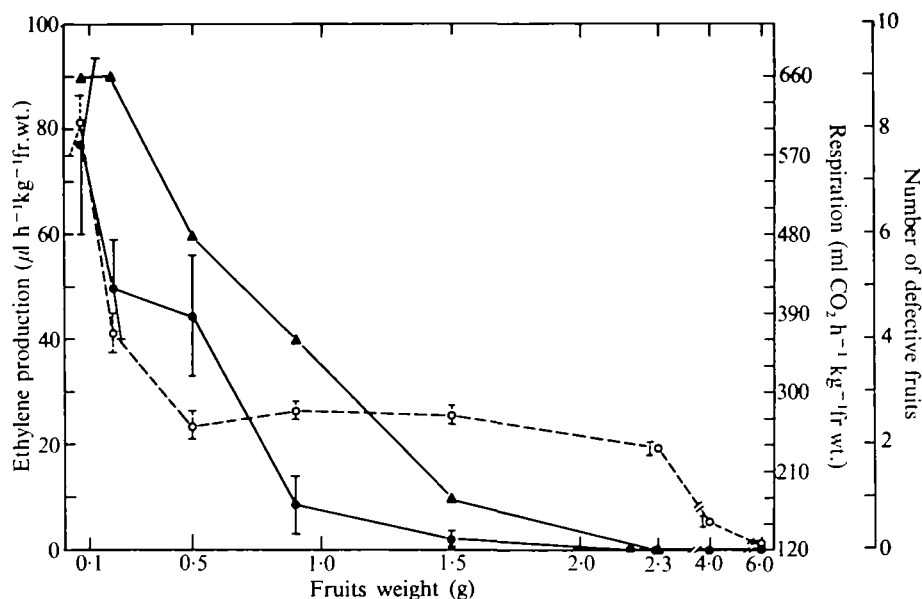
During the same period and from the same trees a large number of untagged fruitlets were picked on one day. They were brought to the laboratory and divided into groups according to weight. Within 2–3 h after harvest, the following determinations were made: concentration of endogenous ethylene, rate of ethylene production, respiration, and the existence of any noticeable defects in the seed.

Endogenous ethylene concentration was very high in very small fruitlets and decreased appreciably in fruits weighing more than 1.0 g (Table 3). A similar pattern was found when ethylene production was determined (Fig. 1).

After completion of the respiration and ethylene production determinations, the same fruitlets were bisected longitudinally. All those in which a black spot was found in the seed were classified as defectives. A positive correlation was found

TABLE 2. *Fruitlet drop of cv. Hass during the period 10 May to 5 June*Results are averages of 15 inflorescences (50–100 fruitlets on each)  $\pm$  s.e.

Days from beginning of survey	Fruit diameter (mm)	Fruit weight (g)	Cumulative fruitlet drop (%)
0	4–5	0.1–0.2	
4	—	—	26 $\pm$ 4
7	—	—	39 $\pm$ 4
13	—	—	76 $\pm$ 2
15	—	—	88 $\pm$ 2
18	8–12	0.6–1.4	94 $\pm$ 1
20	—	—	95 $\pm$ 0
25	—	—	96 $\pm$ 0

FIG. 1. Ethylene production (●—●), respiration (○—○) and number of defective fruitlets (▲—▲) as related to weight of *cv. Hass* fruitlets. Results are averages of 10 fruits (replicates)  $\pm$  s.e.

between the number of defective fruitlets in a sample and its average ethylene production rate (Fig. 1).

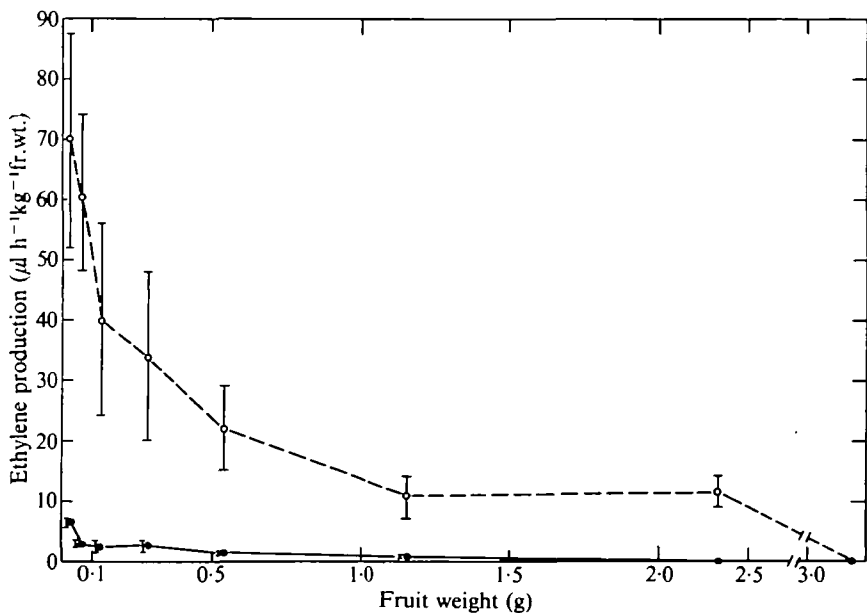
Ethylene production was determined separately in defective and normal-looking fruitlets. It was found that defective fruitlets produced ethylene at a much higher rate than normal ones (Fig. 2). During this period we found similar seed defects in every fruitlet sampled out of the hundreds of fruitlets which dropped from the tree.

