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Stimulating Growth of Quiescent Citrus Buds with 6-Benzylamino Purine[1]

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Abstract. **Application of 6-benzylamino purine (BA) to quiescent citrus buds generally increased the percentage of buds to initiate active growth. Results of 2 years' experimentation were highly variable and revealed several factors, such as season and cultivar, which influence the efficacy of BA for stimulating citrus buds into growth. BA was more effective outdoors and in a screenhouse than in a greenhouse. There was appreciable variation among several citrus cultivars. Early spring applications in a commercial citrus nursery were more effective than late spring or summer applications with 'Owari' satsuma (*Citrus reticulata* Blanco) budded on Troyer citrange (*Poncirus trifoliata* [L.] Raf. x *C. sinensis* [L.] Osbeck) rootstock.**

The utilization of cytokinins for overcoming apical dominance and stimulating quiescent buds into growth has been reported for many crops (2, 3, 5, 7, 8, 9). BA has proved effective with orange (*Citrus sinensis* [L.] Osbeck) (6), tea crabapple (1), and species of *anthurium* (4).

Previous research here showed BA effective in stimulating growth of fall-budded sweet orange trees on Troyer citrange rootstock (6). Further research on the use of BA for citrus bud stimulation was then undertaken for the purpose of adapting this technique ot the production of citrus nursery trees in a commercial citrus nursery in California. It is sometimes difficult to stimulate growth of inserted scion buds by the conventional method of bending over (lopping) the rootstock top. This work encompassed many trials over a period of 2 seasons.

BA is relatively expensive, hence an experiment was initiated to determine the lowest effective concentration. Actively growing sweet orange seedlings in 1 liter containers in a screenhouse were trained to a single leader and were treated when about 80 cm in height. BA in 95% ethanol at concentrations ranging from 0 to 8,000 ppm, with and without 5% dimethylsulfoxide (DMSO) solvent and 2% Tween 20 spreader, was applied to individual lateral buds with a cotton swab. A 10-bud section of stem of each plant was marked off for treatment. Buds 1, 3, 5, 7, and 9 were treated while alternate buds 2, 4, 6, 8, and 10 were untreated controls. Five buds on each of 5 plants, 25 buds total, received each treatment in April of 1979. All of our work involved treatment of individual buds with cotton swabs (Q-tips) rather than spraying, as we felt that spraying would stimulate excessive growth of rootstock suckers, a highly undesirable side effect.

Bud growth approached 100% at 250 ppm and higher BA concentrations with DMSO and Tween 20 (Table 1). Concentrations below 125 ppm were ineffective, as were all concentrations of BA without DMSO and Tween 20. None of the control buds grew, i.e. the alternate untreated buds on each plant, indicating that there was little or no translocation of BA from the treatment sites. Also, it can be concluded that DMSO and Tween 20 have no growth promotion effects *per se*, since there was no bud growth on plants treated with DMSO and Tween 20 plus BA at concentrations below 32 ppm.

Single leader plants of 5 citrus cultivars actively growing in the greenhouse were treated with BA by the same method as in the concentration experiment reported above. Twenty-five buds of each cultivar were treated with BA in 95% ethanol at 4,000 ppm with 5% DMSO and 2% Tween 20.

Cultivar response ranged from no growth to 100% bud growth (Table 2). All treated buds on Mexican lime (*C. aurantifolia* Swing.) grew, most buds of Pineapple sweet orange grew, about half of the rough lemon (*C. jambhiri* Lush.) buds grew, but there was no response to BA treatment by Duncan grapefruit (*C. paradisi* Macf.) or seedlings of Parsons's Special mandarin (*C. reticulata* Blanco).

Whether DMSO or Tween 20 is the more important additive was next investigated. Again, single-leader actively growing sweet orange seedlings in the greenhouse were treated by applying BA to 5 individual lateral dormant buds per plant with a cotton swab. Materials used were 1,000 ppm BA in ethanol, BA plus 5% DMSO, BA plus 2% Tween 20, and BA plus both DMSO and Tween 20. A total of 20 plants were used per treatment at 3 treatment times, April and May of 1979 and February of 1980. Results of 2 of these 3 trials indicate that Tween 20 is more effective than DMSO, and that DMSO could be omitted if not desired as a means of dissolving the BA powder in ethanol more rapidly (Table 3). Timing of treatment was important.

