

**Response of the Endangered Medicinal Plant
Siphonochilus aethiopicus (Schweif.) B.L. Burt.
to Agronomic Practices**

by

James Francis Hartzell

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School of Agricultural Sciences and Agribusiness
Faculty of Science and Agriculture
University of KwaZulu-Natal

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Response of the Endangered Medicinal Plant *Siphonochilus aethiopicus* (Schweif.) B.L. Burt. to Agronomic Practices

Thesis Abstract

This study examines field cropping constraints for domestication of an endangered, wild medicinal plant, *Siphonochilus aethiopicus*, (Schweif.) B.L. Burt. Extensive literature review and careful observations of plant growth behavior during two years of crop trials overturned several long-held but erroneous claims that have consistently appeared in the scholarly literature, and revealed previously undocumented plant growth characteristics. *S. aethiopicus* (Schweif.) B.L. Burt. is a rhizomatous corm, not a rhizome. Field growth observations demonstrated clearly that the false stem and leaves grow continuously from emergence in September to senescence in April-May; the corm retains its tuberous roots during winter senescence, and is genetically preprogrammed to shoot in September. Flowers may emerge throughout the growing season (not only initially prior to shoot emergence), typical leaf count is 11-15, not 6-8 as previously reported, numbers that remain constant even when the plant height increases by 20-30% under shade, and leaf distichy is independent of the sun's course and is unaffected by mother corm orientation. *S. aethiopicus* proved to be unusually resistant to common field diseases and pests, and resilient to severe hail.

The responses of *S. aethiopicus* were tested in a series of field trials to the effects of levels of compost, field spacing, size of planting material, addition of biocontrol agents, different degrees of shading, and factorials of the macronutrients Nitrogen, Phosphorous and Potassium. Spacing-Composted chicken litter combinations were tested in 2005-2006 in factorial combination with Spacing at 15 cm-4.5 kg ha⁻¹, 20 cm-7.5 kg ha⁻¹, 30 cm-10 kg ha⁻¹, and 40 cm-15.5 kg ha⁻¹, and these treatments were randomized with 4 Corm planting sizes (height by base diameter in mm): Small (S, 12.38 mm x 12.6 mm), Medium Small (MS, 29.65 mm x 27.93 mm), Medium Large (ML, 38.48 mm x 37.78 mm) and Large (L, 52.37 mm x 44.10 mm). 2005-2006 ANOVA tests showed significant differences between Spacing-Compost and Corm Size for the total harvest biomass measure, with 30 cm and 40 cm spaces better than 15 cm spacing, and Corm Size MS, ML and L all better than S, and ML better than MS. Total Corms harvested per block and

Survival Percentage were similarly significant for Corm Size, but not Spacing. Corms smaller than the Small criteria were raised separately, under optimal conditions in a nursery. In a separate 2005-2006 Compost-only trial ANOVA tests did not find significant differences between compost levels.

In 2006-2007 we tested Spacing separately at 5, 10, 15, 20, 30 and 40 cm between planted corms in each plot. We tested Compost levels separately, with 0, 5, 10 and 15 kg ha⁻¹ compost per plot. In 2006-2007 only the ML and L sizes were used in an even mix. There were no significant differences between treatments due to high experimental error, but measurement across all production parameters showed a clear trend towards best performance at spacing between 20 and 40 cm. Overall the results from the Spacing, Compost-level and Corm Size trials suggest that 30 cm is perhaps the optimal field spacing, higher compost levels tend to give better results, and the ML and L corm sizes perform better in open-sun field trials. These parameters are recommended for further field studies and production.

The effects of two commercial strains of *Trichoderma* spp were tested at recommended doses applied to *S. aethiopicus*. *T. harzianum* Strain B77 was used as a drench at planting in comparison with a Control and a fungicide in 2005-2006. There were no significant differences between treatments for Harvested Biomass or Survival Percentage. B77 did perform significantly better than the Fungicide in the Total Corm measurement, but neither treatment was significantly different from the Control. In sum, there was a weak trend towards a greater number of output corms as a result of the application of the biocontrol agent. In both 2005-2006 and 2006-2007 we tested *T. harzianum* Strain kd applied as a drench at planting, with a second drench at 4 weeks. In 2006-2007 there were no significant differences between treatments, but the trend was towards better performance as a result of the drench at planting only.

In 2005-2006 open field trials had shown that *S. aethiopicus* is susceptible to sunburn and Erwinia soft rot when grown in the full sun. Therefore, we tested the effect of various shade cloth densities and colours on production performance in 2006-2007. Treatments were Control (full sun), 40% White (TiO₂) (23% shade), 40% Grey (28-30% shade), Light Black (40%), Medium Black (50%), Dark Black (80%), and Red (40%).

There were no significant differences between treatments, but the trends indicated that the Control (full sun) and Dark Black (80% shade) performed the worst. Colour of shade did not appear to be important, and plants under all the shade cloths with 40-50% shade grew best.

In a factorial trial different levels of Nitrogen, Phosphorous, and Potassium (NPK) were tested, over two seasons. Four levels of each input were used: N at 0 (Control), 40 kg ha⁻¹ (N1), 80 kg ha⁻¹ (N2), and 120 kg ha⁻¹ (N3). P levels were 0 (Control) 60 kg ha⁻¹ (P1), 120 kg ha⁻¹ (P2) and 200 kg ha⁻¹ (P3). K levels were 0 (Control), 100 kg ha⁻¹ (K1), 200 kg ha⁻¹ (K2), and 400 kg ha⁻¹ (K3). In 2005-2006 there were no significant differences between treatments. In 2006-2007 data there were significant results for Nitrogen only within each repetition. However, significance disappeared when combining across repetitions. We then ran a Bootstrap re-sampling analysis of both 2005-2006 and 2006-2007 data (data were analyzed separately because of different plot sizes and corm numbers in the two years), looking at the optimal level of each macronutrient tested against all combinations of the other two. Though significant results were obtained for each individual level of each macronutrient against the others in combination, the difference between the confidence intervals was not significant. However, there was a clear trend: the optimum N levels were between 40 and 80 kg ha⁻¹; optimum P level was 0 (the Control) and optimum K levels were between 100 and 200 kg ha⁻¹.

Tests of handling during harvest, storage, and planting yielded additional useful information for small scale commercial farmers. The optimal harvest time is May, when the false stem and leaves are senescing and yellow, but still upright and visible. Harvest is facilitated by moistening the soil to minimize breaking off of tuberous roots, with simple field washing to remove compacted soil highly recommended. Harvested corms and tuberous roots should be stored under air-restricted, cool conditions because the tuberous roots contain high moisture and will shrivel quickly when left exposed to air, and excessively dried corms will eventually die. Senesced mother corms should be discarded at harvest. Corms are genetically preprogrammed to shoot, so should be planted in September in soft soil, with 1-2 cm of soil coverage.

The studies provide a framework for developing the basic agronomy for the domestication and commercial crop production of an endangered medicinal plant species.

DECLARATION

I, James Francis Hartzell, declare that

- (i) The research reported in this thesis, except where otherwise indicated, is my original work.
- (ii) This thesis has not been submitted for any degree or examination at any other university.
- (iii) This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
- (iv) This thesis does not contain other persons' writing unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
 - a) their words have been re-written but the general information attributed to them has been referenced;
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Signed.....
James Francis Hartzell

Signed.....
Professor Mark Laing, Supervisor

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Thesis Introduction: Field Trials of *Siphonochilus aethiopicus* (Schweif.) B.L. Burt., an Endangered Medicinal Plant

Domestication, i.e. developing field cropping information for previously wild-harvested medicinal plants is an important component of medicinal plant conservation strategies. Recent estimates suggest that between 4160 and 10,000 wild medicinal plants are considered threatened with extinction through over-harvesting (Hamilton 2004). Medicinal plant propagation information is available for less than 10% of plants globally, with agro-technology available for only 1% (Kala et al 2006). For example, only 3-6% of medicinal plant species in Germany are cultivated, only 130-140 (out of 1200-1300 in use) in Europe as a whole, and only 100-250 species in China (out of 5000 in use) (Schippmann et al 2002). In South Africa, only about 1% of the 400-550 medicinal species currently sold are cultivated (Hamilton 2004).

Cunningham (1993), commented on the dearth of cultivation of medicinal plants in Africa, due partly to the lack of institutional support and the low prices available for the plants. To these observations we may add the traditional division of labor in African rural communities, where traditional healers have not usually cultivated even their own fields, leaving such strenuous labor to neighboring farmers, while the healers concentrate on tending to patients and gathering materials from the wild (personal communication, members of the Ezemvelo Farmer's Organization, KwaZulu-Natal, 2000-2006). While figures vary, Mander (1998) reported that over 700 medicinal plant species are actively traded in South Africa. Some senior izinyanga (herbalists) in the Durban area report that they use over 1000 plant species in their practice (P. Cele, personal communication), many of which they personally collect from the wild. Wild harvested medicinal plants form for many suburban and rural healers the bulk of the material they use in their practice. Urban healers typically collect from the wild themselves to supplement what is available in the muthi markets. Individual healers may go on collecting trips every week or two, which may take as long as 6-10 hours per trip, depending on the distance to certain natural areas where the plants grow (M.P. Dube, personal communication 2008, members of the KZN Traditional Health Practitioners Council, personal communication 2006-2008).

The local KZN extinction of *S. aethiopicus* is widely noted and cited (Goodman 2004). Cunningham (1993) also noted that as of 1993, 68% of herb traders in South Africa nominated wild ginger as becoming scarce, the third highest plant after *Warburgia salutaris* (Bertol. f.) Chiov.(Canellaceae) and *Boweiea volubilis* L.. The South African National Biodiversity Institute (SANBI) lists the plant's 2007 interim national status as Critically Endangered (though its 2007 Global Status is Not Endangered); the category is reserved for plants facing an extremely high risk of extinction in the wild (<http://www.sanbi.org/frames/documentsfram.htm>). Gordon-Gray et al (1989) noted that the bulk of traded material in the late 1980s was coming from the Transvaal (Limpopo, Mpumalanga, Gauteng, and eastern North West Province) and further east, and that due to depredation of natural stocks, commercial cultivation would be necessary to maintain supply and keep prices reasonable.

While there is very little reliable public information currently available for medicinal plant cultivation in South Africa, some projects are underway. The Medical Research Council's Indigenous Knowledge Systems Lead Program, headed by Dr. Motlalepula Gilbert Matsabisa, has started, in collaboration with DOH and DST, a series of small-scale, poverty-alleviation focused, commercial cultivation projects of "scientifically validated medicinal plants" in community projects in Sengu and Tsolwana Municipalities in the Eastern Cape, Nama Khoi Municipality in the Northern Cape, Mbombela Municipality in Mpumalanga, and Makhudutamanga Municipality in Limpopo (as of this writing, there are no MRC cultivation projects in KZN). The specific plants under cultivation are kept confidential to protect IP leads (M. G. Matsabisa, personal communication and www.mrc.ac.za/iks/iksclinical.htm). Plants under cultivation include those showing promising leads for antimalarials (DACST Annual Report 2000/2001).

Published cultivation information for *S. aethiopicus* is minimal, and limited to horticultural and botanical studies. Nichols (1989) provided useful horticultural notes, McCarten et al (1999) conducted a small one-year cultivation trial near the coast as part of the Silverglen Municipal Nursery medicinal plant conservation effort, and Spring (2002) posted some cultivation notes on the KZN Provincial Department of Agriculture and Environmental Affairs website. Three masters degree theses from the University of Pretoria (Masevhe 2004, Baloyi 2004, and Manzini 2005) provided some additional

information on plant growth characteristics. Perhaps the most useful practical information was provided by Crouch and Symmonds (2002), both botanists.

Motivation for The Current Study

Given the local extinction of *S. aethiopicus* in KwaZulu-Natal, its spreading scarcity in more northern regions of South Africa, the rising price of the corms in the markets, and the dearth of practical farming information for field cropping the corm, the current study was undertaken to develop a preliminary base of agronomic information for *S. aethiopicus*. Several types of trials were designed: to test macronutrient requirements under normal growth conditions, to optimize plant spacing in the field, to test the viability of growing corms with compost, to test practical seed corm sizes, to determine whether the plant performs better under shadecloth, and whether it is responsive to selected biocontrol agents.

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Chapter 1: Literature Review

1.1. Taxonomy of *Siphonochilus aethiopicus* (Schweif.) B.L. Burt.

The Zingiberaceae is a pantropical family in the Zingiberales order, with 53 genera, and over 1200 species. Among the most well-known and commercialized species in the Zingiberaceae spice family are ginger, turmeric, cardamom, large cardamom, and grain of paradise (Ravindran and Babu 2005). The first classification of the family was proposed in 1899, and then refined into four morphology-based tribes: Globbeae, Hedychieae, Alpinieae, and Zingiberaceae. Recent molecular analysis by Kress et al (2002), using DNA sequences of the plastid *matK* and the nuclear internal transcribed spacer (ITS) regions, provided a new classification of four subfamilies and four tribes:

Siphonochiloideae (Siphonochileae), Tamijinoideae (Tamijeeae), Alpinioideae (Alpinieae and Riedelieae) and Zingiberoideae (Zingiberaceae and Globbeae). The new molecular analysis has shown the African genus *Siphonochilus* to be a basal lineage whose sole member is the Siphonochiloideae. The nearly complete fusion of the lateral staminodes to the large labellum is taken as a pleiomorphic character of the basal Zingiberaceae shared with the sister families Costaceae and Tamijia. The Kress et al (2002) study was based on genetic analysis of an East African specimen (not further defined), with a GenBank accession number for ITS sequences GBAN-AF478792, and GBAN-AF478893 for the *matK* sequences. Harris et al (2003) have recently presented chloroplast and nuclear molecular systematic studies evidence resulting in *Aulotandra* Gagnep. (Madagascar) being transferred from the subfamily Alpinioideae, tribe Alpinieae, to the subfamily Siphonochiloideae, due to chloroplast and nuclear molecular systematic studies showing that *Aulotandra* and *Siphonochilus* form a monophyletic group.

The Siphonochiloideae includes as its sole member the 12 members -- not 15 as mistakenly reported by Larsen (2005) -- of the genus *Siphonochilus*, and is restricted to tropical Africa (Lock 1985; Larsen 2005; Newman 2007). *Siphonochilus aethiopicus* (Schweinf.) B.L. Burt (Burt 1982), type *Cienkowski s.n. Steudner s.n.*, is reported to be widespread in the savanna regions of tropical Africa, from Senegal to Ethiopia, and south to Zimbabwe, Mozambique and South Africa. Its habitat is deciduous woodland, bushland, and wooded grassland, from 390-1830 m altitude. The species was first officially identified by J. Medley Wood and Franks (1911a) in Inanda at 540 m, and

'Ngoya, at 450-610 m in 1910, and brought back by the Curator Mr. Wylie from 'Ngoya to the Durban Botanic Gardens (where it flowered) (Woods and Franks 1911b; original description in Latin). The other members of the genus are:

1. *Siphonochilus bambutiorum* A.D. Poulsen and Lock (Poulsen and Lock 1999), Type *Poulsen and Liengola 1146* (holo, C; iso BR, E, K, MO), discovered in the Ituri Forest of Congo, Kinshasa.
2. *Siphonochilus brachystemon* (K. Schum.) B.L. Burttt (Burttt1982), type: *Volkens 201* (B), *Holst 3100* (B), found in Uganda, Kenya, and Tanzania.
3. *Siphonochilus carsonii* (Baker) Lock (Lock 1984), type *Carson s.n.* (K), found in Zambia, Malawi, Mozambique and Zimbabwe.
4. *Siphonochilus decorus* (Druten) Lock (Lock 1999), type *Schweikert s.n.* (PRE). comb. nov. *Kaempferia decora* Druten; *Kaempferia* is now exclusively Asian.
5. *Siphonochilus evae* (Briq.) B.L. Burttt (Burttt 1982), type: *Prosch 12* (G).
6. *Siphonochilus kilimanensis* (Gagnep.) B.L. Burttt, (Burttt 1982).
7. *Siphonochilus kirkii* (Hook.f.) B.L. Burttt, (Burttt 1982), type: *Kirk s.n.* (K), found in Uganda's West Nile District, Kenya's Kwale District, Tanzania, Sudan, Zambia, Malawi and Mozambique.
8. *Siphonochilus natalensis* (K. Schum.) Wood and Franks (Medley-Wood and Franks 1911b), type: *Wood 544* (K) (actually a synonym of *S. aethiopicus*).
9. *Siphonochilus nigericus* (Hepper), B.L. Burttt (Burttt 1982), type: *Dalziel 276* (K).
10. *Siphonochilus parvus* Lock (Lock 1991) type: *Congdon 46* (K), discovered in Tanzania.
11. *Siphonochilus rhodesicus* (T.C.E.Fr.) Lock, (Lock 1984), type: *Fries 1146* (UPS), found in Tanzania, Zambia, and Malawi.

The name *Siphonochilus* comes from Greek *siphono* (tube), and *chilus* (lip), referring to the shape of the flower formed by the fusion of the fertile stamen filament with the labellum base, forming a tube above the corolla lobe insertion point. *Aethiopicus* is the ancient name for Africa (not just southern Africa, as erroneously reported on the SANBI website by Hankey and Reynolds 2002), and is a common botanical name for many African species. The English common name is wild ginger, or African ginger, the Zulu indungulo or isiphephetho, the Afrikaans Wildege mmer; it has had as botanical

synonyms *Cienkowskia aethiopicus* (Schweinf), *Kaempferia aethiopica* (Schweinf), Benth, *Kaempferia ethelae* JM Wood, *Kaempferia natalensis* (Schlecht and K. Schum.), *Siphonochilus natalensis* (Schlecht. And K. Schum), JM Wood and Frank and *Cienkowskeilla aethiopica* (Schweinf) YK Yam. (Smith 1998; Hankey and Reynolds 2002; Harris et al 2004; Hyde and Wursten 2008; Scott and Springfield 2004). Despite the clarification of its taxonomy by Kress et al (2002), Van Wyk (2008) has recently written that no comprehensive taxonomic studies on the genus *Siphonochilus* and related genera has yet been done. Makhuva et al (1997) have shown a wide genetic variation among 50 plants collected from a farm near Tzaneen, and virtually no genetic variation in material collected from the Kirstenbosch Botanical Garden, and suggested that sexual reproduction occurred successfully in the wild population.

1.2. *S. aethiopicus*, African Distribution and Conservation Status

Van Wyk (2008) commented recently that the exact distribution of *S. aethiopicus* is still poorly known, and there are as yet no published comprehensive distribution maps. Some facts are nonetheless well established. As Kress et al (2002) have clarified, *S. aethiopicus* occurs only in Africa. Extended literature search (reviewed below) provides reliable citations of *S. aethiopicus* in Benin, Ethiopia, northern Ghana, Malawi-Mozambique, Nigeria, Niger, Swaziland, Tanzania, and Zimbabwe, in addition to South Africa. Otherwise excellent published research has more than once mis-reported *S. aethiopicus* distribution as restricted to southern Africa, i.e. South Africa, Zimbabwe, Malawi, and Zambia (see for example Holzapfel et al 2002).

In a 2001 biogeographic survey of the Kouffe Mountains in Central Benin, near Kambole on the Togo border, an area of savannah, woodland and patches of dry forests, *S. aethiopicus* is listed among the species constituting the Sudanese-Zambesian vegetation type, itself 13.5% of the vegetation type of the region (Houinato and Sinsin 2001). *S. aethiopicus* appears on the Flora List of the Ethiopian Government Roads Authority in an Assosa-Guba Road Project (north-western Ethiopia, along the Sudan border) (World Bank 2004). In a list of Hausa plant names in use along the northern Ghana, Nigeria, Niger border, *S. aethiopicus* is referred to as a 'resurrection lily', Láminíyár kwààlíí or Lányár kwààlíí (Blench 2003). The plant was positively identified in Miombo woodland between 800-1200 m altitude on the 2000 m high Mt. Chiperone, located about 35 km

northeast of Chilomo, Malawi, right along the Malawi-Mozambique border (Anonymous 2007); the restriction of evidence to higher altitudes (above 900 m) was reported to be due to frequent cutting and/or burning of lower altitude woodlands. In a plant survey of Mahale Mountains National Park, on the western border of Tanzania along Lake Tanganyika, *S. aethiopicus* is included in the list of 1174 plants found in the park (Mahale no date). In the 45000 km² Selous Game Reserve in southeastern Tanzania, 75% of which is Miombo woodland dominated by *Brachystegia* species, *Julbernardia globiflora*, *Isobertinia*, *Pterocarpus angolensis* and *Combretum*, with long dry seasons and between 750-1300 mm rain from late November to May, *S. aethiopicus* is “commonly seen” (Njawa 1999). In Zimbabwe, the plant is said to occur in north, central and east of the country, at altitudes up to 900 m, flowering in November and December, in woodland high rainfall areas along the eastern border, as well as in the Zambezi valley (Hyde and Wursten 2008). Bart Wursten (2008, personal communication), one of the principals of “Flora of Zimbabwe” reported that the plant is common on the slopes of Mt. Gorongosa in Mozambique, and even grows “in quite disturbed cultivated fields”.

