STUDY ON GROWTH, DEVELOPMENT AND SOME BIOCHEMICAL ASPECTS OF SEVERAL VARIETIES OF *Nerine*

by

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Submitted in partial fulfilment of the requirements for the degree of Master of Science Studies

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May, 1993

DECLARATION

To the best of my knowledge and belief, this thesis contains no material which has been submitted for the award of any other degree or diploma, nor does it contain any paraphrase of previously published material except where due reference is made in the text.

Kumala Dewi

ABSTRACT

Nerine fothergillii bulbs were stored at different temperatures for a certain period of time and then planted and grown in an open condition. The effect of the different storage temperatures on carbohydrate content and endogenous gibberellins was examined in relation to flowering. Flowering percentage and flower number in each umbel was reduced when the bulbs were stored at 30° C while bulbs which received 5° C treatment possess earlier flowering and longer flower stalks than bulbs without 5° C storage treatment.

Carbohydrates in both outer and inner scales of *N. fothergillii* were examined semi-quantitatively by paper chromatography. Glucose, fructose and sucrose have been identified from paper chromatograms.

Endogenous gibberellins in *N. fothergillii* have been identified by GC - SIM and full mass spectra from GCMS. These include GA_{19}, GA_{20} and GA_1, their presence suggests the occurence of the early 13 - hydroxylation pathway.

The response of *N. bowdenii* grown under Long Day (LD) and Short Day (SD) conditions was studied. Ten plants from each treatment were examined at intervals of 4 weeks. Photo period did not affect the growth and development of *N. bowdenii* but it influenced the flowering time. SD condition gives earlier flowering than LD condition.

Anthocyanins in *Nerine* have been examined using paper chromatography. The solvents used were BAW and MAW. Five major anthocyanins (pelargonidin, cyanidin, delphinidin, malvidin and peonidin) have been identified from several varieties of *Nerine* .

ACKNOWLEDGEMENTS

Firstly, I thank Almighty God for His grace, protection and guidance during my study in The University of Tasmania.

Secondly, the following people helped in the preparation of this thesis directly or indirectly, and I thank them with my sincere gratitude:

Dr. R.K. Crowden, my supervisor, for his continuous help and encouragement during my study in The University of Tasmania. He also provides me with the plant materials used in this recent works and very special thanks for him in correcting my English.

Dr. J.J. Ross, Mr. Noel Davies and Mr. Omar Hasan for their help and assistance in doing HPLC and GC-SIM.

Mr. Peter Bobbi and Miss K.A. Mc Pherson for their help in looking after the plants in the glass-house.

All staff and post graduate students in Plant Science Department, who give me some helps and good environment for my works.

Setyantono, my lover, friend and husband, whose patient, understanding and continuous encouragement, to me, helped support me through the lonely time of the two years we had been far away.

My mother and two younger brothers, whose encouragement and pray support I do appreciate so much.

Lastly, I thank The World Bank XXI (Indonesia) for financial support.

Kumala Dewi

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CHAPTER I : INTRODUCTION

There are hundreds of thousands of different kind of flowers in the world and many of them have a potential value as ornamental plants. Many kinds of flowering plants have been subjected to experiments aimed at improving their variability and quality as cut-flowers, as well as to elucidate processes involved in flower development.

Amongst the ornamental plants, bulb plants are always popular as garden plants because they are generally easy to grow. Rees (1972), stated that even if the climate is unsuitable for wild plants, the cultivated form can be treated so as to flower in a wide range of environments.

Bulb plants are usually forced for use as cut-flower and flowering pot plants. The value of bulb plants as an export commodity may give benefit to the producing countries, such as The Netherlands with its tulip production.

The growth habits and environmental requirements of various bulb plants are different. Bulb forcing which is aimed at producing high quality potted plants or cut-flowers, as well as selection and breeding experiments have been highly developed for many of the currently important bulb species. Many of these experiments are usually associated with the horticultural practices.

In this paper, several factors involved in the flower development of the bulb species (*Nerine*) have been examined. It is hoped that this paper will be of benefit in improving the scientific knowledge about flower development in *Nerine*.

CHAPTER II : LITERATURE REVIEW

2.1. BULBOUS PLANTS

2.1.1. True bulbs and taxonomy

"Bulbous plants" have the characteristic of specialised plant parts below the soil surface that are modified specifically for storing food. These underground storage organs enable them to survive during the unfavourable season and also function in vegetative reproduction. Plants with this type of modification generally are herbaceous perennials. At the end of each active growing period the above ground parts die down and the underground parts (bulb) enter a dormant stage. This bulb bears the buds which will produce the new shoots at the start of the next favourable season (Hartmann and Kester, 1983).

"Bulbous plants" are defined more accurately as bulbs, corms, tubers, tuberous roots and rhizomes based on whether the specialised organ of the plant is a modified stem or a modified root (Hartmann *et al.*, 1981). The true bulb is a specialised underground stem. It consists of numerous fleshy scales or leaf bases attached to a distinct basal plate that gives rise to roots and shoots. The outer scales are usually fleshy and contain reserve food materials, whereas the scales toward the centre of the bulb are less fleshy and more readily identified as leaves. There is either a vegetative meristem or an unexpanded flowering shoot. Bulblets or miniature bulbs can be produced from the development of the meristem in the axils of these scales (Hartmann *et al.*, 1981).

According to Moore *et al.* (1982), true bulbs can be separated into tunicated bulbs or untunicated bulbs based on the presence or absence of an impervious covering layer. In tunicated bulbs, e.g. *Tulip* and *Narcissus*, the whole structure is enclosed in a dry and membraneous outer sheath that provides protection from drying and mechanical injury to the bulb. The fleshy scales are in continuous concentric layers or laminae. The untunicated bulbs,e.g. *Lilies* do not posses the protective covering. The untunicated bulbs are easily damaged and need more careful handling than tunicated bulbs. They also require continual moisture because they can be injured by drying.

"Bulbous plants" are found almost exclusively in monocotyledons, however, true bulbs also occur in the dicotyledons, e.g. *Oxalis* (Rees, 1972). *Amaryllis belladonna*, *Scilla* spp, *Chionodoxa* spp, *Narcissus* spp, *Galanthus nivalis*, *Erythronium* spp, *Galtonia candicans*, *Muscari* spp, *Hippeastrum* spp, *Hyacinthus* spp, *Lilium* spp, *Nerine* spp, *Polianthes* spp, *Vallota* spp, *Leucojum* spp, *Tulipa* spp, and *Zephyranthes* spp are plants which have true bulbs (Hartmann *et al.*, 1981).

2.1.2. Distribution and climatic relationship to life cycle

"Bulbous plants" can be grown probably in every country in the world since many species are surprisingly adaptable to a variety of climates. However, bulb producing areas usually are found only in a few countries and these are mostly in the cool temperate areas although some are within the tropics at high altitude (Rees, 1972). Many of "bulbous plants" originated from South Africa (Eliovson, 1960). According to Burns (1946) in Rees (1972), climate is one of the important factors in the

evolution of the bulbous habit.. The natural distribution of several families of "bulbous plants" has been described by Genders (1973).

The "true bulb" has a general developmental cycle which is composed of two growth stages namely, the vegetative stage and the reproductive stage. The bulb grows to the flowering stage and then the subsequent reproductive stage includes the induction of flowering, differentiation of floral parts, elongation of the flowering shoot and finally flowering and usually seed production. Various bulbous species show a different environmental requirement for each phase of this cycle. Basically the "bulbous plants" can be grouped into spring-flowering bulbs, summer-flowering bulbs and winter-flowering bulbs according to their blooming time (Hartmann and Kester, 1983).

2.1.3. Annual growth cycle

The "bulbous plants" show a strong periodicity in their life cycle which is closely related to seasonal changes in weather. Flowering is an annual event and the plant has built-in mechanisms to prevent flowering at other times of the year (Rees, 1972). According to De Hertogh (1980), five stages can be distinguished in the growth cycle for bulb production namely, (1) harvesting and preplanting storage, (2) planting, rooting and low temperature mobilisation for flowering and/or bulbing, (3) leaf and flower stalk growth, (4) flowering and (5) increases in bulb size and / or number. After the bulb has been planted, the stored food is used for the production of leaves, flowers and sometimes the seed. However, not all "bulbous plants" follow the same cycle.

In the spring-flowering group, such as tulip, narcissus and hyacinth, they flower in late winter or spring, increase their bulb size during the spring and becomes dormant in the summer. *Lilium longiflorum*, which represents the summer-flowering bulbs produces flower in early summer and becomes dormant in autumn. In tropical areas, there are a number of flowering bulbs whose growth cycle is related to wet-dry rather than a cold-warm climatic cycle, e.g. *Hippeastrum* (Hartmann and Kester, 1983).

2.1.4. Flowering pattern

The studies of flower initiation and development in "bulbous plants" have been largely conducted in The Netherlands since 1920s. It has been noted that "bulbous plants" differ in the time at which floral initiation occurs (Rees, 1972). Based on the pattern of flower formation, Hartsema and Luyten (1961) in [Rees 1972, and Hartmann *et al.*, 1981], categorised the "bulbous plants" into seven groups but only the first five are relevant to "true bulbs". These are :

1. The flower buds are formed during the spring or early summer of the previous year, usually when flowering in the field is nearly over but just before the bulbs are dug from the soil, e.g. *Narcissus, Galanthus* and *Leucojum.*

2. Flowers are formed at the end of previous growing period or perhaps, after they have been harvested and placed in storage. Bulbs of this group have flower buds when they are replanted in autumn, e.g. tulip, hyacinth, *Iris reticulata.*

3. Flowers are initiated some time after replanting and after some low temperatures in winter or early spring, e.g. bulbous iris except *Iris reticulata.*

4. Flowers are initiated more than a year before anthesis, e.g. *Nerine.*

5. Flower initiation occurs throughout the entire growing season but alternately with leaf formation. Undeveloped or newly initiated flower buds may be present when the plants are in full bloom, e.g. *Hippeastrum, Zephyranthes.*

6.Flowers are initiated toward the end of the storage period but development continues after the bulbs have been planted, e.g. *Allium cepa, A. escalonicum, Galtonia, Solanum tuberossum* and *some Lilies.*

7.Flowers are initiated after replanting, e.g. freesia and anemone.

2.1.5. Economic significance and important knowledge about manipulation of the annual growth cycle

"Bulbous plants" are usually grown by temperate zone gardeners because they have several qualities such as, they are easy to grow and colourful and some of them bear fragrant flowers in a large array of shapes, size and colours. Many species of "bulbous plants" are commercially important as cut flowers like tulip, narcissus, iris, etc. Bulbs are also exported in large numbers from the producing countries, especially The Netherlands.

The flowering time in "bulbous plants" is predictable since some flower in late winter, e.g. *Galanthus* and *Chionodoxa.* Flowering begins early in spring with the *Crocus* and *Tulip. Begonia* and *Canna* flower during summer. Fall is the flowering season for *Colchium* (Hartmann *et al.*, 1981). Thus there is a wide choice of seasonal flowering habits among the "bulbous plants".

By knowing their seasonal flowering habits as well as environmental conditions needed during the annual growth cycles, the optimal production

and marketing of bulbs and cut-flowers can be reached. The manipulation of the annual growth cycle is usually termed as bulb forcing. It refers to programming, in which the bulbs are induced to flower (the methods used are precooling, natural cooling, controlled temperature forcing and / or long day treatments) and green house phase which encompasses the time from placing the plants in the green house until flowering.

According to De Hertogh (1980), there are four basic factors influencing flower formation and development of "bulbous plant" namely bulb size, leaf formation, environment (particularly temperature and light) and relationship of flower formation to harvest and low temperature requirement. It has been considered that high temperatures favour flower formation more than low temperatures do but each species has a different optimal temperature (Hartmann *et al.*, 1981). Obviously the advantage of bulb forcing is that flowering can be directed for a specific date such as Christmas or New Year. It means that through bulb forcing precise flowering can be obtained.

2.2. NERINE

2.2.1. Taxonomy and distribution

Nerine is one of the "true bulb" plants. It belongs to the family Amaryllidaceae and there are about 30 species of Nerine. The genus *Nerine* is considered closely related to *Amaryllis* (Genders, 1973) and *Brunsvigia* (Synge, 1961 ; Traub, 1967). The relationship between *Nerine* and *Brunsvigia* has been examined cytologically by Goldblatt (1972). He

found that the species currently known as *Nerine marginata* has a similar karyotype with *Brunsvigia*, thus it is treated as *Brunsvigia*.

The classification of nerine has been done by Hebert (1821 and 1837 in Traub, 1967), Baker (1896 in Traub, 1967), Traub (1967) and Norris (1974). There are some difference between these classifications such as the number of species and the criteria used in each classification. Traub (1967), grouped the species of nerine under 4 sections based on peduncle (short or slender), stamens and style (erect or declineate) and filaments (with appendages or without appendages). These four groups are Laticomae, Nerine, Bowdenii and Appendiculatae. Norris (1974), divided the species of nerine into 12 groups based on the arrangement of the perianth segments which form the individual floret, the presence and form of appendix or modification to the base of stamen and the presence or absence, of hair on the stem, pedicels and ovary. The species typifying each group are *N. marginata, N. sarniensis, N. laticoma, N. ridleyi, N, humilis, N. pudica , N. gaberonensis, N. filifolia, N. pancratuoides, N. platypetala, N. angustifolia, N. appendiculata and N. hesseoides* . Within this classification, *N. fothergillii* as well as *N. corusca* are grouped with *N. sarniensis*.

The genus *Nerine* is native to South Africa and its distribution has been examined and described by Norris (1974). However, nerines are widely cultivated in many countries particularly because the flowers are colourful in the garden and lasting in the vase. According to Smithers (1990), *N. sarniensis* has been grown in Europe since 1680 and in England it has been cultivated since the end of the last century. In some countries like The Netherlands and New Zealand, it becomes an important

commercial ornamental "bulbous plant" and both the bulb and flowers are traded.

Nerines can be grown in cool, sunny green-houses or in out-door gardens. In New Zealand, Australia and similar areas, they are grown out-doors and left in place for several years to develop the bulbs, they might be lifted when too over-crowded or if attacked by pest and disease. On the contrary, in the cold temperate zone of Europe they are grown partially or wholly in the green-houses, since they are not tolerant to below-freezing temperature. Often each bulb is grown in its own container (Salinger, 1985).

2.2.2. Description

The nerine bulb is globose or ovoid pyriform, tunicated, sometimes produced into a neck. The leaves are slender, almost linear and usually come with or just after the flower. The flowers are borne in a 2 to 20 flowered umbel on an erect slender scape up to 60 cm tall. The flowers are funnel-shaped in bright red, pink or white. The perianth is divided nearly to its base into six segments, commonly called petals but more correctly tepals, that are erect or deeply cut and sometimes much recurved. There are six erect stamens, three longer than the others and a style with a three-lobed stigma (Genders, 1973; Everett, 1981 and Rowell, 1986). The description of each species of nerines can be found in Norris (1974).

2.2.3. Structure of the bulb and flowering behaviour

The growth and flowering behaviour in nerines differ for cultivars and by their provenance or their breeding. According to Salinger (1985),

there are three types of nerines which are commercially grown. These are (1) winter foliage, in which the dormancy takes place over summer and flowering will commence in late summer, e.g. *N. fothergillii-major*, (2) summer foliage, in which the leaves senesce just before flowering in autumn or they may persist until the flowering time, however, they do not remain during winter, e.g. *N. bowdenii,* (3), those with foliage persisting for most of the year, e.g. *N. flexuosa-alba* and *N. "Pink Triumph"*

2.3. FACTORS AFFECTING FLOWER INITIATION AND DEVELOPMENT

2.3.1. Introduction

Several factors may affect flower initiation and development in "bulbous plants" such as bulb size, leaf formation, environment (particularly light and temperature) and vernalisation (Rees, 1972; De Hertogh, 1980). It is important to know the effect of these factors, since most of the "bulbous plants" are grown to produce cut-flowers. The bulb then can be treated accordingly and flowering can be retarded or more frequently advanced.

2.3.2. Bulb size and leaf formation

The bulb size may determine whether flowering will occur or not in the flowering season. The bulb size is measured in circumference. Below a certain size the bulb does not flower and each species should achieve a minimum size before the flower initiation can occur. In *N.undulata*, bulbs as small as 8 cm will flower, although the flowers are much smaller

compared with the larger bulbs (Rees, 1972). For *N. sarniensis* and *N. bowdenii*, 12 to 14 cm are the critical size. If the size falls below 14 cm, there is only a small chance that the flower bud will develop to anthesis (Luyten, 1957 in Rees, 1972). The size of the bulb is thus important especially if it is intended to "force" flowering.

Concomitant with bulb size, the number of leaves seems important in determining flower initiation times. In tulip, for example, 3 to 5 leaves (depending on the cultivar) must be formed for flowering to occur (De Hertogh, 1980). In *N. bowdenii* and *N. sarniensis*, 6 to 8 leaves are formed between two inflorescence buds, while in *N. flexuosa-alba* the number is more irregular, from 4 to 15 (Fortainer *et al.*, 1979). It is suggested that in narcissus, the presence of assimilating leaves is essential for flower initiation and that defoliating can prevent flower initiation totally (Hartsema, 1961 in Rees, 1972). However, the number of leaves present in flowering-sized bulb of other "bulbous plants", e.g. hyacinth can be variable.

2.3.3. Temperature

The effect of temperature on initiation and subsequent flower development has been widely examined in many commercial "bulbous plants" such as, tulip, narcissus, iris and nerine. It is known that these "bulbous plants" undergo periodicity of dormancy as well as the timing of flower initiation. The period of flowering can be modified by applying appropriate temperature to bulbs during storage and after planting (Salinger,1985). Each species, however, has a different optimum temperature for flower initiation.

In nerines, the effect of temperature has been examined only in a few species. For *N. flexuosa-alba*, it has been found that good flowering occurred after growth at temperatures of 9° or 13° C. However, based on a high inflorescence number per plant, high flower number per inflorescence and fewer days to flower, it has been noted that the best combination of growing and storage temperature is 9° and 21° C, respectively (Fortainer *et al.*, 1979). In *N. bowdenii*, it has been found that the optimum temperature for bulb storage is 2° C although the planting date rather than the storage temperature governs the flowering time (Sytsema, 1971). For the other species, the optimum temperature for triggering the flowering has not been examined yet.

2.3.4. Light quality and photoperiod

In "bulbous plants", the effect of light and photoperiod has been examined in some species like *Lilium longiflorum* Thunb. (Waters and Wilkins, 1967), *Tulip* (Hanks and Rees, 1979) and *Nerine flexuosa-alba* (Fortainer *et al.*, 1979). In *Lilium*, it has been found that incandescent light accelerates floral differentiation and bloom date as much as 5 weeks over control plants although bud count is usually reduced (Waters and Wilkins , 1966). However, photoperiod has no effect in the flowering date of *Tulip* (Hank and Rees, 1979) or in flower initiation of *Hippeastrum* (Rees, 1972).

The effect of light on flower initiation in nerines has been examined by Fortainer *et al.*(1979), using *N. flexuosa-alba*. The bulbs were subjected to 8 hr or 16 hr of fluorescent light per day with different temperature conditions (9, 13, 17, 21 or 25° C). It was found that the light quantity did not affect the flower initiation although more and longer leaves were formed in 8 hr light than in 16 hr light. This experiment was continued by

exposing the bulb to different photoperiods (8 hr natural day light as basic illumination with 0, 4, 8, 12 or 16 hr supplementary weak incandescent light). It was also found that photo period has no influence on growth or flowering. From the results of van Brenk (1980), it has been suggested that there is no effect of day length on the growth or flowering of *N. bowdenii*, cv. Favourite.

2.4. THE CHEMISTRY OF 'BULBOUS PLANTS'

2.4.1. Carbohydrate metabolism

According to Harborne (1984), carbohydrate in plants can be found in the form of stored energy (as starch), transport of energy (as sucrose) and the building blocks of the cell wall (cellulose). It is suggested that these carbohydrate reserves are mobilised to sustain growth during and after the sprouting of the seeds and tubers (Porter, 1962) or bulbs (Halmer and Bewley, 1982).

The bulbs that are the food-storage organ usually are composed of water, insoluble organic substance of high molecular weight and water soluble substances of low molecular weight (Pate and Dixon, 1982). Amongst the water soluble substances, free-sugars and oligosaccharides have been examined in several food-storage organs such as in onion bulb or some ornamental bulbs. In onion bulb (*Allium cepa*), it has been found that carbohydrate may account about 65 % of the dry weight (Bennet, 1941 in Darbyshire, 1978). From extraction of the onion bulb it has been found that the sucrose concentration as well as glucose and fructose levels tended to increase from the outer to inner leaf bases (Darbyshire, 1978).

Amongst the ornamental bulbs, carbohydrate metabolism has been studied in some species like, *Narcissus* (Chen,1969), *Iris* (Halevy and Shoub, 1964) and *Tulip* (Thompson and Rutherford, 1977; Moe and Wickstrøm, 1973 & 1979; Hobson and Davies, 1978). Thompson and Rutherford (1977) showed a relationship between the carbohydrate metabolism with the temperature treatment in tulip bulb. In tulip bulbs held at 9° C for periods ranging from 8 to 21 weeks, they found that there is a progressive decline in alcohol-insoluble carbohydrates associated with increased duration of low temperature treatment. On the other hand, there is corresponding increase in soluble sugar levels.

Moe and Wickstrøm (1979), considered that the amount of sucrose in the scales and oligosaccharides in the shoots during the first week of precooling is correlated with the capacity of the bulbs for fast growth and flowering. They suggested that after extended precooling, the amount of sugar is no longer a limiting factor. With *Nerine*, there is no literature dealing with the carbohydrate metabolism so far.

2.4.2. Plant hormones

It is known that plant hormones play a role in the growth and development of plants, particularly in directing the movement of organic metabolites and in establishing sinks (Rees, 1972). The four major types of plant hormones (gibberellin, auxin, cytokinin and absisic acid) interact with one another to govern the growth but the mechanism of their interaction is very complex. Although there have been many experiments conducted in dealing with plant hormones, it is still suggested that more

attention should be given to understanding hormones as components of regulatory systems (Morgan, 1990).

In "bulbous plants", only a little work has been done regarding with endogenous plant hormones or the application of plant hormones although it is proposed that all types of plant hormones are involved in bulb growth. According to Rees (1972), the senescence of leaves and the dying of roots in "bulbous plants" means that there are decreases in hormone supplies which are resumed only when new organs are formed during the developmental cycle. The bulbs come to the dormant period when the above ground parts die down. However, some bulbs can be active during the period when they initiate their flower or new leaves(Anon, 1984). Cessation of growth may be caused either by the formation of inhibitor by old leaves or because of a decrease in cytokinin supplied from the root system (Rees, 1972).

It is known that many "bulbous plants" like tulip, require cold treatment to "force" growth and induce flowering. It has been suggested that this cold requirement may be substituted by gibberellin treatment (Hanks and Jones, 1984). For tulip, it has been found that applied GAs advance the date of floral maturation irrespective of the length of previous cold storage (Hanks, 1982). In nerines, the effect of gibberellin has been studied on *N. fexuosa-alba.* After growing in a green-house for 10 months, bulbs were stored at 21° C for 10 weeks and were soaked in GA_3 before or after storage. It was found that no flowers were produced during the next 8 months in a green-house at 17° C. However, GA treated bulbs had split up into 2 - 7 separate bulbs with an accompanying increase in the number of flower buds from 2 to 3 in control, to 4 to 7 in treated bulbs (Fortainer *et al.,* 1979).

It is considered that the endogenous plant hormones are involved in a number of phases in the growth of "bulbous plants". The occurrence of gibberellin-like substances, whether in free or bound forms, has only been studied in a few species such as, *Tulip* (Aung and De Hertogh, 1968) and *Iris* (Pereira, 1964). In both tulip and iris bulbs, the gibberellin contents have been examined in relation with the low temperature treatment.

