J. Amer. Soc. Hort. Sci. 100(5):447-449. 1975.

Studies on the Inhibition of Ripening in Attached Avocado *(Persea americana* Mill.) Fruits[1]

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ABSTRACT. The inability of 'Fuerte" and 'Hass' avocado fruits to ripen on the tree has been attributed to a ripening inhibitor which has been presumed to be transported from the tree to the fruits. These studies, undertaken to explain this phenomenon, indicate that leaves, usually considered to be the source of this substance, did not delay ripening. Fruit on detached branches abscised before ripening and softened later than detached fruit. Leaves on the detached branches accelerated abscission and subsequent ripening. Fruit detached from the branch with the peduncle attached ripened later than when it was removed. The peduncle and stem may supply a ripening inhibitor to the fruit or the stem may act as a sink for a ripening hormone produced in the fruit.

Ripening of some climacteric fruits, e.g., tomato, and of many nonclimacteric fruits occurs on the parent plant following maturity (8). In contrast, the principal CA avocado cvs. Hass and Fuerte, do not ripen or soften while attached (2, 3, 4) but do so a few days after being detached. This lack of ripening is of both practical and theoretical interest and has been attributed to an inhibitory substance, probably hormonal (15), transmitted from the tree to the fruit (2, 3, 4,5). Evidence suggesting the existence of the substance was provided by Burg and Burg (5) who showed that when the stems bearing avocado fruits were girdled between the fruits and the nearest leaves, the fruits ripened as if they were detached; if the girdle was proximal to a cluster of leaves, the fruits remained longer in the preclimacteric stage. In apples also, which can ripen on the tree, it was observed that detaching of the fruits, or severing the branches on which they were borne stimulated the climacteric rise (13). The extent of the resistance to ripening in avocado fruits was demonstrated by Gazit and Blumenfeld (7),where C_2H_4 treatments sufficient to induce prompt ripening in detached fruit elicited no response in mature attached fruit. We examined possible mechanisms by which ripening of attached avocado fruits is prevented.

[1] Received for publication June 13, 1974. This paper represents part of the Ph.D. dissertation of the senior author. Present address: Faculty of Agriculture, P. O. Box 32, Khartoum, North Sudan. The authors wish to thank L. C. Erickson and J. B. Biale for helpful criticisms and suggestions and the technical assistance of Cameron Duncan is gratefully acknowledged.
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Materials and Methods

RIPENING STUDIES IN THE FIELD. The experiments were conducted on 'Hass' avocado trees at the South Coast Field Station, University of California, Orange County, CA. Uniform limbs bearing one fruit each were girdled proximal or distal to the terminal cluster of leaves by removal of a 1-cm wide strip of bark. There were 3 treatments: a) distal girdling; b) proximal girdling with terminal leaves removed; and c) proximal girdling with the leaves left intact. In addition, ripening of detached fruit was observed both in the field and laboratory. Ripening was evaluated subjectively by feeling the softness of the fruits.

RIPENING ON SEVERED BRANCHES. Roughly 1-meter long branches with one fruit each were cut from 'Fuerte' trees and their ends immediately put in water. They were transferred to growth chambers maintained at 20 ± 2°C and 65% relative humidity and kept overnight. Branches that failed to take up water were discarded the following day. The remaining branches were divided into 2 treatments: a) all leaves removed (defoliated branches); and b) about 8 leaves left on the branch (foliated branches). Fruits were then sealed in gas tight polyethylene bags provided with an inlet and outlet for air with the aid of Apiezon Q (a sealing compound). Some detached fruits were similarly placed in bags as controls. Half the number in each treatment was placed in a dark growth chamber and the remainder in another chamber scheduled for 12 hr of light each day. Both CO_2 and ethylene were monitored daily by injecting a 1 ml aliquot of the out-flowing air into a Beckman GC 4 gas chromatograph equipped with Porapak Q column. CO_2 was determined with a thermal conductivity detector and C_2H_4 with a flame ionization system.