Cunningham (1988) reported that *S. aethiopicus* is found in the Zambezi and Mopane Woodlands (AT0725), a biome type dispersed throughout southern Africa, bounded by Pongola River, a tributary of the Maputo River, rising in Utrecht, northern KZN, in the south and the Luangwa River in the north, the southern extension of the Rift Valley, and a tributary of the Zambezi. The Zambezi woodlands in lower-elevation areas mix with Mopane tree (*Colophospermum mopane*) woodlands, typically along the major river valleys. In the southern Africa region these woodlands are found in South Africa, Mozambique, Botswana, Zambia, Zimbabwe, Swaziland, Namibia, and Malawi. The Biome is Tropical and Subtropical Grasslands, Savannas, and Shrublands, comprising about 182,700 square miles (473,190 square kilometers) (Estes and Greyling 2001). A Sasol Gas specialist report on threatened plants along the new pipeline route being constructed from the gas fields of Temane and Pande in the Inhambane Province of Mozambique through Komatipoort (near the Mozambique border) to Secunda (near Johannesburg) noted the IUCN Red Data list endangered status of *S. aethiopicus* among those threatened species in the lower Escarpment region of the project area (Sasol 2001).

The 2000 Swazi Government Flora Protection Act lists the endangered *S. aethiopicus* (Sidvungula) as one of the specially protected flora (Swaziland 2000). According to the Swaziland Flora Database, *S. aethiopicus*'s Red Data Book status is EN A1d, formerly listed as Rare. Within Swaziland it is found in the Malolotja, Balegane, Komati Valley, and Piggs Peak areas, and it is said to be generally heavily utilised everywhere. The Malalotja Nature Reserve in north-west Swaziland extends over 18,000 ha, and its protected area subpopulation is evidently well known and utilised by local herbalists (Swaziland National Trust Commission 2009).

1.3. *S. aethiopicus* South Africa Distribution and Conservation Status

Medley-Wood and Franks' (1911a) original report on *S. aethiopicus* in the Kew Garden Bulletin recorded collection from Inanda at 540 m, and from Zululand at 'Ngoya, at 450-610 m. The specimen that flowered at the Durban Botanic Gardens in December 1910 was brought from 'Ngoya by Mr. Wylie, the Gardens' curator. They mention that the plant was first collected by the then already late W.T. Gerrard, one of a team of botanists working with the Garden in the 1870s (Medley-Wood and Franks 1911a). Crouch et al (2000) reported that historically natural stands of *S. aethiopicus* had been known from the Umhloti, Let and Umtwalume Valleys, and Inanda, Ongoye, Hlophenkulu, Dumisa, and Umbambasa. They consider that the report on its occurrence at Lusikisiki in Pondoland, Eastern Cape, may relate to the recorded trade of Wild Ginger to that region from Inanda in about 1880. According to Crouch and others (2000) and Cunningham (1988), Medley-Wood reported in 1900 the Basuto carrying off pack-horse loads of Inanda corms to Lesotho. *S. aethiopicus* had been one of the first plants recorded as part of the medicinal plant trade since the early 1800s, with another report from Burt Davy in 1910 of trade within the Transvaal (between Mpumalanga and Gauteng). In a separate report, in 1911 Medley-Wood and Franks documented the disappearance of *S. aethiopicus* from the Durban area 11 years earlier (George et al 2001), more particularly from its only known localities in the Inanda and Umhloti valleys, due to the Lesotho trade (Cunningham 1993). Cunningham (1988) in an article on the overexploitation of muthi plants in KZN, quotes Father Jacob Gerstner, who in 1946 wrote of "the lamentable process of extinction" of medicinal plants from overharvesting, and recommended as the solution cultivation, "taken up by state nurseries run on scientific lines." Cunningham (1988) cited the rise in the black population of KZN (the primary users of medicinal plants)

from 2,199,000 in 1960 to 4,766,000 in 1980, to a predicted 9.7 million by 2010, and pointed out that with increased population and continued widespread use of traditional medicine, it was inevitable that popular species would be overexploited in the wild and have to be cultivated or driven into extinction. Cunningham pointed out that *S. aethiopicus* has lost extensive natural habitat, such as through the replacement of 90% of the Coastal Forests with Sugar Cane as of 1980, and a 75% reduction of the Karkloof Forest between 1880 and 1980. In the 1970s *Kaempferia aethiopica* was added to the list of protected plants for the Transvaal (Onderstall 1978). The local KZN extinction of *S. aethiopicus* was widely noted and cited (Goodman 2004). Lawes et al (2007), in their study of the forests of the Maloti-Drakensberg transfrontier region, which joins Lesotho's Sehlabathebe National Park with KZN's Ukhahlamba Drakensberg Park, an area covering 8 113 km², 64% in Lesotho and 36% in KZN, confirmed that as of 2007 that *S. aethiopicus* was extinct in KZN outside protected areas. Cunningham (1993) also noted that as of 1993 68% of herb traders in South Africa nominated wild ginger as becoming scarce, the third most scarce plant after *Warburgia salutaris* (Bertol.f.) Chiov. and *Boweia volubilis* Harv. Ex Hook. The South African National Biodiversity Institute (SANBI) lists the plant's 2007 interim national status as Critically Endangered (though its 2007 Global Status is Not Endangered); the category is reserved for plants facing an extremely high risk of extinction in the wild (<http://www.sanbi.org/frames/documentsfram.htm>). Gordon-Gray et al (1989) noted commonplace transplantation from natural populations to vicinities of habitation either for cultivation or magical protection of inhabitants. They further noted that the bulk of traded material in the late 1980s was coming from the Transvaal and further east, and that due to depredation of natural stocks, commercial cultivation would be necessary to maintain supply and keep prices reasonable.

SANBI's report (Hankey and Reynolds 2002) refers to *S. aethiopicus* as a "forest floor plant." Crouch et al (2000) report that outside of South Africa natural stands have been reported to include up to 4000 plants, though 60% of sites hold fewer than 100. Within South Africa they map the natural stands still existing to northeastern Mpumalanga Province in east central area of Limpopo Province, with a small area in Swaziland. Numerous prior stands in those areas are now denuded of plants, as are all the former sites in KwaZulu-Natal. One unconfirmed site in northeastern Eastern Cape was also

noted. The natural habitat is deciduous woodland, wooded grassland and bushland, Acocks' veld type 9, Lowveld Sour Bushveld, within Tall Open or Closed Woodlands. As of April 2002 *S. aethiopicus* was recorded as extinct in Mpumalanga outside of protected areas in a Department of Water Affairs (DWAf) report. It has a Red Data status of CR A1abcd B1B2abcd (Emery et al 2002). Wild ginger was listed as an endangered species in the NATIONAL ENVIRONMENTAL MANAGEMENT; BIODIVERSITY ACT, 2004 (ACT 10 of 2004), one category lower than critically endangered, facing a 'high risk of extinction in the wild in the near future' as opposed to an "extremely high risk" (pp 4-5). The 2005 National Roads N1 Wild Coast Toll Road between East London and Durban: Environmental Impact Assessment Report for KwaZulu-Natal and Pondoland lists the plant as regionally extinct (National Roads 2005). Anomalously, Hoare's (2006) Pondoland (between the Mtavuna and Mthatha rivers) environmental impact study on proposed upgrading of the N1 Highway between Mthatha and Port Shepstone lists *S. aethiopicus* as "near endemic" and not included on the threatened species list.

The corms, typically without the tuberous roots, are regularly traded in the Durban muthi market. Discussions by this author (Hartzell) with traders in the Durban muthi market indicate that in 2008 corm supplies were coming from the northern border regions of KwaZulu-Natal, northeastern Mpumalanga, and Mozambique, with a less definitive report of some supply from one person in the Eastern Cape, or possibly some wild stands in the northern part of that province.

Citing Mander's earlier (1998) study, and according to data from 2002, *S. aethiopicus* ranked as one of the top ten traded plants in KZN (9th) and Mpumalanga (4th), but not the Eastern Cape (Dold and Cocks 2002). Masevhe's interviews with villagers around Venda in Limpopo found respondents reporting that the plant was becoming so scarce in their area that some were traveling to Zimbabwe for wild-harvesting (Masevhe 2004). The drop in availability of *S. aethiopicus* was reflected in a study of the Gauteng medicinal plant markets: in 1995 20% of the Witwatersrand muthi shops sold the species; by 2001 only 8% of the traders at the Faraday market sold the species. The estimated number of bags (50 kg) bought by 189 shops in 1995 was 20; in 2001 only one 50 kg bag was bought by 164 traders (Williams et al 2007). Manzini's (2005) interviews with 150 traditional healers in Mpumalanga Province villages found that

96% of the respondents said they used the thickness of the corm for determining the selling price of the plants. Seventy-seven percent considered that there was no difference between cultivated and wild plants, while 5% reported growing the plant, 40% purchased it, 47% collected it from the wild, and the others were unclear about the source. Fifty-four percent considered the plant scarce or very scarce in the wild, and 20% thought it extinct. Sixty-one percent of the healers were ignorant of the length of the growing season for *S. aethiopicus*. Mander (1998) ranked *Alepidea amatymbica* var. *amatymbica* Eckl. and Zeyh. and *Siphonochilus aethiopicus* as the most sought-after medicinal plants, based on trade popularity, in the Bushbuckridge area. Mander (1998) calculated that 31.2 tons of corms are traded in the Durban medicinal trade every year.

Crouch et al (2000) gave a comprehensive report on *S. aethiopicus* in 2000. They reported that there were a few wild stands in Limpopo, Mpumalanga and Swaziland, but about 2/3rds of these were outside of nature reserves and so severely threatened. Nichols (1989) first brought back bisexual clones to the Parks, Recreation and Beaches Department, Durban, from Ian Garland's farm at Mtunzini on the Zululand Coast in 1980. Mr. M.P. Cele gave the Parks Department another clone in 1983 (Crouch et al 2000 and Nichols and Cele, personal communication 2000 and 2003). The two plants produced very different flowers in November 1983. Two more clones given by the late John Huntley and Margaret Hoile both turned out to be bisexual and established themselves well in the Silverglen Medicinal Plant nursery. The Silverglen Nursery team continues clonal propagation of *S. aethiopicus*, where corms are still sold to the public every season (the corms used for the crop trials in this MSc project were purchased from Silverglen). The National Botanical Institute produced thousands of plants by tissue culture which were sold and distributed to nurseries (Scott-Shaw 1999).

1.4. Cultivation Information for *S. aethiopicus*

Public cultivation information for *S. aethiopicus* is poorly developed. A search of the FAO database on crops (www.ecocrop.fao.org) provided no information in the Crop Data Sheet for *S. aethiopicus* other than that it is an erect, perennial herb, with roots/tubers, and grown on a small scale. The EcoPort lists the plant's climate zone as "subtropical, dry summer" (Cs), and reported its main use as a food and beverage, containing starch and vitamins.

In 1989 Nichols published "Some Notes on the Cultivation of Natal Ginger (*Siphonochilus Aethiopicus*)" (Nichols 1989) wherein he describes the source of the various clones, and noted the plant "is easily grown in the warm subtropical east coast and lowveld regions of the country." Nichols stated that the dormancy period was "June to November." (Though dormancy did begin in June, we found in our cultivation trials that the plant sprouted in September in the Pietermaritzburg area, suggesting that the dormancy period is more precisely June to September.) Nichols cautioned against removing the tuberous roots ("tuber-like swellings") when splitting the corms as they provide water and nutrient storage for the energy burst that produces the flowers and later the leaves in the spring. Nichols reported that seed from bisexual plants took about a year to germinate. They discovered this in 1987 when, after the seeds from the 1986 crop failed to germinate after four months, they were discarded into the waste pile, but germinated there after a year's time. The next season seeds were replanted along with the corms, and also germinated a year later. Both Kirstenbosch and Durban Botanic Gardens have developed tissue culture, but this is an expensive way to source plant material. Silverglen sells corms, but also expensive at R6.00 each (as of 2006). In Durban, with its sandy, highly leached soils, the plants responded well to high levels of organic matter. (Tests by Gareth Olivier and Mark Laing in the UKZN Controlled Environment Research Unit in Pietermaritzburg have shown that liquid feed also produces substantial root mass.) Nichols also noted that flowering is complete in mid-December, and only after the completion of flowering do the leaves continue to grow and expand. (This was not consistent with our field observations, which showed some plants flowering as late as March, and steady growth and expansion of leaves from the time of first emergence). Nichols suggests "growing the plant as one would grow commercial ginger is the ideal way by digging trenches and filling them with good compost, to encourage good root growth." He suggested the most efficient propagation method by splitting corms or tissue culture, and pointed out that "it has never been attacked by any pests or diseases since I have had it in cultivation." Similar information to the Nichols report appears in the Institute of Natural Resources pamphlet on growing muthi plants (Mander et al 1995). Generally Nichols' observations, valuable and at the time unique, are more useful for horticulturalists than farmers.

There have been several small studies completed on *S. aethiopicus* cultivation. McCarten et al (1999) at Silverglen Nursery published a one-year study examining the effect of propagule size, planting density, and soil type on yield. Source material was the propagules produced vegetatively each year by Silverglen from the original Nichols, Cele etc. clones. The authors found optimal yield at a total propagule planting mass of approximately 3.0 t ha⁻¹, using small propagules (6.2g-7.8 g), compost-enriched soil (250 m³ ha⁻¹ compost, pH 6.4, EC2.2 mS/ cm, AFP 12%), and a planting density at 15 x 15 cm (444,444 plants ha⁻¹). The one time, nine-month trial was initiated 10 October 1997, harvested 15 June 1998, using corms with the roots removed, and dipped in Fonagrid fungicide and air-dried prior to planting. Silverglen is located just in from the coast, 200m above sea level, with 800-900 mm annual rainfall. In the sandy, leached soils of the Silverglen reserve the compost-enriched planting yielded approximately five times (45 t ha⁻¹ vs. 9t ha⁻¹) in the un-enriched soil, and 4-6 corms per propagule vs. 2-3 corms for the un-enriched soil (McCarten et al 1999). The plants were regularly weeded and watered during the growing season.

Spring (2003), an employee of the KZN Provincial Department of Agriculture and Environmental Affairs, conducted a year of medicinal plant cultivation trials including *S. aethiopicus*. Using a split plot design, with three replications, and grading the corms small, medium and large, he tested cultivation manually (hand land preparation, with addition of kraal manure and straw mulch to reduce weed pressure and retain moisture, hand weeding), by machine (tractor and rotovator for fine-tilthed soil, fertilizer as per onions, spraying of herbicides) and in tyres (mixing organic matter and soil, hand weeding and spot herbicide sprays). The manual and rotovated treatments produced more daughter corms than the tyres, and the larger corms produced more daughters than the smaller corms. Spring concluded, "Siphonochilus prefers a warm, well-drained soil as the rotovated treatments had slightly better growth in its medium and large corms, when compared to the other treatments. A rotovated or non-mulched manual treatment would be the best method of cultivation." Spring noted nematode damage at planting, callus formation to combat fungal infection on opened tissue on the split corm, some millipede damage, some eating of foliage by grasshoppers, and some butterfly pupae on plants, but no major, crop-threatening pest or disease pathogen problem. Unlike other authors, he also noted the dying off of the mother corm once it produces

the daughter corms. He suggests replanting of daughter corms as soon as possible after lifting, and taking care not to disturb the roots (Spring 2003).

Mashudu Ronnie Masevhe finished an M. Inst. Agrar. Degree project in 2004 through the University of Pretoria, studying mulching and plant population density of *S. aethiopicus*, using tissue-cultured plants produced by CSIR, and treated with copper oxychloride to prevent fungal growth during storage (Masevhe 2004). Wheat straw mulching was investigated for moisture retention and weed control, and 15 cm, 30 cm, and 45 cm spacing for yield and quality, with field experiments on the Hatfield Experimental Farm, University of Pretoria, at an altitude of 1370 m, with annual rainfall 600-700 mm (Oct-March), and frequent winter frosts. Results showed optimum yield (measured in terms of fresh corm mass) for non-mulched plants at 30 cm, but better yields at 15 cm spacing with mulch. Planted corms were 3 cm (834) and 4 cm (326) with 1160 total planted on the 11-12th of December 2001, in a sandy loam, +/- 7 cm depth. Corms were harvested the week of 19th June 2002, giving a 7-month growing term. Mulching proved to prevent some night-time soil heat loss, reduced day-time soil temperatures, and stabilized maximum daytime temperatures. Mulching was also effective in improving soil water retention, and in reducing weed pressure during the early part of the season. The mulch was not replaced, resulting in lower weed suppression later in the season. However, the 6 cm of mulching of fresh straw apparently resulted in "general poor emergence of the plants" (p. 39). Masevhe erroneously stated on page 3 that although corms harvested during the growing period have roots, "those taken during the dormant period, when the plants are leafless, have no roots on them."

Another study was completed by Tlangelani Cedric Baloyi for his MSc Agric in the Dept. of Plant Production and Soil Science, University of Pretoria, 2004, examining nitrogen, fertigation, and growing medium (Baloyi 2004). Six levels of N were used in the field trial, (0, 50, 100, 150, 200 and 250 kg ha⁻¹), applied at planting as limestone ammonium nitrate. Results showed positive linear relationship in emergence, plant height, fresh corm and enlarged root mass, and length of enlarged roots. Number of corms was not affected by N. A parallel trial in tunnels examined fertigation frequency (0.25 L/day, 1 L/day, 2 L/day, 2 L/2nd day and 2 L/week) in both pine bark and sand growing media,

using 200 total propagules (p. 39). During early growth phase only 2 L/day did not help growth, but during later growth phase only 2 L/week proved inadequate. Pine bark showed increased growth at early stage, but sand showed increased growth in later stage; the author concludes that in fertigation setups, plants should be started in pine bark (up to 112 Days after emergence), then moved to sand for the remainder of the growth cycle. Neither fertigation frequency nor growing medium affected fresh corm and enlarged root yield in the tunnel trial. Laboratory analysis of enlarged roots showed a linear relationship to increased Nitrogen (0, 50, 100, 150, 200 and 250 kg ha⁻¹) with an increase in glandular cells involved in essential oil production (from two at 0 kg ha⁻¹ to eight at 250 kg ha⁻¹). Varying fertigation frequency and growing medium showed no glandular cells in plants with 2 L/day, and sixteen glandular cells in plants grown in sand with lowest fertigation (2 L/week). Baloyi recorded optimum emergence at 250 kg ha⁻¹ N, with the second highest emergence at 50 kg ha⁻¹ N, though he noted that emergence was generally poor which he attributed to corms having already sprouted, and some sprouts drying off before planting. Unfortunately Baloyi did not record either the actual dates of planting, nor the total number of propagules, so it is difficult to assess the value of his data in terms of a full growth season.

Another study from U. Pretoria was T. Manzini's M. Inst. Agrar (Plant Production) "Production of Wild Ginger (*Siphonochilus Aethiopicus*) Under Protection and Indigenous Knowledge of the Plant from Traditional Healers" in 2005. Manzini's cultivation trials, in 4 liter plastic bags with pine bark, were started 20 December 2001, for 100 plants in a 10 x 30 m plastic tunnel (240 micron light grey stabilized polyethylene film, with two top ventilation flaps) and 100 plants under 30% white shade-net, also 10 x 30 m, measuring yield and the effect of different harvesting periods (10 plants each harvested on 28/06/2002 and 29 plants each harvested 28/09/2002). Source material was produced in a CSIR field, and corms were dipped in copper oxychloride prior to planting, with regular fertigation (12 x day, 2 minutes each during daylight hours). The study concluded that plants grown in tunnels and harvested at the end of June produced better results (p. 47), noting that the resumption of vegetative growth after the dormancy period entails usage of the reserves of plant nutrients stored in the corms and roots (p. 51).

