

異なった発育段階で収穫された温州ミカン果実のエチレン生成と呼吸

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Ethylene Production and Respiration of Satsuma Mandarin (*Citrus unshiu* Marc.) Fruit Harvested at Different Stages of Development

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Summary

The young, immature fruit of satsuma mandarin produced a great amount of ethylene. Starting after a few days off the tree, the fruit began producing ethylene at an increasing rate, rising to a maximum and falling off thereafter. The highest rate was obtained for the fruit harvested June 17. The rate of ethylene production decreased as the fruit increased in weight. For the young fruit harvested in May and June, the high rates of ethylene production were associated with browning of the fruit. For the fruit sampled in July browning was not observed and the increasing rate of ethylene production paralleled that of respiration, followed by yellowing of the rind. The fruit harvested in September and those of all subsequent harvests produced no measurable quantities of ethylene.

When young fruits were cut into small pieces, a large quantity of ethylene was evolved during the course of incubation. The smaller the segments the faster the rise in the rate. Flavedo, albedo and pulp tissue separated from the intact fruit also produced ethylene in a great amount. The ethylene production by the excised segments or albedo tissue was markedly prevented by the addition of cycloheximide.

Introduction

Citrus fruits have been classified by Biale as non-climacteric fruit from the evidence that ripe fruits exhibit no post-harvest increase in respiration(4). However, Aharoni has demonstrated that in young, unripe oranges and grapefruit, there was a marked increase in respiration after harvest, paralleling a marked ethylene production, which resembled the climacteric type of respiration and ethylene production(2). Eaks also found the remarkable rise in respiration after harvest for the young fruits of grapefruit and oranges but not for lemon, which he referred to as a pseudo-climacteric(5). But the increase in respiration and ethylene production shown by young and immature citrus fruits were considered to be different from those found in typical climacteric fruits from the response to applied ethylene(3,5). A similar observation was

made by us for satsuma mandarin fruit that the young fruits produced ethylene remarkably after harvest(6). The present paper deals with ethylene production and respiration of satsuma mandarin fruit harvested at different stages of development from May to November. A marked production of ethylene by excised segments of fruit or tissues like flavedo, albedo and pulp is also reported.

Materials and Methods

Satsuma mandarin fruit(*Citrus unshiu* Marcovitch, cv. Owari) were harvested during the months from May to November 1975. Blooming was almost completed by the middle of May. The fruit became almost entirely ripe on the tree by the end of November. Small fruits harvested in May, June and July were placed in 1.5-liter desiccators. Larger fruits harvested from September to November were placed in 10-liter desiccators used as the respiratory chambers. The desiccators were sealed with

covers with one-hole silicone rubber stoppers. They were covered with black cloth to prevent the effect of light. After closure for 2 to 3 hr, the atmosphere in the desiccators was sampled through the stoppers with a plastic syringe, and ethylene and CO_2 concentration were determined. Determinations were carried out daily at 22°C.

Ethylene was measured on a Hitachi 063 gas chromatograph equipped with a hydrogen flame ionization detector and an activated alumina column. This system could detect 0.02 μl ethylene per liter in a 1.0 ml gas sample. CO_2 concentrations were determined by thermal conductivity gas chromatography using a Porapak Q column. Ethylene production and respiration were expressed on fresh weight basis.

For the measurement of ethylene production by excised tissues, fruit were cut longitudinally into halves to eighths. They were dipped in a 1% KCl solution or in that containing

cycloheximide and subjected to vacuum infiltration under 50 mmHg for 5 min. The vacuum infiltration was very effective to administrate inhibitors such as cycloheximide into segments. After the infiltration they were blotted with filter paper and placed in 130 ml Erlenmeyer flasks, then incubated at 26°C in the dark. The flasks were sealed with rubber serum caps (SGA Scientific Inc.) and the gas phase sample in the flasks was periodically determined for ethylene by gas chromatography. After each sampling, the flasks were flushed with air and then resealed. In later experiments as fruit developed, flavedo, albedo and pulp were separated from the fruit and individual tissues were measured for ethylene production.

