

## イチジクの芽の休眠の深さの程度と枝梢内成分の変化

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著者	川俣, 昌大 西田, 悦子 小原, 均 ほか2名,
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## Changes in the Intensity of Bud Dormancy and Internal Compositions of Current Shoot in Fig

Masahiro Kawamata\*, Etsuko Nishida, Hitoshi Ohara, Katsuya Ohkawa and Hiroyuki Matsui

Faculty of Horticulture, Chiba University, Matsudo, Chiba 271–8510

### Summary

To estimate the intensity of bud dormancy in fig (*Ficus carica* L.) 'Masui Dauphine', current shoots in the field were collected monthly from July 1999 to March 2000 and cut at the 5th, 8th, 11th, 14th, 17th and 20th nodes. The cutting were kept in water at 25 °C under fluorescent light (5 klx) continuously. The percentage of budbreak within 20 days and the days to 80% budbreak were recorded.

The water, carbohydrate, and abscisic acid (ABA) contents of each shoot were also measured.

The percentage of budbreak within 20 days at 25 °C on all nodal position was 40%, in late November; the basal nodes tended to have lower % budbreak than the upper ones. Water content increased with the growth of the current shoots, reaching over 60% during the introductory phase of endodormancy. The starch accumulated during this period; it was hydrolyzed to sugar at the onset of budbreak or the awaking phase. The ABA content increased only during the introductory phase of endodormancy.

The endodormancy of the fig bud was classified into three phases, i.e., 1) introductory (early October to early November), 2) deepest (late November) and 3) awaking phases (middle December to late January). The fig shoot can be heated or headed back at any season to get fruit-bearing shoots for double cropping or year-round production. These treatments force most buds to sprout within a short period, even when they are in the deepest phase of endodormancy.

**Key Words:** abscisic acid, carbohydrate, endodormancy, fig, water content.

### Introduction

Woody plants, including deciduous, temperate zone fruit trees exhibit bud dormancy. It can be defined as the phase in which the visible growth is temporarily suspended and is generally awakened by chilling. The chilling, required to break endodormancy, depends on the kind of tree, variety, and place of cultivation (Westwood, 1993). To estimate the intensity of endodormancy and the days to blooming of fruit trees, models, i.e., chill unit (Richardson et al., 1974), developmental rate (Sugiura and Honjo, 1997), and chilling requirement etc., have been used. It is, however, difficult to define the model for budbreak of fruit trees. Because endodormancy of bud is accompanied by changes of internal compositions in the current shoot or root (Westwood, 1993). In previous studies, relationships between bud dormancy and water content (Mochioka et al., 1996) or carbohydrate (Wang et al., 1998) or plant hormone, especially abscisic acid (ABA) (Horiuchi et al., 1981; Powell, 1987; Tamura et al., 1993) in shoot of

fruit trees were discussed on a physiological basis. Carbohydrate content in the shoot changes to withstand freezing (Winkler and Williams, 1938), which is considered as an indicator of budbreak (Tamura, 1999). The research on dormancy was mainly done on peach (Chandler, 1934), pear (Weinberger, 1950; Koma, 1953; Tamura et al., 1998), persimmon (Kang et al., 1998), and grape (Horiuchi et al., 1981). But, no work on dormancy of fig (*Ficus carica* L.) has been done in Japan.

Double cropping of fig under hydroponic culture, which was recently introduced, reveals that the yield increased and fruit quality was highly acceptable (Kawamata et al., 2002). For double cropping or year-round production it is necessary to understand the terms and the intensity of endodormancy of fig.

The objective of this study is to investigate the terms and the intensity of bud dormancy of fig to explain the physiological basis for double cropping as well as year-round production of fig.

### Materials and Methods

Field experiments were conducted at Kashiwa Horticultural Farm of Chiba University, using eight-year-old fig 'Masui Dauphine' (syn. 'Masui Daufin') trees. The trees were trained to flat fan by removing lateral shoots.

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\* Corresponding author.

Five current shoots were randomly collected monthly on July 26, August 26, September 27, October 26, November 24, 1999, and continued on January 10, February 1, and March 1, 2000. Each shoot, which was cut at the 3rd internode from the base, had at least twenty internodes. The remaining 3 nodes were left for next years shoot growth.

