

Histochemical Study of Vascular Blockage in Flower Stems of Orchids in Relation to Vase Life

Saichol Ketsa¹ and Tadashi Nobuchi²

ABSTRACT

Sections were cut from the basal and upper portions above the water level of aged flower stems of *Dendrobium* 'Pompadour' and from freshly cut flower stem. Observation of cross sections by light microscope showed the presence of vascular occlusion in aged flower stems. Occlusions at the basal portion were much more than at the upper portion. Occluding material at both the basal and upper portions above the water level contained both pectin and carbohydrate, while only at the upper portion contained phenol. Lignins were not found in the occluding material.

INTRODUCTION

The most widely accepted theory on extending cut flower vase life is based on the improvement of water relation. Decreased water uptake due to vascular occlusion has been shown to be associated with decreased vase life of cut flowers (Burdett, 1970; Gilman and Steponkus, 1972; Parups and Molnar, 1972; Lineberger and Steponkus, 1976). Gilman and Steponkus (1972) showed that bacterial contamination of stems and of water in which cut flowers are placed can block the xylem and cause early flower senescence. However, even when microorganisms are controlled, vascular blockage, as evidence by resistance to water flow, occurs in cut flower stems. Durkin and Kuc (1966), Burdett (1970), and Marousky (1971) concluded that in addition to microorganism control, some active processes in stem tissue must be inhibited if blockage is to be arrested.

Lineberger and Steponkus (1976) revealed the existence of two types of vascular occlusions in rose sections. Microbial occlusion was located at the base of cut stem, while a second type, gum deposition was always found above the solution level. The materials in blocking xylem vessels of senescing rose stems contained carbohydrate, pectin, lipid, protein and some enzymes. Tannins, lignins and callose were not

found in the blocked vessels (Burdett, 1970; Gilman and Steponkus, 1972; Parups and Molnar, 1972; Lineberger and Steponkus, 1976). Similarly, Fujino *et al.* (1983) reported that vascular blockage in rachides of cut Maidenhair fronds contained pectin while no lignins and tannins were found. The gums or mucilages related to pectic substance was always found in blocked vessels above the solution level (Lineberger and Steponkus, 1976).

Study of vascular blockage in cut orchid flowers had been carried out with *Dendrobium* 'Pompadour' (Amutiratana, 1986) without success. This might be due the lack of good techniques in section preparation and/or staining. At the present study we report the results from histochemical study of vascular blockage in cut orchid flowers of *Dendrobium* 'Pompadour'.

MATERIALS AND METHODS

Inflorescence of *Dendrobium* 'Pompadour' were purchased from a local grower in the Bangkok suburban. They were dry packed and brought to laboratory by truck within two hours. Inflorescences were selected for uniformity of length and floret number. Individual flower stems was slantingly cut to 14-cm length from the first open floret to the basal end. They were held in a glass vase containing tap

1 Dept. of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
2 Dept. of Wood Science & Technology, Kyoto University, Sakyo-ku Kyoto 606, Japan.

water 10 cm up on the flower stem at ambient temperature. Flower stem samples were taken from the basal and the upper portions just above the water level on the flower stem at the 10 th day at which most of orchid flowers terminated their vase life which was based on 50% wilted or abscised open florets. Control samples were taken from flower stems of freshly cut orchids without holding in tap water and immediately prepared for light microscopic study. Flower stem sections 2-5 cm in length were fixed in 15% formaldehyde for at least 24 hours. Cross sections were cut approximately 20-30 μm on a sliding microtome. Since the flower stems of orchids are hard at the outer and soft at the inner, subsequently sections were cut brokenly. Thus sliding microtome and sliding cut flower stem samples were placed in the freezer before making sections. Cross section were stained with a saturated solution of ruthenium red or phloroglucinol (Jensen, 1962), or 0.05% toluidine blue in 0.1 M phosphate buffer pH 6.8 (McCully, 1966). The periodic acid-Schiff's (PAS) reaction was also used in this work (Jensen, 1972). All sections were examined under a compound microscope for the evidence of occlusions in vascular system and for positive reactions to the stains. Determination of water content in both freshly cut and aged flower stems was done according to Ranganna (1978). The data presented represents the means for five segments of flower stems.

