Gibberellin-Delayed Senescence and Essential Oil Changes in the Navel Orange Rind

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Composition of essential oils of the flavedo of navel orange (*Citrus sinensis* L., Osbeck) was examined by gas-liquid chromatography from the beginning of the harvest season until past the normal harvest period. Quantitative changes of several minor constituents occurred during the 28-week interval. Concentrations of linalool and geranial decreased in a linear fashion. The concentration of valencene remained relatively constant through March and then increased and remained high throughout the remainder of the season. Gibberellic acid, which delays senescence of navel orange rind tissue, minimized changes. These data suggest the possibility that a senescence index, based on quantitative changes in certain oil constituents, can be developed.

Under California cultural and marketing conditions the navel orange may be stored on the tree for 5 or 6 months after legal maturity is attained. Subsequent to harvest, many fruit are shipped long distances before they ultimately reach the consumer. Although internal quality remains high during this lengthy preharvest and postharvest period, the rind presents a wide range of problems, especially during the latter half of the harvest season. These problems are attributed to senescence.

Gibberellic acid (GA_3) delays certain aspects of aging of rind tissue when applied preharvest to navel oranges. The following changes, which are characteristic of maturation and senescence of navel orange rind tissue, are delayed by GA_3: loss of chlorophyll (Coggins and Hield, 1962); accumulation of carotenoid pigments (Lewis and Coggins, 1964); softening (Coggins and Lewis, 1965); changes in carbohydrate metabolism and cation composition (Lewis *et al.*, 1967); and development of a structurally weak tissue as determined by histological studies (Coggins and Hield, 1968).

Physiological rind disorders that appear to be associated with aging include rind staining, water spot, increased susceptibility to decay, puffy rinds, and the accumulation of rind exudates. These disorders are reduced by GA_3 (Coggins and Eaks, 1967). Since physical, anatomical, chemical, and physiological rind disorder data indicate that GA_3 delays rind maturation and senescence, it is safe to assume that the effect is real.

Evidence that composition of orange rind essential oils changes during advancing maturity and senescence has been available for some time (Eschinazi, 1952; Kesterson and Hendrickson, 1953). Recent advances in gas-liquid chromatography have made it possible to examine quantitative changes in particular constituents. Data reported for Valencia oranges (Scora and Newman, 1967) show that certain quantitative changes occur during maturation. Similar techniques indicate that concentrations of many rind oil constituents vary during maturation and senescence of navel oranges (Scora *et al.*, 1969).

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Physiological age of the rind is probably the prime factor involved in postharvest problems. These problems vary from year to year and from grove to grove during a particular harvest season. When a particular grove should be harvested, and predictions as to whether the fruit will tolerate long-distance transport, should be based on physiological age or degree of senescence. No objective method, however, is available to determine degree of senescence.

Since rind oil composition appears to change during maturation and senescence, it is possible that a senescence index can be based on evaluations of oil constituents. Furthermore, it should be possible to delay senescence by the use of GA_3 and obtain a reasonably accurate initial evaluation of the magnitude of differences that exist between fruit that are susceptible to physiological rind disorders and those that are considerably less susceptible. Such an evaluation forms the basis for the study reported in this paper.

MATERIALS AND METHODS

Navel orange fruit located on large trees near Riverside, Calif., were selected for uniformity of size, shape, texture, and exposure. The experimental design was a paired comparison, with one member of the pair serving as the untreated control. The second fruit and a few adjacent leaves were submerged for 30 seconds in aqueous solutions of 500 p.p.m. acid equivalent potassium gibberellate containing 0.02% (v./v.) of a wetting agent (X-77). Fruit were dark green in color when treated on October 27, 1965.

Beginning 10 weeks and continuing for 38 weeks after treatment, samples were collected at 4-week intervals—from the beginning until well beyond the normal harvest period. Data for each sampling date were obtained from 20 pairs of fruit representing one pair from each of 20 trees.

