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Long term lily scale bulblet storage: effects of temperature and storage in polyethylene bags

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Summary

Collections of lily genotypes are usually maintained by yearly planting, harvesting and storage of the bulbs. To facilitate this maintenance, a storage method has been developed for a collection of lily genotypes, including Asiatic hybrids, Oriental hybrids, *Lilium longiflorum* and *L. henryi*. Scale bulblets were stored either dry, sealed air-tight in polyethylene bags, or in moist vermiculite in open polyethylene bags for a period of 2 yr. The decrease in mass, sprouting proportion and ion leakage or sprouting proportion alone were determined for treatments carried out at −2°C, 0°C and 17°C. Sealing scale bulblets in polyethylene bags at −2°C resulted in the smallest decrease in mass, the least ion leakage and the highest sprouting proportion after 2 yr of storage.

Key words: Conductivity, ion leakage, Lilium, lily, modified atmosphere, temperature, storage, germplasm collection

Introduction

To support breeding and research, the Centre for Plant Breeding and Reproduction Research (CPRO-DLO) maintains a lily collection of more than 1000 genotypes. Collections of bulbs are usually propagated by yearly planting, harvesting and storage, which involves a considerable investment in labour and space and also introduces the risk of losses through disease (Withers, 1991). Methods for long term storage of vegetative material would make maintenance more efficient. Techniques for long-term storage of clonal material should create conditions for slow growth or use cryopreservation (Grout, 1991; Towill, 1988; Withers, 1991). The most easy way to minimise growth is storage at low temperature. However, this is limited by the sensitivity to frost damage of lily bulbs. Lily bulbs can be stored at −2°C to obtain flowering out of season (Boontjes, 1983; Beattie & White, 1993; Bonnier, Keurentjes & Van Tuyl, 1994).

As well as low temperatures, the rate of metabolism of the material can also be decreased by enriching the atmosphere with CO_2 and decreasing the partial pressure of O_2 under controlled conditions, called controlled atmosphere storage (CAS). Storage under low partial pressure of oxygen may also increase frost tolerance, since an inverse relationship was found between frost survival and atmospheric pressure for five plant and two vertebrate species (Halloy & González, 1993). Modified atmosphere (MA) packaging in polyethylene film bags has been used to extend the storage life of many crops including pre-cooled tulip bulbs (Prince, Herner & De Hertogh, 1981; Prince, Herner & Lee, 1986). An atmospheric equilibrium develops in the bags, which is enriched in CO_2 and diminished in O_2. The equilibrium is dependent on the respiratory rate of the material and the gas-permeability of the bags (Prince *et al.*, 1986). If the temperature decreases, both the respiration of the tissue and the gas-permeability of the bags decrease (Prince *et al.*, 1986). Sealing in gas-permeable bags has several advantages over storage in rooms with a controlled gas composition. Firstly, material is divided into small batches, reducing the risk of quickly spreading diseases. Secondly, gas-permeable bags are much cheaper than CAS. However, one disadvantage is that the atmosphere is less accurately controlled. MA packages have also been shown to create a suitable environment for infection by fungi and bacteria (Prince, Herner & Stephens, 1987). Therefore, material needs to be disinfected before sealing.

Lily bulbs can be propagated by regeneration of scale bulblets on detached lily scales (Van Tuyl, 1983; Matsuo & Van Tuyl, 1984). Regeneration of lily scale bulblets on scales appeared to be possible after 2 to 3 yr storage of bulbs at −2°C in moist peat (Bonnier & Van Tuyl, 1996). Combining storage of bulbs and storage of scale bulblets would further reduce the frequency bulbs need to be planted in the field. Ion leakage has been shown to be a useful criterion to measure viability of lily scales (Bonnier *et al.*, 1994). In this study, storage of lily scale bulblets in open and closed polyethylene bags was investigated. Viability was measured by the sprouting proportion and by ion leakage. Ion leakage was tested as a criterion to measure viability of scale bulblets.

Materials and Methods

Plant material

Lily genotypes representing Asiatic hybrids ('Avignon', 'Connecticut King', 'Enchantment', 'Esther', and 'Mont Blanc'), Oriental hybrids ('Casablanca', and 'Star Gazer'), *L. longiflorum* ('Gelria', and 'Snow Queen'), and *L. henryi* (accession no. 72122) were used. For each genotype, scales from 10 bulbs (circumference 12–16 cm) were detached for propagation. Scales were dipped in fungicide for 10 minutes in 1 g $litre^{-1}$ captan and 0.2 g $litre^{-1}$ prochloraz (Sportak), surface-dried for 1 h, planted in moist vermiculite and placed for 8 wk at 25°C and 4 wk at 17°C, to regenerate scale bulblets (Van Tuyl, 1983). Scale bulblets were subsequently removed from the scales and disinfected again.