#### *Experiment 1. Changes of intensity of bud dormancy*

To determine the percentage of budbreak and the days to budbreak, 5 current shoots, collected monthly, were cut at the 5th, 8th, 11th, 14th, 17th, and 20th nodes and placed in water (Exp. 1). The remaining nodes were kept in a freezer ( $-20^{\circ}\text{C}$ ) (Exp. 2). Leaves, petioles, and fruits were also removed from all nodes and each cutting was placed in a plastic tray, 2 cm deep filled with water. The tray was kept at  $25^{\circ}\text{C}$  under continuous fluorescent light (5 klx); the water was changed every 2 days. The percentage of budbreak was calculated as the number of buds which sprouted within 20 days per total buds. The days to budbreak after the start of the experiment was calculated when 80% of the buds sprouted.

#### *Experiment 2. Changes of water, carbohydrate and ABA content in current shoots*

The 6th nodes of 5 current shoots stored frozen (see above) during were analyzed for water, starch, and sugar contents. The fresh weight of nodes was recorded by electric balance (A and D Co., Ltd., Japan). The nodes were then heated for 15 min at  $100^{\circ}\text{C}$  in a convection oven to stop the enzymatic activity and dried for 48 hr at  $60^{\circ}\text{C}$  in a convection oven (Sanyo, Japan) to obtain their weights and to calculate the water content. To determine the carbohydrate content in current shoots, the dried samples were pulverized (Wonder Blender, Osaka Chemical Co., Ltd., Japan). A 1-g sample was boiled in 80% ethanol for 20 min and filtered. The filtrate was analyzed for sugar content, whereas the pellets were analyzed for starch.

For starch analysis, the pellets were dried in a convection oven for 24 hr and then boiled in 6 ml at 4.6 N perchloric acid twice for 15 min and then the mixture centrifuged twice for 10 min at  $12,000 \times g$  by refrigerated centrifuge (SCR 20B, Hitachi, Japan). The supernatant was brought to 100 ml; a 3-ml aliquot was boiled in a test tube for 2 hr, neutralized with 1 N NaOH, and brought to 25 ml. Reducing sugar was measured by the Somogi-Nelson method and the starch content calculated.

To determine the sugar content, the above filtrate was evaporated in vacuo at  $40^{\circ}\text{C}$  and brought to 10 ml with distilled water. Glucose, fructose, and sucrose contents were determined by HPLC equipped with a Asahipak NH2P-50 column (4.6 mm ID  $\times$  250 mm, Asahi Chemical Co., Ltd., Japan) kept at  $30^{\circ}\text{C}$  and eluted with 75% acetonitrile at a flow rate;  $1.0 \text{ ml} \cdot \text{min}^{-1}$ . The detector was RI Monitor (L-3300, Hitachi, Japan). The total

sugar content was computed from the sum of glucose, fructose, and sucrose.

The 9th nodes of 2 current shoots were analyzed for ABA according to Hu et al. (1999). A 5-g frozen sample tissue was homogenized and extracted for 18 hr by 80% methanol containing 0.5% ascorbic acid and 0.01% butylhydroxytoluene (BHT) at  $0^{\circ}\text{C}$ . The mixture was filtered and the filtrate evaporated in vacuo at  $40^{\circ}\text{C}$  to the water phase, passed through in polyvinylpyrrolidone (PVPP), and centrifuged twice for 10 min at  $12,000 \times g$ . The supernatant was partitioned three times with ethyl acetate at pH 2.8. After adding 0.5 M phosphate buffer (pH 8.0), the aqueous phase was reduced in vacuo at  $40^{\circ}\text{C}$  to the water phase. The water phase was extracted three times with diethyl ether with 0.01% BHT; the ether phase was set aside and 0.5 M phosphate buffer (pH 8.0) added to the aqueous phase which was evaporated in vacuo at  $40^{\circ}\text{C}$  to the water phase. The water phase was passed through a PVPP column and then through a Sep-Pak Plus C<sub>18</sub> cartridge (Waters, U.S.A.) at pH 2.8. The sample was eluted with distilled diethyl ether and the eluate evaporated under a N<sub>2</sub> stream and dissolved in 200  $\mu\text{l}$  methanol. An aliquot of the solution was injected into HPLC according to Yokota et al. (1994). HPLC which was fitted with a Senshu Pak ODS 2201-D column (6 mm ID  $\times$  200 mm, Senshu Scientific Co., Ltd., Japan) was kept at  $40^{\circ}\text{C}$ , and eluted for 5 min isocratically with 45% methanol (0.1% AcOH) followed by a 30-min gradient elution to 100% methanol at a flow rate of  $1.0 \text{ ml} \cdot \text{min}^{-1}$ . The detector was UV Detector (L-7420, Hitachi, Japan).

