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Changes in Leaf, Stem, and Root Anatomy of Chrysanthemum cv. Lillian Hoek Following Paclobutrazol Application

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Abstract. Plants of *Chrysanthemum* cv. Lillian Hoek were treated with a paclobutrazol (PBZ) soil drench and histologically examined after 3 months. PBZ application resulted in thicker leaves, reduced stem diameter, and roots with an increased diameter and an unusual segmented appearance. Increased leaf thickness was partly due to an additional layer of palisade mesophyll, although individual palisade cells were shorter, of smaller diameter, and more tightly packed. Spongy mesophyll depth was also greater and the individual cells were more rounded and the volume of intercellular space was reduced. The narrower stems had an increased development of secondary xylem, but had a marked reduction in the number of sclerenchyma bundle caps. Increased root diameter was due to an increase in the number of rows and diameter of cortical cells. In PBZ-treated plants, root cortical cell length was 50–70% less than in untreated plants, and this reduction appeared to be associated with the segmentation of the roots. PBZ inhibited secondary vascular development in the roots. This study is similar to other relevant studies in recording thicker leaves and roots with PBZ application; however, many of the underlying anatomical changes described above have not been previously reported.

The regulation of plant growth rate and form by application of the plant growth regulator paclobutrazol (PBZ) is widespread in agriculture, production horticulture, and amenity horticulture for both foliage and flowering plant production and turf management. While the changes that PBZ application induces in various plant parts have been well documented (e.g., thicker leaves and larger diameter roots), the underlying anatomical modifications have received little attention. The aim of this study was to document the changes in leaf, stem, and root anatomy of *Chrysanthemum* cv. Lillian Hoek following PBZ application.