Scale bulblets were randomised for each genotype and put into groups of five. For each group the fresh weight was measured and the scale bulblets were then either sealed dry in low-density polyethylene bags, or placed in moist vermiculite in open polyethylene bags, and stored at −2°C, 0°C and 17°C (± 0.1°C). The low-density polyethylene bags (Stibbe bv, Zwolle, The Netherlands) were 11 cm × 12 cm, and had a thickness of 0.05 mm. During storage, the total air volume in the sealed bags decreased until a vacuum was established within 4 months to 1 yr, dependent on the size of the bulblets and the temperature.

For scale bulblets sealed in polyethylene bags, decrease in mass, sprouting proportion, and ion leakage were recorded every 4 months, during the 2 yr storage. After storage for 1 yr, it was noticed that the scale bulblets were drying. The sealed bags were then placed under conditions of 100% relative humidity in a box with moist vermiculite, to prevent further drying out. For scale bulblets stored in open bags in moist vermiculite, only sprouting proportion was observed. Ion leakage and decrease in mass could not be determined, because of the occurrence of rotten scale bulblets in the vermiculite. Scale bulblets of Oriental hybrids, *L. longiflorum* and *L. henryi* were scored only 6 ('Gelria'), 5 ('Casablanca'), 5 ('Snow Queen'), 4 ('Star Gazer'), and 3 (*L. henryi*) times, because of a limited availability of scale bulblets of these genotypes.

Decrease in mass

The sealed bags containing the scale bulblets were weighed before and after storage. The decrease in mass was calculated with respect to the initial fresh mass of the scale bulblets.

Ion leakage

The method of measuring ion leakage of lily scale bulblets by conductivity was modified from that used to measure ion leakage of lily scales (Bonnier, Keller & Van Tuyl, 1992; Bonnier *et al.*, 1994). The roots of the scale bulblets were removed, and after one night at 20°C, groups of five scale bulblets were placed for 1.5 h in 100 ml of distilled water at 20°C. Subsequently, the electrical conductivity of the samples was measured with a digital conductivity meter (Philips PW9526 with electrode PW9514/60) and each sample corrected by subtracting the conductivity of a distilled water control and dividing by the original fresh weight of the scale bulblets.

Sprouting proportion

After measuring conductivity, the same scale bulblets were planted in soil. Those stored at −2°C and 0°C were placed directly in a greenhouse. The scale bulblets stored at 17°C were first placed at 5°C for 12 wk (to break dormancy) and then cultured in the greenhouse. After 6 wk, the sprouting proportion of the scale bulblets was recorded. After a storage duration of 2 yr, the sprouting scale bulblets were kept in the greenhouse for 0.25 yr to check their ability to form new bulblets. All sprouting scale bulblets formed new bulblets.

