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PLANTING DATE, STORAGE AND GIBBERELLIC ACID AFFECT DORMANCY OF ZANTEDESCHIA Spreng. HYBRIDS

A thesis presented in partial fulfilment of the requirements for the degree of

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ABSTRACT

To match the supply of *Zantedeschia* cut flowers and tubers to the demands of the international market, crops have to be timed to a schedule, which requires control of the growth cycle and, in particular, dormancy. In order to improve the predictability and accuracy of timing of *Zantedeschia*, the effect of different planting seasons and two dormancy-breaking treatments were tested on cultivars 'Black Magic' and 'Treasure', which were known to have a contrasting level of dormancy. Tissue-cultured plants were ex-flasked in July and November 1999, and grown for 180 days in a heated glasshouse (first cycle). Between 120 and 180 days of growth, plants were harvested at 15 days intervals, and tubers cured. Subsequently, tubers were stored for 0 or 3 weeks (10 ± 1°C; 70-80% RH) and dipped in 100 mg.L⁻¹ gibberellic acid plus surfactant or water plus surfactant, prior to planting for dormancy assessment (second cycle).

Growing the plants with four months difference in planting date did not cause major alteration in the occurrence of dormancy. Dormancy was brought forward by up to 10 days after the November date of ex-flask, but this was most likely to be due to higher temperatures during that period. In contrast, depth of dormancy varied between cultivars, with 'Black Magic' taking in average 16 days longer to emerge than 'Treasure'. Storage partially released bud dormancy of the tubers. It increased emergence to over 80% regardless of the time of harvest in the first cycle and cultivar, but reduced time to emergence mostly after harvests at 180 days. Furthermore, following storage, time to emergence was reduced to over 50 and 30 days for 'Black Magic' and 'Treasure', respectively, which exceeded the commercially acceptable period to emerge. Gibberellic acid also broke bud dormancy, improving emergence to over 80%, and reduced time to
emergence to between 29 and 57 days, irrespective of the time of harvest in the first cycle and cultivar. The effectiveness of gibberellic acid at any time following harvest during the first cycle, may imply that dormancy of *Zantedeschia* is not as deep as in temperate woody plants.

Cessation of leaf emergence in the first cycle was found not to be directly related to the occurrence of dormancy. Degree-days, on the other hand, presented a possible alternative to predict this process. It was estimated that deepest dormancy of 'Black Magic' occurred between 2614 and 2732 °C-days after planting, while deepest dormancy of 'Treasure' occurred between 2681 and 2839 °C-days after planting.

The present study presents storage and gibberellic acid as possible options to control dormancy, and the use of degree-days to predict the occurrence of this process. Further research is necessary to develop these options as commercially applicable practices, and to further clarify the process of dormancy in *Zantedeschia*.
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1. INTRODUCTION

1.1 Commercial significance of Zantedeschia.

Zantedeschia spp. Spreng. is currently the second largest cut flower export for New Zealand after Cymbidium orchids, with a value of 4.5 million dollars being exported in the year to June 1999 (Topless, 2000). The counter-seasonality of the production in the Southern Hemisphere allows growers to supply lucrative export markets in the Northern Hemisphere during peak demand (Clemens et al., 1999).

Field production of Zantedeschia flowers in New Zealand is highly seasonal, extending from November through to March (Clemens & Welsh, 1993), with a peak in December/January (Muller, 1993). The high supply of flowers during the peak period has a negative impact on international prices, which consequently reach a minimum over the Christmas-New Year period and start to recover from mid-January onwards. Therefore, growers have been encouraged whenever possible to time their plantings to take advantage of the higher prices which occur in the shoulders of the season, i.e. October, November, February, March and April (Muller, 1993).

An additional market for Zantedeschia flower growers and specialist propagators is the sale of tubers on the export market (Clemens & Welsh, 1993). These growers purchase tubers for pot forcing or cut flower production for sale at Christmas, New Year and Valentines, which implies that New Zealand growers need to be able to supply tubers during October-February, i.e. the normal growing season in the southern Hemisphere.
To supply the constant demand of the international market, the *Zantedeschia* cut flower and potted plant industries must aim to attain all year production, which implies the manipulation of the timing and duration of the plant's natural growth cycle (Halligan et al., 1995; Brooking et al., 1998). However, as it will be shown in this introduction, negative results of practices applied to manipulate the duration of the growth cycle of *Zantedeschia*, and a lack of knowledge on the factors that control dormancy, have made accurate timing of the crop difficult to achieve.

### 1.2 Origin and distribution of *Zantedeschia* genus.

The majority of New Zealand's production is limited to the summer calla, a term that groups the species *Zantedeschia jucunda* Letty, *Z. pentlandii* (Wats.) Wittm., *Z. rehmannii* Engl. and *Z. elliotiana* (Wats.) Engl., and the hybrids resulting from interespecific crosses. Therefore, the following review is limited to these species.

The genus *Zantedeschia* corresponds to a group of geophytic plants endemic to the African continent. It is most prevalent to the southern regions (i.e., Cape Province, Orange Free State, Natal, Lesotho, Swaziland, Transvaal), but also extends into Zimbabwe, Malawi, Nigeria and Angola (Letty, 1973). Summer calla plants grow in mountainous regions, at altitudes of 1200 to over 2000 m, generally in grassy slopes and at forest margins.

Distribution of the summer calla is restricted to cool-temperate climates, with a mean air temperature of 11 °C during June-July (min. 2.7 °C; max. 20 °C), and 20 °C (min.
14 °C; max. 27 °C) from October through to March (Funnell, 1993a). Rainfall is predominantly distributed during the summer.

1.3 Cyclic periodicity.

A major feature of geophytes (i.e. plants that survive using specialised underground storage organs) is the development of a cyclic behaviour or periodicity, which typically matches their phenologic cycle to the climatic conditions (Rees, 1972; 1984). It is believed that periodicity evolved as a survival means to overcome unfavourable climatic conditions in seasonal climates, allowing the plant to renew activity when conditions improved (Rees, 1981).

The close relationship between environment and periodicity of a plant is still evident in the behavior of commercial geophytic flower crops. A general example is the division of geophytes into spring-, summer- and autumn-flowering plants made by horticulturists, according to the time of the year when active growth and flowering is expressed (e.g. De Hertogh & Le Nard, 1993). Although broad, this division shows the behaviour that the plants would have had in their natural habitat, and the requirements that have to be met to grow them successfully. A more specific example are the commercial varieties of *Tulipa* sp. L., which retain within their genotype those attributes which fitted their ancestors to the harsh habitat of the uplands of Asia Minor (Rees, 1981). In those areas, winters are severe with deep snow, the springs are short and moist and the summers and autumns dry and hot. Consequently, tulip plants evolved into spring flowering with no aerial growth in summer and the resumption of aerial growth once the cold requirement has been fulfilled.
As a response to cool and dry winters and summer rainfall, *Zantedeschia* species also developed a seasonal periodicity, with complete foliage senescence during winter, while growth and flowering occur during late spring through summer (November-January) (Letty, 1973; Funnell, 1993a; Figure 1). A compact, disc-shaped rhizome -also called a tuber or corm¹- is the structure which survives under the ground during dry periods (Corr, 1993).

As with tulips, the seasonal periodicity of *Zantedeschia* is also evident in commercial crops, where it follows basically the same cycle as in its native habitat. In a normal production cycle in the Southern Hemisphere, *Zantedeschia* tubers are planted in September/ October, flowering from November through to March (Clemens & Welsh, 1993; Halligan et al., 1995). The new foliage stops appearing in February, and by April/ May leaf senescence of the plants begins (Figure 1).

¹ The term tuber will be used in this review to avoid confusion.
1.4 Dormancy.

Dormancy is an integral part of the annual cycle of most geophytes, characterising perennial plants that exhibit seasonal growth (De Hertogh & Le Nard, 1993). Lang et al. (1987) defined dormancy as 'the temporary suspension of visible growth of any plant structure containing a meristem', and divided it into three types. These types are: eco-, para- and endo-dormancy, representing dormancy controlled by conditions outside the
plant, outside the affected organ but within the plant and within the organ, respectively (Dennis, 1996). Although not universally accepted (Rowland & Arora, 1997), Lang's terminology has been qualified as more physiologically descriptive than most of the earlier terminology and, therefore, it will be used in this study.

Additionally, endo-dormancy can be further divided into two consecutive phases. The first corresponds to deep endo-dormancy, and is based on the inability to induce the buds to grow under natural conditions (Faust et al., 1997; Figure 2). The second is a shallow endodormant period, which is the stage where dormancy can be overcome by artificial treatments.

Figure 2. A schematic representation of inhibition of budbreak during dormancy. Dormancy begins with para-dormancy and it deepens during deep endo-dormancy (d-endo-dormancy). When endo-dormancy weakens during shallow endo-dormancy (s-endodormancy), buds respond to dormancy-breaking treatments. The depth and duration of eco-dormancy is environment dependent. (From Faust et al., 1997).
There has been some debate concerning dormancy of Zantedeschia (Kuehny, 2000). Early studies suggested the absence of a dormant period unless it was artificially induced by drying, and that year-round production of flowers could be achieved in greenhouses under protection (Post, 1959; Wilkins, 1985). This theory was later refuted by Corr & Widmer (1988), who demonstrated that Zantedeschia does have a dormant stage, since tubers replanted immediately after leaf removal were not able to sprout even if placed under ideal environmental conditions. Halligan et al. (1995) observed that this dormant period lasted from late summer (January), when leaf production stopped, to autumn (late April/early May) (Fig. 1). During this period, the apical meristem was incapable of continued growth until dormancy was released.

It is not known if bud dormancy of Zantedeschia tubers corresponds to endo- or para-dormancy. Previous studies reported the ability of buds to continue developing once isolated from the rest of the tuber and placed in agar (Halligan et al., 1994), which suggests the occurrence of para-dormancy. However, it has not been possible to confirm these results in later experiments, due to contamination of the material. In order to avoid misinterpretations, the term dormancy in the present study will refer indistinctly to endo- or para-dormancy.

1.4.1 Horticultural importance of dormancy

The existence of a dormant period is convenient for horticultural purposes, since it permits easy handling, storage and transport of the bulbs\(^2\) (De Hertogh & Le Nard, 1993). Furthermore, it can be overcome by natural or artificial means at a predetermined time,

\(^2\) The term bulb here includes all geophytes with diverse storage organs
which allows growers to control timing of production or flowering independently from the natural season to which the plant alone is constrained (Rees, 1981). Means of overcoming dormancy include storage of the bulbs and the use of growth regulators. In addition, timing of the production can be controlled through early lifting practices, which bring forward the occurrence of dormancy, allowing growers to harvest and handle the bulbs earlier in the season.

Although being an extensively used practice, timing of bulb or flower production through storage and early lifting has not been successfully achieved with all geophytes. As it will be explained with more detail in the following pages, these practices have even resulted in negative responses when applied to *Zantedeschia* crops. Since the environmental and/or genetic factors that control dormancy in *Zantedeschia* are not known, it has not been possible to develop the best techniques to control the duration and occurrence of dormancy, and consequently, to accurately time the flower and bulb production. A more complete knowledge of dormancy of this crop would greatly improve possibilities of timing the production to the needs of the market.

From a horticultural viewpoint, not only it is important to control the duration and occurrence of dormancy, but also it is essential to be able to visually detect when dormancy occurs. If detectable, the best lifting time and the most effective moment to apply dormancy-breaking treatments can be programmed. Nevertheless, to date the only visual change that might possibly be linked to dormancy is the cessation of leaf emergence and the subsequent onset of foliage senescence during the summer (Halligan et al., 1995).
1.4.2 Factors influencing dormancy onset.

Environmental factors that induce dormancy vary with the species and their native environment. For the majority of the woody plant species from temperate climates, short days and cold temperatures cause the cessation of extension growth and the formation of resting buds (Wareing & Saunders, 1971; Wareing & Phillips, 1981; Olsen et al., 1995). For some bulbous species, such as *Gladiolus grandiflorus* Hort., corm development is also stimulated by short days (Hartmann et al., 1997). For others, like onion (*Allium cepa* L.) and *Poa bulbosa* L. -a grass geophyte with summer dormancy-, bulb production and leaf senescence, which are considered as indicative of the onset of dormancy, are induced by long days (Ofir & Kigel, 1998). Dormancy of tulip bulbs, on the other hand, is triggered by high temperatures (Rees, 1972).

Although periodicity of growth and development in *Zantedeschia* has been studied (e.g. Funnell & MacKay, 1987; Halligan et al., 1994, 1995), the factors that induce the cessation of leaf production and, possibly bud dormancy, have not been identified yet. In addition, most of the research regarding the influence of the environment on *Zantedeschia* has focused on growth and flowering, and their effect on dormancy can only be inferred.

1.4.2.1 Photoperiod

Photoperiod plays an important role in both vegetative and reproductive growth of plants, since it influences processes such as seed germination, stem elongation, leaf growth, senescence, abscission and dormancy (Coleman & Chen, 1996). The adaptive value of using photoperiod as a timekeeping mechanism for synchronising growth transitions is evident, since it is the one environmental cue that does not vary from year to year.
There is little evidence of photoperiodic control of dormancy in ornamental bulbs, which is in sharp contrast to that shown in the buds of many woody plants (Rees, 1981). Up to now, there is confirmation of day-length effects only on dormancy of *Allium* species (Rees, 1972; Wareing & Phillips, 1981), *Gladiolus* (Hartmann et al., 1997), *Begonia x tuberhybrida* Voss. (Lewis, 1951) and *Dahlia pinnata* Cav. (De Hertogh & Le Nard, 1993).

Early studies by Greene et. al. (1932; cited by Dole & Wilkins, 1999), as well as observations by commercial growers, indicated that there are no photoperiodic effects on *Zantedeschia*’s growth (Ball, 1986; Corr & Widmer, 1990) and dormancy (Funnell, 1988). In a later unpublished experiment, Brooking et al. (1998) confirmed that photoperiod did not affect the induction of dormancy, since plants remaining in a glasshouse under declining daylength, and plants transferred to a long-day environment entered dormancy at the same time. Therefore, *Zantedeschia* plants should be able to grow under any daylength conditions.

1.4.2.2 Light intensity

Light has been related to the release of dormancy in seeds of several species, such as lettuce (*Lactuca sativa* L.) (Wareing, 1982), celery (*Apium graveolens* L.) endive (*Chicorium endivia* L.) (Khan, 1996) and *Arabidopsis thaliana* L. (Cone & Spruit, 1986). However, there is no evidence of this factor affecting the onset of dormancy.