RESULTS AND DISCUSSION

The microscopic examination of cross sections of freshly cut flower stems did not show any occlusion in both xylem and phloem (Figure 1 a), while sections from aged flower stems in tap water showed occlu-

sions in both xylem and phloem of basal (Figure 1 b) and upper portions (Figure 1 c). The basal portions showed more occlusions than the upper portions. This indicates that both pathological and physiological occlusions exist simultaneously in aged flower stems of orchids similar to that found in rose stems (Lineberger and Steponkus, 1976).

Xylem and phloem in cross sections of basal and upper portions from both freshly cut and aged flower stems were not stained with either phloroglucinol or toluidine blue (Table 1). The lack of a positive reaction to the staining indicates that the occlusion at the basal and upper portions above the water level on the aged flowers did not contain lignins (Jensen, 1962). The result obtained in this study was similar to that reported in rose stem (Gilman and Steponkus, 1972; Parups and Molnar, 1972) and fern frond (Fujino *et al.*, 1983).

Xylem and phloem in cross sections of freshly cut flower stems were not stained pink-red of ruthenium red (Table 1 Figure 2 a). Phloem in cross sections from both basal and upper portions of aged flower stems were stained pink-red of ruthenium red, while xylem were not (Figure 2 b and 2 c). Since ruthenium red may be considered rather specific for pectin (Jensen, 1962). Therefore pathological occluding material in phloem at basal and upper portions of aged flower stems of orchid may contain pectin degradation products. This was different from rose stem (Parups and Molnar, 1972; Lineberger and Steponkus, 1976) and fern frond (Fujino *et al.*, 1983), where pectin was found in occlusions occurred only at the basal end.

Table 1 Staining of xylem and phloem in cross sections from freshly cut and aged flower stems held in tap water of *Dendrobium* 'Pompadour'.

	Freshly cut flower stem		Aged flower stem			
	phloem	xylem	Basal	portion	Upper	portion
			phloem	xylem	phloem	xylem
Phloroglucinol	-	-	-	-	-	-
Toluidine blue	-	-	-	-	-	-
Ruthenium red	-	-	+	-	+	-
PAS reaction	-	-	+	+	+	-
Nitroso reaction	-	-	-	-	+	+

(- no staining, + staining)