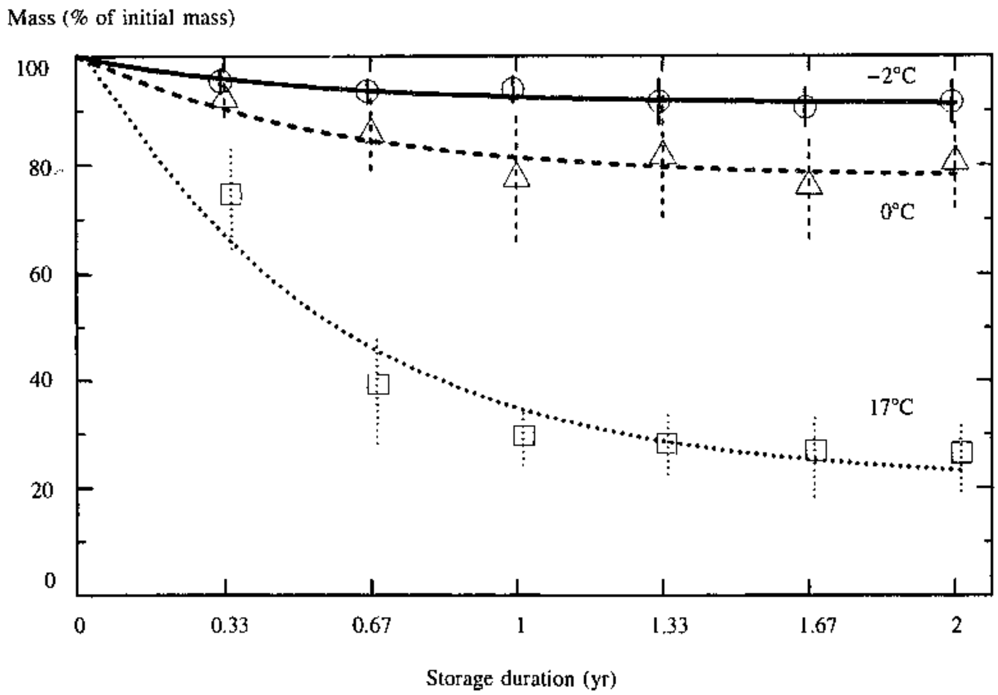

Fig. 1. Mean and fitted decrease in fresh mass for scale bulblets of 10 genotypes (2 × 5 scale bulblets of each) during storage in sealed polyethylene bags at −2°C (○), 0°C (△) and 17°C (□). Decrease in fresh mass was fitted exponentially per temperature ($y = A + B * R^x$) and then expressed on initial mass base. The vertical lines represent standard errors per observation.

Table 1. *Means of initial mass, decrease in mass (percentage of initial mass and angular transformed values), conductivity (original values and log-transformed values), and sprouting proportions (predictions from generalised linear model for proportions + standard errors) of two groups of five lily scale bulblets sealed in polyethylene bags, after storage at −2°C for 2 yr*

Genotype	Mean initial mass (g)	Proportion of initial mass (%)	Proportion of initial mass (angle)	Conductivity (μS cm^{-1} g^{-1}) (original)	Conductivity (ln)	Sprouting proportion % (predicted)	Sprouting proportion % (SE)
Avignon	0.80	92	73.7	0.83	−0.19	94.9	3.6
Connecticut King	0.79	89	70.3	1.19	0.17	100.0	0.0
Enchantment	0.46	84	66.7	0.76	−0.28	97.0	2.2
Esther	1.28	96	78.4	0.51	−0.67	100.0	0.0
Mont Blanc	0.60	90	71.4	1.19	0.17	100.0	0.0
Casa Blanca	1.25	94	76.3	0.86	−0.15	62.1	8.2
Star Gazer	2.20	97	79.6	0.52	−0.66	92.9	4.2
Gelria	0.80	90	72.0	1.27	0.24	93.5	3.7
Snow Queen	0.74	92	73.5	0.28	−1.28	100.0	0.0
L. henryi	1.22	92	73.4	0.71	−0.34	74.6	7.5
SED			2.4		0.70		

Statistical methods

Decrease in fresh mass was fitted exponentially on storage duration ($y = A + B * R^X$) per temperature and then expressed on initial mass base (Fig. 1). Significance levels between cultivars after 2 yr storage at −2°C (Table 1) and between storage durations per temperature (results in text) were calculated using the protected LSD-test after angular transformation of proportions (Snedecor & Cochran, 1980).

Conductivity values were log-transformed to obtain a normal distribution of standard errors. Natural logarithms of conductivity values were fitted linearly on storage duration for the temperatures −2°C and 0°C and by a Gompertz curve ($y = A + C * EXP(EXP(-B * (X-M)))$) (Payne, Ainsley, Bicknell & Franklin, 1993) for the temperature 17°C (Fig. 2). Significances between cultivars after 2 yr of sealed storage at −2°C (Table 1) were calculated for the log-transformed values using the protected LSD-test (Snedecor & Cochran, 1980).

The sprouting proportions of the scale bulblets (Fig. 3) were regressed per treatment on storage duration using a generalised linear model for proportions (Payne *et al.*, 1993). For the treatment with the highest sprouting proportion after 2 yr: decrease in mass, conductivity values and predicted sprouting proportions were summarised per genotype (Table 1).

Results

Decrease in mass

During the first year of dry storage, the sealed scale bulblets lost mass. The decrease was relatively small at −2°C, but at 17°C, the decrease in mass was about 70%. After transfer of the sealed bags to conditions of 100% relative humidity after 1 yr storage, no further significant decrease in mass was found (Fig. 1). Differences between genotypes were mainly caused by differences in the original mass of the scale bulblets. Genotypes with light scale bulblets lost mass quicker than genotypes with heavier scale bulblets. The correlation coefficient between initial mass and mean loss of mass was 0.93 for scale bulblets stored for 2 yr at 0°C.