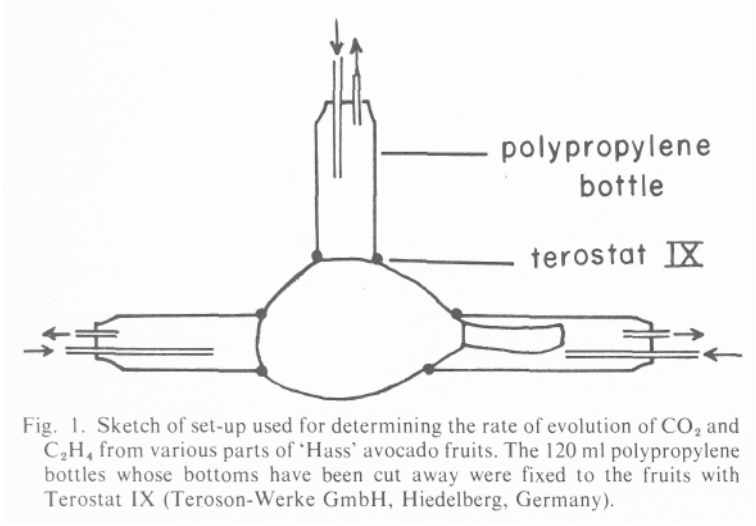

Fig. 1. Sketch of set-up used for determining the rate of evolution of CO_2 and C_2H_4 from various parts of 'Hass' avocado fruits. The 120 ml polypropylene bottles whose bottoms have been cut away were fixed to the fruits with Terostat IX (Teroson-Werke GmbH, Hiedelberg, Germany).

RIPENING OF DETACHED FRUITS. Avocado fruits were harvested with a 6 cm peduncle (including both pedicel and the true peduncle), attached. The peduncles were trimmed under water, and a #20 serum bottle cap with a hole in the top was fitted over the peduncle and placed in a test tube containing water. Another group of fruits with peduncles cut off close to the fruit were divided into 2 lots: in 1 the peduncle scars were sealed with vaseline and in the other they were left untreated. Fruits were placed in 1.8-liter jars and ventilated with humidified air at 70 ml/min. The effluent air was sampled automatically every 4 hours and passed through a Beckman 2I5A infrared analyzer for CO_2 determination, and thence to an Aerograph HY-FI Model 600-D gas chromatograph for C_2H_4 determination. Operation of the gas chromatograph was made automatic through the use of an electrically activated sample loop and a Varian Model 480 digital integrator to record the output. The integrator was set to reject peaks of less than 100

counts, which was equivalent to 13 ppb or a production rate of 0.19 μl/kg-hr. This rejection level was always more than 4 times an average noise peak.

The rate of CO_2 and C_2H_4 evolution from different parts of 'Hass' fruits with and without stems were determined as shown in (Fig. 1). The caps of the bottles were fitted with inlet and outlet tubes so that air would be passed continuously over a definite area of the fruit surface. Carbon dioxide and C_2H_4 production were determined from these areas and from separated peduncles by the system described above.

Table 1. Effect of girdling and defoliation of branches and detaching fruit on abscission and ripening of 'Hass' avocado fruit in May 1972. Average temperatures in the field were: 25.5°C mean maximum and 16°C mean minimum. Fruits in the laboratory were kept at 20°C.

Treatment	Av. days to abscission	Av. days to soften
Proximal girdling defoliated	23	28
Proximal girdling foliated	21	26
Distal girdling	16	21
Detached fruit in field		5
Detached fruit in lab		7

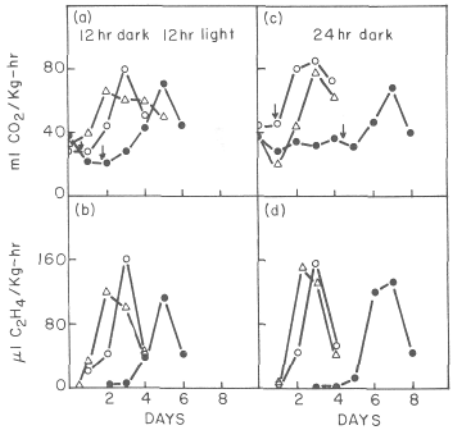

Fig. 2. Respiration pattern (a) and (c), ethylene production (b) and (d) of 'Fuerte' fruits in detached form Δ, on foliated branch O, and on defoliated branch ●; at 20 ± 2°C. Arrows indicate the time at which the fruits abscissed.