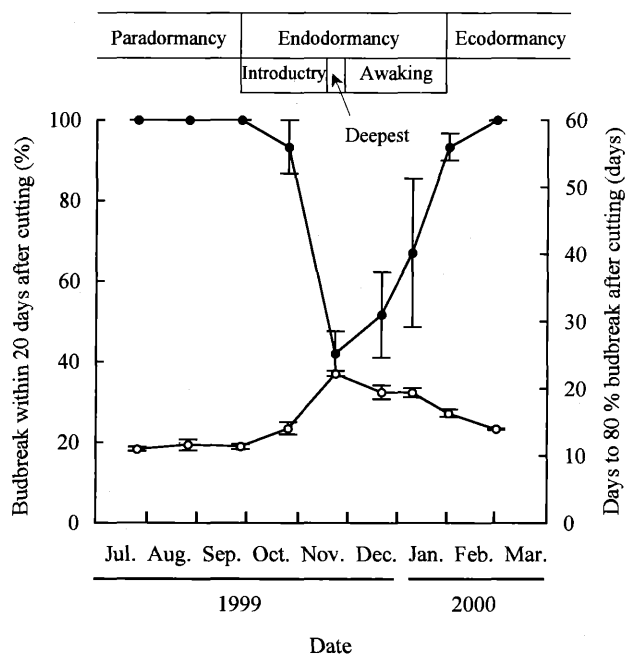
## Results

#### *Experiment 1. Changes of intensity of bud dormancy*

The intensity of bud dormancy of fig was calculated by considering the percentage of budbreak and the days to budbreak at all nodal positions of the shoot (Fig. 1). The percentage of budbreak within 20 days after treatment at  $25^{\circ}\text{C}$  on all nodal positions was 100% from late July to late September; it decreased to about 40% in late November, then increased to 100% in early February. While the days to 80% budbreak after beginning of the experiment was 11 days from late July to late September; it increased to 22 days in late November and decreased to 14 days in early March (Fig. 1).

To estimate the difference of the intensity of bud dormancy on each nodal position of the shoot, the percentage of budbreak (Table 1) and the days to budbreak (Table 2) were calculated for each nodal position. The upper nodes of fig shoot had a low percentage of budbreak, i.e., the deeper phase of endodormancy. The budbreak tended to be slow and the intensity of endodormancy weak as compared to the lower nodes (Table 1). The percentage of budbreak was 20% on the 5th, 8th, and 11th nodes in November. The

days to 80% budbreak at different nodal position differed, the lower nodal positions required more days than did the upper ones (Table 2).



### Experiment 2. Changes of water, carbohydrate, and ABA content in current shoots

The water content of the current shoot was 70% in late July, but decreased linearly to 45% in early March. The water content of the current shoot at the beginning of endodormancy was about 60% (Fig. 2).

The starch content was  $25 \text{ mg} \cdot \text{g}^{-1} \text{DW}$  in late August, it but increased rapidly to  $105 \text{ mg} \cdot \text{g}^{-1} \text{DW}$  in late October when the percentage of budbreak started to decrease, then dropped sharply. The total sugar content was  $35 \text{ mg} \cdot \text{g}^{-1} \text{DW}$  in late July and decreased to  $20 \text{ mg} \cdot \text{g}^{-1} \text{DW}$  in late October, but again increased to  $50 \text{ mg} \cdot \text{g}^{-1} \text{DW}$  in late January, contrary to the starch fluctuation. Fructose and glucose content curves were almost the same on all sample dates. These sugars decreased slightly in late August, but increased afterward. Sucrose

**Fig. 1.** Changes in the percentage of budbreak within 20 days and the days to 80% budbreak at all nodal positions after the fig shoots were excised and kept at  $25^\circ \text{C}$ . Vertical bars indicate SE. ● : Budbreak within 20 days, ○ : Days to 80% budbreak.