General information on *S. aethiopicus* cultivation has been published in a number of sources, predominantly for the small-scale grower, horticulturalist, gardener or nursery manager. Keirungi and Fabricius (2005) reported the preferred growing medium to be slightly acid, with high humus content and excellent drainage, such as three parts well decomposed compost and one part coarse river sand. The corms should ideally be planted 2-3 cm below soil level, and grow well in deep raised beds. An experiment with winter watering of the plants at Kirstenbosch resulted in plants not flowering in spring, and showing delayed vegetative growth. Plants were found to need at least full (not shaded) morning sun in order to flower. De Lange et al (1991) suggested that since little was known about wild ginger's cultivation requirements, one should use commercial ginger cultivation information in South Africa as a guide. They suggested that topsoil should be at least 250 mm deep, the soil loose and friable with high organic material, good drainage, and high water holding capacity, with planting preferably on ridges. Brief small-scale horticulture and gardening instructions appear in the Catchment Action booklet (Mander et al 1995), and on a variety of South African web pages, where the information appears to be largely copied from Keirungi and Fabricius (2005), or adapted from the Nichols (1989) paper.

1.4.1. Vegetative Propagation

Vegetative propagation is the most practical way to produce starting material for *S. aethiopicus*, given the scarcity and long germination time of seeds. Crouch and Symmonds (2002) recommended lifting corms with a fork in mid to late winter (July-August), being careful to include the root tubers, cutting off the residual dry leaves, washing the soil off the corm clump, and storing material in a cool, well aired and dry area. After the first spring rain in September, they recommend soaking the corm clump in water overnight to improve turgidity, so that clean breaks can be made either by splitting off the daughter corms, or longitudinally sectioning the corm, making sure to keep the root tubers attached. They recommend coating exposed surfaces with flowers of sulphur or Benlate, and replanting about 15 cm apart, with immediate watering and fertilizer application. Soil preparation is recommended by fertilizing with well-rotted fowl manure, and an anti-eelworm preparation. Top-dressing with composted fowl manure or 2:3:2 at 60 g m⁻² is recommended in mid-January to help ensure a second

flush of corm segments. Nursery tests and field observations indicate that winter warming of the soil around the corms leads to more profuse flowering.

An interesting angle on natural propagation of *S. aethiopicus* appears in Crouch et al.'s review (2000). Rangers in Kruger National Park observed that the only three wild stands of *S. aethiopicus* were all under marula trees, the favored fruit of elephants. Elephants scoured a large trench through the middle of a *S. aethiopicus* population, and a ranger reported observing that the elephants had traveled large distances to obtain the corm. The authors suggest that elephants may have played a role in the dispersal of large vegetative propagules (Crouch et al 2000:119).

1.4.2. Tissue Culture and Micropropagation

A tissue culture protocol has been developed for *Siphonochilus aethiopicus* and the corms have been grown with this protocol at the UKZN facility in Pietermaritzburg (Nora Choveaux, personal communication 2006.) Another tissue culture protocol was developed by Margaret Appleton of the National Botanical Institute, working in the Durban Botanical Garden nursery (Margaret Appleton, personal communication, 2007). Appleton kindly provided some additional background by email: Plants were first obtained by Silverglen Nature Reserve in the 1980's. With the establishment of their highly endangered status at that stage, plant cultures were initiated both at Kirstenbosch and at the NBI's Micropropagation lab in Durban. Kirstenbosch no longer produces wild ginger. The Durban NBI protocols have been altered several times since first developed, but not published. Most plants cultivated in KZN since the 1980's have originated from Durban's NBI cultures and sold via either the P&D Sales Nursery or the Silverglen Medicinal Plant Nursery. It is virtually impossible to obtain plants from the wild, but since the micropropagation protocol is the property of the Production and Display Nurseries, it remains unpublished.

De Lange et al (1991) reported developing a micropropagation protocol in 1991, using essentially the same procedures as for culturing *Zingiber officinale*, with corm buds sterilized and put into agar-solidified medium. Multiple reesterilizations were required (also typical), then shoot proliferation was obtained using a multiplication medium with relatively high cytokinin content. Some *in vitro* shoots successfully rooted with

commercial rooting powder and mist beds with bottom heating, with best results obtained with *in vitro* root initiation on an auxin-containing medium prior to transfer to the nursery. The Agricultural Research Council in Nelspruit reported in 2003 the development of a tissue culture (micropropagation) and hardening off protocol for *S. aethiopicus*, with plants then multiplied and supplied to conservation authorities. Details were not available.

(<http://www.arc.agric.za/institutes/itsc/main/highlights/biotech.htm> 13 Oct 03).

ARC/VOPI has *Siphonochilus aethiopicus* in its list of genetic material as of 20-03-2008 but the material is not available for exchange (http://www.arc.agric.za/home.asp?pid=4,2.4765_arcvopigeneticmaterials.updated.xls)

Although tissue culture may be popular with biotech funders, it is not really a practical option for most farmers for source material, due to the high costs of purchasing tissue-cultured plants. It has however played a vital role in ensuring the survival of this plant and others.

1.5. Medicinal Plant Biochemistry

Plants are collectively estimated to produce over 100,000 low-molecular mass secondary metabolic compounds, which are usually not essential for the plant's basic metabolic processes. Primary products include carbohydrates, lipids, proteins, heme, chlorophyll, nucleic acids, all common among plants and part of primary metabolic processes required for building and maintaining plant cells (Dixon 1986). Secondary products, while not involved in building and maintaining plant cells, have been shown to function in defensive roles against herbivores and pathogens (sometimes creating localized antimicrobial environments around invading fungal and bacterial pathogens), attractant roles for pollinators and symbionts, management of eco-system stressors (weather, light, mineral nutrients, etc.), as plant growth regulators, and as modulators of gene expression and signal transduction. Most flavonoids, in addition to their antifungal and antibacterial properties, are efficient absorbers of UV light and so protect the plant from UV damage. Secondary metabolites have also been shown to confer frost tolerance, provide allelopathy, be involved in nutrient storage, provide structural reinforcement, mediate stigma-pollen interactions, regulate biochemical processes, and signal to mutualists (Rausher 2001). Secondary metabolism in plants can be influenced by biotic

and abiotic factors, including plant pathogens. Phytopathogens can interfere with secondary metabolite production and storage organelles, forcing the plant to adopt alternative biosynthetic pathways that can alter secondary metabolite chemical structure and availability, causing as much as a 50% drop in therapeutically significant secondary metabolites (Bruni and Sacchetti 2005:120). Endogenous plant-defense molecules, typically secondary metabolites, have been used for millennia in traditional medicine globally, with the medicinal effects typically produced by combinations of secondary plant products. Examples are the antidepressant-associated compounds hypericin and pseudo-hypericin from St. John's Wort which appear to be used by the plant to defend itself from insect herbivory (Briskin 2000). Such secondary plant metabolites are increasingly being shown to have direct homologues in animals (SurrIDGE and Anson 2001).

1.6. Traditional and Contemporary Use of *S. aethiopicus*

The traditional use of *S. aethiopicus* for coughs, colds, asthma, headache, candida and malaria, menstrual pain and dysmenorrhea, as well as horse sickness and for stupefying horses has been reported by several authors (see for instance Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997; Crouch et al., 2000; Van Wyk and Gericke, 2000). Gericke reported in 2001 that *S. aethiopicus* is regarded as a natural anti-inflammatory and is sometimes referred to by indigenous South African healers as "our Panado" (paracetamol). Clinical indications also include tension headache, asthma, sinusitis and sore throat, candida, PMS and menstrual cramps, and fever in children. Treating his own young children (ages 4 and 20 months), Dr. Gericke reported using a 50 mg table crushed as powder and mixed with a little castor sugar to bring down fever from 38.5° C to 37.5-37.7°C, with the advantage over paracetamol of being slightly sedating so the child would tend to rest. Sublingual eight-hourly doses of 30 mg crushed with castor-sugar was effective in reducing acute asthma in his 20-month old daughter, with the effect noticeable in about 15 minutes. In clinical practice with AIDS patients, 100 mg tablets chewed and taken 8-hourly were found effective for oral and oesophageal thrush, with full recovery in 2-3 days; oral treatment was also reported effective by a colleague for vaginal thrush. The juice was reported as traditionally used as a douche, and effectively used by a respected Zimbabwean healer to treat malarial fever and the accompanying severe headaches with a decoction of *Sclerocariya birrea* and

Siphonochilus aethiopicus (Gericke 2001, 2002). Van Wyk et al (1997) indicate that the plant was recorded being as traditionally used for asthma and dysmennorrhoea, but indicate preparation as only by fresh chewing of corms and roots. Discussions by this author (JF Hartzell) with traditional healers in Durban in 2005-2008 indicated that the most common individual usage of *S. aethiopicus* was to chew the freshly harvested corm for sore throats and chest ailments. For larger scale use by professional herbalists, the corms and swollen roots are typically dried, ground up into a powder, and used as an ingredient in a wide variety of *muthi* mixtures (personal communication, P. Cele, and M. Dlamini, 2005-2008). One might suggest *in vitro* or *in vivo* tests of the effects of human saliva on the fresh chewed corms as a promising route for investigation.

Interviews with traditional healers, vendors of medicinal plants, and others in nine villages around Venda in Limpopo in 2004 revealed 84% of 76 respondents using it for stomach pains (Masevhe 2004). One hundred and fifty traditional healers interviewed in Mpumalanga Province villages considered the plant useful for coughs, colds, flu, hysteria, malaria, menstrual disorders, headaches and toothaches, and protection against lightning. Healers reported washing harvested corms and roots, dried them with newspapers, and storing them in airtight containers. Roots and corms were sometimes boiled in water and half a cup of the tea given to patients; other times the root or corm was chewed fresh (Manzini 2005).

According to Siedemann (2005) in his book on world spice plants, *S. aethiopicus* is also used as a spice and flavorant throughout tropical Africa, Senegal and Niger, from East Africa to southern Africa. Crouch et al (2000) report a similar use for *Siphonochilus kirkii* Zingiberaceae in Malawi, where dried and powdered roots are used as an adaptable seasoning, with tubers sometimes used in chicken stuffing.

1.7. Earlier and Recent Commercial Activity

Crouch et al (2000) summarized early 20th century industrial assessment of wild ginger for the perfume and soap industries in Europe (235 lbs were shipped to the Imperial Institute in 1915 for assessment). In both cases the industrial analysts, using steam distillation of dried rootstock, deemed the 1.8% oil extracted “not be of much value” (Crouch et al 2000). Makunga et al.’s (2008) review of South Africa’s emerging natural

products sector mentions in a table that *S. aethiopicus* tinctures, tablets and oils are being sold in Limpopo, Mpumalanga, KwaZulu-Natal and the Eastern Cape through health shops, pharmacies, supermarkets and/or internet-ordering systems.

Phyto-Nova, a modern South African company marketing, among other products, *S. aethiopicus* tablets, says they are indicated for headache, influenza, mild asthma, sinusitis and sore throat, thrush, candidiasis syndrome, PMS, and menstrual cramps (www.phyto-nova.co.za, accessed April 23, 2007). Internet searches turn up several companies selling tablets of *S. aethiopicus*. African Drugs.com (Kommetjie, South Africa) sells African Ginger (Phyto Nova brand) at 60 x 100 mg tablets for Euro 9.99. House of Health sells 100 mg *Siphonochilus aethiopicus* "elite chemotype", 60 tablets for R108.00, stating that "If a person is experiencing a sore infected throat, or oral thrush the tablet can be chewed with a little water, and swallowed," and that the plant is "helpful as a supplement in the following conditions: Arthritis, asthma, *Candida albicans*, colic, constipation, cramps, colds and flu, diarrhoea, digestive disorders, female health problems, halitosis, headaches, heartburn, impotence, indigestion, menstruation, mood swings, oral thrush, premenstrual syndrome, sinusitis, throat infections, thrush." GardeningEden.co.za lists *S. aethiopicus* as one of the plants recommended for asthma, cough and bronchitis. The Big Red Warehouse (<http://www.bigredw.com>) sells 150 mg Vegecaps of *S. aethiopicus*, stating the traditional use as anti-inflammatory for colds and flu, and noting the precaution that pregnant and lactating women and anyone under medical supervision should consult a doctor before use. Helmut Wilderer in Paarl includes *S. aethiopicus* in his product FYNBOS, a healing herb bitter sold at R160 for 500 ml. (<http://www.wilderer.co.za/products.html>). Parceval Pharmaceuticals, Wellington, South Africa, sells a *S. aethiopicus* tincture of 200 mg/ml $\emptyset = 30\%$ (<http://www.parceval.co.za>). Plant products have also appeared in Germany, such as Tropische Pflanzenwelt – Michael Peuthert – Fürst-Ernst-Str. 4 – 31675 Bückeberg, *Siphonochilus aethiopicus* Euro 29,00 (www.tropische-pflanzenwelt.de). In Camps Bay near Cape Town, The Twelve Apostles Hotel and Spa has had on their Azure Restaurant, under starters, Fynbos Button Mushroom Samoosas, made "with tinsel flower essence (*Alepidea amatymbica*), coriander and Wild Ginger (*Siphonochilus aethiopicus*), Served with an Apricot and Lemon Geranium (*Pelargonium betulinum*) Chutney." (<http://www.12apostleshotel.com/dining>). Frequently though web searches on *S.*

aethiopicus turned up websites that purport to present reliable scientific information on *S. aethiopicus*, which is frequently referred to as Wild Ginger or African Ginger. Careful reading shows that source material is usually copied without citation from published academic sources, but then sometimes mixed indiscriminately with information on *Zingiber officinale*, as though the research on the latter plant is equally applicable to the former (see for instance <http://herbalafrica.co.za/HerbsAGinger.htm>, accessed March 14, 2006).

On 8 February, 2002 the US Government's FDA responded to an application by one Ms. Fedra Sembiente, from Power Africa, Inc. of New Jersey, for a product containing *S. aethiopicus* that claimed it to be a natural anti-inflammatory, that may help relieve tension headaches, influenza, sinusitis, sore throats and mild asthma, for fever or colds and flu, anti-candidal, effectively treat the fever of malaria, as well as the severe headache that accompanies the fever, treatment for oral and oesophageal thrush in AIDS patients, and that oral treatment with African Ginger is effective for vaginal thrush. The FDA officer, Rhoda Kane, rejected the application as an "unapproved new drug" (FDA 2002). The International Bulb Society, based in Sanger, California, included *S. aethiopicus* in its 2000 issue (Vol. 55) among other flowering bulbs (www.bulbsociety.org).

Mander (1998) reported in 1998 that *S. aethiopicus* regularly traded at US\$ 100/kg (R 450/kg). Masevhe's interviews in 2004 with villagers around Venda in Limpopo found single corms selling at prices from R5 to R50 (R5-R15 (15%), R16-R20 (24%), R21-R35 (15%), R36-R50 (47%)). In 2007 individual corms were selling for R5 each at the Durban muthi market (JF Hartzell, personal purchases). Makh. Ma Dlamini, the head of the Durban muthi market, reported in 2008 that traders would be willing to buy in organically/naturally-cultivated bulbs at R100 per 2 liter container (personal communication).

Marketing is not necessarily straightforward, any more than is cultivation. One south-coast farmer produced several hectares of wild ginger in 2007 but could not find a market for it (Laing, personal communication 2007). Durban muthi traders interviewed by the author (Hartzell) in 2007 reported buying in wild ginger bulbs from Mozambique

or northern Zululand, or from the Eastern Cape. While these reports are not necessarily reliable, they do confirm the lack of sufficient (or acceptable) local supply of the crop. As of May 2008, reliable estimates are that perhaps 1 out of 20,000 tons traded in Durban is cultivated, from a handful of growers, with a few healers growing for their own needs (Steve McKean, personal communication 2008).

1.8. Biochemical and Toxicity Studies

Gericke (2002) has suggested that the limited range of *in vitro* bioassays used so far to assess traditional medical usage may have led scientists down some false paths. Studies of such indigenous use of *S. aethiopicus*, either by chewing the fresh root, or by making a tea (aqueous extraction) may reveal as yet unidentified compounds to help validate traditional uses. As is clear in the following review of published research, no studies have yet focused on the enzymatic effect from saliva of freshly chewing roots and corms and its health implications. A good comparative example of assessing traditional medicines *based on the actual methods of their traditional use* is provided by attempts to study oxytocic activity of *Montanoa tomentosa*, a plant used by Aztecs and modern Mexicans to stimulate uterine contraction. After a series of failed studies (i.e. no detectable effect) using non-aqueous extracts, in 1979 active components montanol and zoapatonaol were isolated from the plant using aqueous extraction to prepare a tea as per indigenous practice. Zoapatonaol has since been confirmed to cause contractions in uterine smooth muscle (Etkin 1986).

McGaw et al (1997) examined *S. aethiopicus* prostaglandin synthesis inhibition using leaf material dried at 50°C and stored in brown paper bags at room temperature. No information was provided on the growth location of material collected. Ethanol extracts showed greater (86-96%) inhibition of cyclooxygenase compared to water extracts (20-40%) or indomethacin (data not provided).

In 1999 a group in van Staden's lab examined the cyclooxygenase inhibiting activity of several medicinal plants including *S. aethiopicus*, as the excessive production of prostaglandins by the myometrium and endometrium induce the painful uterine contractions characteristic of dysmenorrhoea (Lindsey et al 1999). *S. aethiopicus* was one of three plants whose ethanol extracts from dried and powdered corms and leaves,

dissolved in water and heated to 45°C, showed highest inhibitory activity, though none were able to relax or mitigate pre-contracted uterus muscle. A follow-up paper (Jager and van Staden 2005) suggested that the COX inhibition by *S. aethiopicus* may be due to a combination of compounds, not a single molecule – though no data for this suggestion was provided.

Zschocke et al (2000) conducted a follow-up to McGaw's 1997 work. Three greenhouse-grown, summer-harvested plants from the KZN Nature Conservation Services (2 young, one older) were dried for two days at 50°C, ground and extracted with ethyl acetate. An *in vitro* comparison of leaf, stem, corm and root extracts found that leaf and stem were equal in showing greater cyclooxygenase-1 inhibition than the equally inhibiting corm and root, though the mature corm showed approximately 50% inhibitory activity as compared with approximately 70% activity for the young leaves (highest of all measures; data approximated from bar graph). The authors caution though that the leaf extract activity increased over several weeks (differential data not provided), and suggest this may have been the result of breakdown products. As a result, they concluded that it was not possible to say that the same active principles are present in the leaf/stem and corm/root, and suggested that the COX-1 inhibition by the different plant parts may have been caused by different compounds.

Light et al (2002) showed antibacterial activity from ethanol and ethyl acetate extracts (but not aqueous extracts) of dried, ground material of stock plants summer-harvested (mid-growing season) at the University of Natal Botanical Garden, Pietermaritzburg) at minimal inhibitory concentrations (0.78 to 3.13 mg/ml against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*), and (with lesser effect) against Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*). No activity was found in antiviral (HSV-1, HSV-2, Influenza A), anthelmintic (*C. elegans*), antischistosomal (*Bulinus africanus*) or biochemical induction assays. Seasonal variation was tested by harvesting plants pre- (green leaves) and post-senescence. There was little difference found between leaf and corm extract activity pre- and post-senescence, though leaf antibacterial activity was lost at senescence while alpha-root activity increased. A slight loss of corm antibacterial effect after drying suggested higher activity in fresh corm. The Light group's anti-inflammatory COX-1 and COX-2 assays showed no

activity with aqueous extracts, but high levels of anti-inflammatory activity from ethanol and ethyl acetate extracts. Aqueous extracts tested against Vervet Monkey secondary kidney cell line (VK cells) showed cytotoxicity, a result that has not been followed up by other researchers.

