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Effect of Essential Oils on Ethylene Production in Citrus Peel Discs

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Summary

When assayed with peel discs of satsuma mandarin (*Citrus unshiu* Marc.) fruit picked at various maturing stages, ethylene production was promoted in response to β-pinene, myrcene, p-cymene, limonene and γ-terpinene although their high concentrations were inhibitory. In contrast, linalool invariably reduced ethylene production in the peel discs after August. Furthermore, not only increasing the number of discs but also decreasing the volume of headspace in the incubation vials inhibited ethylene production from peel discs. These results suggest that volatiles including essential oils have some regulatory functions in the ethylene production of citrus fruit. Concerning this, flavedo tissues of mature satsuma mandarin contained 13.5 μl/g f.w. limonene and 0.155 μl/g f.w. linalool.

Introduction

There has been much research work about essential oils in citrus. More than 100 compounds are separated from citrus oils[9]. What food scientists are most interested in is the role of the volatile compounds in the aroma and flavor of citrus products. On the other hand, some workers utilize leaf essential oils for the chemo-taxonomic purpose of citrus plants[7,8,10]. However, very little is currently known about their physiological roles in growth, development and ripening of citrus fruit. Iwanami and Hyodo[5] reported that volatiles from peels of lemon, orange and lime inhibited growth of mung bean seedlings. They also found the volatiles from lemon oils alleviated the inhibitory effect of ethylene on the growth of the seedlings. Citrus are known as typical non-climacteric fruit. However young citrus fruit show a burst of ethylene production after picking like climacteric fruits but become typical non-climacteric fruit as they mature[2]. The mechanism that loses the ability to produce ethylene as they mature remains to be solved. Here we report effects of essential oils on ethylene production of citrus peel discs and discuss their potential roles in the regulation of ethylene production in citrus fruit.

Materials and Methods

Plant tissues

Fruit of satsuma mandarin (*Citrus unshiu* Marc. cv. Miyagawa-wase) were obtained from the orchards of Ehime University. Peel discs of satsuma mandarin fruit picked at different maturing stages were obtained using a cork borer (ϕ 12mm). All assays and determinations below were triplicated.

Essential oil treatment and assay for ethylene production

α-Terpinene was obtained from Tokyo Kasei Co. Inc., γ-terpinene from Sigma Chemical Co. Inc., myrcene from Aldrich Chemical Co. Inc., and the other chemicals from Wako Pure Chemical Industries, Ltd. After freshly prepared peel discs were weighed, they were placed in Elrenmyer flasks or glass vials. The vessels were then immediately sealed with glass or rubber stoppers. Just before the discs were placed, essential oil was injected with a microsyringe into a vial, which was then heated to volatilize the chemical and cooled. They were incubated at 25°C for 20 hours and ethylene concentration in the headspace gas was determined on a Shimadzu GC 9A gas chromatograph equipped with an activated alumina column and a flame ionizing detector.

Self inhibition of ethylene production by volatiles from citrus peels

Satsuma mandarin fruit, collected on November 20 and stored at room temperature, were used in early December. In the first experiment, one, four, eight and sixteen discs were placed in a 138 ml-Elrenmyer flask containing 0.5 ml 1N NaOH in absorbent cotton wrapped with aluminium foil and glass stoppered. After 4-hr incubation at 25°C, internal gas was withdrawn and ethylene and other gases were analyzed by gas chromatography. Concentrations of nitrogen, oxygen and carbon dioxide were determined on a Shimadzu GC 8A gas chromatograph equipped with dual columns of Porapak Q and Molecularsieb 5A and a thermal conductivity detector. In the second experiment, a set of four peel discs were placed in various volumes of Elrenmyer flasks or glass vials containing 0.5 ml 1N NaOH in absorbent cotton wrapped with aluminium foil and then rubber stoppered. After 4-hr incubation, the headspace gas was similarly analyzed.

ACC determination

After the headspace gas withdrawn for ethylene determination, the tissues were taken out and extracted with 70% ethanol containing 0.05% (v/v) 2-mercaptoethanol. The extract was evaporated *in vacuo* to dryness and the residue was then taken in 2 ml distilled water. An aliquot of the solution was used for ACC determination after the method of Lizada and Yang[11].