**Table 1.** The percentage of budbreak occurring within 20 days at  $25^\circ \text{C}$  after cutting at different nodal position in fig.

Nodal position	Budbreak within 20 days after cutting(%)								
	1999						2000		
	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
5	100.0	100.0	100.0	80.0	20.0	60.0	60.0	80.0	100.0
8	100.0	100.0	100.0	80.0	20.0	66.7	80.0	80.0	100.0
11	100.0	100.0	100.0	100.0	20.0	50.0	60.0	100.0	100.0
14	100.0	100.0	100.0	100.0	60.0	40.0	60.0	100.0	100.0
17	100.0	100.0	100.0	100.0	80.0	40.0	75.0	100.0	100.0
20	100.0	100.0	100.0	100.0	80.0	75.0	50.0	100.0	100.0
Avg. <sup>z</sup>	100.0	100.0	100.0	93.3	42.0	51.7	67.0	96.7	100.0

<sup>z</sup>Average was calculated on results of all nodal positions.

**Table 2.** The days to 80% budbreak after cutting at  $25^\circ \text{C}$  at different nodal position in fig.

Nodal position	Days to 80% budbreak after cutting (days)								
	1999						2000		
	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
5	11.0 ± 1.2 <sup>z</sup>	13.8 ± 0.3	13.0 ± 0.0	16.3 ± 0.7	23.3 ± 1.4	18.3 ± 0.9	19.0 ± 0.8	17.8 ± 1.3	15.6 ± 0.2
8	12.0 ± 0.5	10.8 ± 1.5	10.5 ± 0.9	13.3 ± 2.0	28.3 ± 5.7	21.0 ± 3.0	18.8 ± 0.8	17.8 ± 1.0	14.6 ± 0.4
11	10.7 ± 1.2	10.8 ± 0.6	10.3 ± 0.8	16.0 ± 1.3	24.0 ± 3.8	22.0 ± 2.8	20.0 ± 0.6	16.0 ± 1.1	15.0 ± 0.6
14	10.3 ± 0.5	11.3 ± 0.9	12.8 ± 0.5	13.5 ± 1.4	21.0 ± 1.4	17.0 ± 2.0	19.0 ± 1.5	16.3 ± 1.1	14.0 ± 0.4
17	10.3 ± 0.3	10.0 ± 0.6	11.0 ± 1.0	13.0 ± 1.0	19.8 ± 1.6	19.2 ± 1.4	19.3 ± 0.7	14.3 ± 2.3	12.8 ± 0.6
20	10.8 ± 0.9	9.8 ± 1.8	10.5 ± 1.5	9.5 ± 1.5	17.8 ± 1.0	17.7 ± 1.5	19.0 ± 0.0	14.0 ± 1.2	14.0 ± 1.0
Avg. <sup>y</sup>	11.0 ± 0.3	11.6 ± 0.8	11.4 ± 0.4	14.1 ± 0.9	22.2 ± 0.4	19.5 ± 1.0	19.4 ± 0.7	16.3 ± 0.6	14.0 ± 0.1

<sup>z</sup>Mean ± SE.

<sup>y</sup>Average was calculated on results of all nodal positions.

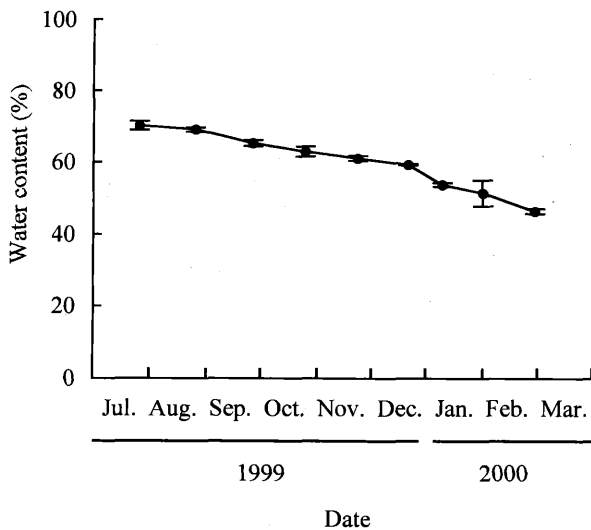


Fig. 2. Seasonal change in water content of current fig shoot. Vertical bars indicate SE.