In iris bulbs, it has been noted that gibberellin-like substances are successively transported from scales to buds depending on the duration of the cold treatment and developmental stage of the primordium (Pereira, 1964). It has been proposed that gibberellin could initiate bulb growth and hence set in motion the events involved in carbohydrate metabolism (Halmer and Bewley, 1982). However, this relationship has not been fully understood yet. It is important to know precisely the inter-relation of carbohydrate and hormones as well as the amount of cold received, so that these processes can be accurately manipulated for the benefit of commercial forcing procedures (Rees, 1975).

2.4.3. The Anthocyanins

The anthocyanins are water soluble flavonoid pigments located in the vacuole of plant cells (Harborne, 1967 ; Proctor, 1971). There are six common anthocyanins, namely pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin. They are an important and widespread group of colouring matters in plants and often responsible for red coloration in plants although pink, scarlet, mauve, violet and blue plant colours are also attributable to the presence of anthocyanins. Pelargonidin is considered as an unusually frequent pigment in *Amaryllidaceae* (Harborne, 1967)

There are some factors that affect the colour of an anthocyanin, such as the acidity of the solution in which it is dissolved, complexing with metals (magnesium, iron or aluminium) and co-pigmentation, particularly with other flavonoid molecules, e.g. flavones. The concentration of the pigment or spectral composition of the light illuminating pigment surface may also modify the colour of plant pigmentsin tissue (Proctor, 1971). Such modificationsare known to be controlled by single gene substitutions in the flower of many higher plants. However, the production of anthocyanin in plants can be induced by several different environmental and developmental stimuli (Jackson *et al.*, 1992).

Anthocyanin identification is important since colour is one of the values for flower marketing. By knowing the anthocyanins present and the genes that control their synthesis, quality improvement of flower crops may be achieved. The anthocyanins in "ornamental bulbous plants" have been examined in several genera, for instance *Tulip* (Halevy, 1962), *Nerine* and *Hippeastrum* (Harborne, 1967) and *Iris* (Yabuya, 1991) . In *Nerine*, the anthocyanins have been examined only for *N. bowdenii* from the red berry and it has been found that the pigments present are pelargonidin 3-glucoside and pelargonidin 3- sambubioside. In this present work, anthocyanins have been examined in several other varieties of *Nerine*.

CHAPTER III : EXPERIMENTS

3.1. INTRODUCTION AND AIMS

Many experiments have been conducted with the "bulbous plants" in relation to the structure of the bulb, flowering , hormone content, etc.With *Nerine*, however, only few papers have been published. The value of *Nerine* as ornamental plants has increased during the last 15 years and it has been noted by Custers and Bergervoet (1992), that the auction turnover of *Nerine* flowers in Dutch ornamental horticulture increased from 3.5 million guilders in 1975 to about 15 million guilders in 1990. It has been suggested that many factors influence the growth and development of "bulbous plants" and such factors can be manipulated to program the flowering (Rees, 1972). It seems that the fundamental knowledge is required on the effects of both internal (hormones, carbohydrates) and external factors (temperature, photoperiod) on the growth and flower development of *Nerine*.

This recent work has been carried out with the purpose of :

- examining the effect of storage temperature on the carbohydrate contents, endogenous gibberellins and flowering of *N. fothergillii*
- examining the effect of photoperiod on the growth, development and flowering of *N. bowdenii*
- examining the anthocyanins from several varieties of *Nerine*.

3.2. THE EFFECTS OF STORAGE TEMPERATURE ON CARBOHYDRATES CONTENT, ENDOGENOUS GAs AND FLOWERING TIME OF *N. fothergillii*

This experiment was carried out to examine a possible correlation between carbohydrate contents, endogenous GAs and flowering time of *N. fothergillii* after the bulbs were stored in different temperature conditions.

Bulbs of uniform size (12 cm and over) of *N. fothergillii* were obtained from Channel Bulbs, Kettering. One hundred and twenty bulbs (120) were used and these bulbs were stored in different temperature conditions namely :

A : 30° C (bulbs were put in the oven) for 6 weeks

A1. 30° C (6 weeks) and then 5° C (2 weeks)

A2. 30° C (6 weeks) and then 5° C (4 weeks)

B : room temperature (± 20°) C for 3 weeks and then 30° C for 3 weeks

B1. room temperature (3 weeks) and then 30° C (3 weeks) and then 5° C (2 weeks)

B2. room temperature (3weeks) and then 30° C (3 weeks) and then 5° C (4 weeks)

C : room temperature (± 20° C) for 6 weeks

C1. room temperature (6 weeks) and then 5° C (2 weeks)

C2. room temperature (6 weeks) and then 5° C (4 weeks)

At the end of each storage treatment, these bulbs were taken out of the store. Five (5) bulbs were used for carbohydrate determination and endogenous GAs while the rest were planted for flowering.

3.2.1. Carbohydrate analysis

3.2.1.1. Materials and methods

Scales from five (5) bulbs of each storage treatment were peeled after removal of the outermost, dry scales and the scales were separated as :

(a) outer scales (S1), those which surround the bulb to the outside of first inflorescence bud

(b) inner scales (S2), those which surround the bulb inside the first inflorescence bud

(c) basal plate.

From each part, 10 grams were used as sample for carbohydrate analysis. Each sample was chopped into small pieces, homogenised in a Waring blender, and treated with 98 % ethanol. The mixture was then filtered through a thin layer of cotton wool 3 or 4 times. Subsequently, the ethanol was evaporated in vacuo at 40° C and the extract was diluted with 5 ml of 10 % isopropanol. This solution was then examined using paper chromatography.

Whatman no. 1 paper chromatography was used for this carbohydrate analysis. Each sample, except those of the basal plate, was diluted with 10 % isopropanol to give an extract concentration equivalent to 0.4 mg fresh weight of scale material per µL. Two (2) µL of this was used for paper chromatogram. The standard solutions used were glucose, fructose, sucrose and maltose. These standard solutions were prepared in 10 % aqueous isopropanol with concentration of 1 mg / ml.

Paper chromatograms were run for 16 hours with the solvent EtAcPy (ethyl acetate : pyridine : water = 120 : 50 : 40 v/v). Spots at the border of the paper sheet were revealed by alkaline silver oxide reagent which consists of :

(a) Ag NO_3 saturated solution in water 0.1 vol.

Acetone 100 vol.

(b) NaOH, 0.5 g dissolved in 5 ml water and diluted to 100 ml with ethanol (Menzies and Seakins, 1969).

The paper was first dipped through the silver reagent and the acetone was blown off. Subsequently, the paper was dipped through the alkali and again this solvent was blown off. The spots appear as dark brown spots on a background which changes through yellow to brown. The distance of the spots moving from the starting point is compared with those of the standards.

3.2.1.2. Results and discussion

Paper chromatograms showed that glucose, fructose and sucrose are present in all extracts examined. The glucose and fructose, however, are not completely separated. According to Darbyshire and Henry (1978), the glucose oxidase and resorcinol technique might be applied to estimate these separately. The amount of each extract used for paper chromatogram were equal (2 μL) but after the spots were revealed, they showed a difference in the colour intensity. The concentration of glucose, fructose or sucrose from each extract can be estimated by comparing the colour intensity of its spot. These results are illustrated in Plate 1.

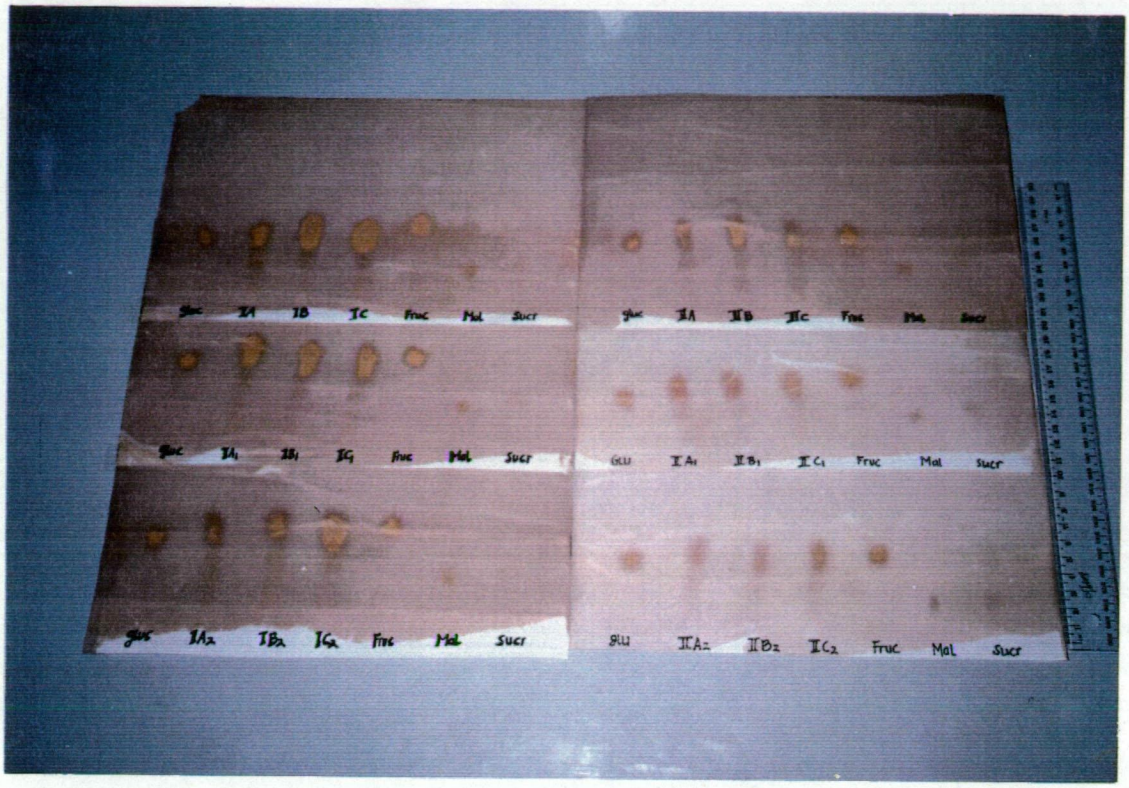


Plate 1. The chromatogram of carbohydrate content from outer (I) and inner (II) scales of *N. fothergillii* after being subjected to different storage temperatures

The colour spots of glucose, fructose and sucrose obtained from the outer scales are more intense compared with those from inner scales and there is only small variation of the colour intensity in the inner scales. In this case , it may be assumed that the carbohydrate storage in the outer scales has not been used or translocated to the inner scales, or that breakdown of storage polysaccharide has commenced in the outer scales. From tissue culture experiments with *Nerine bowdenii* as well as *Hippeastrum hybridum*, it has been found that bulblet regeneration is better in twin scales taken from the outer scales than in those from the inner scales (Grootarts *et al.*, 1981 ; Huang *et al.*, 1990). It seems that as long as the sprouting of the new leaves has not commenced, the outer scales contain more carbohydrate than the inner scales. The different storage temperatures in these experiments, however, seem to influence

the breakdown or hydrolysis of these food storages and it looks like the outer scales are more affected than the inner scales. The effect is more noticeable in bulbs subjected to 5° C for 4 weeks than 2 weeks.

In bulbs exposed to 30° C, the concentrations of glucose and / or fructose are lower compared with those which were subjected to room temperature or combination of room temperature and 30° C. This might be caused by the rapid transpiration at high temperature, as Dufus and Dufus (1984), have mentioned that the temperature between 25° and 30° C is the highest temperature at which a steady state of respiration can be maintained for long periods of time. The cold treatment (5° C) seems to reduce the concentration of glucose and / or fructose. This possibly is due to the absorption of these sugars by the basal plate during the cold treatment, since the basal plate also showed more intense colour than the outer scales (see Plate 2). This finding demonstrate the importance of the basal plate in successful twin scaling propagation.

The sucrose concentration is quite difficult to estimate because the spots only showed a slight difference in colour intensity. In many experiments with "bulbous plants", it has been found that sucrose content increased with the cold treatment. On the contrary, these results showed that the sucrose content seems to decrease with the cold treatment. Probably this is caused by the sucrose hydrolysis or degradation at the lower temperature as it has been found with onion (Darbyshire, 1978). However, this finding needs to be proved quantitatively.

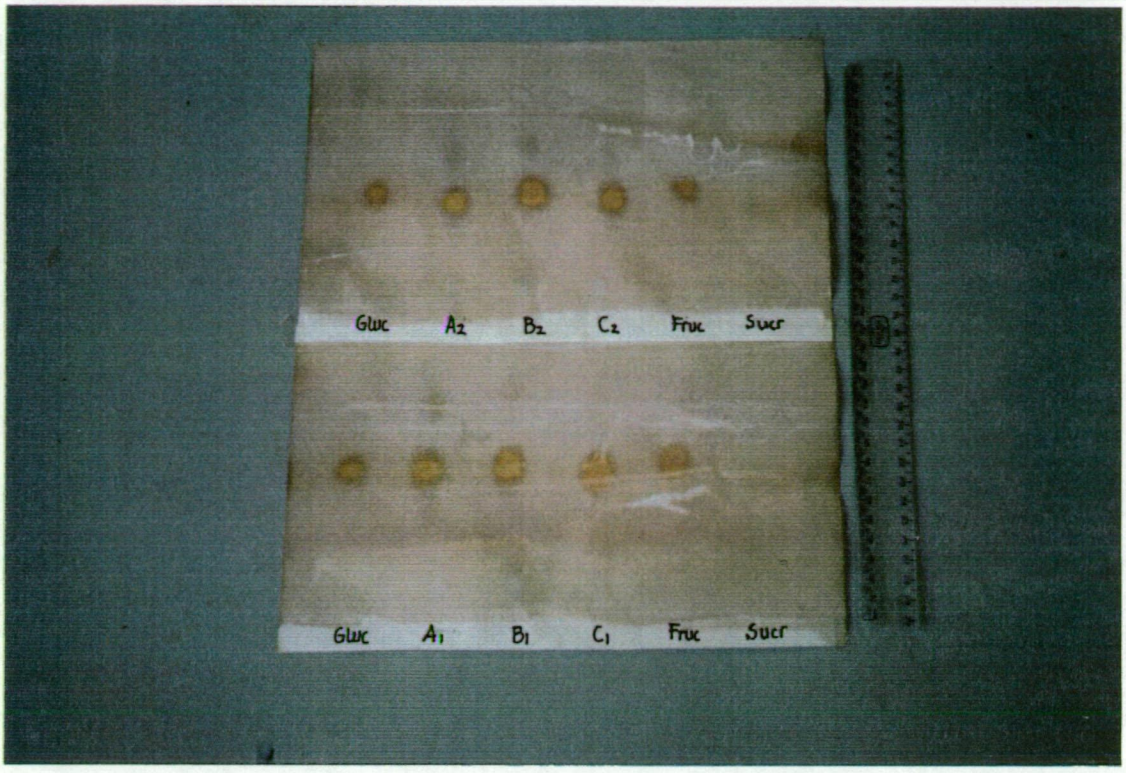


Plate 2. The chromatogram of carbohydrates content from basal plate of *N. fothergillii* after subjected to different storage temperatures

3.2.2. Identification and quantification of endogenous GAs

3.2.2.1. Materials and methods

Plant materials used for this experiment came from the same bulbs used for carbohydrate analysis. These bulbs had been stored in different temperatures as described before. Only the inner scales were examined for their endogenous GAs.

Extraction and fractionation of Gibberellins

Each sample was chopped into a small pieces and then macerated with Waring blender. Tissues were homogenised in absolute methanol (MeOH), approximately 5ml / g fresh weight and kept in the freezer. The concentration of the MeOH was reduced to 80 %, 24 hours before beginning the extraction procedure and each sample was transferred from freezer to fridge. The extracts were then filtered through Whatmann no. 1 filter paper and the residue washed with 80 % MeOH. Dideuterated internal standards were then added. The dideuterated internal standards used were [17,17 $^2H_2$] GA_{19}, [17,17 $^2H_2$] GA_{20}, [17,17 $^2H_2$] GA_1 (provided by Professor L. Mander, Australian National University, Canberra). Table 1 shows the quantities added. The filtrate was diluted to 60 % MeOH with distilled water and was passed through a Sep - Pak C_{18} cartridge (Millipore - Waters, Midford). Subsequently the extract was evaporated in vacuo to reduce the volume to approximately 10 %. An equal volume of sodium phosphate buffer (pH 8) was added and the pH was then reduced to 2.9 by adding 32 % HCl. After that the extract was partitioned against 40 % volume of ethyl acetate. The ethyl acetate fraction was then stored in the freezer.

HPLC procedure

The frozen ethyl acetate fraction was allowed to defrost at room temperature and then the water which had collected at the bottom of the container was discarded. Toluene equal to 5 % of its volume was added and also the tritiated internal standards were added. The tritiated internal standards used were [17,17 ^{13}C, $^{3}H_2$] GA_{20}, [17,17 ^{13}C, $^{3}H_2$] GA_1 and [17,17 ^{13}C, $^{3}H_2$] GA_{19} (provided by Professor B.O. Phiney, University of California, Los Angeles, USA). Table 1. shows the quantities added. The extract was evaporated to dryness on the rotary evaporator. Aliquots of extract were fractioned using C18 reversed-phase HPLC (Potts *et al.*, 1985). The main solvent programme ran from 21 to 70 % MeOH in 0.4 % aqueous acetic acid for 40 min with the solvent programmer (Waters model 660) set on gradient curve 6. The flow rate was 2ml min^{-1} and 1-min fractions were collected. Radioactivity was determined by a Beckman LS 5801 Liquid Scintillation System.

GC-SIM procedure

Samples containing GAs were analysed as the methyl ester trimethylsilyl ethers. Extracts were methylated in a 4 : 1 mixture of ethereal diazomethane and MeOH, dried and then trimethylsilated at 60° C for 10 min in 10 μ l of bis(trimethyl-silyl)trifluoroacetamide, with 3 μ l of dry pyridine to aid solution. GC- High Resolution SIM was performed using a Hewlett-Packard 5890 Series II GC coupled to a Kratos Concept ISQ, operating at a resolution of 10,000 with an accelerating voltage of 8KV and electron energy of 70 eV. A 25 m film BP1-fused silica column with internal diameter of 0.2 mm was coupled to the mass selective

detector via an open-split interface. Helium was used as the carrier gas, with an initial pressure of 25 psi to 40 psi at 30 psi / min, and then 25 psi at 30 psi / min and then 35 psi at 2 psi / min. Samples were injected in the splitless mode. The column was temperature programmed from 60° C to 240° C at 30° C / min, and then 290° C at 3° C / min. The ion pairs monitored for quantification of the respective GAs were : 506 / 508 (GA_1), 434 / 436 (GA_{19}) and 418 / 420 (GA_{20}). Additional ions were monitored to confirm identification. The peak area corresponding to the internal standard was corrected for the contribution by naturally occuring isotopes in the endogenous GA (using a computer program). In addition the peak area corresponding to the endogenous GA was corrected for the contribution by unlabelled material in the internal standard. This gave the corrected ratio of endogenous ion intensity to internal standard ion intensity. The product of this ratio and the amount of internal standard added was divided by the fresh weight of the tissue to give the endogenous GA level (Lawrence *et al.*, 1992).

Table 1. Fresh weight and internal standards added for GAs identification and quantification

Sample	FW (g)	dideuterated int. standard (ng)			tritiated int. standard (dpm)		
		GA_1	GA_{20}	GA_{19}	GA_1	GA_{20}	GA_{19}
A	23.58	20	20	200	20.000	30.000	20.000
A2	27.24	50	50	50	30.000	30.000	30.000
B	27.12	20	20	200	20.000	30.000	20.000
B2	27.24	50	50	50	30.000	30.000	30.000
C	25.49	20	20	200	20.000	30.000	20.000
C2	18.82	50	50	50	30.000	30.000	30.000

Note : Samples were obtained from bulbs that had been stored in different temperature
A = 30° C (6weeks) ; A2 = 30° C (6weeks) and then 5° C (4weeks)
B = room temp (3weeks) and then 30° C (3weeks) ; B2 = room temp. (3weeks) and then 30° C (3weeks) and then 5° C (4weeks)
C = room temperature (6weeks) ; C2 = room temp. (6weeks) and then 5° C (4weeks)

3.2.2.2. Results and discussion

In a preliminary experiment using inner scales from treatment C2, tritiated internal standards GA_1, GA_{20}, GA_{19}, GA_4 and GA_9 were added but after GC - SIM analysis only GA_{19} and possibly GA_{20} and GA_1 were detected as endogenous GAs. Thus with the other samples, analyses were concentrated on GA_{19}, GA_{20} and GA_1. Table 2 presents an assessment of the level of endogenous GA_{19}, GA_{20} and GA_1 from bulbs which had been subjected to differents storage temperatures.

Table 2. GC - SIM analysis of selected gibberellins in *N. fothergillii* bulbs which had been stored in different temperatures.

Sample	Gibberellin concentration (ng / g fresh weight)		
	GA_1	GA_{20}	GA_{19}
A	0.0259	0.0467	10.8969
A2	0.0650	0.0531	10.3209
B	-	0.0619	12.8947
B2	0.0351	0.0274	11.1332
C	0.0537	0.0960	9.1384
C2	-	0.0287	10.4600

Bulbs used in this experiment were still in a dormant condition when they were subjected to different storage temperatures. From the results, it is shown that GA_{19} and GA_{20} occured in all samples while GA_1 was only detected in trace amount from treatments A, A2, B2 and C. The amount of endogenous GA_{19} obtained from treatment B is slightly higher compared to the other treatments while the endogenous GA_1 could not be detected possibly due to the very low level. Steffens and Hedden (1992), have found that in dwarf apple trees, the high temperature of the ramped regime (20 - 30 - 20° C) results in reduced rate of conversion of GA_{19} to GA_{20}. It

seems that with *N. fothergillii*, the higher storage temperature (30° C) also reduces the conversion of GA_{19} to GA_{20}, since the level of GA_{20} in treatment A(bulbs stored at 30° C for 6 weeks) and treatment B (bulbs stored at room temperature for 3 weeks and then 30° C for 3 weeks) are lower than in treatment C (bulbs stored at room temperature for 6 weeks). According to Aung and De Hertogh (1968), the total concentration of the GA- like substances in tulips increased during the dry-cold treatment. The effect of cold temperature in this experiment, however, can not be seen clearly and it is difficult to determine whether cold temperature influences the metabolism of GA_{19} to the active GA_1 in these bulbs.

