

## LOW PRESSURE STORAGE OF HERBACEOUS CUTTINGS

A. Skytt Andersen  
Department of plant Physiology  
Thorvaldsensvej 40, DK-1871  
Frederiksberg C, Denmark

Hanne Grethe Kirk  
same address

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### Abstract

Cuttings of Hibiscus rosa-sinensis L. cv. Moesiana were stored at 15 or 760 mm Hg (2 or 101.3 kPa) for 3, 5, 8 or 11 weeks. No ethylene production could be detected regardless of treatment. No chlorophyll degradation, proline accumulation or respiratory increase were seen during storage. Stomata were open under low pressure and closed under normal pressure. The open stomata caused a great water loss from cuttings after removal from storage because they were unable to respond normally to low humidity. Rooting ability decreased as storage time increased, but satisfactory results were obtained up to a storage period of 5 weeks.

### 1. Introduction

The purpose of post-harvest storage of plant material is to keep the product alive and fresh for an extended period of time. The desired retardation can be accomplished by a combination of low temperature, high carbon dioxide/low oxygen concentration and removal of ethylene. For commodities susceptible to cold damage, the system of low pressure storage (LPS) is highly efficient (Jensen and Rasmussen, 1979).

Under low pressure the partial pressure of oxygen is very low, and those of ethylene and carbon dioxide approach zero (Veierskov and Kirk, 1985). Burg and Burg (1966) ascribed the efficiency of LPS to removal of ethylene combined with a lower activity of ethylene at low oxygen concentrations. Stenvers and Bruinsma (1975) and Bridgen and Staby (1981) found, however, that low oxygen concentration was the only cause of the lower metabolic activity.

Stomatal opening has been reported to control senescence (Thimann and Satler, 1979), and Veierskov and Kirk (1985) found low diffusion resistance values in LPS-treated oat (Avena sativa L.) leaf segments. They therefore ascribed the senescence retarding effect of low pressure to a combination of open stomata and low oxygen concentration.

In order to obtain a more coherent picture of the effects of LPS during and after storage, it was decided to use cuttings as test material, and measure ethylene production, chlorophyll degradation, proline accumulation, respiration rate, stomatal opening and rooting ability.

### 2. Materials and methods

Stock plants of Hibiscus rosa-sinensis L. cv. Moesiana were potted in 13 cm pots in sphagnum. They were placed in

ANDERSEN  
86

a growth room where day/night temperatures were 27/21°C, with a day length of 14 hours. Light intensity was 150  $\mu\text{mol m}^{-2}\text{s}^{-1}$  and relative humidity was about 60 per cent. The plants were watered three times a week with a 0.4 per cent solution of complete fertilizer (Pioner Hornum).

Uniform three-leaved terminal shoots were used as cuttings. Cuttings were rooted in water culture in an aerated nutrient solution (Veierskov et al., 1982a). Cuttings were placed in the same growth room and under the same conditions as the stock plants. The nutrient solution was changed every 2 wks and rooting percentage was noted once a week.

Storage experiments were performed in a cold room at 15°C. The LPS system consisted of 24 l stainless steel milk cans connected to an oil-seal vacuum pump (Leybold-Heraeus S 30 A) which maintained a pressure of 15 to 20 mm Hg (2.0 to 2.7 kPa). Air exchange was 6 l h<sup>-1</sup> as measured with a flowmeter before pressure reduction. After pressure reduction, the air passed through deionized water kept at 17°C to ensure adequate humidification. Air humidity was not measured because of technical difficulties, but since stored cuttings did not lose weight it was assumed to be more than 98 per cent. A normal pressure system (NPS) was used as control. All cuttings were positioned vertically in wire baskets during storage.

#### 2.1. Storage experiments; performance of cuttings during storage

Cuttings were placed in storage under low or normal pressure and 25 cuttings were removed after 3, 5, 8 and 11 weeks. Ten of these cuttings were rooted and the rest were used for determination of the parameters described later. Initial values were determined at the time of excision.

