Response of *Forsythia ×intermedia* 'Spectabilis' to Uniconazole. II. Leaf and Stem Anatomy, Chlorophyll, and Photosynthesis

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Abstract. Uniconazole was applied as a foliar spray at 0, 90, 130, 170, or 210 ppm to rooted stem cuttings of 'Spectabilis' forsythia (*Forsythia xintermedia* Zab.) potted in calcined clay. Uniconazole resulted in higher total leaf chlorophyll (chlorophyll_a + chlorophyll_b) concentration and a decreased ratio of chlorophyll a : b. Stomatal density of the most recently matured leaves increased linearly with increasing uniconazole concentration 40, 60, and 100 days after treatment (DAT). The number of stomata per leaf (stomatal index) increased linearly with increasing concentration of uniconazole throughout the initial 100 DAT. Uniconazole suppressed stomatal length at all sampling dates and the level of suppression increased with increasing concentration of uniconazole from 20 to 100 DAT. Stomatal width was suppressed by uniconazole at 40 DAT. Leaves developed after uniconazole application had higher levels of net photosynthesis when measured 55, 77, and 365 DAT. Stomatal conductance for uniconazole-treated plants was higher compared to nontreated control (0 mg-liter⁻¹) plants when measured 49, 55, 77, and 365 DAT. Initiation of secondary xylem for stem tissues of uniconazole-treated plants was suppressed and expansion of xylem vessel length and width was less. Secondary phloem tissues of stems from uniconazole-treated plants contained larger numbers of phloem fibers having smaller cross sectional areas than phloem fibers of controls. Chemical name used: (E)-1-(p-Chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (uniconazole).

Plant growth retardants (PGRs) have the potential to reduce vegetative growth and pruning frequency of woody landscape species. Uniconazole, a triazole, can effectively retard growth of several woody landscape species including 'Spectabilis' forsythia without injury (Keever and West, 1992; Norcini and Knox, 1989; Thetford et al., 1995; Warren et al., 1991). Little information is available, however, on the effects of foliar application of uniconazole on woody plant morphology or anatomy (Wang and Gregg, 1989 (hibiscus, *Hibiscus rosa-sinensis* L.); McDaniel et al., 1990 (Poinsettia, *Euphorbia pulcherrima* Willd.)) and subsequent changes in physiological processes such as net photosynthesis (P_n). Previous studies of photosynthetic responses to paclobutrazol (another triazole compound) reported results varying from inhibition (Wample and Culver, 1983), to stimulation (Jaggard et al., 1982) to no effect (Wood, 1984).

Triazole application commonly produces plants with darker leaves than nontreated plants. The "greening" effect of triazoles is the result of increased chlorophyll concentration (Wang and Gregg, 1989) and an alteration of chlorophyll synthesis (Dalziel and Lawrence, 1984). While these above mentioned reports provide useful information about the effectiveness of uniconazole following a soil or potting medium application or inclusion within a nutrient solution culture, these application methods allow con-

tinuous exposure to uniconazole through root uptake. Triazoles can be quite persistent with growth retardant effects occurring on plants that were grown in soil treated the previous year (Fletcher and Hofstra, 1985); an undesirable characteristic for plants destined for the landscape. There also exists major differences in effectiveness among the gibberellin biosynthesis inhibitors which result in differences in longevity of effect and extent of inhibition; uniconazole is more effective than paclobutrazol, flurprimidol, and triadimefon in reducing growth (Steffens, 1988). Therefore, the objectives of this research were to: 1) quantify changes in leaf chlorophyll concentration of container-grown 'Spectabilis' forsythia following foliar application of uniconazole, 2) describe accompanying changes in leaf morphology, i.e. stomatal size and distribution, 3) determine if uniconazole affects net photosynthesis or stomatal conductance, and 4) to investigate anatomical changes in secondary stem development for stem tissues initiated following uniconazole application.

