Effects of an organic seaweed growth regulator to enhance root growth parameters in pot-cultured *Erica verticillata* Bergius (*Ericaceae*) in developing a production schedule to support future reintroductions into the wild

T.D. Adams[1], C.P. Laubscher[2], F. Nchu[2] and C.M. Wilmot[2,a]

[1]Cape Research Centre, South African National Parks, PO Box 216, Steenberg, 7947, South Africa; [2]Faculty of Applied Sciences, Cape Peninsula University of Technology, PO Box 1906, Bellville, 7535, South Africa.

Abstract

Erica verticillata **Bergius (*Ericacea*) is a South African indigenous species which is classified as extinct in the wild. Current cultivation practices have focused on reintroducing the coastal fynbos shrub into its original habitat. The use of organic seaweed growth regulators on indigenous fynbos species is unknown. The 18-week study tested two different formulations of a commercial organic seaweed growth regulator at varied dosages on root growth parameters of potted *Erica* plants. Ten replicates of plant growth regulator treatments were used. Treatments of 0 (control), 50, 100, 200, 300 mL liquid stock solution and 1/4, 1/2, 1 and 2 solid disks plant^{-1} were applied to rooted cuttings of ±3 cm in height. The study aimed to develop a production schedule for growing pot-cultured *E. verticillata.* Plants treated with the highest liquid treatment of 300 mL, 1/4 disk and the control treatment induced the highest wet weights. Liquid treatments of 50, 100, 200 mL and 1/4, 1 and 2 disks were all similar in increasing dry root weight compared to the control (0). An application of (100-200 mL) liquid and 1/2 solid disk treatments were most significant in the postharvest root lengths compared to the other treatments at 18 weeks postharvest. Liquid treatments had better results on the root length than did disks. It is recommended that an application of 100-200 mL liquid of organic seaweed growth regulator per plant be used in growing pot-grown *E. verticillata.* This might enhance cultivation protocols of other indigenous fynbos species with similar growing requirements suitable for container growing. The production of *E. verticillata* has the potential to support the effective re-establishment and conservation in the wild.**

Keywords: extinct species, organic seaweed, plant growth regulator, indigenous fynbos, potted plant

INTRODUCTION

Erica verticillata Bergius (*Ericacea*) commonly known as the Cape Flats Erica is a narrow-leaved endemic species formerly restricted to the southern areas of the Cape Flats Sand Fynbos (CFSF) vegetation of the Cape Peninsula (Gibbs 2014). *E. verticillata* is a very tough growing, disease resistant perennial with an average height of 1.5 to 2 m (Hitchcock et al., 2013b). It flowers intermittently throughout the year with peak flowering in mid-summer showcasing its striking pink tubular flowers (Hitchcock, 2001a). *E. verticillata* is a suitable species for containers and indigenous coastal gardens, and it attracts a variety of bees, beetles, moths and birds (Hitchcock, 2001a).

In the past, *E. verticillata* was among the most sought after species in the wildflower picking industry. It became extinct in the wild by the second half of the 20th century and has been listed as “Extinct in the Wild” (EW) on the IUCN Red List. Its habitat on the Cape Flats has been placed under huge pressures from urban expansion, small scale farming, wet land

[a]E-mail: wilmot@telkomsa.net

draining and alien vegetation (Gibbs, 2014). Fortunately, it was re-discovered in Protea Park Botanical Garden, Pretoria in 1984 and is conserved by the Royal Botanic Gardens, Kew (Hitchcock et al., 2013b). As a result, efforts to conserve this species are escalating. More recently *E. verticillata* cultivation has led to the species being re-introduced in the CFSF reserves of Kenilworth Racecourse Conservation Area, Rondevlei Nature Reserve and the lower Tokai Park (Da Bush, 2010). Seeds from Rondevlei have however not been viable to regenerate the species (Hitchcock, 2001a). It is unclear why there is a lack of seed viability. It is suggested that this red listed species will remain extinct in the wild until its survival of three burn cycles without replanting is secured (Hitchcock, 2001a), which is obviously hard to achieve. Hence, the future survival of *E. verticillata* will depend on ex situ conservation and commercialised cultivation (Winter and Botha, 1994).