#### 2.2. Storage experiments; performance of cuttings after storage

Cuttings were treated with 0, 10<sup>-6</sup> or 10<sup>-5</sup> ABA in the nutrient solution. After 48 hrs the cuttings were moved to a fresh solution without ABA. The ABA treatment was given to: fresh cuttings; fresh cuttings before 2 wks in LPS; and cuttings after 2 wks in LPS, the latter with or without a 15 min dip in the solution before final placing. Stomatal resistance and rooting were recorded. In each treatment 4 cuttings were used, and the experiment was replicated 4 times.

Cuttings were placed in LPS for 2 wks. On removal from storage they received air with 5 per cent carbon dioxide during the period of pressure increase. Stomatal resistance and rooting were recorded. The experiment was replicated.

Cuttings were placed in LPS or NPS for 1 wk. Immediately after removal from LPS the cuttings were transferred to NPS and stomatal resistance was measured after 0, 2, 4 and 6 hrs.

#### 2.3. Parameters measured

Ethylene production: 1 to 2 g of leaf material was placed

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Chlorophyll  
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Prolin  
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curve.

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Diffusion  
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All treatments  
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### 3. Results

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Proline

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in darkness at room temperature in rubber-sealed 100 ml serum bottles for 24 h. Two 1 ml samples were analyzed in a gas chromatograph (Hewlett Packard 7620 A) with a Porapak T column at 60°C.

Chlorophyll: A leaf of 10 to 20 cm<sup>2</sup> was extracted with 10 ml 80 per cent ethanol, and the absorbance at 660 nm was measured spectrophotometrically.

Proline: A leaf of 10 to 20 cm<sup>2</sup> was homogenized in 4 ml 0.1 M phosphate buffer pH 6 with a homogenizer (Ultra-Turrax 18-10). The proteins and the low molecular weight components containing the amino acids were separated on a PD 10 column (Pharmacia, Sweden). Two hundred  $\mu$ l of the low molecular weight fraction was mixed with 1 ml of ninhydrine reagent and boiled for 15 min. After cooling, 2 ml of concentrated acetic acid was added and the mixture was boiled for 10 min, recooled and centrifuged at 18000 G for 5 min. The absorbance of the supernatant was measured at 507 nm, and proline concentration was determined from a standard curve.

Respiration: One excised leaf, 15 to 20 cm<sup>2</sup>, was placed in a 45 ml air-tight chamber filled with air-saturated tap water with 20  $\mu$ M NaHCO<sub>3</sub>, and oxygen consumption was measured with a Clark type oxygen electrode at 20°C.

Diffusion resistance: Transpiration and diffusion resistance were measured on an intact leaf with a steady-state porometer (LI-1600, Lambda Inst. Co.) immediately after removal from storage.

All the above measurements were performed on 5 cuttings per treatment at each removal.

Anatomical investigations of stomata were performed with a scanning electron microscope.

### 3. Results

Hibiscus cuttings did not produce any detectable amount of ethylene in 24 hours regardless of treatment, and no degradation of chlorophyll was seen during the storage period. Respiration rate had increased slightly in NPS cuttings after 8 wks, but was unchanged in LPS cuttings. Proline accumulated in small amounts.

Diffusion resistance of LPS cuttings was always very low, whereas it was high in NPS cuttings (Fig. 1). Microscopic investigations showed that the measured values reflected the degree of stomatal opening (Fig. 2). No cuticular damage could be seen.

After removal from LPS, transpiration rate decreased to a minimum value in about 2 hours, and this process could not be hastened by ABA (Fig. 3). A pulse of 5 per cent carbon dioxide in air given at pressure increase caused a higher transpiration rate. Placing LPS cuttings in NPS induced stomatal closure, but the response was much slower than for fresh cuttings and also slower than for LPS cuttings placed in the light in water culture (Fig. 3).

Rooting was adversely affected by storage (Fig. 4). Satisfactory results were obtained after 3 wks in LPS, but not after longer storage periods. Callus developed on the

cut ends when storage lasted for more than 8 wks. This temporarily enhanced the rooting rate (Fig. 4), but after 11 wks in storage there was no rooting and many of the NPS-treated cuttings had died.