Few studies have investigated the effect of light intensity on growth and development of *Zantedeschia*. Warrington & Southward (1989) and Funnell (1993a) found that, under a constant temperature and low light conditions (350 μmol s⁻¹m⁻²), leaf area expansion was extended and leaf senescence delayed, as compared with high light
intensity (700 µmol s\(^{-1}\) m\(^{-2}\)). However, neither of the experiments measured any parameter that could directly be related to the onset of dormancy. In addition, both experiments were carried out in controlled environment rooms, and it has been suggested that the observed response may be related to photoassimilate partitioning and not necessarily to dormancy (Funnell, pers. comm.).

As shown in this section, there is no information that associates light intensity with the induction of dormancy on Zantedeschia. However, studies with other species suggest that these two factors may not be directly related.

**1.4.2.3 Cultivars**

Length and depth of dormancy of many species is largely under genetic control. For example, cultivar- and species-dependent periods of dormancy have been noted in potato (*Solanum tuberosum* L.) and yam (*Dioscorea alata* L.) (Turnbull & Hanke, 1985; Burton, 1989) onion (Carter et al., 1999) and *Dahlia* (Konishi & Inaba, 1967 cited by De Hertogh & Le Nard, 1993).

In *Zantedeschia*, it appears that there is significant variation in both tuber maturation and dormancy among cultivars (Halligan & Fulton, 1998). Reports show that tubers of the cultivars ‘Treasure’ and ‘Cameo’ were able to sprout within 1.5-3 weeks when replanted without a storage period. In contrast, tubers of the cultivars ‘Black Magic’ and ‘Dominique’ sprouted after a minimum of sixteen weeks or failed to sprout altogether.
In addition, a difference of 7-8 weeks in the occurrence of cessation of growth and leaf senescence between cultivars 'Black Magic' and 'Pink Persuasion' (Clemens & Welsh, 1993), suggests that timing of the onset of dormancy may also depend on the cultivar.

1.4.2.4 Temperature

Of the various climatic variables to which a plant is exposed, temperature has been considered as a major environmental factor determining variations in growth (Piñera, 1995) and development (Terry, 1968; Passian & Lieth, 1994). Higher temperatures generally lead to the earlier onset of a phenological event, like time to flower of Hibiscus moscheutos (Wang et al., 1998), Dahlia pinnata (Brøndum & Heins, 1993) and rose (Rosa x hybrida L.) (Pasian & Lieth, 1994), while low temperatures suppress processes such as sprouting of potato tubers (Suttle, 1995).

Warmer temperatures lead to a chronologically earlier onset of all events in growth and development of Zantedeschia, including shoot and leaf appearance, flowering, rapid tuber growth, cessation of leaf appearance and leaf senescence (Funnell, 1993b). For instance, Z. rehmannii plants grown at an ambient temperature of 20 °C flowered approx. 18 days earlier than plants grown at 15 °C (Corr & Widmer, 1990). Similarly, senescence was advanced by 60 days in plants grown at high temperature (28/22 or 22/16 °C day/night), as compared with plants grown at low temperature (16/10 °C) (Warrington & Southward, 1989; Funnell, 1993a). If we assume that there is a relation between cessation of growth and dormancy, then this could indicate that the onset of dormancy is probably also affected by the temperature regime.
1.4.2.4.1 Temperature as a predictor of the occurrence of dormancy

Given the importance of dormancy on timing and scheduling floricultural crops, the ability to predict its occurrence is essential for growers who aim to supply the international market during highest demand. In many temperate woody species, prediction of dormancy does not represent an obstacle, since the factors that trigger the process are well known (refer to section 1.4.2). However, prediction of dormancy in *Zantedeschia* has not been possible since, as shown in this chapter, the environmental and/or physiological factors involved in its induction have not been identified. Thus, the accumulation of temperature by the plant, measured as degree-days, may be a useful tool for the prediction of the occurrence of dormancy.

1.4.2.4.1.1 Degree-Days

Heat units, measured in growing degree-days, relate the accumulation of heat energy by a crop during a given period to the progress in development or growth processes (McMaster & Wilhelm, 1997). This system is currently being used to monitor growth and development of many crops (O'Rourke & Branch, 1987), and has vastly improved description and prediction of phenological events as compared to other approaches, such as time of the year or number of days (McMaster & Wilhelm, 1997).

There are no precedents on the use of degree-days to monitor growth and development, or to predict the occurrence of dormancy in *Zantedeschia* plants. However, interpretations of the studies carried out by Halligan et al. (1995) and Halligan & Fulton (1998), and the temperatures recorded during their experiments, lead to suggest that the onset and release of dormancy may occur between 1000-1600, and between 2600-2900 °C-days, respectively (Appendix 1).
1.4.3 Manipulation of dormancy

1.4.3.1 Early lifting practices

Early lifting of geophytic crops may provide a means of reducing the need for long-term storage, to supply tubers and flowers for early season exports (Funnell & MacKay, 1989). Practices applied for early lifting include mechanical removal of the foliage and promotion of early foliage senescence by means of herbicide application and withholding water.

Studies performed on early lifting of Caladium x hortulanum L. showed that foliage mowing was effective in reducing weight of roots and shoots of the plants (Gilreath & Harbaugh, 1986). The application of herbicides like paraquat, oxyfluorfen and, to a minor extent, ethephon, was equally effective in promoting foliage senescence without affecting growth in the subsequent cycle.

Although both Caladium and Zantedeschia belong to the Araceae family, the use of the same practices, as described above, on Zantedeschia crops has resulted in negative outcomes. Plants subjected to foliage mowing and subsequently left under conditions favorable for sprouting, were not able to resume growth until they were lifted and their roots removed (Corr & Widmer, 1988; Brooking et al., 1998). In contrast, tubers that were lifted and cured or stored grew soon after being replanted (Corr & Widmer, 1988; Brooking, Pers. Comm).

Similarly, the artificial induction of foliar senescence through withholding water caused slow and erratic emergence during the following growth cycle (Funnell & MacKay, 1989). In addition, the application of ethephon did not induce foliage senescence, and
progressive increases in the doses resulted in a reduced increase in tuber dry weight. Since export tubers, like cut flowers, have to meet quality standards such as high quality flowers and productivity and flowering programmability (New Zealand Calla Council, 1994), erratic emergence represents a serious problem for exporters.

Practices to promote early senescence of the foliage are thought to induce dormancy of buds on tubers of Zantedeschia (Corr & Widmer, 1988). This would explain the poor results obtained. Clearly, it will be necessary to fully understand the natural periodicity and dormancy of this species before being able to develop techniques for the early lifting of the plants.

1.4.3.2 Storage duration and temperature

Storage of Zantedeschia tubers is currently used to facilitate production programming for both pot plant and cut flower production (Funnell & Go, 1993). While the primary objective of long term storage is to allow scheduled planting and flowering by inhibiting shoot growth, the aim of short-term storage is to break bud dormancy of the tubers. In the past years, a considerable amount of research focused on the effect of storage duration and temperature on subsequent performance of Zantedeschia, but only a few investigations dealt with its effect on bud dormancy, and mostly non-dormant tubers were used. Only recently dormancy has become a major subject in research related with storage.

Earlier investigations suggested that dormancy of Zantedeschia could be overcome with storage at 20 °C for several months (Cohen, 1981). The effects of a storage period on dormancy release were later confirmed by MacKay (1985), who determined that time to
emergence of cultivars 'Pink Persuasion' and 'Pacific Pink' could be reduced by 45-50 days if tubers were stored for two weeks at 5-7 °C. Likewise, a reduction in time to emergence of approx. 50% was observed in cultivar 'Black Magic', when storage duration was increased from 0 to 3 weeks at 10 °C (Halligan & Fulton, 1998).

As for the optimum temperature to break dormancy, no differences in the release of dormancy were found between tubers stored either at 10 ° or 20 °C (Brooking, Pers. Comm.). Similarly, no significant effects on growth were found on tubers of Z. elliottiana and Z. rehmannii after a storage period of six weeks at 4, 9 or 22 °C, although the highest temperature largely reduced fresh weight of the tubers, and dormancy of the tubers had been already partially released (Corr & Widmer, 1988). Thus, it would be advisable to use temperatures close to 10 °C, in order to avoid high water losses during the storage period.

Storage duration also influences time to sprout and the plant's final performance. Storage of dormant tubers for nine weeks increased time to sprout by 200%, compared with tubers stored for 0, 3 or 6 weeks (Halligan & Fulton, 1998). Additionally, storage for six months reduced flowering potential of the tubers up to 100% if gibberellins were not applied (Funnell & Go, 1993). In contrast, a minimum storage period of three weeks has shown to be effective in breaking dormancy of cultivars with deep dormancy (Halligan & Fulton, 1998), as well as in increasing plant height, number of leaves and shoots per tuber (Corr & Widmer, 1988).

1.4.3.3 Gibberellins

Among the phytohormones involved in growth and development of plants, gibberellins are the hormones most commonly associated with dormancy release (Wareing
& Phillips, 1981). When exogenously applied, they have proved to be effective in breaking dormancy of seeds (Desai et al., 1997), buds (Saure, 1985), *Lilium* sp. L. bulbs (Ohkawa, 1979; Niimi et al., 1988), and potato tubers (Tsukamoto et al., 1961; Wiltshire & Cobb, 1996), among other crops.

In *Zantedeschia*, gibberellin application has been widely used for the promotion of flowering (e.g. Funnell et al., 1988; Corr & Widmer, 1990; Funnell & Go, 1993; Dennis et al., 1994). Its application increases flower production through an increase in the number of buds emerging as primary shoots (Funnell & Go, 1993), and also induces flowering in tubers that otherwise would not be able to flower due to reduced size or prolonged storage (Funnell et al., 1988). Nevertheless, their possible effect on dormancy release has not been studied.

In potato crops, gibberellin concentrations recommended to break dormancy range from 1 mg·L\(^{-1}\) (Contreras, Pers. Comm.) to 50 mg·L\(^{-1}\), depending on the cultivar and storage duration (Dean, 1994; Centro Internacional de la Papa, 1988). On the other hand, concentrations used to promote flowering of non-dormant *Zantedeschia* tubers varies between 50 mg·L\(^{-1}\) and 600 mg·L\(^{-1}\) (Funnell et al., 1988; Reiser & Langhans, 1993; Dennis et al., 1994) when applied as a preplanting dip. However, the concentrations that would be most effective in breaking dormancy of *Zantedeschia* tubers are unknown.

### 1.5 Summary and objectives

One of the aims of the *Zantedeschia* cut flower and potted plant industries is to match the supply of flowers and tubers to the constantly increasing demand of the
international market. To achieve this, crops have to be timed to a schedule, which often implies the artificial shortening of the growth cycle, or the extension of the period of inactivity. In other species, cultural practices to manipulate the duration of the growth cycle such as early lifting and storage are widely used, but their application on \textit{Zantedeschia} crops have not provided the predictability and accuracy desired.

Studies have been carried out to clarify \textit{Zantedeschia} periodicity, aiming to develop more effective techniques of manipulating the growth cycle (Halligan et al., 1994 & 1995). In these studies, special attention has been paid to dormancy, since its occurrence determines the duration of the growth cycle and the storage requirements of the tubers, and consequently, the possibilities of crop scheduling. As shown in this review, the environmental and/or genetic factors that control dormancy have not been determined, but there is evidence that photoperiod and light intensity are not involved in its induction (Brooking et al., 1998; Funnell, 1993a), and that temperature can bring forward or delay its occurrence (Funnell, 1993b). In addition, it is known that the intensity of the process is highly dependent on cultivars (Halligan & Fulton, 1998). Short-term storage has been successfully tested as a treatment to artificially release dormancy of \textit{Zantedeschia} (Halligan & Fulton, 1998), but there is no information on the efficacy of gibberellic acid, which has been used to break dormancy of numerous other crops. In addition, if the occurrence of dormancy could be visually determined or predicted by means of degree-days, this would greatly aid to program lifting of the crop and the application of dormancy-breaking treatments.

Despite the advances that have been achieved in the control of growth and periodicity of \textit{Zantedeschia}, there are several questions that have to be answered before
timing and scheduling can reach the level of predictability and accuracy as in other geophytic crops, e.g. tulips. In order to answer some of them, the following study aims to:

- Quantify what changes in timing and duration of vegetative growth and dormancy result from a modification on growth season of *Zantedeschia* hybrids;
- Determine differences in the occurrence of bud dormancy between two cultivars known to differ in their depth of dormancy;
- Determine if dormancy duration can be modified by storage and gibberellin application and,
- Test the possibility of using cessation of leaf emergence and degree-days as indicators of the occurrence of dormancy.
Chapter 2: Materials and Methods

2. MATERIALS AND METHODS

2.1. Cultural.

Plants of Zantedeschia cv. 'Black Magic' and 'Treasure' received from a commercial laboratory were removed from their tissue culture medium on either July 20 or November 1st 1999 (i.e. date of ex-flask), at Massey University (Palmerston North, New Zealand; 40° 20' S). On arrival, agar was washed from the roots with water at 20 °C, plantlets wrapped in paper towels, and dipped in a fungicide solution of benomyl (Benlate®; Du Pont de Nemours and Co. Inc., USA), at a rate of 0.1 g·L⁻¹ water. Plant material was planted in sterilised trays containing a 50 peat: 30 pumice (v/v) growing medium, plus 3 kg·m⁻³ dolomite lime, 0.5 kg·m⁻³ iron sulfate, and 4.0 kg·m⁻³ Osmocote® 16N-3.5P-10K (Grace-Sierra International, Netherlands). Plant density was 500 plants·m⁻².

For the first three weeks, plants were covered by a knitted polyethylene cloth (evolution cloth TM) and subjected to intermittent fogging. The resulting environment created a relative humidity of approximately 90%. Greenhouse temperature was maintained at 15 °C minimum, with ventilation at 20 °C. After removal of the evolution cloth, irrigation was changed to overhead sprinklers. To overcome probable nutrient deficiencies during later periods of growth of plants ex-flasked in November, liquid feeding with standard Peter's® Professional water soluble fertiliser (20N-8.7P-16.6K) was provided once a week.

During the dormancy assessment phase (i.e., second growth cycle), tubers were planted in individual polythene bags (0.9 L) of the same growing medium as described above, and irrigated via capillary matting on benches. Frequency of irrigation was adjusted...
according the plants' requirements, avoiding periods of water stress. Once a week, overhead irrigation was provided, in order to wash off the salts accumulated on the surface of the growing medium.

Greenhouse temperatures, measured at 9 positions (one for each block) with a shaded sensor at foliage height, were recorded at 30-min intervals during the study with a Squirrel 1200 Digital Meter/Logger (Grant Instruments Ltd., Barrington, Cambridge, U.K.). Additionally, a thermo-hygrograph (C. F. Casella & Co Ltd., London, U.K.) recorded temperatures in the glasshouses and the curing room.

