ウンシュウミカン樹における BA の吸収とその転流

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Absorption and Translocation of 6-Benzylamino Purine in Satsuma [*Citrus unshiu* Marc.] Trees[1]

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Summary

Absorption of 6-benzylamino purine (BA) combined with [8-^{14}C] BA by abaxial and adaxial surfaces of satsuma leaves as well as the effects of the surfactant Tween 20 on BA absorption were studied. In addition, the movement of BA combined with ^{14}C-BA in a one-year-old shoot was examined after application to the surface of buds and leaves with cotton swabs and to the vascular tissues of the shoot by the cotton thread method.

Absorption of ^{14}C-BA began immediately after treatment and reached a maximum level in about 8 hours. Absorption of ^{14}C-BA by the abaxial leaf surface seemed faster than adaxial absorption, although similar time course trends of absorption were shown in both abaxial and adaxial applications. Most of the BA applied seemed to be taken in by penetration, perhaps through the ectodesmata, but also slightly through stomatal pores. Even under low temperature conditions, BA was absorbed either by quiescent axillary buds or their subtending leaves, resulting in the break of bud dormancy. Inclusion of Tween 20 in the treating solutions clearly enhanced the absorption of BA by the epidermal tissues. An optimum concentration of Tween 20 for obtaining maximum BA absorption was about 0.1%. The ^{14}C-BA applied directly to the vascular system by the cotton thread method was translocated mostly toward nearby axillary buds and their subtending leaves, although the amount of ^{14}C recovered from each location was quite limited. Dormancy-release effects of BA applied directly to the bud surface were restricted to the treated bud and not transmitted to untreated neighboring buds.

Introduction

As stated by Nauer *et al.* (6) and Nauer and Boswell(7), benzyladenine (BA) applied by a foliar spray stimulates quiescent citrus buds into growth. Such effects of BA at different concentrations were also examined by other investigators with various kinds of plants. In these experiments, the efficiency of BA absorption by the stem, leaves and axillary buds seemed to depend on the inclusion of solvents and/or surfactants in the treatment solutions, because application of different formulated BA solutions usually produced inconsistent results. Details of the effects of solvents and surfactants on BA absorption must await further experiments.

The extent and direction of cytokinin movement in a plant under different conditions are also important for producing desirable effects. Sachs and Thimann(10) and Williams and Stahly(12) reported that dormancy release by BA application was strictly restricted to the buds treated directly with BA solutions because of very limited movement of BA in plant tissues. On the other hand, Pieniazek and Jankiewicz(8) pointed out that acropetal transmission of BA effects was not inhibited by girdling applied on the distal side of an apple shoot, while basipetal movement was impeded almost completely by basal side girdling. Friedrich *et al.* (3) later confirmed that BA and its metabolites were translocated acropetally through the xylem and basipetally through the phloem. Kender and Carpenter(4) reported that when single leaves were dipped in BA solutions, the buds subtended by the treated leaves initiated growth

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even though the buds per se were not contaminated with BA at all.

The present experiment was designed to study BA absorption by satsuma leaves and to examine the effects of including Tween 20 in the treatment solutions. In addition, BA transport when applied to the stem surface as well as to the vascular tissues of one-year-old satsuma shoots was studied with ^{14}C-labeled BA.