Conservation of red listed species can be achieved through creating awareness of species, developing efficient propagation and cultivation protocols, and re-introduction into the wild. Many efforts have been made to find effective methods for the protection of red listed species (Khoshbakht and Hammer, 2007) however; this will be better achieved with the understanding of an individual species reproductive system and growth pattern. Little has been documented on the cultivation aspects of *E. verticillata*. Cultivation aspects would include growing requirements, i.e., environmental, soil and nutritional requirements and adaptability to container production. Furthermore, not much research has been conducted on the use of organic seaweed growth regulators on Fynbos plants. Kirstenbosch National Botanical Garden (KNBG) has propagated and grown *Erica* species for many years; however, the practise of regular feeding using organic seaweed growth regulators on species such as *E. verticillata* has never been recorded. The aim of this study was to determine an effective dosage of organic seaweed growth regulator for root growth in pot-cultured *E. verticillata* and to develop a production schedule of the species for future cultivation success. We therefore, tested two different formulations of a commercial organic seaweed growth regulator at varied dosages on root growth parameters of potted *Erica* plants.

MATERIALS AND METHODS

Experiment layout

The experiment was conducted at the Disa House of the Living Collections Nursery of KNBG, Newlands, Cape Town, longitude 18.43209E and latitude 33.99039S. The greenhouse was covered with opaque polycarbonate sheeting where average temperatures between 13 and 23°C were recorded. The brick constructed growing benches were covered with 13 mm drainage chip and used as benching for the experiment.

Plant material

Cutting material of *E. verticillata* was collected from KNBG in the early morning and transferred to the propagation house. Small vegetative heel cuttings with tips (30 mm) were made. Cuttings were dipped in Seradix® 2 rooting hormone powder and rooted in a propagation medium consisting of one part fine milled pine bark and one part polystyrene. These were placed in compartmental plug trays. Cuttings were rooted under bottom heat conditions at 25°C and irrigated with misting in an environmentally controlled greenhouse. Cuttings took between three to four weeks to root. Rooted cuttings were moved out of the greenhouse and hardened off in a shade house (50% shading) for two weeks. The cuttings were then transplanted to 15-cm square plastic pots which contained a well-drained nutrient poor fynbos soil mix developed by KNBG. The fynbos soil mix (8:1:2:1) consisted of eight parts, 6 to 12 mm fine milled pine bark, one part milfeed wet Consol® glass sand, two parts "Malmesbury Grof" sand and one part agricultural lime.

Organic growth regulator treatments

The experiment consisted of the control, and the organic growth regulator liquid Kelpak® and Kelpak® Plantit® disks manufactured and supplied by Kelp Products (Pty) Ltd., Simonstown, Cape Town, South Africa. Kelpak growth regulators contain 0-0-0.5% NPK while these bioregulators contain 11 mg L^{-1} auxins and 0.03 mg L^{-1} cytokinins (Kelpak®, 2016). The experimental layout consisted of ten replicates of plant growth regulator treatments laid out in a randomised block design of 90 plants. The once off application of treatments included 0 (control), 50, 100, 200, 300 mL of liquid stock solution and 1/4, 1/2, 1 and 2 solid disks of seaweed growth regulator per plant. The disks were cut into the various size classes according to the different treatments each plant would receive. The treatments that received the half and quarter size disks were cut using a guillotine. The pots were filled half way with soil and the disks were inserted on top and covered lightly. The rooted cuttings were then inserted and the remainder of the soil added to fill the pot. The liquid dosage treatments of seaweed growth regulator were administered to each 15 cm potted plant using a measuring cylinder to ensure accuracy. The plants that contained the control only received water and no growth regulator treatment. Watering during the trial period was conducted, using a hand-held hose with a rose head sprayer twice a week and from the 10th week onwards once a week. Each container received an average of 250 mL of water.