Materials and Methods

Rooted stem cuttings of 'Spectabilis' forsythia were potted 14 May 1991 in 3.8-liter containers with arcillite, a calcined clay substrate (Aimcor Inc., Deerfield, Ill.) and placed on a gravel container pad at the Horticulture Field Laboratory, Raleigh. Uniconazole at 0, 90, 130, 170, or 210 mg-liter⁻¹ was applied to 'Spectabilis' forsythia on 13 June 1991 between 7:00 AM and 8:30 AM with a hand held sprayer to uniformly wet all leaves and stems (12 to 15 ml per plant). Climatic conditions at the time of treatment were clear skies with a dry bulb temperature of 20.8C, a dewpoint of 17.4C, and no wind.

The experimental design was a split plot with four replicates assigned to blocks based on initial primary stem number, relative leaf area (visual assessments), and plant height. Uniconazole concentrations were the main plots and five harvest dates of 0, 40, 80, 120, and 369 days after treatment (DAT) were subplots. The first harvest (0 DAT) consisted of 20 plants (one plant per treatment per replication) and subsequent harvests consisted of 40 plants [two plants (subsamples)

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per treatment per replication]. Plants were destructively harvested to obtain growth data presented in a companion paper (Thetford et al., 1995). General cultural conditions were previously described (Thetford et al., 1995).

Chlorophyll. One 0.76-cm² leaf disc was collected from each of five recently matured, fully expanded leaves from each plant at the time of each destructive harvest. Leaf disks were removed from the center portion of a leaf from either side of the midvein, weighed (fresh weight), placed in glass vials (five leaf disks per plant per vial) to which 10 ml *N, N*-dimethylformamide (DMF) was added, and stored in the dark at 4C before spectrophotometric analyses (Moran and Porath, 1980). Absorbance (*A*) was measured with a spectrophotometer (UV-265FS/FW; Shimadzu Corp., Kyoto, Japan) at 664 and 647 nm on a 3 ml aliquot of the chlorophyll extract. Total chlorophyll [chlorophylla (chl_a) + chlorophyllb (chl_b)] and the chl *a* : *b* ratio were calculated using equations developed by Moran (1982). Chlorophyll concentration was calculated as mg·g⁻¹ leaf fresh weight and mg·cm⁻² of leaf to account for changes in leaf thickness which may have occurred with triazole application (Davis et al., 1988; Gao et al., 1988). Specific leaf fresh weight (g·cm⁻²) was calculated from total chlorophyll data (mg·cm⁻² : mg·g⁻¹).

Stomata. Five recently expanded, mature leaves (fourth visible node from the apex of a stem) were selected from each of five plants within a treatment at 20 day intervals beginning 20 DAT and sampling continued until 120 DAT. Following resumption of growth the following spring, leaves were again collected (365 DAT). Samples were placed in formalin-acetic acid-ethanol (FAA) for later microscopic examination (Johansen, 1940). Beginning at the midvein in the center portion of the leaf and moving randomly to the leaf margin, length and width of 10 stomata were measured per leaf. Stomatal density (number stomata per mm²) and stomatal index [(number stomata : (number epidermal cells + number stomata)) × 100] were determined for four fields (1 mm²) on each leaf sample (Meidner and Mansfield, 1968) using a no. 2 ocular (Weibel; Electron Microscopy Sciences, Fort Washington, Pa.) (Weibel, 1969). Fields were selected at random beginning at the midvein in the center portion of the leaf and moving outward to the leaf margin. Stomata are present only on the abaxial leaf surfaces of 'Spectabilis' forsythia.

