A STUDY OF THE PROPAGATION AND CULTIVATION OF GETHYLLIS MULTIFOLIA AND G. VILLOSA

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2007

A STUDY OF THE PROPAGATION AND CULTIVATION OF *Gethyllis multifolia* AND *G. villosa.*

by

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Thesis presented in fulfillment of the requirements for the

Magister Technologiae: Horticulture

in the
Faculty of Applied Sciences

at the
CAPE PENINSULA UNIVERSITY OF TECHNOLOGY

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Cape Town
June 2007

DECLARATION

I, Christiaan Winston Daniëls, declare that the contents of this thesis represent my own unaided work, and that this thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed ..... Date 1/10/2007

ABSTRACT

Gethyllis multifolia and *Gethyllis villosa* (Family: AMARYLLIDACEAE) are indigenous geophytes, growing naturally in the Worcester area, Western Cape. *G. multifolia* falls in the Vulnerable category of the Red Data List of Southern African Plants while *G. villosa* is not threatened at all. Both these species are winter growers and start their growing phase between March and April. These bulbs start their dormant phase between September and October when their leaves start to die down. Flowers of both species are short-lived and borne towards the end of November and early December when no leaves are present. The leaves and berries are simultaneously pushed above ground at the onset of the new growing phase. The fruit of some *Gethyllis* species is sweet, juicy, pleasantly aromatic and good to eat and has medicinal properties for the cure of various ailments. The genus is difficult to propagate asexually and very little is known about its propagation and cultivation. The fragrance and medicinal value of the fruit of *G. multifolia* necessitates future research in the commercial production of this species.

A habitat observation study of the two species was conducted to assess the vulnerable status of *G. multifolia*. Asexual propagation experiments were conducted to find ways of reproducing these two species successfully. A hydro culture study was also conducted to ascertain whether this method of cultivation could be incorporated in the general cultivation of the two species. Finally an *in vitro* propagation study was conducted to look at faster methods of reproducing these two species. This is of extreme importance in the conservation of the vulnerable *G. multifolia*.

Leaf, root and basal plate cuttings were unsuccessful with no rooting in both species. *G. multifolia* bulbs were propagated successfully using twin scaling, bulb cuttings, scooping and scoring propagation techniques with between 80% and 100% rooting success. *G. villosa* was unsuccessful using the above propagation techniques with a 0 - 40% rooting rate.

Although *Gethyllis* species in general are sensitive to over-watering, the hydro culture experiment with the sub-irrigation system and leca pellet medium proved to be an effective method of cultivating both species throughout the growing phase. *G. multifolia* proved to be unsuccessful during the initial *in vitro* propagation experiments with no surviving explants during the initiation phase. Results improved with an increased number of trials. It is possible to grow

both species by means of *in vitro* propagation, but more emphasis in future research, should be placed on the multiplication aspect of *G. multifolia,* since not many new bulblets were produced.

It was observed through this study that grazing domestic livestock, urban expansion (this includes agricultural extension) and in some cases the lack of interest shown in our indigenous plant species, are some of the main factors influencing the decline in numbers of this species. It is also recommended that more emphasis be placed on the conservation of South Africa's indigenous flora and that the vulnerable status of *G. multifolia* according to The Red Data List of Southern African Plants, be changed to the "Endangered category" as the factors causing its decline continue to increase.

To my wife Naomi
and my son Lance, with love and in gratitude.

ACKNOWLEDGEMENTS

I wish to thank:

- My supervisors, C Laubscher (Cape Peninsula University of Technology) and C Joubert (Cape Peninsula University of Technology), for their guidance and encouragement.
- D Viljoen and W Voight (Karoo Desert National Botanical Garden, Worcester), for their assistance throughout the study.
- D Erasmus (Cape Peninsula University of Technology) for her assistance in the laboratory.
- C Duckitt and M Duckitt (Rondeberg Private Nature Reserve) for their guided tours.
- V Potterton for editing the thesis.
- S Tobias for her assistance in finding the various populations.
- C Botha (Farm: Holland in Rawsonville) for allowing access to his farm.
- The Cape Peninsula University of Technology for the use of the nursery and laboratory facilities.
- The URF for their financial assistance.
- Victor Harley for assisting with the statistical analysis of the thesis.

GLOSSARY

Bokkeveld Escarpment - The region between Vanrhynsdorp and Nieuwoudtville in the northern Cape of South Africa.
Cape - (Cape Peninsula) Is situated at the south-western tip of southern Africa.
Cape Floral Kingdom - (Cape Floristic Region) Covers approximately 0.04% of the total land surface of the earth and is situated at the southern tip of Africa.
Cataphyll - A collar-like sheath from which the leaves emerge.
CPUT - Cape Peninsula University of Technology, Cape Town, South Africa.
Endangered category - Taxa in danger of extinction and whose survival is unlikely if the causal factors continue operating.
Fynbos - Is the collective noun used to describe the vegetation in the Fynbos Biome which is a large group of evergreen plants with small, hard leaves.
Fynbos Biome - Also called the Cape Floral Kingdom
Geophyte - Plant species which contain underground resting buds attached to storage organs such as rhizomes, tubers, bulbs or corms.
Gethullis - A Greek word meaning leek or small onion.
Gethyllids - All the plant species that belongs to the *Gethyllis* genus.
Hardening-off - The process of acclimatizing plants, usually under shade.
Hottentots - A term formerly used to refer to the Khoi people of South Africa and Namibia.
Karoo Gardens - Karoo Desert National Botanical Garden in Worcester, South Africa.
Kirstenbosch Smoke-Plus - A smoke extract disc developed by the South African National Biodiversity Institute, Kirstenbosch for the breaking of seed dormancy in various Fynbos species.
Khoi - Native group of people that are still living in parts of South Africa and Namibia - also referred to as Hottentots.
Kukumakranka - English common name for all *Gethyllis* species (Koekemakranka - Afrikaans; Bramakranka - Hottentots).
Latin Square arrangement - A table used for testing 36 media combinations of auxins and cytokinins in the iniation stage of *in vitro* propagation.
Nama-Karoo Biome - Occurs on the central plateau of the western half of South Africa with most of the biome falling between 1000 m and 1400 m.
Red Data List of South African Plants - The conservation status of the list of plants that is regarded as 'threatened' in South Africa.
Scooping - Removal of the entire basal plate of a bulb with a special curve-bladed scalpel, a round-bowled spoon or a small-bladed knife.
Scoring - Three straight knife cuts across the basal plate towards the growing point of a bulb.
Succulent Karoo Biome - Most of this biome covers a flat to gently undulating plane, with some hilly and "broken" veld, mostly situated to the west and south of the escarpment, and north of the Cape Fold Belt. The altitude varies from below 800 m to 1500 m.
URF - University Research Funding (CPUT).
Vulnerable category - taxa believed likely to move into the Endangered category in the near future if the factors causing the decline, continue operating.

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CHAPTER 1
INTRODUCTION

History

Horticulturists and botanists around the world became aware of the *Gethyllis* bulb when it was first introduced to Europe in 1780. For two centuries this bulb was known as the Cape Crocus in Europe but this name today still remains unused in our Cape Floral Kingdom (Lighton, 1992:103). Because of their long-necked bulb structure, the Swedish taxonomist, Linnaeus formulated the name *Gethyllis* from the Greek word *gethullis*, which means a leek or small onion. It was also mentioned that the genus *Gethyllis*, more commonly known as Kukumakranka (English); Koekemakranka (Khoi, Afrikaans), is one of the most extraordinary and poorly researched of all Southern African amaryllids (Liltved, 1992:104). The meaning of the word kukumakranka is described by farmers as "goed vir my krank maag", meaning cure for an upset stomach (Van der Walt, 2003:19). Vosa (1986:251), mentioned that the edible, pulpy, aromatic berry is also called Bramakranka by the Hottentots in Namaqualand.

Taxonomy

The genus *Gethyllis* (family: AMARYLLIDACEAE) consists of 37 currently accepted species and subspecies (Muller-Doblies, 1986:465). According to Snijman (2004), *Gethyllis* has 32 recognized species, 30 of which are found in the summer-arid areas of Southern Africa. Manning *et al.* (2002:181), reported that the most recent taxonomic literature of *Gethyllis* is an outline by Muller-Doblies (1986:465), and suggested that a key and complete descriptions of the species are still needed. Further literature studies, (Alan Horstmann, 1999:36), divided *Gethyllis* into several groups depending on the leaf shape, hairiness and the absence or presence of a cataphyll. The first group consists of those species with cataphylls visible above the ground (e.g. *G. verticillata* and *G. ciliaris*). The second group consists of species with prostate leaves arranged in a rosette form (e.g. *G. barkerae* and *G. lata*). The third group has no visible cataphyll (e.g. *G. villosa* and *G. multifolia*). The final group are those with non-hairy leaves (e.g. *G. afra* and *G. campanulata*).

According to Snijman (2004), the greatest number of species occurs in the Succulent Karoo Biome, followed by the Fynbos Biome. Habitats range from coastal forelands to South Africa's high-lying, inland plateau. In most instances the plants prefer open sites, free of competition from shrubs and grasses. The majority of species prefer semi-arid habitats but a few Cape species (*G. afra* and

G. kaapensis) are found in seasonally moist sites amongst lowland fynbos vegetation. Only *G. transkarooica* and *G. longistyla* are found in the summer-rainfall region's Nama-Karoo Biome.

Morphological description

G. multifolia is a bulbous geophyte of 120 mm in height, with slightly twisted, lightly hairy leaves which are dry at flowering. The flowers are large and coloured white to cream with 12 anthers (six pairs) and the flowering period is from November to January (Goldblatt & Manning, 2000:25). According to Hortsmann (1999:36), this species has up to 30 leaves, which can either be straight or spiral. The leaves are uniformly green and may have a reddish colouration towards the base. Fine hairs cover both leaf surfaces. Straight leaves are up to 200 mm long and about 1–1.5 mm wide.

G. villosa bulbs are 30-150 mm in height. Leaves are 40-120 mm long, flat and loosely spiralled towards the apex, and covered with soft, white, T-shaped hairs. Flowers are white or pink with tepals 20-40 mm long. The flower consists of six anthers with the style longer than the stamens. The style is curved sideways and has a broad 3-lobed stigma (Manning *et al*, 2004:185; Snijman, 2004). *G. villosa* has 3-10 green leaves, which may be either straight or coiled, and are up to 100 mm long and between 1.5 and 5 mm wide (Hortsmann, 1999:36).

Life cycle

According to Van Reenen (1975:3), *Gethyllis* species are winter growers. Both leaves and fruit reach the soil surface more or less at the same time and this happens in autumn in Southern Africa. Elvin (2000), reported that when the rainy season comes to an end and the temperatures rise towards spring, the foliage dies back to the cataphyll and the bulbs begin to go dormant. Du Plessis & Duncan (1989:104), documented that the bulbs reach their flowering phase when the climate is hot and dry and that the leaves die down before the flowers appear. Flower formation starts in August when flower development takes place at the base of the bulb. The flowering period ranges from October to March (Van Reenen, 1975:2). Once pollinated, the third and final phase, fruiting, begins (Elvin, 2000). Du Plessis & Duncan (1989:105), mentioned that after cross-pollination (probably all species are self-sterile), the ovary begins to swell. The club-shaped berry is gradually formed and pushed above ground during autumn. According to Elvin (2000), it can take more than two months to produce the club shaped, aromatic fruit that ripens just in time to release the seeds for the rainy season. As the fruits ripen, they fall over and, if not eaten or removed, the seeds will germinate to form a dense group of seedlings. Van Reenen (1975:63), reported that seeds of

Gethyllis germinate without a preceding resting period and in exceptional cases germination takes place within the berry.

Predators

According to Liltved (1992:105), tortoises are attracted by the ripening berry and play an important role in the dispersal of the seeds. Snijman (2004), also reported that tortoises, birds and rodents eat the berry's fleshy pulp and act as agents for seed dispersal, but that this remains unconfirmed. Van der Walt (2003:19), mentioned that humans, tortoises porcupines, birds, rodents and sheep are common predators of the berry.

Survival strategies

The hair on the leaves of some species serves to trap moisture, prevents excessive transpiration and helps protect the bulbs from the sun (Liltved, 1992:106; Elvin, 2000). The coiled and curled leaves of geophytes serve the purpose of maximizing photosynthesis by providing leaf surfaces perpendicular to the sun at every hour of the day. Geophytes survive long periods of environmental stress such as summer drought or winter cold by dying back to underground storage organs only to re-sprout the following growing season as in the case with *Gethyllis* species. The same underground storage organs also give geophytes a strong tolerance to other stresses such as fire and grazing (Esler, 1998:7). According to Du Plessis & Duncan (1989:104), the fleshy roots of some gethyllids are extremely long and are attached to an enlarged, fleshy basal plate. Bulbs eaten by moles or rats, regenerate new bulbs from these fleshy eaten basal plates. Van Reenen (1975:2), suggested that the reasons why *Gethyllis* bulbs are found growing in clumps, are because they propagate themselves vegetatively through division. Several authors (Du Plessis & Duncan, 1989:105; Manning *et al.*, 2002:180), reported that young bulbs frequently produce flowers without a style and also with a reduced number of stamens. It was suggested that this is probably a protective mechanism, to prevent the immature plants from being fertilised and use up all its resources to produce berries. The leafing and fruiting stages occur at the same time and all the nutrients and moisture required by the large berry and leaves, have to be drawn from the stored resources in the bulb. It was further suggested by Du Plessis & Duncan (1989:105), that the sheathing neck left over from the dried leaves helps to protect the delicate flower from the hot sand. It was also mentioned that because flowers only last for a few days, it is essential that as many bulbs as possible flower at the same time to ensure cross-pollination.

Distribution

According to Goldblatt & Manning (2000:25), *G. multifolia* occurs naturally on stony clay flats in the Bokkeveld Escarpment, Worcester and Montagu areas. Hortsmann (1999:36), reported the occurrence of this species in the Worcester and De Doorns districts.

G. villosa occurs naturally in the Worcester area, Cape Peninsula, Western Karoo to Mossel Bay, Namaqualand and the Bokkeveld Mountains (Goldblatt & Manning, 2000:25; Pacific Bulb Society, 2004). This species is also found over a wide area from Kamieskroon in the north to Bredasdorp in the south (Hortsmann, 1999:35).

Conservation status

G. multifolia falls in the vulnerable category of the Red Data List of Southern African Plants (Hilton-Taylor, 1996:9). *G. villosa* grows in the same region and is not threatened at all. The term Vulnerable (V) is defined as taxa believed likely to move into the Endangered category in the near future if the factors causing the decline, continue operating. Included here are taxa of which most or all of the populations are decreasing because of over-exploitation, extensive destruction of habitat or other environmental disturbances; taxa with populations that have been seriously depleted and whose ultimate security has not yet been assured; and taxa with populations that are still abundant but are under threat from serious adverse factors throughout their range (Hilton-Taylor, 1996:9).

Propagation and cultivation

Even though it is mentioned that certain *Gethyllis* species can regenerate new bulblets from the basal plate remains after mole or rat predation and that it is possible for *G. ciliaris* to be propagated by basal bulb cuttings, the genus *Gethyllis* is difficult to propagate asexually (Du Plessis & Duncan, 1989:105; Elvin, 2000). Neither *G. multifolia* nor *G. villosa* have ever been propagated by means of *in vitro* propagation and only the publication (Drewes & Van Staden, 1994:295) on the *in vitro* propagation of *G. linearis* L Bol. could be found. Literature studies revealed that *Gethyllis* species are easily propagated from seed (Du Plessis & Delpierre 1973:39; Du Plessis & Duncan, 1989:105). For this reason sexual propagation will not be experimented on in this study.

