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Response of Harvested Avocado Fruits to Supply of Indole-3-acetic Acid, Gibberellic Acid, and Abscisic Acid

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Satisfactory penetration and distribution of growth regulators in harvested avocado (*Persea americana* Mill.) fruits were achieved by infusing growth regulator solutions through the pedicel. Indole-3-acetic acid (IAA) greatly accelerated ripening and inhibited abscission of the ripe fruit but only when applied at a very high dosage (10^{-2} M). Gibberellic acid (GA_3) had no influence upon abscission and ripening processes even though it seemed to inhibit the ethylene production of the fruit at abscission. Abscisic acid (ABA), when given in a 100-ppm solution, accelerated abscission but not ripening, while a 1000-ppm solution accelerated both. The rate of ethylene production at abscission time was in all cases much lower than in control fruits.

Three plant hormones are usually considered to interact or influence the production and effects of ethylene: auxin, gibberellin, and abscisic acid (Dilley, 1969; Leopold, 1971). The effect of auxins on accelerating endogenous ethylene production in plant tissues has been established (Burg and Burg, 1968; McGlasson, 1970). Thus, induction of flowering (Burg and Burg, 1966) and of fruit ripening (Hansen, 1946; Mitchell and Marth, 1944) was achieved indirectly by auxin treatments. On the other hand, auxin delayed ripening of pears in spite of the increased production of ethylene (Frenkel and Dyck, 1973). The gibberellin effects were shown to be antagonistic to those of ethylene (Babbitt et al., 1973; Dostal and Wilcox, 1971; Scott and Leopold, 1967; Sharples, 1973), although in some cases gibberellin application accelerated ethylene production and abscission (Becka, 1973; Wittenbach and Bukovac, 1973). Exogenous ABA promoted ethylene production in fruits (Cooper and Henry, 1971; Cooper and Horanic, 1973; Cooper et al., 1968) and disks of orange peel (Gertman and Fuchs, 1972), while it inhibited ethylene production in cut flowers (Mayak and Halevy, 1972) and pea seedlings (Gertman and Fuchs, 1972).

When studying fruit response to exogenously supplied growth regulators, one of the main problems is its penetration and distribution within the fruit. Insufficient penetration and uneven distribution of an auxin, applied by immersion to banana, accelerated the rate of ripening; however, application by vacuum infiltration to banana slices, a method providing even distribution, inhibited ripening (McGlasson, 1970; Vendrell, 1969, 1970).

In the present work we used an infusion method through the pedicel (Adato and Gazit, 1974a) to achieve satisfactory penetration and distribution of plant hormones in the fruit. Using this method we have attempted to clarify the effect of three hormones on ethylene production, abscission, and ripening behavior of avocado fruits.

MATERIALS AND METHODS

Plant Material and Infusion Method. Ten-month-old mature Hass avocado (*Persea americana* Mill.) fruits were used for the experiments with indole-3-acetic acid (IAA) and gibberellic acid (GA_3); 7-month-old mature fruits of the same variety were used for the abscisic acid (ABA, *R,S*, cis–trans) experiment.

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Table I. Effect of Indole-3-acetic Acid Infusion through Pedicels to Harvested Hass Avocado Fruits, and the Rates of Ethylene Production, Abscission, and Ripening (Results Are Averages of Ten Fruits (Replications) ± Standard Error)

Treatment		Time from beginning of treatment, days		Ethylene production at abscission, μl/kg of fruit h^{-1}	Abscission in relation to the peak day of ethylene production, days
IAA concn in the treatment soln, M	IAA penetration into fruit during 9 days, μmol/kg of fruit	To abscission	To peak of ethylene production		
0	0	10.7 ± 0.8	12.5 ± 0.8	93 ± 37	−1.8
10^{-6}	0.1	10.7 ± 0.6	12.3 ± 0.6	80 ± 18	−1.6
10^{-4}	10	10.9 ± 0.7	12.0 ± 0.9	111 ± 19	−1.1
10^{-2}	870	11.9 ± 1.7	4.0 ± 1.5[a]	40 ± 19[b]	+7.9

[a] In this case only, the day of peak ethylene production was determined by subtracting 2 days from the date of complete softening (cf. Adato and Gazit, 1974b). [b] Fruits were soft and post-climacteric at abscission time.