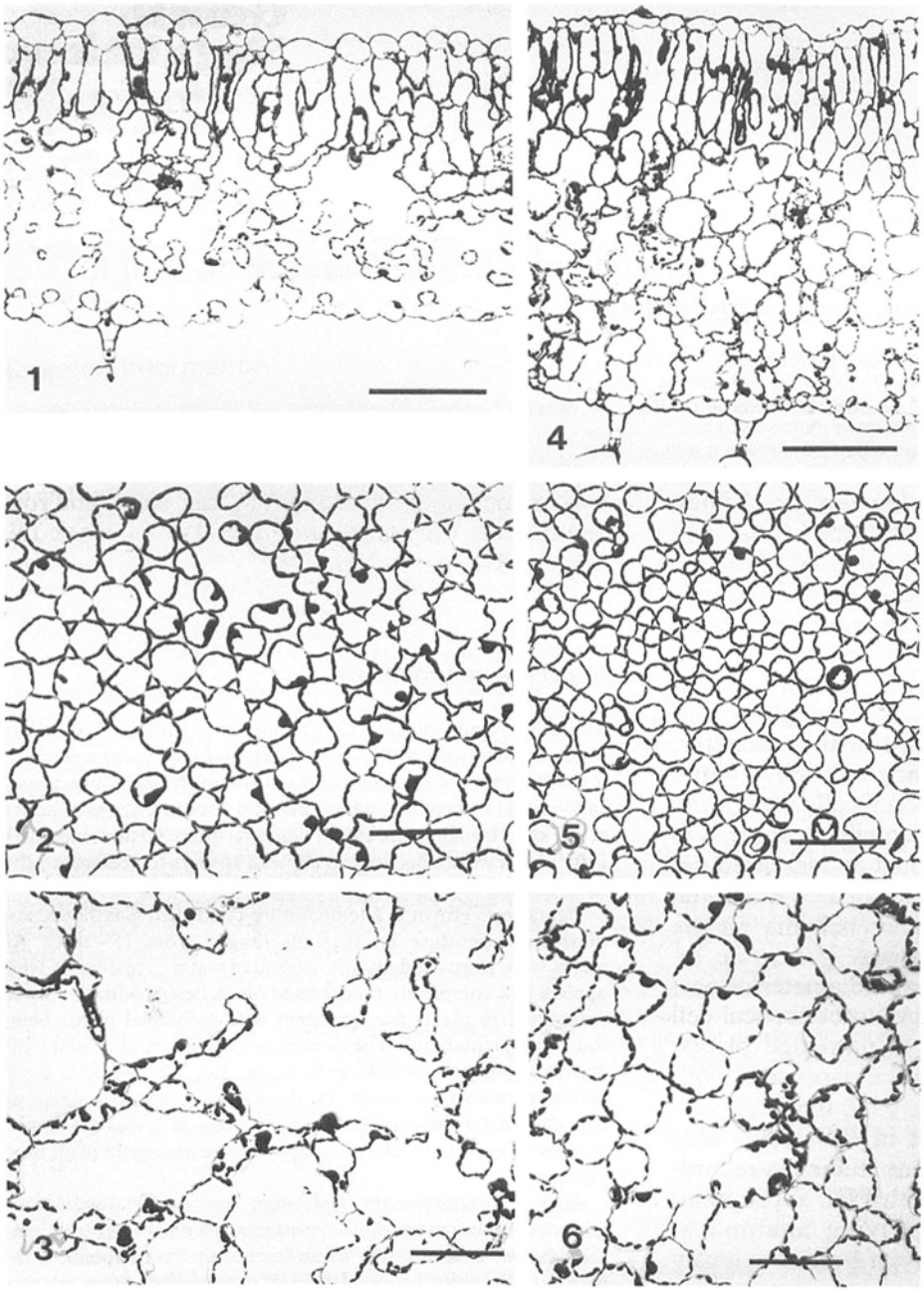
Materials and Methods

Rooted cuttings of *Chrysanthemum* cv. Lillian Hoek were planted individually in 1.25-L black planter bags using a sterile (1 h at 60°C) mixture of composted sawdust:pine bark chips:coarse sand (1:1:1, vol:vol:vol) with a pH of 6.0. Nutrients were added at 6.0 kg^{-3} using a controlled release fertilizer (Osmocote Plus). Fifty plants were selected and allowed 10 days to acclimate prior to treatment.

Plants were grown in a temperature-controlled glasshouse and diurnal temperature fluctuations ranged from 15°–28°C. All plants were irrigated daily with 100 ml of water. Treatments were applied in a completely randomized block design with five treatments and 10 plants per treatment with individual plants being the experimental unit. The treatments consisted of a 50-ml soil drench application of PBZ at 0, 0.125, 1.0, 5.0, and 10.0 mg of active ingredient per plant. Of these treatments, 5.0 mg/50 ml was chosen for intensive anatomical study as it was the lowest concentration that produced an appreciable change in plant morphology.

After 3 months growth, leaf, stem, and root materials were collected from at least three representative plants per treatment for fixation. Leaf material came from near the midpoint of the midrib of the third youngest fully expanded leaf. Stem material came from the internode above where the leaf was collected and root material from the largest diameter laterals.

Tissues fixed in 70% formalin–acetic acid–alcohol were dehydrated through a tertiary-butyl-alcohol series and embedded in paraffin wax (melting point, 58°C). Transverse sections of the leaves, stems, and roots were cut, and radial longitudinal sections were made of the latter two. Sections, 10-μm thick, were cut on a rotary microtome and stained with 0.05% toluidine blue O. Alternatively, leaf tissues were fixed in phosphate-buffered 2.5% glutaraldehyde for 8 h. The tissue was dehydrated in a graded ethanol series, embedded with Spurr's media, and incubated at 70°C overnight. Transverse and paradermal sections, 1–2-μm thick, were cut on a glass knife on an LKB Ultratome 3 and stained with 0.5% toluidine blue O in 0.1% sodium carbonate (pH 11.1).