A long-day photoperiod was provided by night lighting interruption between 22:00 and 02:00 HR. In order to assist temperature control, greenhouse shading of ~50% was used during the high light intensity period (from October through to March).

2.1.1 Pest and disease control.

Plants were regularly monitored for phytosanitary problems and a regular spray and drench program was followed throughout the experiment (refer to Appendix 2 for details).

2.2. Experimental.

After ex-flasking, plants of both cultivars progressed through two consecutive phases: initial plant establishment and growth phase to time to harvest (1st cycle) and
subsequently a dormancy assessment phase (2nd cycle). A summary of the treatments and their timing over these two phases are detailed in Figure 3.

### 2.2.1 Growth phase.

Samples of plants ex-flasked either on July or November were harvested 120, 135, 150, 165 and 180 days after planting. The spread of harvest dates was chosen to encompass the period of dormancy, which occurrence was estimated from accumulated heat units reported previously (Halligan et al., 1995; Halligan & Fulton, 1998) (Appendix 1).

![Figure 3. Schematic outline of the treatments applied in the experiment. The experiment was divided in two consecutive phases: a growth phase (A) (1st cycle) and a dormancy assessment phase (B) (2nd cycle).](image)

At each harvest, one tray per block containing both cultivars each was lifted, and leaf petioles and roots trimmed to equal or less than 0.5 cm in length to reduce water loss during curing. In each replicate, which corresponded to one tray, tubers were divided in 4 treatments per cultivar, leaving the same number of tubers and range of sizes in all treatments when possible. Then, tubers were placed in mesh bags, dipped in 5 g·L⁻¹
Kocide® for 5 min, and cured for 14 days at 25 ± 1 °C and 80-90 % relative humidity. After one week of curing, when tuber's skin had suberised, the remaining leaf petioles and roots were removed. Tubers were subsequently dipped in 0.5 g·L⁻¹ prochloraz (Octave®; BASF) and cured for further 7 days at 25 ± 1 °C and 70-80 % relative humidity.

2.2.2 Dormancy assessment phase.

Once curing was completed, tubers were stored at 10 ± 1 °C and 70-80 % relative humidity either for 0 or 3 weeks. Subsequently, tubers from each storage period were immersed for 10 minutes either in 100 mg·L⁻¹ gibberellic acid plus 0.25 mL·L⁻¹ surfactant (Contact®; Crop Care Holdings Ltd., NZ), or water plus surfactant (no gibberellic acid). As soon as their surface was dry, tubers were planted as described above (section 2.1).

During the dormancy assessment phase, plants were grown under the same cultural and environmental conditions as used in the initial growth phase.

2.3 Recording, sampling and analysis.

2.3.1 Growth phase.

During the period of growth from each date of ex-flask, the number of emerged leaves on the dominant shoot was recorded fortnightly on 35 monitor plants per cultivar (five plants per harvest, per cultivar). A leaf was counted as emerged when its lamina was fully expanded. Each newly expanded leaf was identified with a marker to avoid confusions with already counted leaves. At 60 and 90 days after planting, and subsequently at each date of harvest, dominant buds of 5 monitor plants per cultivar were dissected. All structures enclosed by the base of most recently emerged leaf, down to the apical
Chapter 2: Materials and Methods

meristem, were removed and recorded as leaf primordia, including those leaves that were unfurling at the time of the dissection. Mean cumulative number of expanded leaves and leaf primordia in time were calculated. Subsequently, linear regressions were fitted to the cumulative number of expanded leaves, in order to determine the point of cessation of leaf emergence.

2.3.2 Dormancy assessment phase.

To assess dormancy, date of emergence of individual plants was recorded twice a week. Emerged plants were defined as plants with shoots above the growing medium surface. Mean time to emergence, cumulative emergence and mean percentage of emergence from the alive tubers was calculated from the data set. In addition, median emergence time (T50) and the time interval between the emergence of 10% and 90% of the alive plants (T90-T10) was calculated using equations 1 & 2, respectively (Coolbear, et al., 1984).

\[
T_{50} = t_i + \frac{2}{n_j - n_i} x(t_j - t_i),
\]

where:

\( n_i \) and \( n_j \) are cumulative emergence counts at adjacent counting times \( t_i \) and \( t_j \), where \( n_i < \frac{N+1}{2} < n_j \); \( N \) being the total number of alive tubers.

\[
(T_{90} - T_{10}) = \left[ t_a + \frac{9(N+1)}{10} \left( \frac{n_a}{n_b - n_a} \right) x(t_b - t_a) \right] - \left[ t_c + \frac{10}{n_d - n_c} x(t_d - t_c) \right]
\]
where:

\[ n_a \text{ and } n_b \text{ are cumulative emergence counts at consecutive times } t_a \text{ and } t_b, \]

where \( n_a < \frac{N+1}{10} < n_b \) and \( N \) is the final number of alive tubers. Similarly, \( n_c \) and \( n_d \) are cumulative emergence counts at consecutive times \( t_c \) and \( t_d \), where \( n_c < \frac{n+1}{10} < n_d \).

Degree-days accumulated during this phase were calculated from the recorded temperatures, using the standard growing degree-day formula (Perry, et al., 1986; McMaster & Wilhelm, 1997):

\[
GDD = \sum [T_{\text{MEAN}} - T_{\text{BASE}}]
\]

where the mean temperature was replaced by the daily average. The base temperature was 2.1 °C (Funnell, 1993a).

### 2.4 Experimental design.

The experiment consisted of four factors arranged in a nested factorial treatment structure, laid out in the greenhouse in a randomised complete block design. Nine replicates with seven samples per treatment were used. Replicate and sample number were estimated based on variance within a prior experiment (Halligan & Fulton, 1998) (Appendix 3). During the initial growth phase, one tray per harvest was assigned to each block.

To determine the existence of temperature gradients inside the glasshouse, 6 plants from the replicates with the most extreme temperatures were sampled at the first
harvest, and leaf area and tuber diameter subjected to a t-test (Appendix 4). Since no significant differences were noted, subsequent analysis did not include temperature effects as a source of variation.

Data were analysed with the Statistical Analysis System version 8 (SAS Institute, Cary, N.C.). Due to the non-normal distribution of percentage of emergence, this variable was analysed with a generalised linear routine, in which the data were fitted to a binomial distribution (proc genmod). Days to emergence were subjected to a standard analysis of variance (proc nested).

For the prediction of dormancy with degree-days, a quadratic function was fitted to the percentage of emergence of the controls (plants not subjected to any of the dormancy-breaking treatments), to determine the point of maximum dormancy.
3. RESULTS

3.1 Growth cycle.

3.1.1 Leaf emergence.

Leaf emergence showed three distinctive phases during the growth cycle following the July date of ex-flask. Firstly an initial lag phase, where no new leaves emerged; secondly a phase of rapid leaf emergence, and finally a plateau phase, where the emergence of new leaves ceased and older leaves started to senesce (Figure 4 A & B).

Figure 4. Number of emerged leaves (■) and leaf primordia (▼) formed by cultivars Black Magic (A) and Treasure (B) after the July ex-flask, in relation to calendar days (days from treatment). Arrows indicate 50% of the next emerging leaf primordia senesced. Broken lines indicate phase changes. Vertical bars denote ± SE. Values are means of 7 samples.
During the rapid phase, the rate of leaf emergence was one leaf every 18 days for 'Black Magic' and one every 15 days for 'Treasure'. 'Black Magic' plants ceased further leaf emergence ~115 days after ex-flask, with a total leaf number of 8.0 ± 0.6. Plants of 'Treasure' ceased leaf emergence ~5 days later, with a total of 9.9 ± 0.4 leaves. There were significant differences in the total number of leaves between cultivars (t-value=2.5, df=12, P=0.027).

Figure 5. Number of emerged leaves (■) and leaf primordia (▼) differentiated by cultivars 'Black Magic' (A) and Treasure (B) after the November ex-flask, in relation to calendar days. Arrows indicate when 50% of the next emerging leaf primordia senesced. Broken lines indicate phase changes. Vertical bars denote ± SE. Values are means of 22 samples for 'Black Magic' and 17 samples for Treasure.
In contrast to the July ex-flask, no lag phase was observed after the November ex-flask (Figure 5A & B). The rate of leaf emergence during this phase was one leaf every 14 days for 'Black Magic', and one leaf every 16 days for 'Treasure'. Cessation of leaf emergence occurred at ~72 days after the ex-flask for 'Black Magic' and at ~83 days after the ex-flask for 'Treasure'. Despite this, no significant differences in the number of emerged leaves were found between cultivars (t-value= 0.1486, df=37, P= 0.8827), which formed a maximum of 7 ± 0.7 leaves each.

Possible points of intersection between linear fits to the rapid leaf emergence and plateau phases determined that cessation of leaf emergence after the July ex-flask occurred between 1856.9 (r²= 0.46;P<0.01) and 2044 °C-days (r²= 0.52;P<0.01) for 'Black Magic', and between 1957 (r²= 0.80; P<0.01) and 1959 °C-days (r²= 0.80; P<0.01) for 'Treasure'. In contrast, cessation of leaf emergence after the November ex-flask occurred between 1266.1 (r²= 0.44; P<0.01) and 1366.8 °C-days (r²= 0.46; P<0.01) for 'Black Magic', and between 1498.0 (r²= 0.65; P<0.01) and 1591.7 °C-days (r²= 0.62; P<0.01) for 'Treasure'.

3.1.2 Leaf primordia.

For both cultivars, the number of leaf primordia continued to increase throughout the period of observation after both dates of ex-flask (Figure 4 & Figure 5). The number of differentiated primordia became highly variable after 150 days in 'Treasure' plants that were ex-flasked in November, but complete cessation of differentiation was not observed (Figure 5B). The maximum number of differentiated primordia for the July ex-flask was 8.4 ± 0.5 for 'Black Magic' and 8.8 ± 0.4 for 'Treasure', and 9.3 ± 0.3 and 8.5 ± 0.9, respectively, for the November ex-flask. There were no significant differences among
cultivars either after the July ex-flask ($t$-value = 0.6, $df$ = 8, $P$ = 0.5), or the November ex-flask ($t$-value = 0.8, $df$ = 5, $P$ = 0.5).

Changes in the appearance of the leaf primordia were observed as the growth cycle of the plants progressed. Approximately 20 days (~250 °C-days) after the cessation of leaf emergence on 'Black Magic' and 'Treasure' plants ex-flasked in July, a senesced petiole and/or lamina on the outermost leaf primordium was observed on 50% of the dissected buds when the last emerged leaf was removed (arrows in Figure 4A & B; Plate 1). No senesced tissue had been observed at earlier harvests. The percentage of buds with this senesced tissue increased in subsequent dissections for both cultivars, and at 180 days, 100% of the samples exhibited a senesced leaf primordium. For samples ex-flasked in November, 50% of 'Black Magic' buds had dry leaf primordia ~30 days (389.54 °C-days) after the cessation of leaf emergence, while 'Treasure' buds had reached the same condition ~20 days (350 °C-days) after the cessation of leaf emergence (arrows in Figure 5A & B). All the leaf primordia were senesced 9 and 33 days later for 'Black Magic' and 'Treasure', respectively.
Plate 1. Dissected apical bud on a *Zantedeschia* tuber showing a senesced leaf primordium in the outermost position. The primordium was exposed when leaf petioles of expanded leaves were removed.

It should be noted that at any harvest time after both dates of ex-flask, a number of small tubers (usually pea-sized) presented only one or two emerged leaves, a proportion that did not alter as the growth cycle advanced, although the rest of the plants continued to produce 7 or more leaves each (Figure 4 & 5). In general, bud dissections of the pea-sized tubers showed the same number of leaf primordia as the rest of the tubers, but already with a senesced outermost leaf primordium.
3.2 Dormancy Assessment Phase

3.2.1 Dormancy-breaking treatments

In this section, only mean time to emergence, cumulative emergence and percentage emergence are reported. Due to the low number of plants which emerged in some of the treatments (mainly plants not subjected to a storage period or gibberellic acid application), the calculation of T50 and T90-T10 was not possible for each of the treatment combinations. Therefore, both parameters were excluded from this section.

In order to simplify their interpretation, results are grouped according to the significance of the treatments' main effects and interactions. Summary tables detailing results for all treatment combinations are included in Appendix 5, while significance of main factors and interactions are summarized in Table 11.

3.2.1.1 July ex-flask

Plants ex-flasked in July (first cycle) were replanted between November 1999 and February 2000 for the dormancy assessment phase (second cycle), that extended until April-May 2000.

During the second cycle, cumulative emergence of 'Black Magic' and 'Treasure' harvested at 135 and 180 days, respectively, followed a triple sigmoid pattern (Figure 6 A & B). For 'Black Magic', the first phase of the sigmoid plateaued at ~56 days, where ~37 % of the plants had emerged; the second phase plateaued 34 days later, while the last phase plateaued after further 26 days, with a total emergence of 81 %. For 'Treasure', the first phase plateaued after 39 days, when 34 % emergence had been reached; the second phase plateaued 26 days later and the last phase plateaued after further 62 days, when
73% of the plants had emerged. Cumulative emergence of ‘Black Magic’ after the harvests at 120 and 180 days and ‘Treasure’ after the harvest at 150 days presented a double sigmoid pattern (data not shown). At all other harvest times, the pattern was a single sigmoid.

Figure 6. Cumulative emergence of *Zantedeschia* cv. ‘Black Magic’ (A) and of ‘Treasure’ (B) harvested at 135 and 180 days, respectively. July date of ex-flask. Values represent only the controls (gibberellic acid and storage treatments were not included).

### 3.2.1.1.1 Time to harvest and cultivars

Time to emergence during the second cycle increased as time to harvest increased, reaching a peak at 150 days (Table 1). A second increase was observed at 180 days. Irrespective of the cultivar, differences between harvest dates were significant ($P <$
However, there was a difference of only 15 days between the longest and the shortest time to emerge. ‘Black Magic’ took, on average, 23 days longer to emerge than ‘Treasure’ \((P < 0.05; \text{Table } 1)\), irrespective of the harvest date.