Table II. Effect of Gibberellic Acid Infusion through Pedicels to Harvested Hass Avocado Fruits, on the Rates of Ethylene Production, Abscission, and Ripening (Results Are Averages of Ten Fruits (Replications) ± Standard Error)

Treatment		Time from beginning of treatment, days		Ethylene production at abscission, μl/kg of fruit h^{-1}
GA_3 concn in the treatment soln, M	GA_3 penetration into fruit during 9 days, μmol/kg of fruit	To abscission	To peak of ethylene production	
0	0	10.7 ± 0.8	12.5 ± 0.8	93 ± 37
10^{-6}	0.07	11.7 ± 0.7	12.5 ± 0.7	80 ± 21
10^{-4}	8	11.9 ± 0.4	12.4 ± 0.5	77 ± 17
10^{-2}	700	10.0 ± 0.6	11.4 ± 0.6	34 ± 14

Table III. Effect of Abscisic Acid Infusion through Pedicels to Harvested Hass Avocado Fruits, on the Rates of Ethylene Production, Abscission, and Ripening (Results Are Averages of Ten Fruits (Replications) ± Standard Error)

Treatment		Time from beginning of treatment, days		Ethylene production at abscission, μl/kg of fruit h^{-1}
ABA concn in the treatment soln, ppm	ABA penetration into fruit during 4 days, μg/kg of fruit	To abscission	To peak of ethylene production	
0	0	10.0 ± 0.5	11.5 ± 0.4	44 ± 20
100	6 150	8.6 ± 0.6	11.6 ± 0.5	0.5 ± 0.3
1000	26 630	6.4 ± 0.2	8.6 ± 0.2	1.0 ± 0.5

Infusion was accomplished by applying the solution to the fruit through its stalk (Adato and Gazit, 1974a). The fruits were picked with a 25- to 50-cm length of stalk plus branch attached. Glass tubes filled with 10 ml of the treatment solution were attached to the branches of the fruits with the aid of latex tubing. Air pressure of 0.5 atm was applied through the free end of the tubes, to the surface of the solution. Vital dyes applied to the fruit by this method penetrated within 1 h throughout its vascular system.

Hormone Treatments and Ethylene Determination. The hormones were dissolved in a few droplets of 2 N NaOH and then IAA and GA_3 were diluted to 10^{-2}, 10^{-4}, and 10^{-6} M. Two ABA concentrations were tested, 100 and 1000 ppm. All of the solutions, including the control (water), were brought to a uniform pH of 10.8 with 2 N NaOH. Each treatment consisted of ten fruits (replications). The relative humidity (R.H.) of the ambient air was about 40–50% and the temperature during treatments and determinations was 21 °C.

For the determination of ethylene production, the fruits were sealed (after their abscission) in 750-cm^3 glass containers for 15 min, after which a sample from the atmosphere was injected into a Packard gas chromatograph (Adato and Gazit, 1974b). The peak day of climactric ethylene production was found to be a good and objective index for avocado fruit ripening (Adato and Gazit, 1974b). Therefore, the time from harvest to peak of ethylene production was used to present ripening rate.

RESULTS AND DISCUSSION

IAA (Table I). The absolute amounts of IAA which actually penetrated the fruits during 9 days of infusion were considerable and fairly well correlated with the concentrations of the treatment solutions. Fruits responsed to IAA, either in terms of ethylene production or by abscission and ripening rates, only at the highest concentration, 10^{-2} M, which is undoubtedly a supraoptimal one. At this concentration ripening of the fruits was advanced by about 8 days in comparison with the control. This finding refutes the possibility that acceleration of fruit ripening caused by auxin is a result of uneven penetration and dispersal within the fruit (McGlasson, 1970), as found for banana (Vendrell, 1969, 1970). From Table I it can be seen that abscission occurred about 1–2 days before the fruit reached the climacteric peak in ethylene production. It is therefore noteworthy that fruits which were infused with 10^{-2} M IAA abscissed about 8 days after the fruit had reached the climacteric peak of ethylene production. This means that the fruits infused with 10^{-2} M IAA apparently resisted 10 days of high levels of endogenous ethylene more than control fruits, before they abscissed.