The rind from each fruit was macerated in cold water, the slurry was transferred to a large round-bottomed flask, and the

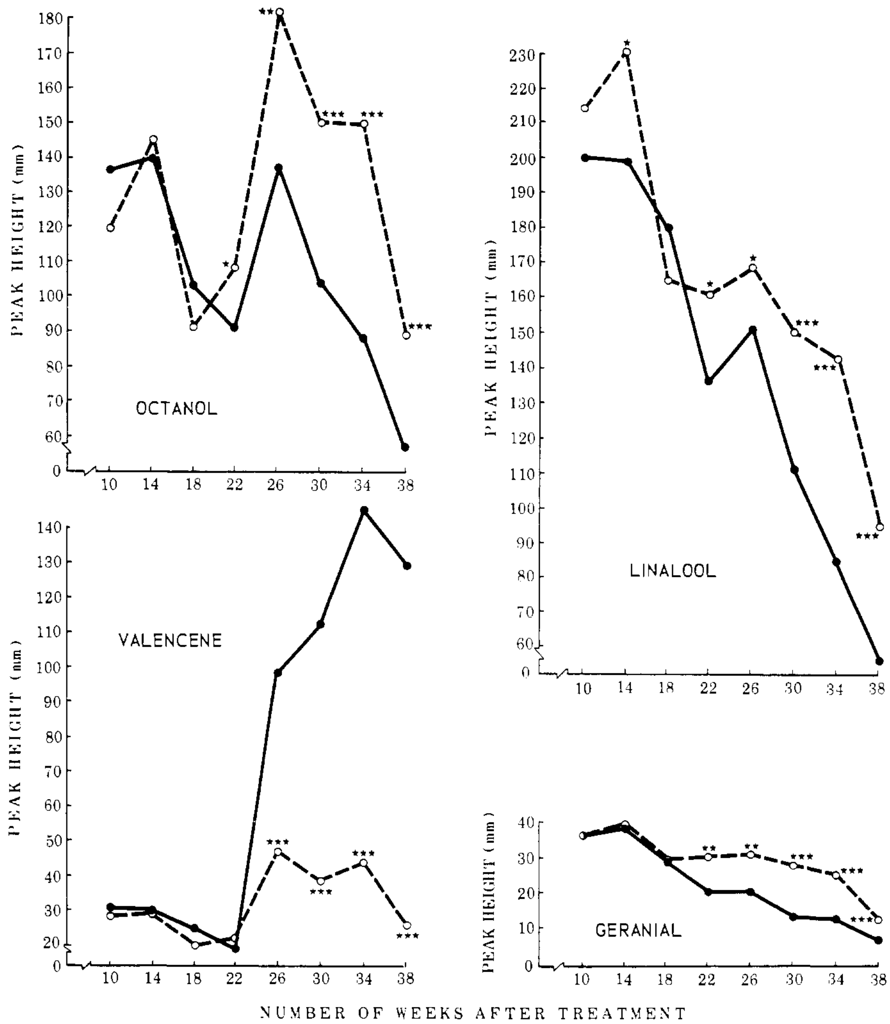

Figure 1. Changes in concentrations of essential oil constituents of navel orange flavedo as related to time and to GA_3 treatment

Samples collected January 3 (10 weeks after treatment) through July 18.
—— Untreated fruit
- - - Fruit receiving GA_3
*, **, ***. Means significantly different at 0.05, 0.01, and 0.001 levels of probability, respectively.

oil was removed by steam distillation. Oil samples were immediately injected into a gas-liquid chromatograph (Varian Aerograph A-90-P). The conditions were those described by Scora and Bitters (1967), except that the sample injected was 20 μl., to obtain relatively large peaks for the minor constituents. Data were subjected to statistical evaluations.