Hutchings (1996) and Van Wyk and Gericke (2000) have suggested that monoterpenoids and sesquiterpenoids in the volatile oils in *S. aethiopicus* may be active against colds, cough, and influenza (see also Light 2002). Using material from the commercial herbal supplier Parceval, Wellington (near Cape Town), a group in van Wyk's University of Witwatersrand lab hydro-distilled the fresh roots and corms for 3 hours, yielding a yellow oil (0.1% wet weight for both corms and roots). The major compounds in the oil of both roots and corm are 1,8 cineole, (E)- β -ocimene, *cis*-allocimene, and (roughly 20% of the oil composition) the furanoterpenoid (in two derivatives) as reported by Holzapfel et al (2002) who distilled crushed fresh roots from several plants for just one hour): the major compound 4aaH-3,5a,8ab-trimethyl-4,4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one (C₁₅H₁₈O₂) and the minor compound 2-hydroxy-4aaH-3,5a,8ab-trimethyl-4,4a,9-tetrahydronaphtho[2,3-b]-furan-8-one (C₁₅H₁₈O₃). In total, the roots yielded 70 compounds, the corms 60 of virtually identical composition (Viljoen et al 2002). Jager and van Staden later showed antibacterial activity of the furanosesquiterpenes isolated in the Holzapfel (2002) experiment: furanoeremophil-2-en-1-one (8,12-epoxy-2,7,11-eudesmatrien-1-one), though the specific bacteria species, required concentrations, etc. were not identified in the paper (Jager and van Staden 2005). Freeze-dried and powdered corms were extracted with ethyl acetate by a group in Peter Smith's lab at UCT (Lategan et al 2009) and three additional novel furanoterpenoids (pale yellow solids and oily solids) showed *in vitro* activity against *Plasmodium falciparum*, and statistically significant *in vivo* activity in a mouse strain, validating the traditional use against malaria, and Gericke's (2001) report on the Zimbabwean healer, and his (2002) suggestion to seriously investigate this potential use. Solid phase extraction fractions showed greater activity than isolated compounds. None of the isolated compounds showed significant activity against *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* or *Candida albicans*. *S. aethiopicus* contains none of the terpenoids of the oil of *Zingiber officinale* or 'real ginger'.

Verschaeve's lab (Taylor et al 2003) tested apolar (dichloromethane) and water-soluble (methanol/water (9:1)) compound extracts of 51 South African medicinal plants for genotoxicity in the micronucleus and alkaline comet assays using human peripheral blood lymphocytes. For both extracts *S. aethiopicus* was non-toxic to human peripheral blood lymphocytes in the micronucleus test, but results showed genotoxicity of methanol extract of *S. aethiopicus* ($p < 0.01$) in the comet assay. Later studies in Verschaeve's lab using the dichloromethane and methanol-water extracts with the well-known bacterial anti-mutagenicity Ames test showed no toxicity (Verschaeve and Van Staden 2008).

Steenkamp et al (2005) conducted a study on the anti-oxidant or pro-oxidant effect of 13 medicinal plants including *S. aethiopicus* using hot-water infusions and methanol extracts, and measuring hydroxyl radical (HO*) scavenging and protection ability of normal human peripheral blood mononuclear cells against lipid peroxidation and DNA damage. The specimens were provided by University of Witwatersrand Medical School's Adler Museum, though no further provenance information was provided. Plant material was suspended in water and then brewed as a tea for 15 minutes. Overnight incubation with 10 ml methanol provided the methanol extraction. *S. aethiopicus* water extract showed the highest HO* scavenging activity (84%) of all the plants; its methanol extract showed approximately 72% scavenging, the third lowest of the plants. The water extract showed the highest lipid peroxidation, which is initiated at the membranes of human peripheral blood mononuclear cells at just over 50%, with its methanol extract showing only about 5% activity. The methanol extract of *S. aethiopicus* showed the highest DNA damage to the human peripheral mononuclear cells. The authors suggest that this DNA-damaging ability may be one of the mechanisms of the plant's anti-bacterial activity.

Gaidamashvilli and van Staden (2002) analyzed *S. aethiopicus* for lectin-like proteins from late-autumn harvested fresh corms and roots of plants grown under shade at the University of Natal Botanical Garden. Material was cut up, and homogenized in a blender with phosphate buffered saline solution (0.15M NaCl, 40 mm KH₂PO₄, pH 7.4). Relative to the other plants tested, *S. aethiopicus* showed high minimal concentration of protein to cause visible agglutination, and low specific activity after fractionation with a

mmonium sulphate. It was also on the low end of sugar-binding specificity of plant hemagglutinins among the plants tested.

Water, ethanol, and hexane extracts of fresh and 90-day old *S. aethiopicus* were tested by Stafford et al (2005) against four bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*) and for COX-1 inhibitory activity. Ethanol extract of *S. aethiopicus* (along with several other plants) showed a doubling (i.e. half the minimum inhibitory concentration required) of anti-bacterial activity against three of the four test bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*) at 90 days' storage compared with fresh material, with no change of activity against one bacteria (*Escherichia coli*). Longer-term (1 and 5 years) antibacterial storage effects were not determined for *S. aethiopicus*. After 90 days' storage, the water extracts of *S. aethiopicus* lost approximately half their percentage inhibition prostaglandin synthesis effect, but the ethanol extracts retained approximately the same percentage inhibition. The authors note that the drying process itself (oven drying at 50°C for 24h) may have had biochemical effects that were not separated from the storage effects (20°C in brown paper bags). One might also mention that normal traditional-healer drying method, using open air drying in Sun or shade, shielded from the rain (personal communication 2006, MP Cele) does not involve intense heat as in the Stafford et al (2005) experiment.

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Chapter 2: *S. aethiopicus* (Schweif.) B.L. Burt. Growth Behaviour and Field Observations



Figure 2.1 2006-2007 Field Trial of *Siphonochilus aethiopicus*; shade trial to the left, compost and spacing trials in foreground; fertilizer trial to the right.

2.1. Introduction and Overview of Literature on Botany and Growth Behavior

The name *Siphonochilus* comes from the Greek *siphono* for tube, and *chilus* for lip, referring to the shape of the flower. *Aethiopicus* refers to Africa, as the species and the genus are abundant in Africa (see Distribution section, Chapter 1) (Van Wyk and Gericke 2000). Though many authors have referred to *S. aethiopicus* as a 'rhizome,' presumably due to the similarity of its aroma to that of common ginger, *S. aethiopicus* is a geophyte, and is in fact a corm, a rhizomatous corm, or a conical corm, the last being the preferred term by some botanists. Geophytes are herbaceous plants with underground storage organs. Typically the underground storage organs contain reserves of carbohydrates, nutrients, and water, and evolved as a mechanism for plant survival through adverse climatic conditions, with perennial life cycles in their natural habitats. During the dry, cold winter months, the above ground portion dies back, with the corm remaining physiologically active

(<http://www.hort.cornell.edu/department/faculty/wmiller/bulb/what.html>). The natural behavior of *S. aethiopicus* is consistent with other geophytes, the leaves dying

back during the cool, dry winter months, and re-emerging with the onset of spring. The corm remains alive throughout the year. Accordingly, earlier references in the literature to 'rhizome' are henceforth replaced by 'corm.'

Information from Durban-area healers (P. Cele, M. Dlamini, 2004 and 2008, personal communication), and researchers in other southern African countries (Hyde and Wursten 2008) indicate that *S. aethiopicus* naturally grows both in partial shade at the edge of forests, and in the open in the veld. Light (2002a), citing Onderstall (1978) and Scott-Shaw (1999), noted that *S. aethiopicus* grows in savanna and coastal grasslands, and in the ecotone of forests and bush clumps in damp and partially shady sites.

Medley-Wood and Franks' (1911z) original report in the Kew Garden Bulletin in 1911 recorded collection from Inanda at 540 m, and from Zululand at 'Ngoya, 450-610m (the specimen that flowered at the Durban Botanic Gardens in December 1910 was brought from 'Ngoya by Mr. Wylie, the Gardens' curator). Medley-Wood and Franks (1911b) originally described the subglobose corm as 4-8 cm in diameter, with filiformes roots, *Caulis foliosus* 6-8 cm altus, *Folia* 5-10, and the stamen *O. Pistillum* floris hermaphrodit. They described the plant as a new genus, with the characters of *Kaempferia*, and polygamous, monoecious flowers, and a 4-6 lobed long narrow tube containing the staminodes. "Corm subglobose, 23 inches diameter, aromatic, roots filiform. Leafy stem 1 to 3 feet high. Leaves 5 to 10 or more, their petioles sheathed into a false stem, blades lanceolate, the middle ones largest, 12 to 14 inches long, 3 to 3.5 inches broad, the lowest 3 inches long, with a one inch, broad, midrib prominent beneath. Flowers 3-6, proceeding from the corm, pedunculate, solitary or very shortly racemose, bracteate at the base, bracts oblong, obtuse, 9 to 14 inches long, 3 to 7 lines broad, pedicels 5 to 12 lines long; peduncles, bracts, pedicels and ovary subterranean". *S. aethiopicus* is a gynodioecious flowering plant (sexual reproduction via hermaphroditic and female flowers only). Notably, Medley-Wood and Franks (1911b) also identify the plant as a corm, and not a rhizome.

The plant produces flowers at ground level, directly from the corm, both bisexual and female, faintly ginger-scented purple and white flowers with a yellow spot, with a white corolla tube 30-40 mm long, and petal lobes 60-80 mm wide (Pooley 1998). The

inconsistent polygamous production of hermaphroditic and female flowers, sometimes on the same corm (noted by Medley-Wood and Franks, 1911b) has apparently been part of the cause for the earlier confusion of the taxonomy of the species. Edwards et al (2004) examined this issue, and reported that while the plants in cultivation normally produce “perfect” (hermaphroditic) or female flowers, individual corms may produce from the same plant both hermaphroditic and female flowers (from different inflorescences), with a predominance of female flowers. The evidence presented dispelled the notion that “the female-flowered and bisexual-flowered plants of *Siphonochilus* represent different taxa, *S. natalensis* and *S. aethiopicus* respectively.” The authors also noted that “the occurrence of female and bisexual flowers on single plants is commonly encountered in the daisies but is otherwise rare” (Edwards et al 2004:29). Remarking on the body of evidence showing that “the environment drives shifts in the equilibrium between plant hormones”, and that “the observed sex ratios [of *S. aethiopicus*] make no evolutionary sense,” the authors suggest that “*S. aethiopicus* was moved with southward migrating human populations, [and] the species may have passed an environmental threshold which has altered the hormonal balances. Because of the more temperate conditions of KwaZulu-Natal the production of cytokinins may have been elevated, resulting in the suppression of hermaphrodite flowers” (Edwards et al 2004:29). The flowers are “fugacious” (short-lived) (Edwards et al 2004), lasting only about a day or two at the most. Nichols (1989) reported that emerging leaves only continue to grow and expand once flowering is completed in mid-December, and other researchers have uncritically repeated his view (see for example Crouch et al 2000:122). Our field observations during the two years of crop trials showed rather different behaviors: in both years of trials the leaves of all plants grew and expanded continuously from the first emergence of the shoots until the time of senescence in early winter. On at least one occasion a perfectly formed flower emerged in March in Pietermaritzburg from an otherwise nearly full-grown plant (Fig 2.6).

Although as many as 20 flowers can develop from one plant, only one flower is reported fully open at a time and lasting for only one day (Onderstall 1978); this observation was consistent with what we observed during field trials. Pictures from wild plants in Mozambique suggest however that more than one flower can appear simultaneously (Fig 2.1). Small, berry like fruits are born below or above the ground

(Gordon-Gray et al 1989), though we did not observe any fruits in the field trials.



Fig 2.1 (a) *S. aethiopicus* flowers on the lower slope of Mt. Gorongosa, Mozambique. (b) and (c) Note the presence of multiple flowers apparently simultaneously from individual corms in wild plants, apparently contradicting Onderstall's (1978) claim that only a single flower at a time opens. Photos courtesy of Bart Wursten (2008).

Crouch et al (2000, following Smith 1998) reported the plant to be highly polymorphic, with labellum color, size and lobing depth varying even within single populations, as does the size of the 'conical corms and tubers'. Crouch et al (2000) and Smith (1998) accurately describe the plant as a herb with a false stem up to 60 cm tall, in that it lacks the nodes and internodes of a true stem, with long, radical, tapering leaves. The South African Traditional Medicines Research Group (SATMERG)'s monograph (Scott and Springfield 2004) reported that the 'corm' tissue is golden brown cork, with suberised cell walls that stain with Soudan IV. Abundant oval or kidney starch grains are found in the central stele, and bright yellow-brown oleoresin cells are scattered in the parenchyma. Crouch et al published in 2003 a study of the pollination of *S. aethiopicus*. While no definitive conclusions are yet possible, based on all available evidence they suggest that the local anthophorine bee *Amegilla caelestina* (Apidae, Anthophorinae) could be a pollinator. The tiny *Drosophila calignosa*, observed visiting both female and bisexual flowers of *S. aethiopicus* in the Durban Botanic Gardens, showed under electron microscopy to be a poor pollinator, and so an unlikely candidate. Crouch et al also noted that in addition "the structure of the flower indicates that a larger-bodied insect is more probably involved" (Crouch et al 2003:18). Successfully pollinated plants have been observed to produce epigeal (i.e. at or just below the soil surface) plum-colored fruits (Nichols 1989, Edwards et al 2004:28, showing G. Nichols' photo). The seeds, which are

produced in small quantities and difficult to obtain (Crouch and Symmonds 2002) can take up to a year to germinate, as observed by Nichols and others at Silverglen. Seedlings are also subject to damping off (confirmation by D. Moon, personal communication 2007).

Gordon-Gray et al (1989) and Smith (1998) reported between 4 and 8 leaves developing on the unbranched false stem up to 60 cm tall during or after flowering. Our field observations during two years of crop trials showed the majority of plants consistently produced higher numbers of leaves, closer to the ratios originally described by Medley-Wood and Franks (1911a), though the Gordon-Gray et al (1989) height observations were largely confirmed for open-field grown plants. Plants grown under shade-cloth exceeded the 60 cm height (see leaf number and height tables below), and two clusters of long-established plants on the farm (not part of the field trials), both incidentally partly shaded, annually reached approximately 1.4 meters. Plant leaf numbers did not increase with increased plant height under shade.

Table 2.1, Plant Heights, Leaf Counts, March-April 2007

2006-2007 March '07	No. of Plants	Mean Height (cm)	Max Height (cm)	Min Height (cm)	Mean Leaf No.	Max Leaf No.	Min Leaf No.
Fert Trial	247	29.2	66	40	8.15	14	2
Spacing Trial	101	27.9	56	30	8.2	14.	1
Compost Trial	74	33.6	56	110	9.4	14	3
Biocontrol Trial	44	22.7	50	40	7.4	12	2
Shade Trial	64	42.8	83	17	9.2	12	5

2006-2007 April '07	No. of Plants	Mean Height (cm)	Max Height (cm)	Min Height (cm)	Mean Leaf No.	Max Leaf No.	Min Leaf No.
Spacing Trial	103	35.5	65	4.5	9.66	16	1
Compost Trial	75	41.8	66	11	10.84	14	3
Biocontrol Trial	44	30.4	51	6	9.05	15	2
Shade Trial	64	51.4	90	10	10.3	14	5

Table 2.1. (continued), Plant Heights, Leaf Counts, March-April 2007

March-April Growth '07	Mean cm Height March	Mean cm Height April	% increase	Mean Leaf Count March	Mean Leaf Count April	% increase
Spacing Trial	27.9	35.5	12.72	14	16	11.43
Compost Trial	33.6	41.8	12.44	14	14	0
Biocontrol Trial	22.7	30.4	13.39	12	15	12.5
Shade Trial	42.8	51.4	12	12	14	11.67
Overall Mean	31.75	39.78	12.53	13	14.75	8.9

Gordon-Gray et al (1989) reported that little was known regarding the population biology of *S. aethiopicus*, though they investigated some aspects of the floral and reproductive biology. The complete reproductive cycle is known for corms that produce bisexual flowers, and details of the vegetative organs, flowering and growth cycle also apply to corms that produce female flowers. The corms of *S. aethiopicus* grow either on the soil surface or underground to a depth of 150 mm. During winter, no above ground parts are visible, but with the beginning of spring rains the aerial shoots develop. Our field trials showed that plants are pre-programmed to shoot regardless of rains, as we found consistent shoot development in corms in storage prior to planting (see Fig 2.3). Gordon-Gray et al (1989) report that the shoots continue to elongate after flowering but growth gradually ceases after the December solstice, and that yellowing of the leaves occurs quite quickly, usually during April, and by May the aerial parts have fully senesced (Gordon-Gray et al 1989, Light 2002a). Our field trials in Pietermaritzburg suggest a modification of these statements: growth, and often vigorous growth, continued well past December, with plants in the field showing substantial gains in height and leaf numbers between March and April (see tables below). Even though we planted perhaps two months later than ideal, i.e. November instead of September, this two-month planting delay would not account for continued leaf growth as late as March-

April, particularly since the plants shoot already in September. Growth in early April (at least in the Pietermaritzburg area) then seems to cease, and then plants immediately begin to yellow and senesce in the latter part of April, with full senescence reached by the end of May.

Crouch’s suggestion, and that of the Kirstenbosch authors, that exposure of the subterranean corms to winter sun appears to affect flowering is supported by the comments of Hyde and Wursten, who note that in Zimbabwe the plant often flowers after fires (which clear the ground, thus exposing the soil to direct sunlight), “when the large mauve flowers make a short but spectacular display on the bare ground” (Hyde and Wursten 2008). They describe *S. aethiopicus* as “small perennial herbs, often flowering before the leaves are fully developed. Lamina elliptic, glabrous. Inflorescence racemose, lateral. Corolla with a tube and 3 petals. Androecium composed of a 3-lobed labellum (the central lobe usually deeply divided) and a single stamen with basal anthers and a long petaloid apical part, at least twice as long as the basal anther. Stigma glabrous” (Hyde and Wursten 2008).

2.2. Propagation

Vegetative propagation of *S. aethiopicus* is fairly simple, involving splitting off the daughter corms from the necrosed mother corm either at harvest time or shortly before planting. The plants used in these two years of field trials were from the corms sold every winter by the Silverglen Nursery team. Approximately 1000 corm clusters which had been partially pre-split, and were lacking the necrosed mother corm and lacking the tuberous roots, were purchased directly at the nursery in the winter of 2005, then brought in paper bags to the Ukulinga Seed House where they were further split by hand. After splitting, the individual corms available for planting numbered 2850, which were sorted into four different sizes (see Table 2.2).

Table 2.2. Initial Size of Planted Corms

Initial Corm Size	Mean Weight (g)	Mean Height (mm)	Mean Base (mm)
Large	29.64	52.37	44.10
Medium Large	19.82	38.48	37.78
Medium Small	8.87	29.65	27.93

Small	2.28	12.38	12.6
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A small number of split off cormels were judged too small to be planted in the field; some of these were no larger than one's small fingernail. Outside of the actual field trials, these cormels were propagated in the farm's nursery by David Moon and his team. Every single one of them germinated, and produced a healthy plant (see Fig 2.2; plants courtesy David Moon).



Fig 2.2 Cormels in Nursery, after sprouting.

2.3. Initial Growth Behavior

The rhizomatous corms typically produce one or more shoots, each shoot feeding the development of a new corm from the planted mother corm. Corms also develop tuberous roots that extend down into the soil. New shoots emerge from the plant in KZN in September (Fig 2.3), whether or not the corms have been planted in the soil (Fig 2.4). Both from individual corms, and from the multiple plants that emerge from the mother corm, it is clear that some plants emerge late into the season, and are outcompeted for nutrients. In the field trials, new shoots were still emerging from some new daughter corms as late as March. These much smaller plants were also the first to show signs of winter senescence (yellowing and die back).



Fig 2.3 Sprouting corms in insulated storage room, early October 2006. (a) Note long initial shoots and multiple shoots from corm. (b) Note how size and vigor of initial shoots appear fairly independent of corm size.



Fig 2.4 Inconsistently re-planted corms after incomplete harvesting from 2006-2007 field trial. Note long initial shoots (1-4 per corm) forming first leaves at apparently predetermined heights, both beneath and above soil surface.