Figs. 1–6. Transverse and paradermal sections of leaves from untreated and PBZ-treated plants of *Chrysanthemum* cv. Lillian Hoek. Scale bars for Figs. 1 and 4 are 200 μm, whereas scale bars for Figs., 2, 3, 5, and 6 are 100 μm. **(1)** Transverse section of control leaf showing the single palisade layer and large air spaces in the spongy mesophyll. **(2)** Paradermal section of the palisade mesophyll of a control leaf. **(3)** As per Fig. 2 except the spongy mesophyll is sectioned. **(4)** Transverse section of a leaf from a PBZ-treated plant showing the two to three layers of palisade mesophyll and the greater depth of spongy mesophyll. **(5)** Paradermal section of the palisade mesophyll from a treated plant showing the smaller diameter of the cells and the greater packing density. **(6)** As per Fig. 5 except the spongy mesophyll is sectioned. Note the denser packing of the cells and their more rounded appearance.

Results

Leaf Anatomy

Chrysanthemum leaves from untreated plants exhibited typical mesophyte anatomy (Fig. 1). They possessed a thin-walled upper epidermis with a very thin cuticle, a single layer of relatively loosely packed palisade mesophyll cells (Fig. 2), several loosely arranged layers of spongy mesophyll cells (Fig. 3), and a lower epidermis that possessed the majority of the stomates and three-celled trichomes. Chloroplasts in both the palisade and spongy mesophyll had obvious starch deposits.

The leaves of treated plants were a darker green and were thicker than those of the control (Fig. 4). The increased thickness was due to 64 and 72% increases in palisade and spongy mesophyll thickness, respectively (Table 1). Individual palisade cells were shorter in the PBZ-treated plants (Fig. 4, Table 1), but as two to three layers were present as opposed to the single layer of the control, this re-

Table 1. Mean (±SE) for dimensions of various anatomical structures in control and PBZ-treated *Chrysanthemum* plants.

	Control	PBZ
Leaf		
Total leaf thickness	427 ± 19.0	706 ± 10.7
Palisade mesophyll thickness	129 ± 3.1	211 ± 3.8
Spongy mesophyll thickness	248 ± 15.0	427 ± 10.5
Palisade cell diameter	42 ± 1.3	27 ± 0.9
Palisade (top layer) cell length	129 ± 3.1	96 ± 2.6
Palisade cells, per 0.04 mm^2	20.5 ± 1.0	44.0 ± 3.5
Stem		
Diameter (mm)	5.0 ± 0.3	2.5 ± 0.1
Pith cell length	199 ± 13.5	77 ± 9.1
Root		
Diameter	1415 ± 75.1	1715 ± 70.0
Stele diameter	565 ± 13.2	355 ± 18.1
Cortex width	420 ± 32.4	675 ± 23.5
Cortical cell length	168 ± 9.2	43 ± 1.7
Cortical cell diameter	40 ± 1.8	57 ± 2.3

All measurements in μm unless otherwise indicated. All comparable means are significantly different at the 1% significance level, except for root diameter which is significant at the 5% level (*t* test).

sulted in the thicker palisade. Individual palisade cells were a smaller diameter in the PBZ-treated plants and this was associated with higher cell density (Fig. 5, Table 1). The spongy mesophyll was more densely packed in treated plants and, as measured in transverse section, intercellular air spaces accounted for 40–60% of the spongy mesophyll in control plants, but only 20–30% in treated plants. Spongy mesophyll cells had long tubular projections in the control (Fig. 3), but were more rounded in treated plants (Fig. 6).

Stem Anatomy

In control plants the primary vascular tissues were arranged in numerous oval-shaped vascular bundles separated by wide parenchymatous medullary rays, and the vascular bundles were capped by large hemispherical sclerenchyma groups. Chlorenchyma, with a well-developed system of intercellular spaces, was present in the subepidermal layers (Fig. 7).

With the start of secondary growth, the arcs of fascicular cambium were linked by interfascicular cambium to give a complete but slightly convoluted ring (Fig. 7). However, only the arcs of fascicular cambium produced secondary phloem and secondary xylem with vessel elements, as the interfascicular arcs produced only xylem parenchyma and fibers (Fig. 7).