It was reported that *Gethyllis* bulbs require soil with excellent drainage and can be difficult to grow for those who like to water plants too frequently. It was also mentioned that they are poor vegetative producers but seed readily when cross-pollinated, and that a fairly large population is needed to

ensure large seed quantities (Du Plessis & Duncan, 1989:105; Elvin, 2000). According to Du Plessis & Duncan (1989:33), the parent plants must not be allowed to dry out unnecessarily, and that they benefit from additional feeding during fruit formation. They also mentioned that full sun is required, and that the soil must be deep enough to accommodate the long cataphyll, bulb and fleshy roots. After the leaves have died down all watering must be stopped. Overhead shade is recommended in hot climates during the dormant stage. Watering can be carefully resumed after the leaves and/or berry appear at the onset of the new growing season. Apart from neck and root rot, *Gethyllis* is not susceptible to other serious pests and diseases (Buckly, 1999:3; Du Plessis & Duncan, 1989:33).

Buckly (1999:3), also suggested the following series of cultivation practices. The medium for growing bulbs consists of 6 parts sand, 1 part fine decomposed pine needles and 1 part fine peat. It was also mentioned that all the *Gethyllis* species flowered and grew in this medium and none have ever rotted. A layer of small pebbles can be placed at the base of the pots over the drainage holes. Bone meal can be added to the soil medium and it is not necessary to sterilize the soil medium. Bulbs must be kept out of the rain, especially during the dormant phase. Give a good watering once every two weeks and in cooler climates every three weeks. Do not leave the bulbs covered up during high humidity conditions as this will result in rotting and death of the plants. *Gethyllis* species thrive in areas with a good airflow and low relative humidity.

According to Du Plessis & Duncan (1989:33), the process of seed maturation in *Gethyllis* exhausts the resources within the parent plants and they are easily lost if allowed to set seed heavily for two consecutive years. Du Plessis & Delpierre (1973:39) mentioned that seedlings must be kept moist (the winter rain is normally adequate) and overhead shade is advised. Young seedlings must also be protected against the harsh summer sun even though they are dormant. Young plants can be transferred to their permanent stations towards the beginning of the third growing cycle. It takes about six years or even longer before bulbs reach maturity and are able to flower and produce seeds. According to Snijman (2004), the cultivation of *Gethyllis* is best left to a bulb specialist and their use in gardens should be avoided.

Medicinal value and other uses

Several authors (Van Wyk *et al.*,1997:1; Watt & Breyer-Brandwijk, 1962:32), reported that koekemakranka brandy, made of the fruit of *G. afra* and *G. ciliaris*, is one of the early Cape remedies

for colic and indigestion and that the edible fruit was highly valued for perfuming rooms and linen. It was learnt that the fruit of this plant is sweet and juicy, pleasantly aromatic and good to eat (Fox & Norwood Young, 1983:67). Some authors (Rood, 1994:4; Watt & Breyer-Brandwijk, 1962:32) reported that the early Cape colonists used an alcoholic infusion of the fruit of *G. linearis* and *G. spiralis* as a remedy for digestive disturbances and that this remedy is still used in Europe. In more recent times a diluted infusion of the flower has been used for teething troubles and the skin of the fruit as a local application on boils, bruises and insect bites. Du Plessis & Delpierre (1973:37), reported that the club-shaped fruit was used to perfume rooms and was often dried in newspaper for use as a bookmarker. It was also mentioned by Rood (1994:4), that the fruit was boiled by the Khoi and used as an aphrodisiac. Van der Walt (2003:19), reported that *G. ciliaris* was used for fatigue and gave a recipe for the kukumakranka brandy. Van Wyk *et al.* (1997:1) reported that no published information on the chemistry of *Gethyllis* could be found and that preliminary tests on the fruit indicated slight analgesic effects, but no details were available. Through later studies, it was found by Elgorashi & Van Staden (2003:28), that the anti-inflammatory antibacterial activities found in *G. multifolia*, *G. villosa* as well as other *Gethyllis* species, are in line with their uses as traditional medicines.

Need for research

The following statement was made by Saunders (2004:2): "many of us have quite extensive collections of *Gethyllis*, and none of us know what they are, due to the lack of literature on these plants". According to the literature survey conducted, it was observed that limited literature is available on the genus *Gethyllis*; therefore a serious need for further research on all aspects of this genus still exists. Liltved (1992:106) stated that much variability exists between members of the same species in different localities and that it is often not possible to truly verify the identity of species. It was also mentioned that it is feasible to assume that unidentified species do exist and that the identification thereof rests in the isolation and examination of genetic material of *Gethyllis*. Liltved (1992:106) also mentioned that problems are associated with researching members of the genus *Gethyllis,* for the reason that the flowering period is extremely short-lived and that the bulb remains dormant for almost half the year. For flowering to be introduced, certain species require very specific environmental growing conditions and are not at all suited to cultivation.

Future of the species

The conservation of *Gethyllis* is crucial, in that this strange indigenous geophyte does not appear to

have a bright future. The use of land for agricultural development has had a negative impact on the survival of these species. Farmers are appealed to strictly protect and allow left over plants to fruit and seeds to germinate (Du Plessis & Delpierre, 1973:39). This fact is further supported by the statement made by Malan (2000) "I live in the Southern Cape of South Africa, grow a lot of bulbous plants from seed and also collect a lot of things around here from building sites – where they tend to just bulldoze the lot before building!" According to the following authors (Du Plessis & Delpierre, 1973:37; Van Wyk *et al.*, 1997), due to the fragrance and taste of the berry, children were gathering the berries as part of a game. It was also mentioned that the child who found the most berries was regarded as the champion of the year and that this innocent game did not benefit the existence of the species. Liltved (1992:106) reported that alien vegetation spread and agricultural and urban expansion, are contributing factors to the destruction of natural habitats of genera such as *Gethyllis*. According to a report by Townsend & Viljoen (1997), *G. multifolia* bulbs were collected by staff members of the Karoo Desert National Botanical Garden in 1997 from the Osplaas farm in De Doorns, because the land was required for agricultural development. Similarly, *G. villosa* bulbs were collected by the same staff in 1994 from the Hartebeesrivier farm (Fairway Heights) in Worcester, because this land was required for urban expansion (Townsend & Viljoen, 1997).

The aims of the study are:

1 To explore explanations for the vulnerable status of *G. multifolia,* which grows in the Worcester area.

2 To observe the natural habitats and the impact it could have on the survival of the two species. Factors will include climatic conditions, soil analysis (pH as well as the availability of mineral elements necessary for plant growth), surrounding vegetation, as well as predators and agents responsible for pollination and seed distribution.

3 To survey five populations within the study area and observe population sizes during the study period. The study areas for both *G. multifolia* and *G. villosa* fall in the Worcester area. Populations of *G. multifolia* occur near De Doorns, Orchard (a residential area outside Worcester) and in the Karoo Desert National Botanical Garden, while those for *G. villosa* are near Rawsonville and in the Brandwag mountains. Specific localities of the studied populations have been deliberately omitted in this text to protect the two species.

4 To test propagation techniques for reproducing *G. multifolia* and *G. villosa* asexually. Because of the medicinal properties of their fruit and flowers (Elgorashi & Van Staden, 2003:28; Van Wyk *et al.*, 1997:1; Watt & Breyer-Brandwijk, 1962:32), these species have the potential to be grown by traditional healers and pharmaceutical companies. The genus *Gethyllis* is also known by collectors as sought after plants, especially the rare *Gethyllis* species (Van der Walt, 2003:19). No information could be found on the *in vitro* propagation of *G. multifolia* and *G. villosa,* therefore a study using this technique, will be conducted. This study is important for the conservation of our indigenous flora. A further important characteristic of several species is the valued taste and fragrance of their fruit and flowers (Du Plessis & Delpierre, 1973:37; Van Wyk *et al.*, 1997:1). The agricultural sector could also benefit from successful and proven *in vitro* propagation techniques, for the commercial production of viable indigenous fruit with export potential.

5 To find methods in which *G. multifolia* and *G. villosa* can be cultivated successfully, using the hydro culture cultivation technique. In this case the responses of the two species will be observed and compared.

CHAPTER 2
STUDY AREA

2.1 GEOGRAPHICAL DISTRIBUTION, MORPHOLOGICAL DESCRIPTION, HABITAT, POPULATION SIZES, SOIL AND VEGETATION TYPE

2.1.1 Introduction

Gethyllids thrive in a variety of well-drained, deep sand or stonier habitats on lowland flats or high altitude mountainous environments (Liltved, 1992:105). Exact locations of populations are omitted for conservation purposes. All the populations occur naturally in the Worcester area (position 33°S 19°E CB), which is a winter rainfall area characterised by cold and wet winters and hot and dry summers. Data on the geographical position of Worcester was obtained from the Karoo Desert National Botanical Garden. The Worcester area is frequented by a cold North Westerly wind in winter and a light South Easterly wind in summer (Agricultural Weather Station- Worcester). Data on the rainfall as well as maximum and minimum temperatures at the Worcester recording station (see Tables 2.1 and 2.2), was obtained from the South African Weather Service in Pretoria (www.weathersa.co.za). Two soil samples were taken at the root zone of four of the five populations. Only one soil sample was taken at the Karoo Garden population, because the size of the area (1.3x2.5 m) justified only one soil sample. The soil samples taken, were analysed for pH, soil texture, concentration of calcium, magnesium, phosphorus, potassium and sodium (see Table 2.3). The analyses were done at CAL Laboratories in Somerset West, Western Cape.

Gethyllis species have four distinct phases i.e the fruiting (seeding) phase, the vegetative growing (leafing) phase, the reproductive (flowering) phase and the dormant phase (not growing). Visits to the various populations were limited to one per growth phase and were done over a period of three and a half years. Detailed studies of populations could not be done because of time constraints; observations were only done at the time of the visit. More or less three hours were spent at each population on the day of the visit. For the purpose of this study, single bulbs that were scattered will be termed single bulbs. Bulbs that were attached or semi-attached to each other will be

termed clumps. Single bulbs and clumps that were growing together as a group will be termed a colony.

2.1.2 Morphological description

G. multifolia

The stamens of open flowers are arranged in six pairs of two, flat against the tepals. The stamens are 10-12 mm long. Flowers are mostly white or light pink in colour and the tips of the tepals are curved downwards. See Figure 2.1. Flowers measure 60–80 mm in diameter. The flower petiole measures 3 mm in diameter and 20-30 mm in length. The fragrance of flowers varies from not scented to lightly scented, but not significantly. The leaves are covered by fine hairs, linear, 1-1.5 mm in diameter and vary in length from 85-240 mm. The leaves in young bulbs are completely spiral compared to straight with a slight twirl towards the end in older bulbs. See Figure 2.2. In mature bulbs, leaves are completely straight. Leaves tend to spiral up more intensely under extreme dry conditions and are dry at flowering. Leaves spirals are not flat on the ground but have an upright tendency. See Figure 2.2. Younger bulbs have grey coloured foliage, older bulbs grey-green foliage and mature bulbs green foliage. The bulb consists of fleshy roots, which varies between 2-4 mm in diameter. The bulbs in general are small, measuring from 30-40 mm in diameter. Berries are produced from mid-March to mid-April at the onset of the new growing season (Van Reenen, 1975:3). Seeds are ripe when the soft berry pushes above ground and topples over (Du Plessis & Duncan, 1989:105). See Figure 2.3. The berry of *G. multifolia* is highly aromatic and has a passion fruit (more towards pineapple) fragrance. The colour of the berry varies from maroon on the wide end to creamy white towards the narrow end. The berry is club-shaped and varies in size from 45-80 mm in length and 8-13 mm in diameter on the wide end. Seeds are fleshy and suspended in an even more aromatic and sticky pulp. Seeds are round to slightly oval in shape with the diameter varying from 2-3 mm. Seed numbers per berry of *G. multifolia* vary from 13 to 85 per berry.

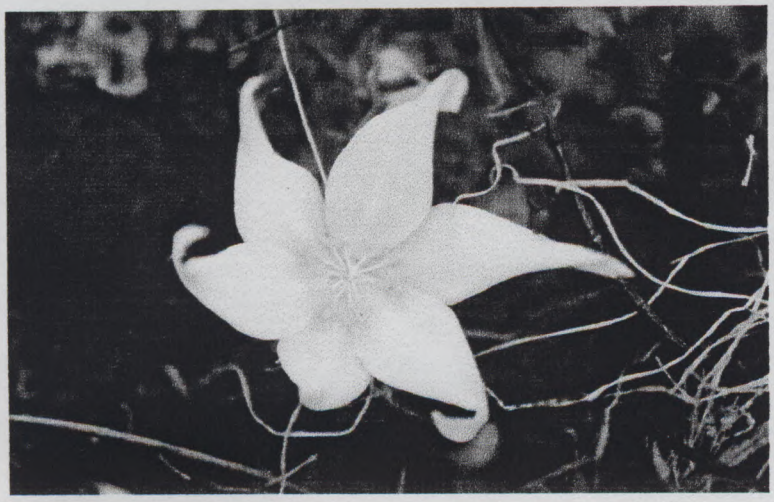

Figure 2.1: Flower of *G. multifolia.*

Figure 2.2: Leaves of *G. multifolia.*

Figure 2.3: Berry of *G. multifolia.*

2.1.3 Population descriptions of *G. multifolia*

Population A: De Doorns

De Doorns (500 m above sea level) is a small town located 30 km outside of Worcester. This population was found with the assistance of staff from the Karoo Desert National Botanical Garden. This site is flat, open, full sun (approximate size 2400 m²) with no trees or structures casting shade over the populations. The soil texture of the site is mainly clay (see Table 2.3) with many underground stones up to 0.75 m in diameter, which made digging with a spade extremely difficult. The soil is acid with an average pH of 5.5 (see Table 2.3).

This was the only population where a clump, consisting of a relatively large number of seedlings (22), was found next to the parent plants (27/04/2003). However, these seedlings were never seen again throughout the duration of the study. It must be noted that these seedlings were well protected by parent plants and a few bigger stones. The following neighbouring plant species: *Felicia filifolia* (small shrub), *Galenia africana* (medium shrub), *Rhus lucida* (large shrub) and *Ornithogalum thyrsoides* (bulbous), were found growing in this population. Bulbs appeared healthy with vigorous growth. Generally all bulbs were growing under full sun with the exception of a few single ones

that were growing in the shade of *Galenia africana*. Bulbs were either growing in clumps or as single plants. There were ± 9 single bulbs and 7 clumps growing in a colony. Single bulbs and clumps within colonies were spaced ± 3 m apart. Three colonies were found, which were spaced ± 60–100 m apart. Forty-six plants were found in this population. It was observed that *G. multifolia* suffered more under drought stress, compared to *G. villosa*. After the dormant season (16/03/2002) a low rainfall period was experienced and *G. multifolia* bulbs produced new leaves three weeks after *G. villosa*. *G. multifolia* bulbs also produced less leaves than *G. villosa* bulbs. Nine *G. multifolia* bulbs did not re-shoot after the dormant period compared to all *G. villosa* bulbs that re-shooted after the dormant period. *G. multifolia* bulbs produced less flowers (29/11/2003) than *G. villosa* and in some cases no flowers. During this period *G. multifolia* bulbs produced flowers that died during the bud stage. See Table 2.1. This phenomenon did not occur amongst *G. villosa* bulbs. No natural elements that could threaten the existence of the species in this population, were found.