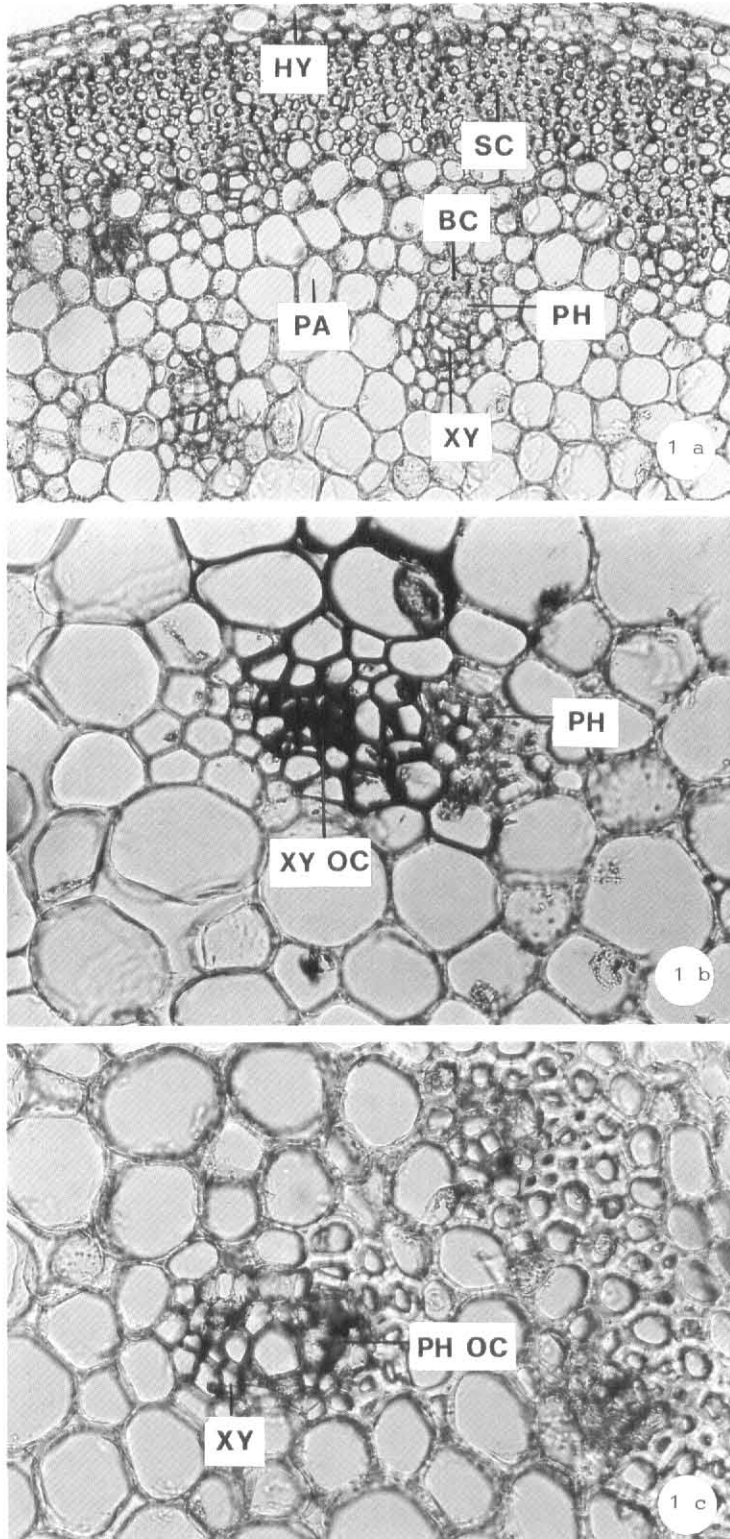


Figure 1 Non-stained cross sections of freshly cut and aged flower stems held in tap water for 10 days of *Dendrobium* 'Pompadour' a, freshly cut flower stem (X 125, BC = bundle cap, OC = occlusion, HY = hypodermis, PA = parenchyma, PH = phloem, SC = sclerenchyma, XY = xylem); b, basal portion of the aged flower stems (X 250); c, upper portion above the water level on the aged flower stem (X 250).