### Table 1. Time to emergence (days) and emergence (%) of *Zantedeschia* cvs. ‘Black Magic’ and ‘Treasure’ following sequential harvests. July ex-flask date.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time to emergence (days ± sem)</th>
<th>Emergence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cultivar</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black Magic</td>
<td>(57.5 \pm 0.8^z)</td>
<td>86.0^y</td>
</tr>
<tr>
<td>Treasure</td>
<td>(34.5 \pm 0.6)</td>
<td>87.8</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td><strong>Time to harvest (days from the 1st cycle)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>(36.6 \pm 1.4^x)</td>
<td>91.6^w</td>
</tr>
<tr>
<td>135</td>
<td>(44.9 \pm 1.3^c)</td>
<td>89.2</td>
</tr>
<tr>
<td>150</td>
<td>(50.5 \pm 1.2)</td>
<td>81.8</td>
</tr>
<tr>
<td>165</td>
<td>(46.6 \pm 1.2)</td>
<td>80.1</td>
</tr>
<tr>
<td>180</td>
<td>(51.5 \pm 1.2)</td>
<td>91.8</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td><strong>Significance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>C</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>H x C</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^z^\) Values are means of 1257 samples and are averaged across harvests, storage and gibberellic acid treatments.

\(^y^\) Values are means of 180 samples and are averaged across harvests, storage and gibberellic acid treatments. No standard errors and mean separation tests are presented due to the non-normal distribution of the data.

\(^x^\) Values are means of 503 samples and are averaged across cultivars, storage and gibberellic acid treatments.

\(^w^\) Values are means of 72 samples and are averaged across cultivars, storage and gibberellic acid treatments. No standard errors and mean separation tests are presented due to the non-normal distribution of the data.

\(^y^\) Means followed with the same letters are not significantly different according to the Least Significant Difference test (LSD).

\(^u^\) Statistical significance of effects in the ANOVA and Generalised Linear Model are indicated as follows: C, cultivar; H, time to harvest; H x C, harvest by cultivar interaction. NS, not significant; +, significant at \(P< 0.10\); *, significant at \(P< 0.05\); **, significant at \(P< 0.01\).

Mean percentages of emergence ranged between 80.1% and 91.8% (Table 1). Although differences between harvest times were not significant, percentage of
emergence declined as time to harvest increased, plateauing between 150 and 165 days before rising again following the harvest at 180 days. Differences between cultivars and the interaction between cultivars and harvest dates were not significant. Therefore, mean values across cultivars and harvests are presented in Table 1.

3.2.1.1.2 Storage

The effect of storage duration on time to emergence in the second cycle depended on time to harvest and on the cultivars ($P < 0.05$). For 'Black Magic', storage reduced time to emergence by 12 and 22 days at harvests at 135 and 180 days, respectively (Figure 7A). In contrast, it delayed emergence by 7 and 16 days at harvests at 120 and 150 days, and had no effect at harvest at 165 days. Storage reduced time to emergence of 'Treasure' only after 180 days, where a difference of 15 days between storage treatments was observed (Figure 7B). With all other harvests, storage increased time to emergence of 'Treasure'.
Figure 7. Time to emergence (days) of *Zantedeschia* cvs. ‘Black Magic’ (A) and ‘Treasure’ (B) following sequential harvests and storage for 0 or 3 weeks. July ex-flask date.

Values are mean of 126 samples each. Standard error of the mean is indicated by vertical lines.

Irrespective of time to harvest, percentage of emergence after the storage treatments varied with cultivars ($\chi < 0.05$). Storage increased emergence of ‘Black Magic’ by 24.6% compared with unstored tubers, while it increased emergence of ‘Treasure’ by 12.4% (Table 2). As a result, across all harvest dates, emergence of ‘Black Magic’ after storage was ~4% higher than of ‘Treasure’, although it was ~8% lower if tubers were not stored.
Table 2. Emergence (%) of *Zantedeschia* cvs. ‘Black Magic’ and ‘Treasure’ following sequential harvests and storage for 0 or 3 weeks. July ex-flask date.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Storage (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Black Magic</td>
<td>73.7&lt;sup&gt;z&lt;/sup&gt;</td>
</tr>
<tr>
<td>Treasure</td>
<td>81.6</td>
</tr>
</tbody>
</table>

Time to harvest (days from the 1<sup>st</sup> cycle)

<table>
<thead>
<tr>
<th>Time to harvest</th>
<th>Storage (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>85.3&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>135</td>
<td>84.7</td>
</tr>
<tr>
<td>150</td>
<td>70.3</td>
</tr>
<tr>
<td>165</td>
<td>63.9</td>
</tr>
<tr>
<td>180</td>
<td>84.0</td>
</tr>
</tbody>
</table>

Significance<sup>x</sup>

- **S**
- **C**
- **H**
- **S x C**
- **S x H**
- **S x H x C**

<sup>z</sup> Values are means of 90 samples and are averaged across harvests and gibberellic acid. No standard errors and mean separation tests are presented due to the non-normal distribution of the data.

<sup>y</sup> Values are means of 36 samples and are averaged across cultivars and gibberellic acid.

<sup>x</sup> Significance refers to statistical significance of effects in the generalised linear model, as indicated in Table 1. Additionally, the following symbols are included: **S**, storage; **S x C**, storage by cultivar interaction; **S x H**, storage by harvest interaction; **S x H x C**, storage by harvest by cultivar interaction.

Irrespective of the cultivar, the interaction between storage and time to harvest was also significant (*χ<sub>2</sub> < 0.05*) (Table 2). Although storage increased emergence to over 90% after all harvests, highest improvements occurred at harvest times where unstored tubers had lower emergence. For example, storage increased emergence by 32.5% when plants were harvested at 165 days, where only 64% of the untreated plants emerged. In contrast, it increased emergence by only 12.6% at 120 days, where 85% of the unstored plants emerged. Highest percentage emergence after the storage treatment was observed in tubers harvested at 180 days.
3.2.1.1.3 Gibberellic acid

Irrespective of the cultivar, time to emergence was significantly reduced with the application of 100 mg·L⁻¹ of gibberellic acid, compared with 0 mg·L⁻¹ (P<0.05). However, the magnitude of the reduction depended on the date of harvest (P<0.05). As time to harvest increased, the effect of gibberellic acid on emergence declined. For example, 100 mg·L⁻¹ of gibberellic acid reduced time to emergence by 17 days following the harvest at 120 days, but they only reduced time to emergence by 4 days following the harvest at 180 days (Table 3). The shortest time to emergence occurred in tubers treated with 100 mg·L⁻¹ gibberellic acid after the harvest at 120 days.

Table 3. Time to emergence (days ± sem) of *Zantedeschia* cvs. ‘Black Magic’ and ‘Treasure’ following sequential harvests and a pre-planting dip of 0 or 100 mg·L⁻¹ gibberellic acid. July ex-flask date.

<table>
<thead>
<tr>
<th>Time to harvest (days from the 1st cycle)</th>
<th>Gibberellic acid concentration (mg·L⁻¹)</th>
<th>0</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>45.7 ± 2.2</td>
<td>28.5 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>135</td>
<td>49.8 ± 1.9</td>
<td>40.6 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>54.5 ± 1.8</td>
<td>46.9 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>165</td>
<td>49.7 ± 1.6</td>
<td>43.9 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>53.7 ± 1.7</td>
<td>49.3 ± 1.6</td>
<td></td>
</tr>
</tbody>
</table>

Significance:
- C
- H
- G
- H x G
- G x C

<table>
<thead>
<tr>
<th>Significance</th>
<th>0</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>H</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>H x G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G x C</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

Significance refers to statistical significance of effects, as indicated in Table 1. Additionally, the following symbols are included: G, gibberellic acid; H x G, harvest by gibberellic acid interaction; G x C, gibberellic acid by cultivar interaction.

Y Values are means of 251 samples and are averaged across cultivars and storage.

The interaction between gibberellic acid concentration and storage was not significant.
Application of 100 mg·L⁻¹ gibberellic acid significantly increased percentage emergence compared with the control ($\chi < 0.05$). However, the magnitude of the increase depended on the cultivar ($\chi < 0.05$). When 100 mg·L⁻¹ of gibberellic acid were applied, emergence of 'Treasure' increased ~12% over that of the control, while emergence of Black Magic increased only ~6% (Table 4). The effect of gibberellic acid on percentage emergence was independent of the storage treatments and time to harvest.

Table 4. Emergence (%) of *Zantedeschia* cvs. ‘Black Magic’ and ‘Treasure’ following a pre-planting dip of 0 or 100 mg·L⁻¹ gibberellic acid. July ex-flask date.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Gibberellic acid concentration (mg·L⁻¹)</th>
<th>0</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Magic</td>
<td>83.0$^Y$</td>
<td>88.9</td>
<td></td>
</tr>
<tr>
<td>Treasure</td>
<td>81.6</td>
<td>94.0</td>
<td></td>
</tr>
</tbody>
</table>

Significance$^z$

<table>
<thead>
<tr>
<th>C</th>
<th>G</th>
<th>C x G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>$^*</td>
<td>$^**</td>
<td>$^*</td>
</tr>
</tbody>
</table>

$^z$ Significance refers to statistical significance of effects in the generalised linear model, as explained in Table 2.

$^Y$ Values are means of 90 samples and averaged across harvests and storage. No standard errors or mean separation tests are presented due to the non-normal distribution of the data.

Although the statistical analysis did not detect a significant interaction between harvest dates and gibberellic acid concentration, the effect of the latter on emergence of 'Black Magic' tended to change with time to harvest. Application of 100 mg·L⁻¹ gibberellic acid increased percentage of emergence to ~91% at 135 days, but was not effective at 165 days, where only ~55% of the plants emerged (Table 5). In contrast, it increased percentage of emergence of ‘Treasure’ to over 85% at any of the harvest times.
Table 5. Emergence (%) of *Zantedeschia* cvs. 'Black Magic' and 'Treasure' following sequential harvests and a pre-planting dip of 0 or 100 mg·L⁻¹ gibberellic acid. July ex-flask date.

<table>
<thead>
<tr>
<th>Harvest date (days from the 1st cycle)</th>
<th>Cultivar</th>
<th>Black Magic</th>
<th></th>
<th></th>
<th></th>
<th>Treasury</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gibberellic acid (mg·L⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td>Gibberellic acid (mg·L⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td></td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>120</td>
<td></td>
<td>70.8&lt;sup&gt;y&lt;/sup&gt;</td>
<td>84.3</td>
<td>90.7</td>
<td>95.6</td>
<td></td>
<td>90.7</td>
<td>95.6</td>
<td>91.5</td>
</tr>
<tr>
<td>135</td>
<td></td>
<td>81.5</td>
<td>90.5</td>
<td>75.4</td>
<td>91.5</td>
<td></td>
<td>75.4</td>
<td>91.5</td>
<td>85.5</td>
</tr>
<tr>
<td>150</td>
<td></td>
<td>57.1</td>
<td>74.6</td>
<td>64.0</td>
<td>85.5</td>
<td></td>
<td>64.0</td>
<td>85.5</td>
<td>85.2</td>
</tr>
<tr>
<td>165</td>
<td></td>
<td>55.2</td>
<td>54.5</td>
<td>60.3</td>
<td>85.2</td>
<td></td>
<td>60.3</td>
<td>85.2</td>
<td>85.2</td>
</tr>
<tr>
<td>180</td>
<td></td>
<td>77.8</td>
<td>90.2</td>
<td>76.6</td>
<td>91.3</td>
<td></td>
<td>76.6</td>
<td>91.3</td>
<td>91.3</td>
</tr>
</tbody>
</table>

Significance<sup>z</sup>

- **H**
- **G** NS
- **H x G** NS
- **H x G x C** NS

<sup>z</sup> Significance refers to statistical significance of effects in the generalised linear model, as explained in Table 1.

<sup>y</sup> Values are means of 9 samples and represent samples without storage treatment. No standard errors or mean separation tests are presented due to the non-normal distribution of the data.

### 3.2.1.2 November ex-flask

In contrast to the July date of ex-flask, cumulative emergence of 'Black Magic' and 'Treasure' harvested at 180 days followed a double sigmoid pattern during the second cycle (Figure 8). For 'Black Magic', the first phase plateaued at 80 days, when 54% of the population had emerged, while the second phase plateaued ~40 days later (Figure 8A).
Figure 8. Cumulative emergence of *Zantedeschia* cv. 'Black Magic' (A) and 'Treasure' (B) harvested at 180 days after the November date of ex-flask.

Values represent only the controls (gibberellic acid and storage treatments were not included).

For 'Treasure', the first phase plateaued at ~80 days, where 51% of the plants had emerged, while the second phase plateaued 45 days later (Figure 8B). A similar pattern was also observed after harvests at 165 days for both cultivars (data not shown). After the rest of the harvests, emergence followed a single sigmoid pattern.

Time to, and percentage emergence of *Zantedeschia* plants ex-flasked in November followed a similar pattern during their second cycle to those ex-flasked in July (Table 1; Table 6 & Table 7). However, as will be detailed in this section, time to
emergence was longer, and percentage emergence slightly lower, than as observed in the July ex-flask.

3.2.1.2.1 Cultivars and harvest dates

Plants ex-flasked in November had a slower emergence compared to the July ex-flask, with 'Black Magic' and 'Treasure' taking 6 and 20 days longer to emerge, respectively. Mean time to emergence ranged between 48 and ~68 days (Table 6).

Table 6. Time to emergence (days) of Zantedeschia cvs. 'Black Magic' and 'Treasure' following sequential harvests. November ex-flask date.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time to emergence (days ± sem)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td></td>
</tr>
<tr>
<td>Black Magic</td>
<td>63.9 ± 0.8</td>
</tr>
<tr>
<td>Treasure</td>
<td>54.0 ± 0.8</td>
</tr>
<tr>
<td>LSD0.05</td>
<td>2.6</td>
</tr>
<tr>
<td>Time to harvest (days from 1\textsuperscript{st} cycle)</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>48.0 ± 1.4 e worthless</td>
</tr>
<tr>
<td>135</td>
<td>55.0 ± 1.3 d</td>
</tr>
<tr>
<td>150</td>
<td>67.9 ± 1.2 a</td>
</tr>
<tr>
<td>165</td>
<td>63.2 ± 1.1 b</td>
</tr>
<tr>
<td>180</td>
<td>59.1 ± 1.1 c</td>
</tr>
<tr>
<td>LSD0.05</td>
<td>3.3</td>
</tr>
<tr>
<td>Significance\textsuperscript{x}</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>**</td>
</tr>
<tr>
<td>C</td>
<td>**</td>
</tr>
<tr>
<td>H x C</td>
<td>NS</td>
</tr>
</tbody>
</table>

\textsuperscript{x} Values are means of 1124 samples and are averaged across harvests, storage and gibberellic acid treatments.

\textsuperscript{y} Values are means of 453 samples and are averaged across cultivars, storage and gibberellic acid treatments.

\textsuperscript{x} Significance refers to statistical significance of effects in the ANOVA, as indicated in Table 1.