Population B: Karoo Desert National Botanical Garden (Worcester)

Bulbs in this population, were collected on 29/05/1997 from a farm named Osplaas (Worcester) by staff of the Karoo Desert National Botanical Garden because the land was required for agricultural development. The bulbs were then planted in a single raised bed, size 1.3x2.5 m by the same staff on the grounds of the Karoo Desert National Botanical Garden (Worcester - 300 m above sea level). A clay soil texture with a pH of 6.3 (see Table 2.3) was used with no underground stones surrounding the bulbs. This is an open, full sun flowerbed with no plants or structures casting shade over the bulbs. The spacing of plants was ± 0.15 m and the number of individual bulbs as well as clumps added up to 95 in total. Growth of the bulbs was vigorous and luscious and they seemed to have adapted well after the transplanting process. Throughout the study, this population produced the highest number of berries compared to other *G. multifolia* populations. The number of berries counted at the onset of the growing season (27/04/2003) was 23 from the 95 plants. It must also be mentioned that this is not necessarily a true reflection of the amount of berries produced, because berries are pushed above ground over a period of 10 to 12 days and the number of visits were limited to only one per growing phase. It was also noticed that plants in clumps generally produced more berries compared to single bulbs.

According to the management staff of this garden, the removal of berries by staff and visitors was a regular occurrence. Ants were found to be very active amongst the fruit over all populations. Caterpillars were found eating the flower petals of both species. The following insects (that could be possible pollinators): small beetles, midges, ants and bees, were found on flowers of thisll species. The following neighbouring plant species: *Gazania lichtensteinii* (ground cover), *Bulbinella graminifolia* (bulbous), *Lampranthus* sp. (succulent), *Cotyledon orbiculata* (succulent) and *Haworthia* sp. (succulent), were found growing next to this population. No other natural elements, that could threaten the existence of this population were found.

Population C: Orchard

Orchard (500 m above sea level) is a small town located 28 km outside of Worcester. This is an open flat site (approximate size 1380 m^2), surrounded by a residential area. The environmental conditions are full sun with no trees or structures casting shade over the bulbs. The soil conditions are sandy clay (see Table 2.3) with many underground pebbles, which made digging with a spade extremely difficult. Compared to the Karoo Gardens population, which was watered weekly by the staff of the Karoo Gardens, the Orchard population was characterized by very dry soil conditions. This is also due to the sandy and pebble texture of the soil profile. The average soil pH of this site was measured at 6.3 (see Table 2.3). This population did not consist of colonies, only single bulbs and clumps of bulbs spaced on average 3-4 m apart were recorded. The number of individual bulbs and clumps added up to 127 in total.

Fifteen people from the surrounding residential area were interviewed on the existence of *G. multifolia* (Koekemakranka, as it is commonly known to the residents of this area). Eight people were between the ages of 25 and 35, while the other seven were between 35 and 55 years old. Comments from the younger group were, that they have heard about the berry but had never seen it while the older group claimed that they remembered collecting and eating it as children, but had not seen the berry for a number of years (the exact number of years was not specified). Goats were found eating the berries and leaves of bulbs in this population (see Figure 2.4). No other natural elements, that could threaten the existence of this population were found.

Figure 2.4: *G. multifolia* leaves eaten by goats in the Orchard population.

The following neighbouring plant species: *Hessea* sp. (bulbous), *Galenia africana* (medium shrub), *Asparagus rubicandice* (medium shrub), *Hermania* sp. (medium shrub), *Dodonaea angustifolia* (large shrub) and *Rhus lucida* (large shrub), were found growing in this population. Bulbs that grew in the shade of bigger shrubs (*Galenia africana*) appeared more vigorous and seemed to benefit from this favourable microclimate.

Unfortunately this whole population was destroyed by bulldozers during the growing season of 14/6/2005 when the land was claimed for a residential development. According to the project manager on site, permission was granted to continue with the project. The staff of the Karoo Desert National Botanical Garden were asked about the site being claimed for residential development and they stated that they were uninformed about the project. Permission was obtained from the site manager to rescue any bulbs that could be saved. These bulbs were used for this study and were planted out in a garden at the nursery of the Cape Peninsula University of Technology, Cape Town.

2.1.4 Morphological description

G. villosa

The flowers of *G. villosa* are star-shaped, mildly scented, white or pink in colour and measure 40–90 mm in diameter. See Figure 2.5. The length of the flower petiole varies from 35–80 mm and the diameter of the flower petiole varies from 2.5–3.5 mm. The flower consists of six distinct tepals, which vary from 26–40 mm in length. The number of stamens are six and ± 6 mm long. The style is 15 mm in length and is flexed to the side of the flower, away from the stamens. The growth habit of bulbs varies from spiraled leaves flat on the ground to spiraled leaves above ground. See Figure 2.6. The leaves are flat, hairy, green in colour and vary from 1.5-5 mm in diameter and are dry at flowering. The average diameter of single plants is 60 mm and the average height (excluding the bulb and roots) varies from 30–90 mm (without leaves being straightened). The diameter of bulbs varies from 18-24 mm. *G. villosa's* seed numbers vary from 34 to 115 per berry. *G. villosa*'s berries have no fragrance and are white/cream in colour. See Figure 2.7. The size and shape of the berry and seeds are similar to those of *G. multifolia*.

Figure 2.5: Flowers of *G. villosa*.

Figure 2.6: Leaves of *G. villosa.*

Figure 2.7: Berry and seed of *G. villosa.*

2.1.5 Population descriptions of *G. villosa*

Population D: Brandwag Mountains (Worcester)

This population was found ± 650 m above sea level in the Brandwag mountain range, behind the Karoo Garden with the help of staff from the Karoo Desert National Botanical

Garden. Bulbs were located on both sides of an uphill pathway. This is an open, full sun and mountainous site with only shrubbery casting shade over some of the bulbs. The approximate size of this site is 2200 m^2. This habitat was characterized by rocky clay soil with an average soil pH of 6.2 (see Table 2.3).

This is a small population, which consists of only three clumps and eight single bulbs. No bulbs growing in colonies could be found. All bulbs were spaced from 3–22 m apart. The general appearance of growth in this population was not very vigorous and no trace of berries or germinated seedlings were found next to parent plants throughout the study period. All the bulbs did flower during the flowering stage. Only one bulb was lost throughout the study period. A hole that resembled that of a mole was found next to the marking peg of the bulb. No other natural elements, that could threaten the existence of this population were found.

The following neighbouring plant species: *Drimia capensis* (bulbous), *Felicia filifolia* (small shrub), *Galenia africana* (medium shrub) and *Eriocephalus* sp. (medium shrub), were found growing in this population. Also in this population *G. villosa* benefited from the shade provided by *Galenia africana* shrubs.

Population E: Rawsonville

Rawsonville (260 m above sea level) is a small town located 16 km outside of Worcester. This population was found on a flat open area on an uncultivated section of a farm. Across both species this was the biggest population. Soil conditions are clay, with an average pH of 4.9 (see Table 2.3) and no underground stones or rocks around the bulbs. Throughout the study, this area appeared to be moister than all the other study areas. No trees, shrubbery or other structures were found casting shade over the bulbs.

This population consisted of colonies, clumps, single bulbs and seedlings. The number of bulbs was estimated between 300 and 400 bulbs. On average bulbs were spaced from 0.2-0.25 m apart. During the flowering period, the area was characterized by a carpet of *G. villosa* flowers. The sizes of plants varied from seedlings to mature bulbs. This is the only population (amongst all five populations), where seedlings were found surviving next to mother plants.

The following neighbouring plant species: *Cynodon dactylon* (grass), *Euphorbia crispa* (ground cover), *Oxalis flava* (ground cover), *Mussonia depressa* (bulbous), *Eriospermum* sp. (bulbous), *Veltheimia capensis* (bulbous), *Babiana* sp. (bulbous) and *Stoebe plumosa* (medium shrub), were found growing in this population. Cows were found grazing on leaves, flowers and berries of this population. Holes dug by rodents were a common sight in this population, however no evidence was found that these rodents were actually eating the bulbs. See Figure 2.8. No other natural elements, that could threaten the existence of this population were found.

Figure 2.8: A rodent hole dug around a *G. villosa* bulb.

2.2 THE EFFECT OF CLIMATE ON *G. multifolia* and *G. villosa*

According to some authors (Du Plessis & Duncan, 1989:105; Elvin, 2000) passing cold fronts, accompanied by a definite drop in atmospheric pressure, irrespective whether there are showers or not, have a definite effect on the production of flowers in *Gethyllis*. Elvin (2000) further reported that *Gethyllis* grows in areas where little rain falls, mainly in the winter and that with greater decrease in atmospheric pressure and temperature during cold fronts, more and more bulbs flower, which increases the likelihood of cross-pollination.

According to these observations, both species had similar reactions to climatic changes. Both species produced new leaves from the end of March to mid-April. This normally happened between one and three weeks after the first rains. *G. multifolia* was always two to three weeks slower to react to these climatic impulses. The reproductive growth phase was from mid-November to early December when both species produce flowers simultaneously. This normally happened on average two weeks after the first summer rain. It was noticed that when the summer rains were absent or delayed, the number and quality of flowers produced in *G. multifolia* bulbs, were drastically reduced. Bulbs produced flowers which died during the bud stage. This phenomenon was discussed in the De Doorns population. See Figure 2.9. When flower buds opened the tepals appeared shrivelled up. Under these conditions flowers of both species appeared smaller and *G. multifolia* produced fewer flowers.

It was observed that as the temperature increased from the end of August, leaves of both species gradually started yellowing from the tips down to the cataphyll and this was followed by senescence. This normally lasted until the end of October. *G. multifolia* was seen to be always the first to react to this climatic impulse. A good example of this phenomenon is that when *G. multifolia*'s leaves have reached their final stages of senescence, *G. villosa*'s leaves were only at the beginning stages of senescence. This completed the winter growing cycle of the two species.

According to the data on the rainfall pattern, there is an indication that the absence of moisture has a definite effect on the production of leaves at the onset of the new growing season. Leaf formation is either delayed or does not appear at all in the case of *G. multifolia* bulbs. *G. multifolia* bulbs also produces less flowers or flowers of inferior quality during the absence of moisture. See Figure 2.9. This will result in limited cross-pollination taking place and consequently the formation of a decreased number seeds for the existence of populations and the species.

Figure 2.9: Dry flower buds of *G. multifolia* (29/11/2003).

Table 2.1: The monthly maximum, average monthly maximum, monthly minimum, average monthly minimum temperatures in °C and the rainfall in mm for the Worcester area from January 2002 to December 2003.

	2002					2003				
	Temperature (°C)				Rainfall	Temperature (°C)				Rainfall
Month	Max.	Av. Max.	Min.	Av. Min.	Total (mm)	Max.	Av. Max.	Min.	Av. Min.	Total (mm)
January	37.7	30.5	11.5	16.4	34	42.7	33.2	11.8	17.1	1.2
February	39.8	33.1	12.2	17.4	25.4	41.3	33.9	11.4	18	0.2
March	39.8	32.3	7.3	16	4	40.5	30.8	10.1	16.6	29
April	33.6	26.7	7.6	12.6	20.8	36.5	28	6.9	14.2	17.4
May	31.8	21.6	3.4	9.3	37	30.7	23.4	3.7	9	11.4
June	24.1	18.6	-0.7	7.2	18.8	26.5	20.7	-1.4	4.3	0.2
July	24.4	17.6	1.2	6	58	28.7	20	-0.8	5.1	5.8
August	31.9	19.5	1.7	7	54	27.6	18	-1	5.8	52.6
September	31.7	24.4	5.9	10.5	13.8	28.5	21.1	4.2	9	53.4
October	35.4	26.3	3.3	11.1	13.8	34.8	25.3	8.4	11.7	11.6
November	37.4	29.1	8	11.5	7.2	37.5	29.5	8.8	13.6	0
December	41.2	32.5	9.2	17.2	7.8	34.3	28.8	9.7	14.1	10.2

Table 2.2: The monthly maximum, average monthly maximum, monthly minimum, average monthly minimum temperatures in °C and the rainfall in mm for the Worcester area from January 2004 to October 2005.

	2004					2005				
	Temperature (°C)				Rainfall	Temperature (°C)				Rainfall
Month	Max.	Av. Max.	Min.	Av. Min.	Total (mm)	Max.	Av. Max.	Min.	Av. Min.	Total (mm)
January	38.5	32.2	12.9	17.4	2.2	36.2	31.1	13.8	17.7	21.8
February	38.3	32.3	15.5	18.1	0.4	40.8	33.1	13.7	17.5	11.6
March	38.2	28.5	9	12.8	1.8	38.4	30.7	11.5	16.1	0.6
April	35.7	26.5	5.5	12.2	17.8	36.2	25.1	8.1	12.6	63.2
May	32	24.8	3.2	9.8	0.4	29.6	21.1	4	9.9	7.4
June	30.2	20.1	-0.3	6.6	34.8	22.3	17.7	-0.7	5.9	30.2
July	24.7	19.2	-0.4	3.4	27.8	25.9	20.3	2	5.6	15.2
August	30.2	20.4	2	8	10.6	25.2	18.1	-1	5.9	33.2
September	31.5	23.7	2.2	8.4	11.2	32.9	23.1	2.5	9	13.2
October	34.1	26	4.7	11.6	69.8	32.6	24.5	3.5	9.7	2.6
November	35.8	30.8	11.2	14.9	5.8					
December	38.7	31.7	12.4	16.9	3.2					

Table 2.3: Soil analysis report for populations of *G. multifolia* and *G. villosa.*

Area and sample no.	Species	Texture	KCl pH	P Bray I	Macro-elements in mg / kg			
					K	Ca	Mg	Na
De Doorns 1	*G. multifolia*	clay	4.7	3	70	452	277	62
De Doorns 2	*G. multifolia*	clay	6.3	5	87	556	258	98
Karoo Garden 1	*G. multifolia*	clay	6.3	19	41	864	226	60
Orchard 1	*G. multifolia*	sandy clay	6.4	14	45	420	54	6
Orchard 2	*G. multifolia*	sandy clay	6.3	10	33	465	46	4
Brandwag Mountains 1	*G. villosa*	clay	6.4	3	275	1166	366	39
Brandwag Mountains 2	*G. villosa*	clay	5.9	8	107	1293	359	20
Rawsonville 1	*G. villosa*	sandy clay	4.8	19	127	395	69	12
Rawsonville 2	*G. villosa*	sandy clay	5.0	18	44	215	37	7

CHAPTER 3
MATERIALS AND METHODS

3.1 ASEXUAL PROPAGATION

3.1.1 LEAF CUTTINGS OF *G. multifolia* AND *G. villosa*

Sterilization, media and containers

All seed trays and tools used were dipped in a 1% Sporekill solution. All working surfaces and hands were sterilized with the same Sporekill solution. The secateurs were dipped in the same solution after every ten cuttings to prevent the spread of disease to other cuttings. All plant material was rinsed under running tap water and then soaked in a 0.04% Captab solution for ten minutes. The rooting medium used for this experiment was fine river sand (particle size 0.3-0.5 mm), which was watered with the same 0.04% Captab solution to kill soil-borne fungal spores. The containers used were black plastic seed trays size 150x230x65 mm.

Technique

Cuttings of *G. multifolia* and *G. villosa* were taken at the onset of the new growing season, which is from mid-March to mid-April (Van Reenen, 1975:3). Only healthy plant material was selected and leaves were taken from the most mature bulbs (Browse, 1995:157). Mature bulbs of *G. multifolia* have a diameter of 30 mm and mature bulbs of *G. villosa* a diameter of 18 mm. *G. multifolia* bulbs were obtained from the Orchard (Worcester) population where a new road was planned through part of the existing population. *G. villosa* bulbs were obtained, with permission, from the owner of a farm in Rawsonville. Bulbs of both species were collected from their natural habitats (27/04/2003) and planted in pots at the nursery of the Cape Peninsula University of Technology. Leaves for leaf cuttings were then harvested from this material on 30/04/2003. Parts of the leaves used, were tips and middle sections of the leaves of both species. The average length of cuttings was limited to 40 mm, because longer cuttings were too soft. The rooting hormones used were No.1 indole butyric acid (IBA) powder and Dip & Gro liquid at a rate of 1:10. Cuttings were planted, with their proximal

end up, in the rooting medium and watered weekly with the 0.04% Captab solution to prevent rotting. See Figures 3.1 and 3.2. The duration of the experiment was 12 weeks. See Table 3.1.