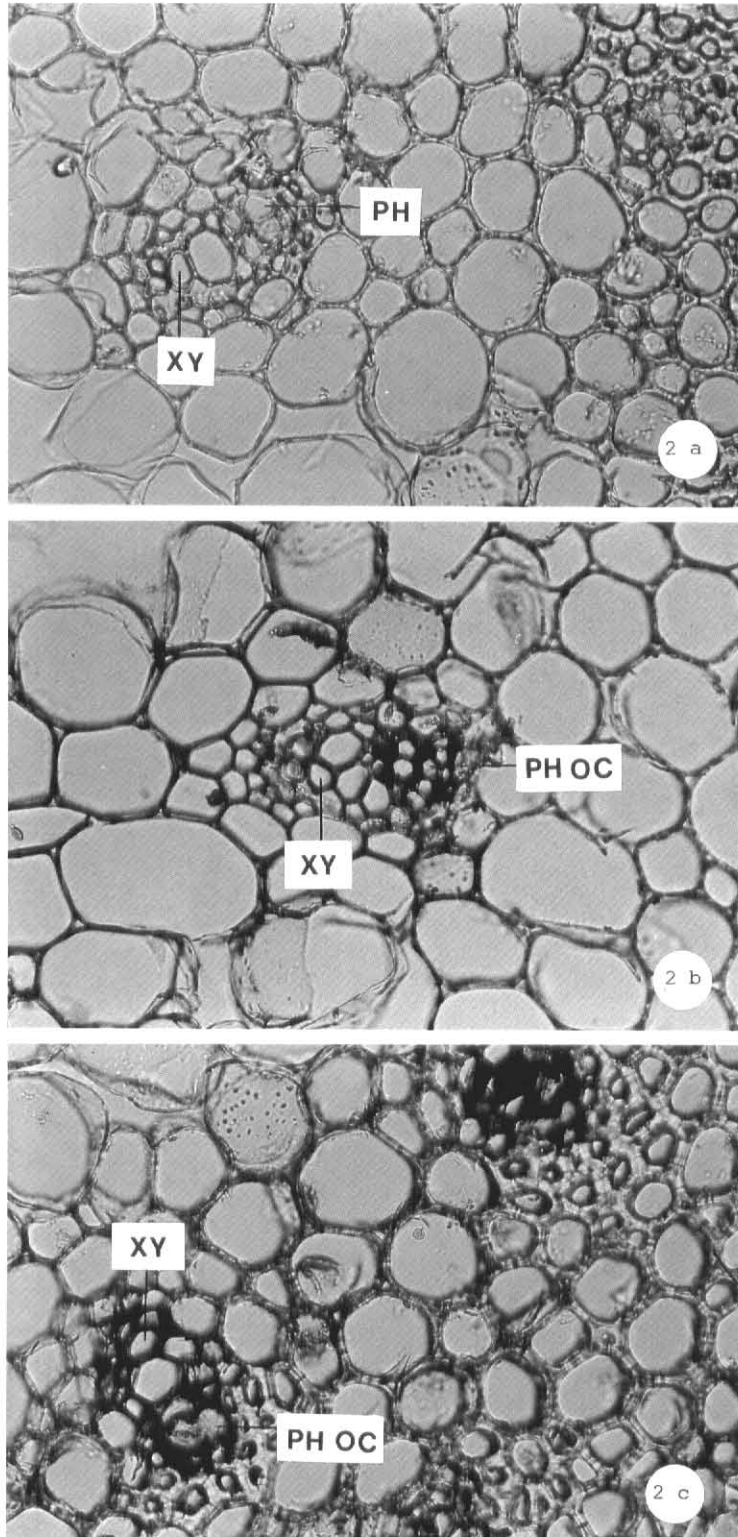


Figure 2 Cross sections of aged flower stems of *Dendrobium* 'Pompadour' stained with ruthenium red. a, freshly cut flower stem (X 125); b, basal portion of aged flower stems (X 250); c, upper portion above the water level on the the aged flower stem (X 250).

