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Effect of GA_{4+7} on Growth and Cellular Change in Uniconazole-Treated Hibiscus

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Abstract. *Hibiscus rosa-sinensis* 'Jane Cowl' in 1.5-l pots were given a soil drench of 0.2 mg uniconazole, pruned 2 weeks later, and treated with a foliar application of GA_{4+7} at 0, 25 (once or four times every 2 weeks), 50 (once or twice every 4 weeks), or 100 mg L^{-1}. One application of GA_{4+7} at 100 mg L^{-1}, two applications at 50 mg L^{-1}, and four applications at 25 mg L^{-1} were more active in partially restoring stem elongation and caused nearly normal leaf production than other GA treatments, but promoted the abscission of the lower leaves. The size of the individual leaves, but not stem diameter, increased following GA_{4+7} application. Multiple applications of GA_{4+7} stimulated flowering of the retarded plants. Uniconazole resulted in short pedicels bearing short cells with increased diameter, as well as larger pith, vascular, and cortical tissues than the untreated control. Four applications of 25 mg L^{-1} GA_{4+7} to uniconazole-treated plants resulted in long pedicels, having long cells similar to the control. Results of the histological study suggest that uniconazole either slowed cell division or caused cell division to cease early.

Uniconazole [(E)-1-(p-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazole-1-yl)-1-penten-3-ol] interferes with the biosynthesis of gibberellic acid in plants. Whether applied to the root medium or shoots, uniconazole is very effective in suppressing shoot elongation and limiting the rate of leaf production and leaf size in hibiscus (Wang and Gregg 1989, 1991). Occasionally, plants are overdosed with a growth retardant, resulting in an unmarketable crop. Cox (1993) recently reported that the application of 50 mg L^{-1} GA_3 reversed the dwarfing effects of paclobutrazol, a retardant structurally similar to uniconazole, on poinsettia. The dwarfing effects of paclobutrazol on apple (Privé et al. 1989), marigold (Moore and Schekel 1985), and sunflower (Wample and Culver 1983) were overcome by GA_3 or GA_{4+7}. Foliar treatment with 50 mg L^{-1} GA_3 reversed the dwarfing effects of uniconazole on hibiscus shoots (Wang 1991).

It has been shown that uniconazole inhibits the development of vascular tissue, resulting in thinner, weaker stems in hibiscus (Wang and Gregg 1989) and poinsettia (McDaniel et al. 1990). Phloem fiber cells were either absent or few with thin walls in stems of uniconazole-treated plants. The effect of growth-retarding substances on the pedicel has received less attention. The short apple pedicels on paclobutrazol-treated plants had fewer but otherwise normal length cells compared to those in untreated plants. The inhibition of cell division by paclobutrazol in apple pedicel was reversed by GA_{4+7} (Privé et al. 1989). The elongation of a hibiscus pedicel was limited by treatment with uniconazole (Wang and Gregg 1989). Structural changes in the pedicels of plants treated with uniconazole and its potential reversal with gibberellins have not been investigated.

The objectives of this study were to determine the effects of uniconazole on pedicel growth and development in hibiscus and evaluate the potential for reversing the dwarfing response with GA_{4+7}. Histological procedures were used to examine changes in the pedicel tissue associated with uniconazole and gibberellin treatments.

Materials and Methods

Plant Material

The cultivar used in this study was *Hibiscus rosa-sinensis* L. 'Jane Cowl' with orange, double flowers. Plants were grown in 1.5-l pots filled with a bark-free medium (Sunshine No. 1; Fisons

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Table 1. Effect of single or multiple applications of GA_{4+7} at several concentrations on growth and flowering of hibiscus previously treated with a soil drench of uniconazole at 0.2 mg/pot[a]

Uniconazole	GA_{4+7} Concentration (mg l^{-1})	Times	Height (cm)	Width (cm)	Stem Elongation (cm)	Stem Diameter (mm)	No. lateral shoots[b]
Yes	0	0	15.3 d	23.3 d	1.3 d	5.72 b	0 b
	25	1	18.0 bcd	24.5 d	1.8 d	5.53 b	0 b
	50	1	17.5 cd	27.8 d	3.8 d	5.79 b	0.1 b
	100	1	21.9 bc	32.9 cd	8.1 c	5.51 b	0.3 b
	25	4	23.0 bc	46.6 b	12.5 b	5.79 b	0.6 b
	50	2	23.8 b	42.1 bc	10.1 bc	5.92 b	0.4 b
No	0	0	74.6 a	78.9 a	42.4 a	7.35 a	13.3 a

[a] Mean separation within columns by Tukeys test at p = 0.05.
[b] Per selected shoot.

