METHODS OF APPLYING CYTOKININS TO LEAF CUTTINGS OF RIEGER BEGONIAS

F. T. DAVIES JR. AND B. C. MOSER

Department of Horticulture & Forestry
Rutgers University
New Brunswick, New Jersey

Certain species of plants can be reproduced asexually by means of leaf cuttings. A leaf cutting is composed of an entire leaf (lamina and petiole), or part of a leaf, which is removed from a plant — without an auxillary bud or a piece of stem — for the purpose of propagation. Of great importance in the propagation of leaf cuttings is the development of numerous adventitious buds at the base of the petiole. These buds develop into full, bushy self-supporting plants more readily than from a bud on a stem cutting.

There are two types of Rieger begonias *Begonia bertini* 'Compacti' x *B. socotrana*: The Schwabenland type is commercially propagated from leaf cuttings which produce multiple vegetative basal shoots and has an upright form. The Aphrodite type is propagated by vegetative stem cuttings and is a pendulous form; leaf cuttings do not consistantly produce adventitious buds at the base of the leaf petiole.

The cytokinins are a growth regulator group reported to stimulate bud initiation from leaf cuttings (2, 3, 4, 5). Experiments were conducted to examine possible methods of applying cytokinins for commercial propagation of leaf cuttings.

MATERIALS AND METHODS

Experiments were performed with 2 cultivars of Rieger begonias *Begonia bertini* 'Compacti' x *B. socotrana*, cultivars: 'Aphrodite Cherry Red (AR) and 'Schwabenland Red' (SR).

Stock plants were grown from rooted cuttings supplied by Mikkelsens, Inc., Ashtabula, Ohio, which were potted in a 4:1:1 peat:perlite:soil mix and maintained in the greenhouse under 70°F night temperature with 3 hr. night light interruption. Noon temperatures were 72-91°F and light intensity was no greater than 2500 ft-c at mid-day.

Cuttings were inserted in flats containing a 1:1 peat:perlite mix with 1 lb. of dolomitic limestone/ft^3, and were grown in the greenhouse under the same temperature regimes as the stock plants. Cuttings were intermittently misted during daylight hours for the first 13 to 16 days, at which time rooting had taken place.

The crystalline forms of 6-furfurylamino-purine (kinetin), 6-benzylamino-purine (BA), and 6-benzylamino-9-(tetrahydro-2-pyryl)-purine (PBA) were brought into aqueous solution. For