It has been suggested that GA_{19} is the precursor of GA_{20} while GA_{20} is 3 β - hydroxylated to GA_1 which is thought to be the biologically active GA controlling internode elongation in pea, maize and other species (Graebe, 1987). From the results, it was found that GA_{19} is present in higher quantity and may serve as a precursor 'pool' from which the bioactive GA_1 is produced. The same results have been found in Safflower (Potter *et al.*, 1993). The level of GA_1 was found to be very low and as Potter *et al.* (1993) have suggested, this lower level of GA_1 may reflect its faster metabolism or metabolic branching between GA_{19} and GA_1. This finding also demonstrates that rate of turnover to, or through, GA_1 may be physiologically more important than the GA_1 level (Graebe, 1987 ; Rood *et al.*, 1990 ; Potter *et al.*, 1993). The occurence of endogenous GA_{19} and GA_{20} in *N. fothergillii* has been identified by full scan GC - MS. GA_{19} gives quite clean spectra while clean spectra of GA_{20} can be obtained after the sample was run again through the HPLC. The GA_{19} and GA_{20} spectrum is presented in the following figure :

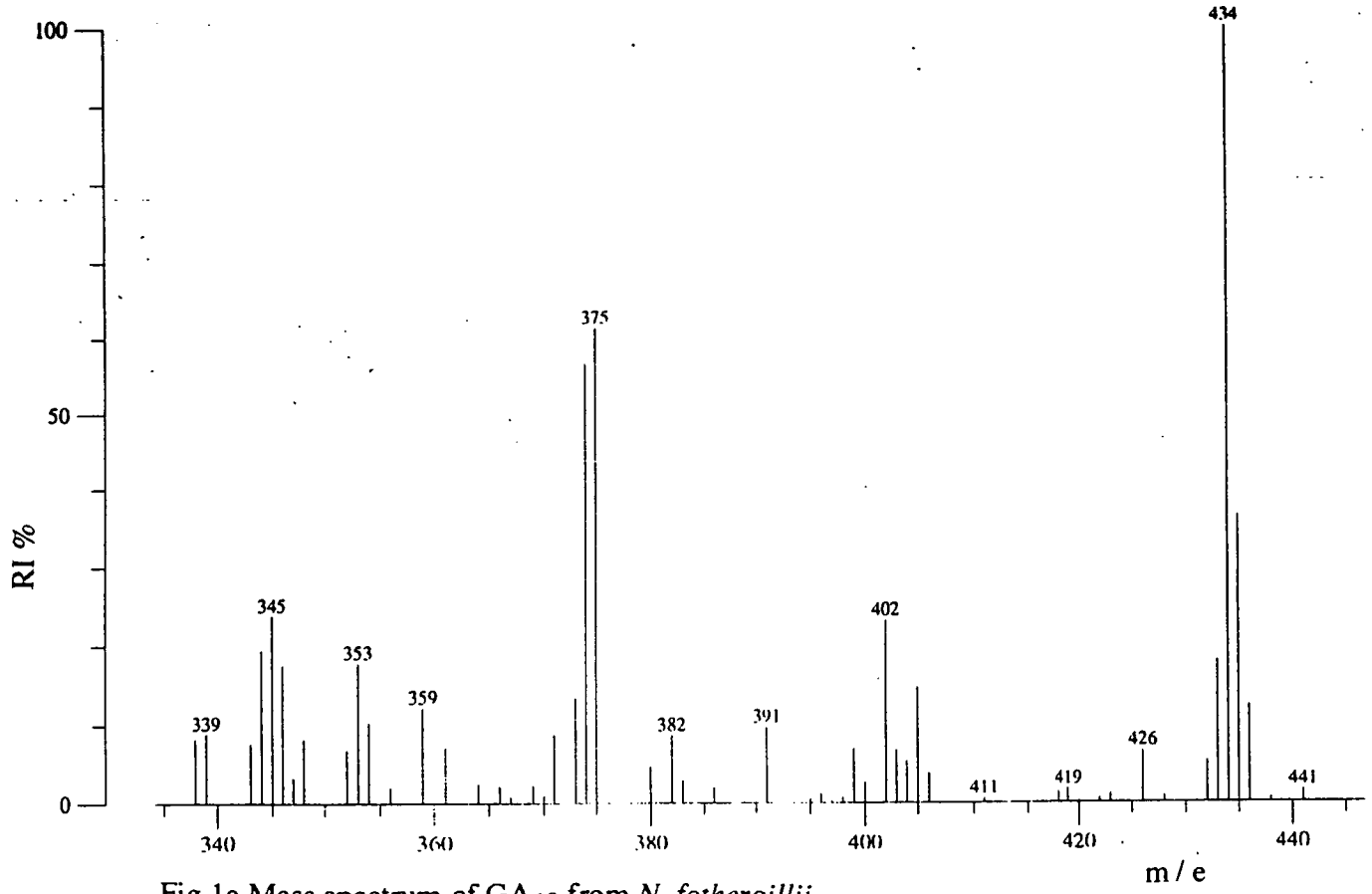


Fig.1a Mass spectrum of GA_{19} from *N. fothergillii*

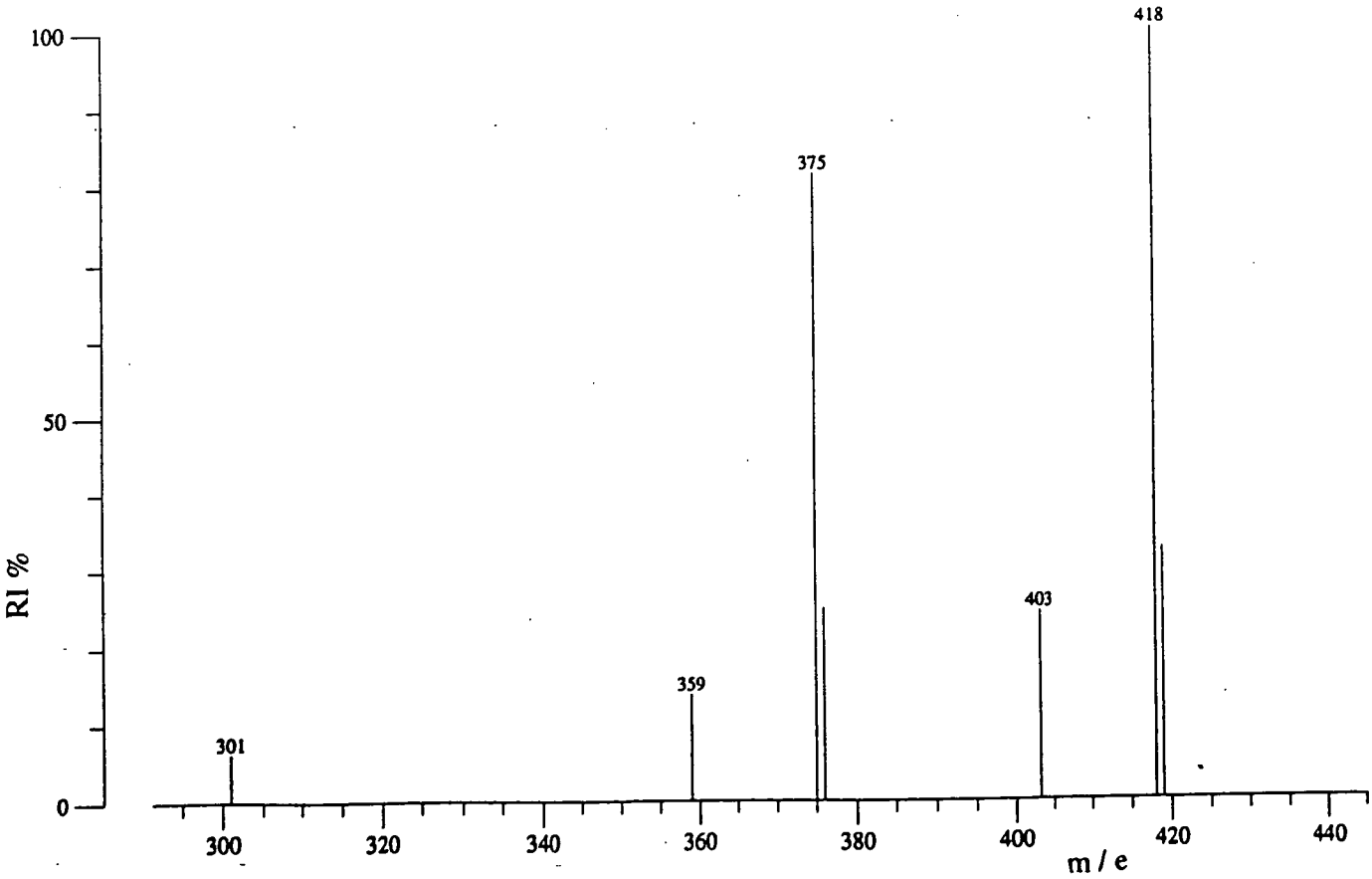


Fig.1b Mass spectrum of GA_{20} from *N. fothergillii*

Gibberellin A_{19} was the first GA to be identified from a plant belonging to the family Gramineae and together with GA_{20} and GA_1, they have been found to occur in sorghum, barley, rice and maize (Schliemann and Scneider, 1993). In dormant bulbils of Chinese Yam (*Dioscorea opposita*), endogenous GA_{19} and GA_{20} have also been detected (Tanno *et al.*, 1992). The present identification of GA_{19} gives another example of its occurence in monocotyledons.

It has been suggested that the GA biosynthetic pathway bifurcates at GA_{12}-aldehyde, giving rise to the so-called 'early 3β-hydroxylation pathway' and the 'non-3β-hydroxylation pathway'(Goodwin and Mercer, 1983). In addition there is a third pathway, the 'early 13-hydroxylation pathway', which operates in french bean, pea and maize (Phinney and Spray, 1982 ; Goodwin and Mercer, 1983). The 'early 13-hydroxylation pathway' also operates in several species of Gramineae as it has been summarized by Schliemann and Schneider (1993). The occurence of GA_{19}, GA_{20} and GA_1 in *N. fothergillii* indicates that in this species the 'early 13-hydroxylation pathway' is active.

Lin *et al.* (1975), have found that in *Lilium longiflorum* bulbs, the maximum GA levels occured 30 days after planting, or 7 days before shoot emergence and that inner scales contained a greater GA concentration than outer scales. In this experiment only inner scales were examined for their GA-content. However, an additional examination has been done with the outer scales from treatment C2 and it was found that GA_{19}, GA_{20} and GA_1 occured with the level of 16.9211, 0.0471 and 0.0558 ng / g fresh weight, respectively. Further examination of endogenous GAs from both outer and inner scales or others plant parts and from bulbs harvested at different

growth stages need to be conducted in order to get complete identification and quantification of endogenous GAs and also to get better understanding of GA biosynthesis in "bulbous plants".

3.2.3. Flowering time and leaf growth of *N. fothergillii*

3.2.3.1. Material and methods

The objective of this experiment was to examine the effect of different storage temperatures on flowering of *N. fothergillii*. The bulbs used in this experiment were stored either at room temperature (treatment C), 30° C (treatment A), or a combination of room temperature and 30° C (treatment B). Cold treatment (5° C) was applied after those storage treatments with a duration of 2 weeks (treatments A1, B1 and C1) or 4 weeks (treatments A2, B2 and C2). These bulbs were then planted, one per pot into a medium of "eucalypt soil" : gravel = 3 : 1 (v / v) and were placed in an open position to grow. The plants were watered lightly every second day and no additional nutrient or fertiliser was applied.

Leaf growth was recorded during the first four months after planting and its maximum length was measured. Flowering time was assessed when almost all of flower buds in each inflorescence were fully open and flowers were harvested. The days to flowering / anthesis were counted from planting date to the harvesting date. The number of flowers and stalk length from the nose of bulb to the base of the umbel were recorded.

3.2.3.2. Results and discussion

Leaf growth

Growth of the leaf is illustrated in Fig. 2 (a - c). From those figures, it is shown that the different storage temperatures (A, B and C) give a similar growth rate of the leaves. However, bulbs given treatment C, had

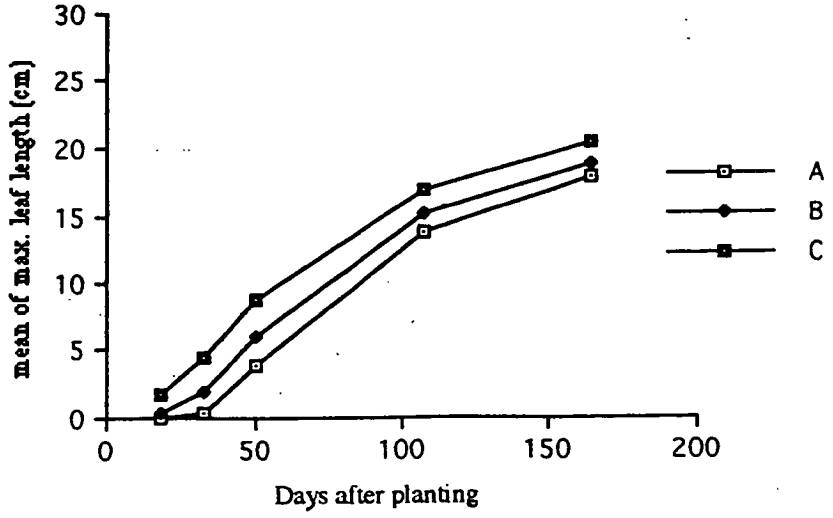


Fig. 2a Effect of different storage temperatures on leaf growth

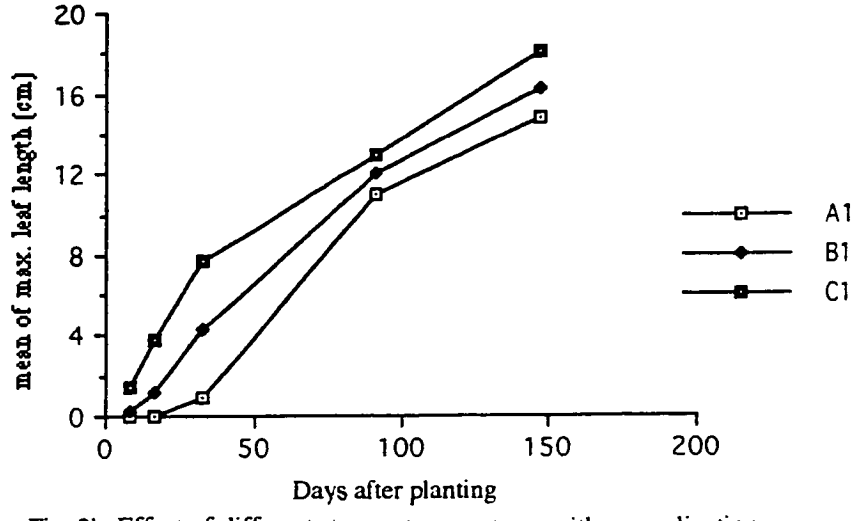


Fig. 2b Effect of different storage temperatures with an application of 5° C for 2 weeks on leaf growth

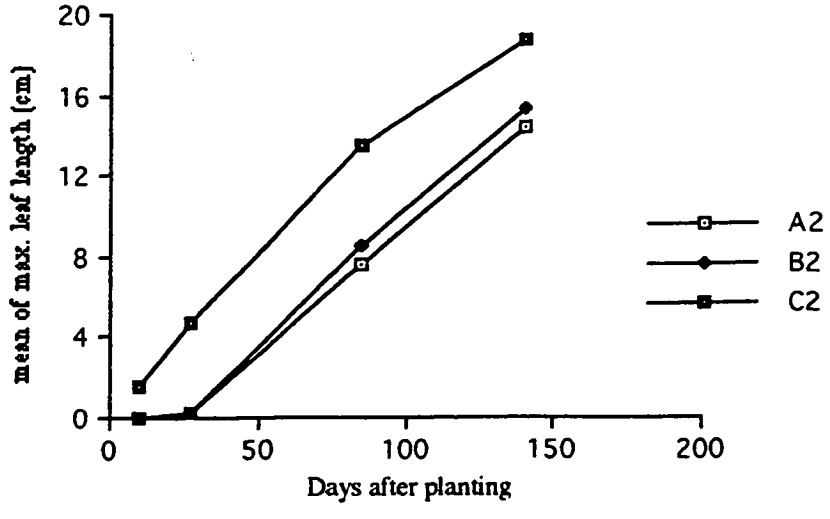


Fig. 2c Effect of different storage temperatures with an application of 5° C for 4 weeks on leaf growth

the greatest mean leaf length followed by treatments B and A. Application of cold temperature (5° C) with different duration (2 and 4 weeks) gives a similar leaf growth pattern. It seems that higher storage temperature (30°, A) slightly decreases leaf length, possibly through the alteration of hormone content. Kamerbeek and De Munk (1976), have noted that growth of roots , leaves and flowers in "bulbous plants" can be influenced by the exposure of the bulbs to ethylene during storage. In this experiment, ethylene may be produced by those bulbs subjected to 30° C.

Flowering percentage and days to anthesis

From the results, it was found that not all of the plants from each treatment come to flowering (see Table 3).

Table 3. The numbers of plant used and their flowering percentage

Treatment	No. of plant used	flower percentage (%)
A	15	73.3
A1	5	60.0
A2	5	80.0
B	15	80.0
B1	5	100.0
B2	5	40.0
C	15	100.0
C1	5	80.0
C2	5	80.0

From the table above, it is shown that flowering percentage decreases with treatment B and A respectively. Treatment C, in which bulbs were stored at room temperature (6 weeks) before planting gives 100% flowering, while treatment A, in which bulbs were stored at 30° C (6 weeks) before planting has less percentage of flowering compared with

treatment B, in which bulbs were stored in room temperature (3weeks) following at 30° C (3weeks) before planting. It seems that higher storage temperature (30° C) affect this flowering percentage.

Cold treatment (5° C) was applied with duration of 2 weeks (treatments A1, B1 and C1) and 4 weeks (Treatments A2, B2, and C2). It is shown that there is a variation in flowering percentage between treatments A1 and A2 as well as treatment B1 and B2, while in treatment C1 and C2 different duration of 5° C gives the same flowering percentage. Unfortunately, in these treatments (A1, B1, C1 and A2, B2 C2) only 5 bulbs were used per treatment, thus the number is not enough to make strong assumptions about effects the 5° C storage. However, the indications from this experiment warrant a further investigation. According to Hartsema (1961), bulb size is important in determining flower formation.With treatment B2, only 2 of 5 bulbs flowered and the three non-flowering bulbs were small (circumference less than 11 cm) when they were planted.

The effect of different storage temperature on the days to anthesis is illustrated in Fig.3 below :

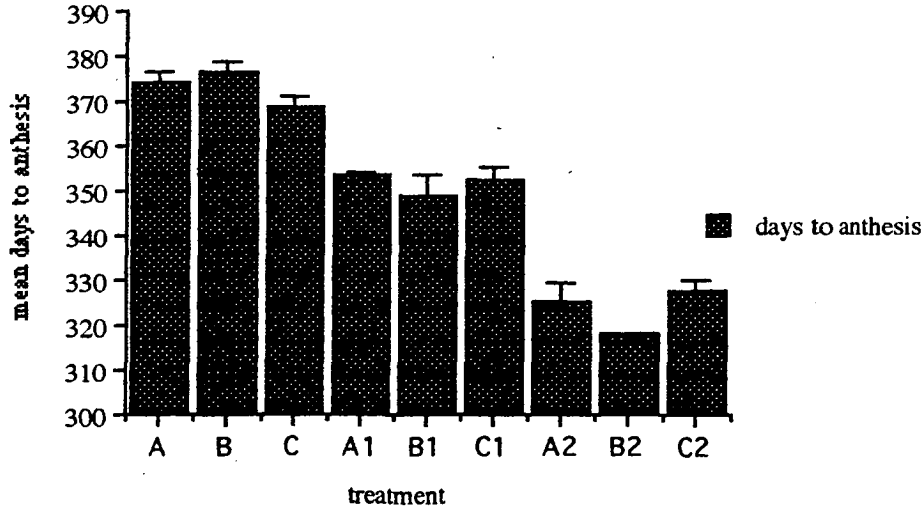


Fig.3 Effect of different storage temperatures on flowering of *N. fothergillii*

From the results, it was found that cold treatment (5° C for 2 weeks) causes earlier flowering and the longer exposure to cold (4 weeks) gives the earliest flowering time. The analysis of variance revealed that there is a significant difference in flowering time between 2 and 4 weeks duration of cold treatment (Table 4).

Table 4. Mean ± SE of days to anthesis, flower stalk length and flower number of *N. fothergillii* after being subjected to different storage temperatures.

Treatment	days to anthesis	flower stalk length (cm)	flower number
A (n=10)	374.2 ± 2.00^{ab}	23.15 ± 1.360	9.6 ± 0.87*
B (n=10)	377.2 ± 2.33^{a}	26.15 ± 0.890	10.7 ± 0.70
C (n=10)	368.7 ± 2.24^{b}	23.95 ± 0.620	11.7 ± 0.86*
A1(n= 3)	353.7 ± 0.67^{c}	25.50 ± 2.520	4.3 ± 0.33*
B1(n= 5)	349.0 ± 4.51^{c}	26.30 ± 1.540	8.8 ± 0.80
C1(n= 4)	352.2 ± 3.07^{c}	27.62 ± 1.910	11.2 ± 0.75
A2(n= 4)	325.2 ± 3.92^{d}	30.25 ± 1.510	10.0 ± 0.71
B2(n= 2)	318.0 ± 0.00^{d}	36.10 ± 2.100	10.5 ± 0.50
C2(n= 3)	327.7 ± 2.19^{d}	30.33 ± 0.930	10.0 ± 0.58

Note : values marked with the same letter show no significant difference, while the values marked with an asterisk (*) show significant difference (LSD, significant at 95%)

Treatment C possesses slightly but not significantly less days to anthesis compared to treatment A. It seems that 30° C storage temperature delays anthesis as it was found that there is a significant difference between treatments C and B. The numbers of days from inflorescence bud emergence to anthesis was counted and it was about 25 to 30 days in all treatments except in treatment B2, which took only 21 days. Erwin and Heins (1990), have found that in *Lilium longiflorum*, flower bud development rate from the visible bud stage until anthesis is influenced by day and night temperature. In this experiment, however, day and night

temperature was not recorded. Further experiments need to be conducted to examine the effect of temperature treatment after planting on flowering time in *N. fothergillii* since treatment B2 gives a more rapid flower bud development rate compared with the other treatments. Possibly with the appropriate combination of storage temperature and temperature after planting, flower bud development rate can be hastened.

Flower stalk and flower numbers

Mean length of flower stalk from the different treatment is presented in Fig. 4 below :

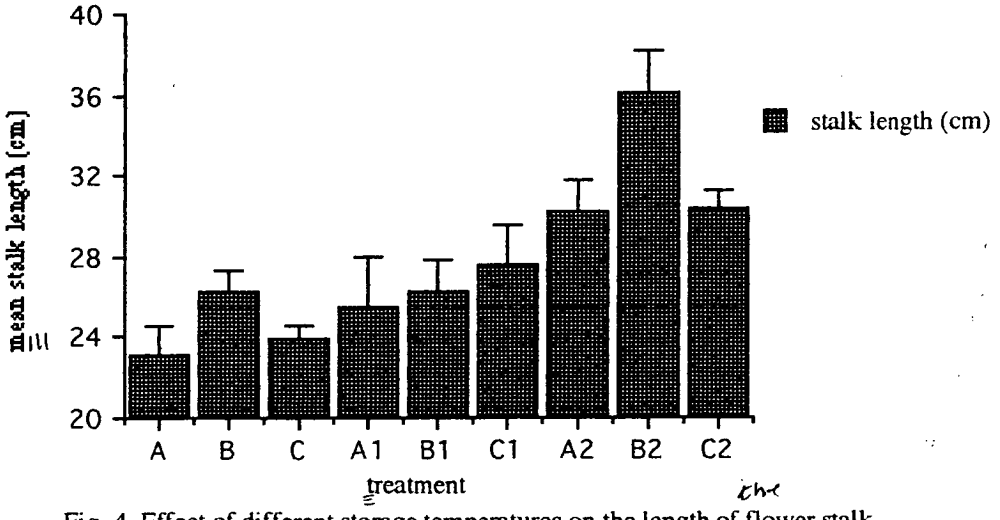


Fig. 4 Effect of different storage temperatures on the length of flower stalk

From the results, it was found that bulbs which had been subjected to 5° C for 2 weeks posses slightly, but not significantly, longer flower stalks compared to those bulbs without 5° C treatment. The longer exposure to 5° C, however, increases flower stalk length significantly compared with those bulbs without 5° C treatment (see Plate 3 and 4). It is suggested that for *N. fothergillii,* 5° C storage treatment for 4 weeks lengthened flower stalk. This effect is possibly due to GAs formed by floral organ, since bulbs which received 5° C for 4 weeks also flower earliest .

