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Endogenous Abscisic Acid in Relation to Bud Growth in Alternate Bearing 'Valencia' Orange[1]

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ABSTRACT

An investigation was conducted into the relation of ABA (*cis-trans*-abscisic acid) in the dormant buds of alternate bearing 'Valencia' orange (*Citrus sinensis* [L.] Osbeck) trees. ABA did not appear to be related to alternate bearing but *t*-ABA (2-*trans*abscisic acid) did. There was 5- to 10-fold more *t*-ABA than ABA in the buds. There was more *t*-ABA in the buds of the "on" trees than in the buds of the "off" trees, and a drastic drop in *t*-ABA in both types of buds as spring growth approached. Bud dormancy and readiness for growth as related to *t*-ABA are discussed.

Alternate bearing is characteristic of the 'Valencia' orange especially in areas where the flowering-to-harvest cycle may be as much as 18 months. The severity of alternate bearing may be influenced by the amount of fruit on the tree and the length of time of on-tree storage of mature fruit (4). Large amounts of carbohydrates are utilized in the process of flowering, fruit growth and development, and during on-tree storage of mature fruit. However, in a recent study of alternate bearing, it was found that carbohydrates were probably not limiting for vegetative and subsequent reproductive growth (4).

In late winter, the dormant axillary buds (a dormant shoot has no terminal bud) begin growth as vegetative shoots (8). These new vegetative shoots then produce flowers. The most terminal axillary bud begins growth followed by the bud next down the stem. In comparing "on" trees (trees which enter winter with a full load of fruit, hereafter designated "on" buds) with "off" trees (trees which enter winter with few or no fruit, hereafter designated "off" buds) (4), it was found that the beginning of growth was delayed by about 3 weeks, that fewer buds grew, and that the buds that did grow were shorter and produced fewer flowers on the "on" trees. For the purpose of study, the alternate bearing problem may be divided into two parts: first, the problem of bud dormancy; and second, the problem of flower production after the beginning of vegetative growth.

The purpose of this investigation was to determine any changes in abscisic acid in dormant buds of the Valencia orange in response to fruit load.

MATERIALS AND METHODS

Sampling and Extraction Procedures. Large Valencia orange (*Citrus sinensis* [L.] Osbeck) orchard trees were used as sample sources. Samples were obtained each of 3 years but since results were similar only the results for 1973 to 1974 will be presented. The three pairs of trees used were part of a thinning study (4). The 1974 yield for the "on" trees was 345 kg/tree and for the "off" trees 53 kg/tree. Bud samples were obtained on December 20, 1973 and on January 18 and February 4, 1974. On the last date, a few buds on the "off" trees were showing visible growth. These buds were not sampled. The three axillary buds most terminal on the shoots (Fig. 1) were sampled with approximately 500 buds/sample. The buds were frozen with Dry Ice, brought to the laboratory, fresh weight obtained, and held at −20 C until needed.

Each sample was freeze-dried and ground in a mortar under liquid N_2, extracted with 80% methanol, and the extract reduced to the water phase in a Buchi evaporator. The water phase was adjusted to pH 9 with KOH and extracted with ethyl acetate. The water phase was then adjusted to pH 2.5 with HCl and again extracted with ethyl acetate. The volume of the ethyl acetate was adjusted to 10 ml and stored in a freezer at −20 C until needed.

Gas-Liquid Chromatography (GLC) and Mass Spectrometry (MS). For GLC, a 1-ml aliquot of the extract was dried with a stream of N_2, and silylated with 200 μl of *N,O*-bis-(trimethylsilyl)-acetamide (BSA from the Pierce Chemical Company). A 2-μl sample was injected into a Hewlett-Packard 7620A gas chromatograph equipped with a dual hydrogen flame detector and a dual glass column (1.83 m × 0.64 cm o.d.) packed with QF-1 on 80- to 100-mesh, high performance, Chromosorb W. Operating conditions were: oven temperature isothermal at 125 C for 4 min; increased at 6 C/min to 190 C; increased at 4 C/min to 245 C; injection temperature 260 C; detector temperature 300 C; helium-carrier gas flow, 75 ml/min; hydrogen flow, 30 ml/min; and air flow, 300 ml/min. Adonitol was used as an internal standard in all samples. A Hewlett-Packard 3370A integrator was used. Standard curves were prepared with synthetic, mixed isomers (1:1 2-*cis*-4-*trans* [ABA] and 2-*trans*-4-*trans* [*t*-ABA]) of ABA. Retention time was 24.96 min, and 28.19 min, respectively. A synthetic racemic (±) ABA co-chromatogramed with the first peak of the mixture. Plant samples co-chromatogramed with both the first and second peaks. In the plant samples, the amount of *t*-ABA was about 10-fold more than the ABA. No bound (3) ABA was detected.

For MS, the *t*-ABA peak was collected, the TMS-*t*-ABA hydrolyzed, esterified with diazomethane, dissolved in pyridine, and injected by probe into a Finnigan mass spectrometer. This produced the same parent ion at m/e 278 and fragmentation pattern similar to the authentic ABA.