Horticulture, Seattle, WA) and pinched to promote lateral shoot development. The maximum photosynthetic photon flux at plant level was 1000 μmol m^{-2} s^{-1}. Pots were irrigated with water containing 1.5 g l^{-1} 20N-8.7P-16.6K soluble fertilizer (Grace Horticultural Products, Foglesville, PA) and flushed periodically with water to avoid accumulation of excessive salts in the medium.

Effect of Gibberellins on the Growth of Plants Dwarfed with Uniconazole

On June 25, 1990, twenty-four plants were trimmed, and the medium was drenched with a dose of uniconazole above the optimal rate (Wang and Gregg 1989), 0.2 mg in 200 ml water. The four control plants were drenched with water at the same time. All plants were transferred into 2.6-l pots on August 16. Both the untreated and treated plants were pruned again on August 27, leaving two nodes on each branch. This practice was done to ensure that all new growth was under the influence of uniconazole. Foliar applications of GA_{4+7} were initiated on September 19 at rates of 25 (once only or four times every 2 weeks), 50 (once only or twice every 4 weeks), or 100 mg l^{-1}. There were no visible flower buds at this time.

The date that the first flower opened and its diameter and pedicel length were recorded for each of three selected shoots per pot. The number and areas of the abscised leaves, if any, were recorded for each shoot. Total flower number for each plant was monitored. In mid-December, leaf number and lateral branches per shoot were counted, and the area of each leaf and the length and diameter (the middle of the second internode from the base) of each shoot were measured. Plant height and width (the average of two measurements made at perpendicular angles) were measured. Treatments were replicated four times in a randomized complete block design.

Histological Studies

In mid-November, open flowers were collected from control, the retarded, and those plants treated with uniconazole and GA_{4+7} four times in the above experiment. Tissue samples 1–1.5 mm were taken from the middle of the pedicels. All tissues sampled were killed and fixed in FAA, dehydrated, embedded in paraffin, and sectioned to 10 μm transversely and longitudinally. Tissue sections were dewaxed and stained with safranin and fast green.

Results

Reversal of Uniconazole Treatments with Gibberellins

Foliar application of GA_{4+7} at 25 mg l^{-1} four times, 50 mg l^{-1} twice, or 100 mg l^{-1} once increased plant height and width, stem elongation (Table 1), and pedicel length (Table 2) of plants treated with uniconazole. The dwarfed plants had narrower stem diameters and very limited or no lateral shoots, regardless of the GA treatments. Multiple applications of GA_{4+7} (25 or 50 mg l^{-1}) tripled the flower number of those receiving uniconazole alone, but still resulted in 50% fewer flowers than the untreated control (Table 2). The greater total flower numbers in the control plants were partially the result of additional flowers on the lateral shoots.

Single application of 25 or 50 mg l^{-1} GA_{4+7} had no or limited effect on increasing leaf size and number (Tables 3 and 4). Although 100 mg l^{-1} GA_{4+7} resulted in an immediate enlargement of leaves, its effect did not last beyond the fifth leaf (Table 4). Multiple applications of GA_{4+7} were needed to result in total leaf areas similar to that of the untreated control by accelerating leaf production (Table 3) and enlarging individual leaf size (Table 4). Increasing concentration and repeated applications of GA_{4+7} promoted the abscission of lower leaves in plants previously treated with uniconazole (Table 3).

Histological Changes in the Pedicel

The pith, cortical, and epidermal cells of pedicel tissue from plants treated with uniconazole (Fig. 1A) were only one-half to one-third the length of cells from the same tissues in untreated control (Fig. 1B). Cells in plants previously treated with

Table 2. Effect of uniconazole and GA_{4+7} on hibiscus flowering[a]

Uniconazole	GA_{4+7} Concentration (mg l^{-1})	Times	Days to first bloom	Total flower no.	Flower no./shoot	Pedicel length (cm)
Yes	0	0	78 ab	8.0 c	1.3 d	2.3 e
	25	1	80 a	8.3 c	1.4 d	2.4 e
	50	1	76 abc	13.8 bc	2.8 c	2.7 de
	100	1	74 abc	14.5 bc	3.3 bc	3.9 d
	25	4	71 bc	25.3 b	4.6 a	7.3 a
	50	2	69 c	24.3 b	4.4 ab	5.9 c
No	0	0	77 abc	49.0 a	4.5 a	9.7 a

[a] Mean separation within columns by Tukeys test at p = 0.05.