#### 4. Discussion

The fact that no ethylene production could be detected in any of the *Hibiscus* cuttings makes it unlikely that the ethylene removing ability of LPS reported by Burg and Burg (1966) exerted any effect on the performance of the cuttings during storage. This is in accordance with Stenvers and Bruinsma (1975) and Bridgen and Staby (1981). They found that reduction in oxygen concentration, but not ethylene removal was the factor responsible for the slower metabolism in LPS.

In *Hibiscus* cuttings, no significant differences were observed in chlorophyll content or respiration rate during the storage period, indicating a very low senescence rate even under normal pressure. In oat leaves, senescence is completed in a few days in NPS, and a significant delay of the senescence syndrome can be obtained by LPS (Veierskov and Kirk, 1985). In an experiment with cuttings of *Euphorbia pinnatum* (L.) Engl., yellowing was observed after 6 wks in NPS but not after 8 wks in LPS. Unfortunately, it was not possible to measure a decline in chlorophyll content in this species because of the variegated leaves (data not shown).

The lack of proline accumulation showed that the cuttings were not water-stressed (Dashek and Erickson, 1981).

The open stomata are the most striking feature of the LPS-treated cuttings (Figs. 1, 2 and 3). This confirms the results obtained by Veierskov and Kirk (1985) who measured a low diffusion resistance in LPS-treated oat leaf segments floating on water. Burg and Kosson (1982) assumed in their calculations of heat transfer under LPS conditions that stomata were closed because the product was harvested and kept in darkness, but they did not measure stomatal parameters.

Stomatal opening in LPS is probably caused by the low partial pressure of carbon dioxide in the container (0.0008 mm Hg = 0.1 Pa, equal to the pressure of 1 ppm in normal air). Stomatal sensitivity to carbon dioxide is reported by various authors and seems to depend on the hormonal balance of the leaves (Blackman and Davies, 1984).

After removal from LPS, stomatal closure will eventually occur. When cuttings are placed in water culture in the light, closure proceeds relatively rapidly (1 to 2 h, Fig. 3). This happens because of excessive water loss - up to 50 per cent of the initial fresh weight during the first hour - and is probably a passive process. The absence of a response to ABA treatment supports this view. Apparently stomata in LPS-treated cuttings do not respond to a low humidity or a high carbon dioxide concentration, and need some hours to regain normal function.

The high water loss from LPS cuttings will affect rooting

adversely and thereby Photosynthesis stomata re better root lower light stomata will should be

It can prolonged on two factors the low partial pressure of the open stomata unsatisfactory

**Acknowledgments**  
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Veterinary  
for inspiration  
the SEM.

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adversely (Fig. 4). Low photosynthesis limits carbohydrates and thereby rooting capacity (Veierskov et al., 1982b). Photosynthesis is limited in LPS-treated cuttings because stomata remain closed until well after root formation. Under better rooting conditions - higher relative humidity and lower light intensity - the adverse effects of the open stomata will be much less pronounced and better rooting should be obtained.

It can be concluded that the increased survival after prolonged storage in LPS relative to NPS probably depended on two factors: A decreased metabolic rate in LPS caused by the low partial pressure of oxygen, and an additional effect of the open stomata. The deteriorated control functions of the stomata in LPS-treated cuttings caused water loss and unsatisfactory rooting results.

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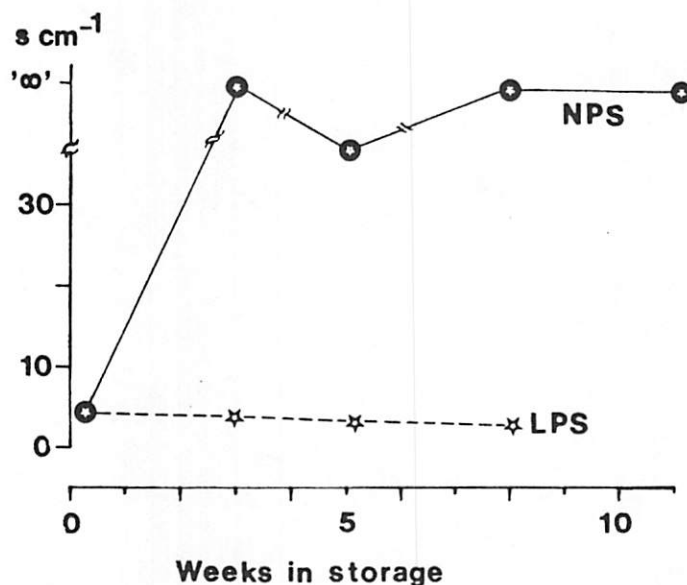
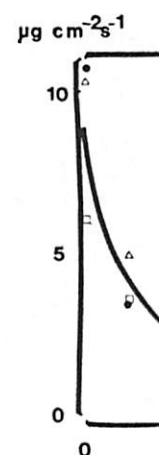