Photosynthesis. Leaf gas exchange was measured 14, 36, 49, 55, 62, 77, 120, and 365 DAT with a portable closed photosynthesis system (LI-6200; LI-COR, Lincoln, Neb.) between 1:00 PM and 3:00 AM. Photosynthetic photon flux (PPF, 400-700 nm), air and leaf temperature, and relative humidity inside the leaf chamber were measured concurrently with gas (CO₂) exchange. Net photosynthesis (P_n), and stomatal conductance (g_s) were calculated using the LI-6200 measurements. An attached leaf was placed in a 0.25-liter chamber and measurements commenced for 30 sec immediately after the CO₂ concentration started to decrease. All plants were irrigated 1 h before measurement of P_n. Levels of PPF, air temperature, relative humidity, and CO₂ at the time of photosynthesis measurements were as follows: 14 DAT, (1079-2288 μmol·m⁻²·s⁻¹, 35 to 39C, 29% to 40%, and 296 to 360 ppm); 36 DAT, (1178-1773 μmol·m⁻²·s⁻¹, 33 to 34C, 40% to 50%, and 307 to 329 ppm); 49 DAT, (1480-1967 μmol·m⁻²·s⁻¹, 34 to 37C, 29% to 42%, and 301 to 369 ppm); 55 DAT, (1099-1971 μmol·m⁻²·s⁻¹, 35 to 38C, 36% to 45%, and 300 to 330 ppm); 62 DAT, (1721-1966 μmol·m⁻²·s⁻¹, 37 to 41C, 24% to 34%, and 308 to 331 ppm); 77 DAT (1426-1830 μmol·m⁻²·s⁻¹, 35 to 39C, 32% to 44%, and 300 to 329 ppm); 120 DAT, (1425-1865 μmol·m⁻²·s⁻¹, 25 to 28C, 35% to 42%, and 327 to 352 ppm); 365 DAT, (1608-1953 μmol·m⁻²·s⁻¹, 29 to 32C, 31% to 34%, and 330 to 370 ppm).

Histological procedures. Stem tissue samples from the first visible internode to elongate after uniconazole application were collected from primary stems 120 DAT. Tissue samples were fixed by vacuum infiltration with FAA, dehydrated and infiltrated in a series of ethanol and tertiary butyl alcohol (Johansen, 1940), and embedded in TissuePrep (Fisher Scientific Co., Fairlawn, N.J.) using a histomatic tissue processor (model 166; Fisher Scientific Co.). Tissue samples were sectioned serially on a rotary microtome at 10 μm thickness, affixed to slides using Haupt's adhesive (Jensen, 1962), and stained with safranin and fast green (Johansen, 1940).

Statistical analyses. Data were subjected to analysis of variance procedures (SAS Institute, Cary, N.C.) to determine sources of experimental variation. Uniconazole concentration response (0 mg·liter⁻¹ excluded) was analyzed by general linear regression. A planned orthogonal contrast to test for a difference between a pooled uniconazole treatment effect and nontreated control (0 mg·liter⁻¹) was also conducted. A significant uniconazole treatment by time of harvest interaction (*P* ≤ 0.05) was present only for P_n, stomatal density, and stomatal index. Hence, these data were analyzed by harvest date while remaining data were pooled across harvest dates.

Result's and Discussion

There was no uniconazole by harvest date interaction for chlorophyll concentration or chl *a* : *b* ratio data. Uniconazole resulted in higher leaf chlorophyll concentration on an area or fresh weight basis while decreasing the ratio of chl *a* : *b* (Table 1). Wang and Gregg (1989) found the level of chlorophyll in leaves from uniconazole-treated hibiscus increased with increasing concentration following a drench application at 0.1, 0.2, or 0.4 mg/2.6-liter pot. In contrast, Steinberg et al. (1991) reported uniconazole did not affect chlorophyll content of 'Texanum' privet (*Ligustrum japonicum* Thunb.). Similar results for chlorophyll data expressed on the basis of tissue fresh weight or tissue area are indicative of leaf tissues similar in thickness. This is supported by similar specific leaf fresh weight for nontreated (27.36 g·cm⁻²) and uniconazole-treated (25.76 g·cm⁻²) plants indicating uniconazole did not influence leaf thickness throughout the experimental period.

The chlorophyll *a* : *b* ratio decreased as the concentration of total chlorophyll increased. Decreased chl *a* : *b* ratio and increased chlorophyll concentration present for leaves of uniconazole-treated plants are characteristics indicative of leaves produced in shade conditions. The relative amounts of pigments can be influenced by irradiance levels during development (Glazer and Melis, 1987) and chloroplasts in organs of plants developing in shade tend to have more photosynthetic pigments per unit volume than chloroplasts developing under high irradiance (Haehnel, 1984). However, the ratio of chl_a to chl_b may be altered by a change in light quality or irradiance subsequent to leaf development and expansion (Burkey and Wells, 1991). The present change in the ratio of chl *a* : *b* may result from

Table 1. Leaf chlorophyll concentration of leaves of 'Spectabilis' forsythia following uniconazole application.