Determination of essential oils

A 100 mg of flavedo tissues of satsuma mandarin fruit was dissected with a surgical knife from the equatorial part of the fruit and placed in a glass vial containing 10 ml of methylene chloride. The tissue was mashed with a pair of surgical nippers in the vial. This procedure enhanced the diffusion of essential oils into the organic solvent. Otherwise the rate of diffusion was very slow. A 1.0 μl aliquot of the solvent was injected to a gas chromatograph (Shimadzu GC-9A) equipped with an SE 30 15% coated Chromosorb WAWDMCS (80-100 mesh) column (0.26 x 300 cm) and a flame ionization detector. Column temperature was programed from 50 to 250°C at an increment rate of 3°C/min, and detector temperature was 260°C. Carrier gas was nitrogen at a flow rate of 38 ml/min. Peak identification of the samples was carried out by comparing their retention time and gas chromatography-mass spectrometry (GC-MS) profiles with those of authentic compounds. GC-MS spectrometry (Shimadzu Auto GC-MS 9020-DF) was carried out by using a capillary column (OV 101 0.25mm x 20m), helium as carrier gas at a flow rate of 10 ml/min, and ionizing voltage at 20 eV.

Results

Effect of essential oils on ethylene production and ACC content of satsuma mandarin peel discs at different mature stages

The peel discs from the fruit picked on July 11 promoted ethylene production in response to 36 nl/ml myrcene (Fig. 1). After August 7, similar dose-response patterns were observed in the discs exposed to β-pinene, p-cymene, limonene and γ-terpinene; the ethylene production was enhanced at 3.6 or 7.2 nl/ml whereas inhibited at higher concentrations. No conspicuous promotion in ethylene production in response to α-pinene, myricene or linalool was noted in the discs after August 7 but the exposure to increasing concentraions of α-pinene and linalool inhibited ethylene production. Linalool showed the greatest inhibition (Fig. 1). On the other hand, ACC content showed increasing tendencies in respose to higher dosages of all essential oils (Fig. 2).

Self inhibition of ethylene production by volatiles from satsuma mandarin peels

Increasing numbers of peel discs in an incubation flask as well as decreasing volumes of headspace of incubation flasks inhibited ethylene production from the peel (Tables 1 and 2). At the end of incubation, carbon dioxide concentrations were less than 0.6% as 0.5 ml of 1 N NaOH had been included in order to prevent the accumulation of carbon dioxide in the flask.

Essential oil content in flavedo of maturing satsuma mandarin fruit

Fig. 3 shows the content of essential oils in the flavedo tissues of satsuma mandarin fruit during maturing period from August 30 to November 12. The average contents of limonene and linalool were 13.5 μl/g f.w. and 0.155 μl/g f.w., respectively. The average amount of total essential oils was 15.6 μl/g f.w. The flavedo tissues from fruit picked August 30 showed lower content than those from more mature fruit.