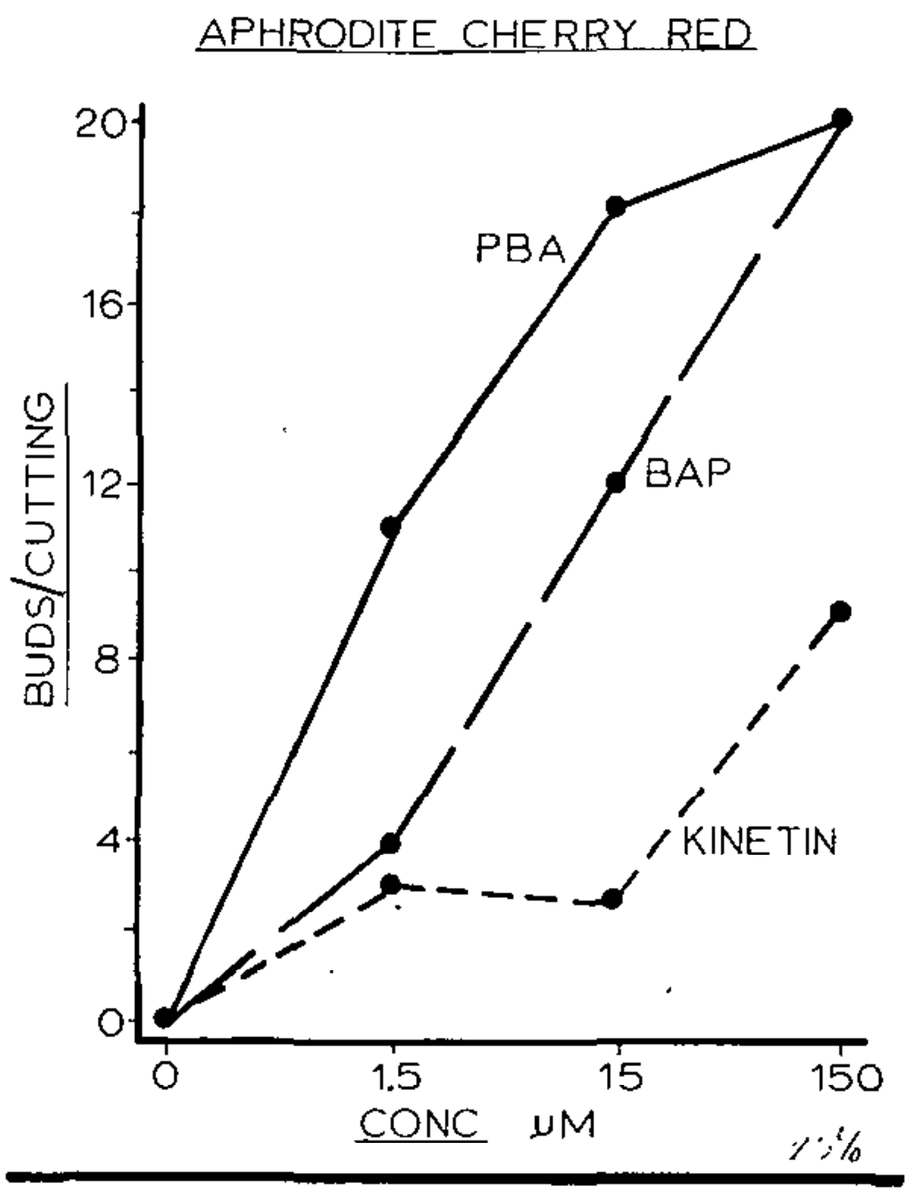

Figure 1. Effect of kinetin, BA, and PBA on the number of buds per cutting. 'Aphrodite Cherry Red'.