Chlorophyll	Nontreated	Uniconazole ^y	Significance
mg·cm ⁻²	0.069	0.083	0.001
mg·g ⁻¹	1.90	2.10	0.001
<i>a</i> : <i>b</i> ratio	3.84	3.60	0.001

^yTotal chlorophyll (fresh weight) = chl_a + chl_b = (0.0202A₆₄₇ + 0.00802A₆₆₄); ratio = chl_a (0.0127A₆₆₄ - 0.00269A₆₄₇) : chl_b (0.0229A₆₄₇ - 0.00468A₆₆₄).

Uniconazole = mean across all uniconazole concentrations (90, 120, 170, and 210 mg·liter⁻¹).

Table 2. Stomatal density and stomatal indices of the abaxial surface of leaves of 'Spectabilis' forsythia following uniconazole application.

Uniconazole concn (mg·liter ⁻¹)	Days after treatment					
	20	40	60	80	100	120
	<i>Stomatal density (no./mm²)</i>					
0	424	575	485	394	318	485
90	441	495	565	515	471	518
130	491	535	622	545	468	501
170	485	668	595	488	511	508
210	488	688	702	582	592	595
Significance						
Uniconazole (U) ^z						
Linear	NS	0.001	0.02	NS	0.01	NS
Quadratic	NS	NS	NS	NS	NS	NS
U vs. control ^y	NS	NS	0.002	0.02	0.001	NS
	<i>Stomatal index (%)^x</i>					
0	17	20	18	17	12	21
90	17	24	23	24	20	22
130	20	24	26	25	21	22
170	21	28	25	25	21	24
210	20	29	28	23	26	23
Significance						
Uniconazole						
Linear	0.02	0.02	NS	NS	0.02	0.05
Quadratic	0.02	NS	NS	NS	NS	NS
U vs. control	0.03	0.005	0.001	0.001	0.001	NS

^xControl (0 mg·liter⁻¹) excluded from regression analysis.

^yU vs. control = orthogonal contrast; U = mean across all uniconazole concentrations (90, 120, 170, and 210 mg·liter⁻¹) for a given harvest date (20, 40, 60, 80, 100, or 120 days after treatment); control = 0 mg·liter⁻¹ uniconazole.

^zStomatal index = [number stomata : (number epidermal cells + number stomata)] × 100.

^{NS}Nonsignificant at $P > 0.05$.

uniconazole directly affecting synthesis of chlorophyll such that synthesis of Chl_a is reduced relative to Chl_b. Another possibility is that uniconazole may indirectly influence Chl a : b ratios by reducing irradiance or quality of light intercepted by the leaves of treated plants as a result of suppressed internode length (Thetford et al. 1995).

A uniconazole by harvest date interaction was present for all stomatal measurements; thus, data are presented by DAT. Stomatal density increased linearly with increasing uniconazole concentration 40, 60, and 100 DAT (Table 2). Similarly, a drench application of uniconazole increased stomatal density of 'Texanum' privet 28% (Steinberg et al., 1991). Stomatal density at 20, 40, and 120 DAT (Table 2) and that of leaves produced the following spring (365 DAT) (data not presented), was similar among treated and nontreated plants. Stomatal indices were increased by uniconazole treatment compared to the controls throughout the initial 100 DAT (Table 2) indicating uniconazole-treated plants had a greater percentage of stomatal cells per leaf. Stomatal indices were not affected by uniconazole the following spring (365 DAT) (data not presented) indicating the percentages of epidermal cells that developed as stomata were similar for leaves of both uniconazole-treated and nontreated plants.

Stomatal length was 12% to 21% less than controls with uniconazole at all sampling dates and the level of suppression increased with increasing uniconazole concentration from 40 to 120 DAT (Table 3). Stomatal width 40 DAT was 20.15 µm and 21.8 µm for uniconazole-treated and nontreated plants, respectively, a decrease of 14%. Stomatal width was similar among all treatments from 60 to 369 DAT ranging from 19.4 to 23.1 µm (data not presented). Morphological changes in stomata have previously been reported in species such as wheat (*Triticum aestivum* L.