The optimum percentage of Tween 20 for maximum bud stimulation was then investigated, using a range between 0 and 5% Tween 20 with 2,000 ppm BA in ethanol. Three greenhouse trials were undertaken, the first 2 with sweet orange and the third with grapefruit seedlings. One and 2% Tween was more effective than 5% in the first trial. The second experiment was inconclusive because only 6 buds out of a total of 275 treated buds initiated growth. The third trial was duplicated on grapefruit seedlings growing in the greenhouse and in a screen house, essentially under outdoor conditions except for reduced light. In this case, 132 out of 250, or 52.8% of the treated buds initiated growth on the screenhouse plants but no buds initiated

Table 1. Influence of BA concentration with and without DMSO and Tween 20 on sweet orange seedling bud stimulation in a screenhouse in April 1979.

BA concn in alcohol	Quiescent buds stimulated into growth[z] (%): 5% DMSO, 2% Tween 20	No DMSO or Tween
Control	0c	0b
≤16	0c	0
32	4c	0b
64	20b	0b
125	88a	0b
250	96a	0b
500	100a	0b
1000	96a	0b
2000	100a	8b
4000	100a	28a
8000	100a	40a

[z]25 buds each treatment.
[y]Mean separation within columns by Duncan's multiple range test, 5% level.

Table 2. Citrus cultivar response to application of BA to quiescent lateral buds of greenhouse seedlings in May 1979[z].

Cultivar seedlings	Quiescent buds stimulated into growth [y] (%)
Mexican lime	100a[x]
Pineapple sweet orange	88a
Rough lemon	52b
Duncan grapefruit	0c
Parson's Special mandarin	0c

[z]BA at 4,000 ppm with 5% DMSO and 2% Tween 20.
[y]25 buds each treatment.
[x]Mean separation by Duncan's multiple range test, 5% level.

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growth on the greenhouse plants. Tween 20 concentrations between 1.2 and 2.0% were satisfactory, concentrations below 1.2% resulted in poorer bud break.

There were some BA-treated buds which initiated growth but stopped growing when 1 to 10 mm in length in all our experiments. These buds did not resume growth and were not included in the data. This phenomenon only occurred when Tween 20 was in the BA treatment solution.

Seedling tops are normally lopped in California commercial citrus nurseries to force growth of the scion bud in the spring. Scion buds inserted in the fall remain dormant until the following spring. There is usually no problem of buds failing to initiate growth except with a few cultivars, particularly 'Owari' satsuma, a popular cultivar. Up to 50% of satsuma buds, depending upon the season and weather conditions, may fail to initiate growth under standard nursery procedure, according to citrus nurserymen.

A series of trials using BA to force stasuma buds was carried out in 1979 and 1980 in a commercial citrus nursery in the southern end of California's San Joaquin valley. Concentrations of BA ranged from 250 to 4,000 ppm with concentrations of 5% and 10% DMSO and 1% and 2% Tween 20. The material was applied with a cotton swab to individual stasuma buds, all on Troyer citrange rootstock, one bud per plant as is normal commercial nursery practice.

BA treatments were applied following healing in of the satsuma bud and lopping of the rootstock seedling. Only those buds which were still quiescent a month or more following lopping were used in these trials except in the final trial in April 1980 when treatments were applied immediately following lopping. Some budded seedlings were left untreated as controls in each trial. Treatments and controls were randomized in the nursery rows. Repeated applications were tried both over a short period of time to keep the buds wet with BA for up to one hour, and at weekly and longer intervals. Applications were made from April to August 1979 and February to April 1980 with repeated applications on still quiescent buds through June 1980.