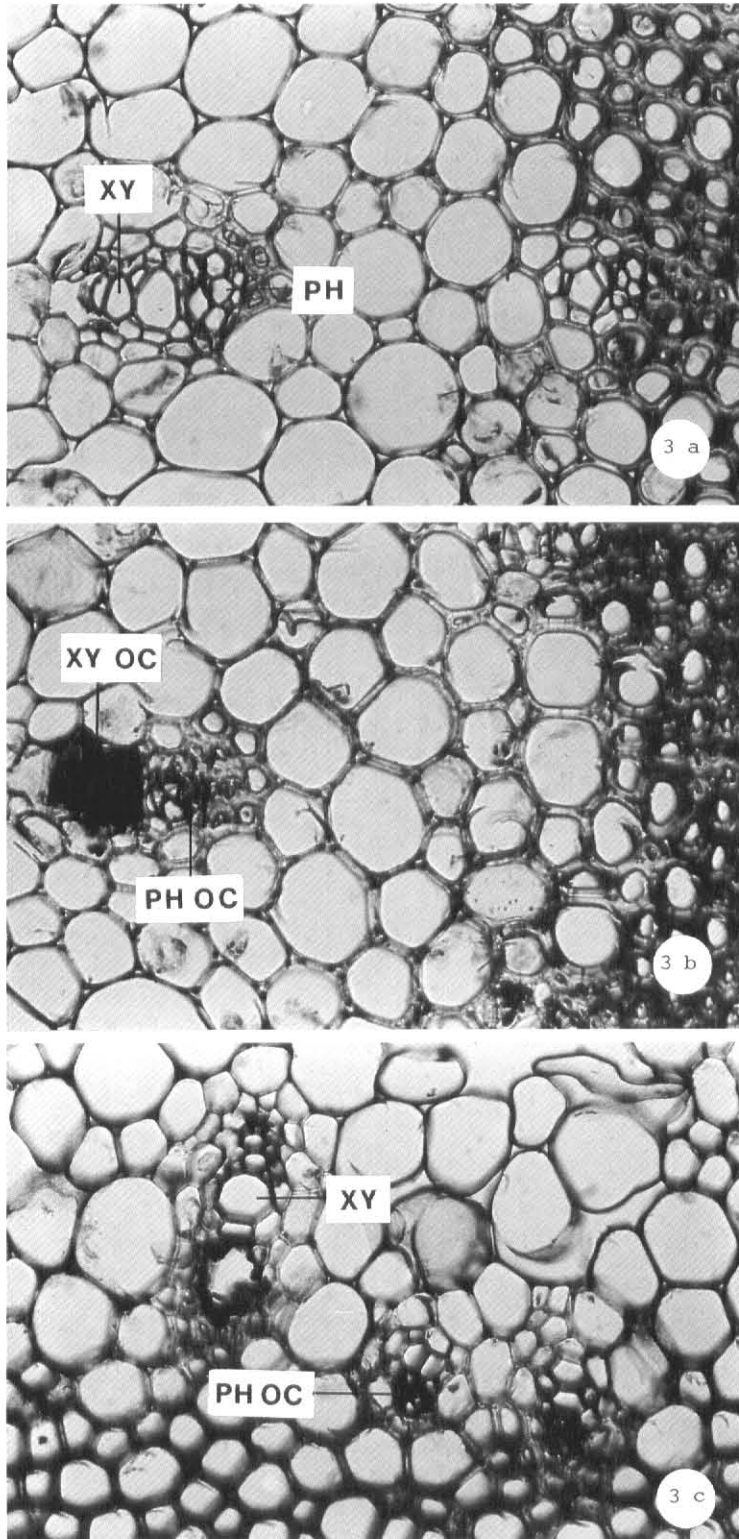


Figure 3 Cross sections of aged flower stems of *Dendrobium* 'Pompadour' stained with PAS reaction. a, freshly cut flower stem (X 250); b, basal portion of the aged flower stem (X 250); c, upper portion above the water level on the aged flower stem (X 250).

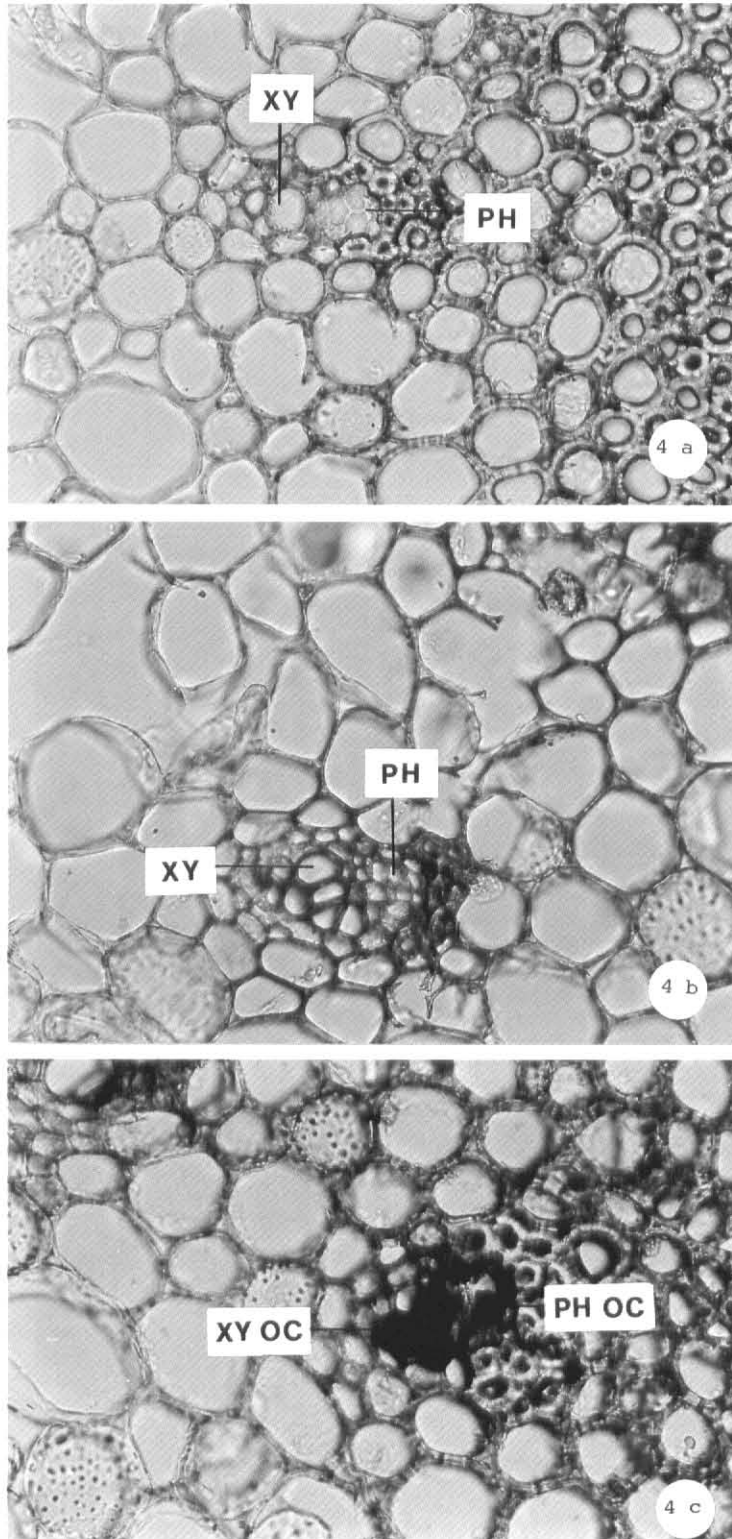


Figure 4 Cross sections of aged flower stems of *Dendrobium* 'Pompador' stained with nitroso reaction. a, freshly cut flower stem (X 250); b, basal portion of the aged flower stem (X 250); c, upper portion above the water level on the aged flower stem (X 250).