'Glenlea') and are attributed to triazole induced changes in the balance of endogenous hormones, including gibberellins, abscisic acid, and cytokinins (Fletcher and Hofstra, 1985; Gao et al., 1988).

Photosynthesis data collected 62 DAT were determined to be invalid since cuvette temperatures reached 41°C. Plants treated with uniconazole had an elevated level of P_n55, 77, and 365 DAT (Table 4). Similarly, Dalziel and Lawrence (1984) reported higher

Table 3. Stomatal length for leaves of 'Spectabilis' forsythia following uniconazole application.

Uniconazole concn (mg·liter ⁻¹)	Days after treatment			
	40	80	120	365
	<i>Stomatal length (µm)</i>			
0	32.2	31.9	31.4	33.5
90	29.2	30.7	29.1	30.4
130	26.5	28.5	28.6	32.1
170	26.4	27.5	28.3	29.0
210	25.7	27.1	27.5	29.4
Significance				
Uniconazole (U)				
Linear	0.001	0.001	0.001	NS
Quadratic	0.01	NS	NS	NS
U vs. control ^y	0.001	0.001	0.001	0.02

^yControl (0 mg·liter⁻¹) excluded from regression analysis.

^yU vs. control = orthogonal contrast; U = mean across all uniconazole concentrations (90, 120, 170, and 210 mg·liter⁻¹) for a given harvest date (40, 80, 120, or 365 days after treatment); control = 0 mg·liter⁻¹ uniconazole.

^{NS}Nonsignificant at $P > 0.05$.

Table 4. Net photosynthetic rates of 'Spectabilis' forsythia following uniconazole application."

Uniconazole concn (mg liter ⁻¹)	Days after treatment				
	49	55	77	120	365
0	19.9	19.9	17.1	8.7	9.2
90	25.6	25.6	25.6	9.8	12.0
130	15.2	22.5	23.9	10.2	16.9
170	22.8	22.5	22.9	12.2	13.2
210	25.7	27.3	25.1	9.2	9.2
Significance					
Uniconazole (U) ^y					
Linear	NS	0.01	NS	NS	NS
Quadratic	NS	NS	NS	NS	NS
U vs. control ^x	NS	0.002	0.001	NS	0.01

^zCarbon exchange rate (net photosynthesis) = $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.^yControl (0 mg-liter⁻¹) excluded from regression analysis.^xU vs. control = orthogonal contrast; U = mean across all uniconazole concentrations (90, 120, 170, and 210 mg-liter⁻¹) for a given harvest date (49, 55, 77, 120 or 365 days after treatment); control = 0 mg-liter⁻¹ uniconazole.^{NS}Nonsignificant at $P > 0.05$.

photosynthetic rates for leaves of 'Bush Mono 6' sugar beets (*Beta vulgaris* L.) treated with paclobutrazol. While forsythia did not exhibit an increase in P_n before 55 DAT, other researchers have demonstrated triazole-induced increases in P_n for 'Cardinal' strawberry (*Fragaria × ananassa* Duch.) within 12 DAT (Deyton et al., 1991). However, both strawberry (Deyton et al., 1991) and forsythia had higher levels of P_n when measured on the new leaves produced the following growing season. Increased chlorophyll concentration and decreased ratio of Chl_a and Chl_b resulting from uniconazole application may influence CO_2 exchange and assimilation and may contribute to the P_n increase reported herein. It is important, however, to note that these changes in chlorophyll concentration and composition occurred prior to the significant increases in P_n which indicates other factors may have contributed to increased P_n . Levels of P_n were 21.4 and 20.3 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 14 DAT and 27.6 and 30.34 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 36 DAT for nontreated and uniconazole-treated plants, respectively.