During the time the plant is generating new leaves above the soil surface and photosynthesizing, below the surface the new corms are developing. The general view among horticulturalists in the Durban area, the nurserymen at Silverglen, and some of the local traditional healers consulted, is that a single planted corm should produce between 5 and 8 new corms from the mother corm. Our trial results produced fewer new corms on average, and we suspect that it was because we planted late (late November both seasons) instead of in September, as recommended.

2.4. The Flower

The famous purple, pink and sometimes yellow *S. aethiopicus* flower appears at

ground level, early in the growth season (Fig 2.5). The logistics of field monitoring for this MSc. study did not permit daily visits to the field, so we cannot confirm the assertion that flowers last only a day, though the flowers are certainly short-lived. Field records indicate that the flower also emerges in an initial tubular shape, similar to the leaves, and then unrolls. The flowers are produced as separate shoots directly from the corm, independent of the leaves (Fig 2.6).



Fig 2.5 (a) flower shoots separately from false stems, early plant growth phase; (b) and (c) flowers at plant base, later growth phases; (d) prior day's flower wilted, new flower opened.



Fig. 2.6 (a) March 2006 photo, late in the growing season, showing fully-emerged flower from plant in full growth. (b) Inset highlighting white flower bud emerging separately from false stems. (c) Close-up of fully-emerged flower.

During the growth phase, approximately September to May in the Pietermaritzburg area, KwaZulu-Natal, South Africa, the plant produces a single solid green leaf from the corm that emerges from the ground as a rolled up, tubular shape. Once the single leaf emerges from the soil, it begins to unroll and flatten. From the same tubular growing point the subsequent leaves emerge, singly, each again in an alternating sequence, producing the false-stem (Fig 2.7).



Fig 2.7 (a) Initial shoot, and (b) subsequent alternating leaf pattern, and (c) Growing tip showing unfurling new leaf.

The multiplying corm cluster produces multiple shoots, each one feeding what will eventually become a distinct but attached daughter corm (Fig 2.8). These shoots emerge closely spaced, and some will successfully compete to become major plants. Usually the initial shoot forms the main daughter plant, with the secondary shoots emerging later, at first smaller, then sometimes catching up in height with the initial shoot, sometimes remaining smaller.



Fig 2.8 Typical multiple shoot pattern from mother corm.

Scott-Shaw reported that *Siphonochilus aethiopicus* produces 4-8 leaves on an unbranched stem up to 400 mm tall (Scott-Shaw 1999). As noted in the Table 2.1, our field trials showed that the healthy plant produces as many as 12-15 leaves, as illustrated in the photos below (Figs 2.9 and 2.10). As the plants get older, but before winter senescence, the bottom leaves frequently begin to senesce and fall away from the main stalk. These separating and senescing lowest leaves were a common sight during both growing seasons of the field trials.



Fig 2.9 (a) *S. aethiopicus* planar leaf orientation and new, tubular leaf unfurling at

center of false stem; (b) Bottom right leaf initiating typical senescence and false stem separation; (c) Standard leaf pattern at *S. aethiopicus* false stem.



Fig 2.10 (a), (b), and (c) Typical healthy plant clusters in field trial; (b) Plant with 11 easily countable leaves.

2.5. Leaf Orientation

The stalk and leaf orientation from a single shoot from a corm is always planar: leaves alternate along a central stalk, and are uniformly in the same plane on both sides of the stalk (Fig 2.11a). Second or more shoots from the mother corm, which will subsequently produce individual corms themselves, may be at any angle (Fig 2.11b-d). It is the multiple shoots forming multiple daughter corms that gives the plant the “cluster” like appearance (Fig 2.11e) in the leaves above the soil surface. Each new shoot adopts a particular angle, and all the subsequent leaves of that new false stem maintain this particular horizontal plane. Multiple shoots from the same corm do not necessarily adopt the same plane of growth. This creates a crowded, competitive growth environment, as daughter plants may block their sisters from photosynthesizing properly.



Fig 2.11 (a) Single shoot leaf pattern in horizontal plane; note second daughter corm shoot at slightly different angle; (b) neighboring plants at orthogonal angles; (c) Clear side view of plant orientation; (d) Three daughter plants all with

the same orientation; (e) Typical cluster presentation, multiple shoots of daughter corms with varying planar leaf orientation.

The base of the plant widens in one plane only (the plane of distichy), within the plane of the leaves, but not much in the other plane, the non-leaf plane. The orientation of the growth of the leaves is not necessarily towards the sun in the first instance, and the plant leaves do not turn towards the sun at any point in the day. The plane of distichy appears to be based on the corm orientation itself. For maximum growth, and consistency of spacing, it might be relevant to ensure the plants are all oriented optimally. For maximum productivity, one might need to consider separating and replanting the growing daughter corms.

2.6. Pest and Disease Susceptibility

Field observations showed a number of specific disease susceptibilities and resistances. Evolution-driven natural selection has honed plant defenses for 1.6 billion years (SurrIDGE and Aronson 2001). There is a generally held belief that years of selective breeding in domesticated crop plants have removed natural products found in their more pathogen-resistant wild counterparts (Dixon 1986). As of 2001 there are for instance 72 different defined genera of plant viruses, containing over 500 species, vectored by insects, nematodes, fungi, pollen, seeds, and/or humans (Waterhouse et al 2001). Plants also release a wide range of volatile compounds which may attract herbivore predators, repel moth egg-laying, or signal uninfested plants to increase their own resistance response proactively (Farmer 2001). In farming one deals with both necrotroph (invade and kill plant tissue) and biotroph (parasitic) pathogens. Microbes often produce toxins, and bacteria and fungi can take either approach to the plant. Chewing of plant tissue by insects induces wound response, and other insects may be drawn to released volatiles (Dangle and Jones 2001).

As no major field trials of *S. aethiopicus* have been reported previously, information on *S. aethiopicus* plant pathogen susceptibility and response is scarce. Crouch et al (2000:125) report observations of grasshoppers and other insects eating leaves of wild plants, but the extent of the eating is not reported. Nichols (1989) had noted no pest or disease attacks on the plants. Spring (2003) did report some nematode damage at

planting, callus formation to combat fungal infection on opened tissue on the corm, some millipede damage, some eating of foliage by grasshoppers, and some butterfly pupae on plants, but no major crop-threatening pest or disease pathogen problem. Keirungi and Fabricus' work (2005) with the plant at the Kirstenbosch Botanical Gardens found the mature plants generally free of pest and diseases, with the exception of small grey snout beetles which hid between leaf bases during the day (also on *Clivia*, *Crinum* and *Nerine*), and then damaged the upper leaf surfaces and leaf margins during the night. Excessively wet soil medium over long periods could also lead to fungal rotting of rootstock. Our own two years of field trials in Pietermaritzburg revealed a number of previously unreported susceptibilities.

2.6.1. Cut Worm

The first leaf emerging from the ground proved (unsurprisingly) to be susceptible to cutworm damage. Partial damage from the cutworm appeared later on the leaves as a striation pattern. When sufficiently damaged, these sections of the leaves later broke off partly or completely (see Fig 2.12). Some type of cutworm control measure must be applied at emergence.



Fig 2.12 (a) and (b) Late stage leaf growth showing residual damage from cutworm attack on primary shoot.

2.6.2. Black Spot

Black spot fungus attempted to colonize some leaves, but the spots were limited both in total number of spots per leaf, in number of leaves per plant, and in number of plants attacked (Fig 2.13a-b). During two seasons of field trials, only a handful of plants showed black spot, and only on one or two leaves of those plants.



Fig 2.13 (a) and (b): Black spot on leaves.

No significant damage either to the leaves or to the plant itself was obvious from these minimal infestations. Plants appeared to contain the initial infection, and as the plants matured these spots disappeared, suggesting that once contained, the fungus did not find sufficient nutrition and was eventually destroyed by host defenses.

2.6.3. Erwinia

Apparent *Erwinia* infection (Figs 2.14, 2.15, and 2.16) during the first growing season appeared to be heat stress or sun stress-related. During the 2005-2006 growing season, a significant infestation of *Erwinia* threatened to destroy the entire field trial, during the hottest and sunniest months of the summer. As soon as the weather cooled down, with several days of rain, the infection abated, with no further loss of plants. This alerted us though that *Erwinia* could pose a major crop-limiting disease if plants are grown in areas of high heat and solar radiation. Since the natural environment of the plant appears to be at least partially shaded environments, this probably explains the heat/sun-stress vulnerability of *S. aethiopicus*.



Fig 2.14 (a) Overhead view of Erwinia-infected plant (top) next to healthy plant; (b) March '07 isolated Erwinia attack; (c) March '06 Erwinia attack. Note entire leaf senescence.



Fig 2.15 March 2006 Erwinia attack. (a) Plant senescing; (b) Two daughter plants have senesced, two daughter plants remain healthy; (c) Complete senescence.

Although the pathogen proved itself capable of killing entire plants, initial infestation appeared to occur in individual corms of the corm cluster, before spreading to the adjacent corms and eventually killing off the entire cluster (Fig 2.16). Characteristic

“soft-rot” appeared in the corms, with the leaves withering, losing color, and dying off. Necrotic leaves were subsequently digested by phagocytic fungi.



Fig 2.16 Selectivity of Erwinia progression. (a) Only middle daughter plant is senescing, other two daughter plants remain healthy; (b) Overhead view showing single Erwinia senescence among cluster of healthy daughter plants; (c) Full cluster Erwinia-induced senescence, with one plant partly alive.

2.6.4. Sun Damage

S. aethiopicus proved itself susceptible to sun damage when grown in full sun. Early phase sun damage appears as mild-severe leaf chlorosis, with characteristic striation patterns, followed by actual leaf burning, and complete burn through, independent of field trial treatments (Figs 2.17, 2.18, and 2.19).





Fig 2.17 Chlorosis due to Sun exposure: (a), (b) and (c) Initial chlorosis, Fertilizer plots, 2006-2007; (d) Complete burn through of a leaf; (e) Burnt leaves from Spacing plot.



Figs 2.18 (a-e) 2005-2206 Compost and Spacing trial plants, exhibiting individual leaf sunburn damage on otherwise healthy plants.



Fig 2.19 (a) Plant from 29% shade exhibiting sun damage; (b) Burnt leaf tip at edge of shade plot; (c) Another 29% actual shade plant with sun damage.

As of 14 April 2007, chlorosis from the intense sun had disappeared from the entire set of trials. The winter die-off of leaves had already started, but primarily only in the smaller plants, and most particularly in those in the fertilizer trial plots.

2.6.5. Caterpillar damage

Fortuitously, a volunteer Swiss chard plant in the trial plot clearly showed that *S. aethiopicus* exhibited complete resistance to caterpillar predation (Fig 2.20).



Fig 2.20. Caterpillar damage

2.6.6 Possible Fungal infection

None of the plants showed any signs of fungal infections on the above ground portions during the two seasons of trials. Only one plant, once, showed some temporary hosting of what appeared to have been fungal spores, on just one leaf (Fig 2.21).



Fig 2.21 Unidentified white spotting on a single leaf from one of the shade cloth plots. These spots rubbed off easily with the finger, leaving no visible damage to the leaf surface.

2.6.7. Unidentified leaf discoloration/necrosis and striations

In both trial seasons we occasionally found some slight discoloration of leaves with brown spots, and some striations (Figs 2.22 and 2.23). Neither was identified, and

neither seemed to cause any growth problems.



Fig 2.22 Unidentified brown spotting (a) Superior leaf surface, edges; (b) Close-up showing necrosis; (c) Superior central leaf surface.



Fig 2.23 Unidentified leaf striations: (a) Inferior leaf surface; (b) Superior leaf surface; (c) Full leaf view.

2.6.8. Eating of leaves

Although the eater in question was not observed in action, individual leaves of a few plants showed signs of having been eaten by a bird or insect (2.24).



Fig 2.24 2006-2007 leaf damage: (a) Central section of leaf apparently eaten; (b) multiple sections of leaf apparently eaten; and (c) some other type of leaf damage, possibly from insects.

2.6.9. White Ants

Early in the 2006-2007, one corm and plant were eaten in their entirety by a colony of white ants (observed by farm crew), leaving a hole in the ground (Fig 2.25). One spray of Metasystox® R Liquid (Bayer Environmental Sciences) eliminated the problem.



Fig 2.25 Hole in soil after white ant consumption of plant.

2.6.10. Hail

A severe hailstorm hammered the research farm in mid-season one year. The force of the hailstones was sufficient to break a corner off of one of the white plastic plot markers (Fig 2.26).



Fig 2.26 Hail damage to plot marker.

Most of the plants on the farm were shredded, and golf-ball size holes were punctured through the leaves of the older aloe plants on neighboring terraces. The *S. aethiopicus* plants were also hammered, and in the immediate aftermath plants showed shredded, broken and torn leaves, and some broken false stems (Figs 2.27 and 2.28). This resulted in some leaf die-off over the next couple of weeks, and some individual plant death. However, the soft, flexible nature of the leaves, and the generally low profile of the plants seems to provide some natural protection from hail storms. Despite the heavy storm and the heavy immediate damage, almost all of the plants recovered completely and continued their growth until the natural winter senescence.



Fig 2.27 Trial plots shortly after hailstorm: false stems are still standing, but leaves are uniformly knocked down.



Fig 2.28 Hailstorm damage: (a) and (b) Clusters of plants showing hail damage; (c) Individual damaged plant; (d) Nearby aloe damage for comparison: note holes in leaves, with travel mug for scale.

2.7. Field Effects

Every field trial is potentially subject to experimental error due to the “field effect,” i.e. uneven distribution of residual nutrients in the soil, or uneven distribution of clay or shale or other mineral structure in the soil that can have strong interaction effects on nutrient availability for the plant. The fertilizer plot in the 2006-2007 trial showed a clear field effect. The western or upper end of the field had less clay and considerably more shale. Plants on this end of the field performed more poorly, regardless of the plot treatment (Fig 2.29a). Plants on the eastern portion of the field performed well, also regardless of plot treatment, this area was slightly lower in elevation, and richer in clay content and nutrients (Fig 2.29b).



Fig 2.29 Field Effects, March 2007: (a) Upper, shale-heavy portion of field; (b) Lower, clay-heavy portion of field.

2.8. Maturation and Winter Senescence

The plant grows steadily until reaching maximum leaf number and stalk height in late March, early April. Then the leaves begin to senesce, turning first yellow, then brown, and the entire stalk and leaves die back, leaving withered light brown material on the soil surface (Fig 2.30). According to Ma Dlamini, head of the Durban muthi market, when the false stems turn a lemony colour, this is the ideal time for harvest to get maximum effectiveness from the corms and tuberous roots (personal comm. to JF Hartzell). During the die-back period, the leaves separate progressively from the stalk until there is no stalk remaining, and are progressively colonized by phagocytic fungi that digest the necrotic tissue (Fig 2.30.c). Eventually there is no plant material left on the soil surface.



Fig 2.30 End of growing season senescence: (a) Individual plant clump senescence, with uniform leaf dieback and stem yellowing; (b) Uniform winter senescence

across trial plot. dieback of plants at time of winter senescence. (c) Phagocytic fungi colonization of necrotic leaves.

2.9. Harvest Botany and Harvest Methods

When growing the corms for a single season, harvest time is when the leaves have all senesced naturally, usually June, July, or August in the Natal Midlands. Makhosi Ma Dlamini, who runs the Durban muthi market, advises that the best time to harvest for optimum corm quality is actually when the leaves have all turned yellow, so have lost their chlorophyll, but just before they turn brown and become colonized by phagocytic fungi. Harvesting at this time provides bulbs with the maximum medicinal content. (Ma Dlamini, personal communication, 2008). Ignorant of this advice, we harvested both years' trials when the leaves had fully senesced and turned brown (Fig 2.32). In the first season we harvested when the senesced leaves were still fully visible on the soil surface. This aided in locating the corm clusters. In the second season, delays meant that in some trial plots the senesced leaves had disintegrated or blown away. This made it more difficult to locate corm clusters, despite regular spacing, and meant that the harvesters missed some 200 of the corm clusters from the total field trial.

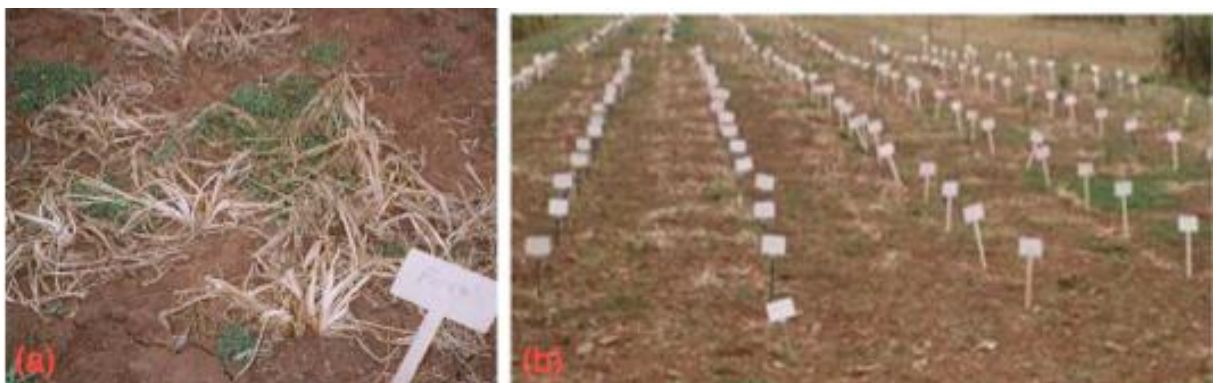


Fig 2.31 End of season senescence: (a) Close-up of sample individual trial plot; (b) 2005-2006 Fertilizer trial plots, illustrating uniform complete above-soil plant senescence.

The small size of plots and the fact that these were field trials made it advisable to harvest corms by hand, using garden forks. One has to be careful to dig under the entire corm and tuberous roots, and it is very easy to break off the tuberous roots when taking the corm cluster from the soil. One must then sift through the soil carefully to find the additional tuberous roots from the one corm cluster. To determine numbers of viable corms and tuberous roots, one needs then to separate the corm clusters by hand,

carefully dis-entangling the tuberous roots, and removing the senesced mother-corm which was the original corm planted at the beginning of the growing season. These senesced mother-corms have a spongy, soft texture, compared to the firm, hard texture of the living corms. The size and number of tuberous roots attached to each individual corm appeared to vary independently of the size of the viable corm, i.e. small corms might have large or small, many or few tuberous roots, large corms might also have large or small, many or few tuberous roots (Figs 2.32, 2.33, and 2.34). Where corms are separated from each other, either during harvest or afterwards, the break points form calluses in a short time.



Fig 2.32 Harvested corm clusters, 2005-2006: (a) Individual corm clusters single trial plot (F007); (b) F007 plot harvest, with corms separated into larger and smaller corms; note cluster of senesced mother corms top right, dirt from between corm clusters, bottom centre, and varying sizes of resulting corms and their associated tuberous roots.



Fig 2.33 Senesced mother corm and daughter corms: (a) Senesced mother corm left; (b) Senesced mother corm right, viable daughter corm left; (c) Corm cluster at harvest, with its tuberous roots; (d) The same cluster, corms now separated; note the break points where the corms were separated from each other.



Figs 2.34 Harvested corms and tuberous roots, 2006-2007 (a) FF135 and (b) FF137.

Experiments with harvesting techniques showed that moistening the soil before digging helps tremendously in harvesting complete clusters with most of the tuberous roots attached. Very dry soil can result in substantial breakage during harvest. Depending on the clay content of the soil, the corms and roots need to be dug out as a single clump, then separated from the attached soil. The harvested corm clusters have, either with dry or damp soil, substantial packed dirt in between the tuberous roots and up inside the spaces between the corm clusters (Figs 2.35 and 2.36). This is very difficult to remove by hand, and in the first season, with dry soil, the team ended up using brushes to remove this soil, which proved an impractically time-consuming process.

