and branch lesions (Dianese, Ribeiro & Moraos 1985) of *Eucalyptus*. In South Africa, this fungus seems to be well established, causing post harvest fruit rot of avocado (Darvas & Kotze 1987) and mango (Darvas 1991), as well as die-back of indigenous *Protea* spp. (Benic & Knox-Davies 1983, Serfontein & Knox-Davies 1990).

Colletotrichum gloeosporioides preferentially infects young succulent tissue (Dodd *et al* 1991), which is consistent with field observations on young *Eucalyptus* shoots in South Africa. It has been shown to be present as quiescent infections in avocado and mango (Prusky & Plumbley 1992) and also to occur in asymptomatic leaves and twigs of Citrus and Rhododendron (Von Arx 1957) and leaves of *Eucalyptus nitens* (Deane et Maid.) Maid. and *E. grandis* (Smith, Wingfield & Petrini 1996). Such latent infections could give rise to the die-back of stressed shoots observed in this study. Although the impact of *C. gloeosporioides* on the *Eucalyptus* industry in South Africa seems to be relatively insignificant at present, we consider this fungus to be a pathogen worth noting in disease surveys.

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In vitro propagation of some *Cyrtanthus* species

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Shoots and roots were initiated on bulb explants of *Cyrtanthus brachyscyphus, C. elatus, C. falcatus, C. guthrieae*, and *C. mackenii* var. *mackenii*. *C. breviflorus* produced small amounts of wound callus only. The species differed in their response to the different levels of plant growth regulators used. In general shoot formation was most favourable with high concentrations BA (2 mgl^{-1}) and lower concentrations NAA (1 mgl^{-1}). Best root formation was obtained with low BA and NAA (0–0.5 mgl^{-1}) concentrations. *Cyrtanthus brachyscyphus* was the most prolific shoot producer, with a 3-fold increase at every sub-culture. *C. elatus, C. guthrieae*, and *C. mackenii* var. *mackenii* were less vigorous and on average showed a 1.5-fold increase at every sub-culture. *C. falcatus* produced a low number of shoots from the explants and this did not increase with subsequent sub-cultures. Rooted plantlets were successfully acclimatized in vermiculite in a mist house (100% survival).

Keywords: Bulb explants, *Cyrtanthus*, *in vitro* propagation, shoot and root formation.

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Cyrtanthus L.f. is a member of the family Amaryllidaceae and is mainly a southern Africa genus (Olivier 1980; Du Plessis & Duncan 1989). There are fifty-one species in southern Africa (Dyer 1976; Du Plessis & Duncan 1989). This bulbous herb may be evergreen, winter- or summer growing. The leaves differ significantly among the species, from slender to strap-shaped. The flowers are single to many and umbellate, tubular and pendulous to widely bell shaped. The colour of the flowers ranges from white and cream to shades of pink, red, orange, and dark maroon (Figure 1 A–C). The seeds are black, flattened and somewhat winged.

The six species studied in this paper are described (Duncan 1990a, 1990b; Du Plessis & Duncan 1989) as follows: *Cyrtanthus guthrieae* L. Bol. is endemic to Bredasdorp. This plant is deciduous and mainly winter-growing. Flowering occurs in March to April. The plant is 10–12 cm in height and the large

flowers are bright red (Figure 1A). *Cyrtanthus brachyscyphus* Bak. occurs in the eastern Cape and KwaZulu-Natal. This plant is evergreen and flowers in September to March. Plants are 25–35 cm in height and the flowers are bright orange (Figure 1B). *Cyrtanthus breviflorus* Harv. is found along the eastern Seaboard. It is deciduous or evergreen and mainly summer-growing (September to December). The plant is 25–45 cm tall and the flowers are bright yellow (Figure 1C). *Cyrtanthus elatus* (Jacq.) Traub is distributed in the southern and eastern Cape. It is evergreen and flowers in October to March. Plants are 25–45 cm in length and the large flowers are scarlet or pink. *Cyrtanthus falcatus* R.A. Dyer occurs in KwaZulu-Natal and is deciduous and summer-growing. Flowering is in September to October. The plants are 25–35 cm tall and the pendulous flowers are red and green. *Cyrtanthus mackenii* Hook.f. var. *mackenii* is found in the eastern Cape and KwaZulu-Natal. It is evergreen and flowering occurs in July to February. The plants are 30–45 cm tall and the flowers are white. Kukulczanka and Kromer (1988) reported on the twin scale propagation of *Vallota purpurea* (*Cyrtanthus elatus*). The objective of this study was to develop a micropropagation system to facilitate the increase in plant numbers for horticultural and conservation purposes.