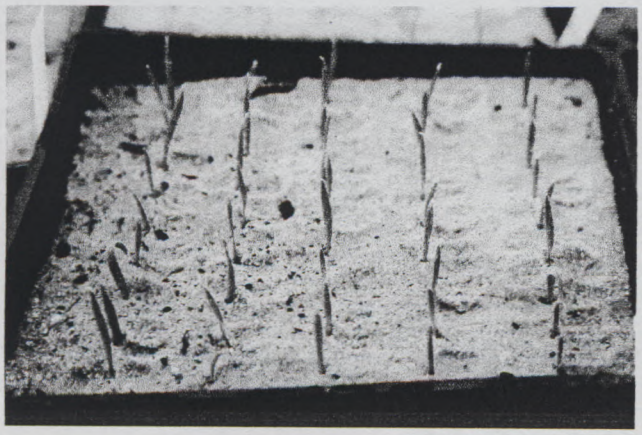

Figure 3.1: Leaf cuttings of *G. villosa.*

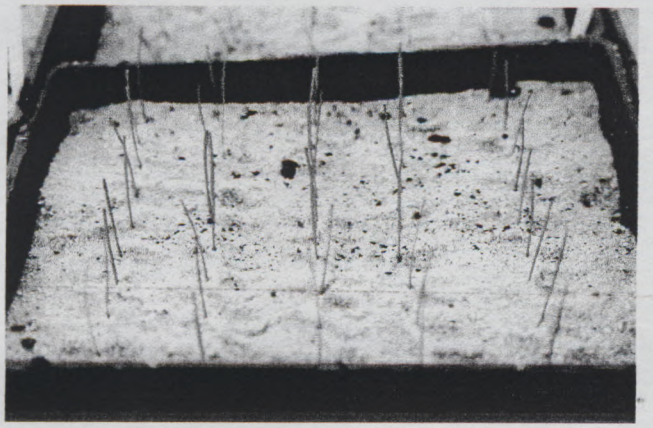

Figure 3.2: Leaf cuttings of *G. multifolia*

Environment

Trays with cuttings were placed on propagation beds with a bed temperature that varied from 20-23°C (Browse, 1995:158). The mist irrigation system was controlled by an automatic intermittent irrigation controller with watering intervals of 15 sec./20 min.

(Hartmann *et al.*, 1997:365). The average light intensity was measured with a Toptronic T630 digital lightmeter and the reading was 16800 lux at midday. The relative humidity reading was measured (Majortech CE Digital relative humidity meter and thermometer) above the cuttings and varied from 74-100%. This reading was influenced by the on and off nature of the intermittent irrigation. The greenhouse temperature varied from 20-28°C. See Table 3.1.

Table 3.1: Leaf cuttings and hormone treatments of *G. multifolia* and *G. villosa* in river sand in a controlled greenhouse at 20-28°C with bottom heat and 15 sec./20 min. misting irrigation. (n=45)

Species	Type of cutting	Hormone treatment
G. multifolia	tip of leaf	no.1 IBA powder
G. villosa	tip of leaf	no.1 IBA powder
G. multifolia	middle part of leaf	no.1 IBA powder
G. villosa	middle part of leaf	no.1 IBA powder
G. multifolia	tip of leaf	Dip & Gro
G. villosa	tip of leaf	Dip & Gro
G. multifolia	middle part of leaf	Dip & Gro
G. villosa	middle part of leaf	Dip & Gro

3.1.2 ROOT CUTTINGS OF *G. multifolia* AND *G. villosa*

Sterilization methods, time of year, selection of material and containers were similar to those used for leaf cuttings.

Environment

Root cuttings were placed in a mini tunnel (size 1000x2000x300 mm) covered with greenhouse plastic. The mini tunnel was placed inside a polycarbonate greenhouse. The use of the mini tunnel was necessary to have more effective control over the relative humidity above the root cuttings. Root cuttings were irrigated by hand when the medium appears dry compared to the intermittent irrigation of leave cuttings by an irrigation controller. Root cuttings require less irrigation and a lower relative humidity (compared to leave cuttings) because of its fleshy nature. Temperatures inside the mini tunnel varied from 20-28°C. The average relative humidity and light intensity recorded inside the mini tunnel at midday was 65% and 16400 lux respectively.

Technique

Bulbs of both species were collected from their natural habitats (27/04/2003) and planted in pots at the nursery of the Cape Peninsula University of Technology (as previously discussed). Bulbs were removed from their pots on 1/05/2003 after which root cuttings were prepared in the greenhouse of the same institution. *G. multifolia* has fleshy roots with an average diameter of 4 mm. The diameter of bulbs and roots of both species were measured with a caliper. Thinner roots were used for *G. villosa* with an average diameter of 2 mm. The average length of root cuttings was 40 mm. No rooting hormones were used. All root cuttings were planted, with their proximal end up, in the rooting media (Browse, 1995:78). Two rooting media were used for this experiment and consisted of 2:1 sifted bark/river sand and river sand only. Root cuttings were irrigated by hand with the 0.04% Captab solution. The duration of the experiment was eight weeks. See Table 3.2.

3.1.3 BULB CUTTINGS OF *G. multifolia* AND *G. villosa*

Due to the limited availability of plant material, bulb experiments were restricted to ten repetitions. Sterilization methods, time of year, containers, the environment and selection of material were similar to those used for leaf cuttings.

Preparation

Bulbs of both species were collected from their natural habitats (27/04/2003) and planted in pots at the nursery of the Cape Peninsula University of Technology. Bulbs were removed from their pots on 2/05/2003 after which bulb cuttings were prepared in the greenhouse of the same institution. Bulbs varied in size with the average bulb size of *G. multifolia* (30x40 mm) compared to the slightly smaller bulbs of *G. villosa* (18x24 mm). All bulbs were washed under running tap water to remove all soil and old bulb scales. Bulbs were then soaked in a 0.04% Captab solution for ten minutes.

Technique and media

The cataphyll of the bulb was removed and the roots trimmed. Each bulb was cut longitudinally into five sections (Hartmann *et al.*, 1997:532). Five bulb cuttings could be cut from one bulb. Each cutting consisted of five to six scales 15-20 mm in length and

part of the basal plate. Bulb cuttings were allowed to dry for 10 minutes after which they were dusted with dry Captab powder to prevent rotting (Hartmann *et al.*, 1997:531). Bulb cuttings were allowed to callus for two weeks in a dark, dry cupboard at a temperature of 21°C and a relative humidity of 60% (Hartmann *et al.*, 2002:573). After two weeks the bulb cuttings were planted in the rooting media. A second technique was applied, whereby bulb cuttings of both species were planted immediately after cutting without allowing a callusing period. Bulb cuttings were planted vertically with only the basal plates inserted in the rooting media. The three rooting media used for this experiment were 1:1 river sand and perlite, 2:1 river sand and peat moss and vermiculite only (Hartmann *et al.*, 1997:532 and Symmonds *et al.*, 1997:26). The media were kept moist by applying the 0.04% Captab solution only when necessary. The duration of the experiment was 16 weeks. See Table 3.2.

3.1.4 TWIN SCALING OF *G. multifolia* AND *G. villosa*

Due to the limited availability of plant material, the number of repetitions were restricted to 15. Sterilization methods, preparation, time of year, containers, environmental conditions and selection of material were similar to those used for bulb cuttings.

Technique and media

Bulbs of both species were collected from their natural habitats (27/04/2003) and planted in pots at the nursery of the Cape Peninsula University of Technology (as previously discussed). Bulbs were removed from their pots on 3/05/2003 after which twin scales were prepared in the greenhouse of the same institution. Each bulb was cut longitudinally into five sections with a budding knife. Each section was then cut up longitudinally to form twin scales. Eight to ten twin scales (15-20 mm long) could be cut from one bulb. Each twin scale consisted of two to three scales and part of the basal plate. Twin scales were allowed to dry for 10 minutes after which they were dusted with dry Captab powder to prevent rotting (Hartmann *et al.*, 1997:531). Twin scales were allowed to callus for two weeks in a dark, dry cupboard at a temperature of 21°C and a relative humidity of 60% (Hartmann *et al.*, 2002:573). After two weeks the twin scales were planted in the rooting media. As an alternative propagation technique, twin scales were placed immediately in clear self-seal plastic bags with fine moist vermiculite for

three to four weeks (Hartmann *et al.*, 1997:532). The duration of the experiment was 12 weeks. See Table 3.2.

Table 3.2: Bulb cuttings (n=10), twin scaling (n=15), callusing period and rooting media of *G. multifolia* and *G. villosa* in a plastic mini tunnel inside a polycarbonate greenhouse at 10-28°C.

Species	Technique	Callusing period	Media
G. multifolia	bulb cuttings	two weeks	1:1 perlite:river sand
G. villosa	bulb cuttings	two weeks	1:1 perlite:river sand
G. multifolia	bulb cuttings	two weeks	2:1 river sand:peat moss
G. villosa	bulb cuttings	two weeks	2:1 river sand:peat moss
G. multifolia	bulb cuttings	two weeks	vermiculite
G. villosa	bulb cuttings	two weeks	vermiculite
G. multifolia	bulb cuttings	none	1:1 perlite:river sand
G. villosa	bulb cuttings	none	1:1 perlite:river sand
G. multifolia	bulb cuttings	none	2:1 river sand:peat moss
G. villosa	bulb cuttings	none	2:1 river sand:peat moss
G. multifolia	bulb cuttings	none	vermiculite
G. villosa	bulb cuttings	none	vermiculite
G. multifolia	twin scaling	two weeks	1:1 perlite:river sand
G. villosa	twin scaling	two weeks	1:1 perlite:river sand
G. multifolia	twin scaling	two weeks	2:1 river sand:peat moss
G. villosa	twin scaling	two weeks	2:1 river sand:peat moss
G. multifolia	twin scaling	none	1:1 perlite:river sand
G. villosa	twin scaling	none	1:1 perlite:river sand
G. multifolia	twin scaling	none	2:1 river sand:peat moss
G. villosa	twin scaling	none	2:1 river sand:peat moss
G. multifolia	twin scaling (self-seal bags)	none	vermiculite
G. villosa	twin scaling (self-seal bags)	none	vermiculite

3.1.5 SCOOPING AND SCORING OF *G. multifolia* AND *G. villosa*

Due to the limited availability of plant material, the number of repetitions was limited to only five cuttings per experiment. Sterilization methods, preparation, time of year, containers, environmental conditions and selection of material were similar to those used for bulb cuttings and twin scaling.

Technique and media

As previously discussed, bulbs were obtained from their natural habitats on 27/04/2003 and removed on 4/05/2003 from their pots at the nursery of the Cape Peninsula

University of Technology. Bulbs were then prepared for scooping and scoring in the greenhouse of the same institution. For scooping, the basal plate was scooped out with a budding knife and for scoring, two cuts were made with the same instrument across the basal plate, to damage the growing point (Browse, 1995:96; Hartmann *et al.*, 1997:531). Bulbs were allowed to dry for ten minutes. Dry Captab powder was forced into all cut surfaces to prevent rotting. All the bulbs were then placed in a dark cupboard at a temperature of 21°C and a relative humidity of 60% for two weeks to allow drying and callusing (Hartmann *et al.*, 2002:573). After two weeks wounded bulbs were planted in a 1:1 river sand and perlite medium. The bulbs were planted upright, with only their necks exposed, in the sterilized propagating medium. To prevent rotting, the medium was only watered with the 0.04% Captab solution before it showed signs of drying out. See Table 3.3. The duration of this experiment was 16 weeks.

3.1.6 BASAL PLATE CUTTINGS OF *G. multifolia* AND *G. villosa*

Sterilization methods, preparation, time of year, containers, environmental conditions and selection of material were similar to those used for scooping and scoring.

Technique and medium

Bulbs were obtained from the same source and on the same date as earlier discussed. Basal plate cuttings were prepared at the greenhouse of the same institution on 4/5/2003. Each basal plate was carefully removed from the bulbs and individually cut up into smaller sections measuring 6x6 mm. The basal plates were allowed to dry for 10 minutes after which all sides were dusted with dry Captab powder to prevent rotting. The basal plates were then lightly embedded in moist fine grade vermiculite (not covered). To prevent rotting, the medium was only watered with the 0.04% Captab solution before it showed signs of drying out. The duration of the experiment was 12 weeks.
See Table 3.3.

Table 3.3: Scooping (n=5), scoring (n=5), basal plate cuttings (n=20) and root cuttings (n=35) of *G. multifolia* and *G. villosa*.

Species	Environment	Technique	Media
G. multifolia	plastic mini tunnel in greenhouse	scooping	1:1 river sand:perlite
G. villosa	plastic mini tunnel in greenhouse	scooping	1:1 river sand:perlite
G. multifolia	plastic mini tunnel in greenhouse	scoring	1:1 river sand:perlite
G. villosa	plastic mini tunnel in greenhouse	scoring	1:1 river sand:perlite
G. multifolia	plastic mini tunnel in greenhouse	basal plate cuttings	vermiculite
G. villosa	plastic mini tunnel in greenhouse	basal plate cuttings	vermiculite
G. multifolia	plastic mini tunnel in greenhouse	root cuttings	2:1 sifted bark:river sand
G. villosa	plastic mini tunnel in greenhouse	root cuttings	2:1 sifted bark:river sand
G. multifolia	plastic mini tunnel in greenhouse	root cuttings	river sand
G. villosa	plastic mini tunnel in greenhouse	root cuttings	river sand

3.2 *IN VITRO* PROPAGATION

3.2.1 STERILIZATION AND INITIATION OF *Gethyllis villosa*

Sterilization

According to Richard (1990:18), there is a high risk of contamination from soil organisms, because the bulb tissue is in direct contact with the soil medium. *G. villosa* bulbs were collected (with permission from the staff of the Karoo Desert National Botanical Garden - Worcester) on 26/4/2003 from their natural habitat in the Brandwag Mountains and transferred to the nursery of the Cape Peninsula University of Technology (CPUT). This time of the year represents the onset of the new vegetative growing season, which is between mid-March and mid-April (Van Reenen, 1975:3). The bulbs were washed under running tap water until all excess soil was removed. The approximate size of the bulbs used was 18 mm in diameter x 24 mm in height. All leaves, roots and outer scales were pruned away. Bulbs were placed into glass jars with clean tap water and transferred to the *in vitro* propagation laboratory of the CPUT. After this, they were rinsed again with running tap water for 20 minutes. Bulbs were cut longitudinally into four equal sections. These smaller sections were then dipped in a 70% ethanol solution for two minutes (Fennell *et al.*, 2001). McAlister *et al.* (1998) suggested soaking *Cyrtanthus* bulb scales in a 3.5% sodium hypochloride (NaOCl) for 10 minutes. The bulb sections were surface sterilized in a solution of 2% NaOCl (Richard, 1990:19) and two drops of Tween for 20 minutes. As a precaution, all jars and lids were rinsed with the NaOCl solution prior to use. Bulb sections were then rinsed three times for 20 minutes each with sterile distilled water in a laminar flow bench (Richard, 1990:38).