Experiment I BA Absorption by Satsuma Leaves

Materials and Methods Three-year-old satsuma trees grafted on *Poncirus trifoliata* rootstock were grown in pots 30 cm in diameter and transferred to a greenhouse in April, 1985. Four one-year-old spring cycle shoots 10–15 cm in length were used in the BA absorption experiment. Generally, 200 ppm BA solutions combined with a small amount of ^{14}C-BA plus 0.1% Tween 20 were applied to either the abaxial or the adaxial surface of the leaf with cotton swabs. The treated leaves were collected at intervals of predetermined time periods. Surfaces of the treated leaves were washed carefully with 30 % isotope cleaner A solution (Chiyoda Hoan Corp., Tokyo) and then rinsed with distilled water. This procedure was repeated 3 times. Moisture remaining on the leaf surface was blotted dry. Discs were excised from both washed and nonwashed leaves. Nonwashed leaf discs were prepared to determine the amount of radioactive BA applied. Washed leaf discs were ground with a mortar. The ground samples were placed in a 25-ml stoppered glass scintillation vial and digested by the method of Egami and Yoshida(2), which was modified in part by the authors of this paper. In order to promote digestion, 0.5 ml of 30% H_2O_2 and 1 ml of 2 *N* NaOH were injected into the vial, then heated in a water bath at 80°C for 3 hours. The solution was cooled to room temperature, 10 ml of Univer-Gel II scintillation fluid was added, and the vial was shaken vigorously for good mixing. The radioactivity of each sample was determined with a Packard Tri-Carb liquid scintillation spectrometer, model 2425. Two vials with 10 ml of Univer-Gel II solution alone were used for background determination. The radioactivity of each sample in dpm was calculated from counting rates in cpm with detection efficiency.

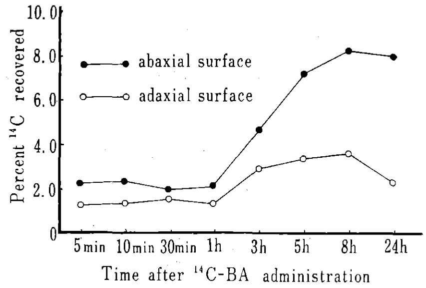

Fig. 1. Time course trend of ^{14}C-BA absorption by abaxial and adaxial surfaces of satsuma leaves.

Results Absorption of ^{14}C-BA by either the abaxial or the adaxial surface of the leaf commenced shortly following the treatment. From 5 minutes to 1 hour after treatment, ^{14}C-BA absorption by the abaxial surface increased very slowly, followed by a rather rapid increase. Rate of absorption reached a maximum ^{14}C level about 8 hours after treatment. This time course trend was similar to that of adaxial absorption (Figure 1), although the amount of ^{14}C-BA absorbed by the abaxial surface was greater than that of the adaxial surface. Eight hours after the treatment, radioactivity recovered from the abaxial-surface-treated leaves was about 2.5 times that of adaxial surface. However, the sum totals of ^{14}C-BA adsorbed by the epidermal ectodesmata and that absorbed by the inner leaf tissues were similar in both treatments.

Experiment II Effect of Tween 20 on BA Absorption

Materials and Methods Optimum concentrations of the surfactant Tween 20 for maximum absorption of BA were examined, using a range between 0 and 5% Tween 20 plus 200 ppm BA, which was combined with a small amount of ^{14}C-labeled BA. Treatment solutions were applied to 3-year-old satsuma trees grown in the greenhouse. Fully developed leaves were selected on 12 uniform one-year-old shoots. Prior to the treatment, new cycle flushes emerging from the shoots were entirely removed. The se-

Table 1. Effect of the inclusion of Tween 20 in treatment solutions on the absorption of BA by satsuma leaves.

Treatment	Radioactivity recovered (dpm)		
	1h	24h	240h
^{14}C-BA+200ppm BA (BA)	100.1	108.9	53.4
BA+0.01% Tween 20	82.7	179.5	84.3
BA+0.1% Tween 20	66.9	214.7	168.8
BA+1.0% Tween 20	37.1	142.8	63.7
BA+2.0% Tween 20	62.1	97.7	84.9
BA+5.0% Tween 20	85.0	64.3	106.0

lected leaves were tagged and dipped for 15 seconds in treatment solutions containing Tween 20 at different concentrations. After 1 hour, 24 hours and 10 days, 3 leaves per treatment were collected, washed and digested as described. Radioactivity recovered from each sample was determined with the liquid scintillation spectrometer as above.