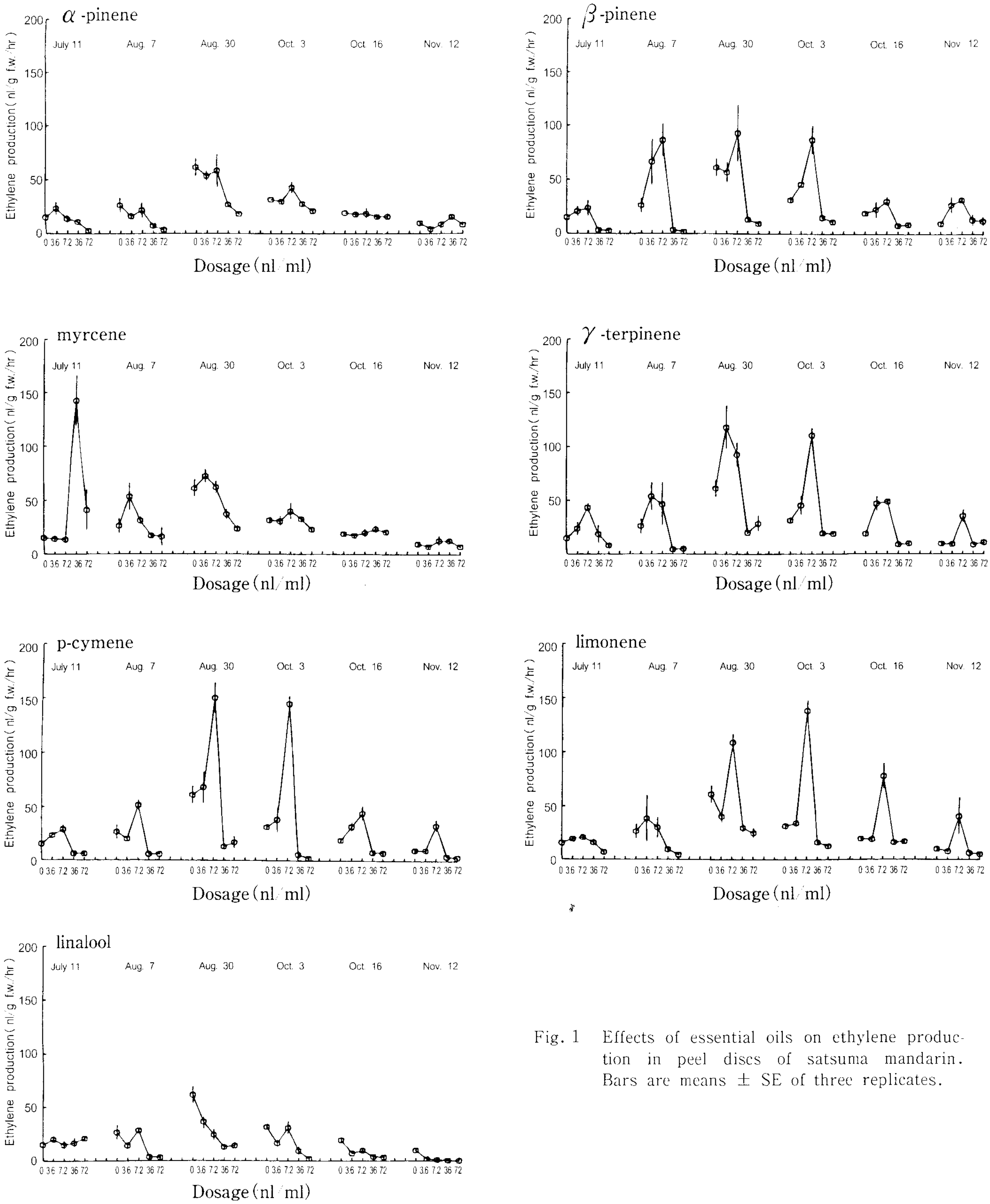
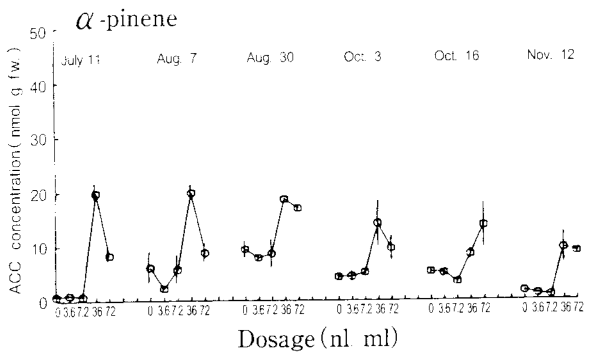
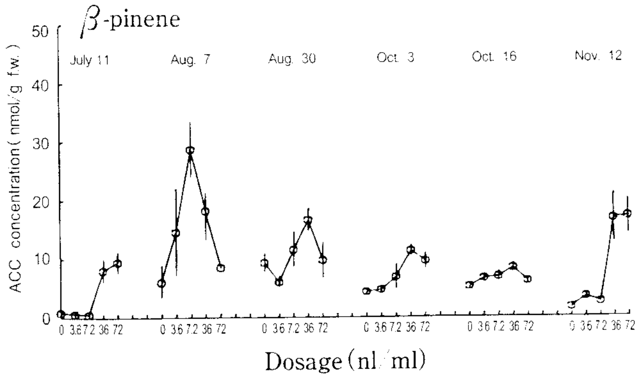
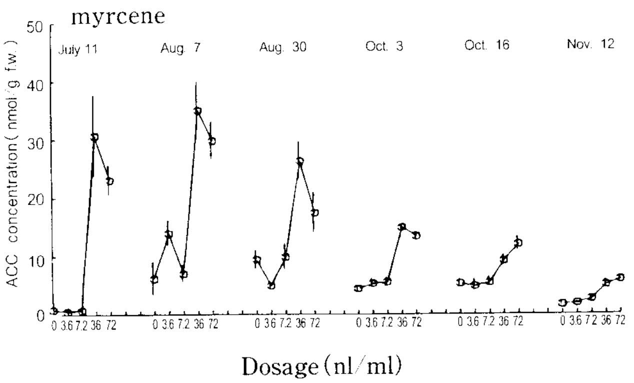
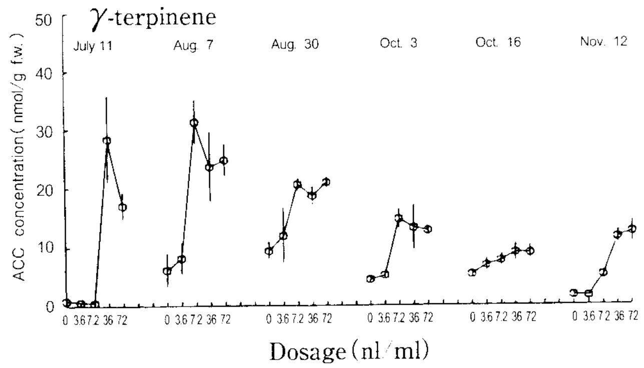