Plant material was obtained from the National Botanical Institute, Kirstenbosch and the garden of the Botany Department, University of Natal Pietermaritzburg. The outer dead or damaged bulb scales and the roots were removed prior to washing and sterilization. The bulbs and leaves were then washed in running tap water. The bulbs were separated into individual scales and together with the leaves immersed in 70% ethanol containing two drops of Tween 20 for 2 min. This was followed by a 5-min soaking in 1% Benlate solution (containing Tween 20) and subsequent soaking in 0.1% mercuric chloride (containing Tween 20) for 10 min. The leaves and bulb scales were then rinsed 3 times in sterile distilled water, cut into 1 cm^2 explants and placed into prepared culture tubes. Twenty five replicates per treatment were used. This experiment was repeated twice.

The culture medium consisted of Murashige and Skoog medium (1962) supplemented with 3% sucrose, and 100 mg l^{-1} myo-inositol. Various concentrations of α-naphthaleneacetic acid (NAA) at 0, 0.2, 0.5, 1 and 2 mg l^{-1} in combination with 6-benzylaminopurine (BA) at 0, 0.2, 0.5, 1 and 2 mg l^{-1} were added to the medium. Medium pH was adjusted to 5.8 prior to autoclaving and the medium solidified with 2 gl^{-1} Gelrite. The leaf and bulb explants were placed into tubes containing 10 ml medium and sealed with parafilm. Cultures were maintained in a growth room with a 16 h light and 8 h dark cycle at 25°C under cool fluorescent light at 35 μmol quanta $m^{-2}s^{-1}$. The number of shoots and roots produced per treatment and the number of shoots and roots per explant were recorded on a monthly basis. Rooted plantlets were acclimatized by exposing opened tubes to constant incandescent light for 24 h. The plantlets were planted in moist vermiculite and kept in a mist house with bottom heat of 30°C for three weeks. They were then transferred to pots containing 1:1 potting soil:sand and grown under normal greenhouse conditions.

Sterilization was a major problem with the plant material but the procedure mentioned above resulted in 70–80% decontamination, with the exception of *C. breviflorus* where only 20% decontamination was achieved. Leaves and bulb scales were also soaked in 1% Benlate for 5 min and subsequently in 3.5% NaOCl for 10 min, but this was unsuccessful and resulted in a high percentage of contamination. Although leaf and bulb material was initially used, the leaf material did not yield any positive results while the bulb material resulted in shoot and root formation (Figure 1D). Bulb scales were therefore, used in all further experiments.

The species differed in their reaction to the different hormone concentrations used (Table 1). The results were taken after eight weeks in culture. Optimal multiple shoot formation of *C. brachyscyphus* occurred on 0.5 mgl^{-1} BA and 0.2 mgl^{-1} NAA (Figure 1E). There was a decrease in shoot production with increased concentrations of BA. Root formation was best on low NAA (0.2 mgl^{-1}) with no BA. *C. breviflorus* showed no shoot or root formation on any of the hormone combinations used. There was however, a small amount of wound callus produced on the explants. *C. elatus* formed shoots on high and low BA (0.2 and 2 mgl^{-1}) with low or no NAA. Optimal root formation was observed on a combination of low NAA (0.2 mgl^{-1}) with no BA. *C. falcatus* formed shoots on 2 mgl^{-1} BA with 1 mgl^{-1} NAA but no root growth occurred on any hormone combination. *C. guthrieae* gave shoots on a high and low BA:NAA, but root formation was best on low BA and NAA (0.5 mgl^{-1}: 0.2 mgl^{-1}). *C.*

Table 1 Shoot (S) and root (R) formation (%) of six Cyrtanthus species on different BA and NAA combinations after 8 weeks in culture

	BA:NAA combinations (mgl^{-1})										
Species	0:0	0:0.2	0:0.5	0:1	0.5:0	0.5:0.2	0.5:0.5	0.5:1	1:0.2	1:0.5	2:1
C. brachyscyphus	-	83 S	91 S	-	-	96 S	-	-	95 S	-	88 S
		43 R	9 R			9 R			8 R		20 R
C. breviflorus	-	-	-	-	-	-	-	-	-	-	-
C. elatus	-	80 S	-	-	95 S	86 S	83 S	-	-	-	98 S
		60 R			35 R	20 R	13 R				40 R
C. falcatus	-	-	-	-	10 S	-	20 S	-	-	-	70 S
					-		-				-
C. guthrieae	-	-	85 S	-	-	78 S	75 S	-	80 S	-	95 S
			20 R			63 R	15 R		38 R		23 R
C. mackenii	-	-	90 S	65 S	-	-	-	94 S	-	76 S	90 S
			8 R	35 R				4 R		10 R	8 R