When ethylene production was determined separately for the intact seed and the pericarp, the seed was found to be responsible for most of the ethylene produced by the whole fruitlet (Table 4).

TABLE 3. Endogenous ethylene concentration in cv. Hass fruitlets as related to their weight

Results are averages of 5–10 fruit lots (replicates)  $\pm$  s.e.

Fruit weight (g)	Endogenous ethylene concentration (parts $10^{-6}$ )
0.05	$8.5 \pm 1.0$
0.15	$9.5 \pm 1.5$
0.4	$11.5 \pm 1.7$
0.6	$8.0 \pm 1.7$
0.8	$6.5 \pm 2.0$
1.3	$1.0 \pm 0.1$
2.0	$0.6 \pm 0.05$
3.0	$0.4 \pm 0.04$
6.2	$0.3 \pm 0.02$

FIG. 2. Ethylene production of normal (●—●) and defective (○--○) cv. Hass fruitlets as related to weight. Results are averages of 5 fruitlets (replicates)  $\pm$  s.e.

Whereas the seed was found to be the main site for ethylene production in fruitlets, it plays a minor role with regard to respiration rate of the whole fruitlet. Although the respiration rate was 2–3 times higher in seeds than in the pericarp, its total contribution to fruitlet respiration was much less than that of the pericarp since the latter forms the predominant part of the fruit (Table 4).

#### *Fruits: ethylene concentration and production, level of ABA, and drop rate*

Drop rate and endogenous ethylene concentration during the season are presented in Fig. 3. for 'Hass' fruits. Certain definite patterns and trends can be discerned. First, fruit dropped continuously from the time of fruit set (Table 2) until September (Fig. 3A). Second, the rate of fruit drop decreased continuously throughout the

TABLE 4. Ethylene production and respiration rates of pericarp and seed in young *cv. Hass* fruitlets of two sizes picked in MayResults are averages of 5 fruitlets (replicates)  $\pm$  s.e.

Av. weight of whole fruit (g)	Tissue type	Tissue/whole fruit (%)	Ethylene production rate ( $\mu\text{l h}^{-1} \text{kg}^{-1} \text{fr. wt.}$ )	Respiration rate ( $\text{ml CO}_2 \text{h}^{-1} \text{kg}^{-1} \text{fr. wt.}$ )
0.20	Seed	7.5	374 $\pm$ 103	747 $\pm$ 54
	Pericarp	92.5	9.2 $\pm$ 4.9	352 $\pm$ 32
0.48	Seed	6.3	188 $\pm$ 55	1000 $\pm$ 87
	Pericarp	93.7	1.6 $\pm$ 1.6	270 $\pm$ 37

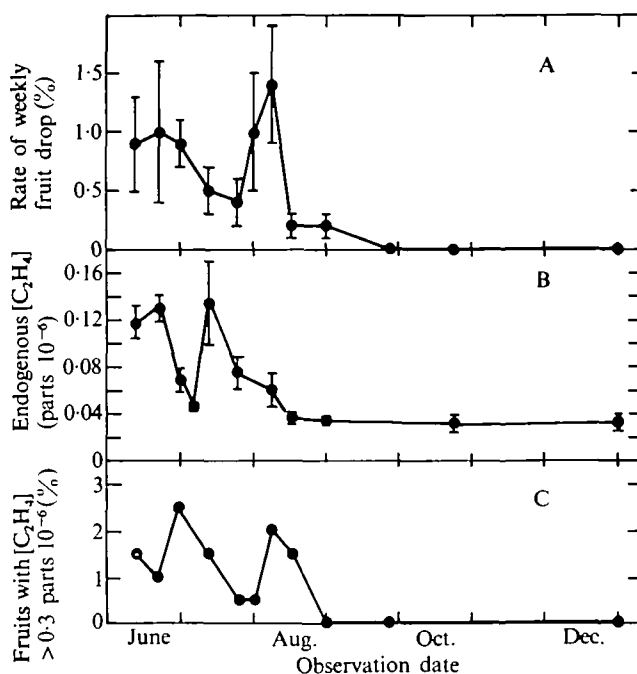


FIG. 3. Rate of weekly fruit drop (A), endogenous ethylene concentration (B), and percentage of fruits with ethylene concentration over 0.3 parts  $10^{-6}$  (C), of *cv. Hass* avocado fruits during the season. (A) is the average drop from 5 trees (40 tagged fruits per tree)  $\pm$  s.e.; (B) is a 20-fruit sample.

season, apart from a distinct increase in late July and early August (Fig. 3A). The endogenous ethylene concentration pattern found in developing avocado fruits (Fig. 3B) was similar to that of the fruit drop rate (Fig. 3A), but the curves do not conform to the same dates—the rise in endogenous ethylene concentration apparently precedes the rise in fruit drop by 3 weeks. This may be explained by the fact that the average ethylene concentrations found were lower than 0.15 parts  $10^{-6}$ , a concentration which may not be capable of causing immediate abscission but rather indicates a tendency, in the population examined, of increasing ethylene production. This increased ethylene production occurred in only a small number of fruits which probably dropped at the end of the 3-week-long process (Fig. 3). The

TABLE 5. *Ethylene production of seeded and seedless cv. Fuerte fruits as related to their tendency to abscise*Results are averages of 10 fruits (replicates)  $\pm$  s.e.

