carnations (Fig. 1). This observation is explained by the rapid movement reported for the STS complex in carnation stems (7). In this experiment, 5 cm of the stem was removed immediately after pulsing. Since recutting did not eliminate the effect of STS on the vase life of the flowers, silver must already have moved further than 5 cm even after a 1 min pulse in the complex (4 mM Ag) indicating a transpiration velocity greater than the 3 cm/min determined by other workers using radioisotope techniques (7). Because the benefits of the silver treatment are not lost when the stem of the flower is recut, the silver thiosulfate complex offers considerable practical advantages over the silver nitrate pretreatments that have previously been proposed (4).

In their work, Veen and van de Geijn (7) used a treatment of 24 hr in STS containing 2 mM Ag at room temperature. We found that this treatment (which under our conditions would result in a silver content of about 50 μmol per stem) caused severe petal burn and pitting and necrosis of the leaves. The safe maximum application in our experience was about 5 μmol of silver, and the optimal treatment resulted in between 0.5 and 2 μmol per stem. The method of applying the silver need not be a short term pulse. Overnight treatment at 2°C with 1 mM Ag in the STS form has also proved highly effective. It appears probable that the application conditions could be tailored to suit any particular commercial operation to ensure that the flowers received the optimal application of silver.

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Stimulation of Bud and Shoot Development of Rieger Begonia Leaf Cuttings with Cytokinins[1]

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Abstract. **Leaf cuttings of Rieger elatior begonias (*Begonia hertini* 'compacti' × *B. socotrana* cvs. Aphrodite Cherry Red and Schwabenland Red) were treated with 6-furfurylamino purine (kinetin), 6-benzylamino purine (BA), and 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA). BA and PBA enhanced bud and shoot regeneration in 'Aphrodite Cherry Red,' while kinetin showed no activity. All cytokinins tested reduced shoot development in 'Schwabenland Red.' PBA stimulated optimal bud and shoot development when applied to 'Aphrodite Cherry Red' leaf cuttings as a 12 hour 15 μM basal-petiole dip, 1000 μM spray, and 0.01% talc-petiole-dip. Cuttings taken from 'Aphrodite Cherry Red' stock plants treated with 1000 μM PBA successfully generated new plants.**

Certain herbaceous species (2, 7) are propagated from leaf cuttings (lamina and petiole) through initiation of adventitious buds at the base of the petiole. Growth regulators most closely associated with bud initiation have been the cytokinins (3, 5, 9, 12, 13), but abscisic acid (5), (2-chloroethyl)trimethyl ammonium chloride (chlormequat, CCC) (6) and auxin (1) also induced buds under certain conditions.

Rieger elatior begonias consist of 2 types in regard to propagation: 1) 'Schwabenland' group is propagated by leaf cuttings and produce multiple vegetative basal shoots, whereas 2) 'Aphrodite' group does not consistently produce adventitious basal buds at the petiole (8) and is, therefore, propagated by vegetative stem cuttings.

This investigation was conducted to characterize effects of cytokinins on bud and shoot regeneration of Rieger elatior begonia leaf cuttings.

Methods and Materials

Stock plants of 'Aphrodite Cherry Red' and 'Schwabenland Red' were grown from rooted cuttings supplied by Mikkelsens, Inc., Ashtabula, Ohio, potted in a 4 peat:1 perlite:1 soil mix and maintained in the greenhouse under long day conditions by supplementary incandescent light of 120 lux from 11 PM to 2 AM.

Leaf cuttings with 2.5 cm petioles were planted in flats containing 1 peat:1 perlite mix (by volume) amended with 16 kg dolomitic limestone/m^3 and grown in the greenhouse under the same conditions as stock plants. Cuttings were intermittently misted during daylight hours for 13-16 days at which time rooting had taken place.

Crystalline forms of kinetin, BA, and PBA were dissolved in water. When applied with a talc carrier PBA was measured, brought into solution with 95% ethyl alcohol and weighed

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talc added. The slurry was mixed until it became a dry powder. No surfactants were used.