Stomatal conductance of water vapor (g_s) differed from P_n in that the uniconazole by harvest date interaction was not significant indicating a similar response to uniconazole for all sampling dates. Levels of g_s for treated plants were as much as 26% greater than controls with values of 0.00023 and 0.00028 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for nontreated and uniconazole-treated plants, respectively. Increased stomatal density could potentially allow leaves of uniconazole-

treated plants to have a relatively greater internal surface area exposed to the atmosphere leading to increased levels of conductance compared to controls. Similarly, paclobutrazol application to 'Fantasia' nectarine [*Prunus persica* (L.) Batsch.] increased leaf conductance to water vapor for most of the growing season (DeJong and Doyle, 1984). Since conductance of water through the epidermis depends mainly on the number and dimensions of stomata, increases in stomatal density and decreases in stomatal length or width could result in increased g_s (Nobel, 1991).

Microscopic examination of cross-sections of stems indicated uniconazole treatment influenced stem tissue development. Stems from nontreated plants were composed of 10 to 12 bands of cortical parenchyma cells, few bundles of phloem fibers, 11 to 13 bands of phloem cells, and 63 ± 9 bands of xylem cells surrounding the chambered pith region (Fig. 1A). The chambered pith of 'Spectabilis' forsythia consisted of sparse and dense regions of parenchyma cells which were not affected by uniconazole application (Fig. 2).

The number of bands of cortical parenchyma from nontreated and uniconazole-treated plants did not differ. Forsythia treated with < 210 mg-liter⁻¹ uniconazole contained a greater number of phloem fibers having a smaller cross sectional area than those from stem tissues of nontreated plants (data not presented). Lumen of phloem fibers from treated plants appeared larger than lumen of

Table 5. Effect of uniconazole on mean size (μ) of cells within stems of 'Spectabilis' forsythia 120 days after treatment.

Cell type	Uniconazole(U) concn (mg-liter ⁻¹)					Significance ^z		
	0	90	130	170	210	L	Q	U vs. 0
Xylem vessel								
Length	38.62	17.81	21.00	17.31	14.25	0.003	0.002	0.001
Width	2.34	2.19	1.84	2.15	2.01	NS	NS	0.01
Ray parenchyma								
Length	4.65	4.41	4.53	3.97	3.47	0.003	NS	NS
Width	2.59	2.44	1.96	2.31	2.06	NS	NS	0.01
Epidermal cell								
Depth (radial sections)	3.81	4.56	4.56	5.06	4.41	NS	NS	0.001
Width (radial sections)	3.80	1.61	1.76	1.68	1.44	0.002	0.001	0.001
Width (cross sections)	4.17	2.45	2.46	2.03	2.72	0.001	0.001	0.001

^zL = linear, Q = quadratic regression, and U = uniconazole (mean of 90, 130, 170, and 210 mg-liter⁻¹) vs. 0 mg-liter⁻¹ orthogonal contrast, respectively.^{NS}Nonsignificant at $P > 0.05$.

Fig. 1. Cross-sections of stems of 'Spectabilis' forsythia 120 days after treatment with uniconazole at concentrations of (A) 0, (B) 90, (C) 130, (D) 170, and (E) 210 mg·liter⁻¹. Tissue regions are identified as follows: e = epidermis, c = cortex, pf = phloem fiber, p = phloem, cr = cambial region, x = xylem, and pi = pith. Scale bar = 100 μ m.

Fig. 2. Radial sections of stems of 'Spectabilis' forsythia 120 days after treatment with uniconazole at concentrations of (A) 0, (B) 90, and (C) 210 mg·liter⁻¹. Tissue regions are identified as follows: e = epidermis and xv = xylem vessel. Scale bar = 100 μ m.

phloem fibers of nontreated plants (Fig. 1). Wang and Gregg (1989) reported uniconazole resulted in fewer and smaller phloem fibers while lumen size for phloem fibers increased for 'Jane Cowl' hibiscus. Similarly, uniconazole resulted in limited fiber wall thickening within stems of 'Annette Hegg Dark Red' poinsettia that were described as having very few thickened phloem fiber cells in bundle caps (McDaniel et al., 1990).

While phloem tissue width varied within each stem sample, the number of bands of cells ranged from 11 to 13 regardless of

uniconazole concentration. The similar numbers of phloem cells indicate uniconazole did not suppress phloem cell initiation while the smaller cross-sectional areas of phloem cells of uniconazole-treated plants suggests uniconazole suppressed phloem cell expansion.