Initiation

The three different media described by Drewes & Van Staden (1994:295) for the initiation of *G. linearis* L. Bol. bulbs, were used for *G. villosa*. In all three cases the lower pH of the media was adjusted to 5.8 with NaOH. The media were heated to 95°C (when agar liquefies) before it was decanted into test tubes (25x100 mm) which contained 10 ml of medium. The pre-mixed Murashige and Skoog (1962) macro-and micro-element mixture was used as a base for all the media in the following set of experiments (Richard, 1990:21). Medium 1 consisted of 4.3 $g1^{-1}$ MS basal medium (Murashige and Skoog, 1962) supplemented with 0.1 $g1^{-1}$ *myo*-inositol and 30 $g1^{-1}$ sucrose which was solidified

with 10 $g1^{-1}$ agar. No hormones were added to this medium. In medium 2, the hormones NAA (naphtaleneacetic acid) at 0.2 $mg1^{-1}$ and BA (benzyladenine) 0.2 $mg1^{-1}$ were added. For medium 3, NAA at 2 $mg1^{-1}$ and BA 0.2 $mg1^{-1}$ were added. All media were sterilized in an autoclave for 20 minutes at 120 °C (Nicol, 1993:78).

In this study, the ultra violet light in the laminar flow bench was switched on for 30 minutes to sterilize all surfaces prior to cutting. All surfaces, glassware and equipment inside the laminar flow bench were wiped with a 70% ethanol solution prior to cutting (Wetherell, 1982:34). One bulb section was removed at a time using one pair of instruments. Van Aartrijk & Van Der Linde (1986) suggested that twin scales were frequently used in micropropagation of amaryllidaceous species, and for that reason only twin scales were experimented on for this part of the study. Bulb sections were finally cut into twin scales while trimming all NaOCl damaged areas away. Each bulb section was split into four twin scales, size 5 mm in diameter x 8 mm in height. Only one explant was inserted upright per test tube with the bottom end (containing part of the basal plate) in contact with the medium. All test tubes were sealed with a Parafilm tape to prevent environmental contamination and placed in the growing room (McAlister *et al.*, 1998). The duration of the experiment was 12 weeks.

The environmental temperature in the growing room varied from 14-16°C at night and 22-24°C during the day (Drewes & Van Staden, 1994:295). Fluorescent lights (Grolux lamps) were positioned 20 cm above the explants and the light intensity reading was measured at 5100 lux. Explants were exposed to a photoperiod of 16 hours (Richard, 1990:38).

3.2.2 MULTIPLICATION AND ROOTING OF *G. villosa*

The medium for multiplication is similar to that used for initiation, except that hormones were adjusted to NAA at 0.2 $mg1^{-1}$ and BA 0.2 $mg1^{-1}$. All surfaces, glassware and equipment inside the laminar flow bench were wiped with a 70% ethanol solution prior to the cutting procedure (Wetherell, 1982:34). The explants from the initiation stage were used for this experiment. Leaves from explants were trimmed back halfway to encourage new growth. Roots were completely removed. All dead, yellow and stunted growth was cut away. All clumps were split up, explants were placed individually in test tubes and kept under similar environmental conditions as previously described. The duration of this part of the experiment was 16 weeks.

3.2.3 HARDENING-OFF OF *G. villosa*

A medium of 1:3 v/v peat: sand, was suggested by Drewes & Van Staden (1994) for the hardening-off of *G. linearis* L. Bol. plantlets. *Gethyllis* species require a medium with a higher drainage capacity compared to most other members of the Amaryllidaceae family (Christian, 2000). For this reason various media with improved drainage were considered. Two different media were chosen, the first consisted of 1:1:1 v/v sifted bark: perlite: river sand and the second consisted of 2:1 v/v river sand: peat moss. Plantlets were hardened-off in 8 cm round nursery pots, which had been sterilized in a 1% Sporekill solution (Fennell *et al.*, 2001). All plantlets were well watered with a 0.04% Captab solution (Duncan, 2000:55) to prevent fungal infection. Plantlets were placed in a controlled greenhouse with polycarbonate sheeting on beds with intermittent irrigation intervals of 15 sec./20 min. and bottom heating at 21-23°C. Environmental temperatures ranged from 24-28°C and the relative humidity around the explants ranged from 70-80%. After four weeks the plantlets were transferred to a shade house that was covered with 60% shade cloth. The soil media were kept moist, not wet, throughout the acclimatization period. The duration of this experiment was four weeks.

3.2.4 STERILIZATION AND INITIATION OF *G. multifolia*

Sterilization

G. multifolia bulbs were obtained from the Orchard (Worcester) population on 27/04/2003 where a new road was planned through part of the existing population and transferred to the nursery of the (CPUT). The approximate size of the bulbs used for this experiment, was 30 mm in diameter x 40 mm in height. All bulbs were washed under running tap water until all excess soil was removed. All leaves, roots and outer scales were removed. Bulbs were placed into glass jars, transferred to the *in vitro* laboratory (CPUT) and further rinsed with running tap water for 20 minutes. The bulbs were cut longitudinally into four equal sections. The smaller bulb sections were placed in a solution of 70% ethanol for two minutes (Fennell *et al.*, 2001). The bulb sections were then surface sterilized in a 2% NaOCl solution with two drops of Tween for 20 minutes. All bulb sections were then rinsed three times for 20 minutes each with sterile distilled water in the laminar flow bench. All jars and lids were rinsed with the NaOCl solution prior to use (Richard, 1990:38).

Initiation

Eleven different media were prepared and the pH in all cases was adjusted to 5.8 with NaOH. The Latin Square grid for testing media (Collin & Edwards, 1998:32), was used to give combinations of NAA and BA. Twenty explants were used with each combination (Table 3.4). The cutting technique and environment were the same as for the initiation of *G. villosa*. The duration of this part of the experiment was 16 weeks.

Table 3.4: Initiation media for *G. multifolia* on different NAA: BA (mgl^{-1}) hormone combinations. (n=20)

BA	NAA						
0	0						
0.2		0.2	0.4	0.8	1.0	1.5	2
0.4			0.4	0.8	1.0	1.5	

3.2.5 MULTIPLICATION OF *G. multifolia*

Similar test tubes used for *G. villosa*, were used in this experiment. The environmental conditions and the cutting procedure were similar to those used for *G. villosa*. The media for multiplication was similar to those used for initiation, except that hormones were adjusted to NAA at 1.0 $mg1^{-1}$ and BA 0.2 $mg1^{-1}$ or NAA at 1.5 $mg1^{-1}$ and BA 0.2 $mg1^{-1}$ as these two combinations showed best results during initiation. Ninety eight (98) explants from the previous initiation experiment were used for this experiment. The duration of this experiment was 16 weeks.

3.2.6 ROOTING OF *G. multifolia*

For this phase four different media were tested. In all four media only the hormones were adjusted and the pH (5.8) and all other ingredients were unchanged. The first medium consisted of 0.5 $mg1^{-1}$ NAA and no BA (Drewes & Van Staden, 1994). In addition, three trials with various combinations of NAA and BA per $mg1^{-1}$ were used. The NAA:BA combinations were as follows 0.5:0, 0.4:0.2, 0.8:0.2 and 0.8:0.4 $mg1^{-1}$ (Table 3.5). Leaves from explants were trimmed back halfway to encourage new growth. Roots were completely removed. All dead, yellow and stunted growth was cut away. Four to five explants were placed in each of the glass jars containing 25 ml of medium. Thirty one glass jars containing a total of 138 explants were used in this experiment. The duration for this part of the experiment was 15 weeks.

Two different environmental conditions with varying temperatures and light conditions were tested on growth and root development. Environment 1 had a temperature that varied from 14-16°C at night and 20-24°C at daytime. Fluorescent lights were positioned 20 cm above the explants and the light intensity reading was measured at 5100 lux. Explants were exposed to a photoperiod of 16 hours. Environment 2 was a darker corner in the same growing room. Fluorescent lights were not positioned directly above plants and the light intensity reading was measured at 2500 lux. The environmental temperature varied from 14-16°C at night and 20-22°C during the day. The glass jars containing the explants were placed in a tray filled with a film of water 15 mm in depth. The temperature of the water was 4°C lower than the environmental temperature. This served to lower the temperature directly around the explants (simulating winter conditions), in order to encourage root growth and/or multiplication (Richard, 1990:28).

Table 3.5: Rooting media for *G. multifolia* on different NAA: BA (mgl^{-1}) hormone combinations.

BA	NAA		
0	0.5 (n=40)		
0.2		0.4 (n=38)	0.8 (n=30)
0.4			0.8 (n=30)

3.2.7 HARDENING-OFF OF *G. multifolia*

After 15 weeks in the rooting media, individual rooted plantlets were separated and planted out in 6-cavity seed trays. The size of the cavities was 50x50 mm. The plantlets were transplanted during the natural winter vegetative growing period of *Gethyllis* species, which is between mid-March and mid-April (Liltved, 1992). Excessive long roots and leaves of plantlets were trimmed with a pair of sterile secateurs prior to planting out. All trays were sterilized in a 1% Sporekill solution. All plantlets were well watered with a 0.04% Captab solution after planting.

Drewes & Van Staden (1994) recommended a transplanting medium of peat: sand (1:3 v/v). Peat moss and vermiculite have a high water holding capacity and *Gethyllis* species are sensitive to over watering (Christian, 2000). Peat moss and vermiculite were thus avoided for this experiment. Instead, three different media were used and consisted of sifted bark: perlite: river sand (1:1:1 v/v), sifted bark: river sand (1:1 v/v) and perlite:

river sand (1:1 v/v). Fifty percent of plantlets were transferred to a controlled greenhouse (polycarbonate sheeting) at 24-28°C under intermittent mist irrigation (15 sec./20 min.) with bottom heat at 21-23°C (Drewes & Van Staden, 1994). The relative humidity reading was between 70 and 80%. The other 50% of plantlets were placed in mini tunnels, size 1000x2000x300 mm, covered with greenhouse plastic (Wetherell, 1982:72). This second environment had no intermittent misting and an average relative humidity of 68%. The mini tunnels were placed in the same controlled greenhouse. The media were only watered before it dried out. After four weeks the plantlets were transferred to a shade house covered with 60% shade netting. The duration of this experiment was four weeks.

3.3. HYDRO CULTURE OF *G. multifolia* and *G. villosa*

3.3.1 INTRODUCTION

There is no hard evidence to support the fact that a higher yield can be obtained through growing by means of hydro culture, but with most crops it can be estimated that the hydro culture method will produce from two to four times the yield expected in soil (Harris, 1994:30). *G. villosa* and *G. multifolia* are winter growing, deciduous geophytes which are extremely sensitive to watering during their dormant season (Du Plessis & Duncan, 1989:105). This statement is based on soil cultivation, which is completely different to hydro culture. No literature could be found on hydro culture of *Gethyllis*. The hydro culture experiment was designed to test whether it is at all possible to grow the two species by means of hydro culture. Different systems and media will also be experimented on, as possible future cultivation practices. Both species will be irrigated throughout the dormant phase to test whether this will result in rotting of the bulbs compared to a control where irrigation will be withheld throughout the dormant phase. Irrigation will also be withheld for six weeks at the onset of the new growing to test whether these two species are dependent on moisture or a drop in atmospheric temperature, for the production of foliage for the new growing season. The timing (formation of foliage at the onset of the new growing season) of bulbs and the quality of foliage (texture, colour, vigor, length and turgidity) produced will be observed during this experiment. The duration of this experiment is 18 months.

3.3.2 SUB-IRRIGATION SYSTEM

Layout and materials

A wooden boxed bed, measuring 3400x750 mm, was lined with a double layer of clear waterproof plastic and a raised sidewall to 90 mm. The height of the wooden boxed bed was 700 mm with a slight gradient to ensure proper flow of water into the reservoir (Nel, 2001:48; Harris, 1994:30). See Figure 3.3. The reservoir was a square fiberglass tank with a capacity of 120 litres and the size was 500x500x400 mm. A small submersible pump was used to circulate the water from the sump back to the bed. The pump was connected to an electronic timer and the plants were irrigated continuously for 8½ hours per day. The piping consisted of 20 mm low-density polyethylene (LDPE) irrigation pipe

and full flow emjay irrigation fittings. Eight water outlet holes (8 mm in diameter) were drilled into the LDPE pipe.

Figure 3.3: Layout of the sub-irrigation hydro culture system.

Containers and media

The water level inside the bed was maintained at ± 35 mm. The bed could accommodate 48 nursery pots (20 cm). Four slits 5x60 mm in size, were made on the sides of the pots to allow for water passage (Harris, 1994:31). Leca pellets (8-16 mm in diameter) and gravel (13 mm in diameter) were used as the hydro culture media for the sub-irrigation system (Harris, 1994:30-36). See Table 3.6.

Preparation and methodology

The availability of plant material limited the number of repetitions to between eight and ten. Two different media were compared with each other to test which medium would be more suitable for hydro culture growing. Two species were tested in two different hydro culture systems. The bulbs were irrigated throughout the dormant season to test whether bulb decay will take place across both species, media and systems. Another experiment was conducted whereby irrigation was withheld throughout the dormant season as well as for seven weeks at the onset of the new growing season. This experiment was done on both species, both systems and all four media. This test was done to evaluate whether the bulbs are dependent on moisture or a change in temperature for the production of new leaves.

As earlier described, bulbs were collected from their natural habitats on 27/ 04/2003 and planted in pots in the nursery of the CPUT. The bulbs were transferred from the existing potting medium to hydro culture media at the onset of the new growing season (15/4/2004). All excess soil was washed from the roots and the bulbs prior to transferring them to the new media. The bulbs were then left to grow in both hydro culture systems for one dormant and two growing seasons. The duration of the experiment was 18 months. Kompel Chemicult - a fully balanced water soluble hydro culture fertilizer (recommended for sub-irrigation and drip irrigation systems), was used at a rate of 2% per solution (20 g per litre). Kompel Chemicult consists of the following macro elements: N (6.5%), P (2.7%), K (13%), Ca (13%), Mg (2.2%), S (7.5%) and micro elements: Fe (0.15%), Mn (0.024%), B (0.024%), Zn (0.005%), Cu (0.002%) and Mo (0.001%). The pH of the water was not adjusted (Nel, 2001:48; Harris, 1994:35) and varied between 6.8 and 7.9 (measured with model: pHep HI 93127, from Hanna Instruments). The electrical conductivity was not adjusted (Harris, 1994:36) and varied from 1.6-2.5 millisiemens (mS) (measured with model: EC/TDS HI 98311 from Hanna Instruments). Municipal water was used for all experiments (James, 2002:29). The water was not tested but the system was flushed with clean water once every two weeks after which the nutrient solution was replaced (Harris, 1994:36).

Environment

All the plants were grown in a 50% white shaded greenhouse tunnel. The temperature in the tunnel varied between 15-38ºC throughout the growing and dormant seasons. The temperature and light intensity readings were taken three times a day (mornings, midday and late afternoon). The average light intensity in the tunnel was 21000 lux and the average relative humidity varied from 38-76%.

3.3.3 DRIP IRRIGATION SYSTEM

Layout and materials

The drip irrigation bed was also raised to 700 mm with a slight gradient to ensure proper flow of water into the reservoir. See Figure 3.4. The size of the bed was 1700x1500 mm and was lined with a double layer of clear waterproof plastic. The same reservoir, pump, pipe and fittings as for the sub-irrigation system, were used for this system. Both irrigation systems formed one complete unit. Button drippers (delivery rate 2 l/hour) were

connected to the main waterline using 3 mm dripper tubing. Flow control valves were used to separate the water flow between the two different systems.

Figure 3.4: Layout of the drip irrigation hydro culture system.

Containers and media

The bulbs were planted in 20 cm nursery pots. The drainage holes of the pots were covered with large pieces of gravel to prevent loss of media from the pots. The bed accommodated 56 nursery pots (20 cm). Two different media were tested for this experiment and consisted of perlite granules (4 mm) and bark chips (6-13 mm) (Harris, 1994:25). See Table 3.6.