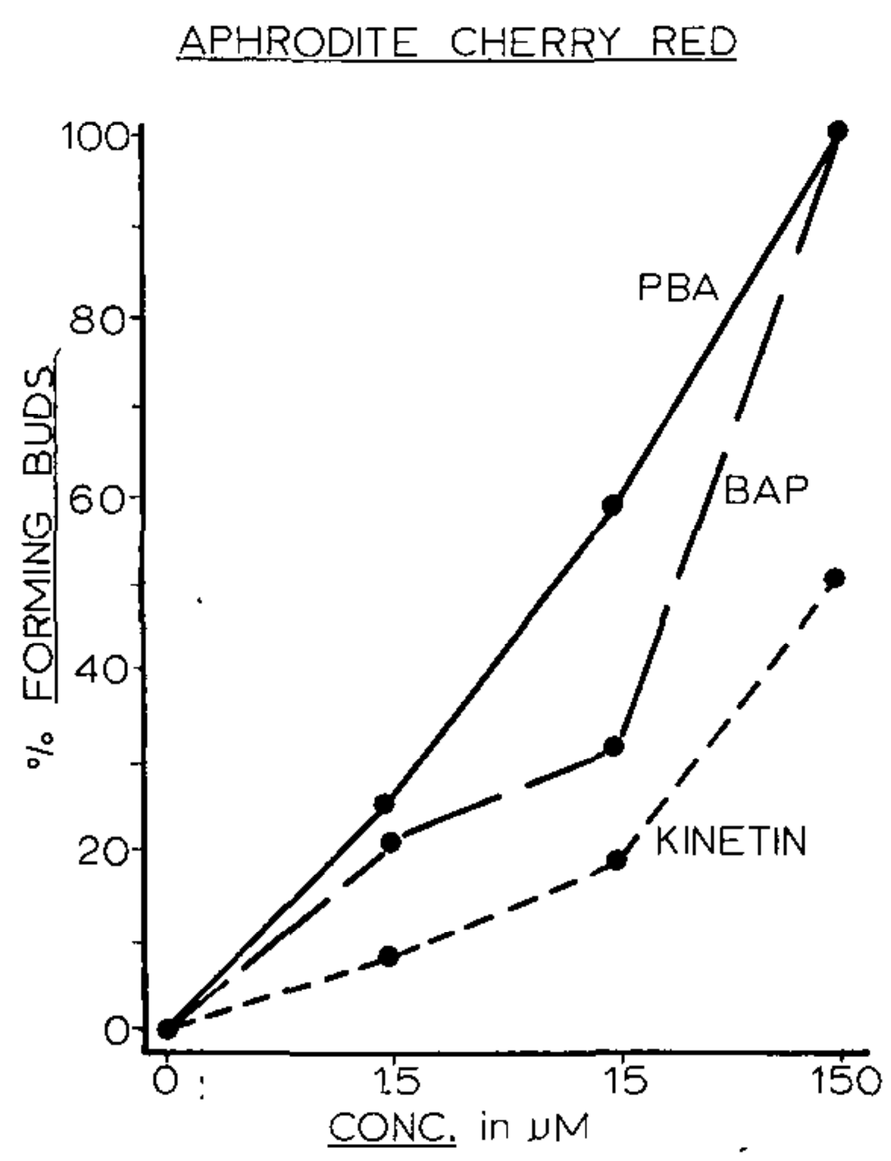

Figure 2. Effect of kinetin, BA, and PBA on the percent cuttings which formed buds. 'Aphrodite Cherry Red'.

"quick-dips" (0.1 min.), a 50% solution of ethyl alcohol was used. When applied with a talc carrier, the cytokinin was measured and brought into solution with 95% ethyl alcohol, and weighed talc was added. The slurry was repeatedly mixed until the mixtures reached a dry powder state. No surfactants were used.

could accurately be measured was twenty. A randomized block design was utilized with 12 observations per treatment. Mean values reflect the average of the twelve cuttings per treatment.

RESULTS

In a preliminary experiment the cytokinins: 6-furfurylamino-purine (kinetin), 6-benzylamino-purine (BA), and 6-benzylamino-9-(tetrahydro-2-pyryl)-purine (PBA) were applied at 1.5, 15, 150 μM to leaf cuttings via a 12 hr basal-petiole soak. Comparing cytokinins (Figs. 1, 2) PBA was the most effective in stimulating bud initiation, followed by BA and kinetin. Too high a concentration (150 μM) of either PBA or BA caused a large proliferation of buds with subsequent poor shoot development (Fig. 3). At 15 μM PBA (Table 1), bud regeneration was reduced as expressed by weight of buds and shoots (Fig. 4). However, 'Aphrodite Cherry Red' responded positively in both bud and shoot regeneration (Table 1).

To determine the relationship between concentration of cytokinin and length of treatment, cuttings were basally soaked for 0.6 to 300 min. at 20 to 1500 μM PBA. With increasing concentration of cytokinin there was a decrease in treatment time to stimulate optimal bud initiation and shoot development (Table 2).