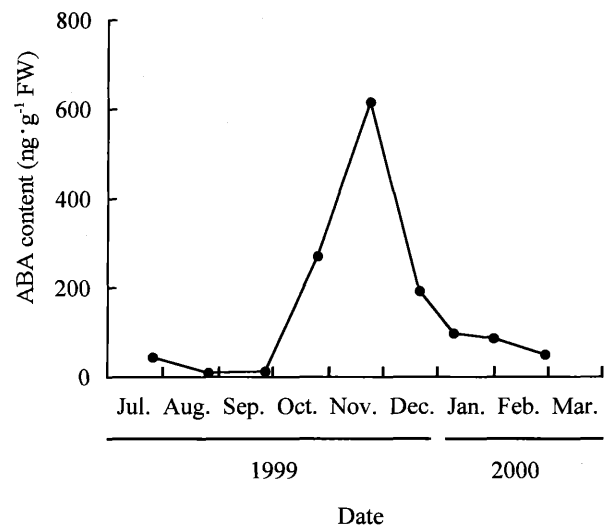


Fig. 4. Seasonal change in abscisic acid content of current fig shoot.

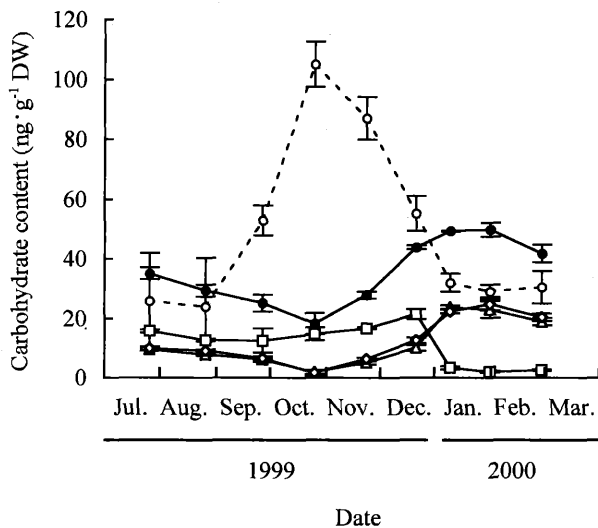


Fig. 3. Seasonal changes in carbohydrate content of current fig shoot. Vertical bars indicate SE.  $\cdots\circ\cdots$ : Starch,  $\bullet$ : Total sugar,  $\square$ : Sucrose,  $\triangle$ : Fructose,  $\diamond$ : Glucose.

content was  $15 \text{ mg} \cdot \text{g}^{-1} \text{ DW}$  in late July and increased slightly to  $20 \text{ mg} \cdot \text{g}^{-1} \text{ DW}$ , but declined from late January to early March (Fig. 3).

The ABA content was low from late July to late September, but increased rapidly to  $600 \text{ ng} \cdot \text{g}^{-1} \text{ FW}$  in late November and then dropped sharply (Fig. 4).

### Discussion

Dormancy has been classified into three phases (Doorenbos, 1953, Samish, 1954; Kondo, 1955; Antcliff and May, 1961) but these names haven't been standardized. Lang (1987) has recommended using para-, end- and eco-dormancy after revising some contradictory terms. Moreover, Horiuchi et al. (1981) classified the endodormancy of grape into three periods: introductory, deepest and awaking. Endodormancy of

fig is typically classified into three periods: introductory, deepest, and awaking as proposed by Lang (1987), Horiuchi et al. (1981) and we are conforming with Lang. In Japan, greenhouses are generally heated for the commercial production of fig after late November that is considered to be the deepest phase of endodormancy. The intensity of bud dormancy of fig seemed to be weaker than that of grape 'Delaware' (Horiuchi et al., 1981) because at the deepest phase of endodormancy the percentages of budbreak never decreased below 40% and the days to 80% budbreak was about 22 days on all nodal positions.

