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Advanced *in Vivo* Propagation Techniques for Specialty Bulbs

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ABSTRACT

Bulbs can be propagated by natural propagation techniques like by seeds and off setts, as well as by techniques like scoring, chipping, scaling and leaf cuttings. These techniques are mostly used for *in vivo* propagation, less for *in vitro* propagation. This article concerns the first case. Specialty bulbs can successfully be propagated by scoring and chipping, either starting at the beginning of the dormant period or at the end of this period. Of various species of the genera *Eucomis*, *Lachenalia* and *Ornithogalum*, it is known that they can successfully be propagated by leaf cuttings. In the presented experiments the techniques scoring, chipping and leaf cuttings were applied on *Eucomis autumnalis* and *Hymenocallis festalis*. Scoring and chipping resulted in new newly formed bulblets with *E. autumnalis*. Propagation by leaf cuttings resulted only with *E. autumnalis* to the formation of adventitious bulblets. Crucial is the timing of propagation, which will count for all genera and species. In previous research with *Lachenalia* species it has been proved that the regeneration potential is highest when leaf cuttings are taken at visible bud stage.. A preliminary conclusion is that with *Eucomis* the regeneration potential is the highest before flowering. It is not possible to draw conclusions for *Hymenocallis*, because this genus belongs to another family than *Eucomis* and *Lachenalia* and the experiment with leaf cuttings failed with this genus. Due to the low number of used bulbs a statistical evaluation was not possible.

Keywords: reproduction, scoring, chipping, leaf cutting, flower bulb, *Eucomis*, *Hymenocallis*

INTRODUCTION

General remarks

Already for decades the most used *in vivo* propagation method for *Lilium* is scaling, as scoring is an important technique used with *Hyacinthus*. On a smaller scale *Narcissus* and *Hippeastrum* are propagated by chipping. Due to the lack of information, the *in vivo* techniques were almost not used for other bulbous crops by commercial bulb growers. Especially in the second half of the 90's of the 20th century and the first years of the 21st century applied research has been done on the *in vivo* propagation of specialty bulbs by chipping, scoring and partitioning. This research has led to protocols that are used on a large scale in practice by the bulb industry nowadays. The propagation techniques are used commercially with *Galanthus* and other members of the *Amaryllidaceae* family and *Eucomis* and *Ornithogalum* and other members of the *Hyacinthaceae* family. The *in vivo* techniques can be applied on a large scale, with low costs, little investments and knowledge, with as a result a relatively great number of bulblets per bulb. There are almost no questions raised which ask for new research on this topic. Let me give an example of this: One of the recent developments in the propagation of *Zantedeschia* corms is the dividing of the corms into several parts, comparable to partitioning with bulbs. The techniques are developed and optimised by the growers themselves, using earlier developed protocols, without the additional support of research.

Where *in vivo* propagation means little investments in equipment and no special knowledge and education, *in vitro* propagation asks for special equipment and knowledge. This means that *in vitro* propagation is more expensive than *in vivo*. *In vitro* techniques are with flower bulbs used for obtaining healthy, disease free material or for propagating material of new varieties on a large scale, as with *Canna*, *Hippeastrum*, *Lilium*, *Dahlia* and *Zantedeschia*.

Scoring and chipping

The aim of this study was to determine the possibilities of propagation of specialty bulbs by scoring, portioning, with as time of propagation the end of the dormant period. Also propagation by leaf cuttings was studied. The possibilities are determined as the propagation rate and the size of the formed bulbs.

Bulbous plants can be propagated by various "natural" propagation techniques like by seed and vegetative reproduction (off sets or division). *Lilium* bulbs can be propagated by taking off the scales to produce bulblets on the scales, this is known for a long period and is practiced since the years before the Second World War (Botke and Van der Slikke 1942). In general "artificial" propagation methods have been developed and improved in order to achieve a greater reproduction rate than can be possible by the natural methods or to obtain plants which are genetic identical to the mother plant. The most well-known propagation tech-

Table 1 Results of the propagation of *Eucomis autumnalis* and *Hymenocallis festalis* by scoring, chipping and leaf cuttings after one growing season: propagation rate and average circumference of bulbs formed.