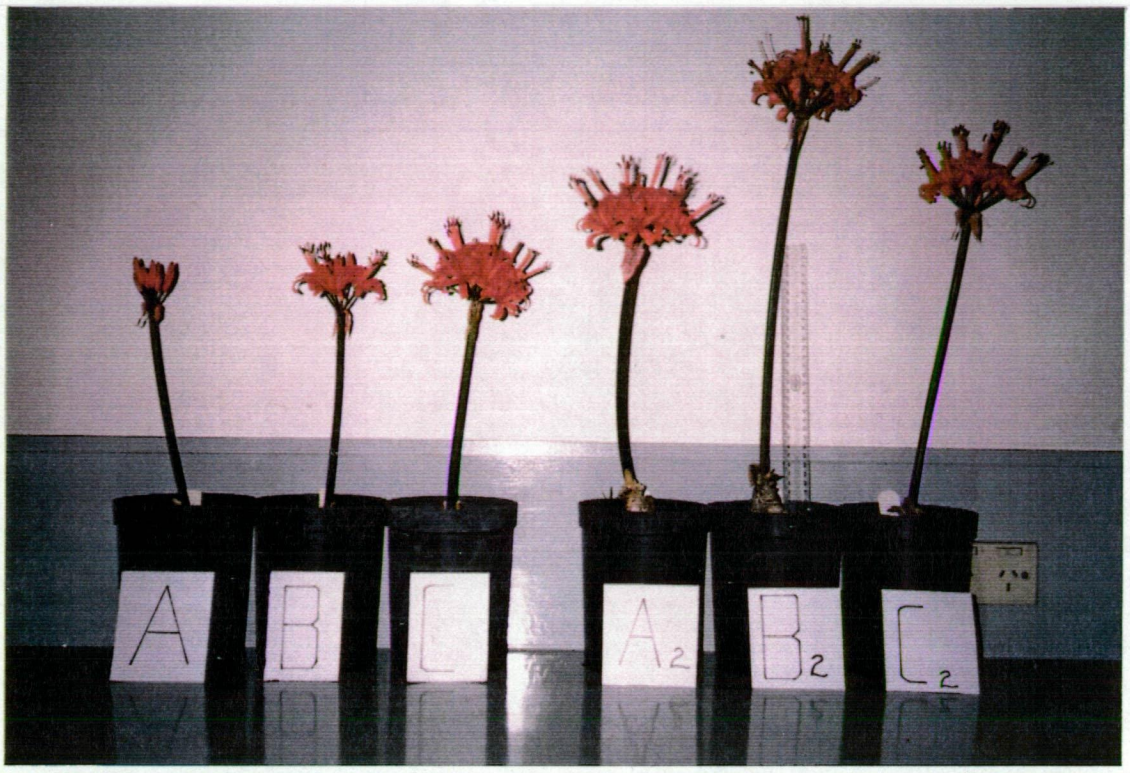


Plate 3. Flower stalk length of bulbs which were treated with 5° C for 4 weeks (A2, B2 and C2) compared with bulbs which were not treated with 5° C (A, B and C)

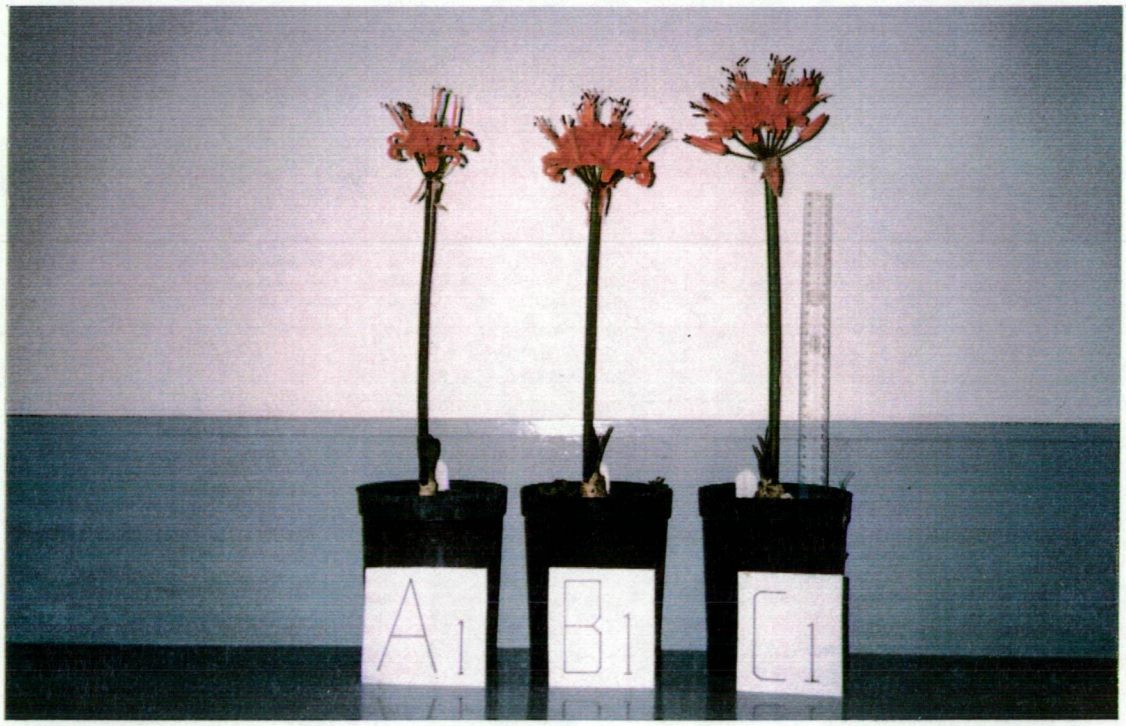


Plate 4. Flower stalk length of bulbs which were treated with 5° C for 2 weeks

It has been suggested that with cut tulips, the perianth appeared to be the primary organ controlling floral stalk elongation followed by the gynoecium and androecium (op den Kelder *et al.*, 1971). The longest flower stalk was obtained from treatment B2, in which bulbs were stored at room temperature (3 weeks) and then 30° C (3 weeks) and then 5° C (4 weeks) before they were planted. These bulbs also posses the earliest flowering time. Unfortunately, only 2 of 5 bulbs from this treatment come to flowering. It seems this storage treatment combination warrants further examination in order to get a good flowering quality (early flowering with long flower stalk) .

Mean numbers of flower from each treatment can be seen in Table 4. It is assumed that all plants will produce same flower numbers since the inflorescence buds had been formed before bulbs were subjected to different storage treatments. From the results, it is shown that there is a significant difference between treatment A and treatment C. It was found that an abortion of some of the flowers in each inflorescence occurred with those bulbs stored at 30° C for 6 weeks, thus reducing the mean number of flower. The effect of 30° C was not moderated by cold treatment applied for 2 weeks (A1), see Fig. 5 below :

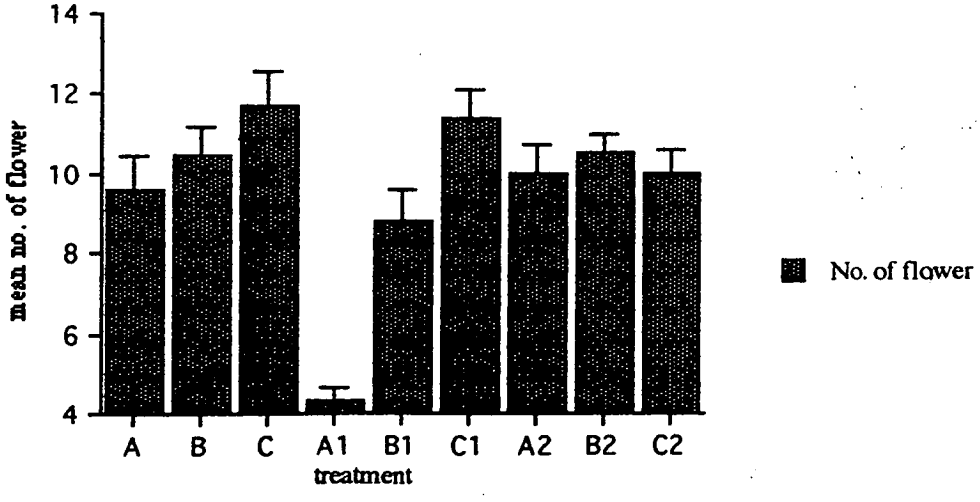


Fig. 5 Effect of different storage temperatures on the number of flowers per umbel

However, the longer exposure to cold treatment (A2) seems to nullify the effect of high temperature since not many abortions occurred, and flower numbers are restored. With *N. bowdenii*, flower bud abortion has been found as an after-effect result of high temperature (25° C) during the growth period. It has been suggested that this abortion is caused by reduction of sink activity of the developing flower (Berghoef and van Brenk, 1983). It could be that in this experiment, 30° C storage treatment inhibits the development of pistil primordia and stamen primordia, which can reduce the sink activity and thus cause flower abortion.

3.3. THE EFFECT OF PHOTOPERIOD ON GROWTH AND DEVELOPMENT OF *N. bowdenii*

3.3.1. Materials and methods

This study was undertaken to determine the effect of photoperiod on the growth and development of *N. bowdenii*. Two hundred (200) bulbs which had been dry stored at 2° C for a period of 4 - 5 weeks and with circumference of 10 - 14 cm were used in this experiment. Ten (10) of these bulbs were utilised as the initial condition. They were weighed, dissected and scales and inflorescence buds were scored. The rest of the bulbs were planted one per pot, in a 1 : 1 (v : v) mixture of vermiculite and gravel. After an initial watering, pots were watered every second day lightly.

Short day plants received 8 hr of daylight at approximately 23°C and they were then transferred to night compartments for 16 hr at 16° C. Long day plants remained on the apron of the glass house where they received normal daylength, supplemented to 18 hrs by fluorescent and incandescent lighting.

Ten (10) bulbs from each treatment (LD and SD) were examined at intervals of 4 weeks. These bulbs were washed free of all planting medium, blotted dry and weighed. The bulb and the basal plate plus roots were weighed separately. The bulbs were then dissected under a binocular microscope to determine the number of new scales formed by the meristem. The number of scales or leaves developed and the

number and position of flower bud was recorded from each bulb. The length of the leaves and inflorescence buds were also measured. The number and the length of the primary roots (those originating from the basal plate) were noted when they had been developed.

3.3.2. Results and discussion

3.3.2.1. Structure of the bulb

The scale arrangement of *N. bowdenii* shows a characteristic pattern. From dissection of many bulbs, it was found that the outer part of the bulb consisted of one or more dry scales which are the bases of former green leaves, then comes several (8 - 10) fleshy circular scales of leaf bases which surround the whole bulb and these scales function for food storage. Depending on bulb age, one or more old, desiccated flower stalks might be found amongst these scales and dry scales. The inflorescence buds are formed between two scales, the outer one is semi circular (it may develop into a leaf) and the inner one quarter circular which never grows into a leaf. Following the first inflorescence bud (± 1 cm in length) there are another 8 - 10 scales progressing towards the bulb centre which develop to leaves after the bulbs have been planted for a few weeks. The second inflorescence bud (2 - 3 mm in length) comes after these scales or leaves. New scale initials are evident after the second inflorescence bud and the meristem is in the centre of the bulb. These new scales are formed continually during the growth period and eventually develop into green leaves. Almost all of the bulbs examined had two inflorescence buds and out of 100 observed inflorescence buds, 58 % were located directly opposite to, and 42 % on the same side as the preceding one and always on direct line. These

results are similar to the descriptions by Sytsema (1982) and Berghoef and van Brenk (1983). The scales pattern of *N. bowdenii* is illustrated in Diagram 1 below :

---

1. o l l q f h l l l L s s s q f h s s s s s s s s s q p h s s d
2. o l l l l q f h l l l l l l l l q f h l L s s s s s q p h s s s s s s s d
3. o l l q f h l l l l l l l l l l l q f h l L s s s s s s s s s s s d
4. o l l l l l q f h l l l l l l l l q f h l L s s s s s s s s s s s s d
5. o l l l l l l L s s s s s s s s s s s s s s s s d
6. o l l q f h l l l l l L s s s q f h s s s s s s s s s s s d
7. o l l l q f h l l l l l l l l l l l q f L s s s s s s s s s s d
8. o l l q f h l l l l l l l l l q f L s s s s s s s s s d
9. o l l q f h l l l l l l l l l l q f L s s s s s s s s s s q p h s s s s s s d
10. o l l q f h l l l l l l l l l l q f L s s s s s s s s d

Diagram 1. The scales pattern of *N. bowdenii* (10 bulbs) with circumference of 10 - 14 cm

Symbols : o = meristem ; l = leaf ; q = quarter scale ; f = inflorescence bud ; h = half scale ; L = first emergent leaf ; s = scale / leaf base ; p = peduncle ; d = dry scale

3.3.2.2.Growth of the bulbs

All of the bulbs were of similar size when first planted on 31 st August 1992. Within 4 weeks the leaves from the centre of the bulb began to emerge and were accompanied by the development of some new primary roots. The first inflorescence buds are about 1.5 cm (stalk ± 0.5 cm ; flower bud ± 1.0 cm) while the second or the inner inflorescence buds are still very small and difficult to separate between stalk and inflorescence bud (their sizes are about 2 - 3 mm). Some daughter bulbs also developed and formed new leaves. There is not

much different in appearance of the bulbs after 8 weeks growth except the leaves and the primary roots that are getting longer (see Plate 5 and Plate 6).

After 12 weeks secondary roots have been developed. The leaves of the mother bulb as well as the daughter bulb continue to elongate. The first inflorescence buds have not shown any difference in size but the second inflorescence buds have developed from about 2 - 3 mm to 3 - 4 mm. By week 16, the secondary roots have developed abundantly and the leaves have reached the maximum length (see Plate 7 and Plate 8). Both the first and second inflorescence buds show development in size especially in the length of the inflorescence bud. The stalk length of the first inflorescence buds is still about 0.5 cm while the flower buds have reached 1 - 1.5 cm. With the second inflorescence buds, the stalk and buds can be distinguished. The lengths of the stalks are about 2 mm while the flower buds are about 3 - 4 mm.

By week 20 the mother bulbs have come to the maximum vegetative growth and some of the leaves have started to senesce (see Plate 9). Daughter bulbs continue developing and new leaf initials are differentiated from the meristem, which will emerge in the next growing period. The stalk lengths of the first inflorescence buds are about 0.7 - 0.8 cm and the flower buds have reached 1.5 - 2 cm. The second inflorescence buds have stalk and bud length of 2 - 3 mm and 5 - 6 mm, respectively. After 24 weeks some of the leaves have dried whilst some of the first inflorescence buds are emerging from the mother bulbs (see Plate 10).

Plate 5. Growth of *N. bowdenii* after subjected to LD and SD condition for 4 weeks

Plate 6. Growth of *N. bowdenii* after subjected to LD and SD condition for 8 weeks

Plate 7. Growth of *N. bowdenii* after subjected to LD and SD condition for 12 weeks

Plate 8. Growth of *N. bowdenii* after subjected to LD and SD condition for 16 weeks

Plate 9. Growth of *N. bowdenii* after subjected to LD and SD condition for 20 weeks

Plate 10. Growth of *N. bowdenii* after subjected to LD and SD condition for 24 weeks

3.3.2.3. The effect of photoperiod on the growth and development of the bulbs

Any effect of photoperiod on the growth and development of the bulbs were determined from several variables namely : fresh weight of the bulb, the weight of basal plate plus roots, circumference, the number of scales per growing period, maximum leaf length, inflorescence bud length, the number and length of primary roots, the chlorophyll content, total soluble carbohydrates and the days from planting to anthesis. The chlorophyll content and total soluble carbohydrate are determined using the methods described by Witham *et al.*(1971). These examinations (the chlorophyll content and total soluble carbohydrate) were only conducted with the bulbs harvested at 24 weeks when the maximum vegetative growth of the mother bulbs had been reached.

Bulb fresh weight and total soluble carbohydrate

Data of bulb fresh weight is presented in Table 5. From these data , it can be seen that bulb fresh weight decreases for the first 4 and 8 weeks after planting and then increases after week 12. This is probably due to utilisation of food reserves from the scales during the early period of growth and development of leaves and new roots. After week 12 the leaves have fully developed and are capable of maximum photosynthesis, hence the photosynthesis products can be stored again and bulb weight is increased. The mean of bulb fresh weight is slightly greater in SD than in LD, however, the significant difference is only found in bulbs harvested at 4 weeks. Total soluble carbohydrate examined from bulbs harvested at 24 weeks shows similar result

Table 5. The mean ± SE (n = 10) of fresh weight of the bulb, basal plate and roots, circumference, number of scales and the number of daughter bulb of *N.bowdenii* grown in LD and SD condition for 4, 8, 12, 16, 20 and 24 weeks

Treatment	fw (gr)	bp+r (gr)	circ (cm)	S1	S2	S3	ndb
Initial	64.89 ± 7.050	4.86 ± 0.720	13.97 ± 0.620	10.67 ± 0.690	9.44 ± 0.500	2.56 ± 0.290	5.10 ± 0.920
4w - LD	40.77 ± 1.546*	2.04 ± 0.190	12.44 ± 0.320	9.20 ± 0.800	8.90 ± 0.820	3.80 ± 0.250	3.70 ± 0.730
4w - SD	50.38 ± 4.607*	3.26 ± 0.570	13.53 ± 0.850	10.20 ± 0.680	9.50 ± 0.220	3.30 ± 0.300	5.10 ± 1.020
8w - LD	36.80 ± 1.704	2.00 ± 0.190	10.99 ± 0.270	9.20 ± 0.360	9.40 ± 0.270	5.30 ± 0.210	5.20 ± 0.740
8w - SD	38.11 ± 3.335	3.07 ± 0.400	11.33 ± 0.510	9.80 ± 0.650	9.50 ± 0.310	4.00 ± 0.450	5.10 ± 1.160
12w - LD	35.97 ± 2.220	3.82 ± 0.420	10.74 ± 0.320	8.50 ± 0.400	9.30 ± 0.520	5.30 ± 0.750	4.40 ± 1.180
12w - SD	40.81 ± 3.228	3.65 ± 0.390	10.95 ± 0.490	9.50 ± 0.970	8.89 ± 0.110	6.20 ± 0.420	3.50 ± 0.310
16w - LD	46.36 ± 2.610	9.75 ± 0.670	11.11 ± 0.300	9.20 ± 0.740	8.89 ± 0.310	7.60 ± 0.670	3.30 ± 0.300
16w - SD	55.33 ± 3.543	8.97 ± 0.840	12.14 ± 0.300	8.50 ± 0.690	8.80 ± 0.290	7.80 ± 0.530	2.70 ± 0.470
20w - LD	56.56 ± 2.970	12.99 ± 0.870*	12.80 ± 0.280	7.20 ± 0.770	9.20 ± 0.360	10.10 ± 0.310	4.50 ± 0.600
20w - SD	52.10 ± 3.460	8.47 ± 0.770*	12.02 ± 0.320	7.90 ± 0.620	9.40 ± 0.220	9.80 ± 0.250	5.00 ± 0.490
24w - LD	48.32 ± 3.620	9.62 ± 1.820	12.73 ± 0.330	5.67 ± 0.870	8.80 ± 0.360	11.60 ± 7.200	3.30 ± 0.400
24w - SD	51.98 ± 4.530	10.80 ± 1.240	12.28 ± 0.470	6.50 ± 0.580	9.80 ± 0.250	10.60 ± 0.310	4.50 ± 0.750

Note : fw = fresh weight of the bulb ; bp + r = weight of basal plate plus roots ; circ = circumference ; S1 = the scales number after the dry scales to the first inflorescence bud ; S2 = the scales / leaves number from the first inflorescence bud to the second inflorescence bud ; S3 = the number of new leaves to meristem ; ndb = the number of daughter bulb.
The values marked with asterisk (*) show significant difference (LSD, significant at 95%).

between LD and SD. The effect of LD and SD on bulb fresh weight during development is illustrated in Fig. 6 below :

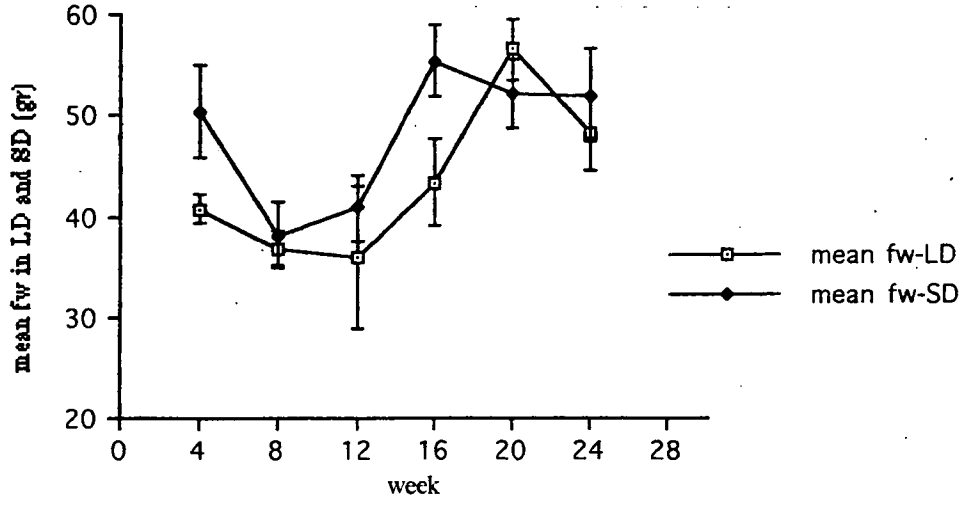


Fig 6. Effect of LD and SD on bulb fresh weight during development

Basal plate & roots weight, primary roots number and maximum length of primary roots.

Data of basal plate & roots mean weight can be seen in Table 5, while the data of mean roots number and roots length can be seen in Table 6. From these data it is found that LD and SD has no effect on these variables except in bulbs harvested at 20 weeks there is a significant difference between LD and SD in mean weight of basal plate & roots. This difference is caused by root rot that occured in some of the SD bulbs. Rees (1972), has noted that fungi and nematodes can cause root rot in some "bulbous plants" like *Tulip*, however, what caused root rot in those SD bulbs has not been determined. The mean weight of basal plate plus roots increases after week 8 as the primary roots grow and develop.