Data collection and analysis

Prior to planting, pre-trial measurements of the longest root length (mm) using a standard ruler for each individual cutting was recorded. On the 18th week of the trial, final readings of the plants were recorded. Plants were carefully harvested and their roots were rinsed with deionised water. The postharvest root lengths (mm) were measured and recorded. The plants were then moved to the Cape Peninsula University of Technology (CPUT), Department of Horticultural Sciences, research laboratory, Bellville campus. The roots and shoots were then separated with secateurs from each other. The fresh root weight (g) of each was weighed and recorded using a Radwag AS 220/C/2 analytical scale. The roots were placed in a manila brown paper bag and placed in a Scientific Series 2000 laboratory oven at 55°C for 48 h. The plants were then removed from the oven and the dry root weight (g) of each was weighed and recorded. The combined total was also recorded. Collected data was analysed using two-way analysis of variance (ANOVA), with the computations being done using the software program STATISTICA (Software Program version 2010 (Statsoft Inc., Tulsa, OK, USA)). Occurrence of statistical difference was determined by using the Fisher least significance difference (LSD) at values of $P \leq 0.05$ and $P \leq 0.001$ levels of significance (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Root weight after organic seaweed growth regulator treatment

The plants exposed to the different Kelpak® organic seaweed treatments were significant ($P \leq 0.001$) in dry weight at 18 weeks post-treatment. However, Kelpak® treated plants had lower dry weight compared to the control plants (Figures 1 and 2). The highest application of liquid seaweed treatment, 300 mL, and the lowest application of solid seaweed treatment, 1/4 disk induced the highest wet weights among *E. verticillata* plants at 18 weeks postharvest (Figures 1 and 2). Liquid treatments of 50, 100, 200 mL and 1/4, 1 and 2 disks were all similar in increasing dry root weight compared to the control (0) (Figures 1 and 2). Plant hormone cytokinins control a range of plant activities shown to increase meristemic cells in roots and shoots (Miransari and Smith, 2014).

Root length before and after organic seaweed growth regulator treatmen

The effect of different dosage applications of Kelpak® organic seaweed growth regulator was significant ($P \leq 0.05$) on the postharvest root length of *E. verticillata*. The application of liquid treatments had an increase in root length compared to the disk treatments on *E. verticillata* (Figures 3 and 4). These results confirm that liquid drench applications are absorbed faster through the roots as it becomes more readily available to the plant (Kelpak®, 2016). Absorption of nutrients from the dissolving disks will be enhanced with sufficient moisture availability in the medium. The application of (100-200 mL) liquid treatment and 1/2 disk solid treatment were most significant in the postharvest root lengths compared to the other treatments at 18 weeks post-harvest (Figures 3 and 4). The application of (100-200 mL) is in line with the recommendation of the manufacturers (Kelpak®, 2016).

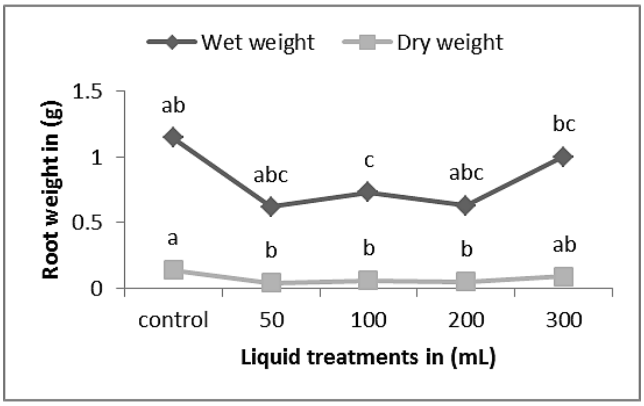

Figure 1. Wet and dry root weight (g) of *E. verticillata* following liquid organic seaweed growth regulator treatment. Each circle represents a mean±SE (n=10) annotated with different letters that differ significantly at P≤0.001.

