Short Communication

Involvement of Endogenous Ethylene in the Induction of Color Change in Shamouti Oranges

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ABSTRACT

Reducing the level of endogenous ethylene in detached Shamouti oranges (*Citrus sinensis* L. Osbeck) by means of subatmospheric pressure did not alter the rate of chlorophyll destruction and color changes during the first 8 days after harvest in the presence or absence of exogenous ethylene. Reducing the activity of ethylene by means of CO_2 — known to be a competitive inhibitor for ethylene — inhibited chlorophyll destruction and color change in Shamouti oranges ventilated with ethylene, but had no effect on these processes in the absence of applied ethylene. The evidence presented indicates that endogenous ethylene may not be the primary inducer for the natural color change in detached Shamouti oranges.

Citrus fruits have generally been recognized as nonclimacteric fruit since an increase in respiration and ethylene production apparently does not occur after the mature fruit has been removed from the tree (1, 5). Orange fruits have been reported previously as not evolving ethylene (1), but the introduction of gas chromatographic techniques revealed measurable, although relatively small, concentrations of ethylene in both emanations and internal atmosphere of mature orange fruits (4). Prominent increases in both respiration and ethylene production have been noted when young citrus fruits are removed from the tree (2).

Exogenously applied ethylene causes rapid degreening of citrus fruit rind (1). Ethylene not only enhances the destruction of Chl but also promotes the subsequent build up of carotenoids (1, 13). Relatively high concentrations of ethylene are required for half-maximal color change response as compared with other ethylene-induced phenomena (1). In addition, exogenous ethylene induces a marked increase in respiration of citrus fruits at various stages of maturity (5).

In view of the complex behavior of citrus fruits, it is difficult to evaluate the role of endogenous ethylene in color change induction of citrus fruits. It seems to be generally accepted, although never experimentally proven, that endogenous ethylene triggers the natural color changes of maturing citrus fruit rind (1, 10).

MATERIALS AND METHODS

'Shamouti' oranges (*Citrus sinensis* L. Osbeck) were picked in a local orchard in a mature green stage. Fruits were selected for uniformity in terms of size and color, weighed, and placed in 10-liter desiccators maintained at 24 C. Each desiccator contained

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25 fruits. In cases where subatmospheric pressure was employed to reduce endogenous ethylene levels, the desiccators were continuously evacuated by means of a vacuum pump. Moisturized pure O_2 or a gas mixture was admitted to the system continuously via a vacuum regulator which maintained a pressure of 150 mm Hg. A rotameter, attached to the inlet of the vacuum regulator, was used to measure the gas flow through the apparatus. In experiments conducted at atmospheric pressure, the fruits enclosed in the desiccator were ventilated continuously with the desired gas mixture. The desiccators were opened periodically, and fruit color was determined according to the color chart of Harding *et al.* (12). The "color degrees" were transformed into a numerical color index of 1 to 12, corresponding to the A to L degrees of Harding's color chart. Chl content in rind samples was determined in 80% acetone extracts according to Bruinsma (6). Air samples were taken from the internal atmosphere of fruits and analyzed for ethylene and CO_2 contents. In some cases fruits were enclosed in sealed jars for several hours, and ethylene and CO_2 emanation was determined by gas chromatography.

RESULTS

Effect of Subatmospheric Pressure. Detached mature green Shamouti orange fruits lose their Chl rapidly, without an apparent lag phase (Fig. 1). However, the change in color (Fig. 2), which includes both Chl destruction and carotenoid accumulation, shows a certain lag during the first 4 days after harvest, but the color changes rapidly subsequently.

Gassing the fruits with either 0.1 or 5.0 $\mu l/l$ ethylene markedly accelerated both Chl destruction (Fig. 1) and overall color change (Fig. 2). Calculating the time to 50% rind Chl destruction shows that 5 days were required for the control fruits, whereas 3 and 2 days were required for fruits gassed with 0.1 and 5 $\mu l/l$ ethylene, respectively (Fig. 1).

Exposing the fruits to subatmospheric pressure (150 mm Hg of water-saturated O_2) did not alter the initial rate of Chl destruction and overall color change, and color changes proceeded normally in both control and ethylene-treated fruits (Figs. 1 and 2). A certain delay in color changes occurred in fruits exposed to subatmospheric pressure during the later stages of color development, from the 8th day after harvest.

The data of Figures 1 and 2 represent one of two experiments which gave essentially the same results, indicating no effect of subatmospheric pressure on color changes of Shamouti orange fruits.