Xylem and phloem in cross sections from basal and upper portions of freshly cut flower stems were not stained purple-red of PAS reaction (Table 1, Figure 3 a), while those from aged flower stems were stained (Figure 3 b,c). Xylem in cross sections from the basal portion were stained more purple-red than the phloem (Figure 3 b), while phloem in cross sections from the upper portion were strongly stained purple-red and xylem were not (Figure 3 c). A strongly positive reaction with PAS stain of cross sections from both basal and upper portions of aged flower stems of orchids indicates that occluding material should be carbohydrate in nature (Jensen, 1962). More occlusions in non-stained sections from the basal portion of aged flower stems of orchid (Figure 1 b) may be due to more carbohydrate in occlusions. However, contrary of previous work (Lineberger and Steponkus, 1976), carbohydrate was found in occlusions occurred only above the solution level on the rose stem. Lineberger and Steponkus (1976) showed that carbohydrate type occlusion in rose stems belonged to the category of plant gums. The presence of both pectin and carbohydrate at basal and upper portions above the water level on the aged flower stems of orchids indicates that both pathological and physiological disturbance could induce the breakdown of the cell walls and release contents of the cells (Esau, 1965; Sutton and Williams, 1970).

Xylem and phloem in cross sections from basal and upper portions of freshly cut flower stems (Table 1, Figure 4 a) and from basal portions of aged flower stems were not stained cherry-red of nitroso reaction (Figure 4 b). This suggested that occlusions at the basal portions of aged flower stems of orchid were not phenolic in nature. Xylem and phloem in cross sections from upper portions of aged flower stems of orchid were stained cherry-red of nitroso reaction (Figure 4 c). This seemed possible that the physiological occluding materials at upper portions above the water level on aged flower stems of orchids may contain a phenolic nature (Reeve, 1951; Burdett, 1970). Water content of freshly cut flower stems of *Dendrobium* 'Pompador' was found approximately 72-75% from basal and to distal end, while that of aged flower stems of terminated orchid decreased starting at the upper portion above the water level. Water content of the upper portion ranged 62-68% and gradually decreased to 46% at the distal end (Figure 5). It is not known if low water content or water stress in aged flower stems of orchid could stimulate the production of phenolic compounds.

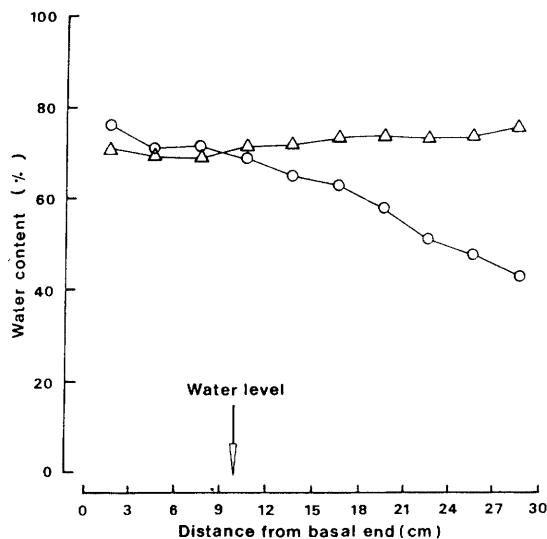


Figure 5 Water content of freshly cut (Δ) and aged flower stems held in tap water for 10 days (\circ) of *Dendrobium* 'Pompador'

Water stress has been reported to increase ethylene production in plants (Apelbaum and Yang, 1981) and ethylene itself can stimulate the production of phenolic compounds (Rhodes and Woollorton, 1973; Sakar and Phan, 1974).