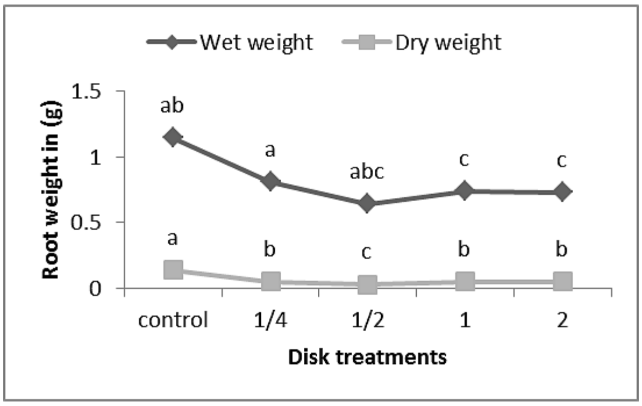

Figure 2. Wet and dry root weight (g) of *E. verticillata* following solid disk organic seaweed growth regulator treatment. Each circle represents a mean±SE (n=10) annotated with different letters that differ significantly at P≤0.001.

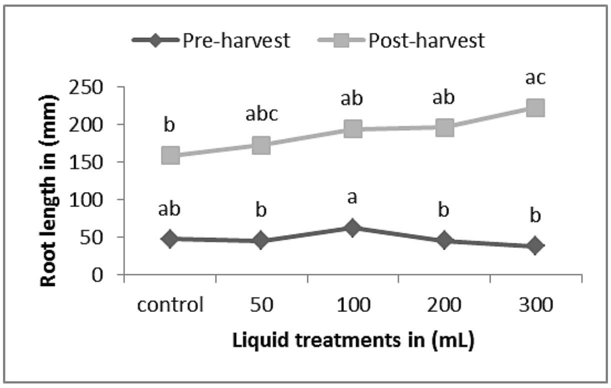

Figure 3. Pre- and postharvest root length (mm) of *E. verticillata* following liquid organic seaweed growth regulator treatment. Each circle represents a mean ± SE (n=10) annotated with different letters that differ significantly at P≤0.05.

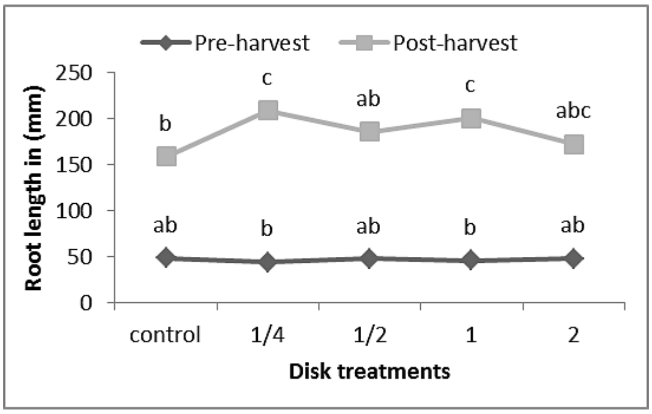

Figure 4. Pre- and postharvest root length (mm) of *E. verticillata* following solid disk organic seaweed growth regulator treatment. Each circle represents a mean ± SE (n=10) annotated with different letters that differ significantly at P≤0.05.

Production schedule

According to Hitchcock et al. (2013b) *E. verticillata* does not freely produce seed unless two or more different clones are in close proximity, for intraspecific cross-pollination and therefore seed production is low when compared to other wild growing *Erica* species. Sexual reproduction cannot maintain these characteristics as each individual offspring is genetically different therefore, vegetative propagation is important to preserve the genetic identity of species where particular characteristics of plants need to be maintained (Hartmann et al., 2014). *E. verticillata* propagated from cuttings taken from mature plants will flower in their first season whereas plants grown from seed usually only flower in their third year (Hitchcock, 2001a).