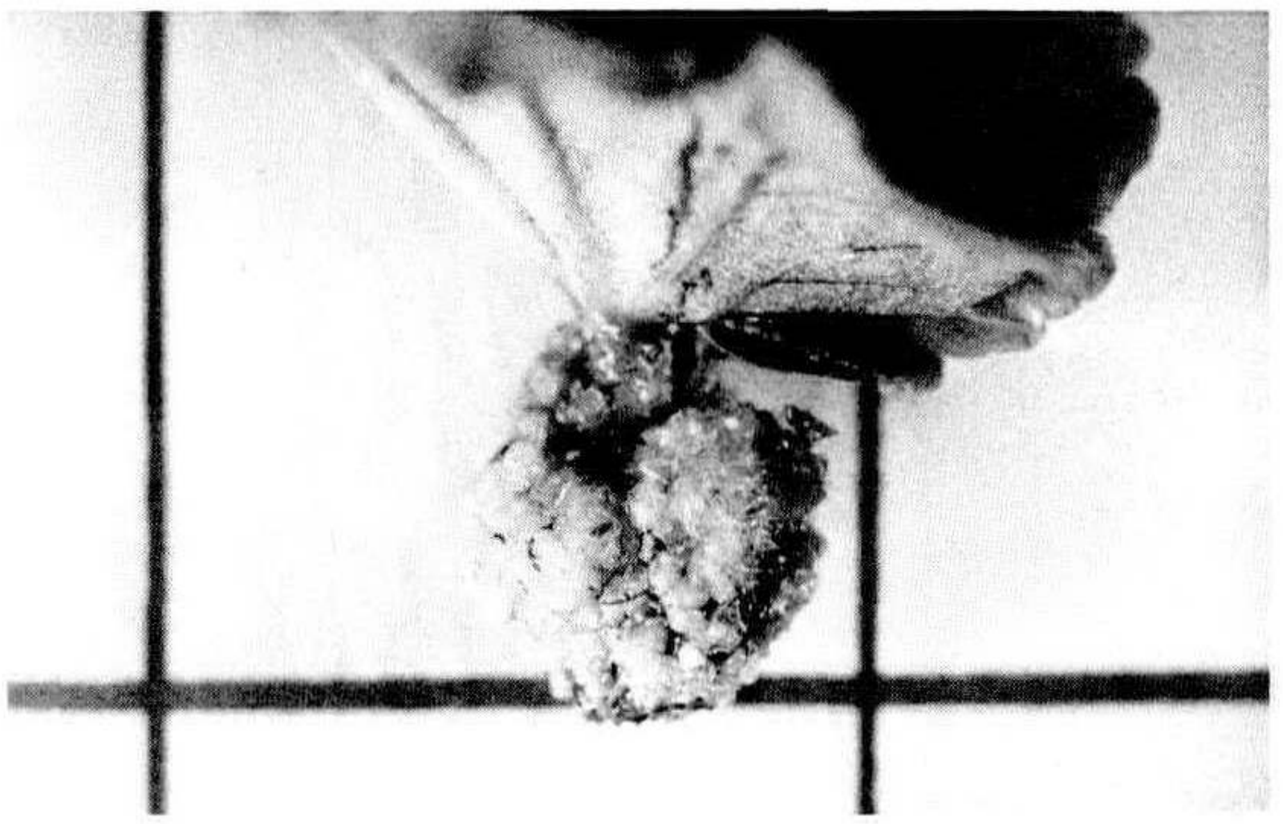

Figure 3. 150 μM PBA. 'Schwabenland Red'. Roots were removed before photograph was taken.

Table 1. PBA at 15 μM. 'Aphrodite Cherry Red' (AR) and 'Schwabenland Red' (SR).

Cultivar	Treatment	% Forming buds	Buds	Wgt of buds & shoots
				(gm)
A.R.	Control	0	0	0
	PBA	58	18.2	0.5
S.R.	Control	92	20.0	2.6
	PBA	100	19.2	1.3
Lsd (0.05)		17	3.9	0.4

Table 2. Optimal duration time for respective PBA concentration. 'Aphrodite Cherry Red'.

Treatment	Time (min.)	Buds	% Buds[1]	Shoots	% Shoots[2]	New green leaves
Control	—	2.6	33	1.3	33	1.0
PBA 20μM	300	7.7	83	9.4	83	4.3
100μM	30	9.3	92	8.1	92	2.3
500μM	10	9.0	92	9.4	83	2.7
1500μM	'Q-dip'	15.0	92	7.8	92	2.5
Lsd (0.05)	5.2	33	3.3	42	1.6	

[1]Percent cuttings which initiated buds.
[2]Percent cuttings which developed shoots.