Table 3. Effect of uniconazole and GA_{4+7} on hibiscus leaf production[a]

Uniconazole	GA_{4+7} Concentration (mg l^{-1})	Times	Leaf no./shoot	Total leaf area (cm^2)	Area of largest leaf (cm^2)	No abscised leaves
Yes	0	0	7.6 e	231 d	49 e	0.8 de
	25	1	7.9 de	274 cd	57 e	1.6 cd
	50	1	9.1 cd	366 bc	76 d	1.9 cd
	100	1	9.9 c	440 b	90 cd	2.4 bc
	25	4	13.0 b	781 a	126 a	4.3 a
	50	2	11.9 b	690 a	109 b	3.4 ab
No	0	0	14.5 a	762 a	102 bc	0.1 e

[a] Mean separation within columns by Tukeys test at p = 0.05.

Table 4. Effect of uniconazole and GA_{4+7} on the size of individual hibiscus leaves[a]

Uniconazole	GA_{4+7} Concentration (mg l^{-1})	Times	Leaf area (cm^2) Leaf no.[b] 1	2	3	4	5	6	7	8	9	10
Yes	0	0	12 b	25 d	36 d	36 d	45 e	39 c	29 b	21 b	—[c]	—
	25	1	12 b	29 cd	43 d	51 cd	44 e	47 c	37 b	20 b	—	—
	50	1	12 b	28 cd	51 cd	67 bc	61 de	49 c	44 b	32 b	23 c	25 b
	100	1	22 a	48 a	81 a	78 ab	63 cd	45 c	35 b	27 b	27 c	27 b
	25	4	16 ab	36 bc	60 bc	84 ab	116 a	112 a	87 a	83 a	63 ab	49 a
	50	2	18 ab	44 ab	66 b	88 a	93 b	91 b	86 a	72 a	56 b	35 b
No	0	0	12 b	34 bc	44 d	70 b	80 bc	92 b	97 a	81 a	73 a	54 a

[a] Mean separation within columns by Tukeys test at p = 0.05.
[b] Counted acropetally.
[c] Fewer than five leaves among all plants.

uniconazole and then 25 mg l^{-1} GA_{4+7} four times (Fig. 1C) were nearly the length of the control.

Transverse sections of pedicels show that plants treated with uniconazole had thicker pith, vascular tissue, and cortex (Fig. 2A) than the control (Fig. 2B). As the result of uniconazole treatment, the inner cortical cells became circular and had larger diameters (Fig. 2A) as opposed to the oval-shaped cells in the cortex of untreated plants (Fig. 2B). Plants treated with uniconazole and GA_{4+7} had large cortical cells and intermediate cortex and nearly normal pith thickness (Fig. 2C). Their cortical cell size was between that of the dwarf and control plants and the pith was thinner. Plants treated