Xylem tissues from stems of plants receiving uniconazole contained 22 ± 3 to 28 ± 5 bands of xylem cells while xylem tissue from stems of nontreated plants contained 63 ± 9 bands of xylem cells. This represented a 65% decrease in the number of bands of xylem cells initiated for uniconazole-treated plants. Suppression

of stem xylem tissues was similar for all plants receiving uniconazole; a response consistent with internode diameter measurements of 2.6 and 2.2 mm for nontreated and uniconazole-treated plants, respectively (Thetford et al., 1995). Individual xylem vessels of uniconazole-treated plants, when viewed in cross sections, appeared to have smaller cross-sectional areas. This was confirmed by measurement of xylem vessels viewed in radial sections (Table 5). Uniconazole-induced suppression of secondary xylem growth was reported for 'Jane Cowl' hibiscus (Wang and Gregg, 1989) and 'Annette Hegg Dark Red' poinsettia (McDaniel et al., 1990). Xylem ray expansion, evaluated from radial sections, was also suppressed with uniconazole application (Table 5). In contrast to our findings, Wang and Dunlap (1994) reported short cells with increased diameter, thicker pith, and thicker vascular and cortical tissues in the pedicels of 'Jane Cowl' hibiscus following a 0.2 mg/1 S-liter pot soil drench application of uniconazole.

While initiation and expansion of secondary xylem was suppressed by uniconazole application, initiation of secondary phloem was not affected. This pattern of development for treated plants resulted in a greater proportion of phloem tissue per xylem tissue than was present in nontreated plants. The preferential suppression of xylem tissues may be related directly to the translocation pattern of triazole compounds which are xylem mobile. When applied to roots, young stems, and the youngest leaves, they quickly enter the plant and are translocated acropetally via the xylem to the leaves (Reed et al., 1989; Sterrett, 1988).

It is not known if epidermal cell numbers were altered by uniconazole application; however, expansion of epidermal cells was affected by uniconazole application resulting in a change in epidermal cell shape (Table 5). While the outermost layers of epidermal cells were square to rectangular when viewed in cross-sections (Fig. 1), these same cells for uniconazole-treated plants were very narrow and elongated when viewed in radial sections (Fig. 2). This change in cell shape is similar to findings of Wang and Dunlap (1994) who noted inner cortical cells in the pedicels of 'Jane Cowl' hibiscus became circular and had larger diameters as opposed to the oval-shaped cells in the cortex of nontreated plants.