Pertinent data are shown in Figure 1. The oils of principal interest occurred in low concentrations (0.1 to 1.3% of total). Since the main objective was to evaluate quantitative changes as a possible index of senescence, we considered it important to express each oil independently rather than as percentage of the summation values for all constituents recovered and accounted for in our chromatographic system. This is important if one considers the possibility that constituents not accounted for vary in quantity with degree of maturity or senescence.

Although we have elected to express concentrations in a form directly related to quantity per unit volume of distilled oil, we cannot assign absolute values to them, because the samples used to determine linearity of peak height to concentrations contained unknown amounts of impurities. It does not detract, however, from the usefulness of these data for the purposes of our study.

Probable identity of the compounds specified in Figure 1 was based on the following comparisons with authentic material: relative retention times; cochromatography; and infrared spectra of collected material. An especially good hand-in-glove fit was obtained for authentic and presumed valencene. This was probably due to the high degree of purity of the two samples.

RESULTS AND DISCUSSION

Preliminary examination of the resulting chromatograms indicated that certain minor constituents were of principal interest. According to data obtained from injection of appropriate dilutions of these compounds, areas under curves, as well as peak height, were lineally related to concentrations. Since two compounds of interest (octanol and linalool) were recorded as somewhat overlapped curves, it appeared that less error would result if corrected peak height rather than corrected area were used. Thus, in spite of the greater influence of temperature on peak height than on area, we feel that peak height is the better way to express our data. The fact that instrument temperature variations were small influenced our decision. Furthermore, in the absence of automatic equipment for integration of area under curves, peak height would be the most useful procedure if such evaluations were to be used routinely.

Concentrations of some of the constituents appeared to change little if any as the season progressed. Examples are those which were tentatively identified as *d*-limonene (the primary component of navel orange oil), nonanal, octyl acetate, decanal, neryl formate, citronellyl acetate, neryl acetate, citronellol, and neral.

The concentration of octanol (Figure 1) decreased substantially from the beginning to the end of the experiment, but concentrations fluctuated widely from one date to the next. Unless degree of senescence is highly reversible, it is unlikely that octanol can be used as a senescence index. Although it is not unlikely that rate of senescence changes with time, the direction probably does not change. Concentrations of linalool and geranial decreased in a fairly smooth fashion as the harvest season advanced. The quantity of valencene remained relatively constant through March and then increased and remained relatively high throughout the remainder of the season. These data suggest that it may be possible to develop a senescence index with any of these compounds, but the extremely small peaks obtained with geranial direct attention to the other two constituents.

The fact that dramatic changes in valencene concentrations occur at approximately the same time reported for certain biochemical and physiological changes (Lewis *et al.*, 1967) suggests that valencene concentrations could be used to determine when navel orange rind tissue has reached a critical stage of senescence. Whether either of these essential oil constituents can be used to predict susceptibility to physiological rind disorders, however, must await studies where correlations with the various disorders are determined. If suitable correlations exist, it would be operationally feasible to utilize such an index in reaching harvest, packing, and marketing decisions.

The magnitude of change for linalool, geranial, and valencene was less in GA_3-treated fruit. Since physical, anatomical, chemical, and physiological rind disorder data indicate that GA_3 delays maturation and senescence, these results strengthen the hypothesis that concentrations of these oil constituents are related to degree of sensescence. The fact that concentration changes of valencene were modified to a greater degree than any of the others, suggests that valencene concentration is a more sensitive index of senescence than the other two. Concentrations of octanol were influenced by GA_3, but because of the cubic nature of the curve, it is difficult to use it as a senescence index. The ideal index requires that samples be collected only once, or at most a few times. Such a procedure would yield useless information if a cubic function were employed.

Qualitative as well as quantitative changes in essential oil composition are being investigated for usefulness as taxonomic tools. These data emphasize the need to evaluate tissues of equivalent physiological age where quantitative data are used, and indicate the need for extreme caution where the concentration of a compound, such as octanol, varies in a complex fashion.

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