Effects of Fruit Removal on Photosynthesis, Stomatal Conductance and ABA Level in the Leaves of Vegetative Shoots in Relation to Flowering of Satsuma Mandarin

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Summary

Photosynthetic rate (Pr) and stomatal conductance (Sc) in the leaves of satsuma mandarin cv. Aoshima were measured from July to November on 1) vegetative shoots of intact (control) bearing branch unit (BBU) consisting of two fruit bearing shoots and a vegetative one, and 2) those of BBU from which fruits were removed in June or September. The data between the bearing and non-bearing vegetative shoots are discussed in relation to the flowering potential for the following spring.

Pr in the leaves of the fruit-removed BBU increased by 24% in September, whereas it increased to 41% by October by fruit removal in June. Pr increased only 30% by November when fruits were removed in September. Sc in leaves of fruit-removed BBU increased by 18% in September; but to 46% by October through fruit removal in June; and 114% by November when fruit removal occurred in September.

In relation to stomatal aperture, ABA concentration in the leaves was measured from September to November during which floral initiation of satsuma mandarin is believed to occur. Leaves of BBU had significantly high ABA concentration in September in the case that the fruits were still remained on the unit, but it was much lower in BBU where fruits were removed in June, and even lower when the fruits were removed in September.

These results indicate a possible contribution of Pr increase during the floral initiation period to the vegetative shoots as well as an involvement of ABA through a regulation of stomatal aperture in the leaves during the period. Conclusively fruit removal could enhance flowering rather effectively.

Introduction

In satsuma mandarin, fruit-bearing shoots do not flower the following spring, contrary to non-bearing vegetative shoots. The potential of the vegetative shoots to flower can be influenced by many factors. In the nutrient diversion hypothesis (Ramina et al., 1979; Sachs, 1977), carbohydrates play a dominant regulatory role in citrus flowering. Fruit load suppresses carbohydrate accumulation in the leaves or buds because the fruits act as a strong sink for carbohydrates, reducing the

flowering potential. We (1995) observed a similar phenomenon in the vegetative shoots of satsuma mandarin. Through a comparison of the vegetative shoots on the non-BBU and the BBU, we confirmed that fruit-bearing suppresses flowering while decreasing Pr, especially during the floral initiation period (Inoue, 1990)

Another factor is some plant hormone exported from the fruit which might be involved in citrus flowering at various stages. Foliar applications of GA under various conditions inhibit flowering in most species (Hirose, 1969; Monselise and Goren, 1969). However, it is not certain whether gibberellin is the critical factor which suppresses citrus flowering because other promoters or inhibi-

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tors might also be involved.

This experiment was performed to clarify the effect of fruit removal on the photosynthetic rate and stomatal conductance of satsuma mandarin leaves. We postulate that fruit removal causes carbohydrate accumulation in the leaves and buds of the vegetative shoots which promote flowers in the following spring. Moreover, ABA in the leaves was quantified during the floral initiation period because it may affect photosynthetic rate through a regulation of stomatal aperture.

Materials and Methods

Twenty-year-old satsuma mandarin (cv : Aoshima) trees on trifoliate orange rootstocks grown in the open field were used. Branches consisting of both vegetative shoots and fruit-bearing shoots were selected (Fig. 1). The BBU (shoot length : 14.3 ± 5.6 cm, avg $\pm$ s.d, $n = 9$; number of nodes : 9.9 ± 1.7 , avg $\pm$ s.d, $n = 9$) served as control. Fruits were removed in mid-June or mid-September from bearing shoots. Thereafter, new shoots did not sprout from this branch unit. Thus, the effects of fruit removal on photosynthetic rate (Pr), stomatal conductance (Sc), and ABA concentration in the leaves of the vegetative shoots were investigated in the current season's growth. Flower counts were made in the following spring. Pr and Sc were measured in triplicate on three terminal leaves of each vegetative shoot at the beginning of each month from July to November. The measurement were made with a portable porometer (Koito industry, KIP-8510) in the morning under a photosynthetically active and saturated radiation above $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$.