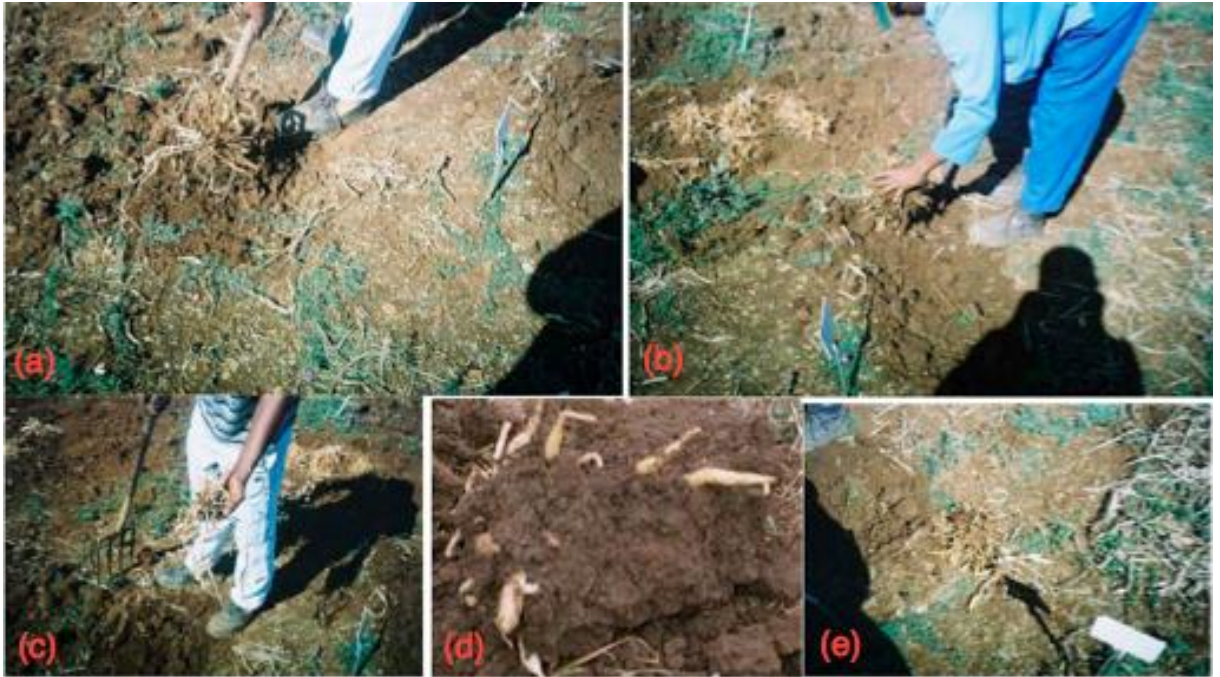


Fig 2.35 Hand-harvesting the corms (a) A corm-root cluster being dug out with a fork; (b) Shaking off the loose dirt; (c) The resulting corm-root cluster; (d) A corm root-cluster close-up shot, taken out of damp clay soil; (e) A corm-root cluster laid to the side prior to washing.



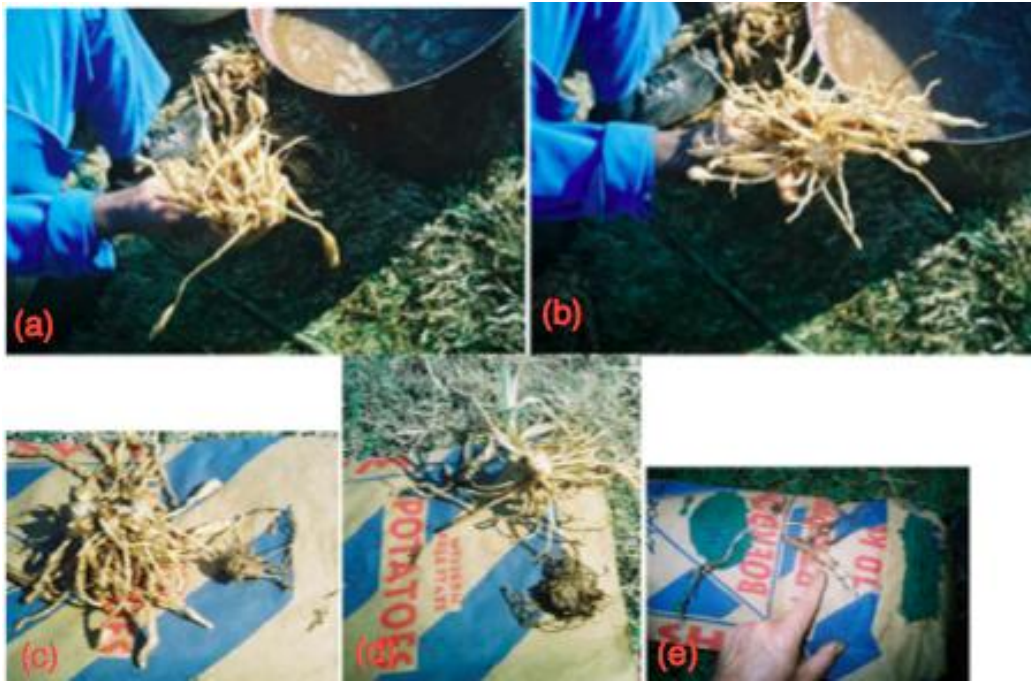
Fig 2.36 Harvest corm cluster details: (a) Same corm cluster and roots as in Fig 2.35, freshly dug from surrounding soil, before cleaning. (b) and (c) Freshly harvested corm clusters (at top of photos), post-cleaning, complete with tuberous roots hanging below.

After some experimentation, a field washing method was developed in the second season. As pictured below (Figs 2.37 and 2.38) small tubs were brought into the field,

and filled with hoses. Harvesters then washed the corm clusters in the buckets, using their fingers to work the dirt loose under water in the barrel. The water quickly became very muddy, but was usable for a long time, and on average it took 3-4 minutes to clean a corm-root cluster. With a bit of practice, this proved a reliable method for producing almost completely (~95%) clean corm and tuberous root clusters, with minimal breakage. Senesced mother corms, which were quite spongy and absorbed water, were readily discarded in the tubs during cleaning.



Fig 2.37 Field washing the corm-root clusters. (a) Pre-washing. (b) and (c) Hand-washing in small barrels.



Figs 2.38 Harvested corm clusters: (a) and (b) Individual corms after field washing; (c) and (d) Corm-root clusters with spongy mother corm removed to one side, on top of potato bags; (e) A separated tuberous root. Extraneous matter is grass.

The tuberous roots are more watery than the corms themselves, and dry out much more quickly. Storage and drying experiments in the Ukulinga Seed House in September-October 2006 (Fig 2.40) indicated that the tuberous roots, left uncovered to air-dry inside will shrivel to a very small size within two to three weeks. If kept in closed bags, the tuberous roots still wither, though much more slowly. Storage experiments with the corms themselves showed that corms left in open air, inside, at room temperature, will dry out in two to three months. Depending on the starting size of the corm, corms may die in two to three months and be no longer viable. Corms stored in small brown paper bags were still viable when planted six and seven months after harvest.



Fig 2.39 Drying room: corm-root clusters laid out on plot bags in the Ukulinga seed house for drying prior to weighing after the first season. The innovation of the second-season in-field weighing apparatus obviated the need for using the Ukulinga facility.

In the second season we weighed and bagged the corms directly in the field (Figs 2.40, 2.41 and 2.42). We took a large step-ladder, attached a hanging scale to the strut, and a bag to the scale. Then we bagged each plot's corm-root production, and weighed total biomass per plot in a potato storage bag, inside the attached canvas bag, subtracted the weights of the canvas and potato bags, and recorded the freshly-harvested plot biomass weight in the field. This second-year innovation ensured we captured the correct biomass at harvest, and did not lose weight from later drying.



Fig 2.40 Field shot of corm-root clusters harvested, washed, and placed back on top of each trial plot, prior to counting, bagging, and weighing.



Fig 2.41 Close-up overhead view of one harvested corm-root cluster resulting from a single-corm at planting time.



Fig 2.42 Field weighing setup.

Once all the corm clusters were weighed and recorded, sample measurements were taken by hand (Fig 2.43), and then the clusters were bagged and stored in a refrigerated container (Fig 2.44) until the following season's planting period.

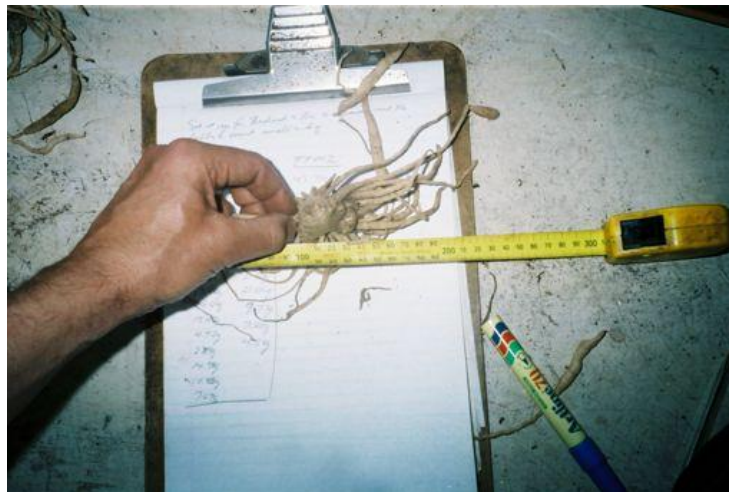


Fig 2.43 A completely cleaned and dried corm cluster and tuberous roots at measurement.



Fig 2.44 Storage of harvested corms, August 2006.

Most likely because of our late planting date both seasons, we found that harvested corms had numerous partially formed mini-corms beginning to form along the outer surfaces of the daughter corms. With the two to three extra growth months afforded by a September planting, we suspect these mini-corms would have developed into full corms. Light (2002b) erroneously reported that corms harvested during the dormant period (after leaf die-back in winter) do not have any roots. As pictured above, our trials showed substantial tuberous roots on corms harvested in July and August, well after complete senescence of the above-ground leaves. While the tuberous roots of our harvested plants were typically no longer than a hand-length at most, the principal author has observed harvested corms, grown ostensibly with only organic composts, with abundant tuberous roots extending more than a meter in length (D. Mitchell, personal communication and demonstration 2003). Plants grown in pots with continuous fertigation throughout the growing season, in a controlled environment at the UKZN Pietermaritzburg campus, also produced extensive, long (6-10 cm, 6 mm diameter) tuberous roots (G. Olivier, M Laing, personal communication 2006). The Mitchell, Olivier and Laing results suggest that with proper planting times and abundant nutrients, the plants will produce a substantial amount of tuberous root material. What is interesting is that the corms grown from the Silverglen clones seem to have a physiological limit in size — i.e. additional nutrients did not produce larger corms. Physiologically, it appears that these tuberous roots provide storage nutrition and water for the plants during the winter senescence, providing the starting nutrition needed for

new growth in the spring, a point noted by other researchers (Nichols 1989).

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Chapter 3: Effect of Compost, Spacing, and Corm Size on Growth of *Siphonochilus aethiopicus* (Schweif.) B.L. Burt.

3.1. Introduction

Finding the correct mix of spacing, fertilizer and planting material is critical for success in field cropping any plant. Since *Siphonochilus aethiopicus* (Schweif.) B.L. Burt. has been traditionally wild-harvested, such basic farming information is lacking. A set of crop trials were designed to test optimal spacing, organic fertilizer levels, and size and viability of seed material.

3.1.1. Spacing

Appropriate spacing for planting field crops of any type is a key agronomic consideration. Planting density directly relates to yield (Benjamin 1999): over-crowding wastes planting material and field space, and can inhibit growth (Ban et al 2006), including by increasing pathogen susceptibility (Bucheli and Shykoff 1999). It can require excess fertilizer and irrigation, and has been shown to affect susceptibility to pests in other crops (Asiwe et al 2005). In some crops we know that spacing is affected by allelo-chemicals (Weston and Duke 2003); excessively wide spacing can also eliminate beneficial self-shading effects and alter microclimate. The *S. aethiopicus* corm has shallow roots, and when soil temperature hits 50-60° C, it has little subsoil reserve to escape from the heat, so one would expect the plant to perform better if the soil surface is kept cool. Well-spaced plants will produce good leaf coverage and a resulting shade effect. These trials aimed to determine optimal field spacing for *Siphonochilus aethiopicus*.

3.1.2. Compost

Traditional healers in KwaZulu-Natal have long argued for organic cultivation of medicinal plants, because they feel that the slower-growing, more 'natural' growth methods produce denser, higher quality, more medicinally effective plants (Cele, personal communication 2004). A recent metanalysis of secondary metabolite production in organically and conventionally cultivated fruits and vegetables found that organic produced secondary metabolite content is 12% higher than that of conventionally produced ($p < 0.0001$, Brandt et al 2011). The Brandt et al analysis lends support to the traditional healer claims: medicinal plant effective compounds are typically secondary metabolites (Surrridge and Anson 2001; Bruni and Sacchetti 2005).

In a 21-year fertilizer experiment, Zhong et al (2010) report that long-term organic manure application increased soil microbial mass, activity and diversity, another factor that may contribute to increased secondary metabolite production by plants through microbial symbiosis in the root zone. To test whether *S. aethiopicus* could be successfully grown without chemical fertilizers, we tried a variety of compost levels over two seasons to investigate optimum compost fertilizer levels. In the first season this trial was combined with the spacing trial. In the second season the spacing trial and the compost trial were separated.

3.1.3. Planting Material

A third factor in optimizing *S. aethiopicus* production is the size of the planting material. As described in Chapter 2 of this thesis, vegetative propagation of the plant has proved to be the most cost-effective and efficient method for producing new planting material, even though tissue culture methods have been developed. Mother corm dimensions have been shown in saffron (*Crocus sativa* L.) to affect stigma yield, daughter corms and quality (Gresta et al 2008, De Mastro and Ruta 1993), and seed rhizome size has been shown to affect yield in tumeric (*Curcuma longa* L.) (Houssain et al 2005) and ginger (*Zingiber officinale* Rosc.L.) (Hailemichael and Tesfaey 2008), suggesting that propagule size may be relevant to effective yield in field-cropped *Siphonochilus aethiopicus*. To test optimum planting material size, we field tested a variety of corm sizes, and experimented with successive splitting of corms to explore the smallest possible viable planting material.

3.2. Materials and Methods

Crop trials were conducted over two seasons on a private farm just northeast of Pietermaritzburg, KwaZulu-Natal, at approximately 675 m altitude, lat-29.6330, long 30.4000. *Siphonochilus aethiopicus* corms were purchased from Silverglen Medicinal Plant Nursery, Chatsworth, Durban. The 1500 partially pre-split corms had had the tuberous roots removed, and were further split prior to planting, yielding 2850 plantable corms. After splitting, corms were separated and laid out to dry in the Ukulinga Research Farm (UKZN) Seed Sorting building. Wounds caused by splitting suberized within one day. Corms were dried at room temperature on tables for several weeks, creating an informal storage-drying test effect for germination, then re-bagged in brown paper bags and stored in metal lockers at room temperature. Corms were then removed from storage and sorted by size and number, yielding results as in Table 3.1.

Some additional miniscule corms deemed too small for field-planting were potted and tended in the farm's nursery (courtesy of David Moon).¹

Table 3.1: 2005-2006 Planting, Corm Counts and Sizes post-split

Total Corms	Number of Corms	Mean Weight (g)	Mean Height (mm)	Mean Base Diameter (mm)
Large (L)	410	29.64	52.37	44.10
Med Lg (ML)	990	19.82	38.48	37.78
Med Small (MS)	610	9.95	29.65	27.93
Small (S) (cormels)	840	2.28	12.38	12.6
TOTAL	2850			

For the 2005-2006 growing season, a combined trial was designed to test the effects of spacing, corm size, and compost amounts. One thousand two hundred corms @ 25 corms/plot were planted in 48 plots according to a Randomized Complete Block design, with 3 reps each of between-corm-spacing and compost level combinations of 15 cm x 15 cm and 4,5 kg ha⁻¹ compost, 20 cm x 20 cm and 7,5 kg ha⁻¹ compost, 30 cm x 30 cm and 10 kg ha⁻¹, and 40 cm x 40 cm and 15,5 kg ha⁻¹ compost. Factorial variation of corm sizes were S, MS, ML and L (as per Table 3.1), tested against each spacing-compost combination, with 4 reps of each treatment (see Table 3.2 below for Randomized Block Design). Rows between the individual blocks were 30 cm each.

¹ 240 Woodhouse Road, Pietermaritzburg, 3201, South Africa.

Table 3.2: 2005-2006 Compost-Spacing-Corm Size Trial, Randomized Block Design

Plot	corm size	Spacing (cm)	Compost (kg ha ⁻¹)	Plot	corm size	Spacing (cm)	Compost (kg ha ⁻¹)
C001	S	15	4,5	C025	S	30	10
C002	L	40	15,5	C026	L	20	7,5
C003	ML	40	15,5	C027	ML	20	7,5
C004	MS	15	4,5	C028	MS	30	10
C005	S	15	4,5	C029	S	30	10
C006	L	40	15,5	C030	L	20	7,5
C007	ML	15	4,5	C031	ML	30	10
C008	MS	15	4,5	C032	MS	30	10
C009	S	15	4,5	C033	S	30	10
C010	L	15	4,5	C034	L	30	10
C011	ML	15	4,5	C035	ML	30	10
C012	MS	15	4,5	C036	MS	30	10
C013	S	20	7,5	C037	S	40	15,5
C014	L	15	4,5	C038	L	30	10
C015	ML	15	4,5	C039	ML	30	10
C016	MS	20	7,5	C040	MS	40	15,5
C017	S	20	7,5	C041	S	40	15,5
C018	L	15	4,5	C042	L	30	10
C019	ML	20	7,5	C043	ML	40	15,5
C020	MS	20	7,5	C044	MS	40	15,5
C021	S	20	7,5	C045	S	40	15,5
C022	L	20	7,5	C046	L	40	15,5
C023	ML	20	7,5	C047	ML	40	15,5
C024	MS	20	7,5	C048	MS	40	15,5

Corm size: S=Small, MS=Medium Small, ML=Medium Large, L=Large, as per Table 3.1

With some remaining corms (after planting all the other trials), we also conducted a smaller Compost-level only trial (Table 3.3), to test the effect of lower levels of compost, independent of corm size and spacing. One hundred and fifty Medium-Small (MS) corms were planted in 10 plots with no compost (3 plots), 2 kg ha⁻¹ compost (2 plots), and 4 kg ha⁻¹ compost (4 plots), at uniform spacing of 30 cm between plants.

Table 3.3: 2005-2006 Compost-only Trial Treatments, using Randomized Block Design, MS corms, 15 per plot.

Plot #	Treatment
P001	NO COMPOST
P002	COMPOST 4KG
P003	COMPOST 2Kg
P004	COMPOST 4KG
P005	NO COMPOST
P006	COMPOST 4KG
P007	COMPOST 2Kg
P008	COMPOST 4Kg
P009	NO COMPOST
P010	COMPOST 4Kg

Total corms used for the two 2005-2006 compost-related trials were 1200 for the Compost, Spacing and Corm Size Trial, and 150 for the Compost Only Trial.

In 2005-2006 all corms except those in the Eco-77² trial were treated with Eco-77 to guard against post-split fungal infection of the wounds, but this was done after suberization. In 2006-2007, calluses were allowed to form without treating the corms with Eco-77 or with fungicide. Normal planting date for *S. aethiopicus* would be September, but for various logistical reasons planting was delayed until November. By the third week of November 2005 all the trials had been hand-planted, so this timing was repeated for the 2006-2007 trials.

In the 2006-2007 season we planted a roughly even distribution of larger and smaller corms in each block. We did not split off the tiny cormels for planting in 2006-2007 as we had done in 2005-2006, given the high mortality rate of the cormels in the first season. We used 3 levels of compost: 5 kg ha⁻¹, 10 kg ha⁻¹, and 15 kg ha⁻¹, plus 0 kg ha⁻¹ as a control, and planted 15 corms per block at uniform 30 cm spacing. Each of the 4 treatments had 4 reps, resulting in 16 trial plots (Table 3.4).

² Plant Health Products, Nottingham Road, South Africa; *Trichoderma harzianum* Strain Eco-77.

Table 3.4: 2006-2007 Compost Trial, using Randomized Blocks

Rep 1	kg ha ⁻¹	Rep 2	kg ha ⁻¹	Rep 3	kg ha ⁻¹	Rep 4	kg ha ⁻¹
CC001	15	CC005	5	CC009	5	CC013	0
CC002	0	CC006	15	CC010	15	CC014	5
CC003	10	CC007	0	CC011	10	CC015	15
CC004	10	CC008	5	CC012	0	CC016	10

The 2006-2007 spacing trial used 15 corms per block, with four reps for spacings of 5 cm, 10 cm, 15 cm, 20 cm, 30 cm, and 40 cm (Table 3.5). Due to an error by the field crew, only 3 of the four reps for the 10 cm spacing were planted.