Fig. 1. Stomatal opening measured as diffusion resistance. Measurements were performed immediately after removal from storage. Each point represents mean of 2 replications with 4 measurements each. Ten "0 wk storage" value was obtained from intact plants before excision of cuttings.  
 ● NPS = normal pressure storage.  
 ☆ LPS = low pressure storage.



Fig. 2. S stored in Distance b



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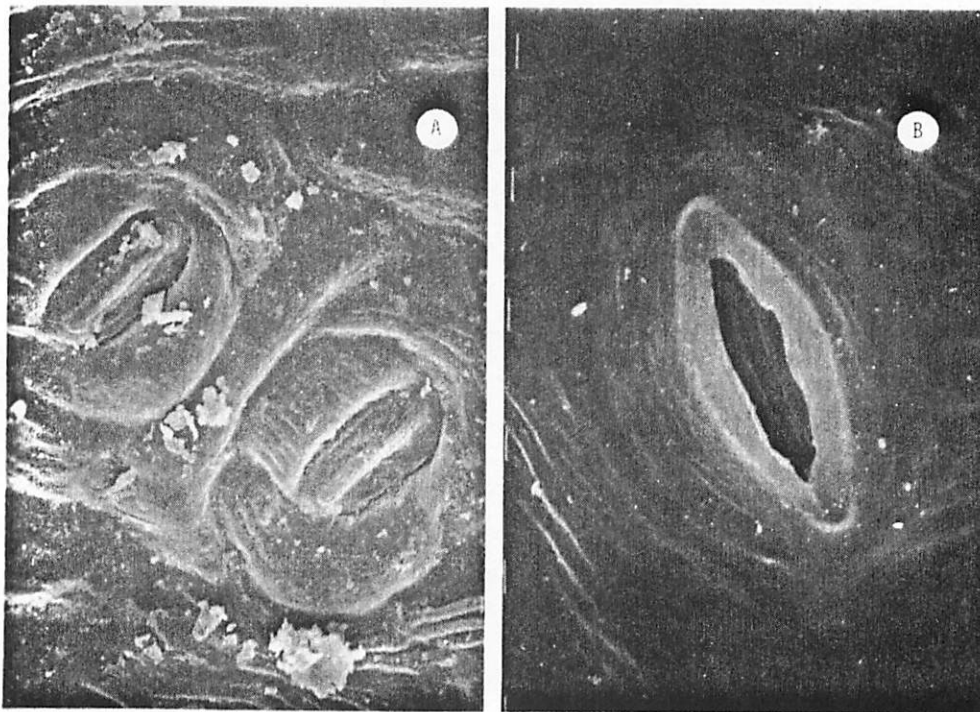


Fig. 2. Scanning electron micrographs of Hibiscus cuttings stored in NPS (A) or LPS (B) for one week. Distance between marks: 100 μm.

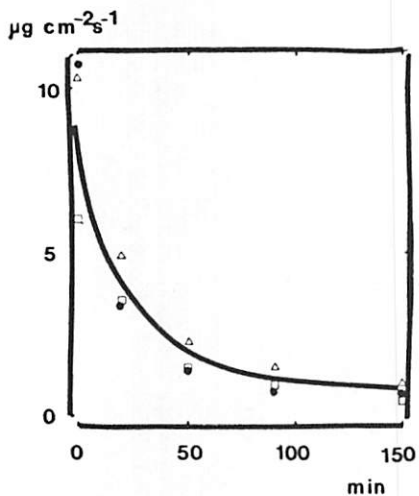


Fig. 3. Stomatal opening in control and ABA-treated cuttings measured as transpiration rate during the first 2.5 h after removal from LPS. Bases were freshly cut under water before placing the cuttings in nutrient solutions. Each point represents mean of 4 measurements.