Irrespective of the cultivar, there were significant differences in days to emergence between harvest dates following the November ex-flask date ($P < 0.05$). Days to
emergence increased as time to harvest progressed, decreasing again after reaching a peak at 150 days (Table 6). Time to emergence increased by 20 days between the harvest at 120 days and the peak, subsequently decreasing by only 9 days following the harvest at 180 days. Across all harvests, 'Black Magic' took in average 10 days longer to emerge than 'Treasure' \((P < 0.05)\) (Table 6).

Percentages of emergence of plants in the second cycle, following the November ex-flask, were in average only 2% lower than those following the July ex-flask. Averaged across cultivars, mean percentages of emergence ranged between \(~79\%\) and 91% (Table 7).

**Table 7. Emergence (%) of *Zantedechia* cvs. 'Black Magic' and 'Treasure' following sequential harvests. November ex-flask date.**

<table>
<thead>
<tr>
<th>Harvest date (days from 1st cycle)</th>
<th>Cultivars</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black Magic</td>
<td>Treasure</td>
</tr>
<tr>
<td>120</td>
<td>83.2(^z)</td>
<td>94.4</td>
</tr>
<tr>
<td>135</td>
<td>76.1</td>
<td>87.8</td>
</tr>
<tr>
<td>150</td>
<td>71.4</td>
<td>86.8</td>
</tr>
<tr>
<td>165</td>
<td>83.0</td>
<td>86.4</td>
</tr>
<tr>
<td>180</td>
<td>93.2</td>
<td>89.0</td>
</tr>
</tbody>
</table>

Significance \(^z\)

<table>
<thead>
<tr>
<th></th>
<th>**</th>
</tr>
</thead>
<tbody>
<tr>
<td>(H)</td>
<td>**</td>
</tr>
<tr>
<td>(C)</td>
<td>**</td>
</tr>
<tr>
<td>(H \times C)</td>
<td>**</td>
</tr>
</tbody>
</table>

\(^z\) Values are means of 36 samples and are averaged across storage and gibberellic acid treatments. No standard errors or mean separation tests are presented due to the non-normal distribution of the data.

\(^Y\) Significance refers to statistical significance of effects in the generalised linear model, as indicated in Table 1.

Percentage emergence declined as time to harvest increased, recovering after a minimum at 150 to 165 days. Differences between harvest dates were significant, but their effect on emergence was cultivar dependent \((\chi < 0.05; \text{Table 7})\). For instance, emergence
of 'Black Magic' was significantly lower than Treasure between harvests at 120 and 150 days inclusive, with a maximum difference of ~15 % at the latter date. In contrast, emergence of 'Black Magic' was 4 % higher than 'Treasure' at 180 days.

3.2.1.2.2 Storage

The effect of storage on time to emergence depended on the harvest date ($P < 0.05$; Figure 9), and was not influenced by cultivars. Storage reduced time to emergence following harvests at 150, 165 and 180 days by 23, 8 and 18 days, respectively. Prior to 150 days, stored tubers took in average 26 days longer to emerge than unstored tubers.

![Figure 9. Time to emergence (days) of Zantedeschia following sequential harvests and storage for 0 or 3 weeks. November ex-flask date.](image)

Values are means of 230 samples each and are averaged across cultivars, storage and gibberellic acid treatments. Standard error of the mean is indicated by vertical lines.

Across cultivars, storage of the tubers significantly increased percentage of emergence ($\chi < 0.05$), with stored tubers achieving an emergence in excess of 89 % at all
harvests (Table 8). In contrast, emergence of unstored tubers ranged between 61 % and ~88%. The magnitude of the improvement of storage on percentage emergence depended on the harvest date ($\chi < 0.05$); highest improvements occurred at harvests where tubers had lower emergence. For example, storage of the tubers increased percentage emergence over unstored tubers by only ~1% when harvested at 120 days, but it improved emergence by ~ 36% after the harvest at 150 days.

Table 8. Emergence (%) of Zantedeschia cv. ‘Black Magic’ and ‘Treasure’ following sequential harvests and storage for 0 or 3 weeks. November ex-flask date.

<table>
<thead>
<tr>
<th>Time to harvest (days from 1st cycle)</th>
<th>Storage (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>0</td>
</tr>
<tr>
<td>135</td>
<td>88.1$^Y$</td>
</tr>
<tr>
<td>150</td>
<td>70.1</td>
</tr>
<tr>
<td>165</td>
<td>60.7</td>
</tr>
<tr>
<td>180</td>
<td>69.8</td>
</tr>
<tr>
<td></td>
<td>83.4</td>
</tr>
</tbody>
</table>

Significance:
- **S**
- **H**
- **H x S**

$^Y$ Values are means of 36 samples and are averaged across cultivars and gibberellic acid treatments. No standard errors and mean separation tests are presented due to the non-normal distribution of the data.

3.2.1.2.3 Gibberellic acid

Application of 100 mg·L$^{-1}$ gibberellic acid significantly reduced time to emergence compared with 0 mg·L$^{-1}$ ($P < 0.05$), but its effect depended on the storage treatment ($P < 0.05$; Table 9). If tubers had not been stored, 100 mg·L$^{-1}$ gibberellic acid reduced time to emergence by 7 days. However, if tubers had been stored for three weeks, 100 mg·L$^{-1}$ gibberellic acid reduced days to emergence by only ~4 days. There was no significant interaction between gibberellic acid concentration and cultivars nor harvest dates.
Table 9. Time to emergence (days ± sem) of *Zantedeschia* following storage for 0 or 3 weeks and a pre-planting dip with 0 or 100 mg·L⁻¹ GA₃. November ex-flask date.

<table>
<thead>
<tr>
<th>Storage (weeks)</th>
<th>Gibberellic acid concentration (mg·L⁻¹)</th>
<th>0</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>62.4 ± 0.9</td>
<td>55.5 ± 0.9</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>60.9 ± 1.4</td>
<td>57.4 ± 1.3</td>
</tr>
</tbody>
</table>

Significance:
- S
- G
- S x G

*Significance refers to statistical significance of effects in the ANOVA, as indicated in Table 1.

Irrespective of the cultivar, there was an interaction between gibberellic acid concentration and storage duration on percentage emergence ($\chi < 0.05$; Table 10). If tubers had been stored, the application of 100 mg·L⁻¹ gibberellic acid reduced emergence by ~1%. However, if tubers had not been stored, 100 mg·L⁻¹ gibberellic acid increased the percentage of emerged tubers by ~14%. No significant differences were found between gibberellic acid concentrations, indicating that changes in the interaction were mainly due to effects of storage duration. In addition, there was no significant interaction between storage duration, time to harvest and gibberellic acid concentration.

Table 10. Emergence (%) of *Zantedeschia* following storage for 0 or 3 weeks and a pre-planting dip with 0 or 100 mg·L⁻¹ gibberellic acid. November ex-flask date.

<table>
<thead>
<tr>
<th>Storage (weeks)</th>
<th>Gibberellic acid concentration (mg·L⁻¹)</th>
<th>0</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>67.7</td>
<td>81.6</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>96.1</td>
<td>95.2</td>
</tr>
</tbody>
</table>

Significance:
- S
- G
- S x G

*Significance refers to statistical significance of effects in the generalised linear model, as indicated in Table 1.

Values are means of 90 samples and are averaged across cultivars and harvests. No standard errors and mean separation tests are presented due to the non-normal distribution of the data.
There was no significant interaction between gibberellic acid concentration and cultivars and/or harvest dates. However, a closer examination of the means for each cultivar and gibberellic acid concentration showed a variation in the emergence of treated 'Black Magic' tubers with harvest time. For example, percentage emergence of tubers treated with 100 mg·L⁻¹ gibberellic acid was ~91% at the harvest at 120 days, but only ~53% at the harvest at 150 days. In contrast, application of 100 mg L⁻¹ gibberellic acid on 'Treasure' resulted in percentage of emergence over 75% after all harvest times.

3.2.1.3 Dates of ex-flask

Significance of the main effects and of the interactions varied between dates of ex-flask and also between parameters measured (Table 11). For example, there was an interaction between harvest times and cultivars for percentage of emergence following the November date of ex-flask, but it was not significant following the July date of ex-flask. Conversely, the interaction between cultivars and gibberellic acid concentration was significant after the July date of ex-flask, but not after the November date of ex-flask.

For the parameter 'time to emergence', detection of significant effects and their interactions also varied between dates of ex-flask. While main effects were the same for both ex-flask dates, differences were evident with 2nd order interactions.
Table 11. Summary table of sources of variation and interactions for days to emergence and percentage of emergence. July and November ex-flask dates.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>July Time to emergence</th>
<th>July Percentage emergence</th>
<th>November Time to emergence</th>
<th>November Percentage emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
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<td>G</td>
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<td>H x C</td>
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<td>H x S</td>
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<tr>
<td>C x G</td>
<td>NS</td>
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<td>NS</td>
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<td>C x S</td>
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<td>S x G</td>
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</tr>
</tbody>
</table>

Significance refers to statistical significance of effects in the ANOVA, as indicated in Table 1.

Significance refers to statistical significance of effects in the generalised linear model, as explained in Table 1.

3.2.2 Prediction of dormancy using degree-days

In sections 3.2.1.1 and 3.2.1.2 it was noted that time to emergence was highly variable, not only among dates of harvest, but also between dates of ex-flask. As will be explained in the discussion, this parameter was apparently influenced by other factors besides the level of dormancy of the buds, which may have masked the progression of dormancy. For this reason, time to emergence was considered less representative of the level of dormancy compared with percentage of emergence, and was excluded from this section.

The inclusion of dormancy-breaking treatments in the dormancy assessment phase could also have masked the evolution of dormancy to some extent. To avoid this situation and emphasize changes in the depth of dormancy through time, only percentages of
emergence of the controls were used in this analysis. Given that onset and full release of dormancy could not be determined (i.e. neither of the cultivars reached 100% emergence after any of the harvest times), degree-days were tested as predictors of the occurrence of deepest dormancy of *Zantedeschia*. It should be noted that it was not possible to fit a curve to the individual percentages of emergence, due to high variability between samples. Instead, a curve was fitted to the mean values of the harvest times.

Percentage of emergence of plants ex-flasked in either July or November followed a similar pattern as that described in sections 3.2.1.1 and 3.2.1.2.1; it initially decreased until reaching a plateau, increasing again thereafter. For example, percentage of emergence of 'Black Magic' after the July date of ex-flask decreased by ~24% between harvests at 135 and 150 days, increasing again by ~23% between harvests at 165 and 180 days (Figure 10). Lowest emergence was estimated to occur at ~158 days ($r^2=0.99$; $P<0.05$) (Figure 12A). Similarly, a reduction of ~43% in percentage emergence was observed between harvests at 120 and 150 days, following the November date of ex-flask (Figure 10). Emergence subsequently increased by ~18% between 165 and 180 days. Lowest emergence was estimated to occur at ~148 days ($r^2=0.96$; $P<0.05$) (Figure 12A).
Figure 10. Percentage of emerged tubers cv. 'Black Magic' harvested at increasing periods of time (days) from date of ex-flask (July [■] or November [△] 1999). Vertical bars denote ± SE. Values are means of 9 samples.

Figure 11. Percentage of emerged tubers cv. 'Treasure' harvested at increasing periods of time (days) from date of ex-flask (July [■] or November [△] 1999). Vertical bars denote ± SE. Values are means of 9 samples.
Changes in emergence of 'Treasure' generally followed the same pattern as 'Black Magic'. Percentage of emergence following the July date of ex-flask decreased by ~32% between the harvests at 120 and 150 days, increasing by ~16% between 165 and 180 days (Figure 11). Lowest emergence was estimated to occur at ~157 days ($r^2=0.98$; $P<0.05$) (Figure 12B). Similarly, percentage of emergence after the November ex-flask declined by ~26% between harvests at 120 and 150 days, and increased again by ~18% between the harvests at 165 and 180 days (Figure 11). Lowest emergence occurred at ~154 days ($r^2=0.97$; $P<0.05$) (Figure 12B).
Figure 12. Percentage emergence (%) of *Zantedeschia* cv. 'Black Magic' (A) and 'Treasure' (B), following sequential harvests (days from ex-flask). July (■) and November (△) dates of ex-flask. Fitted equations for 'Black Magic' are \( y = 1362.3 - 16.5x + 0.05x^2 \) and \( y = 1127.3 - 14.7x + 0.05x^2 \), for the July and November dates of ex-flask, respectively. Fitted equations for 'Treasure' are \( y = 693.3 - 8.1x + 0.023x^2 \) and \( y = 640.1 - 7.5x + 0.03x^2 \), for the July and November dates of ex-flask, respectively. Arrows show estimated point of deepest dormancy. Values are means of 9 samples.

Changing the time scale to degree-days, it was estimated that lowest percentages of emergence of 'Black Magic' occurred at 2614.1 °C-days when ex-flasked in July \((r^2=0.99; P<0.01)\) (Figure 13A), and at 2731.9 °C-days when ex-flasked in November.
In contrast, lowest emergence of 'Treasure' was estimated to occur at 2681.3 °C-days when ex-flasked in July ($r^2=0.99; P<0.01$) (Figure 13B), and at 2839.1 °C-days after the November ex-flask ($r^2=0.96; P<0.05$).

Figure 13. Percentage emergence (%) of Zantedeschia cv. 'Black Magic' (A) and 'Treasure' (B), following sequential harvests (Degree-days from ex-flask). July (■) and November (▲) dates of ex-flask. Fitted equations for ‘Black Magic’ are $y = 1162.2-0.8x + 0.6E-5x^2$ and $y = 1230.3-0.9x+1.6E-4x^2$, for the July and November dates of ex-flask, respectively. Fitted equations for ‘Treasure’ are $y = 616.1-0.4x+8.3E-5x^2$ and $y = 687.2-0.4x+7.8E-5x^2$, for the July and November dates of ex-flask, respectively. Arrows show estimated point of deepest dormancy. Values are means of 9 samples.
4. DISCUSSION

4.1 Growth cycle.

4.1.1 Leaf emergence.

Irrespective of the cultivar, plants ex-flasked in November did not present an initial lag phase. It has been found that the duration of the period between establishment and leaf lamina expansion of Zantedeschia after planting is temperature dependent, with a shorter period occurring at warmer temperatures (Warrington & Southward, 1989). Given that greenhouse temperatures in the present experiment were ~5 °C higher during November compared with July, it is possible that plantlets took a shorter time to establish after the November ex-flask, resuming growth shortly after being ex-flasked.