Genus and species	Propagation technique	Propagation rate	Average circumference of bulbs formed (cm)
Eucomis autumnalis	Scoring		
	Chipping, 4 parts	0	-
	Chipping, 8 parts	0	-
	Leaf cutting, bottom	3	< 2
	Leaf cutting, top	0	-
Hymenocallis festalis	Scoring	9	6
	Chipping, 4 parts	8	7
	Chipping, 8 parts	13	4
	Leaf cutting, bottom	0	-
	Leaf cutting, top	0	-

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Fig. 1 Bulblets of *Hyacinthus* 'Kings of the Blues', propagated by scoring.
Fig. 2 Parts of *Galtonia candicans* in vermiculite with the newly formed bulblets.
Fig. 3 Bulbs of *Eucomis autumnalis* at start of experiment, using scoring.
Fig. 4 Bulb of *Hymenocallis festalis* at start of experiment, using chipping.
Fig. 5 *Hymenocallis festalis* propagated by scoring, at the end of the experiment on 31 October 2010.
Fig. 6 *Hymenocallis festalis* propagated by chipping, 8 parts at the end of the experiment. Note the numerous small bulblets on the bottom part of the bigger formed bulblets on 31 October 2010.
Fig. 7 *Eucomis autumnalis* propagated by leaf cuttings, bottom part, at the end of the experiment on 31 October 2010.
Fig. 8 Leaf cuttings of *Haemanthus albiflos* with newly formed bulblets, four months after propagation. From left to the right: bottom part leaf, middle section and top part.

niques are chipping, scaling and scoring. A number of major bulbous crops such as *Lilium* (scaling), *Hyacinthus* (scoring) (**Fig. 1**) and *Narcissus* (chipping) are mass reproduced in this way. The techniques are for commercial use especially useful for those plants that do not reproduce easily or at all by natural reproduction methods. Little research has been done on the possibilities with the artificial methods for propagation of tender, specialty bulbous plants. Most recent results of experiments with representatives from this group are described by Van Leeuwen and Van der Weijden (1997) and Knippels (2000). Knippels (2000) described a chipping protocol for the propagation of specialty bulbs, which can be used for deciduous or evergreen, summer growing and winter growing bulbs. Bulbs are chipped at the beginning of dormancy and stored, 12 weeks storage in vermiculite (**Fig. 2**), followed by planting in soil at 20-25°C, later between 15 and 20°C.

Van Leeuwen and Van der Weijden (1997) showed that *Eucomis bicolor* and *Eucomis comosa* can be propagated by chipping, when performed in December and in April. For *Eucomis bicolor* there was no difference in propagation rate and yield between chipping in December and storage till planting in April and chipping in April and direct planting. The highest yield (a.o. number of bulbs) for *Eucomis bicolor* was obtained with chipping in December. The tests by Knippels (2000) with *Eucomis bicolor* with propagation at the beginning of the dormant period and storage at 20°C for 13 weeks, gave comparable results as reported by Van Leeuwen and Van der Weijden (1997). The results of Knippels (2000) and Van Leeuwen and Van der Weijden (1997) are presented in **Table 1**.

Leaf cuttings

Various research has been done on the propagation of specialty bulbs by leaf cuttings: Krause (1980); Duncan (1988); Suh and Lee (1997); Ndou (2000); Knippels (2000, 2003); Blomerus and Schreuder (2002) and Niederwieser and Ndou (2002). Duncan (1988) described that *Lachenalia* species can be propagated by leaf cuttings. One or more leaves are taken from healthy, virus-free plants and, depending on the size of the leaf, cut into several parts. The leaves or parts of leaves are planted in a rooting medium. Duncan mentions a medium of equal portions river sand and vermiculite. The bottom centimetre of the leaf parts are put in the medium. The whole is placed in a shady spot and the medium is kept moist. After about a month, the first bulblets and roots will have formed. Suh and Lee (1997) described the results of leaf cutting with *Lachenalia aloides* 'Pearsonii'. The best results were obtained by taking the bottom part of a leaf, by putting that in a potting medium of equal parts peat and perlite and to keep the cuttings at a temperature of 20°C. Most recent research with *Lachenalia* is done by Ndou (2000) and Niederwieser and Ndou (2002). One of the objectives in the research was the timing of the propagation in relation to the regeneration potential. One of the conclusions was that the age of the leaf tissue affected the number of formed buds. Young tissue was the best source of tissue. After flowering the regeneration potential decreased. Blomerus and Schreuder (2002) have described the propagation of *Ornithogalum thyrsoides* by leaf cutting. Objectives were a.o. the influence of bottom temperature on the number of formed bulbs and the size of these bulbs and the used medium. The largest number of bulbs were formed in a peat or sand medium and a bottom temperature of 14°C. The biggest bulbs were produced at a bottom temperature of 24.5°C.