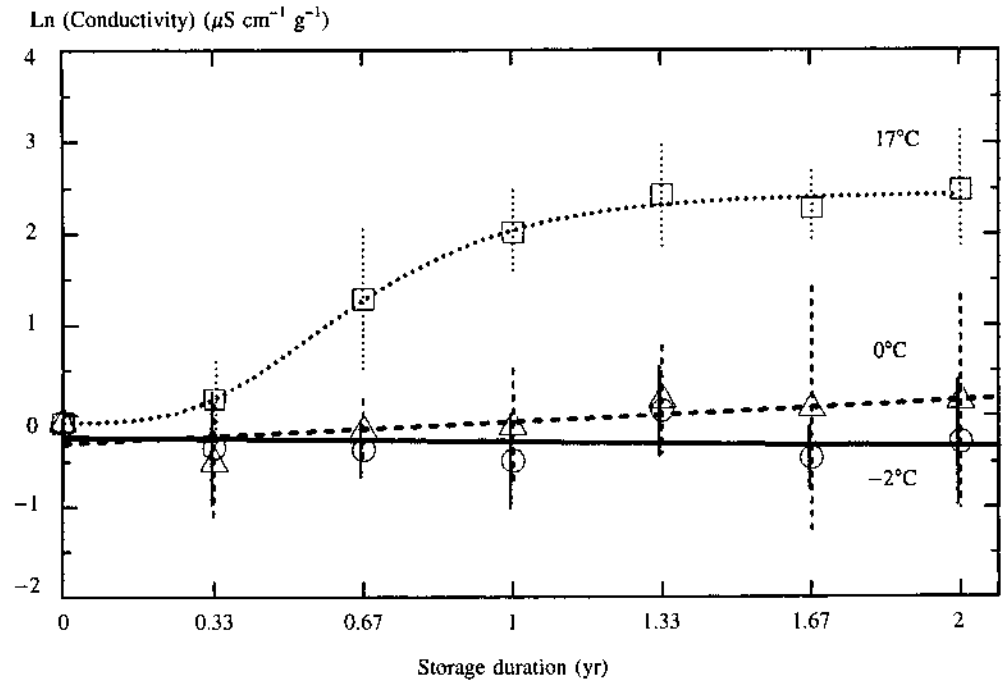

Fig. 2. Mean and fitted electrical conductivity values for scale bulblets of 10 genotypes (2 × 5 scale bulblets of each) during storage in sealed polyethylene bags at −2°C (○), 0°C (△) and 17°C (□). Natural logorithms of condictivity values were fitted linearly on storage duration for the temperatures 2°C and 0°C and by a gompertz curve (y = A + c * EXP(EXP(−B * (X−M)))) for the temperature 17°C. The vertical lines represent standard errors per observation at −2°C, 0°C and 17°C respectively.

Ion leakage

Electrical conductivity values of sealed scale bulblets stored at −2°C were low and no increase in ion leakage was observed during storage (Fig. 2). At the end of the storage period electrical conductivity was still below 1 $\mu S\ cm^{-1}\ g^{-1}$ for all genotypes (Table 1). The mean electrical conductivity of sealed scale bulblets stored at 0°C was higher than at −2°C after 2 yr of storage, but the increase in conductivity at 0°C was small and not significant (Fig. 2). During storage at 17°C, electrical conductivity increased relatively quickly. The increase was significant after storage for 0.67 yr and longer.

Sprouting proportion

After storage for 2 yr, the highest sprouting proportion was found for sealed scale bulblets stored at −2°C (Fig. 3). The two genotypes with the lowest predicted sprouting proportion were *L. henryi* and 'Casablanca' (Table 1). Predictions for their sprouting proportion and standard error indicate that storage is possible for longer than 2 yr. Sealed storage at 0°C was less effective than that at −2°C (Fig. 3). The predicted sprouting proportion of *L. henryi* was not significantly different from zero after 2 yr storage at 0°C (results not shown). A relatively quick decrease in sprouting proportion was observed for storage in sealed polyethylene bags at 17°C (Fig. 3). This was probably caused by drying out of the scale bulblets (Fig. 1).