Root initiation occurred within the first 4 and 8 week of bulb growth but the number of primary roots developed was only counted at

week 12, 16, 20 and 24. Secondary roots also develop during the growth of the bulbs, however, they were not counted. The initiation of primary roots ended within week 16 since after that time the number of primary roots is quite constant. The number of primary roots developed in LD and SD is about 10 - 12 while the maximum length of the primary roots is about 30 cm. The primary roots grow to the maximum length within week 20. The analysis of variance revealed no differences between LD and SD of both the number of primary roots and maximum length of primary root. The number of primary root and maximum length of primary root recorded during bulbs growth in LD and SD are illustrated in Fig. 7 and Fig. 8 below :

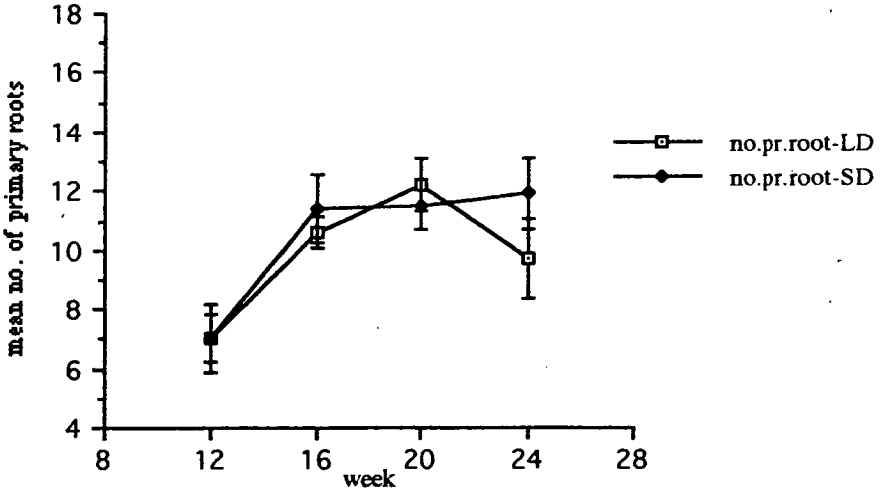


Fig. 7 The number of primary roots from bulbs grown in LD and SD

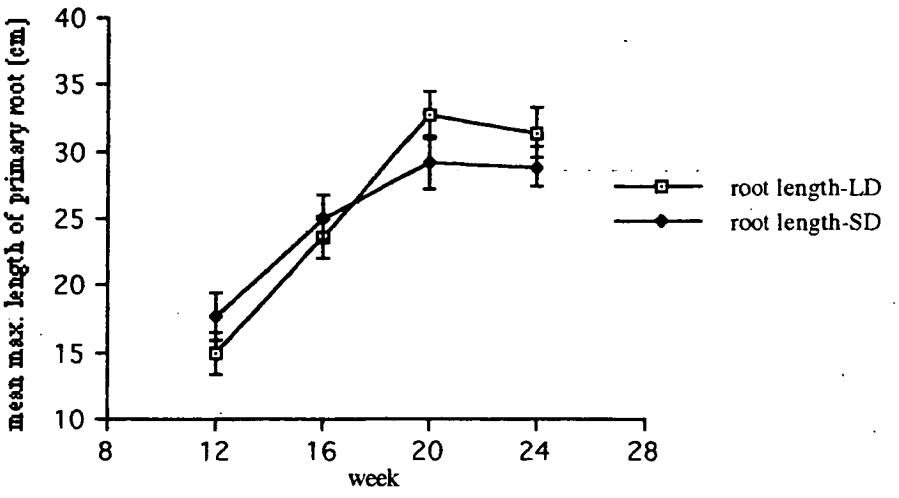


Fig. 8 The maximum length of primary root from bulbs grown in LD and SD

Bulb circumference, scales number and daughter bulb

Data of bulb circumference, scale number and daughter bulb number are presented in Table 5. From these data, it was shown that bulbs harvested randomly from LD and SD at 4 weeks interval are of similar size since the mean circumferences only differ slightly and not significantly. Bulb size seems constant during the vegetative growth period. In this experiment, each bulb was planted individually in a pot and nutrient solution was given fortnightly during the growth. Rees and Torquand (1969), have found that increases in planting density decrease the mean bulb size of *Tulip* cv. 'Apeldoorn' and 'Rose copland'. Possibly bulb size of *N. bowdenii* will differ if they are not grown individually. In *Tulip* cv. 'Apeldoorn', it has been found that bulb size had a positive effect on root number as well as rooting depth (Schuurman, 1971 in Rees, 1972). With *N. bowdenii* it seems that bulb size has positive effect on rooting depth.

Scale number was counted as S1 (those which surround the bulb to the outside of first / outer inflorescence bud), S2 (those which surround the bulb inside the first / outer inflorescence bud and outside

of second / inner inflorescence bud) and S3 (those which surround the bulb inside the second / inner inflorescence bud. From the results, it was noted that S1 and S2 is about 9 - 10. S1 decreases at week 20 since the outer part of these scales become dry. S2 had been formed in the previous growth period thus their number is constant, while S3 gradually increases during this vegetative growth as the result of differentiation of new scales by the meristem. The analysis of variance revealed no significant difference in the number of scales between LD and SD. The scale numbers (S1, S2 and S3) from bulbs grown in LD and SD are illustrated in Fig. 9 below :

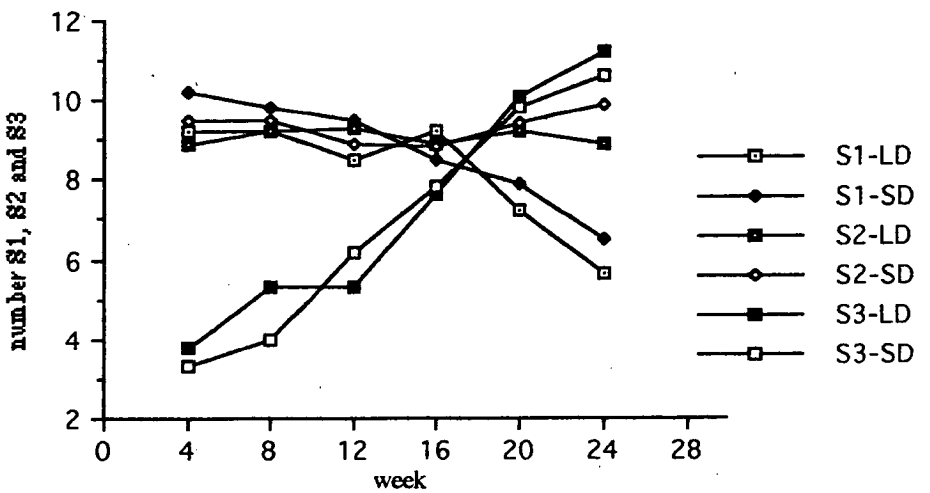


Fig. 9 Mean number of scales (S1, S2 and S3) from bulbs grown in LD and SD

Daughter bulbs are formed in the axils of older scales in the outer part of the mother bulb but some daughter bulbs were already initiated amongst scales which surround the bulb to the outside of first inflorescence bud (S1). These daughter bulbs will be released when the outer part of the mother bulb has dried. From the results, it was found that the mean number of daughter bulb is quite similar between LD and SD (about 3 - 5) and there is no effect of photoperiod on the number of daughter bulb.

Leaf number, maximum length of leaf and total chlorophyll

Leaf growth occurred as soon as the bulbs were planted. From the results, it was found that leaves grew in length from initiation and reached maximum length at week 16. With bulbs harvested at 12 weeks there is a significant different between LD and SD for both leaf number and maximum length. The mean maximum length is greater in SD than LD, this difference probably caused by primary and secondary root growth which is greater in bulbs grown in SD than in LD. At week 24 there is a significance difference in leaf number between LD and SD and this is caused by leaf senescence that is more pronounced in LD than SD. From the result of total chlorophyll content, it was found that chlorophyll content is slightly greater in SD than in LD (see Table 7). Leaf growth of bulbs grown in LD and SD is illustrated in Fig. 10 below :

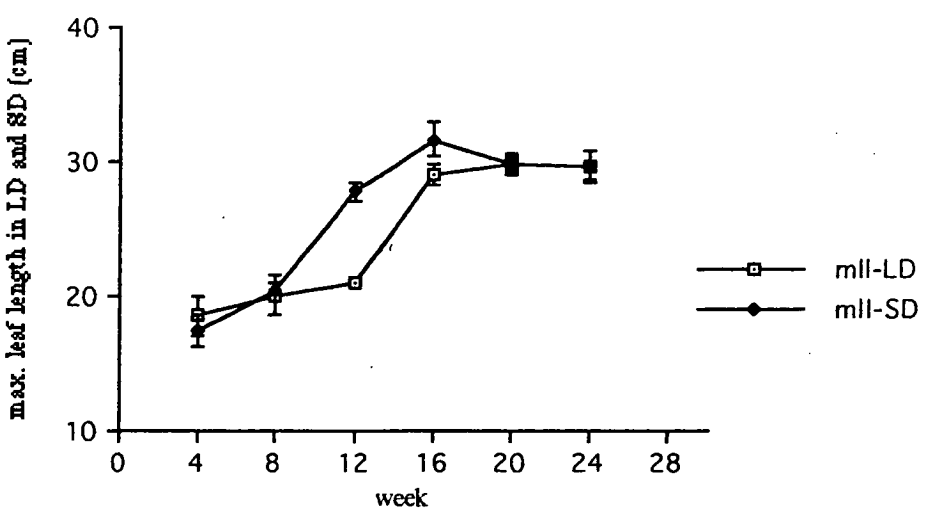


Fig. 10 Leaf growth of bulbs grown in LD and SD

Inflorescence bud

Growth of the outer inflorescence bud is illustrated in the following figure :

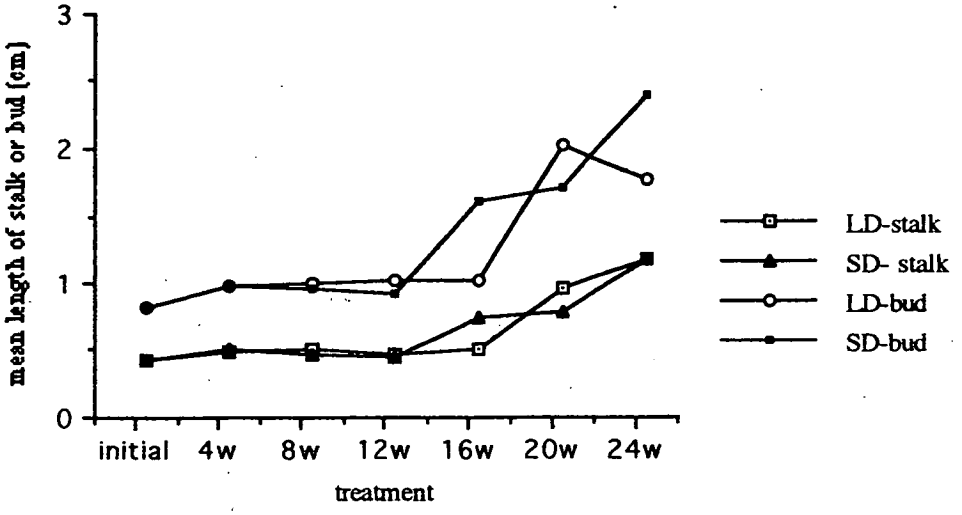


Fig. 11 Mean length of stalk and bud of outer inflorescence buds grown in LD and SD

From the figure above, it is shown that there is only slight growth of outer inflorescence buds during the first three months of growth (week 4 to 12). After week 12, the length of both stalk and bud increase gradually and it was noted that the outer inflorescence buds grow more in SD than in LD. However, from the analysis of variance, there is no significant difference between SD and LD (Table 8). This result demonstrates that bulbs need to establish their growth during the first three months after planting and then they will be able to supply the energy needed for the growth of inflorescence buds.

The inner inflorescence buds are still very small at 4,8 and 12 weeks after planting and their lengths were measured as stalk plus bud. At 16 weeks their stalk and bud can be measured separately. Growth of inner inflorescence bud in LD and SD is illustrated in Fig.12 .

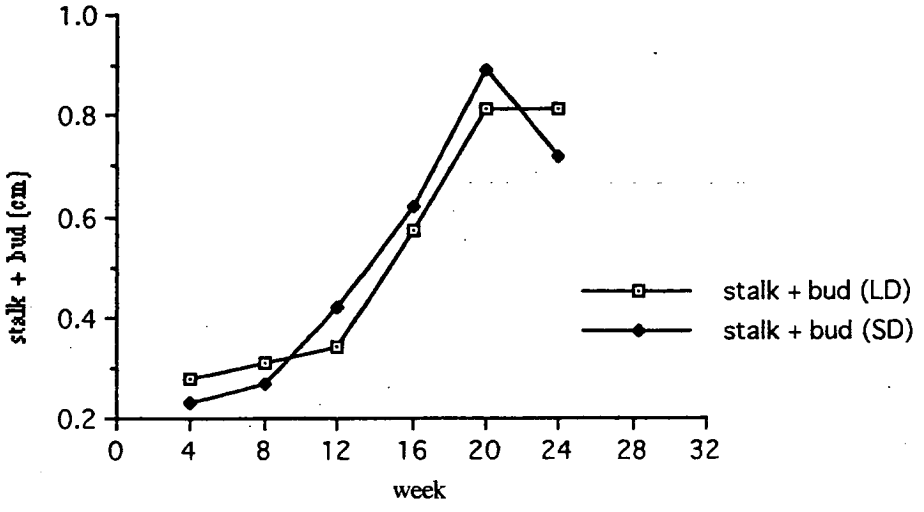


Fig. 12 Growth of inner inflorescence bud in LD and SD

Berghoef and van Brenk (1983), have found that the rate of differentiation of the flower buds as well as the number of leaves initiated are affected by the temperature during growth. They suggested that there is a competition between vegetative and generative growth that is mainly affected by growth temperature. From this experiment, it was found that the growth rates of outer and inner inflorescence buds gradually increase from week 12, when the leaves nearly reach the maximum length. Photoperiod, however, does not influence the differentiation of outer and inner inflorescence bud during the vegetative growth.

Inflorescence bud abortion occurred in both SD and L D conditions. This abortion occurred mainly during the first three months of the growth of the bulbs. An after effect of previous growth conditions could be the cause for this abortion as it has been suggested by Berghoef and van Brenk (1983), that the temperature (within the range 9° C to 25° C) had no effect on flowering during the growth period of *N. bowdenii*, but on the following year the flowering of bulbs previously grown at higher temperature was reduced.

Table 6. The mean ± SE (n = 10) of the number and the maximum length of leaf and primary roots grown in LD and SD condition for 12 , 16 , 20 and 24 weeks

Treatment	roots number	roots length (cm)	leaf number	max. leaf length (cm)
4 w - LD			8.3 ± 0.77	18.54 ± 1.430
4 w - SD			8.7 ± 0.42	17.42 ± 1.240
8 w - LD			9.6 ± 0.40	20.04 ± 1.450
8 w - SD			9.3 ± 0.26	20.36 ± 0.690
12 w - LD	7.0 ± 1.17	14.88 ± 1.570	7.7 ± 0.80*	20.93 ± 0.260*
12 w - SD	7.0 ± 0.79	17.70 ± 1.750	9.0 ± 0.21*	27.78 ± 0.670*
16 w - LD	10.6 ± 0.52	23.56 ± 1.610	8.7 ± 0.30	28.90 ± 0.750
16 w - SD	11.4 ± 1.14	25.09 ± 1.620	9.0 ± 0.39	31.66 ± 1.240
20 w - LD	12.2 ± 0.88	32.68 ± 1.700	8.8 ± 0.36	29.82 ± 0.540
20 w - SD	11.5 ± 0.85	29.12 ± 1.980	9.5 ± 0.22	29.83 ± 0.760
24 w - LD	9.37 ± 1.37	31.39 ± 1.910	6.2 ± 0.59*	29.56 ± 1.150
24 w - SD	11.9 ± 1.20	28.77 ± 1.470	8.4 ± 0.67*	29.66 ± 1.060

Note : The values marked with an asterisk (*) show significant difference (LSD, significant at 95%)

Table 7. The chlorophyll content and total soluble carbohydrate from the bulbs harvested at 24 weeks.

Treatment	chlorophyll content (mg / gr fresh weight)			total soluble carbohydrate
	chl. a	chl.b	total	(µg / g fresh weight)
LD	0.62	0.31	0.93	26.03
SD	0.75	0.35	1.10	26.12

Note : the spectrophotometer used for these quantifications was Spectronic 20 Bausch & Lomb.

Table 8. The mean length ± SE (n = 10) of the outer and inner inflorescence bud grown in LD and SD condition for 4, 8, 12, 16, 20 and 24 weeks.

Treatment	outer inflorescence bud		inner inflorescence bud		
	stalk (cm)	bud (cm)	stalk + bud (cm)	stalk (cm)	bud (cm)
initial	0.35 ± 0.050	0.74 ± 0.090	0.27 ± 0.040		
4w - LD	0.42 ± 0.020	0.91 ± 0.040	0.28 ± 0.020		
4w - SD	0.43 ± 0.030	0.91 ± 0.030	0.23 ± 0.020		
8w - LD	0.43 ± 0.030	0.92 ± 0.020	0.31 ± 0.020		
8w - SD	0.40 ± 0.030	0.89 ± 0.020	0.27 ± 0.020		
12w - LD	0.40 ± 0.040	0.95 ± 0.040	0.34 ± 0.050*		
12w - SD	0.37 ± 0.070	0.85 ± 0.110	0.42 ± 0.030*		
16w - LD	0.44 ± 0.090	0.94 ± 0.140		0.19 ± 0.020	0.38 ± 0.040
16w - SD	0.66 ± 0.070	1.52 ± 0.110		0.20 ± 0.020	0.42 ± 0.040
20w - LD	0.88 ± 0.170	1.95 ± 0.320		0.26 ± 0.020	0.55 ± 0.030
20w - SD	0.71 ± 0.150	1.62 ± 0.810		0.31 ± 0.030	0.58 ± 0.040
24w - LD	1.10 ± 0.650	1.68 ± 0.440		0.25 ± 0.020	0.56 ± 0.030
24w - SD	1.10 + 0.330	2.31 + 0.510		0.22 + 0.020	0.50 + 0.040

Note : The values marked with an asterisk (*) show significant difference (LSD, significant at 95%).

Another possible cause of inflorescence bud abortion could be from virus disease as it has been found in other bulb species like tulip, narcissus, iris and lily (Rees,1972). From the examination of the aborted inflorescence buds, it was found that many callus tissues are scattered in the inflorescence buds and the quarter scales. It is suggested that the higher temperature could act indirectly through the mechanisms that promote virus attack on the bulbs, hence, the abortion is more severe after the bulbs were grown at higher temperature. Alternatively, it might be that an after effect of higher temperature causes the reduction or even lack of supply of substances, such as sugar or hormones which are needed for the growth of inflorescence bud.

Flowering time

Flowering time / anthesis was assessed when almost all of the flowers in each umbel are fully open and the days to anthesis was counted from planting date. From the results, it was found that flowering time is earlier in SD than LD and the analysis of variance revealed a significant difference between SD and LD (see Table 9) below :

Table 9. Mean ± SE (n = 10) of days to anthesis, flower stalk length and flower number of *N. bowdenii* grown in LD and SD condition

Treatment	days to anthesis	flower stalk length (cm)	flower number
LD	207.5 + 1.21*	40.70 + 1.380	6.3 + 0.33*
SD	200.8 + 1.11*	41.95 + 1.050	7.4 + 0.34*

Note : the values marked with an asterisk (*) show significant difference, (LSD, significant at 95%)

Mean flower numbers is slightly, but significantly greater in SD than LD while there is no significant difference in flower stalk length between LD and SD. The difference in flower numbers is mainly caused by abortion of one or two flower in each umbel of those plants subjected to LD. Berghoef and van Brenk (1983), supposed that the absence of androecium and gynoecium is the main cause of abortion in *N. bowdenii* while in tulips, it was found that ethylene can stimulate flower bud blasting (de Munk, 1973).

In this experiment, it was found that leaf senescence is accelerated in LD compared to SD, thus it is assumed that ethylene production could be greater in LD than SD. Flower abortion and delayed flowering time in LD may be caused by this ethylene production. Further experiments need to be conducted to prove this assumption. From the results, it is suggested that different photoperiods do not effect the vegetative growth of *N. bowdenii* but influence the growth rate of inflorescence buds. Short day conditions seem to promote the growth rate of inflorescence buds and possibly the involvement of plant hormones causes the difference in flowering time between LD and SD. However, the after effect of this photoperiod treatments still needs to be examined since it has been found that the reliability of flowering in *N. bowdenii* depends on a wide range of factors like light, temperature and humidity, and such factors often become effective not in the next anthesis but two years later (Fiedler, 1989).

3.4. ANTHOCYANINS OF *Nerine* FLOWERS

3.4.1. Materials and methods

In this experiment, nine species or varieties of *Nerine* were used namely : *N. fothergillii*, *N. flexuosa*, *N. x splendens*, *N. bowdenii*, *N. undulata*, *N. corusca*, *N. sarniensis*, *N. x roseum* and *N. x salmonea* .

For each type, petals were ground and extracted in an excess of methanol containing 1 % of HCl for 1 hour at room temperature. The extract was filtered through a thin layer of cotton wool and the methanolic solution was evaporated in vacuo at 40° C to a minimum volume.

Whatman no. 1 paper chromatography was used for two dimensional chromatography, to display the pigment composition of each variety. Solvent systems used were BAW (butanol : acetic acid : water = 12 : 3 : 5 v/v) and 5% Acetic acid.

Preparative scale paper chromatography using Whatman no. 3 paper was carried out on each sample. The first solvent used was BAW. After development in this first solvent, the separated bands were cut out and the pigments eluted with MAW (methanol : acetic acid : water= 85: 5 : 10 v/v). After concentration under vacuum, they were further purified and separated by redevelopment on paper with 5 % acetic acid. The separated bands were cut out again and the pigments were eluted as before using MAW. The solutions were reduced to a minimum volume in vacuo at 40° C. These pigment samples were put onto watch glasses and dried at room temperature.

The separated and purified pigments were examined by measuring the absorption spectra in methanol containing 0.01 % HCl with a spectrophotometer (Pye - Unicam SP8 - 100).

3.4.2. Results and discussion

Quantities obtained were generally insufficient to allow for hydrolysis and complete identification. However, the spectral and chromatography data when compared with the extensive tables contained in Harborne (1967), allow for some reasonable assumptions to be made regarding the nature of the anthocyanins. These data are given in the following table :

Table 10. The values of λ max, % λ 440 / λ max, Δ λ $AlCl_3$ and anthocyanins identification from several varieties of *Nerine*.

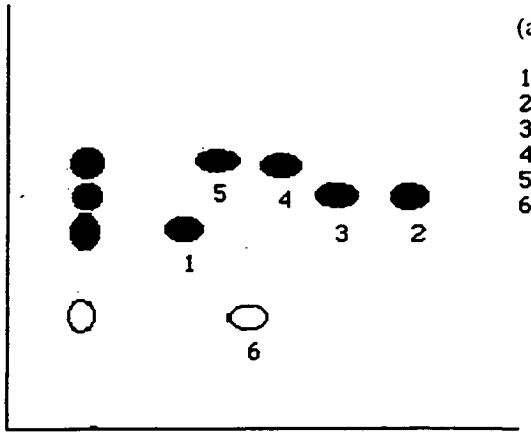
Species	Pigment	λ max	% $\frac{\lambda 440}{\lambda \text{ max}}$	Δ λ $AlCl_3$	Identification
N.fothergillii	1	502	67	0	Pg-3 -?
	2a	507	43	0	Pg-7 glucoside
	2b	503	44	0	Pg-3 -?
	3a	530	20	35	Cy-3 rutinoside
	3b	512	51	0	Pg- ?
	3c	507	45	0	Pg-7 glucoside
	4a	512	43	0	Pg- ?
	4b	510	36	0	Pg- ?
N.flexuosa	1	523	13	47	Pn / Cy- 3,5 diglucoside
	2a	531	36	0	Dp / Mv- ?
	2b	523	27	52	Pn / Cy-3 - ?
	3a	529	34	41	Pn / Cy- 3 - ?
	3b	532	28	38	Dp / Mv- ?
N.x splendens	1a	530	19	40	Pn / Cy-3 glucoside
	1b	527	49	38	Dp / Mv-3 - ?

Species	Pigment	λ max	% λ 440 / λ max	Δ λ AlCl3	Identification
N.x splendens	1c	520	25	0	Cy-3 rutinoside
N.bowdenii	1	527	20	43	Pn / Cy-3 - ?
	2	525	12	45	Pn / Cy-3,5 diglucoside
	3	530	31	45	Dp / Mv- ?
	4	530	36	38	Dp / Mv- ?
N.undulata	1	532	41	28	Dp / Mv- ?
N.corusca	1	502	51	0	Pg-3- ?
	2a	507	43	0	Pg-7 glucoside
	2b	509	47	0	Pg-7 glucoside
	3a	527	45	38	Pn / Cy- ?
	3b	509	38	0	Pg-3,5 diglucoside
	4	510	42	0	Pg- ?
N.sarniensis	1a	519	44	49	?
	1b	502	70	0	Pg-3- ?
	2a	530	27	45	Dp / Mv- 3- ?
	2b	507	47	0	Pg-7 glucoside
N. x roseum	1	529	31	41	Dp / Mv- 3- ?
N. x salmonea	1b	532	23	38	Dp / Mv- ?
	2a	510	40	0	Pg- ?
	2b	507	28	0	Pg-3,5 diglucoside
	3a	507	64	0	Pg- ?
	3b	509	45	0	Pg- ?