A completely randomized block design was utilized with 3 replications of 4 cuttings each. Each experiment was conducted for 10 weeks after which data were collected. Data included number of buds and shoots and fresh weight of buds and shoots (which was used as an index of shoot development). Mean values reflected the average of 12 cuttings per treatment.

In Expt. 1, 1.5-150 μM of kinetin, BA, and PBA were applied as a 12 hr basal petiole soak to 'Aphrodite Cherry Red' and 'Schwabenland Red'. In Expt. 2 the cultivars were treated with a petiole dip in dry talc carrier containing 0.03–1.0% PBA and in Expt. 3 'Aphrodite Cherry Red' was treated with a petiole dip in dry talc carrier containing 0.001–1.0% PBA. In Expt. 4 foliar sprays of 0-1000 μM were applied to 'Aphrodite Cherry Red' leaf cuttings at 4, 13 or 26 days after planting, while in Expt. 5 foliar sprays of 0-1000 μM were applied to 'Aphrodite Cherry Red' stock plants at 1, 3 or 10 days before leaf cuttings were removed for planting.

Results

Expt. 1. Cytokinins applied as a basal petiole soak. Cultivars differed in response to cytokinin (Table 1). Bud initiation in 'Aphrodite Cherry Red' was stimulated at 150 μM BA and 15 and 150 μM PBA, while there was no response to kinetin at any concentration. Bud and shoot weights were also increased at 150 μM BA and PBA.

Cytokinins did not affect the number of countable buds formed in 'Schwabenland Red', however the petioles of treated cuttings often exhibited a proliferation of bud like tissue at the expense of shoot formation (Fig. 1). Kinetin, BA, and PBA reduced bud and shoot weight in 'Schwabenland Red' mainly because of lack of development of buds into shoots.

Expt. 2. PBA applied in talc. There were differences between cultivars in response to PBA application as a petiole dip in talc. In 'Aphrodite Cherry Red', PBA stimulated number of buds and shoots at all concentrations ranging from 0.03–0.1% (Table 2). In 'Schwabenland Red' PBA had no effect on no. of countable buds, and resulted in increased shoot number only at 0.05% and 0.1% (Table 3). Weight of buds and shoots was reduced at all concentrations studied.

Expt. 3. PBA applied in talc. As bud and shoot development was enhanced by talc-applied PBA in 'Aphrodite Cherry Red' (Expt. 2), a larger range was tested to determine optimal concentration. The 0.01% concentration was most effective in producing a horticulturally desirable plant with excellent shoot development (Figs. 2, 3). Concentrations below 0.01% were less effective and concentrations above 0.01% resulted in a proliferation of bud-like tissue at the expense of larger shoots (Fig. 2).

Expt. 4. PBA application as a foliar spray. PBA spray applied to 'Aphrodite Cherry Red' cuttings 4 days after planting was superior to either 13 or 26 days in stimulating no. of buds and shoots (Fig. 4). There was no response to application on the

Table 1. Effects of kinetin, BA and PBA on bud and shoot formation in 'Aphrodite Cherry Red' and 'Schwabenland Red'.

Cytokinin treatment concn (μm)	No. buds/cutting		Bud & shoot wt (g/cutting)	
	Aphrodite Cherry Red	Schwabenland Red	Aphrodite Cherry Red	Schwabenland Red
Control	0	20.0	0.3	2.6
Kinetin				
1.5	0.3	19.6	0	1.2
15	0.4	18.8	0	1.6
150	4.4	16.5	0.1	0.5
BA				
1.5	1.0	16.5	0	2.1
15	4.4	20.0	0	1.3
150	20.0	16.0	1.0	0.9
PBA				
1.5	2.7	17.7	0.1	1.1
15	11.5	19.1	0.5	1.3
150	20.0	18.1	1.0	1.2
LSD 5%	5.1	5.1	0.4	0.4

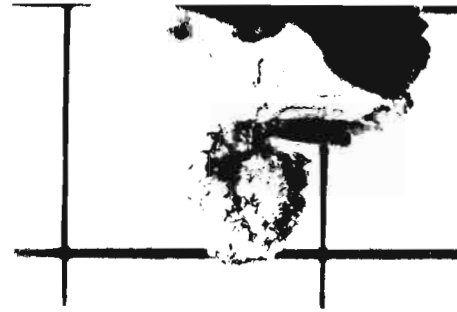

Fig. 1. Cytokinins reduced shoot development in 'Schwabenland Red'. Only buds and bud-like tissue are visible. Roots formed but were removed before photograph was taken.