Cessation of leaf emergence occurred approx. 40 days earlier in plants ex-flasked in November than in those ex-flasked in July. This could be related to higher temperatures recorded after the November ex-flask date, which reflected, as will be explained in section 4.2.3, in a larger quantity of degree-days accumulated for the same amount of calendar days. As a consequence of the shorter period of leaf production after the November ex-flask date, 'Black Magic' formed one leaf less after the November ex-flask compared with the July ex-flask, while 'Treasure' formed three leaves less. This difference shows that cessation of leaf appearance occurred irrespective of the number of leaves expanded by the plant. This is in agreement with previous studies, where it was determined that cessation of leaf emergence does not occur as a result of the exhaustion of a limited number of pre-formed leaves present at planting (Funnell, 1988). In onion plants, leaf production is terminated when bulbing occurs, event that is triggered when plants are
exposed to a combination of 600 °C-days and long photoperiod (Lancaster et al., 1996). When onion plants were sown earlier in the season, they had a longer period to produce leaves before bulbing was initiated. A similar mechanism could be valid for Zantedeschia plants: the occurrence of a factor or combination of factors yet unidentified could induce cessation of leaf emergence. If this factor(s) occur(s) later during the period of growth, then plants would produce leaves for a longer period.

4.1.2 Leaf primordia.

Previous studies determined that the apical meristem of non-flowering Zantedeschia tubers entered a period of inactivity for at least three months, where no further initiation or development of primordia were observed (Halligan et al., 1994; 1995). Opposite results were obtained in the present study, since no arrest of leaf differentiation on the apical meristem was observed, after either of the ex-flasking dates. In a strict sense, this would imply that Zantedeschia buds did not become dormant after cessation of leaf emergence, since this stage implies the suspension of visible growth (Lang et al., 1987). However, the experiments by Halligan et al. (1994 & 1995) were carried out outdoors, where lower temperatures during autumn (when arrest of primordia differentiation was observed) may have been potentially limiting for growth. In addition, Lavée (1973) emphasised that dormancy does not necessarily involve a cessation of biological development, as it is known that important differentiation processes may occur normally in dormant organs, allowing a slow but steady increase in bud weight. For instance, buds of several species are known to continue differentiating and increasing in size throughout dormancy, such as apple (Bubán & Faust, 1995) and Nerine sp. Herb. (Vishnevetsky et al., 1997). Hence, Zantedeschia plants could be considered as dormant once cessation of leaf emergence has occurred.
4.2 Dormancy assessment phase

4.2.1 Dormancy level - population differences

Cumulative emergence of *Zantedeschia* in the present experiment followed a double or even triple sigmoid pattern after several of the harvest times (Figure 6 & Figure 8). This pattern contrasts with the single-sigmoid pattern generally observed in emergence of seeds and bud break (e.g. Bianco et al., 1994; Dokoozlian, 1999), and suggests the presence of more than one population of tubers with different levels of dormancy within a sample. Differences in the degree of dormancy between and within populations, and even individuals, are common to seeds of most plant species, serving as a survival strategy (Gutterman, 2000). The variations are mostly caused by a combination of the microenvironment experienced by the seed due to its position on the parent plant and the abiotic environment of the plant (temperature, day length, water availability, etc). Likewise, it is known that the dormancy patterns of buds in a number of woody species are related to the position of the bud in the plant, or even to its position in the shoot (Saure, 1985).

Although plants from tissue culture are multiplied by means of vegetative propagation, several factors may have altered the degree of dormancy of the plantlets, such as differences in the position of the buds on the tuber from where the ex-plants were made, or changes in the conditions to which plantlets were subjected during the in-vitro period.

There is no previous evidence of the effect of differences in the environment and the culture media on dormancy of ex-flask *Zantedeschia*. However, this hypothesis is also supported by the observation that some of the ex-flasked plantlets in the present experiment only developed at most two leaves and then entered dormancy, while the rest of the plantlets developed normally (refer to section 3.1.2). In addition, it has been noted

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3 The term dormancy refers indistinctly to endo- or paradormancy.
that factors during transplanting, such as the disturbance of the roots, may induce the premature onset of dormancy (Cohen, 1981). Further studies are required to determine the effect of the conditions to which the ex-flask material was subjected during propagation on the plants' final performance and progression of bud dormancy.

Given that the present study only dealt with ex-flask material, it would be of interest to determine if more than one population within a group of plants also occurs in flowering-sized tubers. It is possible that grading of the tubers to an even size would remove this source of variation.

4.2.2 Dormancy-breaking treatments

4.2.2.1 Harvest dates and cultivars

Progressive changes in both percentage and time to emergence observed in the present experiment can be interpreted as indicating that the depth of dormancy of *Zantedeschia* buds was not uniform, but changed throughout the growth cycle examined. After its initiation (which was not observed), dormancy deepened until reaching a maximum between 150 and 165 days of growth, and became progressively shallower from then on. As indicated in the reviews by Saure (1985) and Faust et al. (1997), intensity or depth of dormancy is known to fluctuate with time, initially increasing and then gradually decreasing as time progresses, even if environmental conditions remain constant. This has also been observed in previous studies with *Zantedeschia*, which reported that the depth of dormancy of *Zantedeschia* plants changed during growth (Halligan et al., 1995; Brooking et al., 1998). For example, tubers lifted at 100 days and replanted immediately after curing did not sprout for 42 days, and only ~60 % of the plants showed vigorous
sprout growth when checked ~63 days later (Brooking et al., 1998). In contrast, tubers lifted at ~200 days were able to sprout within 21 days of replanting.

It should be noted that percentages of emergence did not vary significantly with harvest time after the July ex-flask (Table 1), although differences were evident after the November ex-flask (Table 7). In a strict sense, this could imply that plants ex-flasked in July did not present a period of dormancy or, if they did, that it was very shallow. However, changes in days to emergence (Table 1 & Table 6) and percentage emergence of the pooled treatments and in percentage emergence of the controls (section 3.2.2) with harvest time, together with the positive response to dormancy-breaking treatments, indicate that plants ex-flasked in July did become dormant. Moreover, if it is taken into account that 21 days are considered by the industry as the standard time to emergence of non-dormant tubers (Funnell, Pers. comm.), then clearly some degree of dormancy was present in the tubers at all harvest times. As previously mentioned in section 3.2.2, the inclusion of the dormancy-breaking treatments in the analysis may have concealed the progression of dormancy through time.

Averaged across dates of ex-flask, ‘Black Magic’ took 16 days longer to emerge than ‘Treasure’. In addition, percentage emergence of the former was 8% lower than that of the latter after the November date of ex-flask. Differences between cultivars were expected, since ‘Black Magic’ was reported to take in average 46 days longer to emerge than ‘Treasure’ (Halligan & Fulton, 1998). Halligan & Fulton (1998) attributed their results to the presence of a period of “deep dormancy” in ‘Black Magic’, and its absence in ‘Treasure’, which would have allowed ‘Treasure’ plants to resume growth shortly after being replanted. Results of the present study confirm the existence of a dormant period in
'Black Magic', as previously reported by Halligan et al. (1995) and Halligan & Fulton (1998). However, Halligan & Fulton's statement that 'Treasure' lacks a period of dormancy is not supported in the current experiment. Days to emergence and percentage of emergence in the present experiment varied with harvest time (Table 1, Table 6 & Table 7), indicating that plants were not equally able to resume growth through that part of the growth cycle examined. In addition, emergence of 'Treasure' was improved with the storage and gibberellic acid treatments, showing that a percentage of plants were still potentially able to sprout once dormancy was broken. As pointed out by Amling & Amling (1980), the fact that a bud is able to grow within an arbitrary period under temperatures suitable for growth, does not imply the release from, or in this case, lack of dormancy. It only reflects a low enough depth of dormancy to permit bud break. Thus, further reduction in dormancy can be accomplished by additional dormancy-breaking treatments. Moreover, the conclusions in Halligan & Fulton's study were only based on days to emergence, and overlooked the fact that percentage of emergence of 'Treasure' was under 50% for two of the three harvests that they reported.

'Black Magic' plants ex-flasked in November took 6 days longer to emerge in their second cycle than those ex-flasked in July, while 'Treasure' took 20 days more. Percentage of emergence of 'Black Magic' was also 5% lower for plants ex-flasked in November compared with plants ex-flasked in July. Two possible reasons can be presented in an attempt to explain this difference between dates of ex-flask. Firstly, the lower emergence of the plants on the second cycle after the November ex-flask can be related to the greenhouse conditions during growth. Daily average temperatures within the greenhouse were 5 °C lower during the second cycle for plants originally ex-flasked in November, compared with those ex-flasked in July. This may have influenced the level of
dormancy of the tubers. Turnbull & Hanke (1985) and Wiltshire & Cobb (1996) illustrated that temperature is considered as the most important physical factor affecting dormancy. In deciduous fruit trees, low temperatures during dormancy may hasten development (Saure, 1985). Conversely, within a range of 3 to 20 °C, potato tubers stored at lower temperatures have a longer period of dormancy than those stored at higher temperatures (Vegis, 1964; Wiltshire & Cobb, 1996). However, storage of Zantedeschia tubers either at 10 or 20 °C did not show any difference in the time to break dormancy (Brooking, pers. comm.). Hence, it is unlikely that temperature differences between dates of ex-flask in either the first or second growth cycle had an influence in the release of dormancy of Zantedeschia tubers in the experiment reported here.

A second possibility for differences between dates of ex-flask is that tubers were only eco-dormant when replanted, and differences in greenhouse temperature only affected speed of emergence. Funnell & MacKay (1988a) found that an increase of 10 °C in the storage temperature of non-dormant tubers of Z. 'Pink Petticoat' reduced time to emergence by 9 days when stored for four weeks. Based on their results, it can be inferred that a reduction of 5°C in the greenhouse temperature would have delayed time to emergence by 4-5 days. This is similar to the differences found between ex-flasking dates for 'Black Magic', but does not completely account for the larger difference recorded for 'Treasure' between dates of ex-flask. If tubers were only eco-dormant when replanted, then clearly some factor other than temperature must have been involved in the speed of emergence of shoots, like planting depth, or compaction of the growing media.

It is interesting to note that days to emergence recorded in the second cycle following the July ex-flask decreased for tubers harvested between 150 and 165 days, but
increased again at 180 days (Table 1). This response contrasts with emergence of plants ex-flasked in November, where time to emergence progressively decreased from 150 days onwards (Table 6). Air temperature in the greenhouse where the plants harvested following the July ex-flask were placed, with exception of the harvest at 180 days, was constantly 1-2 °C higher than the temperature of the greenhouse where the harvest at 180 days was allocated. As mentioned in the previous paragraph, the lower temperatures to which the last harvest was subjected may have partly accounted for the slower speed of emergence.

4.2.2.2 Storage

Storage has been used to break dormancy of several geophytic ornamental crops. For example, storing tubers of *Aconitum napellus* L. at 2 °C for four weeks led to improved sprouting, with 100% sprouting after storage for 6 weeks (Lurie et al., 1992). Bulbs of *Lilium longiflorum* 'Ace' Thunb. stored for 6 weeks took 18 days less to emerge than unstored bulbs (Wang & Roberts, 1970). Previous studies with *Zantedeschia* have also determined that bud dormancy could be overcome with a storage period (MacKay, 1985; Halligan & Fulton, 1998). This finding is confirmed and extended by the results of the present experiment, since storage increased the percentage of emerged tubers to over 90% irrespective of the harvest- and ex-flasking dates.

Storage was most effective at the harvests where emergence was initially lowest i.e., at harvests at 165 and 150 days in the July and November ex-flasks, respectively. This response was expected, since as buds slowly broke dormancy, the percentage of buds that required a dormancy-breaking treatment to be able to sprout became smaller.
The effect of storage on *Zantedeschia* tubers is somewhat similar to the effect of the after ripening process in seeds, where additional storage in dry conditions is necessary to obtain the relief of dormancy of mature, dehydrated seeds (Bianco et al., 1994). It is believed that storage (or after ripening in the case of seeds) releases dormancy by means of triggering gibberellin biosynthesis in the dormant organs, and/or by increasing the sensitivity of the tissues to the hormone (Karssen & Laćka, 1985; Bianco et al., 1994; Davies, 1995). To be able to differentiate whether storage induces the synthesis 'de novo' of gibberellins in *Zantedeschia*, or if it only increases the sensitivity of the tissues, future studies will have to include the analysis of the endogenous levels of gibberellins in the tubers.

Although storage consistently increased percentage of emergence, irrespective of harvest- or ex-flasking date, it reduced time to emergence only at some of the harvests after both the July and the November dates of ex-flask. For example, storage reduced time to emergence of 'Black Magic' plants ex-flasked in July by up to 22 days if they were harvested at 135 or 180 days, but it increased time to emergence by 16 days if harvested at 150 days. Halligan & Fulton (1998) also observed a variable effect of storage on days to emergence. In their experiment, storage was effective in reducing days to emergence of 'Black Magic' plants harvested at 90 days, but it increased time to emergence of plants harvested at 150 days. In addition, time to emergence was reduced to over 50 days for 'Black Magic' and to over 30 days for 'Treasure', time that is not commercially convenient. The inconsistency of the results may be due to an only partial release of dormancy by storage. As previously mentioned in sections 3.2.1.1 and 3.2.1.2, there was possibly more than one population of tubers with different levels of dormancy in the experiment; each of which may have responded differently to the dormancy breaking treatments. Thus, storage
may have completely released bud dormancy of the tubers with shallower dormancy – which would both increase the percentage of emergence and reduce time to emergence, but only partially released dormancy of the tubers with deeper bud dormancy. The latter situation would increase both percentage of emergence and time to emergence, since the ‘partially dormant’ tubers would emerge over a more extended period of time. In *Lilium x elegans* Thunb., it was observed that individual bulbs within a population responded differently to cold storage, showing high variation in time to emergence (Tammen et al., 1997). This variation within a population was reduced as the period of cold storage was extended, and emergence became more uniform. It is possible that a similar situation occurs in *Zantedeschia*, which implies that a longer period of storage would be necessary to obtain uniform emergence.

The effect of storage on emergence observed in this study is somewhat at variance with the results obtained by Halligan & Fulton (1998). In their experiment, storage for three weeks increased percentage emergence of ‘Black Magic’ and decreased time to emergence only following the harvest at 90 days. At the rest of their harvest times (120 and 150 days), both parameters were negatively affected. Differences between results reported here and those of Halligan & Fulton (1998) may be related to the handling of the tubers during the curing stage, prior to storage. In Halligan & Fulton’s work, plants were cured for the first few days in a glasshouse at a maximum of 25 °C, without control of the relative humidity, with leaves and roots still attached to the tubers. Consequently, plants may have lost high amounts of water through the leaves and the unsuberised skin of the tubers (Funnell & MacKay, 1988b) which, added to the storage period, could have reduced the ability of the tubers to sprout. In addition, a small number of samples (between 12 and 15 per treatment) were used in their study. The greater replication need in the current
experiment (a total of 63 per treatment) is, therefore, more likely to be more representative of the whole population than that used by Halligan & Fulton (1998).