Results

1. Ethylene production and respiration of whole fruit

The fruit harvested May 28 (average fresh weight of fruit : 0.23 g) produced ethylene as

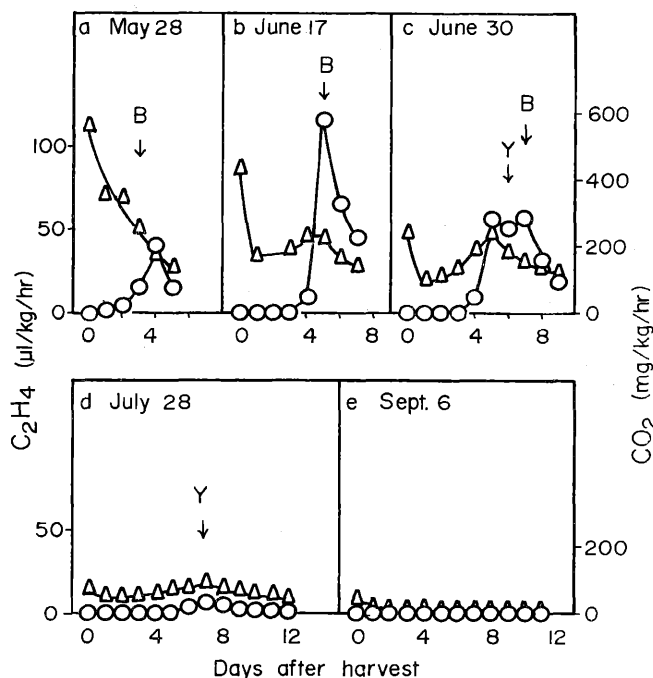


Fig. 1. Ethylene production and respiration of satsuma mandarin fruit at 22°C harvested at different stages of development from May to September. The rate of ethylene production (O—O) was expressed as μl of ethylene per kg fresh weight per hr. The rate of respiration (Δ — Δ) was expressed as mg of CO_2 evolved per kg fresh weight per hr. The points labeled with arrows indicate the times when fruit were turning brown (B) or yellow (Y).

much as $40.7 \mu\text{l}/\text{kg}/\text{hr}$ 4 days after harvest, the rate decreasing subsequently (Fig. 1 a). On the day fruit were harvested (0-day) they produced no detectable ethylene. Three days after harvest the fruit were turning brown. The symptom of necrosis progressed day by day. The rate of respiration was very high initially, but it declined to reach a low level, where almost all fruit became necrotic. The fruit harvested June 17 (average fresh weight: 1.7g) produced much more ethylene than those harvested before. In the early days after harvest they produced no detectable ethylene. The rate of ethylene production began to increase after 4 days, reaching a maximum after 5 days, when it amounted to $116.5 \mu\text{l}/\text{kg}/\text{hr}$ and then fell off (Fig. 1 b). Five days after harvest browning was clearly evident. A high rate of respiration dropped sharply after harvest, which did not keep going down

but rather tended to rise 4 to 5 days. The fruit harvested June 30 (average fresh weight: 4.7 g) produced ethylene greatly after 5 days, but to a less extent than that by the June 17 harvest (Fig. 1 c). The rate of ethylene production exhibited two peaks, the former appearing to coincide with the peak of respiration and relate to degreening (yellowing) of the fruit. The latter peak seemed to be associated with browning. The ability of the fruit to evolve ethylene diminished as the fruit became larger and developed. As seen in Figs. 1d and 2 a, the fruit of July 28 harvest (average fresh weight: 19.2 g) produced only $6.6 \mu\text{l}/\text{kg}/\text{hr}$ at a maximum reached 7 days after harvest. In this case, however, there was a close parallelism between ethylene production and respiration (Fig. 2 a), suggesting that the rise in respiration could be caused by the ethylene produced. The yellowing of the fruit peel