Table 2. Effects of PBA applied in talc carrier on propagational responses of 'Aphrodite Cherry Red'.

PBA treatment (%)	No. buds per cutting	No. shoots per cutting	Bud & shoot wt per cutting (g)
0	0	0	0
0.03	12.1	6.9	12.4
0.05	18.6	8.9	11.1
0.1	13.6	7.6	8.0
0.2	16.7	8.3	5.0
0.4	17.2	10.6	5.7
1.0	10.6	6.3	2.3
LSD 5%	4.6	4.8	6.2

Table 3. Effect of PBA applied in talc carrier on propagational responses of 'Schwabenland Red'.

PBA treatment (%)	No. buds/cutting	No. shoots/cutting	Bud & shoot wt (g/cutting)
0	15.9	7.6	34.9
0.03	16.7	6.6	22.7
0.05	20.0	14.0	16.7
0.1	12.0	12.9	8.4
0.2	17.5	9.3	4.2
0.4	15.4	8.3	2.3
1.0	0	0	0
LSD 5%	4.6	4.8	6.2

Fig. 2. Effects of PBA applied as a talc dip to 'Aphrodite Cherry Red'.

26th day. Maximum promotion of bud and shoot growth was achieved with 1000 μM spray.

Expt. 5. PBA pre-treatment of stock-plant. PBA applied to 'Aphrodite Cherry Red' stock plants 1 day before cuttings were taken stimulated more shoot and bud development (Table 4) than when applied 3 days or 10 days prior to taking cuttings.

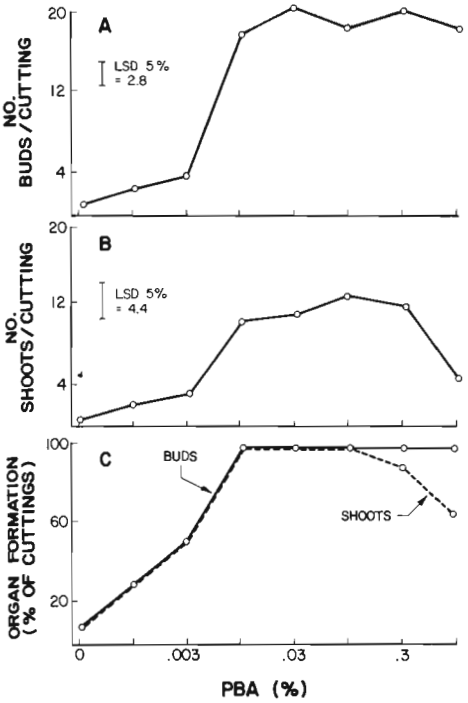

Fig. 3. Effects of PBA applied as a talc dip to 'Aphrodite Cherry Red'.

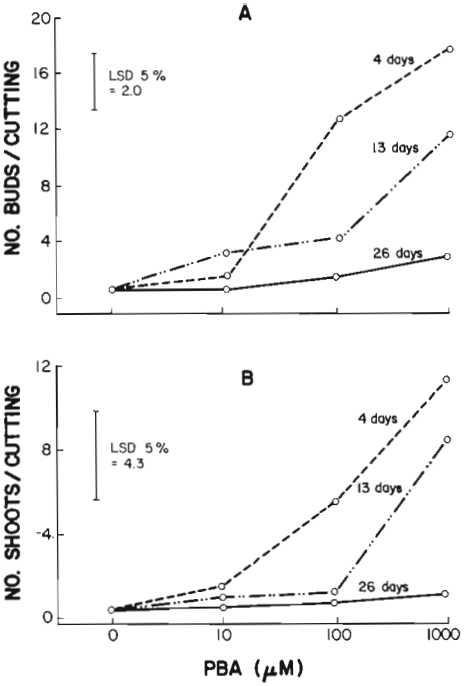

Fig. 4. Effect of PBA applied as a spray to leaf cuttings of 'Aphrodite Cherry Red' on (A) buds and (B) shoots at 4, 13 or 26 days after planting.