In this paper an experiment is described with a representative of the family *Hyacinthaceae*, *Eucomis autumnalis*, and one of the family *Amaryllidaceae*, *Hymenocallis festalis*, which are subjected to three propagation techniques performed *in vivo*: scoring, chipping and leaf cuttings. These techniques can also be done *in vitro*, but this was not within the scope of the experiment. After one growing season, after harvesting the bulbs, the propagation rate for each used technique is determined. The reason to perform this experiment was to answer the above mentioned questions and to verify the results of the experiments of Van Leeuwen en Van der Weijden (1997).

MATERIALS AND METHODS

Scoring and chipping

In the experiments bulbs of *Eucomis autumnalis* (circumference 13 cm) and *Hymenocallis festalis* (circumference 18 cm) were used. The scoring and chipping started on 7 March 2010 with still dormant bulbs. This date is about 4 to 6 weeks prior to normal planting, so at the end of the dormancy period.

For the propagation by scoring and chipping two bulbs of *E. autumnalis* and one of *H. festalis* were used. The applied propagation techniques were:

Table 2 Results propagation experiments Knippels (2000) [1)] and Van Leeuwen and Van der Weijden (1997) [2)]: propagation rate and average circumference of bulbs formed.

	Propagation technique	Propagation rate	Average circumference of bulbs formed (cm)
Hyacinthaceae			
Albuca longipes [1)]	Chipping	6	2
Drimiopsis maculata [1)]	Chipping	9	4
Eucomis bicolor [1)]	Chipping	14	23
Eucomis bicolor, propagation December and April [2)]	Chipping	11	
Eucomis comosa, propagation December [2)]	Chipping	11	
Galtionia candicans [1)]	Chipping	12	8
Lachenalia mathewsii [1)]	Chipping	3	< 2
Lachenalia unicolor [1)]	Chipping	1	< 2
Amaryllidaceae			
Crinum x powellii [1)]	Scoring	3	10
Haemanthus albiflos [1)]	Chipping	6	3
Haemanthus coccineus [1)]	Chipping	2	< 2
Haemanthus albiflos [1)]	Leaf cutting	1 to 2	Up to 4

1. scoring. Eight cuttings were made in the bottom part of the bulbs, each cutting two cm deep (**Fig. 3**);
2. chipping. Bulbs divided into 4 equal parts (**Fig. 4**);
3. chipping. Bulbs divided into 8 equal parts.

The bulbs and the bulb parts were planted in sand with a diameter with a maximum of 3 mm with the lower part of the bulbs and parts in the sand. The pots were placed in a shady place at a temperature of 21-24°C.

Leaf cuttings

For the propagation by leaf cuttings two bulbs of *E. autumnalis* and one of *H. festalis* were planted in two liter pots on 20 March 2010 and grown in a greenhouse. Of each plant leaves were taken on 5 August 2010: of *E. autumnalis* 6 leaves with a length of 15-18 cm and of *H. festalis* 5 leaves of 35-40 cm length. The *E. autumnalis* plants were at that time in full bloom. The *H. festalis* were non-flowering plants. Each leaf was cut into 2 parts of equal length: a bottom part and the top part. The leaf cuttings were with the bottom part placed in moist sand with a diameter with a maximum of 3 mm. The pots with the leaf cuttings were placed in a shady place at a temperature of 22-25°C.

After one growing season all bulbs (end October), bulb parts and leaf sections were observed for the number and size of the newly formed bulbs.

RESULTS AND DISCUSSION

Scoring and chipping

As shown in **Table 2**, *E. autumnalis* did not form any bulbs after scoring and chipping. Part of the used bulbs and bulb parts stayed intact, the other parts died off. The dying off was not caused by a fungal infection, as no symptoms of any fungal infection were visible. The most obvious reason that in case of *E. autumnalis* no adventitious bulblets were formed, is that the bulbs did not receive enough temperature after chipping.

With *H. festalis* scoring and chipping resulted in the formation of new adventitious bulbs (**Fig. 5**). The propagation rate and the average circumference of the formed bulbs were almost the same for scoring and cutting into 4 parts, as presented in **Table 2**. The bulb cut into 8 parts produced more new bulbs compared with the other two techniques: 13 versus 8 or 9. The size of the bulbs however was with this technique (4 cm) smaller than with the other two methods (6 and 7 cm). The reason that more new bulbs are formed when cut into 8 parts, can most probably be explained by the situation that besides buds have sprouted, but also adventitious bulbs are formed. With scoring and cut into 4 parts only buds sprouted.