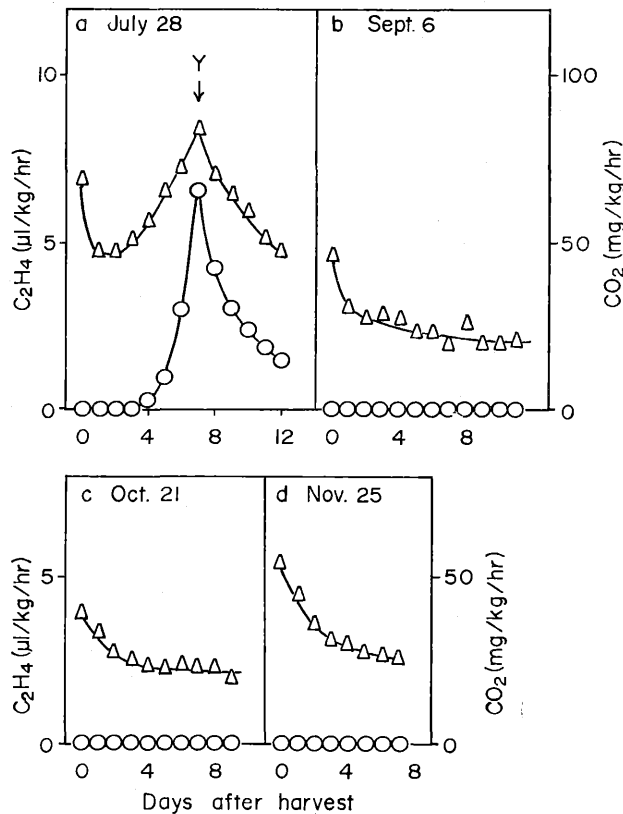


Fig. 2. Ethylene production and respiration of satsuma mandarin fruit at 22°C harvested at various stages of development from July to November. Symbols as in Fig. 1.

was clearly evident as the fruit produced the highest amount of ethylene (Fig. 2 a). The fruit harvested Sept. 6 (average fresh weight: 48.4g) produced no detectable ethylene throughout the post-harvest experimental period (Fig. 2 b). There was also no increase in respiration. A sharp respiratory decline was followed by a subsequent gradual decrease. The same tendency was obtained for the fruit harvested Oct. 21 (111g/fruit) and Nov. 25 (115g/fruit) (Figs. 2 c and 2 b). In neither case was detectable ethylene produced.

2. Ethylene production by excised tissues

When young fruit were cut into small pieces (e. g. quarters) and incubated in the dark at 26°C, a large quantity of ethylene was evolved in a rather short time as shown in Fig. 3. At a peak reached after incubation for about 40hr the rate amounted to 161.0nl per g fresh weight per hr. The great amount of ethylene produced by the excised segments was reduced almost completely by applying 3.6×10^{-5} M cycloheximide known to be an inhibitor of

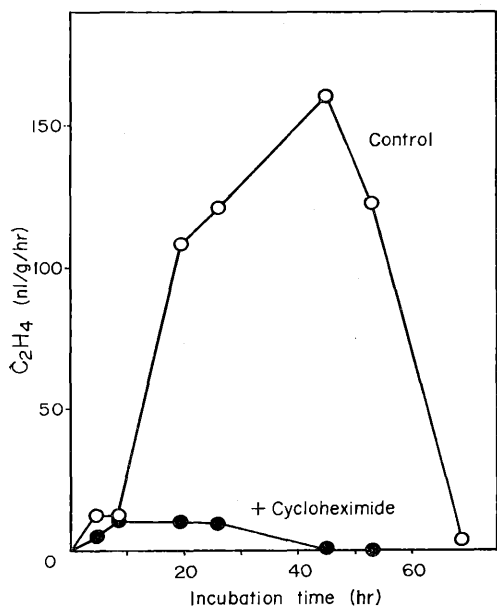


Fig. 3. Ethylene production by excised segments of young fruit of satsuma mandarin harvested June 11 during incubation at 26°C in the dark. Whole fruit were cut into quarters and those measured for ethylene production. The rate of ethylene production was expressed as nl of ethylene produced per g fresh weight per hr. Cycloheximide (3.6×10^{-5} M) was applied to the segments initially by the procedure of vacuum infiltration.