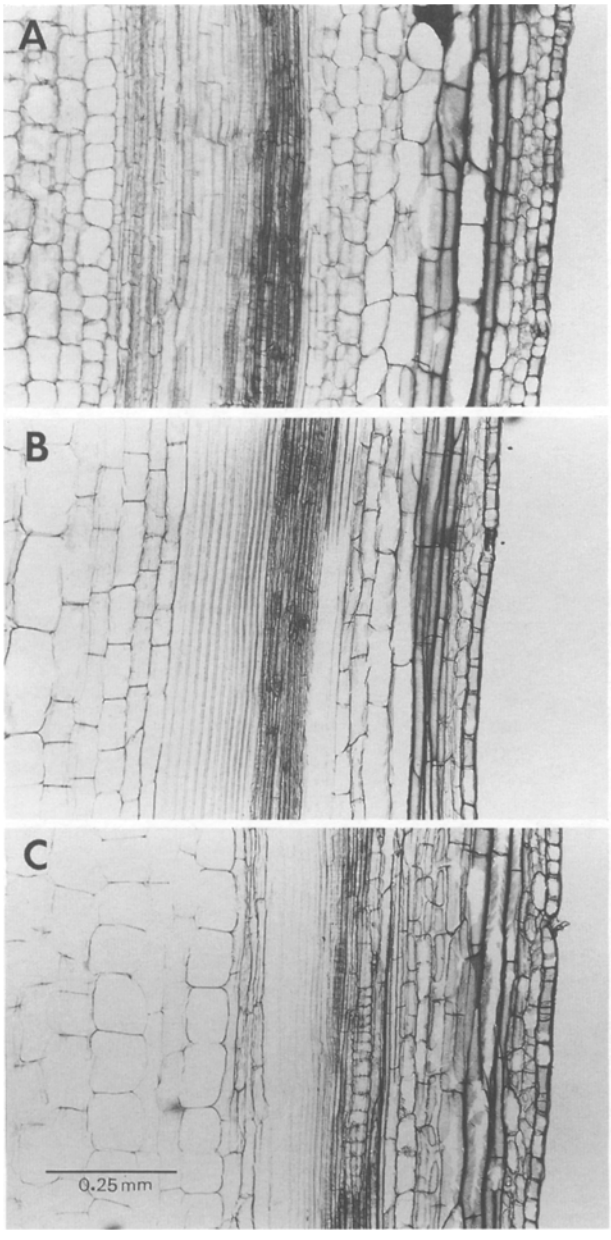

Fig. 1. Longitudinal sections (100×) of hibiscus pedicels from plants receiving uniconazole (A), water (B), or uniconazole followed by four foliar applications of 25 mg l^{-1} GA_{4+7} at 2-weeks intervals (C). Uniconazole was applied as a soil drench at a rate of 0.2 mg per pot.

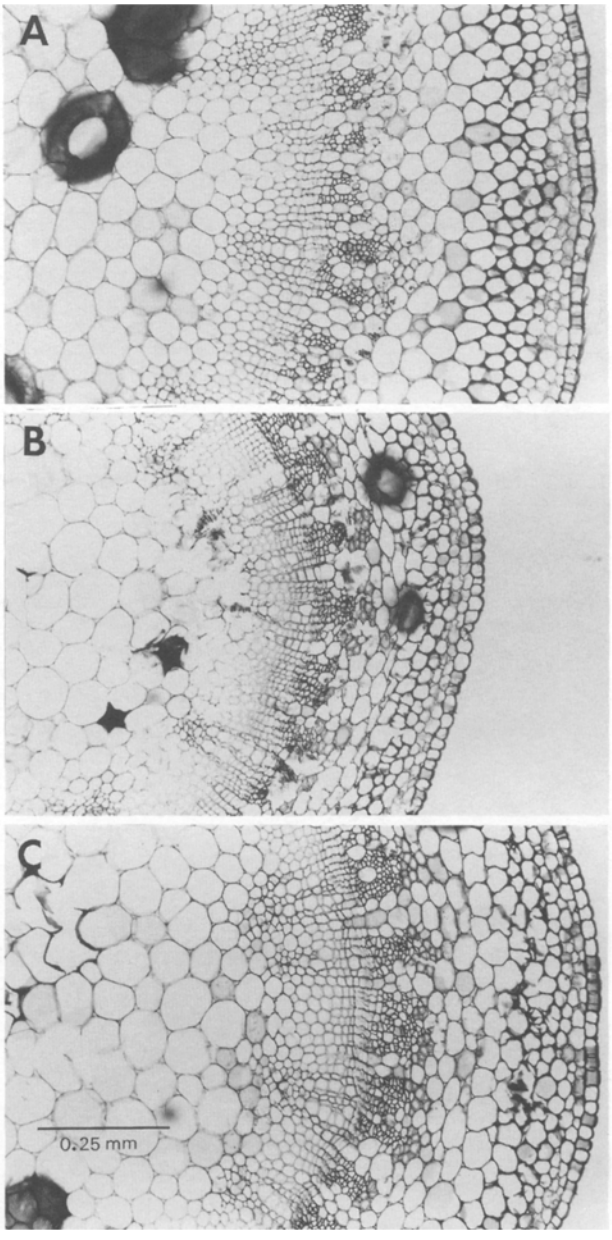

Fig. 2. Transverse sections (100×) of hibiscus pedicels from plants receiving uniconazole (A), water (B), or uniconazole followed by four applications of 25 mg l^{-1} GA_{4+7} at 2 weeks intervals (C). Uniconazole was applied as a soil drench at a rate of 0.2 mg per pot.

with uniconazole, with and without GA_{4+7} had thicker vascular tissues (xylem and phloem, Fig. 2A and 2C) than did the control (Fig. 2B). The abundant fiber cells in the pedicels of untreated plants (Fig. 2B) were entirely missing in the uniconazole-treated plants (Fig. 2A). However, fiber cells were found in the pedicel of plants treated with uniconazole if followed by applications of GA_{4+7} (Fig. 2C).