1. Week 1-4 – cutting rooting stage.

Vegetative propagation of *E. verticillata* was done at week 1 selecting cutting material in the early morning from healthy mother stock, free from disease, pests, deficiencies or environmental stresses. Hartmann et al. (2014) supported this methodology to ensure stress free material to start propagation. According to Hitchcock (2001a), *E. verticillata* is one of the easiest species of *Erica* to grow from cuttings. Selecting heel cuttings with tips is in line with KNBG practices to select cuttings taken from side shoots in autumn (Hitchcock, 2001a, b). Hormone treatment using Seradix® 2 rooting powder with auxin indole-3-butyric acid (IBA) was used to initiate callusing for rooting of semi hardwood cuttings (Hartmann et al., 2014). These practices are recommended at KNBG in using Seradix® 2 for fynbos species (Hitchcock, 2001a). Leakey (2004) reported that cuttings treated with auxins root better than untreated cuttings as photosynthetic periods synthesize endogenous auxins, which is transported to the base of the cutting and promotes root formation. Hitchcock (2001b) also recommended that organic seaweed growth regulators such as Kelpak, is a useful root stimulant during rooting.

Cuttings of *E. verticillata* planted in compartmental plug trays containing a propagation mix of 50% fine milled pine bark and 50% polystyrene granules rooted successfully under intermittent mist on raised heated greenhouse benches (Hitchcock, 2001a, b; Brand and Notten, 2009). This recommended rooting medium is regularly used in the nurseries at KNBG (Hitchcock, 2001a). Similar results of heel and tip cuttings using plug trays with a medium of 50% coarse sand and 50% perlite at a pH level 6 were found to promote root growth (Brand and Notten, 2009). Cuttings should be irrigated with a fine mist every 30 min for a duration of 60 to 120 s at a time or light watering 4 times daily for a duration of 10 min at a time. In following the correct measures during the rooting stage, 80% rooting was obtained in rooting *E. verticillata* at week 4. This rooting time is in agreement with Hitchcock (2001a) where a rooting period between 3 and 6 weeks is suggested (Table 1).

Table 1. Proposed production schedule for the propagation and cultivation of *Erica verticillata*.

Week	Growth stage	Method	Media	Irrigation	Treatment	Environment	Growth dimension
1-4	Cutting rooting stage	Propagate heel tip cuttings in plug trays	50:50 milled bark/ poly styrene	Misting every 30 min for 60-120 s	An application of Seradix® 2 rooting hormone	Controlled greenhouse with bottom heat	Low mortality rate
5-6	Juvenile plant growth stage	Hardening-off of rooted cuttings	50:50 milled bark/ poly styrene	Reduce watering to once daily	Half strength organic liquid growth regulator	50% shaded conditions	Noticeable adaptation
7-8	Plant developing growth stage	Transplant rooted cuttings to individual containers	KNBG Fynbos mixture	Hand watering twice a week	An application of (100 -200 mL) organic seaweed growth regulator per plant	Keep shaded for 2 weeks and move outdoors	Root adaption to new medium. Start of new leaf growth
9 -<	Plant maturing growth stage	Grow mature plant in provision for planting out	KNBG Fynbos mixture	Hand watering once a week reduced to alternative weeks after planting out	Full strength organic seaweed growth regulator	Outdoor conditions	Plant height, leaf colour improve significantly

2. Week 5-6 – juvenile plant growth stage.

Juvenile plants with newly formed roots are relatively fragile by nature as they do not have many roots or leaves to support themselves. Transplanting stresses can have a significant effect on the survival and development of rooted cuttings and care should be taken to impede any damage or exposure to damaging environmental conditions at this crucial stage of growth (Hartmann et al., 2014). Rooted cuttings of *E. verticillata* were removed from the heated beds in the controlled greenhouse and placed in a hardening-off area of 50% shading to acclimatize for a few weeks (Hitchcock, 2001b) weeks 5-6 (Table 1). Acclimatization ensures cutting survival to reduce transplant shock to a new growing environment (Hartmann et al., 2014). Watering of these rooted juvenile plants was reduced to once daily during this period. These juvenile plants were treated with a preventative fungicide to avoid any root rot.