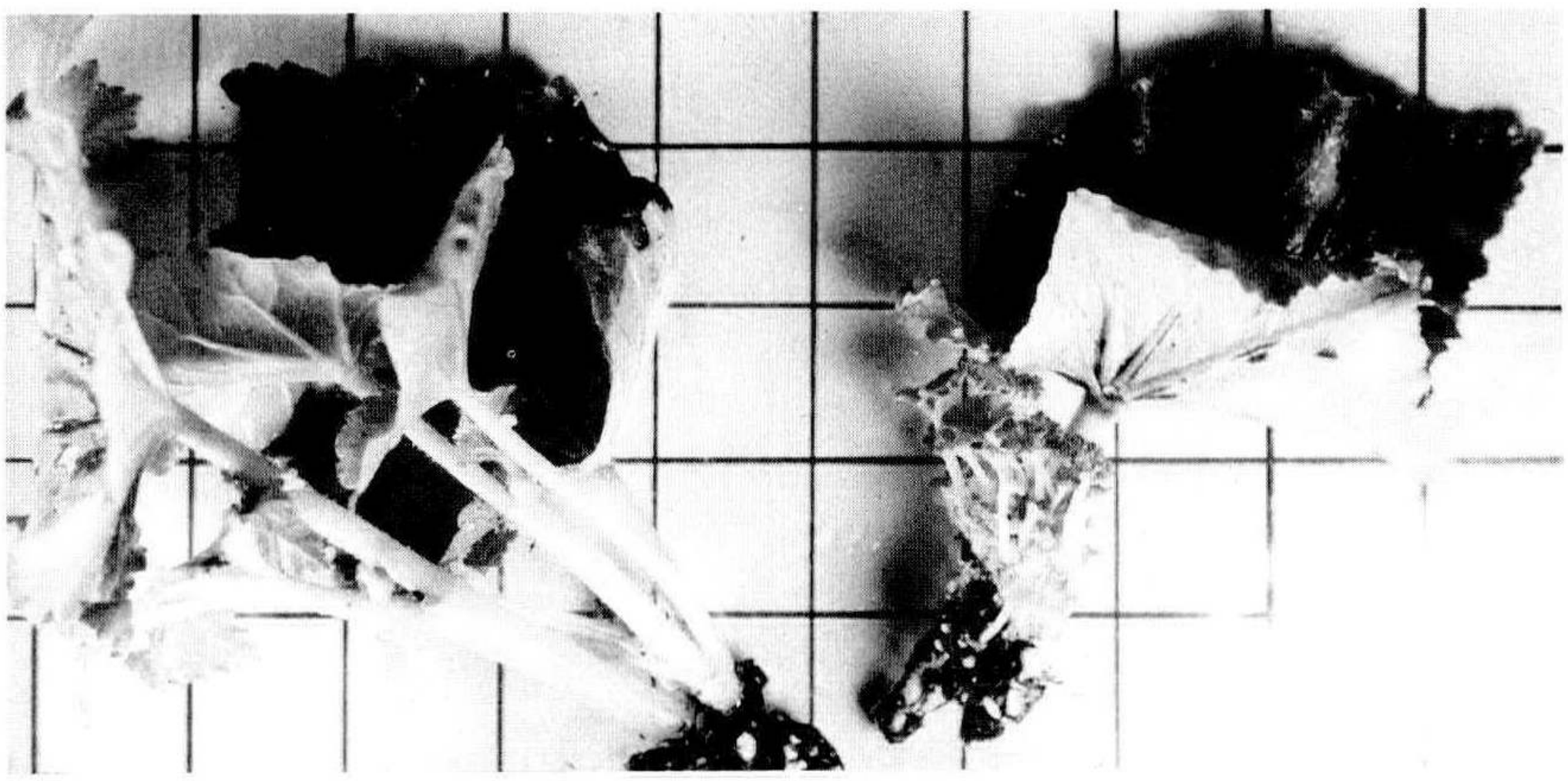

Figure 4. 'Schwabenland Red.' *Left* Control. *Right* 15μM PBA.

Application methods included a basal petiole soak plus a 6-second "quick-dip" treatment, dry talc, and spray applications.

Experiments were terminated 10 weeks after the cuttings were inserted. Data collected included the number of buds, shoots, and new green leaves which developed from adventitious shoots, and weight of buds and shoots. The maximum number of buds which

A more convenient time in employing a basal-petiole soak was via a 6 second "quick-dip." PBA at 4,170 to 12,500 μM was applied to 'Aphrodite Cherry Red'. At the time data was taken (Table 3), 4,170 and 6,250 μM yielded taller, thicker-stemmed, more horticulturally desirable new plants, but when some cuttings were potted and grown on as stock plants, the difference between concentrations was negated in 50 days (Fig. 5).

