

Anatomical Aspects of Chilling Injury to Leaves of *Phalaenopsis* Bl.¹

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Abstract. *Phalaenopsis* species often exhibit light green spots and/or pitting of the upper leaf surface during winter and spring. Results of this study indicate spotting and pitting can be induced by exposure to temperatures of 2°, 4°, and 7°C for 8 hours or less. The spots and/or pitted areas were always found between large vascular bundles. Light green or yellow spots on the leaves were caused by hypertrophied mesophyll cells between the main vascular bundles. Anatomical observations revealed the pitting was due to collapsed mesophyll cells. Severity of response depended on physiological age of the leaf and duration of exposure to the chilling temperature.

Phalaenopsis species and their hybrids have become important commercial orchids during the past 3 decades. During winter and spring growers have reported one or more isolated leaves on their plants with moderate to severe pitting. The pitting may be localized or affect relatively large areas of a leaf surface. Although this pitting initially resembles a virus, reports indicate it only appears on leaves exposed to temperatures below 10°C (1). *Phalaenopsis* spp. are native to tropical regions of the Eastern Hemisphere and should be grown at temperatures above 18° (2). However, they are often grown with other orchids with night controls set at 15°. Chilling injury has been reported on several tropical plants: African violet (4), banana (7), orchids (2), and sanservieria (6). This experiment was conducted to determine the effects of temperatures below 10° but above 0° on leaf pitting of *Phalaenopsis* cv. Pink Chiffon.

A 5 × 3 factorial experiment in randomized block design with 4 replicates was initiated on July 16, 1976. A single 'Pink Chiffon' *Phalaenopsis* in a 15 cm clay orchid pot was considered an experimental unit. Treatment temperatures were 2°C ± 1°, 4°C ± 1°, and 7°C ± 1° for durations of 0, 1, 2, 4, and 8 hr. Plants were placed in unlighted refrigerated units already cooled to experimental temperature at 7:30 AM, 11:30 AM, 1:30 PM, and 2:30 PM. All plants were removed at 3:30 PM and transferred to a shaded glass greenhouse at 35°. Leaf sections were taken 30 days after exposure to low tempera-

ture, killed in FAA, sectioned at 10 μm and stained with safranin O and fast green FCF.

Macroscopic observations. Within 0.5 hr after removal from the refrigerated units, chilling injury was evident by slight water soaked spots between main vascular bundles. After 48 hr these areas were pale green in color. These areas often turned yellow, coalesced and were easily recognized 2 weeks after exposure to low temperature. The yellow spots became almost colorless or dark brown and deeply sunken within 3 weeks. The pitted areas continued to darken and closely resembled virus symptoms. Pitting was usually confined to the upper surface of most leaves, but occasionally, in severe cases, the lower leaf surface was also affected. Total leaf surface damaged by chilling injury did not exceed 25%.

The greatest amount of injury was observed in plants subjected to 2°C for 8 hr. Lyons (5) states that severity of injury to sensitive plant tissue increases as temperature is lowered and/or exposure is extended. Chill damage was confined almost exclusively to those leaves between ½ to ¾ of mature length. Consequently most mature leaves showed no chilling injury even when exposed to 2° for 8 hr. Other investigators have also reported a critical stage in leaf enlargement that most sensitive to chilling injury (7, 8).

Microscopic observations. 'Pink Chiffon' leaves had a distinct cuticle and an epidermis which was characterized by rectangular thin-walled cells slightly thickened on the outer wall, with larger cells in the upper epidermis. Mesophyll cells were relatively uniform in size, isodiametric in shape, and had no distinct arrangement in to an upper and lower mesophyll layer. Equidistant vascular bundles were interspersed midway between the upper and lower epidermis (Fig. 1).

Initial response to chill injury occurred in mesophyll cells between large vascular bundles. One or more layers of mesophyll cells collapsed and formed an internal horizontal necrotic layer (Fig. 2). If only a few cells collapsed, the surrounding mesophyll cells hypertrophied and pitted areas did not develop. Macroscopically these areas appeared as light green to yellowish green spots. More severely injured tissue was characterized by extensive mesophyll collapse which often caused a slight depression of the epidermal cells. These areas were always surrounded

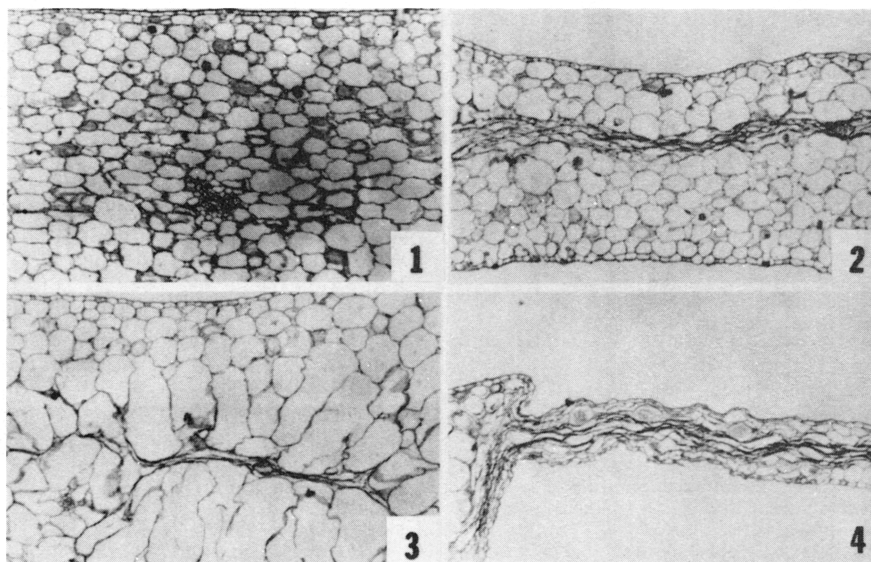


Fig. 1-4. Transverse sections of *Phalaenopsis* 'Pink Chiffon' leaves. (Fig. 1) Untreated leaf. (Fig. 2) 30 days after exposure to 2°C. (Fig. 3) 30 days after exposure to 2°. (Fig. 4) 30 days after exposure to 2°. Fig. 1, 2, 4: × 42; Fig. 3: × 84.