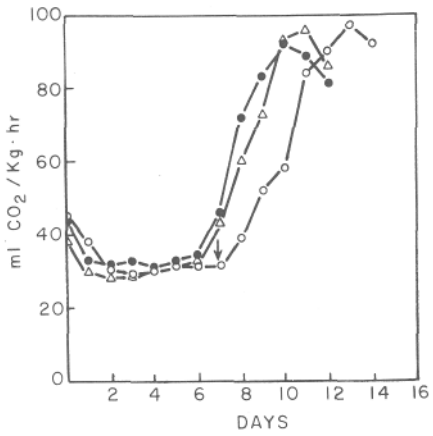

Fig. 3. Respiration pattern of 'Hass' avocado fruits with no peduncle (●), peduncle scar covered with vaseline (Δ) and with 6 cm long peduncle (O) 20°C. Each point represents average of 8 fruits and arrow indicates the time the peduncle separated.

Results

RIPENING IN THE FIELD. Fruit on girdled branches always abscised before any softening could be detected. Once abscission had occurred, prompt ripening was initiated, just as though mechanically detached, which occurred after 5 days in the field.

Days from girdling to abscission was greater by 5 days when the girdle was proximal to a flush of leaves (Table 1) than when girdled distal to the nearest leaves. Removal of the leaves when a proximal girdle was made caused a delay of 2 additional days in the average time to abscission. Clearly, nearby leaves do not delay abscission and ripening. In fact, our results show a slight acceleration of time to abscission when leaves are left attached. It appears that the length of the stem distal to the girdle is the primary factor which regulates the time to abscission.

RIPENING ON SEVERED BRANCHES. Just as in the field studies, ripening was not initiated on fruit attached to branches in the growth chamber, but only occurred after abscission. Here CO_2 and C_2H_4 determinations made on the fruit (Fig. 2) show that the rise in both CO_2 and C_2H_4 occurred just after the fruit abscised. Thus the failure of movement of some inhibitor to the fruit or of a hormone from the fruit to stem must be the signal which initiates ripening. In the branch experiments, fruit on foliated branches always abscised and ripened earlier than fruit on branches from which the leaves were removed. This was especially evident when branches were kept in the dark. The light-dark cycle did not appear to affect the amount of CO_2 and C_2H_4 production but only accelerated the time to abscission.

RIPENING OF HARVESTED FRUITS. When compared to detached fruit without peduncles, the climacteric for fruits with peduncles was delayed by at least one day; but there was no difference in onset of the climacteric between fruits whose stem scar was sealed with vaseline and where the peduncle was cut off as close as possible (Fig. 3). Peduncles separated from the fruit at an early stage of the climacteric rise.

When the rates of evolution of CO_2 and C_2H_4 from the stem end, stylar end and midsection of the fruits were determined per unit area. (Fig. 4), ethylene was always detected first at the stem end of the fruit; this preceded its detection from other parts of the fruits by as much as 8 to 24 hrs. As the climacteric progressed, the rates of evolution of both CO_2 and C_2H_4 from the stylar end and midsection exceeded rates from the stem end. The presence of 6 cm long peduncles delayed onset of the climacteric by 2 days, and also caused reduced C_2H_4 production from all parts of the fruit. No C_2H_4 production could be detected from 2 g of separated peduncles, which indicated that the production rate was less than 0.19 μl/kg-hr.