Results Up to 1 hour after treatment, BA absorption was somewhat slower in leaves treated with plus Tween 20 solutions than in those treated with minus Tween 20 solutions (Table 1). In particular, inclusion of 1.0% Tween 20 in treating solutions caused the lowest absorption of BA, although 24 hours later the radioactivity recovered from leaves treated with solutions containing 0.01 to 1.0 % Tween 20 increased greatly. Ten days after treatment, the recovered radioactivity was highest in the treatment with Tween 20 at 0.1% being about 3 times the amount of ^{14}C recovered from the control sample.

Inclusion of the surfactant Tween 20 in treating solutions seemed to influence the drying process of BA solutions on the plant surface. The higher the concentration of Tween 20, the slower the drying of BA solutions. In other words, the surfactant Tween 20 has wetting properties in addition to its primary function as a spreading agent.

Experiment III Translocation of BA in Satsuma Shoots

Materials and Methods The movement of BA applied to the vascular tissues as well as to the bud surface was studied. Four 3-year-old satsuma trees were treated with 200 ppm BA solutions combined with a small amount of radioactive BA. In May, when the spring growth cycle ceased, BA was applied to the current shoots through the vascular system as described by Shibata and Yamasaki(11). One week after treatment, 30 mg fresh weight of each sample was collected from the stem segments 1 cm above, 1 cm below and at the treatment point along with a nearby bud and its subtending petiole. Plant materials were digested as above. Radioactivity recovered from each sample was determined as described. The results are given in Figure 2.

Transmission of BA effects was investigated with 3-year-old satsuma trees grown in the greenhouse. In this experiment, 200 ppm BA solution with 0.1% Tween 20 was applied with a cotton swab to each alternate leaf blade of 4 shoots on 4 trees. Care was taken to prevent axillary buds and their subtending petioles from comtamination with BA solution. In addition, a 5 μl drop of treating BA solution was applied directly to axillary buds using a microsyringe, to compare bud break effects of BA applied directly to the bud surface with those of BA applied indirectly to its subtending leaf. Every treatment consisted of 2 applications of BA solutions 4 days apart. Untreated shoots were used as controls. Observations on bud break and extension growth of new cycle flushes were carried out over a one-month period.

Results ^{14}C-BA applied to the vascular tissues was translocated both acropetally and basipetally. Redioactivity was detected along the shoot stem as well as in the nearest bud and its subtending petiole (Figure 2).

Axillary buds, whose subtending leaves were treated twice with treatment solutions,

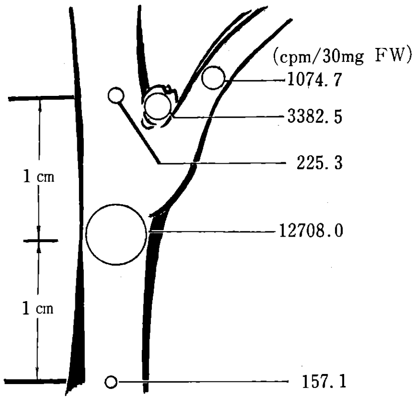

Fig. 2. Translocation of ^{14}C-BA in spring cycle shoots of potted satsuma trees. Acropetal and basipetal movement of ^{14}C-BA was observed. The amount of ^{14}C translocated was much less than that applied, being 2% at most in the stem.

Table 2. Sprouting and extension growth (cm) of alternate bud treated with BA solutions.