Literature Cited

- Burkey, K.O. and R. Wells. 1991. Response of soybean photosynthesis and chloroplast membrane function to canopy development and mutual shading. *Plant Physiol.* 97:245-252.
- Dalziel, J. and D.K. Lawrence. 1984. Biochemical and biological effects of kaurene oxidase inhibitors such as paclobutrazol, p. 43-57. In: R. Menhennet and D.K. Lawrence (eds.). *Biochemical aspects of synthetic and naturally occurring plant growth regulators*. Monogr. 11. Brit. Plant Growth Regulat. Group. Wantage, U.K.
- Davis, T.D., G.L. Steffens, and N. Sankhla. 1988. Triazole plant growth regulators. *Hort. Rev.* 10:63-105.
- DeJong, T.M. and J.F. Doyle. 1984. Leaf gas exchange and growth response of mature 'Fantasia' nectarine trees to paclobutrazol. *J. Amer. Soc. Hort. Sci.* 109:878-882.
- Deyton, D.E., C.E. Sams, and J.C. Cummins. 1991. Strawberry growth and photosynthetic responses to paclobutrazol. *HortScience* 26:1178-1180.
- Fletcher, R.A. and G. Hofstra. 1985. Triadimefon-A plant multiprotectant. *Plant Cell Physiol.* 26:775-780.
- Gao, J., G. Hofstra, and R.A. Fletcher. 1988. Anatomical changes induced by triazoles in wheat seedlings. *Can. J. Bot.* 66:1178-1185.
- Glazer, A.N. and A. Melis. 1987. Photochemical reaction centers: Structure, organization and function. *Annu. Rev. Plant Physiol.* 38:1145.
- Haehnel, W. 1984. Photosynthetic electron transport in higher plants. *Annu. Rev. Plant Physiol.* 35:659-693.
- Jaggard, K.W., D.K. Lawrence, and P.V. Biscoe. 1982. An understanding of crop physiology in assessing a plant growth regulator on sugar beets, p. 139-150. In: J.S. McLaren (ed.). *Chemical manipulation of crop growth and development*. Butterworths, London.
- Jensen, W.A. 1962. *Botanical histochemistry*. W.H. Freeman, San Francisco.
- Johansen, D.A. 1940. *Plant microtechnique*. McGraw-Hill, New York.
- Keever, G.J. and M.S. West. 1992. Response of established landscape plants to uniconazole. *HortTechnology* 2:465-468.
- McDaniel, G.L., E.T. Graham, and K.R. Maleug. 1990. Alteration of poinsettia stem anatomy by growth-retarding chemicals. *HortScience* 25:433-435.
- Meidner, H. and T.A. Mansfield. 1968. *The physiology of stomata*. McGraw-Hill, London.
- Moran, R. 1982. Formulae for determination of chlorophyll pigments extracted with *N,N*-dimethylformamide. *Plant Physiol.* 69:1376-1381.
- Moran, R. and D. Porath. 1980. Chlorophyll determination in intact tissues with *N,N*-dimethylformamide. *Plant Physiol.* 65:478-479.
- Nobel, P.S. 1991. *Physicochemical and environmental plant physiology*. Academic Press, San Diego.
- Norcini, J.G. and G.W. Knox. 1989. Response of *Ligustrum × ibolium*, *Photinia × frazeri* and *Pyracantha koidzumii* 'Wonderberry' to XE-1019 and pruning. *J. Environ. Hort.* 7:126-128.
- Reed, A.N., E.A. Curry, and M.W. Williams. 1989. Translocation of triazole growth retardants in plant tissues. *J. Amer. Soc. Hort. Sci.* 114:893-898.
- Steffens, G.L. 1988. Gibberellin biosynthesis inhibitors: Comparing growth-retarding effectiveness on apple. *J. Plant Growth Regulat.* 7:27-36.
- Steinberg, S.L., J.M. Zajicek, and M.J. McFarland. 1991. Short-term effects of uniconazole on the water relations and growth of *Ligustrum*. *J. Amer. Soc. Hort. Sci.* 116:461-464.
- Sterrett, J.P. 1988. XE-1019: Plant response, translocation and metabolism. *J. Plant Growth Regulat.* 7:19-26.
- Thetford M., S.L. Warren, and F.A. Blazich. 1995. Response of *Forsythia × intermedia* 'Spectabilis' to uniconazole: I. Growth, dry matter distribution, and mineral nutrient content, concentration, and partitioning. *J. Amer. Soc. Hort. Sci.* 120:XX-XX.
- Wample, R.L. and E.B. Culver. 1983. The influence of paclobutrazol, a new growth regulator, on sunflowers. *J. Amer. Soc. Hort. Sci.* 108:122-125.
- Wang, Y.-T. and J.R. Dunlap. 1994. Effect of GA₃ on growth and cellular change in uniconazole-treated hibiscus. *J. Plant Growth Regulat.* 13:33-38.
- Wang, Y.-T. and L.L. Gregg. 1989. Uniconazole affects vegetative growth, flowering, and stem anatomy of hibiscus. *J. Amer. Soc. Hort. Sci.* 114:927-932.
- Warren, S.L., F.A. Blazich, and M. Thetford. 1991. Whole-plant response of selected woody landscape species to uniconazole. *J. Environ. Hort.* 9:163-167.
- Weibel, E.R. 1969. Sterological principles for morphometry in electron microscopic cytology. *Intl. Rev. Cytol.* 40:325-440.
- Wood, B.W. 1984. Influence of paclobutrazol on selected growth and chemical characteristics of young pecan seedlings. *HortScience* 19:837-839.