Note : Pg = Pelargonidin , Dp = Delphinidin, Mv = Malvidin, Pn = Peonidin, Cy = Cyanidin

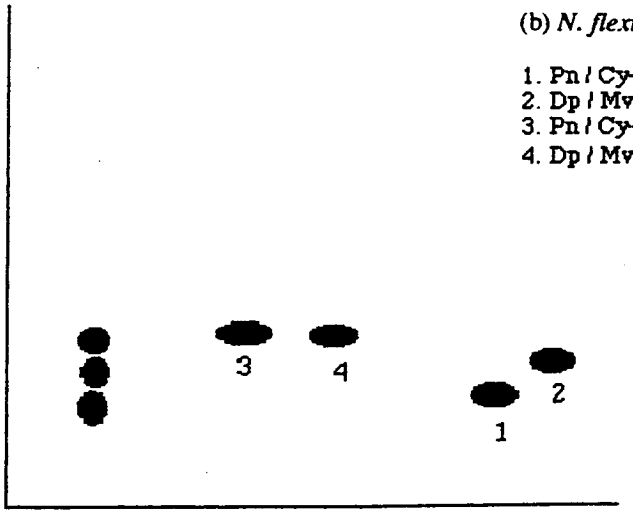
The results of the two dimensional chromatography of *Nerines* are illustrated in Diagram 2. The vertical spots in each diagram show the anthocyanin compounds after development in BAW, while the horizontal spots show the purified anthocyanin gained after redevelopment of those compounds in 5 % acetic acid.

Diagram 2 (a - i). The anthocyanins revealed from two dimensional chromatography of several varieties of *Nerine*



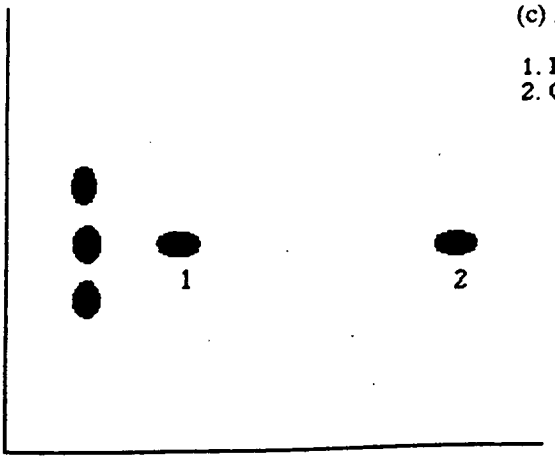
(a) *N. fothergillii*

1. Pg-7 glucoside
2. Pg- ?
3. Cy-3 rutinoside
4. Pg- ?
5. Pg- ?
6 Pg-3-? (estimation spot)



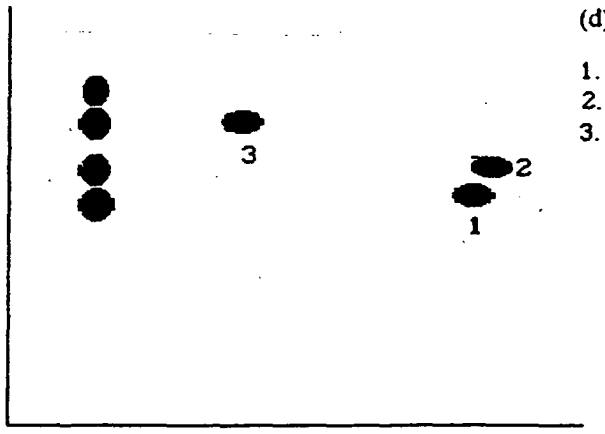
(b) *N. flexuosa*

1. Pn / Cy-3.5 diglucoside
2. Dp / Mv-?
3. Pn / Cy-3 ?
4. Dp / Mv- ?



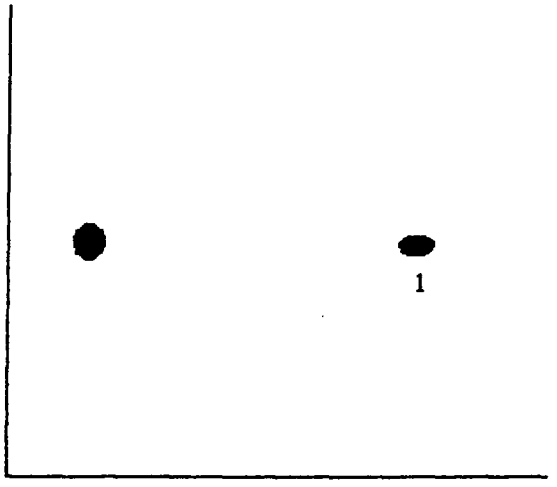
(c) *N. x splendens*

1. Pn / Cy-3 glucoside
2. Cy-3 rutinoside



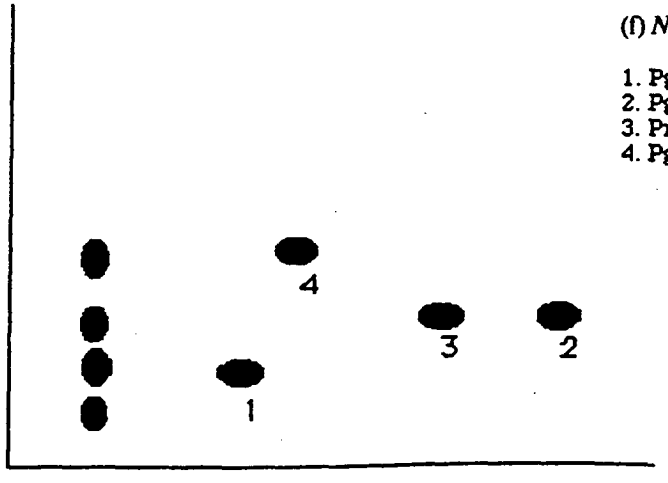
(d) *N. bowdenii*

1. Pn / Cy-3 - ?
2. Pn / Cy-3,5 diglucoside
3. Dp / Mv - ?



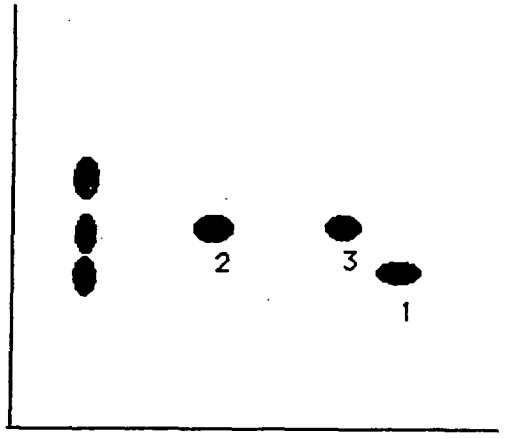
(e) *N. undulata*

1. Dp / Mv- ?



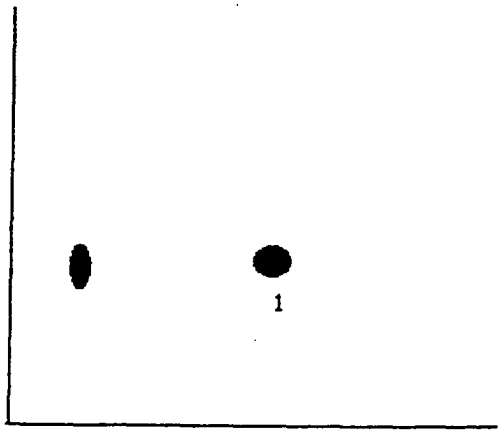
(f) *N. corusca*

1. Pg-7 glucoside
2. Pg-3,5 diglucoside
3. Pn / Cy- ?
4. Pg- ?



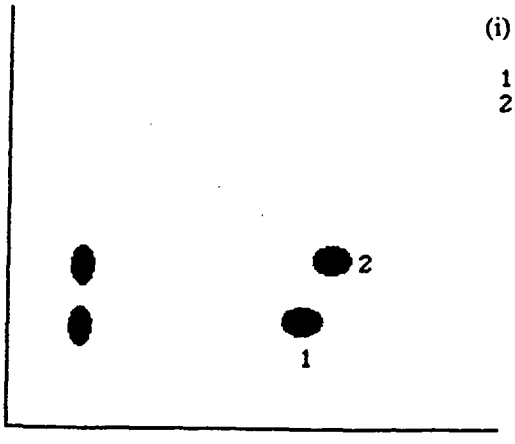
(g) *N. sarniensis*

1. Pg-3- ?
2. Dp / Mv- ?
3. Pg-7 glucoside



(h) *N. x roseum*

1. Dp / Mv - ?



(i) *N. x salmonea*

1. Dp / Mv- ?
2. Pg-3,5 diglucoside

From the results, it was found that five major anthocyanins occur in *Nerines* namely : pelargonidin, cyanidin, delphinidin, peonidin and malvidin. Based on these anthocyanins, *Nerines* which have been examined can be grouped into five classes namely :

(1) Those which contain pelargonidin and peonidin / cyanidin, e.g. *N. corusca*

(2) Those which contain delphinidin / malvidin and peonidin / cyanidin, e.g. *N. flexuosa*, *N. bowdenii* and *N. splendens*

(3) Those which contain pelargonidin and delphinidin / malvidin, e.g. *N. sarniensis* and *N. x salmonea*

(4) Those which contain delphinidin / malvidin, e.g. *N. undulata* and *N. x roseum*

(5) Those which contain pelargonidin and cyanidin, e.g. *N. fothergillii*

The nature of the sugars making up anthocyanin glycosides need to be further examined since the identification above was only based on the comparison data of Rf and λ max with the known anthocyanins as described in Harborne (1967). Pigment 1 of *N. fothergilii* posseses λ max at 502 and % λ 440 / λ max of 67. This pigment is unstable because it was not found in the 2 D chromatogram. However, it seems that this may be a novel pigment from *N. fothergillii.*

The glycosidic variation of pelargonidin of pigment 2 and 4 (*N. fothergillii*) and delphinidin / malvidin of pigment 2a *(N. flexuosa)*, pigment 3 & 4 (*N. bowdenii)*, pigment 1 (*N. undulata, N. x roseum, N. x salmonea)* and pigment 2a *(N. sarniensis)* needs to be determined. The occurence of cyanidin-3 rutinoside in *N. fothergillii* and *N. x splendens*

seems important since it gives magenta colour. Pigment 2 of *N. x salmonea* which has λ max at 507, % λ 440 / λ max = 28 and Rf (BAW) = 32 was identified as pelargonidin-3,5 diglucoside since it appears as an intense fluorescent spot on chromatogram when examined in ultra-violet light. Pigment 1a of *N. sarniensis* has not been identified. It has λ max at 519, % λ 440 / λ max = 44 and Δ λ $AlCl_3$ = 49. Probably it is peonidin / cyanidin. If it is so, *N. sarniensis* is the most varied in anthocyanins since it posseses all anthocyanins found in *Nerine*.

CHAPTER IV : GENERAL DISCUSSION AND CONCLUSION

4.1. GENERAL DISCUSSION

Control of flowering which comprises both stimulation and prevention of flowering has been examined in many "bulbous plants". According to Wellensiek (1961), in most flower bulbs, temperature has a direct role in flower formation and almost complete control of flowering can be obtained by regulating the temperature during storage. The influence of temperatures on flower formation and flowering of bulbous and tuberous plants has been summarised by Hartsema (1961). It has been suggested that the maximum, optimum and minimum temperatures are specific for each genus or possibly species and also specific for different stages of development.

In this present experiment, the effect of different storage temperatures has been examined on flowering of *N. fothergillii.* It was found that bulbs stored at room temperature gives 100 % flowering while higher temperature (30° C) slightly reduced the flowering percentage and leaf length. Application of cold treatment (5° C) causes earlier flowering and increases flower stalk length. The mechanisms of temperature treatment during storage in determining the subsequent growth and flowering in "bulbous plant" have been interpreted in relation to the alteration of bulb hormones content, carbohydrate metabolism, enzyme activities or redistribution of organic nitrogen (Aung, 1971 ; Moe and Wickstrøm, 1973 ; Haaland, 1974; Lambrechts *et al.*, 1992). In this present experiment, the effect of different storage temperatures on flowering in relation to the carbohydrates content and endogenous GAs has been examined.

Rees (1975), has noted that in tulip, the quantity of soluble sugars in the mother bulb related to duration of low temperature to which the plant has been subjected and this quantity determines the shoot growth. Carbohydrate in *N. fothergillii* has been examined only semi quantitatively, however, there is an indication of different concentration of carbohydrate caused by different storage temperatures. It was found that glucose and or fructose are lower in bulbs exposed at 30° C than at room temperature or a combination room temperature and 30° C. This result correlates with the leaf length, flowering percentage and flower number, which are also slightly reduced in bulbs stored at 30° C, but not in bulbs stored at room temperature or a combination of room temperature and 30° C.

It is thought that respiration rate during the storage at 30° C is higher than at room temperature since the source of substrate for respiration activity (sucrose) indicates a higher level at 30° C than at room temperature. In Iris, it has been found that respiratory activity is strongly stimulated by ethylene at temperature of 30° C but ethylene inhibits the growth of the sprout in the bulbs. Both reactions have been proved to be reversible (Kamerbeek and Verlin , 1972). With *N. fothergillii*, it is also thought that ethylene is synthesised by bulbs during storage, especially at 30° C and this ethylene may stimulate the respiration activity, but on the other hand it reduces the leaf length and flower number. This assumption warrants further examination of the occurrence of ethylene and the mechanism of ethylene in influencing the respiratory process since this process remains unclear. The examination of endogenous GAs indicates that 30° C storage temperature reduced the conversion of GA_{19} to GA_{20}, probably the conversion of GA_{20} to the active form (GA_1) is also affected and causes a reduction of leaf length. Alternatively the high storage

temperature (30° C) may be cause of reduction or loss of sensitivity to gibberellin as has been found in dwarf apple trees where both the change in GA level and GA sensitivity may reduce shoot elongation (Steffens and Hedden, 1992). However, in some 'bulbous plants" such as *Narcissus*, tulip, hyacinth and *Iris*, the use of high storage temperature immediately after lifting has been shown to be effective for subsequent flowering.

In a preliminary experiment, *N. fothergillii* bulbs were dry stored at 30° C with different duration at the dormant stage and then were planted. It was found that storage at 30° C for 2 to 6 weeks caused earlier flowering but more than 6 weeks storage at 30° C delay flowering and reduce the flowering percentage (Crowden, data unpublished). In this case the 30° C storage treatment may affect the dormancy breaking rather than the synthesis of hormone or carbohydrate needed for flower development.

The application of cold temperature (5° C) during storage of *N. fothergillii* bulbs causes earlier flowering and increases flower stalk length. Okubo and Uemoto (1985), have examined the changes in endogenous gibberellin and auxin activities during the first internode elongation of tulip flower stalk. They found that gibberellin regulates the elongation of the first internode, possibly through stimulation of auxin transport and auxin response system in the first internode. Endogenous gibberellin in *N. fothergillii* was examined from the inner scales after the bulbs received different storage temperature. The results showed that GA_{19}, GA_{20} and GA_1 were present in the inner scales. The effect of 5° C on endogenous GAs level can not be seen clearly, but it seems that GA_1 level is slightly increased by 5° C treatment.

Aung and De Hertogh (1968), suggested that in tulip, the GA-like substances are both released and synthesised during the dry cold (5° C) treatment, while a very great increase (370 fold) of GA-like substances has been found after 13 weeks in bulbs rooted at 9° C. In *Lilium longiflorum*, it has been found that GA level did not increase during the cold storage at 4.5° C, but increased after the bulbs were planted in the green house for 30 days. In *N. fothergillii*, it seems that gibberellins are not synthesised during the storage treatments since the level of GA_{19} , which is suggested as the precursor of the active GA, differ only slightly amongst the different storage treatments. It remains a possibility that gibberellins are released during the storage treatment.

The results of carbohydrate analysis indicate that during cold (5° C) treatment, translocation of glucose and / or fructose from scales to the basal plate occurred and thus increased carbohydrate concentration in the basal plate. In cereal grain, it has been found that gibberellin stimulates the secretion of hydrolysis enzymes, which catalysed the conversion of storage polymers into sucrose and mobile amino acids or amides (Salisbury and Ross, 1992). In tulips, it has also been thought that carbohydrate metabolising enzyme is activated by low temperature through hormonal control (Aung and De Hertogh, 1968 ; Moe and Wickstrøm, 1973). The increase of carbohydrate content in the basal plate of *N. fothergillii* may also be regulated by GA- induced enzymes analogous to germination in cereal grain.

In *Gladiolus* , it has been shown that both gibberellin and sucrose promote the flower bud growth (Rao and Mohan Ram, 1982), while treatment with GA_3 in several species of "bulbous plants" caused early flowering, such as in *Iris* (Halevy and Shoub, 1964) and *Cyclamen*

(Widmer *et al.*, 1974). In *Hippeastrum*, it has been found that application of GA_3 and IAA by soaking of bulbs or spraying on plants increased the length of flower stalk. With *N. fothergillii*, it is thought that earlier flowering accompanied by longer flower stalks in bulbs which received 5° C treatment, is caused by gibberellin and sucrose which is more available than in bulbs without 5° C treatment. It should be noted , however, that the inflorescence bud of *N. fothergillii* has been formed before the beginning of storage treatment and it remains a possibility that the cold treatment received during storage also accelerate the GA synthesis in the inflorescence bud itself. Thus the effect of preplanting storage treatment in flowering could be indirect or direct (Elphinstone *et al.*, 1987). The level of endogenous GAs in the inflorescence bud during storage and after they emerge from the bulb, and the effect of application of exogenous GA as well as GA inhibitor on flowering need to be further examined in order to understand the GA physiology in *N. fothergillii*.

The effect of photoperiod on growth and flowering has been examined in *N. bowdenii*. Generally there is no effect of photoperiod on the growth of *N. bowdenii*, except on the time when the inflorescence bud start emerging from the mother bulbs. SD condition gives earlier flowering than LD condition. The carbohydrates content and endogenous GAs in *N. bowdenii*, however , were not examined in this experiment. It is possible that GA_{19}, GA_{20} and GA_1, which have been identified in *N. fothergillii* are also present in *N. bowdenii* and perhaps the conversion of GA_{19} to GA_{20} is influenced by photoperiod as it has been found in Spinach (Metzger and Zeevaart, 1980). This assumption warrants further examination.

As an ornamental flower, the flower colour of *Nerine* is important in flower marketing. The anthocyanins from flowers of several varieties of *Nerine* have been examined. It has been found that the five major anthocyanins (pelargonidin, cyanidin, delphinidin, peonidin and malvidin) occurred in *Nerine* , but each variety possesses different compositions of anthocyanins and possibly with different concentration which lead to the various colours such as pale pink, deep pink, magenta or scarlet. However, the anthocyanin identification in *Nerine* still needs to be completed since the nature of sugar making up anthocyanin glycosides was not been examined in this experiment.

It has been suggested that light may influence anthocyanin synthesis as in *Petunia* corollas (Weiss and Halevy, 1991) while in *Anthirrhinum majus*, it has been found that expression of several anthocyanin biosynthesis genes is involved in the production of anthocyanins (Jackson *et al.*, 1992). It has also been suggested that flower morphology is controlled by several genes (Woodson, 1991). With *Nerine* , it is possible that new flower colours can be developed by molecular breeding techniques through genetic manipulation of the floral pigmentation pathway and probably new flower shapes can also be developed since the flower shape in *Nerine* is variable.

4.2. CONCLUSION

From the results and discussion, it could be inferred that in *N. fothergillii*, the preplanting storage treatments influence the growth and development of inflorescence bud. The flowering percentage and the number of flower in each umbel is lower in bulbs stored at 30° C compared to bulbs stored at room temperature. The application of 5° C for 2 or 4 weeks during the storage treatment causes earlier in flowering and increases the length of the flower stalk.

Endogenous GA_{19}, GA_{20} and GA_1 occurred in *N. fothergillii* and it is suggested that GA_{19} act as the precursor of the active GA in *N. fothergillii*. The data indicate that GAs are not synthesised during the storage treatment but the conversion of GA_{19} to GA_{20} seems influenced by 30° C storage temperature.

Glucose, fructose and sucrose occurred in both outer and inner scales of *N. fothergillii*. The changes of their concentration during the storage treatments are apparent on the paper chromatogram but more exact data (quantitative data) are needed to determine the effect of storage temperature on their concentration. The effect of preplanting storage temperature on the inter-relationship of carbohydrate content and gibberellins content also need to be further examined.

Photoperiod does not effect the vegetative growth of *N. bowdenii* but it influenced the growth of the inflorescences bud. SD condition gives earlier flowering than LD conditions.

The five major anthocyanins (pelargonidin, cyanidin, delphinidin, malvidin and peonidin) occurred in *Nerine* with different composition and could be concentration in each variety examined.