Number of node positions	Treatment site			
	Leaf		Bud	
	A	B	A	B
1	2.2	—	17.0	11.2
2	9.3	2.3	3.4	10.6
3	14.6	—	12.7	4.6
4	—	13.5	0.6	12.6
5	13.3	—	12.8	—
6	—	16.4	—	7.7
7	9.2	—	7.2	—
8	—	18.7	—	12.8
9	6.4	—	11.4	—
10	—	13.9	—	9.4
11	—	—	—	—
12	—	11.6	—	3.9
13		—		—
14		5.3		—
15		—		—
16		5.1		
17		—		
18		—		

Notes 1. A : Treatment at odd number node positions.
B : Treatment at even number node positions.
2. Dormancy of some terminal buds was released due to the nature of apical dominance.

initiated growth within 22 days following the first BA application, while untreated neighboring buds showed no sign of sprouting throughout the experiment (Table 2). Dormancy of axillary buds treated directly with

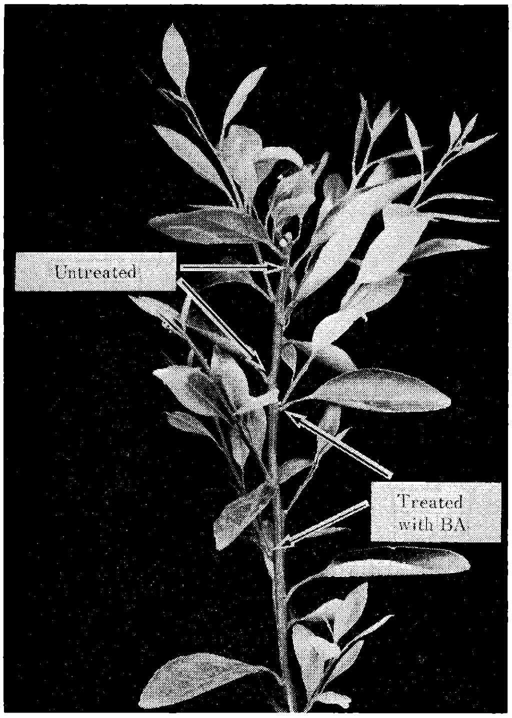

Fig. 3. Sprouting of alternate buds treated with BA at a dormant stage. Only the treated buds initiated growth, while the untreated buds remained dormant.

BA was overcome earlier than in buds subtended by BA-treated leaves. Direct application of BA to the bud surface caused vigorous shoot development 14 days after the first treatment. On the other hand, untreated buds remained dormant even after the growth of nearby shoots ceased (Figure 3). More than 80% of the quiescent axillary buds initiated growth after 2 applications of BA solutions at an interval of 4 days, although the extension growth of new flushes was quite depressed under low temperature conditions.

Untreated buds began to grow in late May, 16 days later than the sprouting of directly treated buds and 8 days later than that of subtending-leaf-treated buds. The shoot sprouting pattern of satsuma trees indicated a typical apical dominance. Most lateral buds treated with BA grew uniformly and no significant difference in the onset of sprouting was observed among different node positions on the stem. However, small-sized basal buds failed to initiate growth even when they were treated with BA by either the direct or the indirect application method. Furthermore, developing shoots located at basal node positions died and fell off after reaching a length of about 2 to 3 cm.

Discussion

As with other growth regulators, information on the time course absorption is an important factor in the effective use of BA. In practice, most plant growers may presume from their experience that application of growth regulators to plants immediately before a rainfall produces undesirable results. However, few papers on the time course trend of BA absorption have been published. In the present experiment, a ready response of leaves to BA application was observed. That is, about 5 minutes after ^{14}C-BA treatment, an appreciable amount of ^{14}C was absorbed by satsuma leaves, and similar time course trends were shown thereafter in both the abaxial and adaxial absorptions, although absorption in the former was somewhat faster than in the latter.

Kuraishi *et al.* (5) reported that stomata of sunflower leaves closed within 1 minute after water spraying. It seems likely, therefore, that the absorption of BA by the plant epidermis is carried out mostly by penetration through the ectodesmata. However, they also pointed out that a latent period of about 2 minutes was required for stomatal opening induced by cytokinin treatment. In the present experiment, BA was absorbed faster or more readily by the abaxial epidermis than by the adaxial surface of the leaves. Therefore, BA absorption by satsuma leaves might be concerned, in part, with the stomatal mechanism and/or the thickness and structure of cuticular layers.