3. Week 7-8 – plant developing growth stage.

During weeks seven and eight the acclimatized juvenile plants of *E. verticillata* were removed from the plug trays, roots were rinsed in deionised water and root lengths were measured and weighed for experimental purposes. Rooted cuttings were then transplanted to 15-cm square plastic pots which contained a well-drained nutrient poor fynbos soil mix of eight parts, 6 to 12 mm fine milled pine bark, one part milfeed wet Consol® glass sand, two parts "Malmesbury Grof" sand and one part agricultural lime, a (8:1:2:1) KNBG fynbos medium developed by the gardens (Hitchcock et al., 2013b). Juvenile plants should be irrigated twice a week to ensure sufficient moisture for root development. In this study the aim was to investigate the effect of two different formulations of a commercial organic seaweed growth regulator at varied dosages on root growth parameters of *E. verticillata* to support the conservation of the species. Physiological responses from various agricultural crops found that auxins and cytokinins within organic seaweed growth regulators are responsible for these growth responses (Robertson-Andersson et al., 2006). While auxins orchestrate almost every aspect of plant growth and development (Overvoorde et al., 2010) this study found an application of 100-200 mL liquid stock solution per plant of an organic seaweed growth regulator should be used in growing pot-grown *E. verticillata.* It also needs to be mentioned that the disks would have a longer supply of auxins as they start breaking down over a longer period. In the presence of water and warmer temperatures, controlled release of growth regulators release nutrients to plants effectively (Hitchcock, 2001b).

4. Week 9-onward – plant maturing growth stage.

The mature plant growth phase for *E. verticillata* involves development of the plant to a growth stage ready for ex situ re-planting to its natural habitat or to produce a marketable plant preferably in flower. A knowledgeable understanding of fynbos species natural growth requirements is necessary to provide specific environmental conditions for species such as *E. verticillata* (Van Jaarsveld, 2010). Many fynbos species of the Cape Floristic Region (CRF) are often cited as shrubs growing on low-nutrient, well-drained sandy soils under 'Mediterranean' climate zones (Sinclair, 2012; Hitchcock, 2001b). These conditions need to be simulated to provide for healthy growth of *E. verticillata*. As the biomass of developing plants increases during the mature growth stage, irrigation frequency too should be increased to meet the crops growth requirements. Insect and environmental damage need to be prevented at this stage as it can diminish the plants marketability or hinder future development. Pruning should be carried out on plants to ensure growth form development and healthy growth (Hitchcock, 2001a; Brand and Notten, 2009) and the *Erica* species should be regularly fed with organic liquid fertilizers or control-release fertilizers low in phosphorus (Hitchcock, 2001a; Van Jaarsveld, 2010). Plants earmarked for the landscape and nursery industries or re-introduction into the wild should be hardened off through decreased irrigation and increased temperatures to prepare them for the transplanting purposed (Table 1). Van Jaarsveld (2010) recommended that it is best to plant *Erica* species during the autumn or winter in the winter-rainfall areas of South Africa. Organic seaweed growth regulator such as Kelpak is recommended to relieve transplanting stress caused by heat and or root disturbance, maintain plant health and eliminate plant losses (Hitchcock, 2001b). This would additionally benefit pot production of *E. verticillata* to support ex situ re-introduction of the species into the wild.

E. verticillata should be planted in full sun, in an acidic (pH 5.5 and 6.7) well-drained medium with minimum disturbance to the sensitive roots (Hitchcock, 2001b). Pine compost can be added to the hole and plants should be well-watered with a basin around the stem (Brand and Notten, 2009). Most *Erica* species form a symbiotic relationship with *Mycorrhiza* species which support the plants survival during hot dry summers. Although *E. verticillata* prefers more water compared to other *Erica* species, *Mycorrhiza* species enter the roots and send out a network of hyphae strands to increase the water-absorbing capacity of the plant during dry periods (Hitchcock, 2001a; Hitchcock et al., 2013a). Mulch the top of the soil to keep the root zone area moist and cool and to reduce weed growth and water plants at two to three day intervals until the rainy season starts (Hitchcock, 2001a).

CONCLUSIONS

The following conclusions can be drawn from the study:

- Use of both liquid and disk organic growth regulators significantly increased the dry root weight and postharvest root length of *E. verticillata*;
- It is recommended that 100-200 mL liquid growth regulator be used in cultivating pot-grown *E. verticillata*;
- The production schedule can be used to propagate and reintroduce *E. verticillata* and as a guide for experimental work on other *Erica* species with similar growing requirements;
- This could unlock the potential for *E. verticillata* to accelerate the natural flowering and seed forming processes to establish new populations at a faster rate in the natural habitat.