Effect of CO_2. Figures 3 and 4 illustrate the effect of CO_2 on the natural and ethylene-induced color change of mature green Shamouti oranges. The natural color change (in the absence of exogenous ethylene) was hardly affected by CO_2 at 0.5 and 1.5%, a slight inhibition effect was noticed in Chl destruction (Fig. 3), while no effect was recorded for the overall color
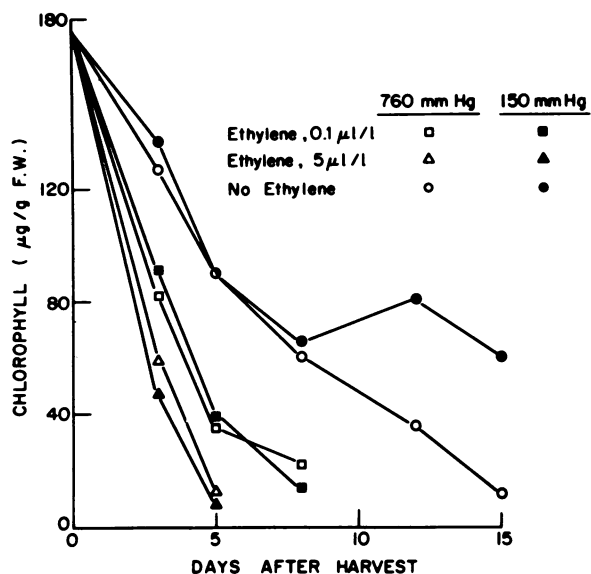
FIG. 1. Kinetics of rind chlorophyll destruction in detached Shamouti oranges. Fruits were maintained at atmospheric or subatmospheric pressure with or without ethylene provision.
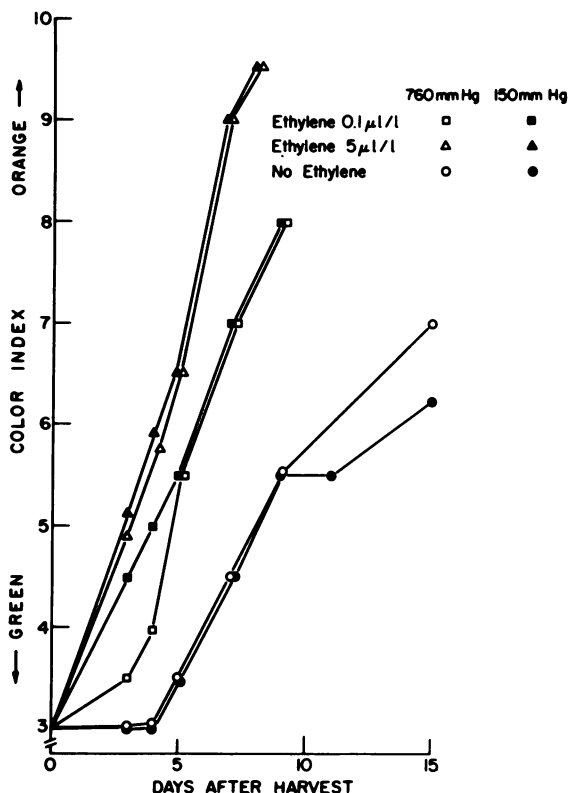

FIG. 2. Kinetics of rind color change in detached Shamouti oranges. Fruit were maintained at atmospheric or subatmospheric pressure with or without ethylene provision.

change (Fig. 4). CO_2 at 5%, however, markedly inhibited both Chl destruction and overall color change (Figs. 3 and 4).

A pronounced inhibitory effect of CO_2 on color change was evident in the presence of 0.1 $\mu l/l$ ethylene. The ethylene-induced color change was partially reversed by 0.5 and 1.5% CO_2, while 5% CO_2 almost completely counteracted this effect of ethylene (Figs. 3 and 4).

Ethylene and CO_2 Production. Low rates of ethylene emana-

tion were recorded during the first day after harvest ranging from 0.007 to 0.010 $\mu l/kg \cdot hr$. Rates of ethylene production increased subsequently reaching peak values of 0.072 to 0.110 $\mu l/kg \cdot hr$ 2 to 4 days after harvest. A moderate peak in respiration, ranging from 9 to 11 ml $CO_2/kg \cdot hr$ occurred on the 4th day after harvest, corresponding roughly to the peak of ethylene production. Ethylene and CO_2 production decreased sharply thereafter, attaining values of the same order of magnitude as fruits at harvest time.

Measurement of ethylene contents in the internal atmosphere of mature green Shamouti oranges at harvest is encountered with difficulties due to the very low concentrations of ethylene, which are often barely detectable even with modern gas chromatographic devices. In addition, determinations conducted with individual fruits immediately after harvest revealed considerable variability, ranging from trace amounts to 0.050 $\mu l/l$ of ethylene. Higher internal ethylene concentrations up to 0.200 $\mu l/l$ were found in fruits 3 to 10 days after harvest.