Fruit type	Abscission of fruit by hand touch	Fruit weight (g)	Ethylene production ( $\mu\text{l h}^{-1} \text{kg}^{-1}$ fr. wt.)
Seedless	Yes	0.7 $\pm$ 0.0	8.0 $\pm$ 1.1
	No	1.0 $\pm$ 0.0	1.8 $\pm$ 1.1
Seeded	Yes	3.1 $\pm$ 0.3	3.6 $\pm$ 1.6
	No	3.9 $\pm$ 0.1	<0.08 <sup>a</sup>

<sup>a</sup> Production of ethylene below 0.08  $\mu\text{l h}^{-1} \text{kg}^{-1}$  fr. wt. was not detectable under our conditions.

changes in the number of fruits having more than 0.3 parts  $10^{-6}$  ethylene (Fig. 3C) were better correlated with the fruit drop pattern (Fig. 3A).

The close link between fruit drop and increased ethylene production can also be seen in Table 5. Increased ethylene production was found in very young 'Fuerte' avocado fruits prior to abscission whether they were seeded or seedless.

Since it is known that ABA plays an important role in abscission (Addicott and Lyon, 1969; Davis and Addicott, 1972; Leopold, 1971; Milborrow, 1974), its level was determined in abscising and attached fruits. The results show that ABA level in abscising fruits, whether determined close to the severed tissues or at a distance, increased 7-fold in comparison with ABA level in attached fruit (Table 6).

TABLE 6. *Ethylene production and the level of ABA in young cv. Hass fruits as recorded on July 5, during the period of their natural drop*Results are averages of 8 fruits (replicates)  $\pm$  s.e.

Abscission of fruit by hand touch	Fruit part	Weight (g)	Ethylene production ( $\mu\text{l h}^{-1} \text{kg}^{-1}$ fr. wt.)	ABA level ( $\mu\text{g kg}^{-1}$ fr. wt.)
Yes	Whole fruit	45.5 $\pm$ 2.2	2.6 $\pm$ 0.3	—
	Proximal part (above the seed)	6.6 $\pm$ 0.3	14.9 $\pm$ 2.7	4724 $\pm$ 143
	Remaining part	38.9 $\pm$ 2.3	1.8 $\pm$ 0.3	4607 $\pm$ 117
No	Whole fruit	37.8 $\pm$ 1.8	<0.08	682 $\pm$ 117

## DISCUSSION

The abscission pattern described in the present work (Table 2, Fig. 3A) reveals two different periods of fruit drop during avocado fruit development: prolific fruitlets drop during the first month, after which fruit continues to drop at a markedly decreased rate. Abscission continued (Fig. 3A) as long as there was rapid fruit growth and the seed coat was growing and succulent (Table 1). As the fruit matured abscission ceased (Table 1, Fig. 3A), but we noticed renewed abscission later, when the fruit could be described as over-mature. A close correlation was found between a high level of endogenous ethylene (Table 3, Fig. 3) and fruit drop during fruit development (Table 2, Fig. 3). It can therefore be suggested that fruit drop in avocado can be attributed to a prior rise in ethylene concentration in the fruit.

The plant part responsible for increased ethylene production and, consequently, abscission of young avocado fruitlets was found to be the seed (Table 4). However, in more developed fruits, most of the ethylene in abscised fruits was produced in the proximal part of the pericarp (Table 6). Nevertheless, it is possible that even here it was the seed which initiated increased ethylene production, a supposition which should be investigated further.

On the basis of results obtained in the present work (Figs 1, 2) it is suggested that the very high ethylene level found in young fruitlets of various species (Akamine and Goo, 1973) is not characteristic of normally developing fruitlets but is found only in those fruitlets which are about to drop. The high ethylene level found in a randomly chosen population of fruitlets may be explained by the fact that most of the fruitlets constituting the population are in various stages of abscission. Consequently, it can be expected that very high ethylene concentrations will not be found in young fruitlets of those species in which natural abscission is rare.

ABA levels in ripening avocado fruits were increased appreciably following the rise in their climacteric ethylene production (Adato, Gazit, and Blumenfeld, 1976). The high levels of ABA found in abscising young avocado fruits (Table 6) may also be the consequence of the elevated levels of ethylene in the fruits, thus suggesting an involvement of both phytohormones in the regulation of the natural drop in avocado.

#### ACKNOWLEDGEMENTS

The technical assistance of Mrs. M. Elimelech and Mr. Z. Ben-Chetz is gratefully acknowledged.

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