The surfactant Tween 20 has often been added to BA solutions as a spreader(1, 4, 6, 7) and caused better adhesion of treating solutions all over the leaf surface, which resulted in greater absorption of BA. In addition, it was proved in this study that the surfactant Tween 20 possesses wetting properties along with functioning as a spreader. Broome and Zimmerman(1) examined the influence of Tween 20 and the solvent DMSO on cytokinin absorption in the tea crabapple. They showed that inclusion of Tween 20 in treating solutions produced greater response of buds to cytokinins. In citrus, Nauer and Boswell(7) showed that Tween 20 enhanced the bud break effects of BA within an optimum range of its concentrations. In the present study, a maximum amount of ^{14}C-BA was recovered when the concentration of added Tween 20 was 0.1%. Optimum concentrations of Tween 20 also depended closely on the concentration of BA per se. High concentrations of Tween 20 produced no desirable results but rather inhibitory effects on BA absorption.

Translocation of cytokinins absorbed depends also on their application sites. Sachs and Thimann(10) found little transport of kinetin in a pea seedling shoot, and only those buds which were directly treated with kinetin were able to sprout. However, our experiment showed that BA absorbed by a leaf blade moved to the axillary bud subtended by the treated leaf. In addition, translocation of ^{14}C-BA was very limited around the treatment point, and bud break effects of BA were restricted to the bud which was treated directly or subtended by a treated leaf. Such tendencies were also pointed out in previous papers(3, 4, 8).

^{14}C-BA applied directly to the vascular tissues of a shoot moved both acropetally and basipetally in this experiment. As compared to a limited transport of ^{14}C along the shoot stem, however, greater amounts of radioactivity were recovered from the nearby bud and their subtending petiole. Based on an earlier report(3), which pointed out that cytokinins moved acropetally in the xylem and basipetally in the phloem, the greater amount of ^{14}C recovered from the petiole in this study is assumed to be due to the translocation of ^{14}C-BA, which was carried with the transpiration stream toward the leaf blade.

In general, dormancy in apical buds of normal satsuma trees is released in spring. In the present experiment, each alternate bud treated with BA initiated growth soon after treatment regardless of its node position, while the untreated buds did not sprout in spring. The presence of quiescent buds between each alternate growing shoot may give a significant clue to the question of bud dormancy. Furthermore, whether summer dormancy, or regularly recurring interruption of cyclic growth in summer, is caused by (1) failure of cytokinin supply, or by (2) exhaustion of some nutritional factors, or by (3) accumulation of some inhibitory substances produced by the buds per se or by other organs remains to be elucidated.

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摘　要

ウンシュウミカンの葉の表裏両面からの ^{14}C-BA の吸収，並びに，界面活性剤 Tween 20 の添加が BA の吸収に及ぼす効果について検討した．さらに，その液を綿棒で1年生枝上の芽と葉の表面に処理したり，あるいは，木綿糸法によって，茎の維管束に処理した場合の ^{14}C-BA の転流についても調べた．

^{14}C-BA の吸収は処理直後に始まり，約8時間かけて最高の濃度に達した．BA の吸収は葉の表よりも裏で速やかに行われたが，その経時的な吸収過程は葉の表と裏でよく似ていた．

処理した BA の大部分は ectodesmata を透過して，また，少量は気孔を通して吸収されたようである．低温条件下でも，BA は休止状態にあるえき芽や葉によって吸収され，その結果芽の休眠が解除された．処理液に Tween 20 を加えると，葉の表面からの ^{14}C-BA の吸収が明らかに増加したが，BA の吸収を最大にする Tween 20 の濃度は 0.1% であった．

木綿糸法で維管束に直接処理した ^{14}C-BA は，主として処理部近くのえき芽や，その基部に着生する葉に向かって流転したが，その量はごくわずかであった．直接芽の表面に処理した BA の休眠打破効果は，その処理芽に限って認められ，隣接した無処理の芽までは及ばなかった．