Discussion

Once applied to the soil, triazole growth retardants can be absorbed by the root system and translocated to other parts of the plant via the transpirational water stream (Reed et al. 1989, Wang et al. 1986). In our experiments, uniconazole exerted prolonged control over the growth of hibiscus. Since triazole growth retardants are relatively im-

mobile after entering the upper portions of the target plant (Barrett and Bartuska 1982, Wang 1991), it is likely that a continuous absorption of the retardant from the potting medium caused the extended inhibition of growth for weeks after treatment (Seely 1982).

Davis et al. (1988) reported that application of GA_3 did not reverse the inhibition on stem growth of poinsettia plants overdosed with paclobutrazol. The growth of less severely retarded poinsettia was fully restored by applying GA_3 at an appropriate stage (Cox 1993). One foliar application of GA_3 fully restored stem elongation and leaf growth of sunflower plants previously treated with a soil drench of paclobutrazol (Wample and Culver 1983). GA_3 was reported to overcome the dwarfing effect of chlormoquat [(2-chloroethyl) trimethylammonium chloride] on hibiscus (Miller 1987).

It is clear from the results of this and other studies (Jiao et al. 1991, Moore and Schekel 1985) that exogenously applied GA will compensate for the decrease in endogenous GA and temporarily support leaf growth. However, GA_{4+7} has only limited capacity to restore stem elongation of plants dwarfed by uniconazole. Single foliar applications of GA_{4+7} did not have a prolonged effect on uniconazole-treated hibiscus, particularly at low concentrations. Multiple applications were needed to maintain the normal leaf production rate and leaf size (Table 3). A threshold level of GA apparently is required for a prolonged period of time to achieve normal leaf growth. It should be noted that foliar applications of GA_{4+7} (up to 100 mg l^{-1}) had no effect on stem elongation or leaf abscission of plants not treated with uniconazole (data not presented).

Uniconazole is a more potent dwarfing compound than paclobutrazol in tests with hibiscus (Wang and Gregg 1991). The dwarfing action of uniconazole in Easter lily was overcome with a foliar application of 200 mg l^{-1} GA_{4+7} (Jiao et al. 1991), but the time of application was critical in restoring full-stem elongation of plants treated with uniconazole. Since Easter lily shoots have a determinant growth, it appears that GA must be applied during the very early part of stem growth to effectively eliminate the effect of uniconazole. Therefore, GA may promote the rate of cell division, cell elongation, or both. GA_3 was reported to induce cell elongation (Davidonis 1990).

The inhibition of pedicel elongation with uniconazole was reversed by the applications of 25 mg l^{-1} GA_{4+7} at 2-week intervals (Table 2). Other treatments in this study resulted in a transient effect that was lost by the end of the study. Privé et al. (1989) reported that the effect of paclobutrazol on apple pedicel length was reversed only when GA_{4+7} (150 mg l^{-1}) was applied prior to the main period of pedicel elongation (before full bloom). They postulated that paclobutrazol limited pedicel length by limiting the cell number and GA_{4+7} increased pedicel length by increasing the total number of cells. The results of our histological study and pedicel length suggest that the short pedicels had fewer cells longitudinally than normal pedicels. Therefore, uniconazole not only resulted in shorter cells, but also adversely affected cell division. In contrast to small stem diameter observed in hibiscus treated with uniconazole (Wang and Gregg 1989), this compound promoted lateral cell division and enlargement in the pedicel, resulting in thicker pedicels (Figs. 2B, 2C). Paclobutrazol was reported to decrease cell length in tomato roots (Barlow et al. 1991). Uniconazole also reduced the growth rate and cell length of maize stem tissue (Katsumi and Tanaka 1992).

It is not known what caused the lower leaves of GA treated plants to abscise. None of the pesticides which were reported to cause the abscission of hibiscus lower leaves in plants treated with ancymidol or GA (McConnell and Short 1987) was used. In addition to inhibition of GA biosynthesis, the triazole growth-inhibitors such as uniconazole reduce the synthesis of auxin in plants (Hamilton and Law 1987, Law and Hamilton 1989). Relatively high levels of IAA are needed to delay the senescence of leaves (Osborne 1967). It is possible that an imbalance of hormones in the GA-treated dwarf plants induced the abscission of the older leaves. Also, since large leaves were produced with short internodes following GA treatment of the dwarf plants, fast new growth and/or severe shading of the lower leaves by the large upper leaves might have induced abscission of the lower leaves.

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