Preparation and methodology

The same methods and materials as for the sub-irrigation system, were used.

Table 3.6: Sub-irrigation and drip irrigation of *G. multifolia* and *G. villosa* throughout the growing and dormant seasons in a tunnel with 50% shade net. Observations were done at the onset of the second growing season.

Sub-irrigation			
G. multifolia		***G. villosa***	
Leca pellets (n=8)	Gravel (n=8)	Leca pellets (n=8)	Gravel (n=8)
Drip irrigation			
G. multifolia		***G. villosa***	
Bark (n=10)	Perlite (n=10)	Bark (n=10)	Perlite (n=10)

Table 3.7: Delayed irrigation at the onset of the new growing season in a greenhouse tunnel covered with 50% shade net. Observations were done at the onset of the second growing season.

Sub-irrigation			
G. multifolia		***G. villosa***	
Leca pellets (n=4)	Gravel (n=4)	Leca pellets (n=4)	Gravel (n=4)
Drip irrigation			
G. multifolia		***G. villosa***	
Bark (n=4)	Perlite (n=4)	Bark (n=4)	Perlite (n=4)

CHAPTER 4
RESULTS

4.1 ASEXUAL PROPAGATION OF *G. multifolia* AND *G. villosa*

The availability of plant material limited the number of repetitions per experiment.

4.1.1 LEAF CUTTINGS

After six weeks, cuttings of both species across both treatments were still green and alive. After seven weeks yellowing and discolouration in 30% of leaf cuttings were noticed. Gradually rotting and collapsing of the cuttings of both species occurred. Cuttings of *G. multifolia* collapsed faster than those of *G. villosa.* After 12 weeks, all cuttings were dead except the leaf tip cuttings of *G. villosa.* Leaf tip cuttings of *G. villosa* remained alive for an additional three weeks before they rotted. No signs of callusing or rooting were noticed in both species across both treatments.

4.1.2 ROOT CUTTINGS

For this study, the root cutting propagation technique is regarded as successful if one or two new bulblets are produced from a single root cutting. This technique is regarded as unsuccessful if the root cutting dies or if no new bulblets are produced. No signs of growth, callusing or rooting on both species across both media were noticed during the first three weeks. After three weeks, 60% of root cuttings were shriveled up and no signs of vegetative growth were noticed. The thinner root cuttings of *G. villosa* were the first to die. After eight weeks all root cuttings were dead. See Table 4.2.

4.1.3 BULB CUTTINGS

Bulbs of these two *Gethyllis* species are relatively small which resulted in small bulb cuttings and twin scales. A sticky sap was noticed on *G. multifolia* bulbs, which made handling of bulb cuttings and twin scales difficult. For this study, the bulb cutting propagation technique is regarded as successful if two or more new bulblets are produced from a single bulb cutting. This technique is regarded as unsuccessful if the bulb cutting dies or if one new bulblet is produced from it. The callusing period of two weeks resulted in moisture loss and death of the bulb cuttings. Although bulb cuttings

appeared extremely dry, they were still planted in the various media. All bulb cuttings died within one week of planting. The second technique, which involved no callusing period proved to be more successful. *G. multifolia* bulbs responded better to this propagation technique whereby eight out of the ten bulb cuttings each produced three new bulblets in the 2:1 river sand: peat moss medium. *G. villosa* bulb cuttings responded poorly to this method of propagation in the 2:1 river sand: peat moss medium with two out of ten bulb cuttings forming three new bulblets. Eight *G. villosa* bulb cuttings died in this medium. *G. villosa* bulb cuttings responded best in the vermiculite medium with four out of ten bulb cuttings forming three new bulblets. In the same medium, five out of the ten *G. multifolia* bulb cuttings, produced three bulblets each. The lowest response was noticed in the 1:1 perlite: river sand medium with one *G. villosa* bulb cutting producing three new bulblets. This propagation technique proved to be most successful for *G. multifolia* in the 2:1 river sand: peat moss medium. This propagation technique is regarded as unsuccessful for *G. villosa* bulbs across all the media tested. Bulb cuttings over both species were slow in responding to this method of propagation. New bulblets only started forming after 13 weeks. See Figure 4.1. On average three new bulblets were formed from one bulb cutting. Statistically, however, there is no significant departure from homogeneity between media and resultant number of bulblets formed for *G. multifolia*, $\chi_2^2 = 0.465$ (NS) and *G. villosa*, $\chi_2^2 = 0.367$ (NS). Only results from experiments with no callusing period were considered.

Figure 4.1: Bulb cuttings of *G. multifolia*.

4.1.4 TWIN SCALING

For this study, the twin scaling propagation technique is regarded as successful if one or more new bulblets are produced from a single bulb cutting. This technique is regarded as unsuccessful if the bulb cutting dies or if no new bulblets are produced from it. The callusing period of two weeks for twin scaling resulted in moisture loss and death of the twin scales. The second propagation technique with no callusing period yielded mixed results. *G. multifolia* twin scales in the self-seal bags in vermiculite were the most successful with 13 out of 15 twin scales each producing one new bulblet. See Table 4.1. Comparatively four out of the fifteen *G. villosa* twin scales each produced one new bulblet in the same medium. *G. multifolia* twin scales in the 1:1 perlite: river sand and the 2:1 river sand: peat moss media were a failure with eight out of fifteen twin scales producing new bulblets. This technique proved to be unsuccessful for *G. villosa* with less than half of the twin scales producing new bulblets per twin scale across all the experiments. Both plant species were slow in responding to twin scaling propagation. New bulblets started to form after 13 weeks. See Figure 4.2. Each twin scale produced one new bulblet. Statistically there is no significant departure from homogeneity between media and resultant number of bulblets formed for *G. multifolia*, $\chi_2^2 = 0.422$ (NS) and *G. villosa*, $\chi_2^2 = 0.925$ (NS), when one considers only experiments with no callusing period.

Table 4.1: Rooting success on bulb cuttings (n=10), twin scaling (n=15), callusing period and rooting media of *G. multifolia* and *G. villosa* in a plastic mini tunnel inside a polycarbonate greenhouse at 20-28°C.

Species	**Technique**	**Callusing period**	**Media**	**2 or more bulblets formed**
G. multifolia	bulb cuttings	two weeks	1:1 perlite:river sand	0 (0%)
G. villosa	bulb cuttings	two weeks	1:1 perlite:river sand	0 (0%)
G. multifolia	bulb cuttings	two weeks	2:1 river sand:peat moss	0 (0%)
G. villosa	bulb cuttings	two weeks	2:1 river sand:peat moss	0 (0%)
G. multifolia	bulb cuttings	two weeks	vermiculite	0 (0%)
G. villosa	bulb cuttings	two weeks	vermiculite	0 (0%)
G. multifolia	bulb cuttings	none	1:1 perlite:river sand	4 (40%)
G. villosa	bulb cuttings	none	1:1 perlite:river sand	1 (10%)
G. multifolia	bulb cuttings	none	2:1 river sand:peat moss	8 (80%)
G. villosa	bulb cuttings	none	2:1 river sand:peat moss	2 (20%)
G. multifolia	bulb cuttings	none	vermiculite	5 (50%)
G. villosa	bulb cuttings	none	vermiculite	4 (40%)
Species	**Technique**	**Callusing period**	**Media**	**1 or more bulblets formed**
G. multifolia	twin scaling	two weeks	1:1 perlite:river sand	0 (0%)
G. villosa	twin scaling	two weeks	1:1 perlite:river sand	0 (0%)
G. multifolia	twin scaling	two weeks	2:1 river sand:peat moss	0 (0%)
G. villosa	twin scaling	two weeks	2:1 river sand:peat moss	0 (0%)
G. multifolia	twin scaling	none	1:1 perlite:river sand	8 (53%)
G. villosa	twin scaling	none	1:1 perlite:river sand	5 (33%)
G. multifolia	twin scaling	none	2:1 river sand:peat moss	8 (53%)
G. villosa	twin scaling	none	2:1 river sand:peat moss	4 (27%)
G. multifolia	twin scaling (self-seal bags)	none	vermiculite	13 (87%)
G. villosa	twin scaling (self-seal) bags)	none	vermiculite	4 (27%)

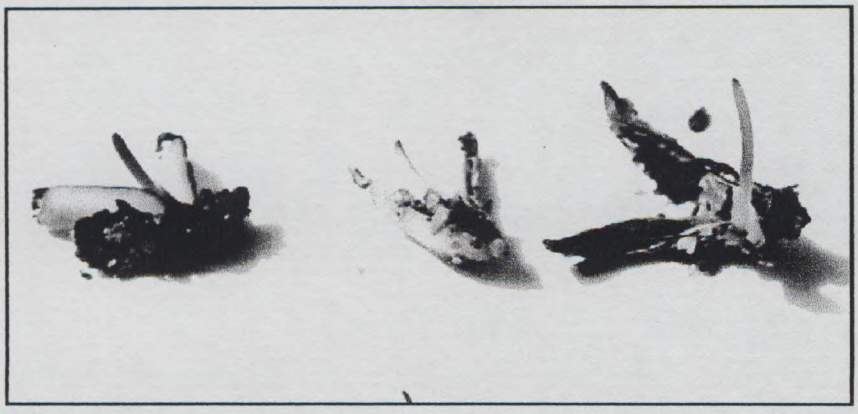

Figure 4.2: Twin scales of *G. villosa*

4.1.5 SCOOPING AND SCORING

For this study, the scooping and scoring propagation technique is regarded as successful if four or more new bulblets are produced from a single scooped or scored bulb. This technique is regarded as unsuccessful if the scooped or scored bulb dies or if three or less bulblets are produced from it. After seven weeks all the scored bulbs of *G. multifolia* showed signs of growth. See Figure 4.3. After 12 weeks all the *G. multifolia* scooped bulbs showed signs of growth. See Figure 4.5. Over the same period all the *G. villosa* bulbs were still alive with two out of the five scooped bulbs that showed signs of growth. See Figure 4.4. Growth is defined as bulbs increasing in size and the formation of roots with no definite bulblets forming. *G. multifolia* bulbs were successfully propagated using both these techniques with all bulbs producing five and more new bulblets. These techniques appeared to be unsuccessful for *G. villosa* with one scooped bulb producing four new bulblets. Bigger and fleshier bulbs were more responsive to this method of propagation and produced new bulblets first. Each scooped or scored bulb on average produced four to six new bulblets. See Table 4.2. Healthy, rooted bulblets were formed after 16 weeks. Due to the low number of repetitions in this experiment did this set of data not lean itself to statistical analysis. The result is very basic and statistical analysis would have made no sense at all.

Figure 4.3: Scoring of *G. multifolia* bulbs.

Figure 4.4: Scooping of *G. villosa* bulbs.

Figure 4.5: Scooping of *G. multifolia* bulbs.

4.1.6 BASAL PLATE CUTTINGS

For this study, the basal plate propagation technique is regarded as successful if one or two new bulblets are produced from a single basal plate cutting. This technique is regarded as unsuccessful if the basal plate dies or if no new bulblets are produced. No drying and callusing period was used for basal plate cuttings. After eight weeks no sign of growth was observed. After 10 weeks all basal plates were shriveled up. See Table

4.2. After 12 weeks all basal plate cuttings were dead.

Table 4.2: Rooting success on scooping (n=5), scoring (n=5), basal plate cuttings (n=20), and root cuttings (n=35) of *G. multifolia* and *G. villosa*.

Species	**Environment**	**Technique**	**Media**	**4-6 bulblets formed**
G. multifolia	plastic mini tunnel in greenhouse	scooping	1:1 river sand:perlite	5 (100%)
G. villosa	plastic mini tunnel in greenhouse	scooping	1:1 river sand:perlite	1 (20%)
G. multifolia	plastic mini tunnel in greenhouse	scoring	1:1 river sand:perlite	5 (100%)
G. villosa	plastic mini tunnel in greenhouse	scoring	1:1 river sand:perlite	0 (0%)
Species	**Environment**	**Technique**	**Media**	**1-2 bulblets formed**
G. multifolia	plastic mini tunnel in greenhouse	basal plate cuttings	vermiculite	0 (0%)
G. villosa	plastic mini tunnel in greenhouse	basal plate cuttings	vermiculite	0 (0%)
G. multifolia	plastic mini tunnel in greenhouse	root cuttings	2:1 sifted bark:river sand	0 (0%)
G. villosa	plastic mini tunnel in greenhouse	root cuttings	2:1 sifted bark:river sand	0 (0%)
G. multifolia	plastic mini tunnel in greenhouse	root cuttings	river sand	0 (0%)
G. villosa	plastic mini tunnel in greenhouse	root cuttings	river sand	0 (0%)

4.2 *IN VITRO* PROPAGATION

All the *in vitro* media were kept constant throughout all experiments and only the hormones were varied to determine the most productive media. Thus, only the hormone combinations will be mentioned for the following set of experiments.

4.2.1 STERILIZATION AND INITIATION OF *Gethyllis villosa*

No hormones were used in the first initiation medium. After five days, eight of the 20 explants showed signs of contamination. This gradually increased over the following number of weeks. Surviving explants were slow to show any form of growth; the remaining *G. villosa* explants took up to nine weeks before the first signs of growth were noticed. After ten weeks 16 of the explants were lost through contamination (Table 4.3). Explants increased in size and the formation of only one bulblet per explant took place. Growth in general was stunted and no roots were formed.

The second initiation medium used, consisted of NAA 0.2 $mg1^{-1}$ and BA 0.2 $mg1^{-1}$. After five days, six of the 20 explants were contaminated. After nine weeks 13 of the *G. villosa* explants were contaminated after which the remaining explants started producing new bulblets. Only 5 of the explants were still alive in this medium after 12 weeks (Table 4.3). In addition, explants were also very slow in the formation of new bulblets. Growth, however, was vigorous compared to the other two media and multiplication did take place. There is a statistically highly significant association between bulb formation and hormone media combinations ($\chi_4^2 = 13.515$, $P<0.01$), especially positive between the NAA: BA combination of ratio 0.2:0.2 $mg1^{-1}$ for this second medium and the two and three bulbs formed per explant.

The third initiation medium consisted of NAA 2 $mg1^{-1}$:BA 0.2 $mg1^{-1}$. After five days, eight out of the 20 explants were contaminated. Explants were gradually lost over the following weeks due to contamination. After 11 weeks, 4 of the *G. villosa* explants were still alive (Table 4.3). No bulblet or root formation took place in this medium. Explants remained alive, increased in size but were characterized by stunted growth.

Table 4.3: Response of *G. villosa* explants in three combinations of initiation media (NAA:BA, mgl^{-1}) (n=20 each) in a controlled growing room at 14-24°C and 5100 lux after 12 weeks. A mean of 15.6 explants were contaminated and survivors (*n*-contaminated) were monitored for bulblet formation or mere enlargement. Total explants or bulblets are those available for multiplication and rooting and include the parent twin scales where no bulblets were formed but enlargement took place.

		Number of bulblets formed				
Hormone ratios	***n*-contaminated**	**Enlargement only**	**1**	**2**	**3**	**Total bulblets**
0:0	4	0	4	0	0	4
0.2:0.2	5	0	0	2	3	13
2:0.2	4	4	0	0	0	4
Total bulblets		**4**	**4**	**4**	**9**	**21**

4.2.2 MULTIPLICATION AND ROOTING OF *G. villosa*

Only one of the 21 explants was lost through contamination during multiplication. After four weeks, explants increased in size and started producing new bulblets (Figure 4.6). In the medium (Table 4.4), eight explants produced one bulblet, seven explants produced three bulblets and two explants produced four bulblets. Simultaneously, explants started producing healthy roots of up to 50 mm long (Figure 4.7). Three explants increased in size but did not produce any bulblets or roots. In total, 37 bulblets with roots were produced and were used for the hardening-off experiment. After 16 weeks explants were ready to be transplanted (Table 4.4). It was noted that bigger twin scales seemed faster at producing new bulblets in this species.