The rate at which dormancy is released by storage is known to depend on the cultivar in species like onion (Abdalla & Mann, 1963) and potato (Pranalli et al., 1994). This also appears to be applicable to *Zantedeschia* since, although emergence of both cultivars was over 90% after the storage treatment, emergence percentage of 'Black Magic' ex-flasked in July improved twice as much as the improvement of 'Treasure' following storage. Since 'Treasure' has apparently a shallower dormancy than 'Black Magic', the maximum emergence that could be achieved with storage would be slightly lower than emergence without storage. This would make the effect of storage in 'Black Magic' more evident.

### 4.2.2.3 Gibberellic acid

Application of gibberellic acid increased the percentage of emergence of plants ex-flasked in both July and November to over 80%, and reduced time to shoot emergence throughout the experimental period. Similar results were obtained by Tsukamoto et al. (1961), who observed that time to emergence of potato was reduced by up to 80 days when gibberellic acid was applied. In the present experiment, as well as in Tsukamoto's study, the effectiveness of gibberellic acid application declined in tubers harvested later in the season, as tubers started to break dormancy naturally.

The statistical analysis did not detect significant interactions between gibberellic acid and time of harvest. However, lower percentage of emergence of 'Black Magic' after the harvest at 150 days for the July ex-flask, and at 165 days for the November ex-flask,
even after gibberellic acid was applied (Table 5), indicate that this treatment was probably not equally effective in breaking dormancy of 'Black Magic' throughout the dormant period. It is possible that the high variability of the results hindered the detection of differences in the analysis. Therefore, further work in this area will be required before gibberellic acid can be safely recommended for breaking dormancy of *Zantedeschia* at the commercial level.

Gibberellic acid has been reported to break dormancy of buds and underground storage organs (Vegis, 1964). However, in species such as sunflower seeds, dormancy can be overcome by gibberellins only after a storage period (Bianco et al., 1994) or, as with deciduous trees, after two-thirds of the cold requirement has been satisfied (Faust et al., 1997). Although apparently not equally effective throughout the growth cycle, gibberellic acid did increase percentage of emergence and decrease time to emergence of *Zantedeschia* (Tables 4-6, 10-12). This shows that dormancy in *Zantedeschia* is probably not as deep as in some species, and that sensitivity to and/or gibberellin synthesis may not be completely inhibited during the process. Only a few other species have been reported to respond to gibberellic acid without requiring storage or chilling period, and they are potatoes (Tsukamoto et al., 1961) and *Paeonia lactiflora* Pall. (Evans et al., 1990).

Both storage and gibberellin treatments increased percentage emergence. However, with exception of 'Treasure' after the July ex-flask, storage was more effective than gibberellic acid. For example, while 98.3% of 'Black Magic' plants ex-flasked in July emerged when stored for three weeks (Table 2), only ~89 % emerged when dipped in 100 mg·L⁻¹ gibberellic acid (Table 4). Likewise, 96 % of the tubers ex-flasked in November and stored for three weeks emerged, while only ~82 % of those treated with gibberellic acid did emerge (Table 10). The fact that both storage and gibberellic acid were effective and did
not interact indicates that both treatments were affecting the same process. However, it is possible that not enough gibberellic acid concentration was absorbed to produce the highest response, and maybe higher concentrations are required. In addition, gibberellic acid may not be the most effective gibberellin, since a number of gibberellins are known to act in dormancy release (Saure, 1985). Future work will have to test different concentrations and combinations of gibberellins to determine the most appropriate treatment to break dormancy.

4.2.2.4 Dates of ex-flask

Under cultivation, some geophytic crops native to temperate and subtropical climates may drastically change their growth habit, especially if the growing conditions are much different than those in their natural habitat (Halevy, 1990). However, as shown in the present experiment, *Zantedeschia* can be successfully grown at different times of the year, without major alterations in its periodicity or the occurrence of dormancy.

Some changes in the effect of the different treatments and their interaction were observed from one date of ex-flask to the other (Table 11). For example, emergence of the plants depended on the cultivar and the harvest date after the November ex-flask, but it was independent of both factors after the July date of ex-flask. These changes may reflect that, although greenhouse conditions were maintained as constant as possible during the experimental period, environmental factors such as the mean air temperature, light intensity, etc. did change as the season progressed. From the physiological viewpoint, it is possible that the duration of the dormancy assessment phase (120 days) was not long enough to record all the plants that could potentially have emerged, which could have masked the effect of some of the treatments. However, it should be stressed that any
treatment that does not reduce time to emergence to \( \sim 21 \) days or \( 30 \) days at the most, is by no means economically viable (Funnell, pers. comm.).

As shown in Table 11, a significant treatment response with days to emergence was not always detected under the same treatment conditions as with percentage of emergence, and results were sometimes contradictory between both parameters. In addition, it was observed that time to emergence was influenced by environmental factors such as temperature, and possibly also by cultural ones, such as planting depth. For these reasons, time to emergence was not considered as accurate a measure of the level of dormancy as percentage of emergence. In contrast, some authors like Borkowska & Powell (1979) have considered percentage of bud break (or in this case emergence) within a certain period as a valid indicator of the degree to which dormancy is completed. Their reasoning was that many dormant buds might fail to grow for reasons other than dormancy. Despite the drawbacks that percentage and time to emergence present as indicators of the depth of dormancy, it is believed that both factors should be considered when dormancy of \( \text{Zantedeschia} \) is assessed; the improvement in the number of emerged plants is of no value if the same plants take a long time to emerge.

4.2.3 Prediction of the occurrence of dormancy using degree-days

Changes in percentage of emergence observed on plants not subjected to dormancy-breaking treatments reaffirm the concept that \( \text{Zantedeschia} \) presents a period of dormancy. In addition, they illustrate changes in the depth of dormancy as this period progresses with time.
Deepest dormancy of 'Black Magic' ex-flasked in November occurred 10 days earlier than in plants ex-flasked in July. However, when the time scale was changed to degree-days, deepest dormancy of plants ex-flasked in November occurred when ~118 °C-days more had been accumulated compared with the July ex-flask. Likewise, deepest dormancy of 'Treasure' ex-flasked in November took place ~3 days earlier than of those ex-flasked in July, but it occurred ~158 °C-days later on a degree-days scale. The accumulation of a higher amount of degree-days after the November ex-flask, although the time elapsed was shorter than in the July ex-flask, indicates that mean temperature during the first cycle was higher after the November ex-flask as compared with the July ex-flask. It also leads to suggest that higher temperatures may bring forward the occurrence of dormancy, which would explain the differences between dates of ex-flask. This is in agreement with previous studies, which determined that higher temperatures led to the earlier onset of phenologic events in *Zantedeschia* (Funnell, 1988). The fact that degree-days did not accurately estimate deepest dormancy of 'Black Magic' and 'Treasure' suggests that the base temperatures may not the appropriate for the cultivars. As noted by Arnold (1959), when the correct base temperature is used, the number of degree-days required by a cultivar to reach a certain phenologic stage does not vary, regardless of the mean temperature. In contrast, if the selected base temperature is too high, the degree-days summation will increase as the mean temperature increases.

Although changing the time scale to degree-days did not completely cause the occurrence of deepest dormancy of 'Black Magic' to coincide between dates of ex-flask, it reduced the difference in time between their occurrence. For example, if the greenhouse had been maintained at constant 18 °C, a difference of 118 °C-days would have implied
only ~7 days between the occurrence of deepest dormancy of plants ex-flasked in July and November. This being 3 days less of that estimated based on a time scale. In contrast, under constant 18 °C, a difference of 158 °C-days would have resulted in 9 days difference between both dates of ex-flask for ‘Treasure’, which is three times higher than the estimations on a time scale basis. The difference in accuracy of degree-days to estimate a same event between cultivars may also be related to the base temperature used. Previous studies have determined that base temperature not only varies between species, but also between cultivars and phenologic stages (McMaster & Wilhelm, 1997). Moreover, differences in base temperatures of up to 6°C have been observed among Zantedeschia cultivars, together with changes in base temperature according to the phenologic stages of the plants (Javellana, pers. comm.). These findings suggest that differences in base temperature between the cultivars tested in the present experiment were likely to exist, and therefore, it would not have been possible to make an accurate estimation for both cultivars with a same base temperature. Additionally, further loss of accuracy in the estimation may have been obtained by calculating degree-days using a single base temperature from ex-flasking until deepest dormancy, without differentiating between phenologic stages.

Despite of the limitations of the present experiment, degree-days at which deepest dormancy was estimated to occur in ‘Black Magic’ (between 2614 and 2732 °C-days) fitted within the degree-days range calculated from previous studies by Halligan et al. (1995) and Halligan & Fulton (1998) (Appendix 1). From these studies, it was estimated that dormancy of ‘Black Magic’ occurred between 1023 and 2961.7 °C-days. Thus, it is suggested that degree-days may offer a means of predicting the occurrence of deepest dormancy on Zantedeschia. To further validate this hypothesis, several considerations
should be taken into account in future studies. Firstly, plants should be grown under a wide range of temperatures, and the base temperature for each cultivar and phenologic stage determined. Secondly, it has been suggested that light intensity and photoperiod may also have an indirect effect in degree-days accumulation (Javellana, pers. comm.), and the possible inclusion of these factors in degree-days calculation should be considered.
4.3 Cessation of leaf emergence as an indicator of dormancy onset.

Even though onset of dormancy could not be determined in the present experiment, the comparison between timing of cessation of leaf emergence and the estimated occurrence of deepest dormancy showed that there was no apparent relation between both processes. For example, the period of time elapsed between cessation of leaf emergence and deepest dormancy was ~43 days for 'Black Magic' plants ex-flasked in July, while it was ~76 days for plants ex-flasked in November. Similarly, the time elapsed between the occurrence of both processes in 'Treasure' plants was of ~37 days for those ex-flasked in July, and of ~71 days for those ex-flasked in November. If both processes were effectively related, the interval of time between their occurrence would have been consistent through time. Therefore, cessation of leaf emergence is probably not an appropriate tool for the assessment of dormancy. Halligan et al. (1995) had previously indicated that, due to an apparent overlap between the onset of dormancy, cessation of leaf emergence and the attainment of the maximum number of structures on the continuation bud, cessation of leaf emergence can not be used as a clear indication of the attainment of bud maturity.
5. HORTICULTURAL AND PHYSIOLOGICAL RELEVANCE

One of the main problems encountered by growers when timing the production of their *Zantedeschia* crops is the inability to control the duration of the growth cycle, without negatively affecting growth and productivity the following season. The present study opens the possibility of growing the plants throughout the year and harvesting the plants at any time during the growth cycle, providing that tubers are stored to break bud dormancy. Thus, tuber growers would be able to harvest whenever the demand is high, or after a desired tuber size has been reached. However, there are still some constraints regarding the use of storage to break dormancy commercially. Although storage for three weeks increased percentage of emergence to over 80% at any harvest time, it reduced days to emergence mostly at later harvests (150-180 days after ex-flasking), and to periods over 50 days for 'Black Magic', and over 30 days for 'Treasure'. Since the standard period between planting and emergence should ideally not exceed 21 days, plants with such a long period of emergence are still far from being commercially acceptable. The present study determined that the partial release of dormancy through storage was related to differences in dormancy levels between plantlets. However, it remains to be tested whether a longer period of storage would completely release dormancy of all the plants, further reducing time to emergence.

The application of gibberellic acid as a preplanting dip represents a possible alternative method to break dormancy, when time constraints do not allow a storage period, or when there are no facilities to store the tubers. It may also be a useful practice to ensure the even emergence of the crop, if the previous handling of the tubers is unknown. It should be noted that different responses among cultivars to gibberellic acid
are likely to be found. Therefore, the effectiveness of gibberellic acid should be tested on several cultivars before general recommendations can be done.

Results of this study showed the existence of differences in the level of dormancy between cultivars. Growers should become aware of these differences, as they potentially may influence the response of the plants to treatments such as gibberellic acid. The extension of dormancy studies to more cultivars may enable growers, in the future, to group cultivars according to their level of dormancy, and to develop the best techniques to control this process for each group. It may also be worthy of mention in marketing catalogues.

The visual detection of the onset of dormancy may be a useful tool to schedule the harvest of a crop, and consequently, to program when tubers can be potentially shipped to buyers. It was found that cessation of leaf emergence, which had been initially suggested as an indicator, is probably not directly related to the occurrence of dormancy. Therefore, the close observation of leaf production during growth would probably be of low value for growers. On the other hand, preliminary results suggest that degree-days may be a means to predict the occurrence of dormancy. Again, further research in this area is required.

Although in the present thesis the control of dormancy of *Zantedeschia* is achieved to certain extent, a deeper insight of the physiology of this process is still necessary. Major concerns are the differences in the level of dormancy between ex-flasking material. Evenness in the performance of a crop (emergence, flowering, senescence) is a vital requirement, and it should be determined if factors during *in vitro* propagation or during transplanting affect the subsequent development of the plants. Additional work with
flowering-sized tubers is also necessary, to determine if similar variations in depth of dormancy as in ex-flasking material are present, and if the treatments tested here could be extended to larger tubers.

Results obtained with the application of gibberellic acid lead to suggest that dormancy in *Zantedeschia* is not as deep as in woody temperate species. However, the effectiveness of other dormancy-breaking treatments will have to be tested before generalisations can be made.

In summary, the present study provides growers with new alternatives for the control and prediction of dormancy of *Zantedeschia*. Although the alternatives proposed here are still in their initial stages, future research will lead to the development of practices commercially applicable, together with providing a better understanding of the physiology of dormancy on this crop.
6. LITERATURE CITED


Dokoozlian, N.K. 1999: Chilling temperature and duration interact on the budbreak of 'Perlette' grapevine cuttings. HortScience 34(6);1054-1056.


APPENDIX 1: Degree-day calculation

According to the experiment performed by Halligan et al. (1995), dormancy onset of 'Black Magic' occurred between 90 and 118 days, which coincided with a degree-day accumulation of ~1024 and 1466 °C-days. Likewise, dormancy was broken between 203-231 days after planting, which coincided with an increase in heat units from 2600 to 2900 °C (Table 12). Similarly, in the experiment carried out by Halligan & Fulton (1998), dormancy release occurred between 120 and 150 days after planting, which coincided with a range of degree-days between 2200 and 2700 °C (Table 13).

Table 12. Degree-days during growth cycle of Z. 'Black Magic' grown in the field from October 1993 to October 1994 (calculated from Halligan et al., 1995).