Table 3.5: 2006-2007 Spacing Trial using Randomized Blocks

Plot #	Spacing (cm)	Plot #	Spacing (cm)	Plot #	Spacing (cm)
SS001	30	SS009	15	SS017	40
SS002	5	SS010	30	SS018	15
SS003	20	SS011	5	SS019	10
SS004	20	SS012	10	SS020	(missing)
SS005	40	SS013	10	SS021	30
SS006	15	SS014	20	SS022	5
SS007	40	SS015	30	SS023	40
SS008	20	SS016	5	SS024	15

In all the trials plants were grown on level terraces in full sun, though a small amount of shade reached the plants in the late afternoon from nearby trees. Organic composted chicken litter was used as fertilizer, supplied courtesy of Eston Organics.³ Field space requirements were determined by simple calculation of plot size and total number of randomized blocks per treatment, with a consistent seed-tray's width (about 30 cm) between blocks. Irrigation was approximately 30 mm of water per week to supplement irregular rainfall (Appendix 3.2).

Field preparation was by manually turning the soil with hoes and forks. The level terrace had previously been limed, and treated in the previous season with 2:3:2 (28) fertilizer for growing cabbages. The P levels were low 10-22 mg l⁻¹ (Appendix 2.3).

³ 1 Nutty Isles Farm 904/9, Umlaas (MR 21/1) Rd, Camperdown, South Africa.

Resident Guinea fowl (*Numida meleagris*, Linn.) controlled cutworm (*Euxoa* and *Agrotis* spp.) and snails. Plots were watered before harvesting to soften the soil. Traditional healers had reported that harvesting when yellowing leaves were still visible above the soil produces corms with the highest medicinal value. In retrospect, it would have been easier to harvest at that time (in June) than later when all the leaves had died off and fallen away, as the lack of surface leaves made it more difficult to ensure one had successfully harvested all the corms present. Simple field-washing by hand was used to remove soil from corms.

Tables of Means and data plots were created using Excel. Statistical analysis was performed using MATLAB (version 7.9.0, ©The Mathworks Inc., Natick, MA, USA). A 2-way, 4x4 Analysis of Variance (ANOVA) was run with Corm Size and Spacing-Compost Level as independent variables against several harvest measures (biomass, corm survival %, total harvested corms). Additional ANOVAs were as follows: One-way ANOVAs for harvested biomass and corms block⁻¹, compost-only trial 2005-2006; a one-way ANOVA for survival percentage was run with an angular transformation. For the 2006-2007 harvest data, one way ANOVAs for both the Spacing-only and Compost-only trials were run for harvested biomass, total corms block⁻¹, and survival percentage (angular transformation). Tukey's least significant difference simple T-tests were performed for harvest biomass against corm size and against spacing for the 2005-2006 results.

3.3. Results

Table 6 is a table of means for the 2005-2006, 2005-2006 Compost-Spacing-Corm size trial. Note that four different spacings were tested for each of the four different corm sizes, with compost levels matched with the spacing levels across all four corm sizes.

Table 3.6: 2005-2006 Compost-Spacing-Corm Size Trial Results (table of means)

Corm Size	Spacing (cm)	Compost (kg ha ⁻¹)	Total Corm Count	Survival %	Total Corm Count (S)	Total Corm Count (L)	Mean biomass (g plot ⁻¹)	Surviving Plants	Mean corm biomass (g orm ⁻¹)
S	15	4,5	18	71%	8	10	24,67	6	1,37
S	20	7,5	11	42%	6	5	21,00	4	1,95
S	30	10	28	113%	17	12	25,33	12	1,73
S	40	15,5	12	47%	6	5	24,67	3	2,23
MS	15	4,5	32	127%	16	16	47,00	11	1,50
MS	20	7,5	39	155%	19	20	74,67	10	1,93
MS	30	10	50	199%	21	28	111,33	14	2,10
MS	40	15,5	64	254%	33	31	131,75	15	2,15
ML	15	4,5	60	241%	32	29	101,67	22	1,67
ML	20	7,5	69	275%	43	25	149,00	25	2,20
ML	30	10	90	360%	52	38	186,00	19	2,07
ML	40	15,5	77	308%	46	32	178,50	17	2,30
L	15	4,5	71	285%	40	31	137,00	26	1,93
L	20	7,5	55	220%	32	23	98,33	18	1,77
L	30	10	65	259%	36	29	159,33	22	2,83
L	40	15,5	106	424%	56	50	267,00	20	2,50

Corm size: S=Small, MS=Medium Small, ML=Medium Large, L=Large, as per Table 3.1

The data show a clear trend for higher survival of planted corms for the ML and L sizes, and for the greater amounts of compost. For small, medium-small, and medium-large corm sizes 30 cm spacing was optimum for survival (Fig 3.1).

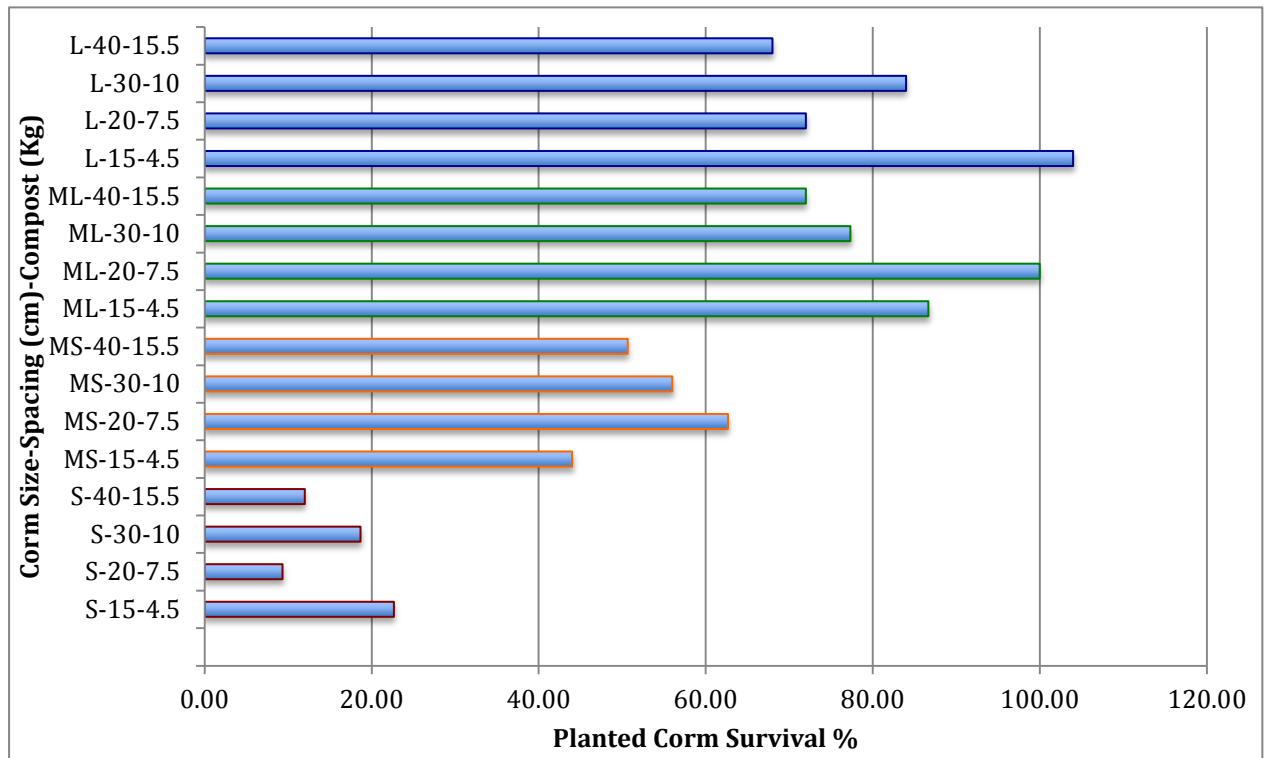


Figure 3.1: 2005-2006 Planted Corm Survival % as Response to Compost x Spacing x Corm Size.

A 2-way, 4x4 ANOVA of Corm Size and Spacing-Compost Level as independent variables against the dependent variable Harvested Biomass showed significance individually for both Corm Size ($F(3,32) = 16.87, p < 0.0001$) and Spacing-Compost Level ($F(3,32) = 3.77, p = 0.02$). Compost levels were treated as a correlated variable, since they were not varied independently of spacing. (The Cormsize x Spacing/Compost interaction effect was not significant: $F(9,32) = 0.57, p = 0.8135$). For Corm Size, MS, ML and L all were significantly better than small, and ML was significantly better than MS (Table 7 and Figure 2). For Spacing, both 30 cm and 40 cm were significantly different to 15 cm (Table 3.8 and Figure 3.3).

Table 3.7: Corm Size LSD Table, 2005-2006 Harvest Biomass (* marks significant)

Comparisons (Corm Size)	Lower bound	Tukey's LSD	Upper Bound
S MS	-1.0424	-0.6658*	-0.2893
S ML	-1.6432	-1.2667*	-0.8901
S L	-1.3084	-0.9318*	-0.5553
MS ML	-0.9774	-0.6008*	-0.2243
MS L	-0.6425	-0.2660	0.1105
ML L	-0.0417	0.3348	0.7114

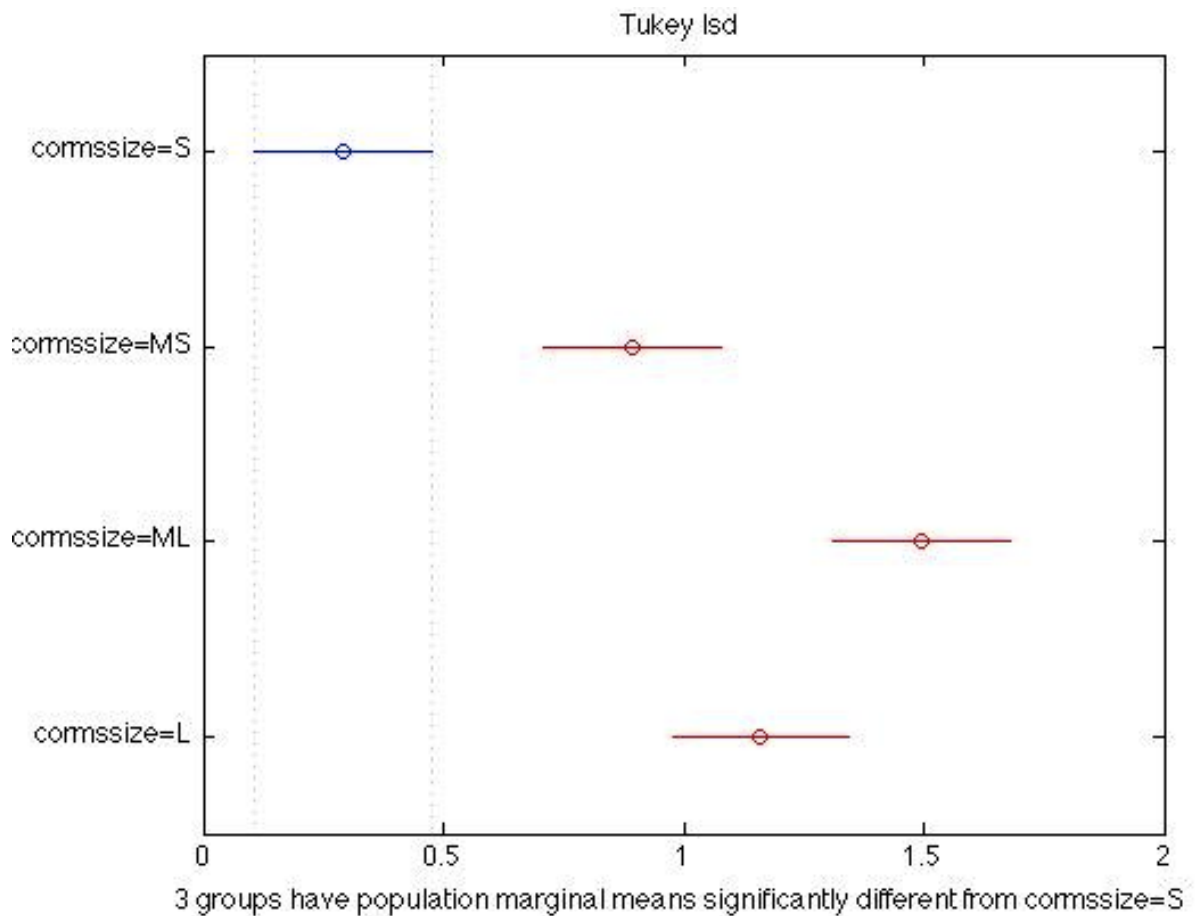


Figure 3.2: Population means and standard error bars for Corm Size x Harvested Biomass (x-axis), showing MS, ML, and L significantly different from S, and ML significantly different from MS.

Table 3.8: Spacing LSD Table, 2005-2006 Harvest (* marks significant)

Spacing-Compost Comparisons		Lower bound	Tukey's LSD	Upper Bound
15 cm-4.5kg ha ⁻¹	20 cm-7.5kg ha ⁻¹	-0.5889	-0.2123	0.1642
15 cm-4.5kg ha⁻¹	30 cm-10kg ha⁻¹	-0.9364	-0.5598*	-0.1833
15 cm-4.5kg ha⁻¹	40 cm-16 kg ha⁻¹	-0.8447	-0.4682*	-0.0916
20 cm-7.5kg ha ⁻¹	30 cm-10kg ha ⁻¹	-0.7240	-0.3475	0.0290
20 cm-7.5kg ha ⁻¹	40 cm-16 kg ha ⁻¹	-0.6324	-0.2558	0.1207
30 cm-10kg ha ⁻¹	40 cm-16 kg ha ⁻¹	-0.2849	0.0917	0.4682

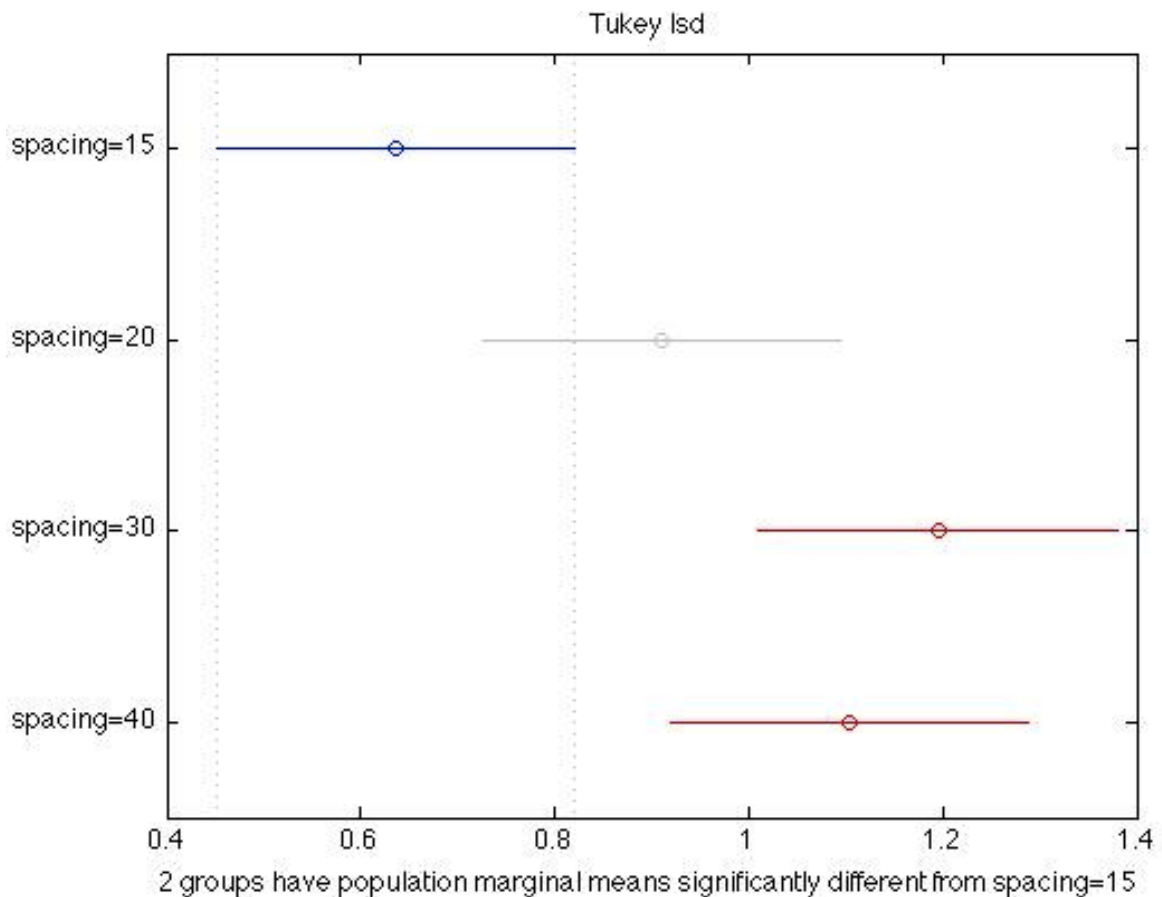


Figure 3.3: Population means and standard error bars for Spacing x Harvested Biomass (x-axis), showing 30 and 40 cm spacing significantly different from 15 cm.

A 2-way ANOVA (4x4) for Total Corms gave significant results for Corm Size but not for Spacing (including any of the standard transformations), $F(3,32) = 47.7, p < 0.00001$. A 2-way ANOVA (4x4) for Survival Percentage produced similar results, i.e. significant results for Corm Size but not for Spacing (including any of the standard transformations) $F(3,32) = 41.74, p < 0.00001$ (See Appendix 3.4). In both cases the Corm Size results matched those reported for harvest biomass, i.e. MS, ML, and L significantly different from S, and ML significantly different from MS. (In neither case was the interaction effect significant—see Appendix 3.4).

The data show a trend to higher biomass harvest per planted corm at the larger spacing (20-40 cm) within the MS, ML and L sizes, though there was a confounding factor because the larger spacing also used larger rates of compost per plot (Fig 3.4).

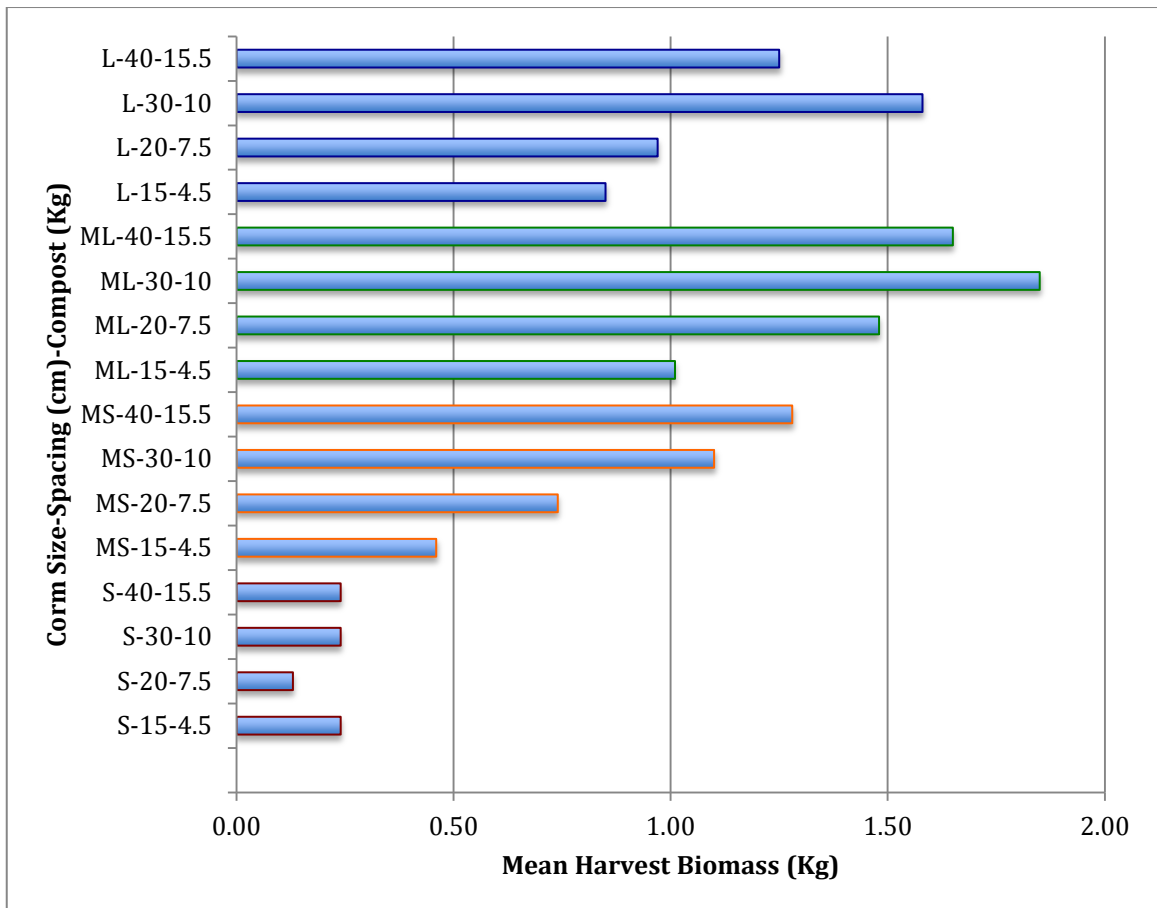


Figure 3.4: 2005-2006 Mean Harvest Biomass (kg) as Response to Compost x Spacing x Corm Size.