PBZ application resulted in shorter stems and a 50% reduction in diameter. In PBZ-treated plants the medullary rays were often narrower and consequently the vascular bundles were closer together. In addition, both the fascicular and interfascicular cambia produced vessel elements. This resulted in an almost continuous ring of secondary conducting tissue (Fig. 8), in contrast to the isolated groups of vessels in the control group. Vessel diameter was similar in untreated and treated plants; however, in the former the vessels were angular in outline, while in the latter they were more rounded.

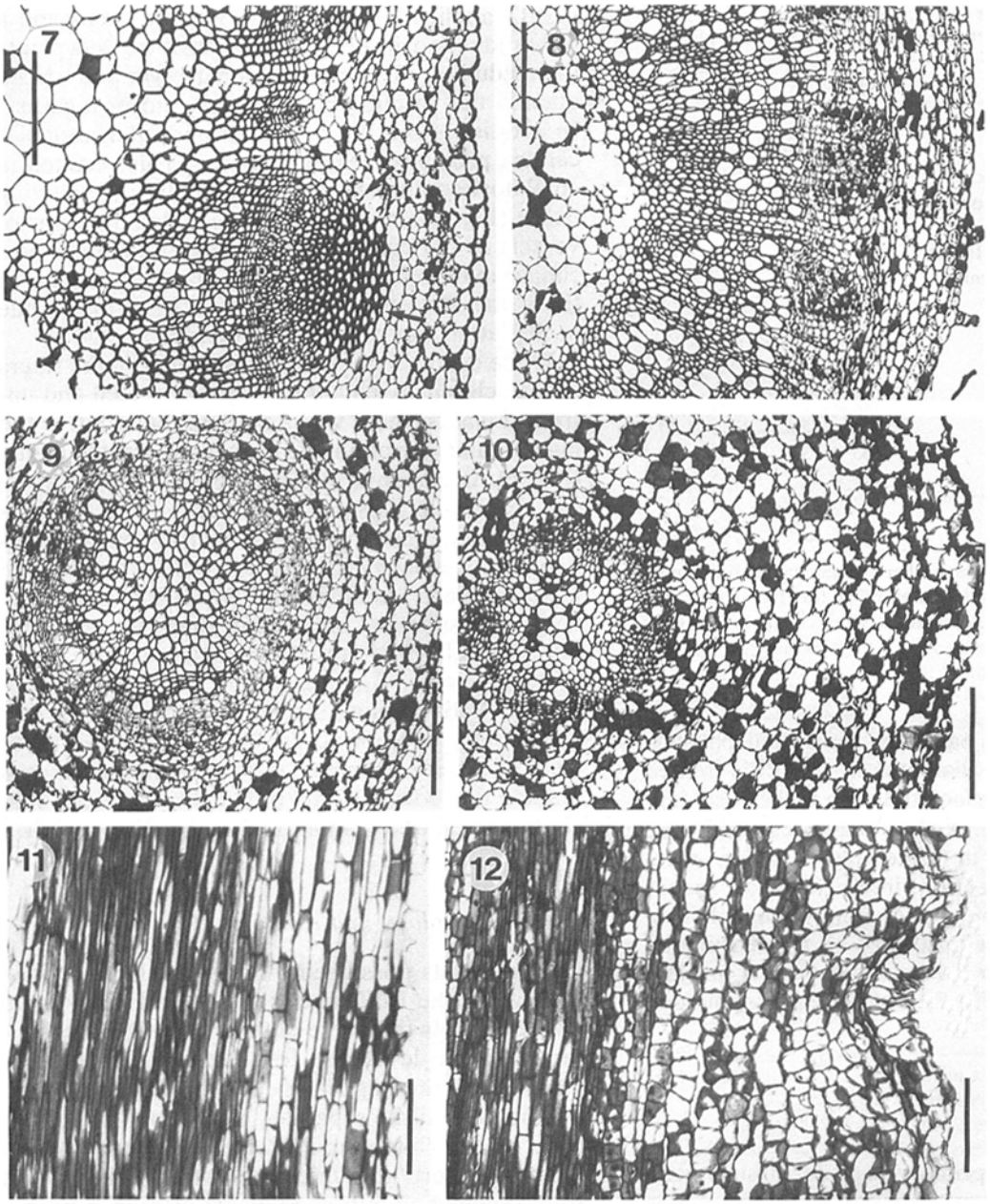
While cell wall thickness was similar in the fibers of the sclerenchyma bundle caps in treated and untreated plants, there was a marked reduction in the number and size of bundle caps in the treated plants (Fig. 8). In the control plants there were, on average, 22 bundle caps per cross section and the caps were 350 μm apart, while in the PBZ-treated plants five to six caps per cross section were present, at an average of 850 μm apart. In PBZ-treated plants the basic hemispherical bundle cap shape was present, but the cells had not differentiated into fibers (Fig. 8).