<table>
<thead>
<tr>
<th>Harvest date</th>
<th>Degrees (^1) (°C)</th>
<th>Harvest (days from planting)</th>
<th>Total degree-days (°C)</th>
<th>Plants' dormancy stage(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27 Oct 1993</td>
<td>10.8</td>
<td>27</td>
<td>291.6</td>
<td>ND</td>
</tr>
<tr>
<td>30 Nov</td>
<td>10.2</td>
<td>61</td>
<td>640.8</td>
<td>ND</td>
</tr>
<tr>
<td>29 Dec</td>
<td>13.2</td>
<td>90</td>
<td>1023.6</td>
<td>ND</td>
</tr>
<tr>
<td>26 Jan</td>
<td>16.0</td>
<td>118</td>
<td>1466.0</td>
<td>D</td>
</tr>
<tr>
<td>22 Feb</td>
<td>17.0</td>
<td>145</td>
<td>1920.0</td>
<td>D</td>
</tr>
<tr>
<td>24 Mar</td>
<td>12.8</td>
<td>175</td>
<td>2329.2</td>
<td>D</td>
</tr>
<tr>
<td>21 Apr</td>
<td>11.7</td>
<td>203</td>
<td>2664.5</td>
<td>D</td>
</tr>
<tr>
<td>19 May</td>
<td>10.1</td>
<td>231</td>
<td>2961.7</td>
<td>ND</td>
</tr>
<tr>
<td>16 Jun</td>
<td>6.4</td>
<td>259</td>
<td>3185.3</td>
<td>ND</td>
</tr>
<tr>
<td>14 Jul</td>
<td>5.8</td>
<td>287</td>
<td>3356.1</td>
<td>ND</td>
</tr>
<tr>
<td>15 Sep</td>
<td>8.1</td>
<td>350</td>
<td>3811.8</td>
<td>ND</td>
</tr>
<tr>
<td>13 Oct 1994</td>
<td>9.8</td>
<td>378</td>
<td>4060.7</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^1\) Base temperature calculated as daily average (AgResearch, P.N.) minus 2.1 °C (Funnell et. al., 1998).

\(^2\) Dormancy stage is indicated as ND = non-dormant; D = dormant.

<table>
<thead>
<tr>
<th>Harvest date</th>
<th>Temperature (^\circ\text{C})</th>
<th>No days from planting</th>
<th>Total degree-days(^\circ\text{C})</th>
<th>Plants’ dormancy stage(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Feb</td>
<td>19.7</td>
<td>90</td>
<td>1654.6</td>
<td>D</td>
</tr>
<tr>
<td>17 Feb</td>
<td>19.7</td>
<td>105</td>
<td>1949.3</td>
<td></td>
</tr>
<tr>
<td>3 March</td>
<td>17.7</td>
<td>120</td>
<td>2238.1</td>
<td></td>
</tr>
<tr>
<td>18 March</td>
<td>17.7</td>
<td>135</td>
<td>2503.3</td>
<td></td>
</tr>
<tr>
<td>12 Apr</td>
<td>15.9</td>
<td>150</td>
<td>2746.6</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^1\) Base temperature same as Table 1.
\(^2\) Dormancy stage as indicated in Table 1.

Based on the previous tables, dates for harvests in the present experiment were planned to occur over the period of heat units between 2200 and 2900 \(^\circ\text{C}\)-days (Table 14 & Table 15).

Table 14. Estimated Degree-days for the present experiment harvest, for planting on 15th July 1999.

<table>
<thead>
<tr>
<th>Harvest date</th>
<th>Temperature (^\circ\text{C})</th>
<th>Harvest (days from planting)</th>
<th>Total degree-days (^\circ\text{C})</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-Nov</td>
<td>17.3(^1)</td>
<td>120</td>
<td>1976.0</td>
</tr>
<tr>
<td>27-Nov</td>
<td>17.3</td>
<td>135</td>
<td>2236.0</td>
</tr>
<tr>
<td>12-Dec</td>
<td>18.6</td>
<td>150</td>
<td>2511.3</td>
</tr>
<tr>
<td>27-Dec</td>
<td>18.6</td>
<td>165</td>
<td>2790.5</td>
</tr>
<tr>
<td>11-Jan</td>
<td>19.0</td>
<td>180</td>
<td>3073.5</td>
</tr>
</tbody>
</table>

\(^1\) Temperatures were estimated from Halligan & Fulton (1998). Base temperature same as table 1.

Table 15. Estimated Degree-days for each harvest for planting on 1st November 1999.

<table>
<thead>
<tr>
<th>Harvest date</th>
<th>Temperature (^\circ\text{C})</th>
<th>Harvest (No days from planting)</th>
<th>Total Degree-days (^\circ\text{C})</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 Feb</td>
<td>19.7(^1)</td>
<td>120</td>
<td>2234.8</td>
</tr>
<tr>
<td>14 March</td>
<td>17.7</td>
<td>135</td>
<td>2501.9</td>
</tr>
<tr>
<td>29 March</td>
<td>17.7</td>
<td>150</td>
<td>2767.1</td>
</tr>
<tr>
<td>13 Apr</td>
<td>15.9</td>
<td>165</td>
<td>3008.6</td>
</tr>
<tr>
<td>28 Apr</td>
<td>15.9</td>
<td>180</td>
<td>3246.3</td>
</tr>
</tbody>
</table>

\(^1\) Temperatures were estimated from Halligan & Fulton (1998). Base temperature same as table 1.
APPENDIX 2: Pest and disease control

After planting, the medium was saturated with water and a fungicide solution of fosetyl-aluminium 80% (Aliette®; Rhône-Poulenc Limited), at a dose of 15 g·5 L⁻¹·10 m⁻². Ten days later, plantlets were sprayed to drip point with cupric hydroxide (Kocide®; Griffin Corporation, USA) at a dose of 1.5 g·L⁻¹. Two and a half weeks after planting, plantlets were drenched with 1 L·m⁻² bed of a solution containing 0.6g·L⁻¹ Benlate®, 0.6g·L⁻¹ thiram 80% (Thiram®) and 1g·L⁻¹ and Aliette®. Subsequently, regular pesticide applications consisted of the products previously mentioned, alternating every other week. For dormancy assessment, pesticides were applied following the same schedule as in the first part of the experiment. Additionally, insecticides for the control of white fly, sciarid fly and aphids were applied when necessary.

During the dormancy assessment phase, tubers were checked periodically, discarding those that were rotten or had not sprouted after 200 and 120 days, for the July and the November ex-flasking date, respectively.
APPENDIX 3: Estimated number of replicates and samples

Using the dataset obtained by Halligan & Fulton (1998), the variation among treatments (residual error) and within treatments (sampling error) in the number of days to emergence was calculated. These values were used for the estimation of the number of replicates (n) for this experiment through the least significant difference (LSD) procedure, rearranged for solving n (Equation 4; Table 16),

$$n = \frac{2s^2 * t^2}{LSD^2}$$

(4)

where $s^2 = \text{variance}$, $t = \text{t-value}$.

**Table 16. Number of estimated replicates (n) necessary for detecting a difference (LSD) of seven days in days to emergence (based on Halligan & Fulton, 1998).**

<table>
<thead>
<tr>
<th>Level</th>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main-plot</td>
<td>Main effect of Cv (c)</td>
<td>(c-1)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Block (d)</td>
<td>(d-1)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Main-plot error (a)</td>
<td>(d-1)(c-1)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>t(0.05)</td>
<td></td>
<td>2.3</td>
</tr>
<tr>
<td>Split-plot</td>
<td>Main effect of Hv (h)</td>
<td>(h-1)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Interaction hv*cv</td>
<td>(h-1)(c-1)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Split-plot error (b)</td>
<td>c(d-1)(h-1)</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>t(0.05)</td>
<td></td>
<td>2.0</td>
</tr>
</tbody>
</table>
APPENDIX 4: T-tests for tuber diameter and leaf area

Table 17. Tuber diameter and leaf area of cultivar 'Black Magic' grown under the lowest and highest mean temperatures in the greenhouse.

<table>
<thead>
<tr>
<th>Block</th>
<th>Tuber diameter (mean ± sem)</th>
<th>Leaf area (mean ± sem)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>14.4 ± 1.0</td>
<td>76.5 ± 23.2</td>
</tr>
<tr>
<td>9</td>
<td>14.9 ± 1.3</td>
<td>93.5 ± 29.4</td>
</tr>
<tr>
<td>T-test</td>
<td>N.S</td>
<td>N.S</td>
</tr>
</tbody>
</table>

Values are means of 7 samples; df = 6

Significant effects in the t-test are indicated as follows: NS, Not significant; *, significant at \( P < 0.1 \); **, significant at \( P < 0.01 \); *** significant at \( P < 0.001 \).

Table 18. Tuber diameter and leaf area of cultivar 'Treasure' grown under the lowest and highest mean temperatures in the greenhouse.

<table>
<thead>
<tr>
<th>Block</th>
<th>Tuber diameter (cm ± sem)</th>
<th>Leaf area (cm² ± sem)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>16.2 ± 1.4</td>
<td>94.2 ± 21.6</td>
</tr>
<tr>
<td>9</td>
<td>12.9 ± 1.5</td>
<td>89.9 ± 18.5</td>
</tr>
<tr>
<td>T-test</td>
<td>N.S</td>
<td>N.S</td>
</tr>
</tbody>
</table>

Values are means of 7 samples. DF = 6

Significant effects in the t-test are indicated as follows: NS, Not significant; *, significant at \( P < 0.1 \); **, significant at \( P < 0.01 \); *** significant at \( P < 0.001 \).
APPENDIX 5: Summary tables of the dormancy-breaking treatment results

Table 19. Time to emergence (days) and percentage emergence (%) of cv. ‘Black Magic’ following sequential harvests, storage for 0 or 3 weeks and a preplanting dip of 0 or 100 mg·L$^{-1}$ gibberellic acid. July ex-flask date.

<table>
<thead>
<tr>
<th>Harvest</th>
<th>Storage (weeks)</th>
<th>% Emergence</th>
<th>Days to Emergence</th>
<th>% Emergence</th>
<th>Days to Emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>0</td>
<td>70.8</td>
<td>84.3</td>
<td>52.4</td>
<td>33.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>93.7</td>
<td>100.0</td>
<td>57.4</td>
<td>38.2</td>
</tr>
<tr>
<td>135</td>
<td>0</td>
<td>81.5</td>
<td>90.5</td>
<td>66.0</td>
<td>58.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>98.4</td>
<td>100.0</td>
<td>52.1</td>
<td>47.3</td>
</tr>
<tr>
<td>150</td>
<td>0</td>
<td>57.1</td>
<td>74.6</td>
<td>54.0</td>
<td>51.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>98.4</td>
<td>96.8</td>
<td>71.7</td>
<td>65.9</td>
</tr>
<tr>
<td>165</td>
<td>0</td>
<td>55.2</td>
<td>54.5</td>
<td>59.1</td>
<td>60.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>98.4</td>
<td>98.4</td>
<td>62.2</td>
<td>58.2</td>
</tr>
<tr>
<td>180</td>
<td>0</td>
<td>77.8</td>
<td>90.2</td>
<td>76.8</td>
<td>74.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>98.4</td>
<td>100.0</td>
<td>56.1</td>
<td>51.1</td>
</tr>
</tbody>
</table>

Table 20. Time to emergence (days) and percentage emergence (%) of cv. ‘Treasure’ following sequential harvests, storage for 0 or 3 weeks and a preplanting dip of 0 or 100 mg·L$^{-1}$ gibberellic acid. July ex-flask date.

<table>
<thead>
<tr>
<th>Harvest</th>
<th>Storage (weeks)</th>
<th>% Emergence</th>
<th>Days to Emergence</th>
<th>% Emergence</th>
<th>Days to Emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>0</td>
<td>95.8</td>
<td>95.6</td>
<td>31.3</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>100.0</td>
<td>97.8</td>
<td>30.9</td>
<td>18.4</td>
</tr>
<tr>
<td>135</td>
<td>0</td>
<td>75.4</td>
<td>91.5</td>
<td>33.1</td>
<td>26.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>77.8</td>
<td>98.4</td>
<td>45.5</td>
<td>30.1</td>
</tr>
<tr>
<td>150</td>
<td>0</td>
<td>64.0</td>
<td>85.5</td>
<td>36.2</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>89.3</td>
<td>88.4</td>
<td>47.8</td>
<td>38.0</td>
</tr>
<tr>
<td>165</td>
<td>0</td>
<td>60.3</td>
<td>85.2</td>
<td>37.0</td>
<td>31.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>88.6</td>
<td>100.0</td>
<td>38.7</td>
<td>31.6</td>
</tr>
<tr>
<td>180</td>
<td>0</td>
<td>76.6</td>
<td>91.3</td>
<td>53.0</td>
<td>41.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>100.0</td>
<td>100.0</td>
<td>32.1</td>
<td>31.5</td>
</tr>
</tbody>
</table>
Table 21. Time to emergence (days) and percentage emergence (%) of cv. 'Black Magic' following sequential harvests, storage for 0 or 3 weeks and a preplanting dip of 0 or 100 mg·L⁻¹ gibberellic acid. November ex-flask date.

<table>
<thead>
<tr>
<th>harvest</th>
<th>storage 0</th>
<th>storage 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% emergence</td>
<td>days to emergence</td>
</tr>
<tr>
<td>120</td>
<td>76.4</td>
<td>90.7</td>
</tr>
<tr>
<td>135</td>
<td>52.3</td>
<td>70.1</td>
</tr>
<tr>
<td>150</td>
<td>33.5</td>
<td>53.0</td>
</tr>
<tr>
<td>165</td>
<td>56.2</td>
<td>77.7</td>
</tr>
<tr>
<td>180</td>
<td>89.7</td>
<td>86.5</td>
</tr>
</tbody>
</table>

Table 22. Time to emergence (days) and percentage emergence (%) of cv. 'Treasure' following sequential harvests, storage for 0 or 3 weeks and a preplanting dip of 0 or 100 mg·L⁻¹ gibberellic acid. November ex-flask date.

<table>
<thead>
<tr>
<th>harvest</th>
<th>storage 0</th>
<th>storage 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% emergence</td>
<td>days to emergence</td>
</tr>
<tr>
<td>120</td>
<td>88.2</td>
<td>97.2</td>
</tr>
<tr>
<td>135</td>
<td>72.9</td>
<td>85.0</td>
</tr>
<tr>
<td>150</td>
<td>62.0</td>
<td>89.4</td>
</tr>
<tr>
<td>165</td>
<td>62.4</td>
<td>83.0</td>
</tr>
<tr>
<td>180</td>
<td>80.1</td>
<td>77.3</td>
</tr>
</tbody>
</table>