DISCUSSION

When fruits are exposed to subatmospheric pressures, the outward diffusion of volatiles is accelerated, thus reducing their levels in internal fruit atmosphere (8). Ethylene concentration in the internal atmosphere of Shamouti oranges at harvest time ranged from trace amounts to 0.04 $\mu l/l$. Reducing the endogenous concentrations through subatmospheric conditions by 80% should have resulted in internal ethylene concentrations below the threshold known for any ethylene-induced phenomenon (1).
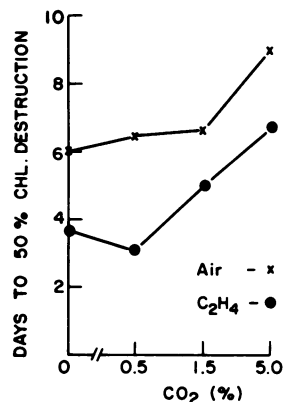

FIG. 3. Effect of CO_2 concentration on the rate of rind chlorophyll destruction in Shamouti oranges in the presence or absence of ethylene gas.
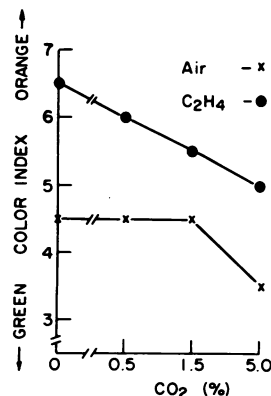

FIG. 4. Effect of CO_2 concentration on rind color change in Shamouti oranges in the presence or absence of ethylene gas. Rind color was determined on the 9th day after harvest.

Half-maximal response for rind color change in citrus fruits is obtained with ethylene concentrations which are higher than those required for most ethylene-induced phenomena (1).

Numerous ethylene controlled processes like fruit ripening (7), leaf abscission (9), and cell division (3) have been altered by reducing endogenous ethylene concentrations through subatmospheric pressure. Our experiments showed that Shamouti oranges held at subatmospheric pressure lost their Chl and changed their color at a rate similar to that of fruit held at atmospheric pressure during the first 8 days after harvest (Figs. 1 and 2). This may indicate that endogenous ethylene does not play a major role in the induction of coloring processes in Shamouti oranges.

The activity of CO_2 as a competitive inhibitor of ethylene action (8) served as another tool for examination of the role of endogenous ethylene in rind color change induction. Our results showed clearly that CO_2 at 0.5 and 1.5% had almost no inhibitory effect on the natural Chl destruction and color change (Figs. 3 and 4). CO_2 at 5% had an inhibitory effect which could be attributed to its general effect on fruit metabolism (14). An inhibition by low CO_2 concentrations was demonstrated clearly in the presence of 0.1 $\mu l/l$ exogenous ethylene (Figs. 3 and 4). This effect is concentration-dependent in accordance with the ethylene-CO_2 competitive antagonism hypothesis (8). The fact that 0.5 and 1.5% CO_2 did not alter the rate of the natural fruit coloration, although it affected the exogenous ethylene induced process, suggests that the natural color change may not be dependent on endogenous ethylene.

Very low rates of ethylene production have been obtained immediately after harvest in our experiments, while barely detectable and highly variable values have been obtained in samples of internal fruit atmosphere. It seems clear that the amounts of ethylene present within the fruit at harvest are not capable of inducing the initial rapid loss of Chl and overall color change. The higher rate of ethylene production found subsequently (days 2–5) may enhance the later stages of detached fruit coloration. It has been shown that the later stages of Chl destruction and color change (from day 8 on, Figs. 1 and 2) were somewhat delayed by subatmospheric pressure.

The fact that a physiological process may be induced by an exogenous supply of a chemical does not in itself prove that this chemical is the inducer of the spontaneous process in nature. Consequently, the induction of both Chl destruction and carotenoid biosynthesis by ethylene (1) cannot be considered as sufficient evidence for the role of ethylene in citrus fruit coloration. The experiments of the present study have not provided rigorous proofs for the role of ethylene in citrus rind coloration. On the contrary, we are left with serious doubts as to the role of endogenous ethylene in our system.

A comprehensive discussion of citrus fruit color regulation must take into account several additional factors. Among these, the sensitivity of the tissue to ethylene seems to be of great importance (7, 8). The flow of growth substances, presumably gibberellins and cytokinins (11), from the tree is believed to maintain the fruit at a low sensitivity to ethylene (7). This flow may be responsible for the slower rate of color changes in fruit on the tree, as compared with detached fruit (11). Measurements of sensitivity to ethylene of citrus fruits are required before a final conclusion on the role of ethylene in citrus rind coloration can be reached.

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