To determine the critical time period in which cytokinin must be applied to cuttings, 'Aphrodite Cherry Red' was treated with 10, 100, 1000 μM PBA, using a spray application at either 4, 13, or 26 days after insertion of the leaf cuttings. Applying PBA at 4 days after

insertion was superior to either 13 or 26 days in stimulating bud, shoot, and formation of new green leaves (Table 4). When PBA was applied at the 26th day there was no response to chemical treatment.

A talc dip is commercially desirable for applying growth regulators during propagation. Leaf petioles were dipped in talc containing PBA. Responses from the talc dip were consistent with other application methods (Table 5), with PBA at 0.01% stimulating optimal responses (Fig. 6).

DISCUSSION

The results demonstrate that the cytokinin PBA can effectively be applied as a basal-petiole dip, "quick-dip," talc dip, or as a spray.

Table 3. "Quick-dip" application of PBA. 'Aphrodite Cherry Red'.

PBA (μM)	Buds	Shoots	New green leaves	Weight (gm)
0	0	0	0	0
4,170	10.0	11.4	12.8	8.8
6,250	16.7	16.1	15.3	10.7
8,338	15.4	16.3	13.8	9.3
10,420	15.4	16.2	15.9	11.7
12,500	19.2	17.7	15.8	11.2
Lsd (0.05)	3.9	4.3	4.4	3.5

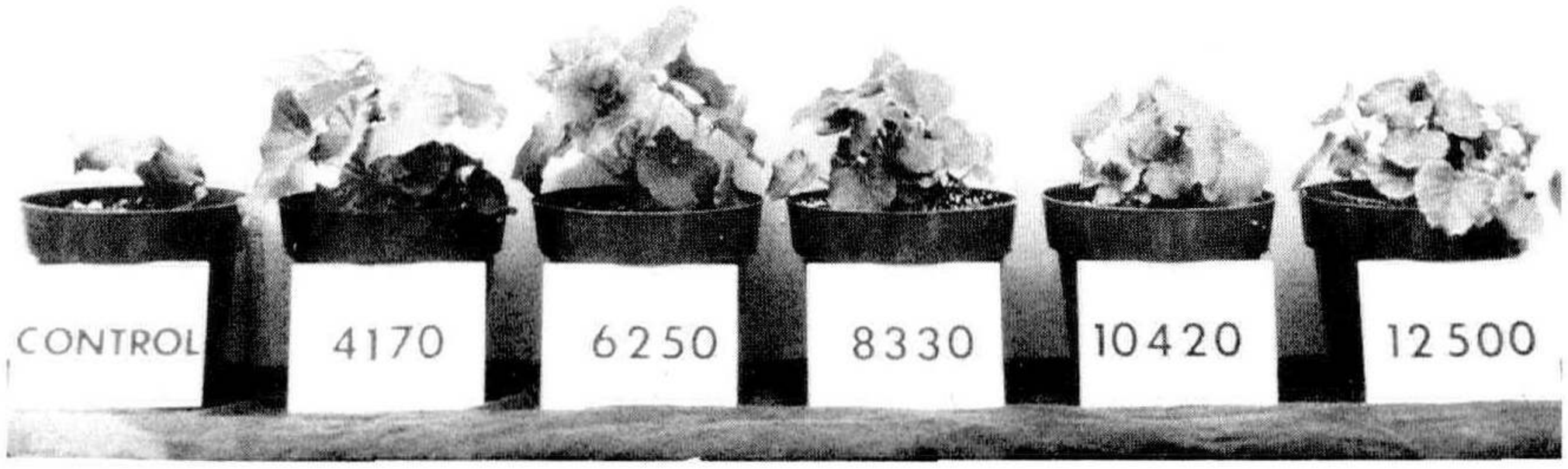

Figure 5. "Quick-dip" application of PBA at 0 to 12,500 μM. 'Aphrodite Cherry Red'.

At a 12-hour basal petiole soak, intermediate levels of PBA (15μM) could effectively stimulate bud and shoot formation in 'Aphrodite Cherry Red'. In 'Schwabenland Red', with increasing PBA concentration shoot development was retarded, as expressed by bud and shoot weight. This would suggest that higher levels of cytokinin:auxin exist in 'Schwabenland Red' vs. 'Aphrodite Cherry Red'. By increasing the cytokinin:auxin level via exogenous cytokinin, a supraoptimal response occurred in 'Schwabenland Red' with a profuse number of buds developing, causing poor shoot development which led to a stunted, less desirable new plant. It became clear that

Table 4. Spray application of PBA at three different intervals after insertion of cuttings. 'Aphrodite Cherry Red'.