Triplicate ABA analyses were performed on the three terminal leaves of the shoots. ABA was ex-

tracted overnight using 20 ml of extraction solvent (ethanol : acetic acid, 98 : 2, v/v) at 4 °C in the dark and purified by the procedure of Okuda et al. (1995). A known amount of ethyl-ABA (Quarrie, 1978) was added to each sample so that the losses of ABA during the purification procedure could be determined. The samples were filtered and the residues were washed three times with the same extraction solvent. Then, 10 ml of distilled water was added and the ethanol was evaporated. The remaining aqueous fraction was acidified to pH 2.5 with 1N HCl and partitioned twice with hexane. The aqueous fraction was then partitioned thrice with ethyl acetate and the organic fraction was further purified by Sep-pack (t-C₁₈, 500 mg) column chromatography, using the procedure of Lewis et al. (1982) with some modification. BHT (2, 6-di-tert-buthyl-4-methylphenol) at the concentration of $100 \text{ mg}\cdot\text{liter}^{-1}$ was added as an antioxidant to all the extraction and purification solvents. The ABA content of the samples was quantified using a Shimadzu gas chromatograph GC-14A equipped with a ⁶³Ni electron capture detector (Okuda et al., 1995).

The numbers of leafy shoots with inflorescences, leafless shoots with inflorescences and current vegetative shoots on each shoot of each treatment were counted in mid-May of the following year. The value is expressed as the mean per one hundred nodes. Each treatment was replicated fifteen times.

Results

1. Effect of fruit removal on flowering of the vegetative shoots of the bearing branch unit

Fruit removal in June and September resulted in a return bloom of 36.2 ± 7.7 and 37.2 ± 8.1 flowers, respectively, compared to 24.0 ± 3.1 for the control. This increase was primarily in leafy shoots and not in leafless ones (Table 1). Moreover, fruit removal in June resulted in 4.8 ± 2.2 vegetative shoots, whereas fruit removal in September resulted in 12.8 ± 3.7 vegetative shoots, compared to the control which produced 14.4 ± 2.7 vegetative shoots.

2. Effect of fruit removal on photosynthesis (Pr) and stomatal conductance (Sc) in the leaves of the vegetative shoots of the bearing branch unit

Fig. 1. Schematic presentation of a bearing branch unit (BBU) before fruit removal.

The Pr in the control leaves remained about $7 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ from July to October and declined in November (Fig. 2). Fruit removal in June increased Pr by 24% in September and 41% by October, whereas fruit removal in September increased Pr by 30% in November (Fig. 2).

The Sc in the control leaves peaked at $0.55 \text{ cm} \cdot \text{sec}^{-1}$ in September (Fig. 3). Fruit removal in June increased Sc in the leaves by 18% in September and 46% in October, respectively, whereas fruit removal in September increased Sc by 114% by November (Fig. 3).

3. Effect of fruit removal on ABA concentration in the leaves of the vegetative shoots of the bearing branch unit

ABA concentration in the control leaves peaked at $8.9 \text{ nmol} \cdot \text{g}^{-1} \text{fw}$ in September and then de-

creased rapidly and remained almost constant from October to November. When fruits were removed in June, the ABA concentration in September was low; fruit removal in September resulted in a rapid decrease of ABA to a lower degree by October (Fig. 4). Consequently, the leaves of fruit-removed BBU had lower ABA concentration from October to November than those of BBU, independent of when the fruits were removed.

Discussion

Alternate bearing in citrus trees might be improved through control of flowering. But the control of flowering is rather difficult due to an annual fluctuation of fruit load on a tree. Excessive fruiting in one year suppresses flowering in the following spring, so that fruit thinning may enhance flowering. Kihara et al. (1995) found that

Table 1. Effect of fruit removal on flowering on the vegetative shoots in the bearing branch unit (average $\pm$ standard deviation, $n=15$).

Treatment	Number of flowers or shoots per 100 nodes			
	Leafless shoots inflorescences	Leafy shoots inflorescences	Total flowers	Current vegetative shoots
Fruit removed in June	4.8 ± 2.2	31.4 ± 8.5	36.2 ± 7.7	4.8 ± 2.2
Fruit removed in September	3.5 ± 3.3	33.7 ± 7.8	37.2 ± 8.1	12.8 ± 3.7
Control (unremoved)	3.9 ± 0.9	20.2 ± 3.0	24.0 ± 3.1	14.4 ± 2.7

Fig. 2. Effect of fruit removal on apparent photosynthesis in the leaves of the vegetative shoots.