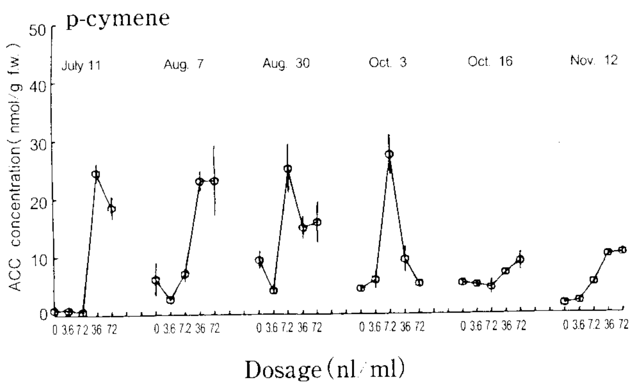
Fig. 1 Effects of essential oils on ethylene production in peel discs of satsuma mandarin. Bars are means ± SE of three replicates.

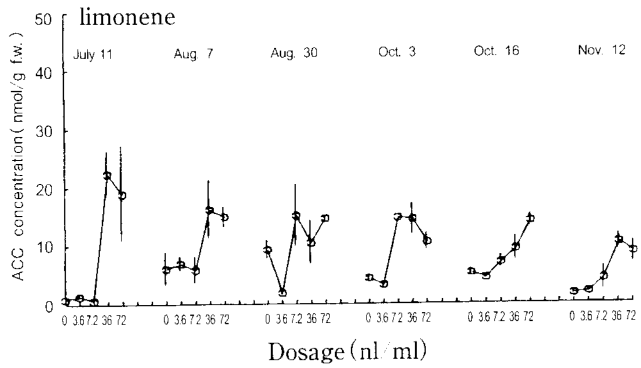












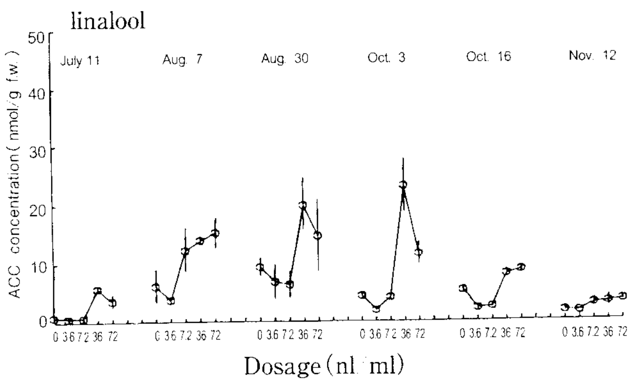


Fig. 2 Effects of essential oils on ACC content in peel discs of satsuma mandarin. Bars are means ± SE of three replicates.

Table 1 Effect of number of peel discs of satsuma mandarin fruit in the incubation vial on their ethylene production. After 4-hr incubation, ethylene, nitrogen, oxygen and carbon dioxide concentrations in the vial were determined by GC. A 0.5 ml of 1N NaOH solution was included in the incubation vial for absorbing carbon dioxide. Results are means ± SE of three replicates.

No. of discs	Weight (g)	C_2H_4 (nl/g f.w./hr)	N_2	O_2 (%)	CO_2
1	0.265±0.030	3.85±0.21	78.70±0.01	20.75±0.01	0.55±0.01
4	0.898±0.043	1.45±0.18	78.87±0.03	20.53±0.01	0.59±0.03
8	1.716±0.088	1.06±0.16	79.35±0.08	20.13±0.08	0.52±0.02
16	3.709±0.217	0.77±0.18	80.15±0.06	19.28±0.07	0.50±0.00

Table 2 Effect of volumes of incubation vial on ethylene production of peel discs of satsuma mandarin fruit. After 4-hr incubation with four discs, ethylene, nitrogen, oxygen and carbon dioxide concentrations in the vial were determined by GC. A 0.5 ml of 1N NaOH solution was included in the incubation vial for absorbing carbon dioxide. Results are means ± SE of three replicates.