The 10^{-2} M concentration, which affected the ripening rate and abscission, is presumed to be supraoptimal, and it is therefore difficult to draw conclusions about the role of auxins in tree-attached fruits.

GA_3 (Table II). A good correlation was found between the declining concentrations of GA_3 solutions and the decrease in the rate of ethylene production by the fruit at abscission. Despite this decrease in ethylene production the abscission and ripening processes were not affected. This fact casts doubt on the significance of the role of this hormone in the natural abscission and ripening processes of mature avocado fruits, especially since it was found that its level in mature fruits is not high (Blumenfeld and Gazit, 1972).

ABA (Table III). A 100-ppm ABA solution caused a significant promotion of abscission without marked acceleration of ethylene production, which naturally characterized the abscissing fruits of the control. Moreover, the 100-ppm infused fruits ripened simultaneously with the control fruits, that is, abscission occurred during the preclimacteric phase.

The 1000-ppm solution was apparently supraoptimal and considerably advanced the ripening date. From our work and the results of Cooper and Horanic (1973), it may be claimed that ABA can regulate fruit abscission independently and not necessarily through the acceleration of ripening or ethylene production.

A more detailed elucidation of the role of ABA in the hormonal regulation of the abscission process will be possible after studying the level of endogenous ABA during the abscission process of fruits, and the influence of ethylene in such systems.

Satisfactory penetration and distribution of the plant regulators, which we believe we achieved, help in the understanding of their role in the natural abscission and ripening processes of the fruit. However, this is insufficient for drawing any conclusions about the role of the plant regulators in tree-attached fruits.

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Biosynthesis of Aflatoxin B_1. Conversion of Versicolorin A to Aflatoxin B_1 by *Aspergillus parasiticus*

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Forty-six percent of added [^{14}C]versicolorin A was efficiently converted to aflatoxin B_1 by a resting cell culture of wild-type *Aspergillus parasiticus*. The labeled pigment was isolated from [1-^{14}C]acetate enriched cultures of a mutant of *A. parasiticus* that elaborates several versicolorin pigments, but no aflatoxins. The high level of incorporation into aflatoxin B_1 and the relative specific activity of 0.475 of aflatoxin B_1 isolated from the culture of *A. parasiticus* enriched with [^{14}C]versicolorin A indicate that this C-18 polyketide-derived hydroxyanthraquinone is a precursor to aflatoxin B_1.

Aflatoxins, closely related secondary metabolites produced by certain strains of *Aspergillus flavus* and *A. parasiticus*, are of considerable importance because of their toxicity (Turner, 1971) and their carcinogenicity (Butler, 1969), but until recently little experimental evidence has been accumulated for their biogenesis. Studies on the incorporation of acetate by Hsieh and Mateles (1971) and detailed degradation studies by Biollaz et al. (1968a,b, 1970) indicate that the carbon skeleton of aflatoxin B_1 is derived entirely from acetate. This evidence and the similarity in structure between many anthraquinones, sterigmatocystins, and aflatoxins led to speculation, without experimental evidence, that these compounds share a common pathway (Holker and Underwood, 1964). In the biogenetic scheme hypothesized by Thomas (1965) two hydroxyanthraquinones, averufin (I, Figure 1) and 6-deoxyversicolorin A (III, Figure 1), and sterigmatocystin (IV, Figure 1) are proposed as intermediates in the biosynthesis of aflatoxin B_1 (V, Figure 1).

Versicolorin A (II, Figure 1) has specifically been proposed as such an intermediate by Heathcote et al. (1973). Versicolorin A, sterigmatocystin, and aflatoxins contain a di- or tetrahydrofurobenzofuran group. With one exception (Bassett et al., 1970) the occurrence of this furano group in natural products is peculiar to these or related mold metabolites. *O*-Methylsterigmatocystin (Burkhardt and Forgacs, 1968), aspertoxin (Rodricks et al., 1968), versicolorin C (Heathcote and Dutton, 1969), versicolorin A (Lee et al., 1975), and sterigmatocystin (Schroeder and

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