A quicker decline in the sprouting proportion was found for unsealed rather than sealed storage at −2 °C (Fig. 3). During unsealed storage at −2°C, soft brown scale bulblets were often observed for genotypes of Oriental hybrids, *L. longiflorum* and *L. henryi*. Scale bulblets

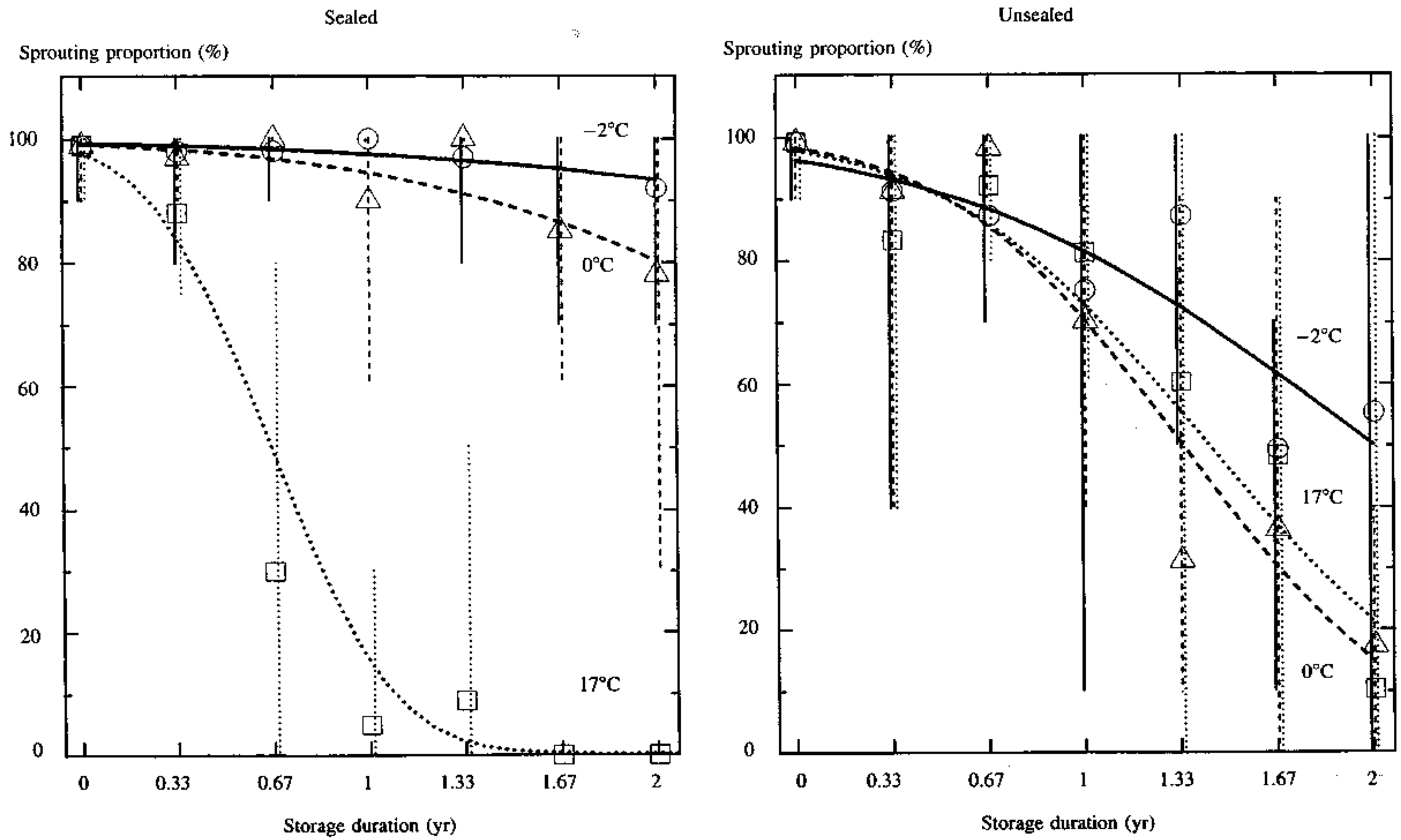

Fig. 3. Mean and fitted sprouting proportions for scale bulblets of 10 genotypes (2 × 5 scale bulblets of each) during dry storage in (*a*) sealed polyethylene bags and storage in moist vermiculite in (*b*) open polyethylene bags at −2°C (○), 0°C (△) and 17°C (□). Sprouting proportions were fitted on storage duration using a generalised linear model for proportions. The vertical lines represent the differences between the genotypes with maximum and minimum sprouting proportion per observation at −2°C, 0°C and 17°C respectively.

stored unsealed at 0°C and 17°C often started sprouting during storage. The sprouting proportion declined in an almost equal rate during storage for both treatments (Fig. 3). Unsealed storage at 0°C and 17°C was less effective than that at −2°C (Fig. 3).