protein synthesis. Fig. 4 shows the increasing patterns in the rate of ethylene production by the excised segments of young fruit harvested June 16. The whole fruit, referred to as 1/1 in Fig. 4, evolved a slight amount of ethylene shortly after incubation, followed by a marked increase. A much less lag period compared to that of Fig. 1 b could be due to that in this experiment stem ends were removed from the fruit, which may have caused some injury. By cutting into half the rate of ethylene production was greatly stimulated. The smaller the segments the faster the rise in the rate. The ethylene production fell off as necrosis progressed. The fruit harvested July 14 were cut and separated to flavedo, albedo and pulp and those measured for ethylene production. The rate of ethylene production rose first for the pulp, followed by the albedo and flavedo (Fig. 5). The rapid rise induced in the pulp tissue was markedly arrested by cycloheximide.

Discussion

The young, immature fruit of satsuma

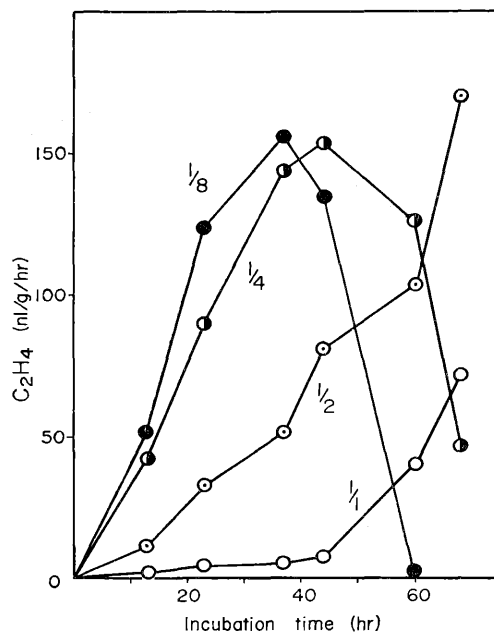


Fig. 4. Ethylene production by excised segments of satsuma mandarin fruit harvested June 16 during incubation at 26°C in the dark. Whole fruit (1/1, ○—○) were cut into halves (1/2, ●—●), quarters (1/4, ●—●) and eighths (1/8, ●—●). The small pieces placed in 130 ml Erlenmeyer flasks were measured for ethylene production.

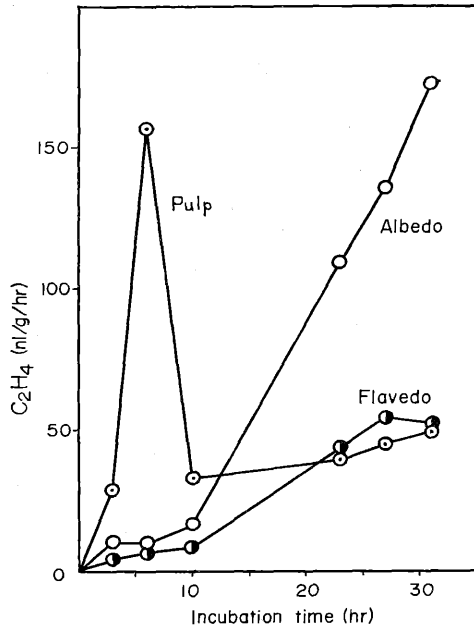


Fig. 5. Ethylene production by flavedo (◐—◐), albedo (○—○) and pulp (●—●) tissue separated from the fruit harvested July 14 during incubation at 26°C in the dark.