Volume of vial (ml)	Weight (g)	C_2H_4 (nl/g f.w./hr)	N_2	O_2 (%)	CO_2
27	1.147±0.034	0.37±0.06	81.01±0.28	18.55±0.31	0.49±0.09
60	1.107±0.012	0.50±0.05	79.82±0.04	19.76±0.03	0.43±0.03
136	1.164±0.055	0.72±0.09	79.36±0.02	20.24±0.05	0.40±0.03
245	1.064±0.009	1.36±0.20	79.20±0.04	20.39±0.11	0.42±0.07

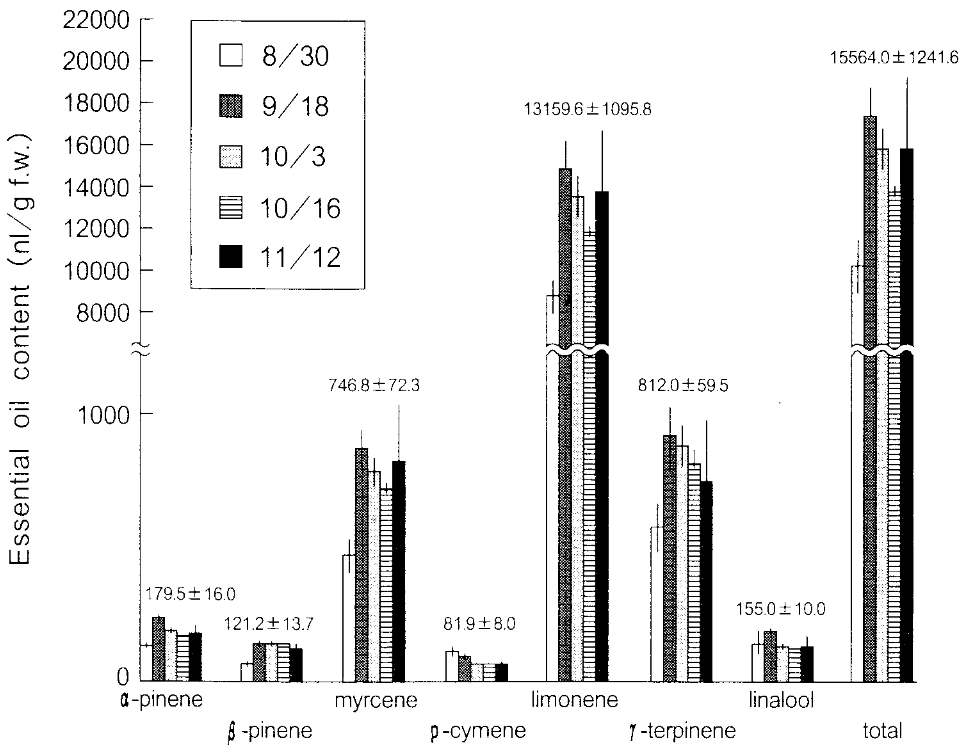


Fig. 3 Changes in the content of essential oils in peels of maturing satsuma mandarin fruit. (Bars are means ± SE of three replicates.)

Discussion

Citrus peels are known to contain essential oils. Although much work has been done about citrus essential oils, little is known about their physiological roles in the fruit growth, development and ripening. Citrus are typical non-climacteric fruit. They show neither climacteric rise in respiration nor a burst of ethylene production in their ripening processes. However, young citrus fruit, when detached from the tree, show a burst in ethylene production like climacteric fruits. The ability of fruit to produce ethylene gradually decreases as the fruit mature[4]. For example, the maximum ethylene production of satsuma mandarin fruit was 230, 2, 0.6, 0.04 nl/g f.w./hr, after picked on June 30, August 11, September 21 and November 30, respectively (unpublished data). However, exogenously applied ethylene can induce a climacteric-like rise in respiration of citrus fruits even at ripening stages[1]. Therefore, non-climacteric behavior of citrus fruit is due to their inability to produce ethylene. However, even the peel discs of mature fruit, when dissected, exhibit ethylene production, probably due to the injury stress.