With PBZ application the chlorenchyma was poorly developed or absent. While most cell types were of similar diameter in treated and nontreated plants, the cortical and pith cells were 50–60% shorter in treated plants.

Root Morphology and Anatomy

Control plants possessed a finely divided root system, while the PBZ-treated plants had many large diameter roots that were untapered over many centimeters and developed few laterals. These roots had a segmented appearance, with numerous 0.5–1.0-mm long disks separated by narrow, dark constrictions. While the PBZ-treated plants had a greater proportion of thick roots, the thickest roots from treated and untreated plants were of a similar diameter, although secondary vascular development was less advanced in the PBZ-treated roots and there was a corresponding increase in cortical thickness (Figs. 9 and 10). PBZ-treated roots had a more aerenchymatous nature, with larger intercellular air spaces in the cortex.

In the treated plants the diameter of the cortical cells was slightly larger than that of the control plants (Table 1); however, they were 50–70% shorter (Figs. 11 and 12). Epidermal cells did not appear to display a size difference of this magnitude. It appeared that there were regions of active cell division in the epidermis and perhaps the subepidermal layer that were not accompanied by



Figs. 7–12. Transverse and radial longitudinal sections of the stem and root from treated and control plants of *Chrysanthemum* cv. Lillian Hoek. All scale bars, 200 μm. **(7)** Transverse section of the stem of a control plant showing the wide medullary rays (small arrow) and the large sclerenchyma bundle caps (large arrows) (p, phloem; x, xylem). **(8)** As per Fig. 7 except from a PBZ-treated plant showing the near continuous ring of secondary xylem and the almost complete absence of sclerenchyma bundle caps. **(9)** Transverse section of a large diameter root from a control plant. **(10)** As per Fig. 9 except from a PBZ-treated plant. Note the wider cortex and the lack of secondary vascular development. **(11)** As per Fig. 9 except a longitudinal section. **(12)** As per Fig. 10 except a longitudinal section. Note the much shorter cortical cells compared to Fig. 11 and the concave depression in the epidermis which resulted in the roots having a segmented appearance.

divisions in the cortical cells beneath. These additional divisions caused the epidermis to arc inwards, distorting several of the subepidermal layers and forming the segmented root morphology (Fig. 12).

Discussion

Leaves

While it is a common observation that leaves from PBZ-treated plants are smaller, thicker, and a

darker green than those from untreated plants (e.g., Wood 1984, Ziv et al. 1986), there have been relatively few studies of the anatomical basis of these changes.

Studies, such as Jaggard et al. (1982) and Dalziel and Lawrence (1984) on sugar beet, Hawkins et al. (1985) on soybean, and Barnes et al. (1989) on soybean and corn, have shown differing anatomical mechanisms associated with increase in leaf thickness. In sugar beet a 100% increase in leaf thickness was associated with a three- to fourfold increase in the length of the individual palisade cells, although there was no increase in the number of rows of palisade (Dalziel and Lawrence 1984). Similarly, Hawkins et al. (1985, Figs. 1 and 2) show that the 15–25% increase in soybean leaf thickness was almost entirely due to elongation of palisade cells, with no increase in the number of palisade rows and no increase in spongy mesophyll thickness. In contrast, in another study of soybean, Barnes et al. (1989) found that a 101% increase in leaf thickness was primarily due to a doubling in the number of palisade rows and their Figure 1 shows the individual palisade cells to be a similar length when treated and untreated. The present study is similar to Barnes et al. (1989) with PBZ application inducing an increase in the number of palisade rows, but no elongation of the individual palisade cells. The present study is unusual in recording a decrease in palisade cell length with PBZ application, although Table 3 of Hawkins et al. (1985) also records a decrease, but their Figs. 1 and 2 indicate otherwise.