mandarin produced a large amount of ethylene. Starting after 2 to 3 days off the tree, the fruit began producing ethylene at an increasing rate, rising to a maximum by 4 to 7 days and then decreasing to a low level. The highest peak was obtained for the June 17 harvest. Similar ethylene productions were observed on successive harvests, but the peak rates decreased as the fruit increased in weight. The fruit harvested Sept. 6 and those of all subsequent harvests produced no measurable quantities of ethylene. For the young fruit harvested by June, the high rates of ethylene production seemed to be closely associated with tissue browning. No browning was observed for the fruit sampled in July and the production of ethylene paralleled the respiratory increase. The increase in ethylene production was also accompanied by yellowing of the rind. For production of ethylene there seem to be three stages: I) high rates of ethylene production with tissue browning; II) relatively lower rates of ethylene production accompanied by respiratory increase and chlorophyll breakdown; and III) no measurable ethylene production with no respiratory increase. It is of

interest to ask how ethylene is caused to produce in the young fruit during the post-harvest period. It may be supposed that removal of fruit from the tree may stop the supply of a substance which could inhibit the functioning of ethylene-producing system and result in the initiation of ethylene evolution. However, this hypothesis is difficult to explain the facts that when young fruit are cut into segments immediately after harvest, ethylene is evolved rapidly and that for the fruit which do not produce ethylene when intact, harvested in September, October and November, a large amount of ethylene is produced by wounding. But by assuming an existence of two systems for ethylene production in the fruit, i. e. one is for intact whole fruit and the other for wounded tissues, these observations would be reconciled.

Wounding is known to cause plant tissues to evolve ethylene(1). Riov *et al* found that grapefruit flavedo disks produced a great amount of ethylene during incubation(7). The separated tissue of satsuma mandarin fruit, namely flavedo, albedo and pulp, produced ethylene with respective increasing patterns. Albedo has been used for the subsequent experiments for studying the mode of ethylene production, because albedo is easy to manipulate, quite a homogeneous tissue and it produces ethylene at a greater rate during the course of incubation. Ethylene production induced in the excised albedo tissue was also completely inhibited by cycloheximide, suggesting that the formation of ethylene-producing system involves *de novo* synthesis of protein. Using labeled methionine by ¹⁴C, it appeared possible that the precursor of ethylene in the albedo tissue was methionine (Hyodo, manuscript in press). Little is known about what kind of substance or condition is responsible for the induction of the ethylene production in the wounded tissue of citrus fruit.

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異なる発育段階で収穫された温州ミカン果実のエチレン生成と呼吸

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摘 要

若い、未熟な温州ミカン果実は著しい量のエチレンを生成した。採取後2、3日して、果実はエチレンを生成し始め、生成速度は増加し、最大に達したのち、減少した。エチレン生成量は6月17日に採取したものが最も大きく、その後果実が生育するにつれ減少した。5月、6月に収穫された若い果実では、著しいエチレン生成は果実の褐変と関連があるように思われる。7月に収穫された果実では褐変はみられず、エチレン生成量の増加は呼吸量の増加と平行しており、果皮の黄化を伴った。9月以降に収穫された果実ではエチレン生成は検出されなかった。

若い果実を小さく切ると(例えば1/4, 1/8にする)非常に大きな量のエチレンの生成が切片を放置(26°C)しておく間にみられた。切片を小さくすればするほど生成速度は速くなった。果実個体から分離されたフラバド、アルベド、果肉も同様にエチレンを生成した。果実切片やアルベド組織から生成されるエチレンはタンパク質合成阻害剤であるシクロヘキシミドを与えることによりほぼ完全に抑えられた。9月以降の果実で収穫後エチレンを生成しなくなったものでも、果実からアルベドを分離して、その切片を26°Cで放置しておくとき著しいエチレンの生成が起きた。