BA treatment generally increased bud growth but the percentage of buds growing was still unsatisfactory in hot weather months (Table 4). There was no significant effect among BA, DMSO, or Tween concentrations within the ranges used. Repeated applications did not increase bud stimulation appreciably and application of BA to buds beginning to sprout out burned the tender leaves and growth often ceased.

The use of BA to stimulate quiescent citrus buds would appear to be a practical, although not always reliable, aid to the citrus nurseryman. There were no detrimental side effects provided only fully dormant buds were treated. Better results were obtained in spring than in summer and outdoors rather than in a greenhouse; and there were pronounced differences among the 5 citrus cultivars tested. BA in ethanol at 250 to 500 ppm with 1% to 2% Tween 20 should be satisfactory for commercial use; the addition of DMSO aids in dissolving BA but does not appear necessary.

Table 3. Influence of DMSO and Tween 20 on stimulation of quiescent sweet orange buds in a screenhouse with BA at 1,000 ppm.

Treatment	Buds stimulated into growth (%) Applied April 30, 1979	Applied May 23, 1979	Applied February 20, 1980	Total
1000 ppm BA	8	4	16	8a[y]
1000 ppm BA + 5% DMSO	60	36	12	36b
1000 ppm BA + 2% Tween 20	56	68	48	60c
1000 ppm BA + 5% DMSO + 2% Tween 20	68	48	36	50bc

[z]25 buds each treatment 4/30/79 and 2/20/80, 50 buds each treatment 5/23/79.
[y]Mean separation by Duncan's multiple range test, 1% level.

Table 4. Summary of results of BA application to quiescent scion buds of 'Owari' satsuma budded on Troyer citrange seedlings in a commercial citrus nursery.

Application date	Bud growth of 1 cm or more: BA treated: Total buds	No. growing	%	Controls: Total buds	No. growing	%
April 1979	57	54	94.7	58	43	74.1**[z]
June 1979	49	35	71.4	46	25	54.4
July 1979	108	23	21.3	66	8	12.1
August 1979	55	17	30.9	25	3	12.0
February 1980	245	235	95.9	49	37	75.5**
March 1980	40	35	87.5	39	24	61.5*
April 1980	249	248	99.6	119	113	95.0**
All combined	788	646	82.0	397	251	63.2

[z]Significantly lower than Ba treated at 5% level (*) and at 1% level (**).

Literature Cited

1. Broome, O. C. and R. H. Zimmerman. 1976. Breaking bud dormancy in tea crabapple [*Malus hupehensis* (Pamp.) Rehd.] with cytokinins. J. Amer. Soc. Hort. Sci. 101:28-30.
2. Davies, F. T., Jr. and B. C. Moser. 1980. Stimulation of bud and shoot development of Rieger begonia leaf cuttings with cytokinins. J. Amer. Soc. Hort. Sci. 105:27-30.
3. de Elizalde, M. M. B. and M. R. Guitman. 1979. Vegetative propagation in everbearing strawberry as influenced by a morphactin, GA_3, and BA. J. Amer. Soc. Hort. Sci. 104:162-164.
4. Higaki, T. and H. P. Rasmussen. 1979. Chemical induction of adventitious shoots in Anthurium. HortScience 14:64-65.
5. Kender, W. J. and S. Carpenter. 1972. Stimulation of lateral bud growth of apple trees by 6-benzylamino purine. J. Amer. Soc. Hort. Sci. 97:377-380.
6. Nauer, E. M., S. B. Boswell, and R. C. Holmes. 1979. Chemical treatments, greenhouse temperature, and supplemental day length affect forcing and growth of newly budded orange trees. HortScience 14:229-231.
7. Ohkawa, K. 1979. Promotion of renewal canes in greenhouse roses by 6-benzylamino purine without cutback. HortScience 14:612-613.
8. Parups, E. V. 1971. Use of 6-benzylamino purine and adenine ot induce bottom breaks in greenhouse roses. HortScience 6:456-457.
9. Sachs, T. and K. V. Thimann. 1967. The role of auxins and cytokinins in the release of buds from dominance. Amer. J. Bot. 54:136-144.