One of the objectives of this part of experiment was to answer the question 'Will chipping at the end of the dormant period followed by immediate planting lead to other results compared to propagation during the first half of the dormant period followed by a storage period in vermiculite or sand of 13 weeks before planting in soil?' (Knippels 2000). With the performed tests with *H. festalis* and *E. autumnalis* this answer cannot be given. According to Van Leeuwen and Van der Weijden (1997) at least for *Eucomis* a positive answer can be given on the above raised question, but can vary for the various species.

In the experiment with *H. festalis* a not known phenomenon was observed. In the case of the treatment of division into 8 parts two newly formed bulbs were harvested with numerous 'side bulbs' (**Fig. 6**). At the bottom part, at the basal plate of the newly formed bulbs various small bulblets are formed. The side bulblets were not formed on the basal plate of the old mother bulb. The most probable explanation for this behaviour is the type of regeneration. Plant hormones in the basal plate and the bulb parts induce adventitious bulblets, and subsequently the bulblets are formed on the new bulblet: a form of rejuvenation of the newly formed bulblets. Further research has to be done to verify the observed phenomenon.

Leaf cuttings

In the here described experiments the leaf cuttings were taken in the case of *E. autumnalis* during flowering and with *H. festalis* of a not flowering plant. The period between the start of propagation by leaf cuttings and the time of the end of the experiment was 12½ weeks. During this period in the case of *E. autumnalis* all top leaf parts died off, as well as a part of the bottom leaf parts, most probably they dried. There is no explanation why all top leaf parts died off. Four bottom leaf parts could be examined at the end of the experiment. In week 12 of the experiment 2 small bulbs were formed (**Table 2; Fig. 7**). At the bottom side of the 4 bottom leaf parts a callus like tissue was visible. It looked like a thickening of the bottom side. At first this callus like tissue is produced before adventitious bulbs are formed. Part of the cells in this callus tissue transformed into adventitious bulbs.

On internet various information (International Bulb Society 2004) has been published on the possibilities of propagation of *Eucomis* by leaf cuttings. The results of the described experiment show that *Eucomis* can successfully be propagated by leaf cuttings. The timing of the propagation is not indicated. In this paper propagation of *Eucomis autumnalis* started during flowering and resulted in a limited number of small bulbs. Based on the experiments with *Lachenalia* by Ndou (2000) and Niederwieser and Ndou (2002), the most likely conclusion can be drawn that the start of the experiment with *E. autumnalis* was too late. The limited number of formed bulbs was caused by a low propagation potential, which seems to decrease with the flowering. This has to be confirmed by further experiments with other *Eucomis* species. Propagation of bulbous plants by leaf cuttings is in general a potential propagation technique

with commercial potentials, at least for *Eucomis*, *Lachenalia* and *Ornithogalum*. Whether it offers possibilities for other genera has to be investigated.

The propagation of *H. festalis* by leaf cuttings resulted in dying of the leaf parts soon after the experiment started. There was during the period until the leaf parts died no symptoms of any fungal infection visible.

CONCLUSIONS

Scoring and chipping

It was not possible to propagate *E. autumnalis* by scoring and chipping, most probably due to the point that the used bulbs did not receive enough temperature after chipping. The conclusion can be drawn that all three techniques can be applied successfully when propagated at the end of the dormant period with at least *H. festalis*. A preliminary conclusion is that in general the here mentioned representatives of the family of *Amaryllidaceae* can be propagated by scoring and chipping, with as most suitable timing the beginning and the end of the dormant period. Experience in practice shows that with the commercial crops *Narcissus*, *Galanthus* and *Hippeastrum* the best results are obtained with propagation at the beginning of the dormant period. These are crops that require cold during their dormant period. There seems to be a difference in the most appropriate time for propagation, at the beginning or at the end of the dormant period, between *Amaryllidaceae* genera that need cold during their dormant period and those genera that do not require such a period (pers. com. Van Leeuwen 2010).

Leaf cuttings

The preliminary conclusion with *H. festalis* is that the dying of the leaf parts had a natural cause and that this propagation method is not suitable for *H. festalis*. More research is needed to confirm this. Knippels (2000, 2003) describes experiments of propagation by leaf cuttings with *Haemanthus albiflos* (**Fig. 8**), another member of the *Amaryllidaceae* family, of which results are presented in **Table 1**. With this species adventitious bulblets were formed. The remark has to be made that the leaves of *Haemanthus albiflos* are succulent and won't dry easily like with *H. festalis*. This is what most likely has happened with this species in this experiment.

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