The results of microscopic examination of cross sections of aged flower stems of orchid obtained in this study demonstrated clearly that pathological and physiological occlusions appeared in aged flower stem of *Dendrobium* 'Pompador'. It should keep in mind that the vase life of cut orchids did not depend on only water supply, but also on the respiratory substrate or food reserve (Ketsa and Boonrote, 1990). Silver ions and 8-hydroxyquinoline sulfate in holding solution for cut orchid (Ketsa, 1989; Ketsa and Boonrote, 1990) may play an important role in extending the vase life of cut orchid by prohibiting the occurrence of occlusions in flower stems similar to that in rose stems (Marousky, 1969; Marousky, 1971) and fern fronds (Fujino *et al.*, 1983).

ACKNOWLEDGEMENT

The authors gratefully acknowledge grant support from the KU-JICA Phase II.

LITERATURE CITED

Amutiratana, A. 1986. Effect of 8-Hydroxyquinoline, 8-Hydroxyquinoline Sulfate, Silver Nitrate, Sil-

- ver Thiosulfate, Glucose and Sucrose on Vase-Life of *Dendrobium* 'Pompadour' Cut Flowers. M.S. Thesis, Kasetsart University, Bangkok. 91 p.
- Apelbaum, A. and S.F. Yang. 1981. Biosynthesis of stressed ethylene induced by water deficit. *Plant Physiol.* 68 : 594-596.
- Burdett, A.N. 1970. The cause of bent neck in cut roses. *J. Amer. Soc. Hort. Sci.* 95 : 427-431.
- Durkin, D. and R. Kuc. 1966. Vascular blockage and senescence of the cut rose flower. *Proc. Amer. Soc. Hort. Sci.* 89 : 683-688.
- Esau, K. 1965. *Plant Anatomy*. John Wiley & Sons, Inc., New York. 767 p.
- Fujino, D.W., M.S. Reid and G.E. Vandermolen. 1983. Identification of vascular blockages in rachides of cut Maidenhair (*Adiantum raddianum*) fronds. *Scientia Hort.* 21 : 381-388.
- Gilman, K.F. and P.L. Steponkus. 1972. Vascular blockage in cut roses. *J. Amer. Soc. Hort. Sci.* 97 : 662-667.
- Jensen, W.A. 1962. *Botanical Histochemistry*. W.H. Freeman and Company, London. 408 p.
- Ketsa, S. 1989. Vase-life characteristics of inflorescences of *Dendrobium* 'Pompadour'. *J. Hort. Sci.* 64 : 611-615.
- Ketsa, S. and A. Boonrote. 1990. Holding solutions for maximizing bud opening and vase-life of *Dendrobium* 'Youppadeewan' flowers. *J. Hort. Sci.* 65 : 41-47.
- Lineberger, R.D. and P.L. Steponkus. 1976. Identification and localization of vascular occlusions in cut roses. *J. Amer. Soc. Hort. Sci.* 101 : 246-250.
- Marousky, F.J. 1969. Vascular blockage, water absorption, stomatal opening and respiration of cut 'Better Times' roses treated with 8-hydroxyquinoline citrate and sucrose. *J. Amer. Soc. Hort. Sci.* 94 : 223-226.
- Marousky, F.J. 1971. Inhibition of vascular blockage and increased moisture retention in cut roses induced by pH, 8-hydroxyquinoline citrate and sucrose. *J. Amer. Soc. Hort. Sci.* 96 : 38-41.
- McCully, M.E. 1966. Histological studies on the genus *Ficus*. *Protoplasma* 62 : 287-305.
- Parups, E.V. and J.M. Molnar. 1972. Histochemical study of xylem blockage in cut roses. *J. Amer. Soc. Hort. Sci.* 97 : 532-534.
- Ranganna, S. 1978. *Manual of Analysis of Fruit and Vegetable Products*. Tata McGraw-Hill Publishing Co., Ltd., New Delhi. 340 p.
- Reeve, R.M. 1951. Histochemical tests for polyphenols in plant tissue. *Stain Technol.* 26 : 91-96.
- Rhodes, M.J.M. and L.S.C. Woollorton. 1973. Stimulation of phenolic and lignin biosynthesis in swede root tissue by ethylene. *Phytochem.* 12 : 707-718.
- Sakar, S.K. and C.T. Phan. 1974. Effect of ethylene on qualitative and quantitative composition of the phenol content of carrot roots. *Physiol. Plant.* 30 : 72-76.
- Sutton, J.C. and P.H. Williams. 1970. Relation of xylem plugging to black rot lesion development in cabbage. *Can. J. Bot.* 48 : 391-401.