RESULTS

Changes in fresh weight in buds from December 20 to Jaunary 18 were very small (Fig. 2A). Between January 18 and February 4, there was a marked increase in bud weight. The increase was 71% for the buds on the "off" trees and 29% for the "on" trees. This was before any visible growth had occurred. As reported earlier (4), the beginning of visible growth in the spring was delayed several weeks by an "on" crop. At each sampling date,

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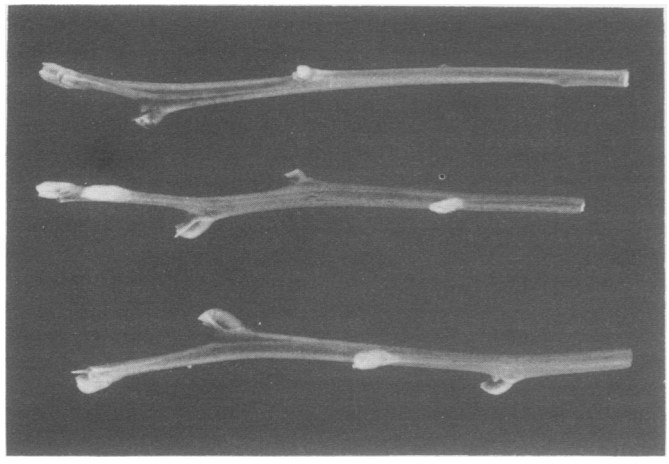

FIG. 1. Kind of shoots from which bud samples were obtained. The leaves were removed before photographing in order to show the buds and thorns. The three buds closest to the terminal end of the shoot were combined into the same sample. The thorn, when present, was discarded.

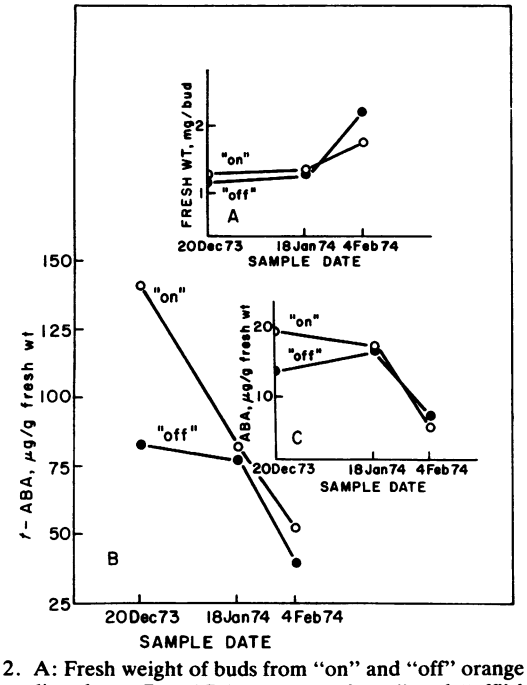

FIG. 2. A: Fresh weight of buds from "on" and "off" orange trees at three sampling dates; B: *t*-ABA contents of "on" and "off" buds; C: ABA contents of "on" and "off" buds.

there was a high level of *t*-ABA (Fig. 2B) in "on" and "off" buds. At the first sampling date, there was almost twice the concentration of *t*-ABA in "on" buds as in "off" buds. At later sampling dates, the highest concentration was always in the "on" buds, but the difference was much less. In all cases, the level of *t*-ABA was five to 10 times the level of ABA (Fig. 2C). Levels of both ABA and *t*-ABA declined in both "on" or "off" buds as the season progressed. These findings are in dramatic contrast to most reports where the principal compound found has been ABA rather than *t*-ABA (2, 3).

DISCUSSION

The time at which citrus buds begin growth in the spring in California depends on temperature as well as on a readiness for growth. Such buds do not require chilling. It appears that readiness for growth must depend on some internal factor. In the spring, buds on "on" trees are less ready to grow than buds on "off" trees. This could be because of more inhibitors in the "on" buds. The source of inhibitors may be the fruit. Goldschmidt *et al.* (3) reported that fruit contains mostly neutral inhibitors and only small amounts of free and bound ABA. They found only traces of *t*-ABA. In the buds, we found no bound ABA but large amounts of *t*-ABA. The difference in ABA between "on" and "off" buds was found to be very small. It is unlikely that these small differences account for the difference in readiness to grow. The readiness to grow of the "off" buds as contrasted with the "on" buds appears to be related to the lower level of *t*-ABA in the "off" buds. Cornforth *et al.* (1) reported that *t*-ABA has little or no biological activity as a growth inhibitor. However, Sondheimer and Walton (9) found *t*-ABA 6% as active as ABA when tested by inhibition of growth of bean embryonic axes. With the large amounts of *t*-ABA in citrus buds (up to 140 µg/g fresh weight in the most inactive buds), even this low inhibitory action could be effective in holding buds dormant. Also, since the biosynthesis of ABA involves the synthesis of *t*-ABA and its subsequent conversion to ABA (7), it is possible that *t*-ABA simply represents a continuous source of ABA precursor for maintenance of dormancy.

We are confident that the high ratio of *t*-ABA to ABA represents the *in vivo* situation. The high ratio cannot be due to photoisomerization during extraction and subsequent laboratory manipulation. When ABA is exposed to light, it isomerizes to give a 1:1 equilibrium mixture of ABA and *t*-ABA (5). As we reported above, we found *t*-ABA to be 5- to 10-fold greater than ABA in dormant citrus buds. If *t*-ABA had arisen *in vitro* from photoisomerization of ABA, we would have found no more than a 1:1 ratio of *t*-ABA to ABA. On the other hand, we must admit that our values for ABA may be in error due to possible *in vitro* conversion of *t*-ABA to ABA.

Speculations concerning the high level of *t*-ABA in citrus buds are in order. We offer the following possibility. Citrus buds are protected by several prophylls (8) which are probably opaque to UV. Since the biosynthesis of ABA involves the synthesis of *t*-ABA and its subsequent conversion to ABA (5) and since *t*-ABA is isomerized to ABA by light (6), the conversion of *t*-ABA to ABA in the citrus buds would be slow, hence, the high level of t-ABA in citrus buds as we have reported.

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