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APPENDIX

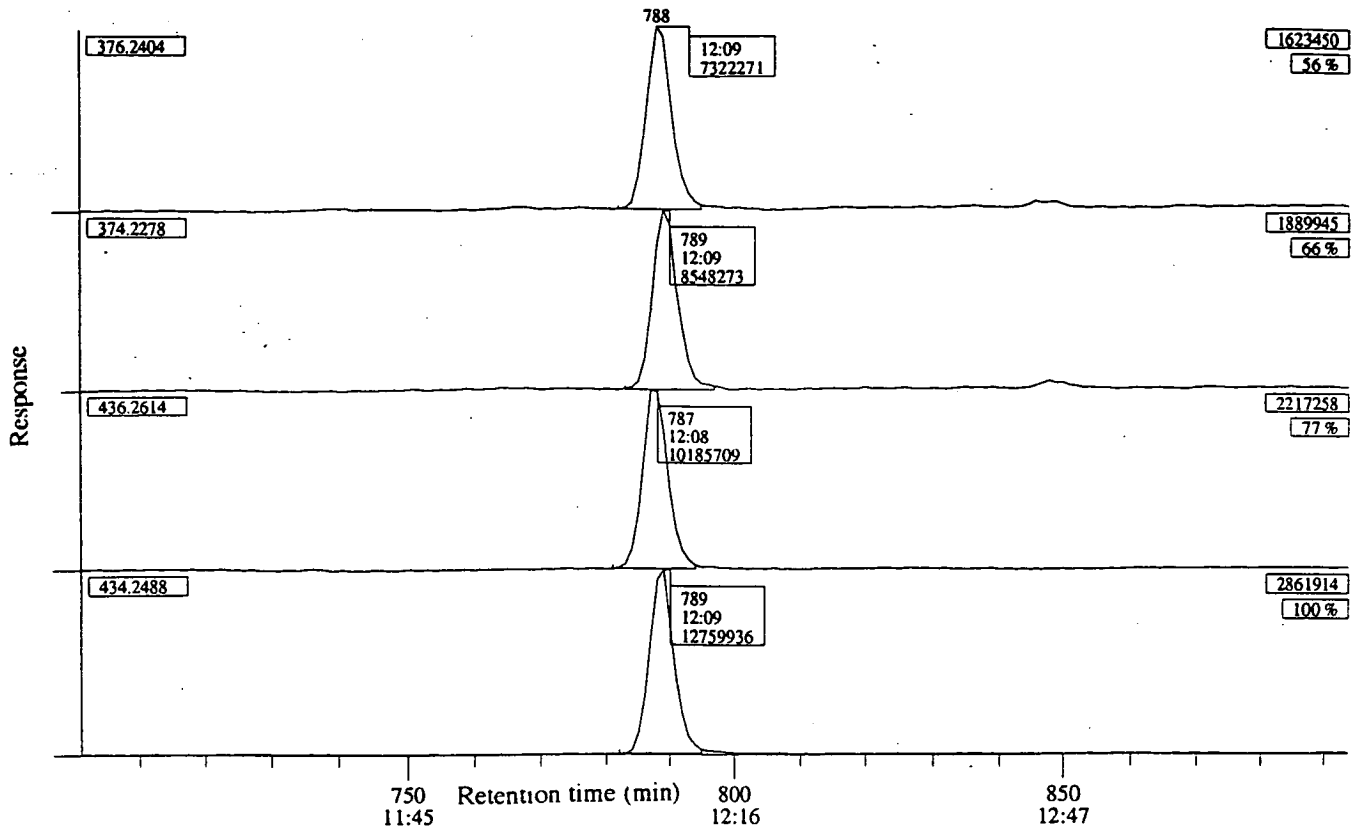

Fig. 13 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_{19} from inner scales of *N. fothergillii* (treatment A)

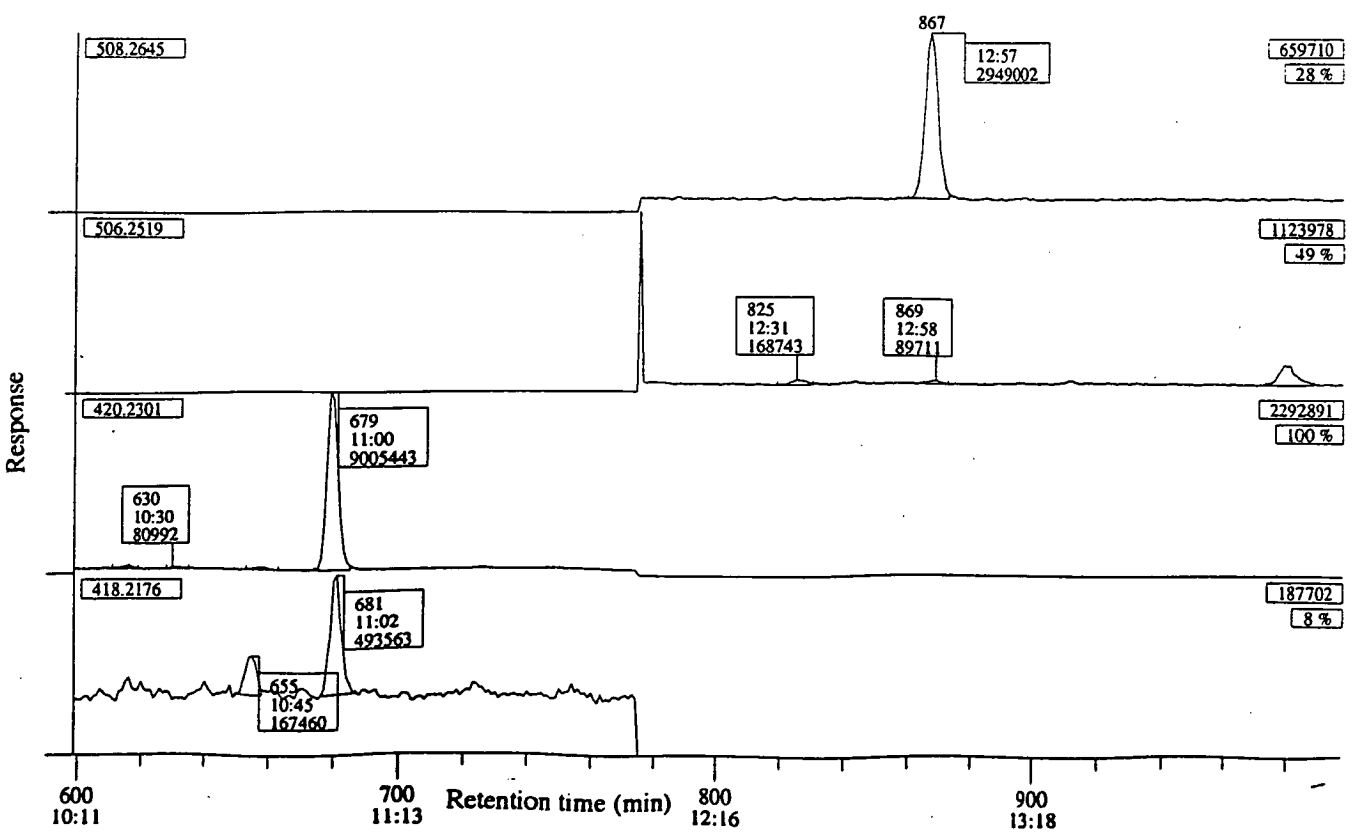

Fig. 14 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_1 and GA_{20} from inner scales of *N. fothergillii* (treatment A)

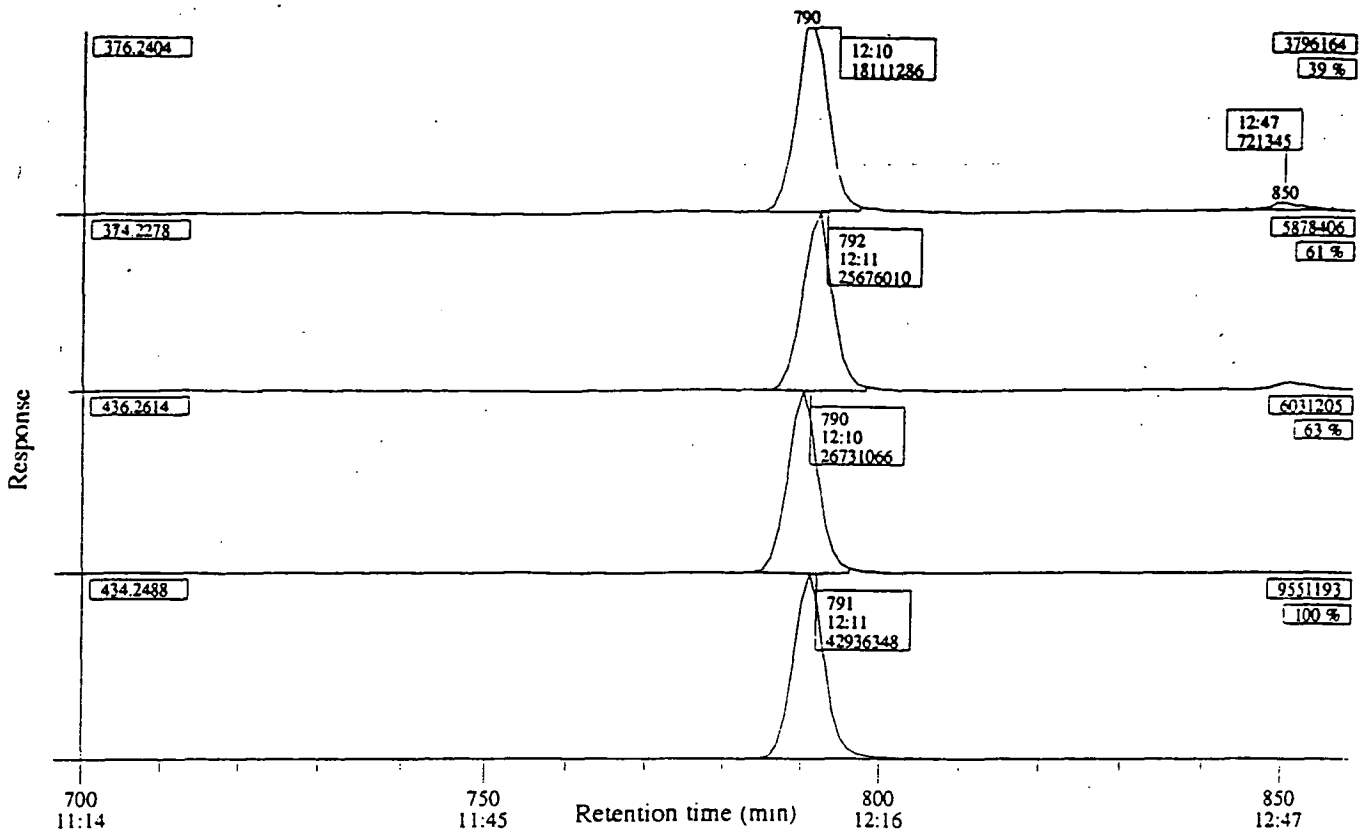

Fig. 15 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_{19} from inner scales of *N. fothergillii* (treatment B)

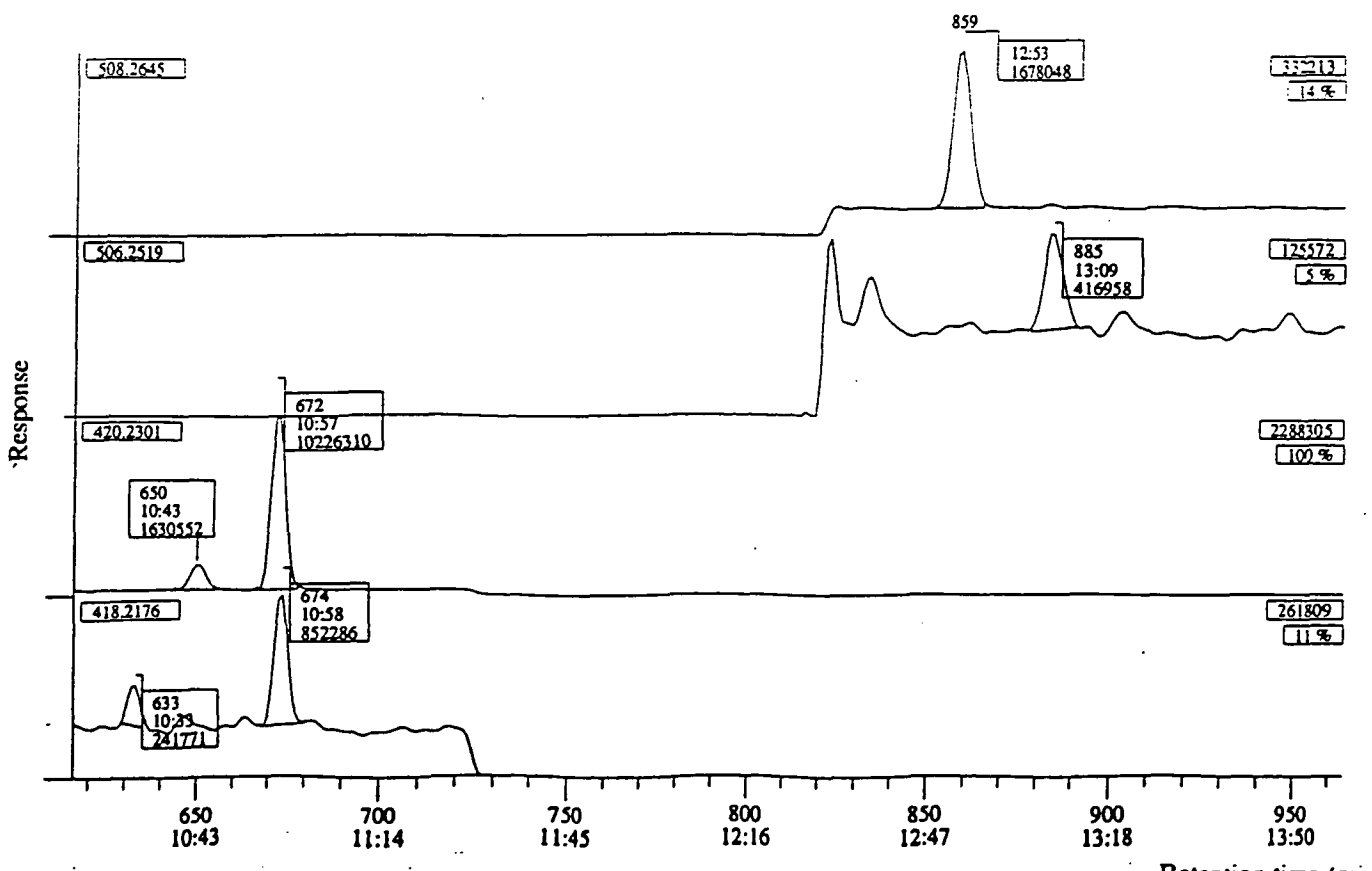

Fig. 16 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_1 and GA_{20} from inner scales of *N. fothergillii* (treatment B)

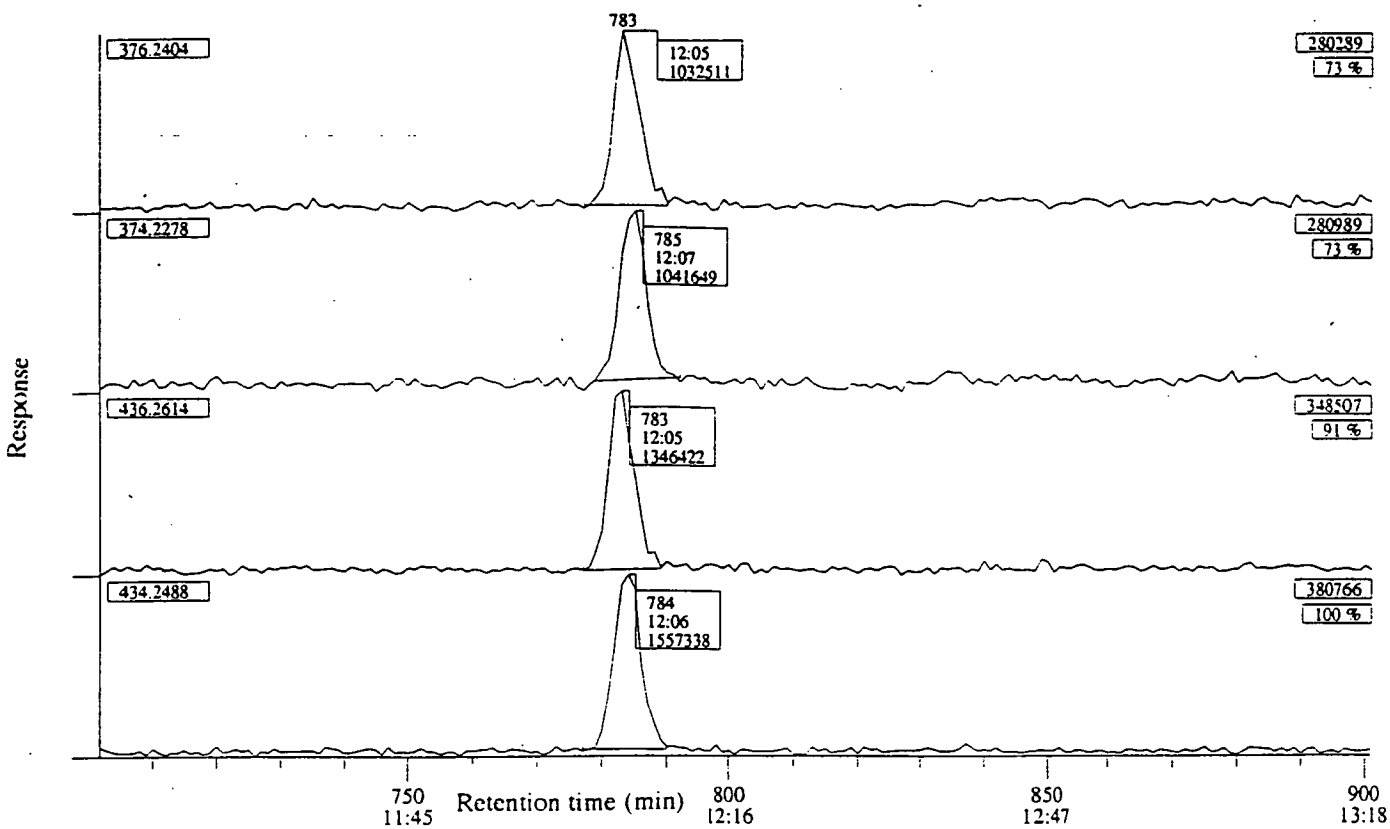

Fig. 17 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_{19} from inner scales of *N. fothergillii* (treatment C)

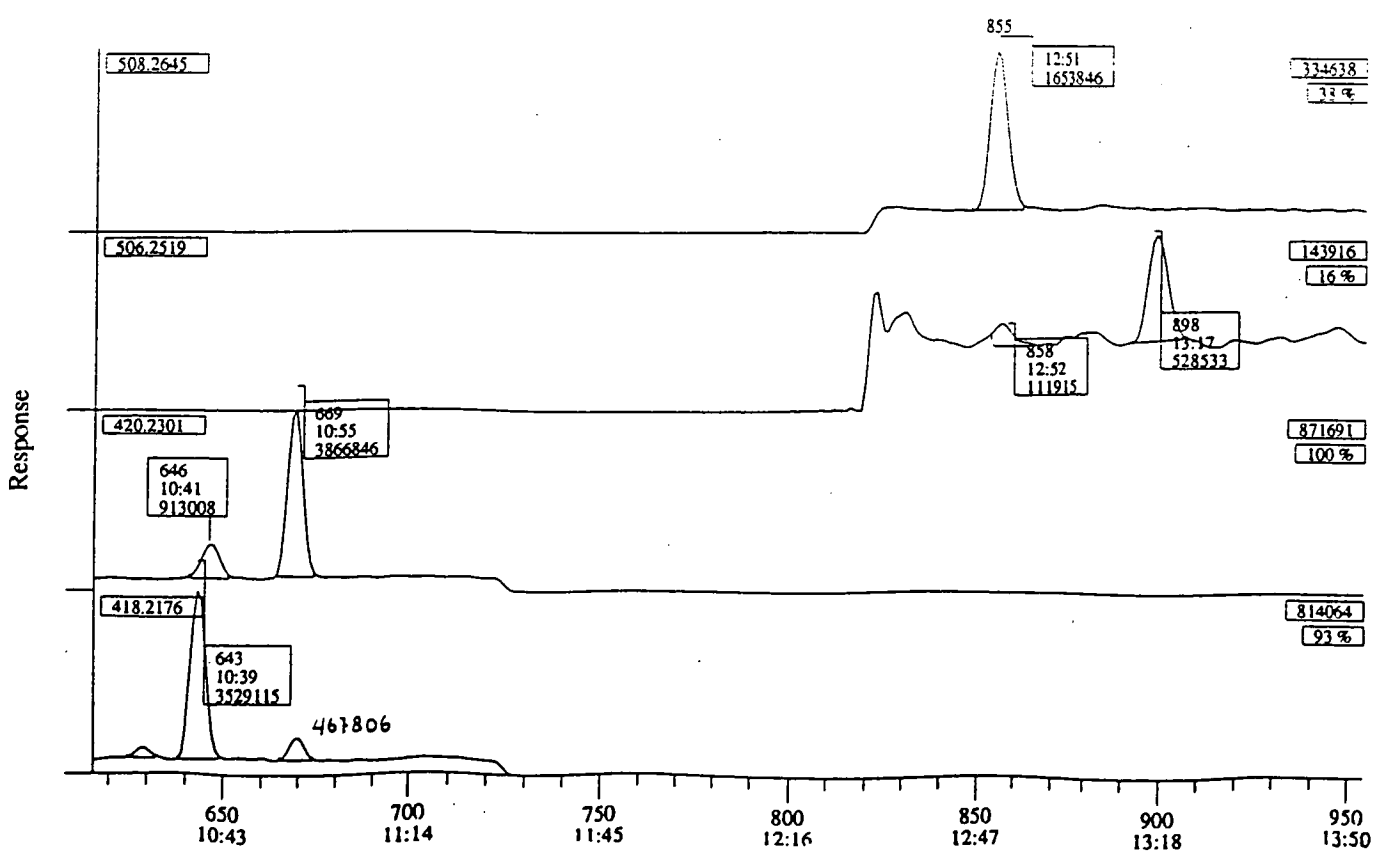

Fig. 18 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_1 and GA_{20} from inner scales of *N. fothergillii* (treatment C)

Retention time (mi

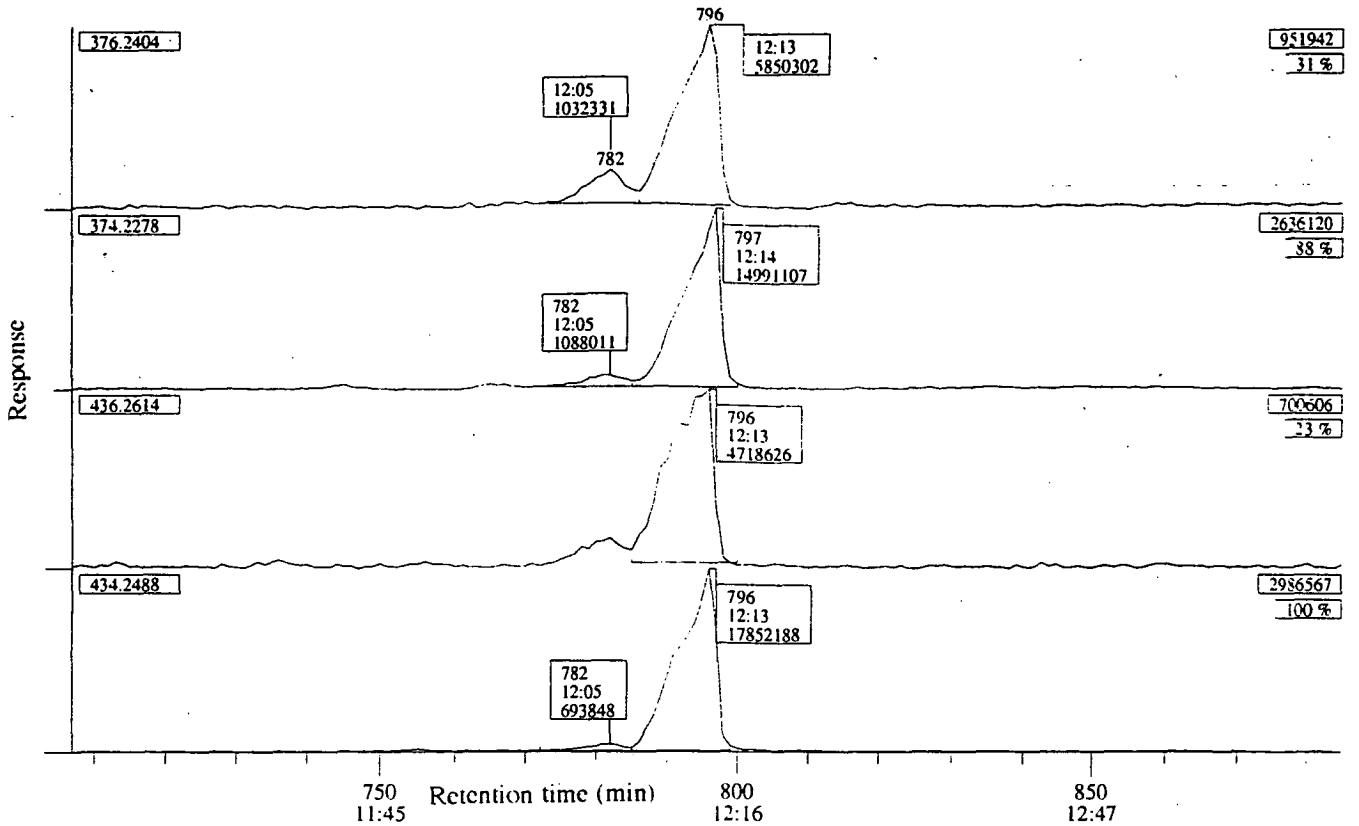

Fig. 19 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_{19} from inner scales of *N. fothergillii* (treatment A2)