Horiuchi et al. (1981) reported that the onset and depth of dormancy differed greatly among the positions of buds on the shoot. The intensity of dormancy was low in the upper buds, compared to the basal ones during endodormancy in grape. Our data (Table 1, 2) indicate that the intensities of bud dormancy differed greatly at each nodal position of the shoot in fig. The intensity of bud dormancy on the basal nodes tended to be weaker than that on the upper one.

Mochioka et al. (1996) reported that the water content of shoots of wild grape (*Vitis ficifolia* Bunge var. *ganebu*), which originated in a warm, tropical region and, thus having a shallow endodormancy, was over 60% during its deepest phase, whereas other wild grapes from the more cooler regions decreased suddenly and resumed during the same phase. We found that the intensity of endodormancy in fig is similar to wild grape from the warmer region in that the water content of shoot of fig was over 50% to 60% during the phase of endodormancy and never decreased suddenly.

Ogawa (1966) proved that the conversion into sugar from starch depended on the de novo synthesis of amylase controlled by gibberellin in rice. Horiuchi et al. (1981) reported that the endogenous substances present in the dormant buds of grape inhibited the bud burst of

vine. In fig, starch was accumulated during the deepest phase of endodormancy and then converted into sugar during the awaking phase of endodormancy to alleviate freeze damage of buds.

The relationships between bud dormancy and ABA content of bud were investigated in apple (Seeley and Powell, 1981), grape (Emmerson and Powell, 1978) and Japanese pear (Takami et al., 1988). In these reports, free ABA level of buds increased suddenly during the introductory phase of endodormancy and decreased afterward. Budbreak was inhibited by ABA treatment (Sterret and Hipkins, 1980). Many investigations demonstrated that ABA induces endodormancy and maintains its intensity, but has no effect on budbreak. In our study ABA similarly increased sharply during the introductory phase of endodormancy and decreased afterward. Hence, ABA may be related to the induction of endodormancy of fig.

Consequently, endodormancy of fig bud has been classified into three phases, i.e. introductory (early October to early November), deepest (late November), and awaking phases (middle December to late January). The fig shoot can be heated up or headed back in the greenhouse at any time to induce budbreak and subsequent shoot growth for double cropping or year-round production. In this study budbreak occurred at almost all basal buds and the days to 80% budbreak after cutting was about 28 days, even when the shoots were headed during the deepest phase of endodormancy.

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## イチジクの芽の休眠の深さの程度と枝梢内成分の変化

川俣昌大・西田悦子・小原 均・大川克哉・松井弘之

千葉大学園芸学部 271-8510 千葉県松戸市松戸

### 摘 要

露地栽培の4年生イチジク '榊井ドーフィン' より、1999年7月下旬から2000年3月上旬にかけて毎月当年生枝を採取し、1節ごとに切り分けた5, 8, 11, 14, 17および20節目の枝梢を恒温室(25℃, 5 klx 蛍光灯下)に置いた水床に挿し木し、20日後における萌芽率および萌芽率80%に達するまでの萌芽所要日数を調査した。また、同時期に採取した枝梢内の水分、炭水化物およびABA含量を測定した。

全ての節位の萌芽率の平均値は、11月下旬に約40%と最も低下した。また、萌芽率は低節位ほど低下する傾向がみられた。水分含量は、枝梢の発育に伴って漸減し、萌芽率が最

も低下した11月下旬には約60%となった。また、デンプン含量は10月下旬を境に急減し、全糖含量は増加した。ABA含量は10月下旬に急増した。

以上の結果より、イチジクの芽の自発休眠の形態は、10月初旬~11月初旬が導入期、11月下旬頃が最深期および12月中旬~1月下旬頃が覚醒期であることが明らかとなった。また、最深期であってもほとんどの節位で萌芽し、萌芽所要日数が最長で約28日とそれほど長くないことから、二期作や周年栽培のための加温や枝の切り戻しせん定はいつ行っても支障がないことが明らかとなった。