Table 4.4: Bulblet formation of *G. villosa* explants (n=21) in one medium (NAA:BA, mgl^{-1}) in a controlled growing room at 14-24°C and 5100 lux after 16 weeks.

	Bulblets formed					
Hormone ratio	**0**	**1**	**2**	**3**	**4**	**Total bulblets**
0.2:0.2	3	8	0	7	2	**37**

Figure 4.6: Multiplication of *G. villosa* twin scales.

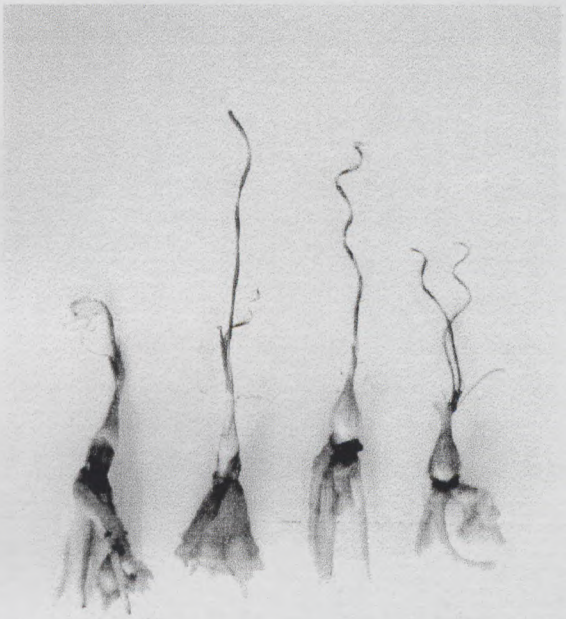

Figure 4.7: *G. villosa* explants ready to be hardened-off.

4.2.3 HARDENING-OFF OF *G. villosa*

The sifted bark: perlite: river sand medium was lighter, better aerated and well drained as compared to the river sand: peat medium (Hartmann *et al.*, 2002:364). Rotting was the reason for the low number of plantlets that survived in the river sand: peat medium (Table 4.5). In December the plantlets were planted out i.e. during the natural dormancy period (November to March) of these winter growing bulbs.

Plantlets were placed in a controlled greenhouse (with polycarbonate sheeting) on beds with intermittent irrigation intervals of 15 sec./20 min. and bottom heating at 21-23°C. In contrast with what was expected, the plantlets did not become dormant. Instead they continued growing until the following growth season i.e. from mid-March to mid-April. The sensitivity of *Gethyllis* species to moisture (Du Plessis & Duncan, 1989:105; Elvin, 2000) was yet again demonstrated in this experiment, even with a hardier species like *G. villosa*.

Table 4.5: Plantlet survival during hardening-off and various media for *G. villosa* in a controlled greenhouse at 24-28°C with 15 sec./20 min. misting irrigation after 4 weeks.

		Plantlets	
Growth media	**Growth media mixture**	**Introduced**	**Survived**
Sifted bark:perlite:river sand	1:1:1	19	16
River sand:peat moss	2:1	18	8

4.2.4 STERILIZATION AND INITIATION OF *Gethyllis multifolia*

After 12 weeks all *G. multifolia* explants (60 out of 220) were lost due to contamination in the media with the following hormone NAA ($mg1^{-1}$):BA ($mg1^{-1}$) combinations 0:0, 0.2:0.2 and 2.0:0.2 (Table 4.6). After 10 days 34 out of 160 explants were lost due to contamination across the other eight media combinations. After four weeks an additional 28 explants were lost after which contamination ceased. In the remaining eight media, the highest number of explants were contaminated in the media with the NAA 0.4 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ and NAA 1.0 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ hormone combinations (24 out of 40 explants) (Table 4.6).

Table 4.6: Of the 20 (*n*) *G. multifolia* explants in each initiation medium, a mean of 11.09 were contaminated. The remaining 98 explants in the various media (*n*-contaminated) of the hormones NAA:BA (mgl^{-1}), responded by either showing no growth, enlarging or producing only roots in a controlled growing room at 14-24°C and 5100 lux after 16 weeks.

		Explant response		
Hormone ratios	***n* - contaminated**	**No growth**	**Enlargement**	**Roots only**
0:0	0	-	-	-
0.2:0.2	0	-	-	-
0.4:0.2	8	0	5	3
0.4:0.4	12	1	11	0
0.8:0.2	11	1	8	2
0.8:0.4	14	4	10	0
1.0:0.2	8	0	7	1
1.0:0.4	16	1	13	2
1.5:0.2	12	0	11	1
1.5:0.4	17	3	11	3
2.0:0.2	0	-	-	-
Totals	**98**	**10**	**76**	**12**

The media with the least number of contaminations were the NAA 1.0 $mg1^{-1}$: BA 0.4 $mg1^{-1}$ and NAA 1.5 $mg1^{-1}$: BA 0.4 $mg1^{-1}$ hormone combinations (7 out of 40 explants). Although the media with the NAA 1.0 $mg1^{-1}$: BA 0.4 $mg1^{-1}$ and NAA 1.5 $mg1^{-1}$: BA 0.4 $mg1^{-1}$ hormone combinations had the lowest number of contaminated explants, proportionately the most vegetative growth occurred in the media with the NAA 1.0 $mg1^{-1}$: BA 0.2 $mg1^{-1}$, NAA 1.5 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ and NAA 0.4 $mg1^{-1}$: BA 0.4 $mg1^{-1}$ hormone combinations where explant enlargement and the formation of roots occurred (Table 4.6). The media with the NAA 1.0 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ and NAA 1.5 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ hormone combinations were selected for the multiplication experiments. Analysis of plant response data in Table 4.6 suggests a significant correlation between the hormone coefficient ratio (NAA/BA) and both plant response in terms of enlargement (r_s=0.894, n=8, P<0.01) and root formation (r_s=0.871, n=8, P<0.02). The inference from analysis is that a strong positive correlation exists between NAA:BA (mgl^{-1}) ratios 1.0:0.2 and 1.5:0.2 and plant vigour. No bulblet formation had taken place in any of the media, but the highest

number of explants forming roots (3 out of 8) took place in the medium with the NAA 0.4 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ hormone combination (Table 4.6). Henceforth, where explants showed no signs of growth or increase in size, it will be referred to as no growth, and where explants increased in size with no distinct bulblet formation, it will be referred to as explant enlargement. After 16 weeks all the explants (98) that were not contaminated were transferred from the initiation media to the multiplication media (Table 4.6).

4.2.5 MULTIPLICATION OF *G. multifolia*

The 98 uncontaminated explants from the initiation media were transferred to the multiplication media as selected from the analysis of results of Table 4.6. The first bulblet formation in both media occurred after nine weeks. Both media were in addition more or less similar in the number of new bulblets formed, though the medium with the NAA 1.5 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ hormone ratio, demonstrated a slightly higher productivity. Out of 49 explants 66 new bulblets were formed in the NAA 1.0 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ medium compared to the 72 bulblets out of 49 explants in the NAA 1.5 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ medium (Table 4.7 and Figure 4.8). No contamination occurred during this part of the experiment, but 34 explants did not form any new bulblets (Table 4.7). Following these results 138 explants were transferred to the rooting stage.

Table 4.7: Bulblet formation of *G. multifolia* explants and the two respective hormone combinations of NAA:BA (mgl^{-1}), (n=49 for each), in a controlled growing room at 14-24°C and 5100 lux after 16 weeks.

	Bulblets formed						
Hormone ratios	**0**	**1**	**2**	**3**	**4**	**5**	**Total new explants**
1.0:0.2	20	13	7	6	4	1	66
1.5:0.2	14	15	11	6	3	1	72
Totals		**28**	**36**	**36**	**28**	**10**	**138**

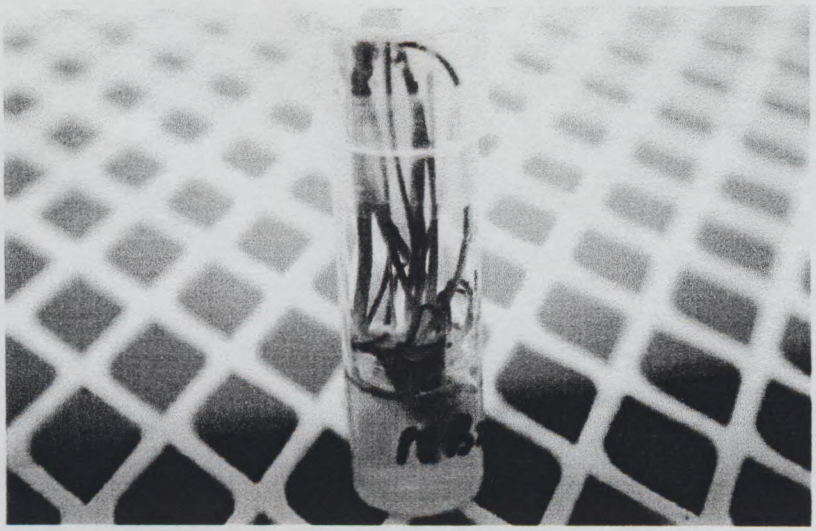

Figure 4.8: Multiplication of *G. multifolia* twin scales.

4.2.6 ROOTING OF *G. multifolia*

G. multifolia explants were divided amongst four different hormone combinations of NAA:BA for the rooting stage. Samples from each were exposed to two different lux settings (Environment A: 5100 lux and Environment B: 2500 lux), and resultant reactions compared (Table 4.8). Explants in the medium with the NAA 0.4 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ hormone combination exposed to 5100 lux, only started forming roots after six weeks. No rooting was observed amongst explants under the lower light intensity environment over the same period. Explants under the lower light intensity environment in the media with the NAA 0.5 $mg1^{-1}$: BA 0.0 $mg1^{-1}$ and NAA 0.4 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ hormone combinations only started forming roots after eight weeks. Delayed rooting (after 10 weeks) was observed in the media with the NAA 0.8 $mg1^{-1}$: BA 0.2 $mg1^{-1}$ and NAA 0.8 $mg1^{-1}$: BA 0.4 $mg1^{-1}$ hormone combinations under both environmental conditions. After 12 weeks almost all the explants in the medium with the NAA 0.5 $mg1^{-1}$: BA 0.0 $mg1^{-1}$ hormone combination under both environmental conditions, had formed roots i.e. 19 out of 23 explants under the higher light intensity environment and 14 out of 17 explants under the lower light intensity environment. After 15 weeks explants were ready to be transplanted. One hundred explants were transferred to the hardening-off stage.

Table 4.8: Rooting response of *G. multifolia* explants in two environments (A, lux 5100, n=73; B, lux 2500, n=65) and various rooting media combinations of NAA:BA (mgl^{-1}), in a controlled growing room at 14-24°C after 15 weeks.

Environment	Rooting medium ratio	Explants (n) per medium	Explants forming roots	Mean number of roots/explant	Mean maximum root length (mm)
A	0.4:0.2	21	16	9.1	19.9
A	0.5:0.0	23	19	6.3	14
A	0.8:0.2	15	8	8.6	18.6
A	0.8:0.4	14	11	5.5	10.9
B	0.4:0.2	17	13	11.5	14.1
B	0.5:0.0	17	14	10.5	12.1
B	0.8:0.2	15	7	9.6	7.7
B	0.8:0.4	16	12	5.5	14.8

There is no significant correlation between an increase or decrease in the NAA component of the media and the proportion of explants forming roots nor between the BA hormone component and the proportion of explants that form roots ($r_s = 0.25$, $n = 8$, $P > 0.05$ each). There is, however, a modest correlation between a low dosage of hormone NAA and high mean number of roots per explant ($r_s = 0.62$, $n = 8$, $P < 0.20$). Significance in variation of mean root diameters (as expression of root vigour) were not attempted as many of the root formations were tightly clustered and measurement was thus limited to those where each root could be measured without harming the explant. The sample does not lean itself to statistical analysis.

ANOVA was executed to investigate significant variation in mean maximum root length (mm) as expressions of vigour for various combinations of hormones NAA:BA under the two mentioned light intensities. Both dataset variances were heterogeneous and both were log transformed to achieve normality. The differences in Environment A between hormone combinations for mean maximum root length were not significant with unequal n ($F_{3,50} = 1.802$, $P = 0.158$) but highly significant for Environment B also with unequal n ($F_{3,41} = 3.139$, $P = 0.035$).

ANOVA between mean maximum root lengths (mm) for all hormone combinations within both Environments simultaneously, indicate highly significant differences with unequal n ($F_{7,91} = 2.224$, $P = 0.039$) once data has been log transformed to achieve normality. Based on the results from a Tukey test, it can be concluded that the mean

of Environment A, with rooting medium 0.5:0.0 is significantly different from the mean for Environment B with rooting medium 0.8:0.2 ($q_{0.05,91,8}$)

It would seem then, that the most vigorous rooting results could be achieved with a hormone combination where the NAA component is between 0.4 to 0.5 $mg1^{-1}$ under a lux of 5100.

4.2.7 HARDENING-OFF OF *G. multifolia*

After one week, plantlets in the controlled greenhouse with intermittent misting across all three media started rotting rapidly. As a result, after two weeks, all plantlets in all three media for this environment were lost. However, only 7 out 49 plantlets rotted across all three media in the 1000x 2000x 300 mm plastic mini tunnels under hand watering conditions. No further plantlets were lost after they were transferred to the 60% shade house. Survival was strongly correlated with the conditions created in the 2nd environment (r_s=0.92, n=6, P<0.05), (Table 4.9). All three media were properly drained and aerated.

Table 4.9: Hardening-off of *G. multifolia* in various media under different irrigation applications (Environment 1: controlled greenhouse with intermittent misting; Environment 2: plastic mini tunnels with hand watering) and resultant plantlet survival after 4 weeks.

Environment	n	Media	Media combinations	Plantlet survival
1	17	Bark:perlite:river sand	1:1:1	0
1	17	Bark:river sand	1:1	0
1	17	Perlite:river sand	1:1	0
2	17	Bark:perlite:river sand	1:1:1	15
2	16	Bark:river sand	1:1	14
2	16	Perlite:river sand	1:1	13

4.3 HYDRO CULTURE OF *G. multifolia* and *G. villosa*

4.3.1 SUB-IRRIGATION

All the hydro culture observation experiments was conducted over 18 months (15/4/2004 – 15/10/2005). The observation of the formation of leaves started from month12 - 18. The start of the new growing season was determined by the formation of new leaves of *G. multifolia* and *G. villosa* bulbs grown in soil under the same environmental conditions as the hydro culture observation experiments (used as a control). No rotting took place although the bulbs were irrigated throughout the growing and dormant seasons. In *G. villosa* bulbs, two of the eight in the leca pellet medium and one of the eight in the drainage chips medium formed new leaves after the first week of the new growing season. Over the same period no growth was noticed in *G. multifolia* bulbs. *G. multifolia* bulbs started forming leaves across both media after three weeks. After four weeks no significant difference in the formation of leaves were noticed amongst both species in all media. After seven weeks all the bulbs across both species and media formed new leaves. See Table 4.10. It was noticed in the natural habitat of both species, that under favourable environmental conditions, both species had healthy twirls and a compact overall shape. The same compact yet vigorous appearance of bulbs of both species was noticed in the leca pellet medium. This was not true for the drainage chips medium and the appearance of new leaves was slow. Weeds were not considered to be a problem in this system.