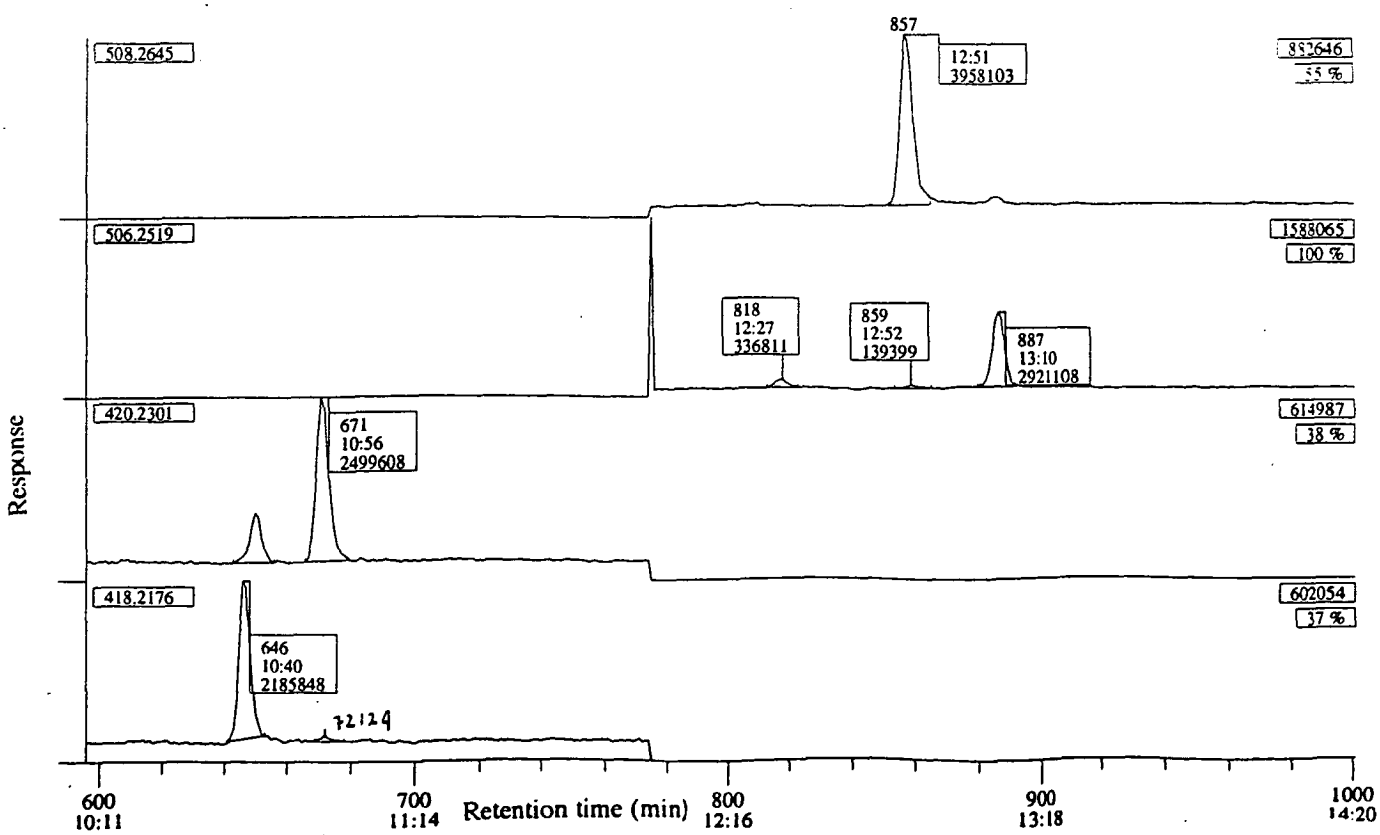

Fig. 20 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_1 and GA_{20} from inner scales of *N. fothergillii* (treatment A2)

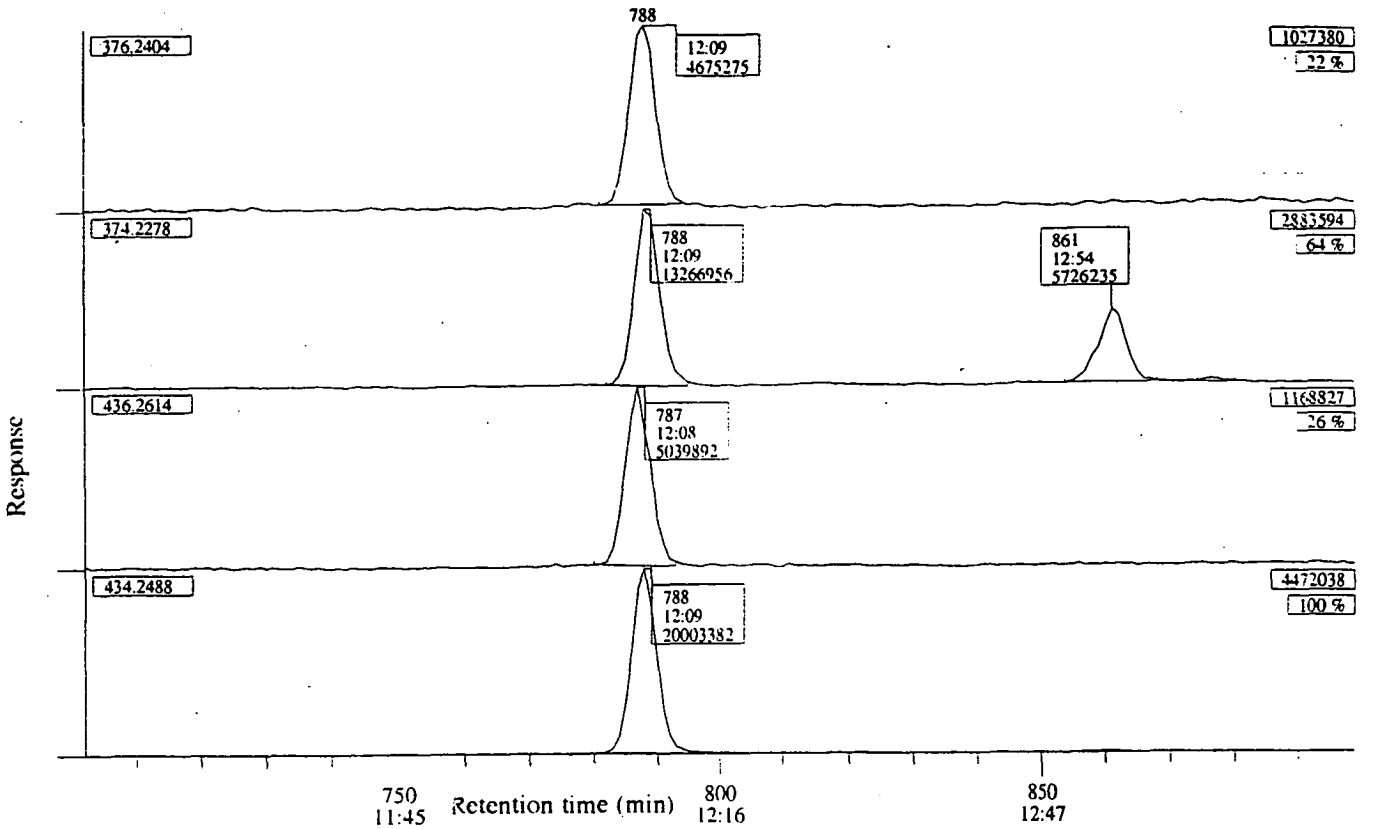

Fig. 21 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_{19} from inner scales of *N. fothergillii* (treatment B2)

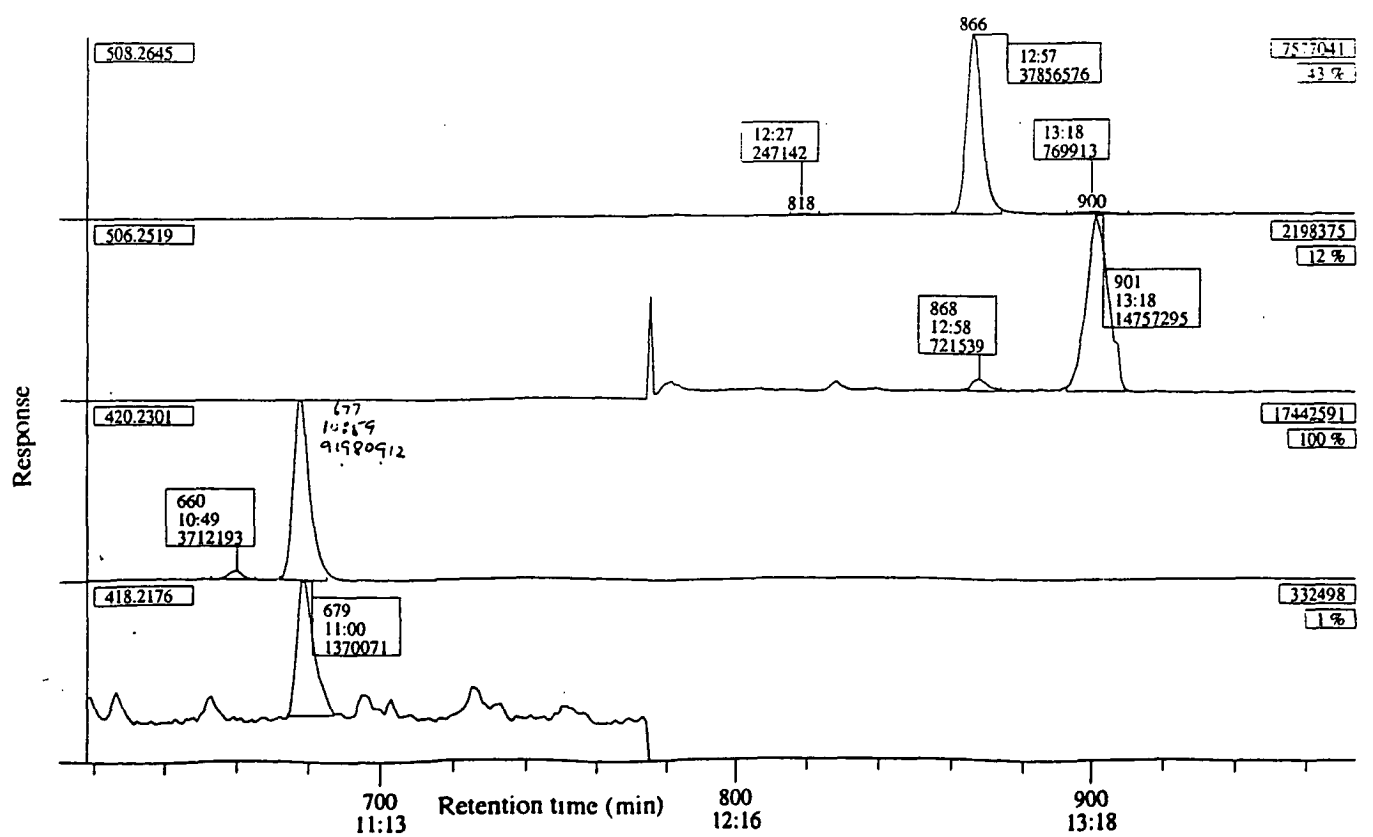

Fig. 22 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_1 and GA_{20} from inner scales of *N. fothergillii* (treatment B2)

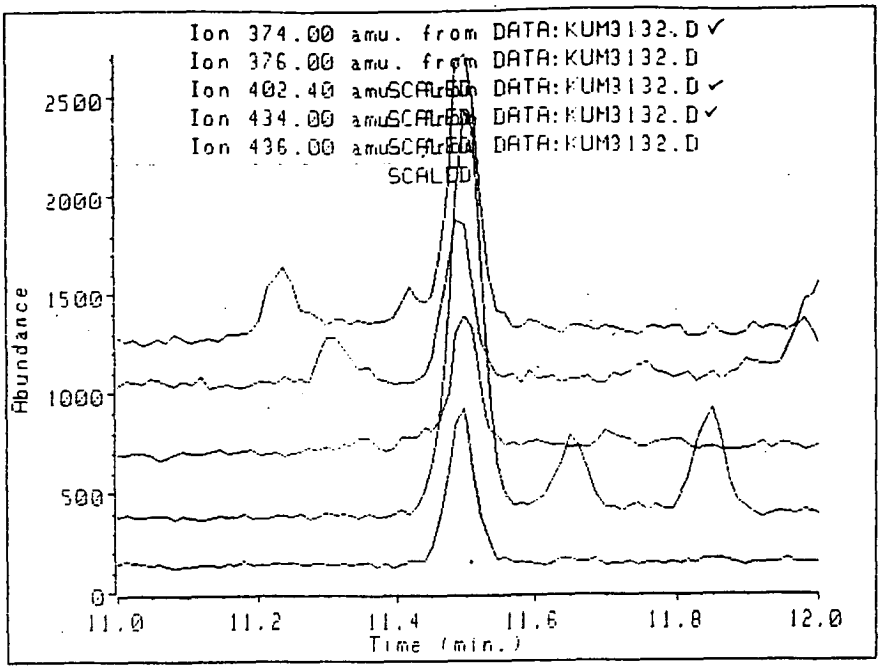

Fig. 23 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_{19} from inner scales of *N. fothergillii* (treatment C2)

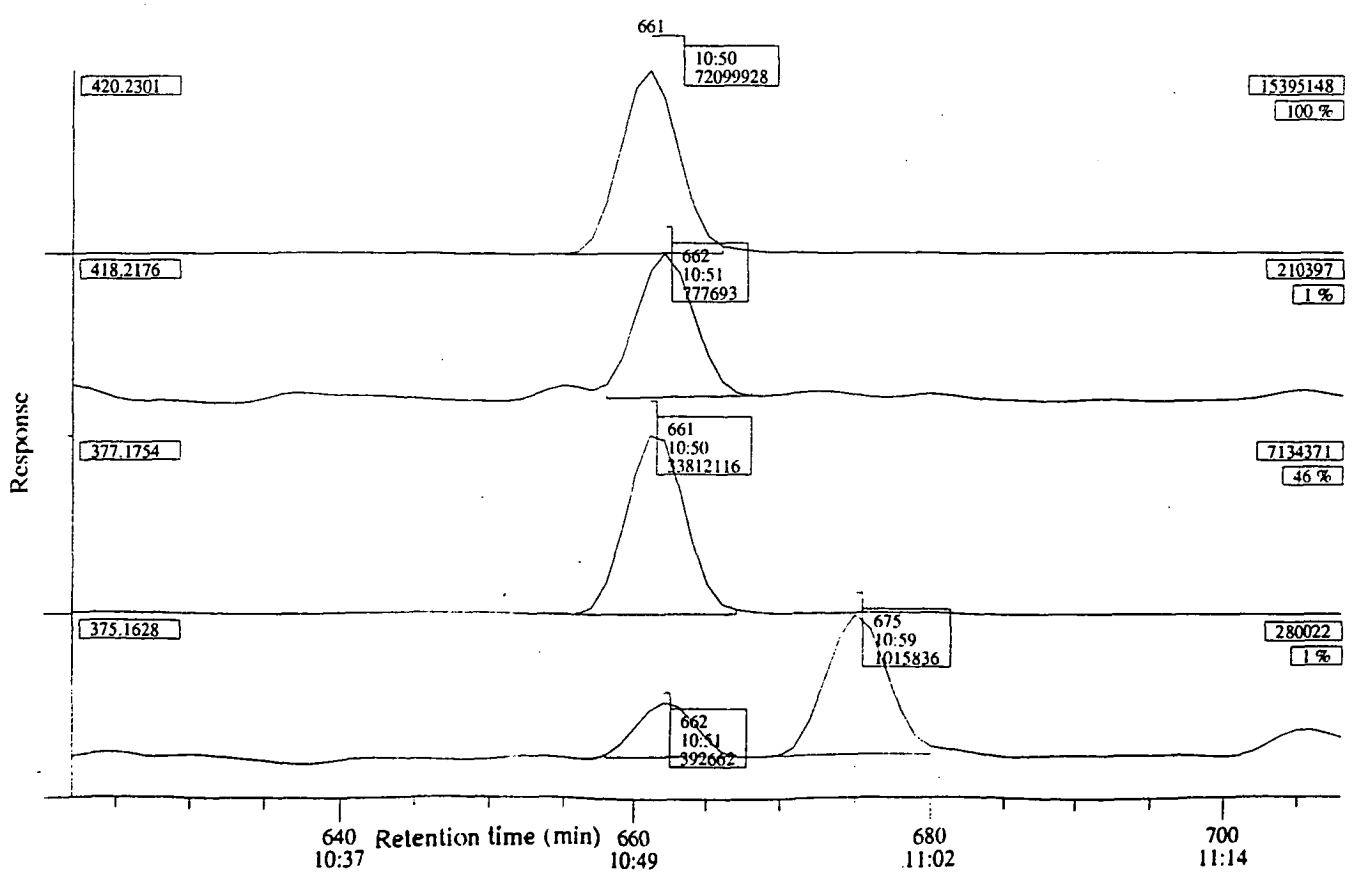

Fig. 24 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_{20} from inner scales of *N. fothergillii* (treatment C2)

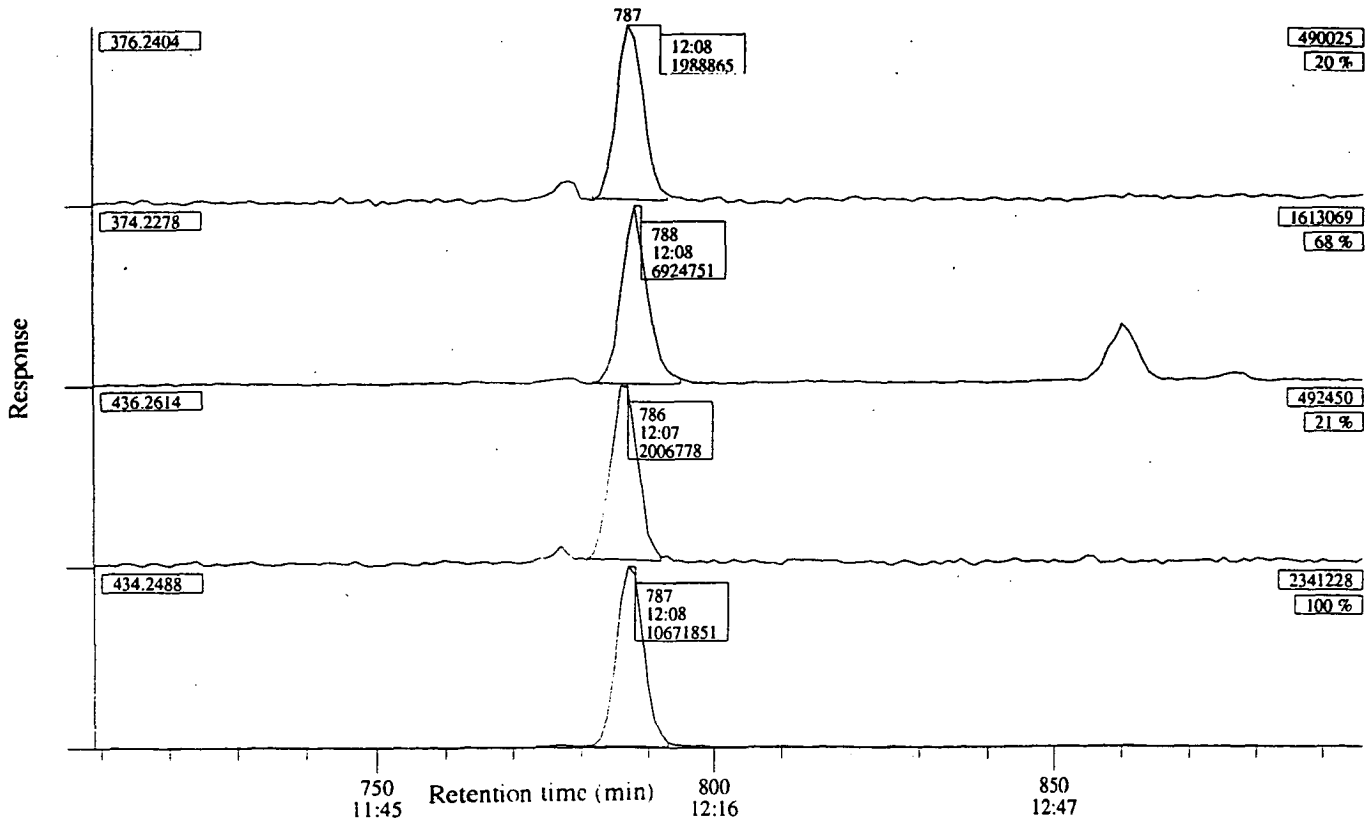

Fig. 25 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_{19} from outer scales of *N. fothergilii* (treatment C2)

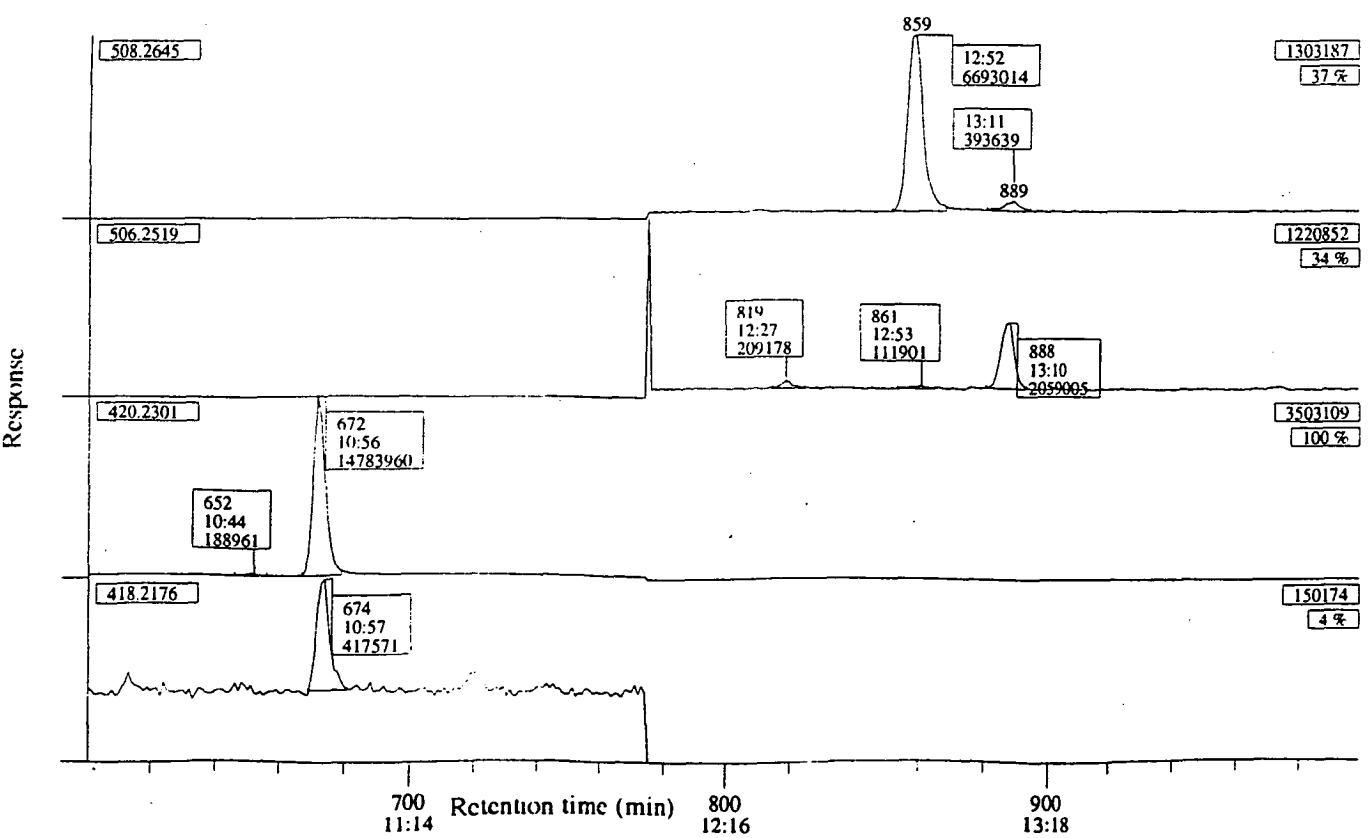

Fig. 26 SIM traces of characteristic fragment ions of MeTMSi derivatives of GA_1 and GA_{20} from outer scales of *N. fothergilii* (treatment C2)