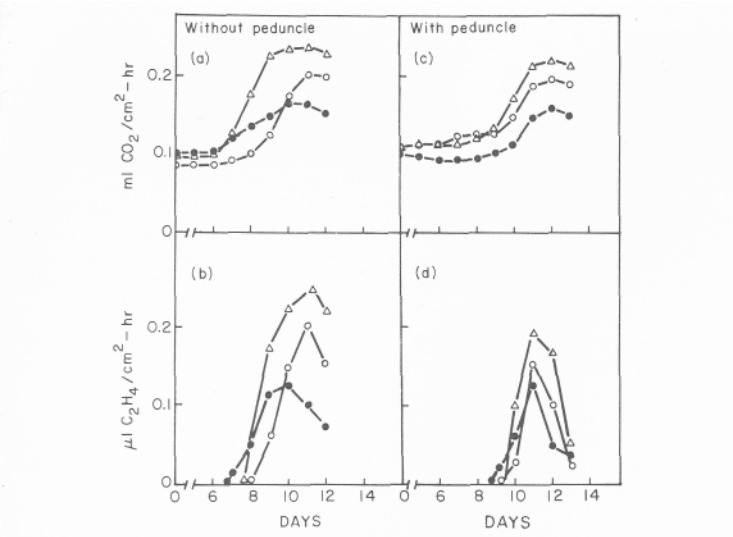

Fig. 4. Respiration rate (a) and (c) and ethylene evolution (b) and (d) from different parts of 'Hass' avocado fruits at 20°C. Symbols represent: ● = stem end, Δ = midsection and ○ = stylar end.

Discussion

The results show that abscission and subsequent ripening of attached avocado fruits was not delayed by the presence or proximity of the leaves (Table 1 and Fig. 2), and therefore, do not support the hypothesis that leaves are a source of ripening inhibitor (5, 15). On the contrary, ripening was hastened when a few leaves were left on the severed branches (Fig. 2c and d) causing these fruits to ripen as if they were detached. Although water loss was reduced to a minimum, the negative effect of leaves may partially be due to greater water loss from these branches and, consequently, from the fruits. Littmann (9) and recently, Adato and Gazit (1) have shown that increased water loss hastened ripening of avocado fruits. The important issue is, however, that leaves do not contribute

to extending the time to abscission and ripening over that provided by the peduncle and branch alone.

Experiments of Burg and Burg (5) focused attention on the leaves as the source of ripening inhibitors. It is, however, equally possible that the peduncle and stem may act as a sink for a ripening hormone produced in the fruit, as was suggested earlier by Biale (2). At the present, we cannot say whether substances controlling ripening are being moved into or from the fruit, or in fact whether movement of substances in both directions is necessary. We have been unsuccessful in making any extract of peduncles which, when vacuum infiltrated into preclimacteric fruit, exert any effect on ripening (14). This leaves open the possibility that the peduncle may act as a sink for hormones produced in the fruit. On the other hand, we have shown (14) that low concentration of IAA (1 μM) infiltrated into preclimacteric avocado fruit does delay ripening and reduce the amount of C_2H_4 produced during the climacteric, much as does attachment of the peduncle. This observation suggests that the peduncle is supplying an auxin to the fruit and ripening is initiated only when the source of auxin is depleted and the level in the fruit is reduced to some critical value. Treatment with 1 mM IAA caused earlier and higher ethylene production compared to control fruit, which induced ripening earlier. Ethylene production in response to auxin treatment has been reported for a number of tissues (10, 12).

Ethylene production and softening of the fruit was always detected earlier in the stem end than in other parts of the fruit. This was not due to production of C_2H_4 by the peduncle which we found to produce no C_2H_4 detectable in our flow system. Earlier ripening in the stem end may be due to the extensive vascular system transferring a ripening regulator more effectively to or from the tissue of this part of the fruit.

Light has been reported by Peacock (11) to accelerate ripening in banana fruit and far-red light to accelerate senescence in *Marchantia polymorpha* (6). While our results (Fig. 2) show some acceleration of ripening on alternating 12 hr light-dark cycles, we were (as was Peacock) unable to keep the fruit tissue under light at exactly the same temperature as fruit held in the dark. We believe that the slightly more rapid ripening in light was mainly a temperature effect. We have not been able to demonstrate any effect of red or far-red light on ripening.

We conclude that attachment of avocado fruit to a branch or even the peduncle, delays abscission and reduces ethylene production during the climacteric. Ripening occurs only after the fruit has abscissed from the peduncle. Nearby leaves on the branch either have no effect or actually accelerate the time to abscission and subsequent ripening. We believe that an auxin is at least one factor involved in the inhibition of ripening of attached avocado fruit.

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