Table 4.10: Observations on leaf formation *G. multifolia* and *G. villosa* under sub-irrigation throughout the growing and dormant seasons in a greenhouse tunnel covered with 50% shade. Observations were done at the onset of the second growing season.

Sub-irrigation (n=32)			
G. multifolia		***G. villosa***	
Leca pellets (n=8)	**Drainage chips (n=8)**	**Leca pellets (n=8)**	**Drainage chips (n=8)**
Week 1 (13/04/2005)			
0	0	2	1
Week 2 (20/04/2005)			
0	0	3	2
Week 3 (27/04/2005)			
1	2	5	3
Week 4 (4/05/2005)			
3	5	6	4
Week 7 (25/05/2005)			
8	8	8	8

4.3.2 DRIP IRRIGATION

Compared to the sub-irrigation system, rotting took place in the drip irrigation system. See Table 4.11. The general appearance of plants in the perlite and bark media, were stunted and bulbs showed signs of yellowing. Six of the ten *G. villosa* bulbs formed new leaves after the first week in the bark medium. *G. multifolia* bulbs were the slowest to react with four of the ten bulbs across both media forming leaves after four weeks. After seven weeks eight of the ten *G. multifolia* bulbs in the perlite medium and the bark medium formed new leaves. Over the same period eight of the ten *G. villosa* bulbs formed new leaves in the bark medium and six in the perlite medium. No further formation of leaves occurred across both species and both media. Bulb decay occurred across both species and both media with the highest percentage of bulb decay (40%) in *G. villosa* bulbs in the perlite medium.

G. multifolia appeared slower to react to the environmental stimuli compared to *G. villosa*. Weed growth appeared to be more of a problem in media in the drip irrigation system. Blockage of drippers was a regular problem in this system. It must be noted that bulbs appeared healthy and vigorous in the bark medium during the first

growing season but failed (bulb decay) at the onset of the new growing season.

Table 4.11: Observations on leaf formation of *G. multifolia* and *G. villosa* under drip irrigation throughout the growing and dormant seasons in a greenhouse tunnel covered with 50% shade net. Observations were done at the onset of the second growing season.

Drip irrigation (n=40)			
G. multifolia		***G. villosa***	
Bark (n=10)	**Perlite (n=10)**	**Bark (n=10)**	**Perlite (n=10)**
Week 1 (13/04/2005)			
1	0	6	2
Week 2 (20/04/2005)			
1	1	7	3
Week 3 (27/04/2005)			
2	1	8	5
Week 4 (4/05/2005)			
4	4	8	6
Week 7 (25/05/2005)			
8	8	8	6

4.3.3 DELAYED IRRIGATION

No irrigation was applied for seven weeks after the first winter rains (onset of the new growing season). This experiment was done to determine whether temperature or moisture is the stimulus for growth amongst *Gethyllis* species. The highest number of bulbs forming new leaves (three of the sixteen) was noticed on *G. villosa* bulbs in the drip irrigation system after the first week of the new growing season. *G. multifolia* bulbs responded slower with one bulb of sixteen forming leaves after four weeks across all media and both systems.

No rotting amongst both species, both systems and all media was observed. Bulbs were slower in the formation of new leaves due to the lack of moisture compared to the drip and sub-irrigation systems. The delay in growth compared to bulbs under irrigation was more or less three weeks. The new leaves that were produced were stunted compared to other bulbs under irrigation. Even though bulbs under the

delayed irrigation observation experiment appeared stressed and stunted, this was gradually corrected as bulbs were irrigated. At the end of the growing season, plants under the delayed irrigation as well as continuous irrigation observation experiments appeared more or less the same size. All bulbs across all media and both systems did form new leaves even though moisture was withheld. See Table 4.12.

Table 4.12: Observations on leaf formation of *G. multifolia* and *G. villosa* with delayed irrigation at the onset of the new growing season in a greenhouse tunnel covered with 50% shade net. Observations were done at the onset of the second growing season.

***G. multifolia* (n=16)**				***G. villosa* (n=16)**			
Bark (n=4)	**Perlite (n=4)**	**Leca pellets (n=4)**	**Drainage chips (n=4)**	**Bark (n=4)**	**Perlite (n=4)**	**Leca pellets (n=4)**	**Drainage chips (n=4)**
Week 1							
0	0	0	0	1	2	0	0
Week 2							
0	0	0	0	1	2	0	0
Week 3							
0	0	0	0	1	3	1	1
Week 4							
0	1	0	0	2	3	2	1
Week 7							
4	4	4	4	4	4	4	4

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

Leaf, root and basal plate cuttings

Veltheimia and *Lachenalia* species are successfully propagated by means of leaf cuttings (Lennox, 1990:100). *Aralia* and *Viburnum* species are successfully propagated using the root cuttings technique (Hartman *et al*, 2002:344)). Most *Gethyllis* species have fleshy basal plates that regenerate new bulblets after mole or rat predation (Du Plessis & Duncan, 1989:104). For this reason basal plate cuttings were experimented on as an attempt to strengthen this statement. *G. multifolia* and *G. villosa* could not be propagated by means of leaf, root and basal plate cuttings using the media and techniques applied in this study. Bulbs of both species are very small especially those of *G. villosa*, which resulted in small basal plate cuttings. Basal plates showed signs of moisture loss shortly after cuttings were made. This resulted in drying out and death of cuttings. This technique would probably be more successful using *in vitro* propagation techniques. Leaves of both species are very thin and this propagation technique of leaf cuttings could be further researched in *in vitro* propagation.

Bulb cuttings and twin scaling

The small bulb sizes of both species necessitated the use of small bulb cuttings and twin scales. The consequence of this was drying out of bulb cuttings and twin scales and eventually death of all the material during the two-week drying and callusing period. Thus, it can be concluded that a callusing and drying period prior to planting of the cuttings, as performed in this study, should be avoided. Asexual propagation by means of bulb cuttings and twin scaling was slow but proved to be successful with *G. multifolia* bulbs but less effective with *G. villosa* bulbs. *G. multifolia* bulb cuttings in the 2:1 river sand: peat moss medium were successful with 8 out of 10 bulb cuttings forming 3 to 4 new bulblets after 16 weeks compared to *G. villosa* bulb cuttings with 2 out of 10 bulb cuttings each forming three new bulblets. Statistically, however, there is no significant departure from homogeneity between media and resultant number of bulblets formed for *G. multifolia* and *G. villosa*. Twin scales of *G. multifolia* were also successfully propagated in the vermiculite medium in self-seal bags with 13 out 15 twin scales each forming one new bulblet after 12 weeks. This was however, not the case with *G. villosa*

twin scales, where 4 out of 15 twin scales each produced one new bulblet after 12 weeks. See Table 4.1. Also in this case there is statistically no significant departure from homogeneity between media and resultant number of bulblets formed for *G. multifolia* and *G. villosa*. For some unknown reasons only bulblets formed from bulb cuttings and twin scales of both species continued to grow during the dormant period, while bulblets from scooping, scoring and seedling bulbs went into dormancy at the onset of the dormant period. This behaviour of twin scales was also noticed in *in vitro* culture where the environmental temperature and illumination were kept constant throughout the growing and dormant periods.

Scooping and scoring

G. multifolia have slightly larger and fleshier bulbs compared to *G. villosa*. The fleshier and larger bulbs were more responsive to these methods of propagation whereby these fleshier bulbs produced new bulblets faster and of a bigger size. This could be the reason why only *G. multifolia* bulbs produced new bulblets using scooping and scoring propagation techniques. All *G. multifolia* bulbs produced an average of four new bulblets per bulb using scooping and scoring propagation techniques in a 1:1 river sand: perlite medium after 16 weeks See Table 4.2. These propagation methods were unsuccessful for *G. villosa* with 1 out of 10 bulbs producing four new bulblets in the same medium after 16 weeks. See Table 4.2. Scooping and scoring could be recommended as a propagation method for increasing *G. multifolia* bulbs. Though not rapidly, this method proved to be effective. Due to the low number of repetitions in this experiment did this set of data not lean itself to statistical analysis.

Environmental factors and general cultivation

The soil analysis report (see Table 2.3) for *G. multifolia* and *G. villosa*, taken at the root zone of the bulbs, revealed no factors that could cause toxicity or deficiency and eventually challenge the survival of the two species from the various populations. According to the temperature and rainfall charts (see Tables 2.1 and 2.2) as well as the natural habitat observations, it is evident that there were climatic changes over the four-year period. Climatic changes especially drought have a definite negative effect on the production of leaves (16/03/2002), flowers (29/11/2003) and berries (not observed) of the two species. See Table 2.1. This behaviour of the two species was also evident in

the hydro culture experiment where irrigation at the onset of the new growing season was withheld. Under very dry conditions, flowers and berries are few or of inferior quality, sometimes none amongst weaker plants, and the production of new leaves at the onset of the growing season are delayed and less turgid. This reaction was found to be more intense amongst the *G. multifolia* species. Over the four-year period of observations, no fires were noticed in any of the five populations. Thus, the reaction of *G. multifolia* and *G. villosa* to veld fires could not be recorded.

This research found no real threat in terms of insects and diseases to the natural populations as well as bulbs under cultivation. The only pests found, were caterpillars eating leaves and flower tepals and snails eating bulb seedlings of both species under cultivation, but these can be easily controlled. *Gethyllis* species can be successfully cultivated if irrigation is withhold during the dormant phase and is controlled (once a week by hand) during the growing phase. This can be achieved by copying the natural environment successfully, understanding the life cycle of these bulbs and applying or withholding moisture at the right time. This study confirms the statement made by Snijman (2004), that because of their sensitivity to irrigation, would *G. multifolia* and *G. villosa* bulbs not be regarded as ideal garden specimens.

In vitro propagation

For future *in-vitro* propagation, environments for *G. villosa* and *G. multifolia* throughout all stages should be as summarized in Table 5.1. These results by no means exclude the potential for further investigation into optimum hormone ratios in combination with varying lux for maximum vigour and explant survival.

Table 5.1: Best environment as concluded from experimental data for the *in vitro* propagation of *Gethyllis villosa* and *G. multifolia*. Initiation, multiplication and rooting should take place under 5100 lux.

Species	Initiation	Multiplication	Rooting	Hardening-off		
	Hormone ratios (NAA $mg1^{-1}$: BA $mg1^{-1}$)			Medium	Medium ratios	Watering environment
G. villosa	0.2:0.2	0.2:0.2	0.2:0.2	Sifted bark:perlite:river sand	1:1:1	Controlled greenhouse, intermittent irrigation (15 sec./20 min.)
G. multifolia	1.0:0.2 1.5:0.2	1.0:0.2 1.5:0.2	NAA ≤ 0.5 NAA ≥ 0.4	Sifted bark:perlite:river sand	1:1:1	Mini tunnels, hand watering, 60% shade netting
				Bark:river sand	1:1	
				Perlite:river sand	1:1	

Hydro culture and drought stress

Statements by Du Plessis & Duncan (1989:105) and Elvin (2000) revealed that *Gethyllis* species are sensitive to over watering during the dormant season (in soil cultivation). As no results were ever published on the hydro culture of *Gethyllis* plant species, this study proved that not only were bulbs grown successfully by using this method, but also survived irrigation throughout the dormant season. To prevent possible rotting, irrigation throughout the dormant season, however, is not recommended and should be commenced after the first winter rains at the onset of the new growing season. In this study, the leca pellet medium using a sub-irrigation system has proven to be the best medium and system for hydro culture. Even though it was not measured or proven, the reason for poor flowering could be ascribed to the natural poor responsiveness of *G. multifolia*, maturity of bulbs, delayed irrigation, irrigating throughout the growing season, transplanting shock or the use of municipal water. The reason for no fruit formation could be ascribed to the same above-mentioned factors as well as the fact that not enough flowers were produced to allow cross-pollination. The use of distilled or rainwater can be incorporated into this system as a future possibility to try and improve on existing results. It is also recommended that mature bulbs be used (which are set to produce flowers) and that large quantities of bulbs are grown together and in open structures to ensure cross-pollination. According to Du Plessis & Duncan (1989:105) and Elvin (2000) bulbs growing in large quantities and in close proximity yield more berries.

The drought stress (delayed irrigation) experiment in hydro culture revealed that if irrigation is withheld from *G. multifolia* and *G. villosa* bulbs at the onset of the new growing season, the bulbs are approximately two to three weeks slower in forming new leaves, compared to bulbs receiving irrigation. The new leaves were stunted compared to other bulbs subjected to irrigation. Even though bulbs under the delayed irrigation observation experiment appeared stressed and stunted, this was gradually corrected as bulbs were irrigated. Therefore, it appears that the initiation of new leaves at the onset of the new growing season of *Gethyllis* species, is not dependant on moisture and could be initiated by atmospheric pressure. This study revealed that moisture speeds up the growing process and improves the quality and number of leaves, fruit and flowers. Because *G. multifolia* bulbs under this observation experiment formed new leaves

approximately three weeks after *G. villosa*, it could be concluded that *G. multifolia* bulbs comparatively, suffers more under drought stress. This phenomenon was however only observed over a period of 18 month in hydro culture.

During the 2005 growing phase of *Gethyllis* species, bulldozers destroyed the Orchard population of *G. multifolia*, and the site is now being used for urban expansion. The Karoo Desert National Botanical Garden staff was not informed about this project and was unable to rescue this *G. multifolia* population. More *G. multifolia* clumps and single bulbs were found close to the area currently under construction, but the population size was small and consisted of 30 clumps and single bulbs. These bulbs are surrounded by a residential area and are definitely under threat of being destroyed sooner or later. This study has also revealed that especially in this population, grazing animals are eating the leaves, flowers and berries of these bulbs. According to the interviews conducted, it can be concluded that the younger generation are unaware or has lost interest in the collection of the berries of *Gethyllis* species in this area. Even though it was not a great threat, it will benefit the conservation of *Gethyllis* species.

The De Dooms *G. multifolia* population size was also found to be smaller than 100 clumps and single bulbs and is unfortunately growing in areas that are currently under threat of either being developed for housing or used for agricultural extension in years to come. This can lead to complete removal of this species from this area. According to Louw (1996:150), a population with fewer than 100 individuals is regarded as too small for long-term survival. Therefore, a concerted effort must be made to conserve the remainder of the *G. multifolia* bulbs in these areas.

Apart from one single bulb being removed, the *G. villosa* population sizes remained constant over the study period. It was also observed that *G. villosa* bulbs propagated itself through division of bulblets as well as self-seeding. Compared to *G. multifolia* bulbs, the *G. villosa* bulbs were not growing close to residential areas and population sizes were in excess of 300 clumps and single bulbs. Thus, the *G. villosa* species is not regarded as vulnerable or threatened in any way in this area.

This study was conducted as an attempt to conserve the *G. multifolia* species and find the reasons behind its vulnerable status. It was observed throughout the study period that traditional healers, bulb collectors, environmental factors such as pollinators, predators, soil and weather conditions have not had a serious effect on the decline in numbers of *G. multifolia*. It was observed through this study that grazing domestic livestock, urban expansion (this includes agricultural extension) and in some cases the lack of interest shown in our indigenous plant species, are some of the main factors influencing the decline in numbers of this species. Facts to back these statements are mentioned earlier in this study.

This study revealed that the vulnerable status of *G. multifolia* according to The Red Data List of Southern African Plants (Hilton-Taylor, 1996:9), should be changed to "Endangered" and that more emphasis should be placed on the conservation of our indigenous flora. The availability of plant material for conducting these experiments was limited, which resulted in a limited number of repetitions per experiment. With limited resources it was managed to conduct as many different experiments as possible to test the reaction of these two species. This was important, since the literature study revealed that very little research has been done on the genus *Gethyllis* and therefore this study is a good platform for new research.

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