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by hypertrophied cells (Fig. 3). Macroscopically these areas were yellowish green to yellowish tan spots. The most severe injury occurred when there was a total collapse of the mesophyll tissue and the upper and lower epidermal cells were separated by a layer of necrotic cells (Fig. 4). These cells accounted for the brown to black elongated pitted areas which were up to 2 mm in length.

Surface pitting is a common response of both attached and detached plant parts to chill injury (5), but it is often associated with subsequent bacterial or fungal diseases (3). Sections of *Phalaen-*

opsis leaves did not reveal any diseased tissue, apparently because the epidermal cells were unaffected by chill injury.

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Influence of Salinity Levels on Growth and Chemical Composition of *Livistona chinensis*¹

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Abstract. The effect of 0, 250, 1000, 3000, 5000, 7500, 10,000, and 15,000 ppm solution of NaCl:MgCl₂ in a 10:1 ratio was determined on Chinese fan palms [*Livistona chinensis* (Jacq.) R Brown] grown in soil or nutrient solution. Plants grown in soil and drenched weekly with 10,000 ppm ceased growth within 2 months, while palms grown in nutrient solution exhibited a reduced growth rate with increasing salinity levels. Tissue analysis showed increased levels of Na and Mg in plant tissue with increased saline substrate levels, with highest Na and Mg tissue levels in fronds from container-grown palms.

Salinity levels in irrigation waters have increased during the past 25 years especially in coastal areas where salt water intrusion is a major problem. Investigations have shown that specific ions in saline water change the nutritional status of plants. Increasing substrate Na may decrease Ca and/or Mg absorption (2, 3, 10). High soil Na levels are known to disperse soil colloids which reduces soil aeration and moisture transmission (1). Even Na tolerant plants may show reduced growth due to poor soil structure (1, 8).

This study was conducted to determine effects of increasing salt concentrations on growth and chemical composition of a slightly salt tolerant plant, Chinese fan palm (7) grown in aerated Hoagland's solution and in a soil mix.

Seedling container-grown Chinese fan palms were transferred to 4-liter

plastic containers having 2 liters of half-strength Hoagland's solution (nutrient solution) or repotted into 20-cm-diameter plastic containers in a mix of 1 Florida peat: 1 washed mason sand: 1 pine bark (by volume) amended with 4.2 kg/m³ dolomite, 0.6 kg/m³ Perk² and 2.9 kg/m³ of 14-6-12 (N-P-K) Osmocote. Hoagland's solutions were maintained at 2 liters with de-ionized water and changed weekly. Container-grown palms were refertilized every 3 months with 6.6 g 14-6-12 (N-P-K) Osmocote until experiment initiation. Plants were grown in a greenhouse with temperatures of 13°C minimum and 38°C maximum and container-grown plants were irrigated weekly during cool weather and twice weekly during warm weather. After an 18-month growth period the Hoagland's solution was adjusted to contain 0, 250, 1000, 3000, 5000, 7500, 10,000 and 15,000 ppm of a 10:1 ratio NaCl:MgCl₂. Two liters of 0, 250, 1000, 3000, 5000, 7500, 10,000, and 15,000 ppm of a 10:1 ratio of NaCl:MgCl₂ were used to drench container plants weekly. A 10:1 ratio of NaCl:MgCl₂ was used to duplicate the relative proportions of these cations in sea water. Treatments were replicated 6 times with 1 palm per container constituting the ex-

perimental unit. Plant heights were measured at initiation and termination of the experiment 8 weeks later to determine new growth. The most recent fully expanded frond from each plant was used for tissue analysis.

As salt levels in the substrate increased, the amount of new growth decreased for all palms. Growth of container-grown plants was less at each salt level compared to non-saline treated plants and plants grown in Hoagland's solution (Table 1). Growth completely ceased at saline levels of 10,000 ppm for container-grown plants, but growth was not completely inhibited for Hoagland's solution plants even at the highest salt level (15,000 ppm). Palms grown in Hoagland's solution treated with 3000 ppm saline solution had a growth rate of 65% of untreated palms, while container-grown plants treated with the same saline level had a growth rate of 33% of untreated plants. When *Phoenix dactylifera*, a salt-tolerant palm, was irrigated with a 3000 ppm saline solution, its growth rate was reduced to 90% of the control (4).

Container-grown plants drenched with 5000 ppm and higher saline solutions showed frond tip necrosis after 4 weeks. Necrosis occurred first on newly expanded fronds and progressed from apical portions of the frond tip toward the hastula. Similar reactions have been reported for other plants (1). At salinity levels of 10,000 ppm some fronds were completely necrotic and 3 of the 6 palms treated with 15,000 ppm saline solution died at experiment termination. In contrast, necrotic areas never exceeded 10% of the leaf surface of Hoagland's solution grown plants even at the highest rate and lower matured leaves did not show tip burn.

The large increases in substrate concentration of Mg and Na were reflected in analysis of fronds with maximum tissue levels of 0.20% Mg and 0.49% Na for Hoagland's solution palms and 0.37% Mg and 1.72% Na for container-grown palms (Table 1). Furr and

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²A micronutrient blend manufactured by Kerr-McGee, Inc., Jacksonville, Florida.