Discussion

Sealing scale bulblets in polyethylene bags at −2°C resulted in the least decrease in mass, the highest sprouting proportion and the least ion leakage after 2 yr of storage. Storage for longer than 2 yr appears possible. Storage of scale bulblets sealed in polyethylene bags at 17°C resulted in relatively quick drying out of the scale bulblets, leading to a quick rise in conductivity values and a fast decline in sprouting proportion. Sealed storage at 17°C would probably have scored better, if the scale bulblets had been protected against drying out from the beginning by placing the polyethylene bags under conditions of 100% relative humidity.

Prince *et al.* (1986) found gaseous equilibrium levels of both O_2 and CO_2 between 3% and 5% for tulip bulbs stored in a low-density polyethylene package. The equilibrium levels were hardly affected by temperature. The level for N_2 was not stated, but it must have been between 90% and 94%. Under such circumstances, N_2 would diffuse out of the package. We did not measure gaseous levels in the packages, but we used a similar low-density polyethylene film for lily scale bulblets at −2°C, 0°C, and 17°C. Therefore, the establishment of a vacuum in the sealed polyethylene bags was probably caused by diffusion of N_2 from the package during storage.

During unsealed storage at −2°C, decrease in sprouting proportion of genotypes of the Oriental hybrids, *L. longiflorum* and for *L. henryi* was probably due to injury caused by freezing, since soft brown scale bulblets were observed and genotypes of Oriental hybrids and *L. longiflorum* are known to be less freeze tolerant than Asiatic hybrids (Boontjes, 1983). The scale bulblets sealed in polyethylene bags were more tolerant to freezing, which can be explained in two ways. Firstly, a reduced oxygen pressure in the bags might have increased freezing tolerance, supporting the results of Halloy & González (1993). Secondly, the scale bulblets sealed in polyethylene bags were partly dried out. It is known that a lower water content increases freezing tolerance (Lång *et al.*, 1994; Maier, Lang & Fry, 1994; Pearson & Davison, 1994). The ability to store partially dried material is therefore an advantage of storing scale bulblets in sealed polyethylene bags.

Scale bulblets stored unsealed at 0°C and 17°C, tended to sprout during storage, which probably exhausted the carbohydrate resources and reduced their viability. Scale bulblets stored dry, and sealed in polyethylene bags, did not sprout during storage at 0°C and 17°C, probably because of lack of water. The prevention of sprouting in the package is another advantage of storing in this way.

When tissue dies, cell membranes deteriorate and the tissue starts to leak nutrients. Therefore, a negative correlation was expected between electrical conductivity values and sprouting proportions. During sealed storage at 17°C, an increase in electrical conductivity values was accompanied by a decrease in sprouting. However, during sealed storage at −2°C and 0°C a decline of the sprouting proportion was often recorded without an increase in electrical conductivity. This might be explained by a different viability of the sprouts and the scales of the scale bulblets. The sprouting proportion is determined by the health status of the meristems, whereas ion leakage is determined by the health status of the scales. If the meristem in a scale bulblet is damaged and the surrounding scales of the scale bulblet are still healthy, the scale bulblet will not sprout, but will leak few ions. Ion leakage was therefore concluded to be a less useful criterion to measure the viability of lily scale bulblets than that of lily scales (Bonnier *et al.*, 1994).

The maintenance of a lily collection can further be improved, if storage of scale bulblets for 2 yr is combined with storage of larger (circumference between 12 and 16 cm) bulbs in moist peat. Bulbs of Asiatic hybrids can be stored for 3 yr and bulbs of Oriental hybrids and genotypes of *L. longiflorum* for 2 yr in moist peat (Bonnier & Van Tuyl, 1996). Combining these storage methods gives the following maintenance scheme: 1) Storage of bulbs in moist peat for 2 yr at −2°C, 2) Generation of scale bulblets, 3) Storage of scale bulblets sealed in polyethylene bags for 2 yr at −2°C, and 4) Production of larger bulbs out of the scale bulblets in 2 yr. Bulbs will only need to be planted in the field twice in 6 yr, using this maintenance scheme.

The storage methods used in the present study were tested using 10 lily genotypes. Since the genetic backgrounds of the genotypes included a wide genetic variation, it is likely, that storage at −2°C in polyethylene bags is also suitable for other genotypes of lily.

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