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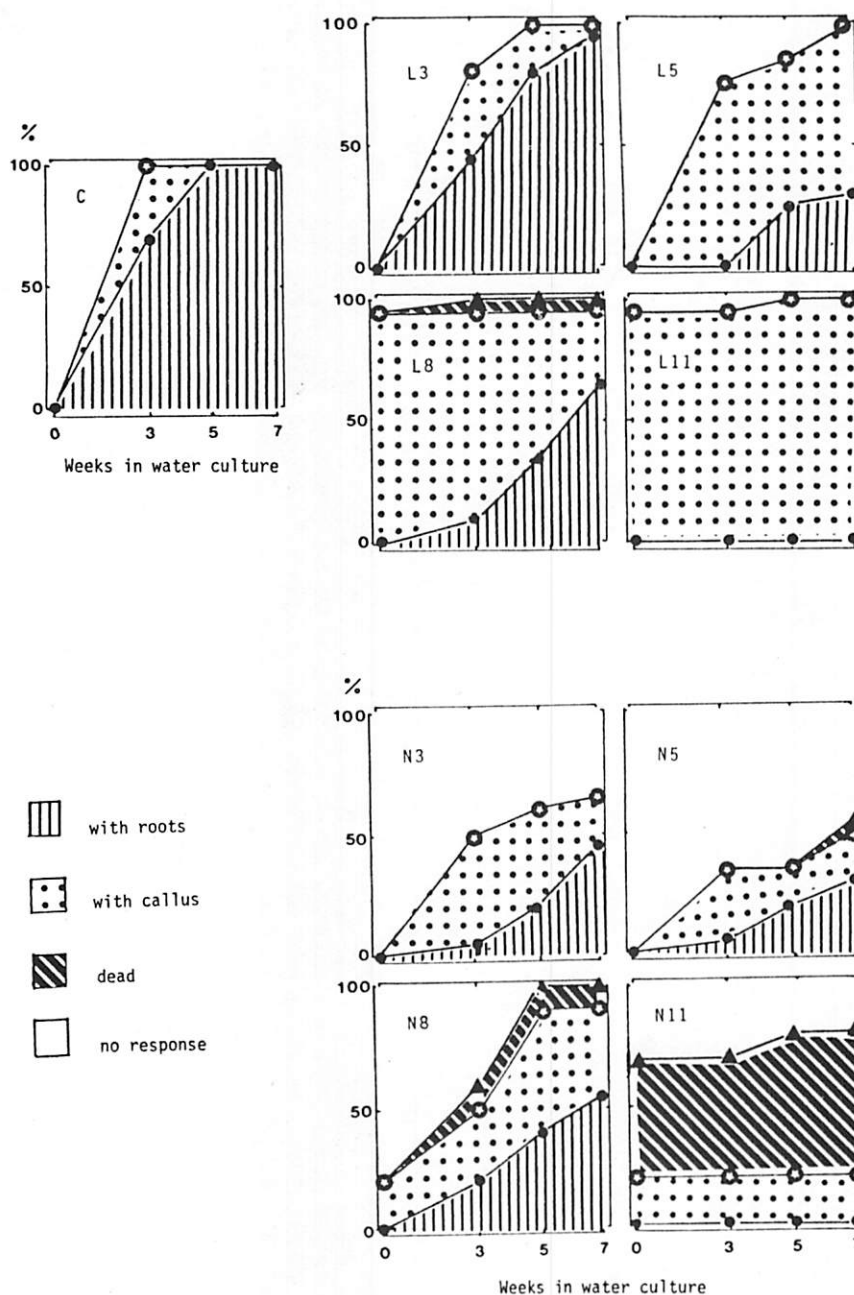


Fig. 4. Rooting results of stored Hibiscus cuttings. C: Freshly cut control. L3, L5, L8, L11: Cuttings stored in low pressure for 3, 5, 8 or 11 wks. N3, N5, N8, N11: Cuttings stored in normal pressure for 3, 5, 8 or 11 wks. Figures are based on two replications of 10 cuttings each.