Treatment	Buds	Percent Forming buds	Shoots	New green leaves
Control	0.8	17	0.3	0.3
Day #4				
10μM	3.3	17	0.1	0.4
100μM	12.5	100	5.8	4.0
1,000μM	20.0	100	11.7	5.8
Day #13				
10μM	0.8	42	0.6	0.2
100μM	4.1	33	1.5	0.3
1,000μM	11.7	100	8.6	3.9
Day #26				
10μM	0.4	8	0	0.1
100MM	0.8	17	0.3	0.1
1,000μM	2.9	33	0.5	0.2
Lsd (.05)	4.0	22	4.3	3.6

Table 5. Applying PBA with talc as a carrier. 'Aphrodite Cherry Red'.

Percent PBA	Buds	Percent Buds[1]	Shoots	Percent Shoots[2]	New green leaves	Weight (gm)
0	0.4	8	0.7	8	0	0.4
0.001	2.5	25	2.0	25	2.0	1.3
0.003	3.3	50	2.7	50	3.0	2.2
0.01	17.9	100	10.5	100	10.1	8.5
0.03	20.0	100	10.8	100	7.7	3.8
0.1	18.3	100	12.6	100	7.1	2.7
0.3	20.0	100	11.7	92	1.1	2.7
1.0	18.8	100	4.3	67	1.4	2.5
Lsd (.05)	2.8		4.4		4.0	1.9

[1]Percent cuttings forming buds.
[2]Percent cuttings forming shoots.

stimulating large numbers of buds was not desirable from a horticultural standpoint.

The 4th day after insertion was the optimal time to apply PBA via spray; 100 μM stimulated the more desirable results. At the 13th day 1,000 μM PBA stimulated optimal results, while at the 26th day after insertion no concentration of PBA stimulated a response. This would suggest that there is a critical time in which a mechanism(s) must be triggered if optimal bud initiation and subsequent shoot development is to be achieved. High concentration (1,000 μM) of PBA could trigger this process through day #13, but later application proved ineffective. With other species of begonias, investigators