ACKNOWLEDGEMENTS

This study was supported by the South African National Botanical Institute and funded by the Cape Research Centre, Kelpak® (Pty) Ltd. and the Cape Peninsula University of Technology.

Literature cited

Brand, L., and Notten, A. (2009). Growing *Erica sessiliflora* (Kirstenbosch National Botanical Garden), http://www.plantzafrica.com/plantefg/ericasessil.htm (accessed October 28, 2016).

Da Bush, J. (2010). A felling tale: restoring critically endangered lowland fynbos in the Tokai plantation. Veld & Flora. *96*, 134–135.

Gibbs, D. (2014). Baptism of Fire. Veld & Flora. *100*, 30–32.

Hartmann, H.T., Kester, D.E., Davies, F., and Geneve, L. (2014). Plant Propagation: Principles and Practices, 8th edn (Essex: Pearson Prentice Hall), p.280–344.

Hitchcock, A. (2001a). Growing *Erica verticillata* (Kirstenbosch National Botanical Garden), http://www.plantzafrica.com/plantefg/ericaverticil.htm (accessed October 28, 2016).

Hitchcock, A. (2001b). Growing *Erica cerinthoides* L (Kirstenbosch National Botanical Garden), http://www.plantzafrica.com/plantefg/ericacerinth.htm (accessed October 28, 2016).

Hitchcock, A., Mcquillan, M., and Notten, A. (2013a). Centenarians and champions on sale. Veld & Flora. *99*, 10–15.

Hitchcock, A.N., Oliver, E.G.H., and Thomas, V. (2013b). *Erica verticillata*. Flower. Plants Afr. *63*, 104–119.

Kelpak®. (2013). Kelpak. http://www.kelpak.com/index.html# (accessed June 8, 2013).

Kelpak®. (2016). Liquid seaweed. http://www.kelpak.com/pdf/publications/kelpak-Liquid-Fertilisers.pdf (accessed October 27, 2016).

Khoshbakht, K., and Hammer, K. (2007). Threatened and rare ornamental plants. J Agr Rural Dev Trop. *108*, 19–39.

Leakey, R.B. (2004). Physiology of vegetative reproduction. In Encyclopaedia of Forest Sciences (London: Academic Press), p.1655–1668.

Miransari, M., and Smith, D.L. (2014). Plant hormones and seed germination. Environ. Exp. Bot. *99*, 110–121 https://doi.org/10.1016/j.envexpbot.2013.11.005.

Overvoorde, P., Fukaki, H., and Beeckman, T. (2010). Auxin control of root development. Cold Spring Harb. Perspect. Biol. *2* (*6*), a001537 https://doi.org/10.1101/cshperspect.a001537. PubMed

Robertson-Andersson, D.V., Leitao, D., Bolton, J.J., Anderson, R.J., Njobeni, A., and Ruck, K. (2006). Can kelp extract (Kelpak®) be useful in seaweed mariculture? J. Appl. Phycol. *18* (*3-5*), 315–321 https://doi.org/10.1007/s10811-006-9030-1.

Sinclair, S.J. (2012). Low-nutrient soils, pollination and plant diversity. J. Veg. Sci. *23* (*3*), 585–589 https://doi.org/10.1111/j.1654-1103.2011.01371.x.

Steel, R.G.D., and Torrie, J.H. (1980). Principle and Procedures of Statistics: a Biometrical Approach, 2nd edn (New York: McGraw Hill), p.20–90.

Van Jaarsveld, E.V. (2010). Waterwise Gardening in South Africa and Namibia (Cape Town: Struik Lifestyle), p.66–73.

Winter, J.H.S., and Botha, D.J. (1994). The release of endangered plants into the horticultural; trade: conservation or exploitation? Int. J. Biodivers. Conserv. *3* (*2*), 142–147 https://doi.org/10.1007/BF02291884.