Hawkins et al. (1985) recorded a decrease in palisade cell diameter with PBZ application and an associated increase in palisade-packing density, similar to the present study. Although not specifically measured by Barnes et al. (1989) and Dalziel and Lawrence (1984), their photomicrographs do not appear to show a similar trend. Hawkins et al. (1985) recorded 36 and 16% intercellular space in control and treated plants, respectively, which is again similar to the present study.

PBZ application did not alter the leaf anatomy of corn (Barnes et al. 1989).

Stem

In an investigation of poinsettia stem, McDaniel et al. (1990) found that PBZ application suppressed cell wall thickening in the phloem fiber caps, decreased the width of the xylem ring, and interfascicular supporting tissues did not differentiate. The overall result was that stems were structurally weaker. In the present study, PBZ caused a marked reduction in the number of bundle cap fibers produced, but when present their wall thickness was similar in treated and nontreated plants. In *Chrysanthemum* and unlike poinsettia, the xylem ring in PBZ-treated plants was a similar width to that in untreated plants and, in addition, it was continuous and constituted a much greater proportion of cross-sectional area. This resulted in what was probably a structurally stronger stem.

In a study of peach shoots, Aguirre and Blanco (1990) found that PBZ treatment produced a decreased proportion of xylem, with a corresponding increase in the amount of phloem and cortex. There was also an increased density of smaller diameter vessels and greater starch storage in parenchymatous tissues. Stems of *Chrysanthemum* treated with PBZ had a greater proportion of their cross-sectional area composed of xylem and there was no decrease in vessel diameter.

In the only other anatomical study of PBZ effects on stem or stem-like structures, Privé et al. (1989) found that PBZ caused little or no change in the cell size of apple pedicels, but noted the epidermal and cortical cells were arranged in a less orderly manner and their cell walls appeared thicker.

Roots

Thickening of the roots of PBZ-treated plants is a common observation (Barnes et al. 1989, Bausher and Yelenosky 1987, Sankhla et al. 1986, Wample et al. 1987, Williamson et al. 1986) and inhibition of the formation of lateral roots has also been reported (Bausher and Yelenosky 1987). The present study adds further support to these observations, although the increase in root diameter in *Chrysanthemum* was not as great as in the above studies.

Most of these studies describe or illustrate only a minor change in stele diameter, with the increase in root diameter being attributable to cortical widening. In some species the widening is primarily due to an increase in the number of rows of cortical cells with little or no change in cell size (Wample et al. 1987), while in others the number of rows was little changed but cortical cell size was greater, especially in the inner rows (Barnes et al. 1989, Williamson et al. 1986). In *Chrysanthemum* the wider cortex was due to an increase in the number of rows and cell diameter, which is similar to the situation illustrated by Bausher and Yelenosky (1987, Fig. 5).

In the only other study to investigate cortical cell length, Williamson et al. (1986) found, as per the present study, a marked reduction in cell length with PBZ application, although in peach segmented root morphology was not recorded. Compared to the present study there have been no reports that

make specific mention of a reduction in secondary vascular growth with PBZ application, although a similar situation appears to occur in *Citrus* (Bausher and Yelenosky 1987, Fig. 5).

This study confirms that PBZ application can induce similar morphological changes (e.g., thicker leaves and wider diameter roots) in a wide range of species. The anatomical modifications responsible for these changes have been, in the species described to date, quite variable. While some of this variation is probably due to the method of PBZ application, concentration of PBZ applied, age of plant material, etc., there can be species-specific responses to PBZ application, which in turn indicates varied physiological responses to PBZ application.

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