Hyodo[3] reported that albedo tissues prepared from fruit harvested from August to November showed similar burst patterns in ethylene production during incubation period. Ethylene produciton by pulp, albedo and flavedo of satsuma mandarin fruit picked on July 14 was compared and the flavedo showed the least production[2]. Riov *et al.*[16] similarly reported that flavedo tissues of mature grapefruits also show a gradual increase, clearly not a burst, in ethylene production during incubation. These facts indicate both albedo and flavedo tissues have the potential of ethylene production even at mature stages. Furthermore, the whole lemon fruit can exhibit rapid increase in rates of respiration, ethylene production and degreening in response to gamma radiation[12].

Riov and Yang[17] reported autoinhibition of ethylene production in citrus peel discs and that ethylene inhibited ACC synthesis but not the conversion of ACC to ethylene. Therefore the self-inhibition of ethylene production by volatiles from peel discs of satsuma mandarin in our present work may be due to ethylene released from the tissues (Tables 1 and 2). Iwanami and Hyodo[5] found that lemon oils alleviated the inhibitory effect of ethylene on the growth of mung bean seedlings, although they were equally inhibitory to the growth of the seedlings. Further, Iwanami and Ishibashi[6] reported that the active component of lemon oils was limonene, and that limonene also alleviated the inhibitory effect of citral, an essential oil, on the growth of mung bean seedlings.

Our present results suggest the possible involvement of essential oils in the regulation of ethylene production of citrus fruit. No conspicuous promotion of ethylene production was noted in apple fruit tissues or peach seeds by essential oils[15]. However, essential oils promoted or inhibited ethylene production in the peel discs of satsuma mandarin depending on the age of fruit, the kind and concentration of essential oil employed.

The average limonene content in flavedo tissues of mature satsuma mandarin reaches as high as 13.5 μl/g f.w. (Fig. 3). Linalool, the most inhibitory in our experiments, was 0.155 μl/g f.w. Yajima et. al[19] also reported that 98% of the peel oil of satsuma mandarin was hydrocarbons such as limonene (88.4%), γ-terpinene (4.8%), myrcene (1.0%) and α-pinene (0.64%), and that 2% was oxygenated compounds such as linalool (0.82%) and α-terpineol (0.21%). Sawamura *et al.*[18] reported that rind oil contents of fresh flavedo of 7 different kinds of citrus fruit including satsuma mandarin gradually incereased from middle July to maturation. It is interesting to note that this corresponds to the period during which fruit gradually decrease in the ability to produce ethylene production[4].

Emanation of essential oils from the oil gland may depend on cell permeability of gland tissues. Norman and Craft[13] reported that yellow lemon emanated volatiles greater that green lemon. They also found that exogenous application of ethylene enhanced emanation of essential oils of both types of lemon although green lemon responded to ethylene greater than yellow lemon. This suggests permeability of volatiles through gland cell membranes increases with the ontogeny of fruit. High temperatures also enhance the emanation of volatiles from injured and uninjured Valencia oranges[14].

Therefore, one of probable explanations for the reason why whole intact mature fruit produce only small amounts of ethylene but the dissected discs exhibit a burst rise in ethylene production is as follows : If the autoinhibition of ethylene production as suggested by Riov and Yang[17] is involved in the non-climacteric behavior of citrus fruit, essential oils, like limonene, may alleviate the autoinhibition, which leads to further ethylene production induced by injury stress, when peel discs were dissected from fruit or whole fruit was gamma-radiated. However, whether limonene actually alleviates the action of ethylene in citrus fruit ramains to be investigated. Another explanation is that certain specific essential oils, such as linalool, may be involved in the inhibition of ethylene production during maturation as suggested by our present results.

Riov *et al.*[16] reported that 20% carbon dioxide inhibit ethylene production of flavedo discs, resulting in only 7.5% of air control. Thus, possible involvement of internal carbon dioxide concentrations, combined with ethylene and essential oils, cannot be excluded in the regulation of ethylene production.

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精油成分がカンキツ果皮のエチレン生成に及ぼす効果

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摘　　　　要

精油成分が成熟段階の異なる温州みかん果皮のエチレン生成に及ぼす効果を調査したところ、β-pinene、myrcene、p-cymene、limonene、γ-terpinene は果皮からのエチレン生成を促進したが、高濃度では抑制した。８月以降、linalool はどの段階でもエチレン生成を抑制した。容器に入れる果皮ディスクの数を多くしたり、容器内のヘッドスペースの容量を小さくすると果皮からのエチレン生成は抑制された。これらの事実はカンキツ果皮からの揮発性成分がエチレン生成の調節に関与していることを示している。また、温州みかんの成熟果実のフラベドに含まれる limonene は13.5 μl/g f.w.、linalool は0.155 μl/g f.w.であった。