Table 4. Effects of PBA foliar sprays applied to 'Aphrodite Cherry Red' stock plants at 3 intervals before cuttings were taken.

Days before cuttings removed	PBA treatment (μm)	No. of buds/ cutting	No. of shoots/ cutting	Bud and shoot wt (g/cutting)
-	Control	0	0	0
1	30	0	0	
	100	10.4	8.3	2.9
	300	12.9	6.3	3.1
	1000	18.3	9.7	4.2
3	30	0.8	0.7	0.1
	100	1.3	0.7	2.9
	300	10.8	9.6	1.6
	1000	16.7	8.7	2.3
10	30	0.1	0.3	0.1
	100	0.9	0.5	0
	300	5.4	1.7	0.4
	1000	13.3	0.8	0.5
LSD 5%		4.7	4.0	0.5

A time-treatment relationship was evident in that PBA applied at day 1 stimulated increases in bud and shoot number and bud and shoot weight at 100, 300 and 1000 μM vs day 3 where cuttings showed similar responses at only 300 and 1000 μM and day 10 where only 1000 μM stimulated responses in bud number and bud and shoot weight but not shoot number.

Discussion

PBA and BA successfully stimulated bud and shoot formation in leaf cuttings of 'Aphrodite Cherry Red'. Once optimum shoot development was obtained further increased PBA concentration reduced bud and shoot weight (Table 2, Fig. 2, 3). PBA was more active than BA since at 15 μM PBA there was increased bud and shoot formation while BA elicited no response (Table 1). Kinetin produced no activity. These results agree with other reports (4, 9, 14) on bud regeneration in leaf propagation that ranked PBA most active followed by BA and kinetin.

Exogenous application of cytokinin at any concentration restricted shoot development which reduced natural regeneration ability of 'Schwabenland Red' (Table 1, 3 and Fig. 1). The fact that cytokinins stimulated bud and shoot regeneration in 'Aphrodite Cherry Red' while retarding shoot development in 'Schwabenland Red' suggested that lower endogenous cytokinin:auxin levels may occur in 'Aphrodite Cherry Red' relative to 'Schwabenland Red'. By increasing endogenous levels of cytokinin the ratio of cytokinin:auxin might have been raised to a level needed for desirable responses. High levels of cytokinin retarded shoot development and reduced root systems. This is in agreement with the relationships found between cytokinin and auxin levels as a major factor controlling bud and root differentiation in other tissues (11, 12).

The talc dip method of applying cytokinins proved easy to use and might be commercially desirable.

There is a critical period in which a mechanism(s) must be triggered for bud and shoot regeneration. Foliar application of PBA to cuttings after they had been placed in flats in greenhouses stimulated maximum responses when applied at day 4, intermediate at day 13, and no response at day 26. Investigators (4, 10) working with other species of begonia observed that the first 10–26 days were critical for bud initiation. Thus, to be effective, cytokinin applications should be made as soon as practical after cuttings are taken.

Cuttings taken from PBA treated stock plants were able to generate greater responses than controls, but there was a reduction in cytokinin effects with time since at day 10 only high concentration (1000 μM) was effective. Reduction in stimulation from 1 to 10 days (Table 4) indicated that cytokinin was partially anabolized, catabolized, and/or transported to other areas of the plant.

There is good evidence that with PBA application 'Aphrodite Cherry Red' could be commercially propagated by leaf cuttings. We suggest that cytokinins may be helpful in other species where leaf propagation is marginally effective.

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