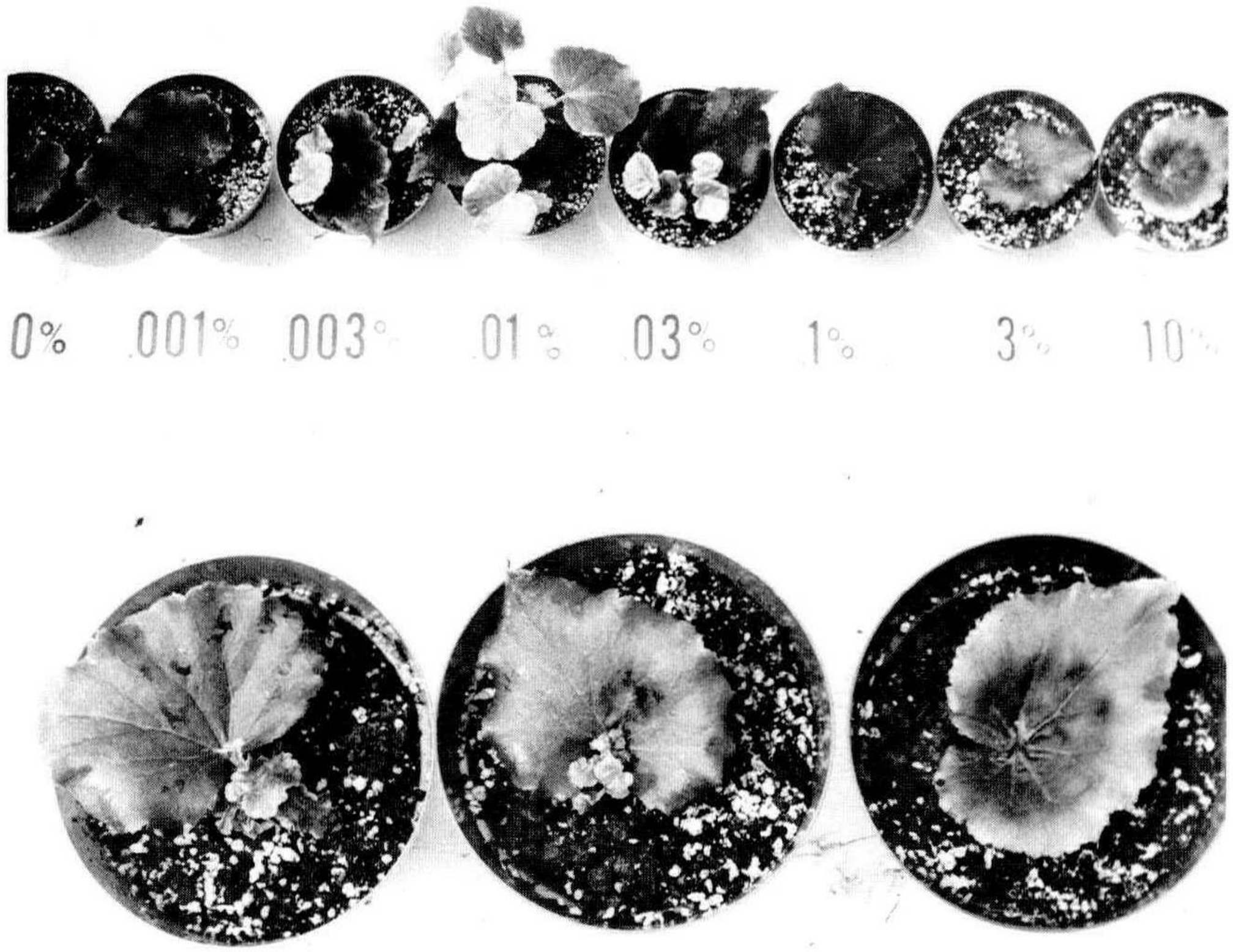

Figure 6. Effect of PBA applied as a talc dip to 'Aphrodite Cherry Red'. *Top:* 0 to 1.0%. *Bottom: left to right:* 0.1%, 0.3%, 1.0%.

(3) have observed that the first 10 to 20 days were critical for bud initiation.

Use of a talc dip is a common method of applying auxins to stem cuttings. However, no attempts have been reported in the literature where cytokinin was successfully applied as a talc in bud and shoot regeneration of leaf cuttings. Responses from the talc dip were consistant with other application methods of PBA.

There are many species where a multi-stemmed plant would be an alternative and/or more desirable form for commercial propagation. With certain species of *Begonia, Peperomia, Saintpaulia,* etc., use of leaf cuttings is the common method of propagation. With other species, leaf cuttings form roots but bud formation is poor or nonexistant. The chemical PBA represents a cytokinin with good mobility, sufficient self-integrity when in the plant system, and/or ability to get to the metabolic site of action. With the good success obtained with PBA in the Rieger begonia system, avenues are opened for testing other species of plants whose propagation by leaf cuttings would be highly desirable.

LITERATURE CITED

1. De Stoppelar, G. and R. W. Lightly. 1967. The Longwood Gardens method of propagating winterflowering begonias. *The Begon.* 34:104-107.
2. Handro, W. and P. S. Rao. 1972. Hormonal control of the formation of callus, buds, roots, and embryos in leaf and stem explants of petunia grown in vitro. *Com. Res. Hebs. Sean. d. 1. Acad. d. Sce.* 275:2861-63.
3. Heide, O. M. 1965. Interaction of temperature, auxins, and kinins in the regeneration ability of begonia leaf cuttings. *Physiol. Plant.* 18:891-920.
4. Rao, P. S., W. Hondor, and H. Harada. 1973. Hormonal control of differentiation of shoots, roots, and embryos in leaf and stem cultures of *Petunia inflata* and *Petunia hybrida. Physiol. Plant.* 28:458-63.
5. Thakur, S. 1973. *In vitro* foliar shoot and bud formation in *Begonia semperflorens. Current Sci.* 42:430-32.