Fruits were removed in June or September. Measurements were made on the terminal three leaves of each vegetative shoot on the BBU in the beginning of each month with three replications. Each column represents the mean ($\pm$ S. D., $n=3$) of the measurements.

Fig. 3. Effect of fruit removal on stomatal conductance in the leaves of the vegetative shoots.

Fruits were removed in June or September. Measurements were made on the terminal three leaves of the vegetative shoots on the BBU in the beginning of each month with three replications. Each column represents the mean ($\pm$ S. D., $n=3$) of the measurements.

Fig. 4. Effect of fruit removal on abscisic acid concentration in the leaves of the vegetative shoots.

Fruits were removed in June or September. Measurements were made on the terminal three leaves of each shoot on the BBU in the beginning of each month. Each value represents the mean ($\pm$ S. E., $n=3$) of the measurements.

thinning alternate branches stabilized annual production, whereas conventional fruit thinning did not offset alternate bearing. Thus, relieving the

fruit load induces flowering of vegetative shoots, whereas cropping inhibits floral initiation, if we assume the following three points; 1) the number

of the vegetative shoots that are the principal source to flower is equal between both branches described above; 2) both bearing shoots of BBU and fruit-removed BBU do not flower; and 3) branches older than an year old may have equal flowering potential. The followings support this viewpoint. We (1995) demonstrated that a reduction of flowering of vegetative shoots was related to excessive fruit load by comparing BBU with non-BBU. In this experiment, we showed that the return bloom of vegetative shoots was enhanced by early fruit thinning. Conversely fruit bearing negatively influenced flowering to a critical level if thinning was delayed. This may be the reason why alternate branch thinning stabilizes cropping more than does conventional thinning, although the leaf number per fruit is almost the same.

An increase in Pr during floral initiation period and an enhancement of flowering were observed when fruits were removed early in the season. An increase in Pr of non-BBU was also correlated to an enhancement of flowering (Okuda et al., 1995). Factors influencing photosynthesis including atmospheric CO₂ level or solar radiation (Friend et al., 1984; Quedado and Friend, 1978) are known to influence flowering, even if a direct relation between photosynthesis and flowering is not proven.

An increase of stomatal conductance was always accompanied by Pr enhancement attributable to fruit removal, which suggests that stomatal apertures may affect Pr through fruit removal. That fruit removal reduced ABA level in the leaves which may lead to cause stomata closing suggests an interrelationship among stomatal aperture, ABA level, crop load, and Pr.

ABA may affect flowering not only by its influence on Pr but also by some direct ways. Goldschmidt et al. (1984) observed a slightly higher ABA concentrations in leaves, shoots and buds in 'Wilking' mandarin trees in 'On' year. An exogenous application of ABA to citrus buds at different stages reduced sprouting, increased flower buds, and decreased the number of vegetative shoots (unpublished data). Similar results were also observed in other plants (Kamuro et al., 1990, 1992).

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果実除去がウンシュウミカンの発育枝の光合成速度、気孔伝導度および ABA 濃度ならびに翌春の着花に及ぼす影響

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摘 要

ウンシュウミカン（‘青島温州’）の成木（20年生）を材料として2本の有葉果枝と1本の発育枝からなる側枝に6月もしくは9月に摘果を行って着果負担を除いた処理区（6月摘果区、9月摘果区）と無摘果の対照区を設けた。これらの処理区の発育枝成葉について光合成速度（Pr）と気孔伝導度（Sc）を7月から11月にかけて測定し、これらの発育枝の翌春の着花性との関連について検討を加えた。

光合成速度は、6月摘果により9月に24%、10月に41%それぞれ対照区より高く、また9月摘果区では11月に対照区よりも30%高くなった。同様に、気

孔伝導度も6月摘果区では9月に18%、10月に46%それぞれ対照区より高くなり、9月摘果区では11月に対照区よりも114%高くなった。

次に、ABAの葉中濃度を9月から11月に比較したところ、無摘果の対照区では9月のABA濃度がそれ以降に比較してかなり高かった。これに対し、6月摘果区ではABAレベルはさほど高くなり、9月摘果区では、摘果に伴ってABA濃度が急減した。

以上のことから花芽分化に及ぼす光合成の関与、とくに気孔開度の調節を通じたABAの関与が示唆されたが、翌春の発育枝の着花は摘果により促進された。