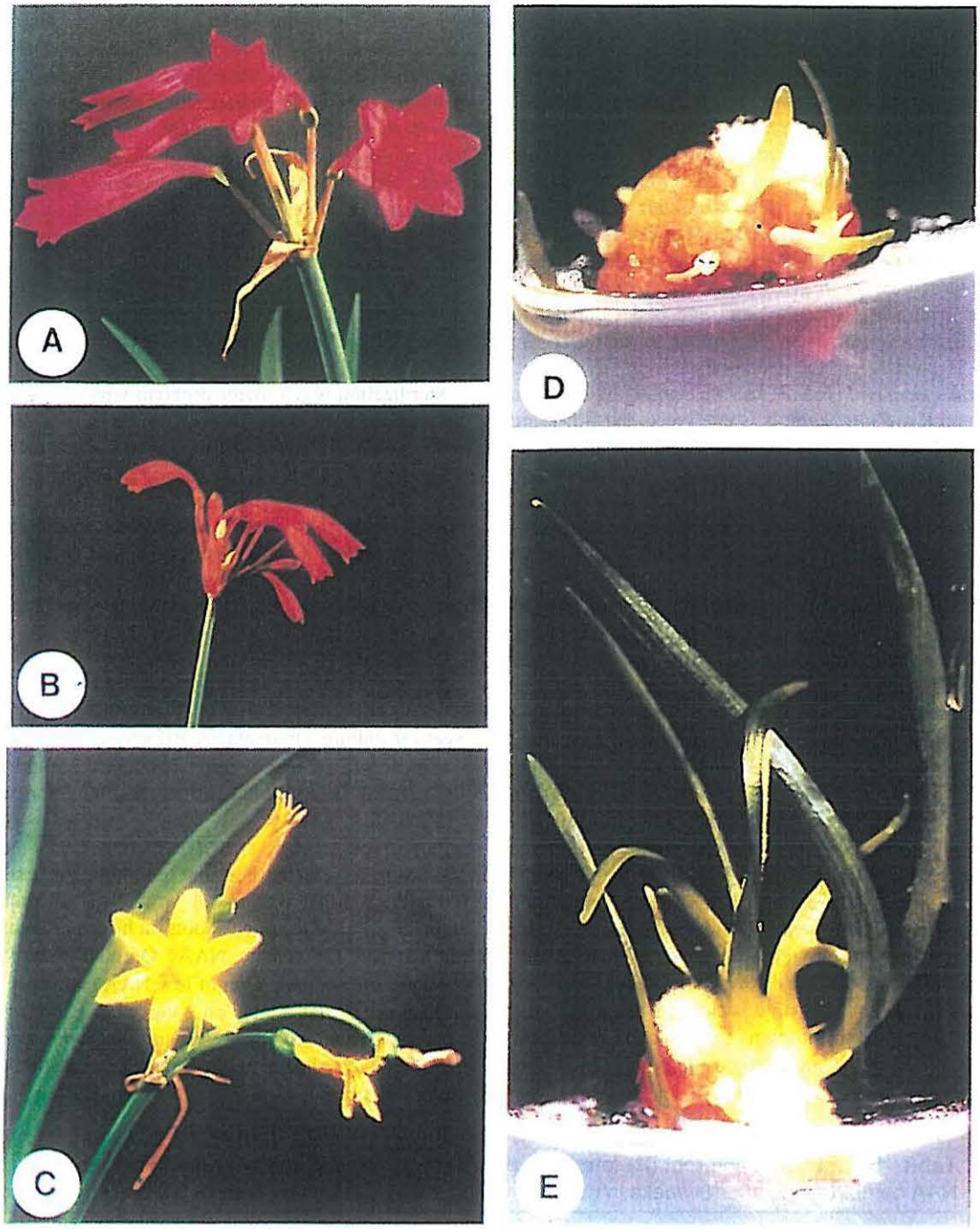

Figure 1 **A–C.** The variety in colour and chape of the different *Cyrtanthus* species. **(A)** *C. guthrieae,* **(B)** *C. brachyscyphus,* **(C)** *C. brevi-florus.* **(D)** The production of shoots by bulb explants. **(E)** Multiple shoots which formed on an explant of *C. brachyscyphus.*

mackenii yielded shoots on high and low BA:NAA concentrations. Root formation occurred on 1 mgl^{-1} NAA with no BA.

C. brachyscyphus multiplied at a faster rate (3-fold increase) than *C. guthrieae, C. elatus* and *C. mackenii* (on average 1.5-fold increase). *C. falcatus* did not show shoot multiplication after the original production of shoots from the explants.

The plants were bulked up on the optimal shooting media and were then rooted on the optimal rooting medium for each species. These plantlets were then acclimatized. There was a 100% survival of all the plantlets of all the species that were acclimatized. *C. brachyscyphus* flowered after a year, in the first growing season. The other species have not yet flowered. There is no reason why they should not do so in future. A micropropagational system has been developed for five of the six species studied. This system can be used for the conservation of the species and for horticultural purposes. The number of plants produced by the *in vitro* system is higher and far faster than the production of plants by conventional methods of propagation. These plants have a high horticultural potential as they make good garden and pot plants. (Duncan 1990a, b).

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