The data show a trend towards higher total number of harvested corms with larger spacing, larger compost amounts, and larger corm sizes (Fig 3.5).

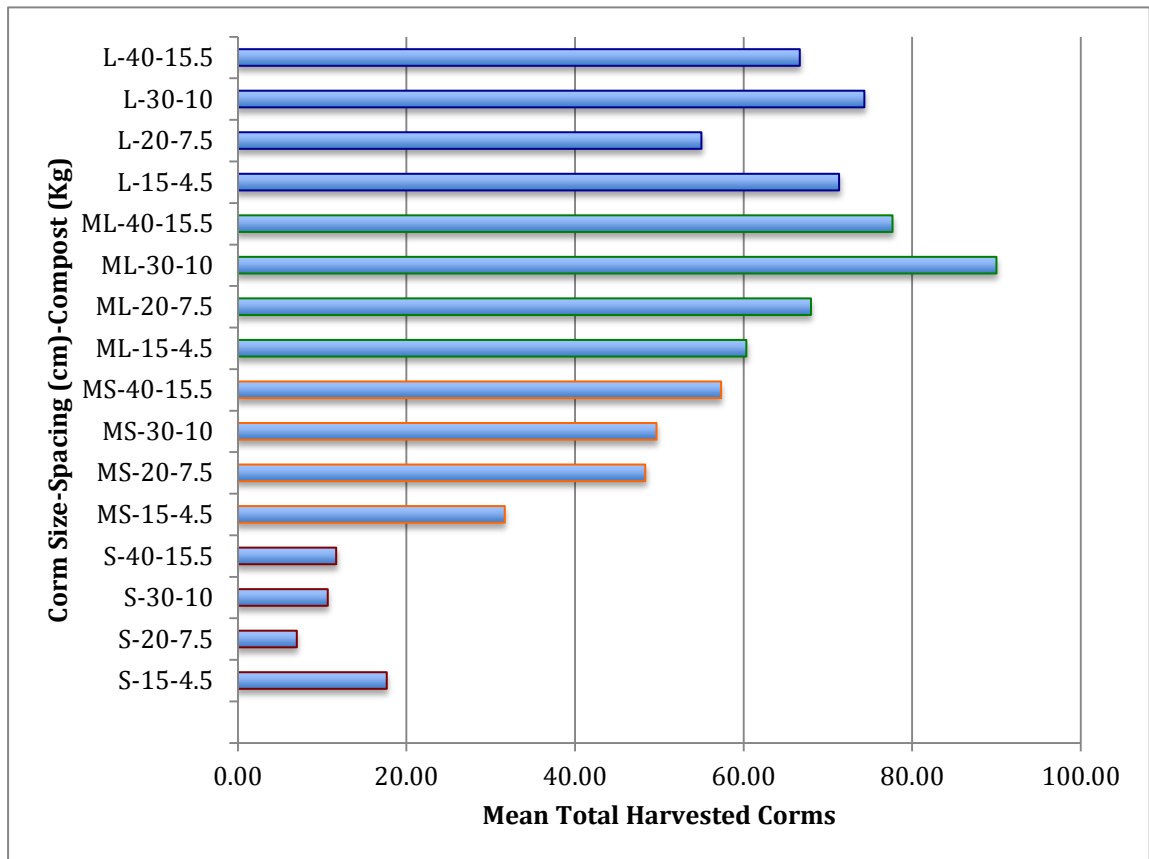


Figure 3.5: 2005-2006 Mean Total Harvested Corms as Response to Compost x Spacing x Corm Size.

The 2005-2006 Compost-Only trial gave inconclusive results, due to its small size. A one-way ANOVA testing harvested biomass against compost levels approached but failed to reach significance (Appendix 3.4). The data suggest better results with more compost, but are insufficient to draw any solid conclusions, even from the trend (Table 3.7 and Fig 3.6).

Table 3.7: 2005-2006, Compost Only Trial Harvest (15 corms plot⁻¹), Table of Means

Plot	Size	Treatment (Compost amount, Kg ha ⁻¹)	Treatment		Mean kg plot ⁻¹	Mean kg		Mean kg bulb ⁻¹ post split
			Small	Large		Plot ⁻¹	Post- split	
P001	MS	None	10	24	1,12	0,67	11	0,02
P005	MS	None	5	5	0,29	0,2	6	0,02
P009	MS	None	14	9	0,4	0,27	7	0,012
P003	MS	2	4	7	0,39	0,23	3	0,021
P007	MS	2	3	13	0,71	0,44	10	0,028
P002	MS	4	5	21	1,13	0,73	8	0,028
P004	MS	4	5	19	0,93	0,6	8	0,025
P006	MS	4	12	25	1,33	0,75	11	0,02
P008	MS	4	2	10	0,5	0,36	5	0,03
P010	MS	4	2	8	0,4	0,25	3	0,025

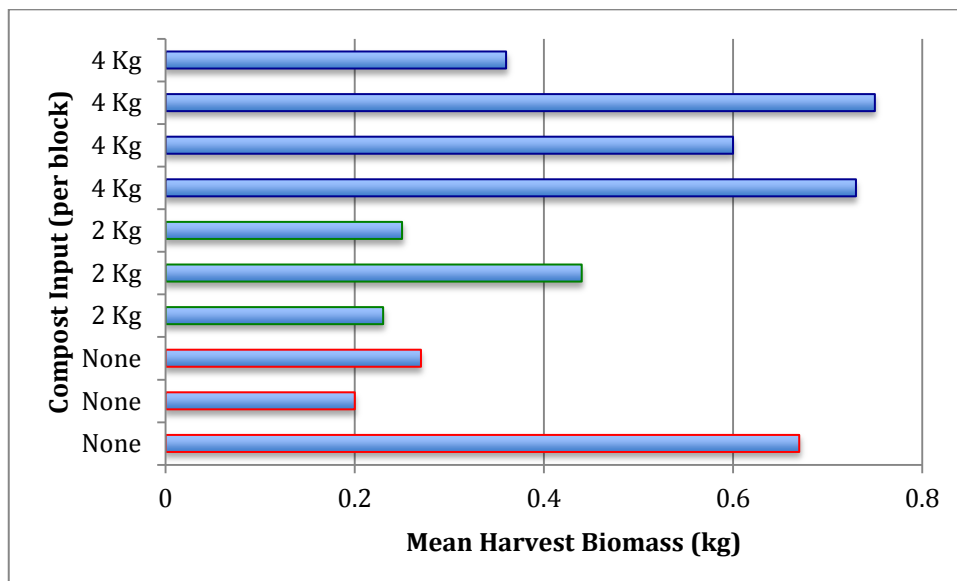


Figure 3.6: 2005-2006, Compost-Only Trial, Mean Harvest Biomass (kg) x Compost Input per plot.

In 2006-2007, the trends from our spacing-only trial confirmed our observations from 2005-2006, that the 20-40 cm spacing provides the best yield. In this particular trial, using 15 corms per plot, we measured a steady increase in overall biomass as the corm spacing increased, but plant survival among the 20-40 cm spacing was even. Field

observations at the time of harvest showed stunted growth, 50% or lower survival, and lower total biomass per plot for the 5 cm and 10 cm spacing, with some improvement in these measures with the 15 cm spacing.

For the 2006-2007 Spacing Trial an ANOVA test for biomass (Appendix 3.4) did not show significant differences among the treatments, but the data show a clear trend towards larger biomass per plot, larger number and survival percentage of plants, and larger mean weight of corms at a spacing of 20-40 cm (Table 3.8 and Fig 3.7).

**Table 3.8: Spacing only Trial 2006-2007, Table of Means
Corm Yield as a Result of Compost x Spacing (Equal mix of corm sizes, Medium and Large)**

Spacing (cm)	Small Corms plot ⁻¹	Large Corms plot ⁻¹	Harvest			Mean corm weight (g corm ⁻¹)
			biomass (g plot ⁻¹)	No. of plants	Survival %	
5	15,25	1,25	50,25	7,50	0,50	34
10	13,00	4,00	54,67	7,33	0,49	30
15	19,50	2,75	79,25	8,50	0,57	33
20	16,50	3,25	87,00	9,25	0,62	44
30	20,75	3,25	91,25	9,75	0,65	36
40	19,75	4,00	100,25	9,00	0,60	40

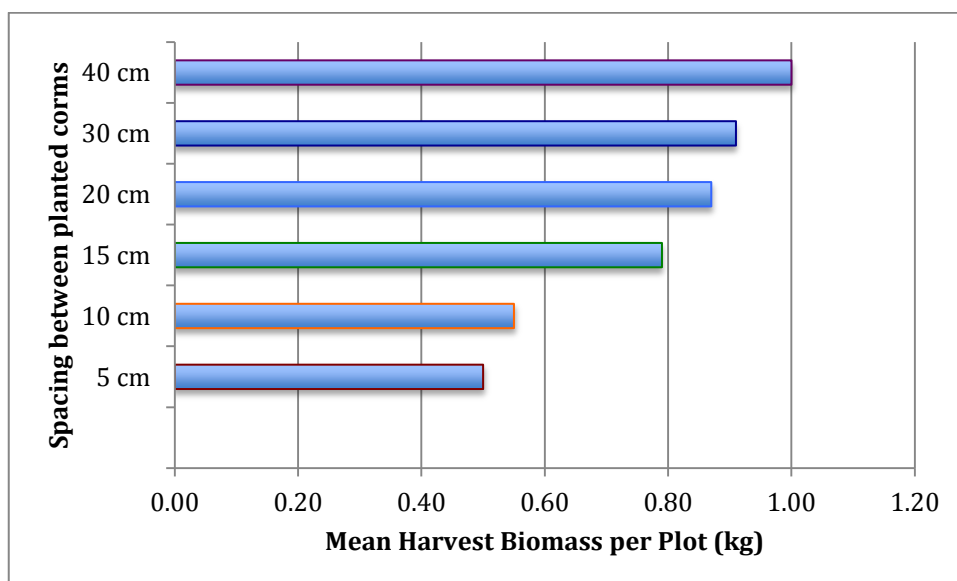


Figure 3.7: 2006-2007 Spacing Trial, Corm Yield (kg) x Spacing per plot

For the 2006-2007 Compost Only Trial, with 15 plants per block, with a 30 cm spacing, the data trends suggest a steady increase in productivity for total corms and for total biomass per block with a increase in the compost levels. Mean size of corm and roots was even, and survivability % was not informative (Table 3.9 and Figs 3.8 and 3.9).

Table 3.9: 2006-2007 Compost Only Trial, Table of Means

Treatment (Kg ha ⁻¹ compost)	No. of corms, small	No. of corms, large	Total No. of corms	Total corm weight Kg block ⁻¹	No. of Plants	Survi- val %	Biomass (g corms and roots)	No. of corms per plant
0	25,33	5,67	31	0,99	9,67	65	0,033	3,21
5	41,33	2,67	44	1,63	13,67	91	0,037	3,22
10	31	3,33	34,33	1,21	8,67	58	0,035	3,96
15	47,33	5,33	52,67	1,9	12	80	0,036	4,39

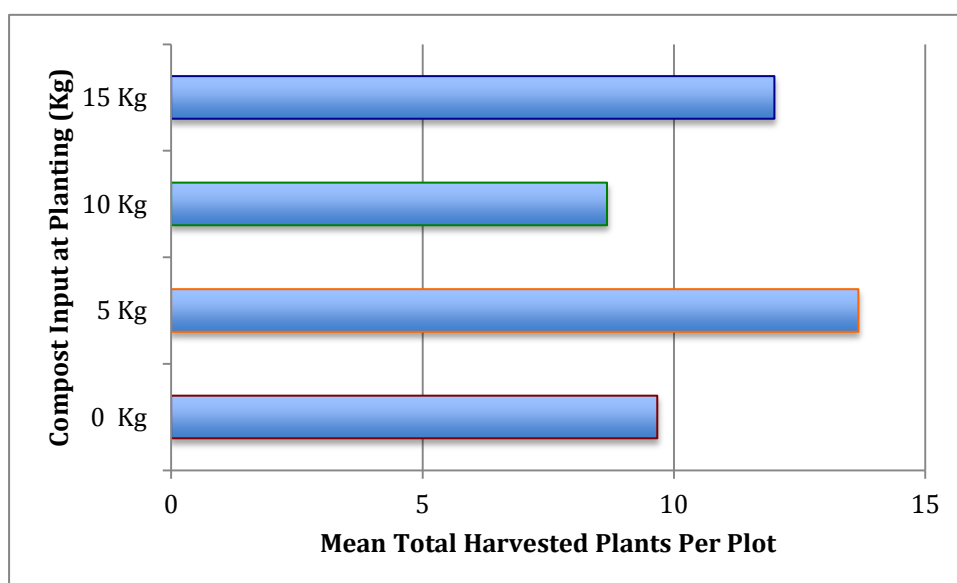


Figure 3.8: 2006-2007 Mean Harvested Plants Per Plot x Compost Level

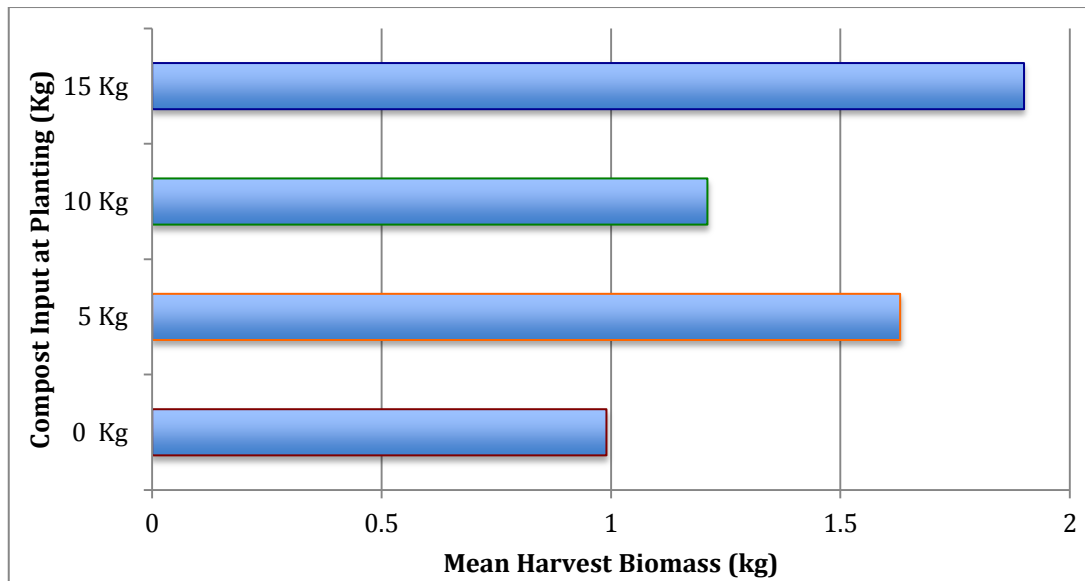


Figure 3.9: 2006-2007 Mean Harvest Biomass (kg) x Input Compost Level per plot.

3.4. Discussion

For each of the trials we performed an analysis of variance (ANOVA), with standard transformations. For the 2005-2006 trial data, a 2-way (4x4) ANOVA for Corm Size and Spacing-Compost level yielded significant differences. For Corm Size, there was clearly superior performance in Harvest Biomass, Survival Percentage, and Total Corms harvested for the MS, ML and L as compared to the S Corm Size, and for the ML as compared to the MS Corm Size. For Spacing-Compost Level, the ANOVA clearly showed 30 cm Spacing with 10kg ha⁻¹ Compost, and 40 cm Spacing with 16kg ha⁻¹ provided significantly better performance in the Biomass measure (only) than 15 cm Spacing with 4.5kg ha⁻¹ Compost, though the differences of the 15 cm, 30 cm and 40 cm Spacing from the 20 cm Spacing with 7.5kg ha⁻¹ were not significant (perhaps due to reduced sample size for the 20 cm Spacing). ANOVA tests of Survival Percentage (with angular transformation) and of Total Corms (including with standard transformations) did not show significant differences for Spacing (Appendix 3.4).

For the smaller 2005-2006 trial (Compost Only) and the 2006-2007 Trials (Compost Only, Spacing Only), however, none of the effects were statistically significant (Appendix 3.4). Subjective examination of the means suggests some trends. For corm size at field planting, corms below 10g weight, and smaller than a 30 mm base and height should probably be established in the nursery prior to hardening off and planting in the field.

Tiny cormels, smaller than 3grams each, and 12 mm height and base, can be raised successfully under ideal conditions in a nursery. For spacing in the field, between 20-40 cm between plants appears optimal. Larger field trials with more corms per block (e.g. 30-35) and more reps would be needed to fine-tune this finding. The trials did not provide clear indications of optimal compost levels, given the quantities of the compost we had available for the trials, and the levels we tested. It does appear that retaining the tuberous roots on the corms after harvest, and planting corms with tuberous roots attached provided for better growth and survival.

The late dates for planting both seasons undoubtedly skewed the results, and a better growth response would be obtained if trials were to be planted in September in KwaZulu-Natal. In all the trials it was evident that new daughter corms were still developing at the time of winter die-back, and we suspect this was because of the delayed planting times. The consistency of new daughter corm production across all the treatments at harvest suggests that this plant behavior is genetically pre-programmed. Final corm size of a full-grown corm also seems to be genetically predetermined. In none of our crop trials (Compost, Spacing, Corm Size, Shade, Biocontrol, and Fertilizer) were there any noticeable increases in corm size as a result of the treatments. Earlier planting would have provided an additional two or more months of growth, and this would have had a substantial effect on most of the tested parameters.

For harvesting, it is suggested that the clumps of tuberous roots and corms be field washed and air dried before bagging for storage. Moistening the soil prior to harvest assisted the ease and completeness of harvest of corms. Senesced mother corms should be removed during field washing, as they otherwise absorb water, and this will prevent the harvested corm and tuberous root cluster from drying out completely.

For storage, it is suggested that stored corms and tuberous roots be kept in closed containers at slightly cooler than room temperature, if possible. Tuberous roots left to air dry shriveled to a fraction of their original size within a few weeks, suggesting a high original moisture content. It may be that physiologically these organs serve for both nutrient and water storage during the dry winter months. Corms left to air dry for extended periods (4-6 months), gradually lost weight and some appeared to senesce.

Our colleague David Moon's successful revival of tiny, very dry cormels suggests that those corms we discarded as apparently senesced after the pre-season trials may still have been capable of growth. However, we were not able to repeat that experiment in the field.

A mould was observed colonizing the corms during storage. It is not clear whether this fungus had any effect on germination or growth. Further research might be conducted to identify the mould, and to determine whether it might have medicinal properties itself.

Our research into composted mulch identified a key quality issue. Ideally one would use a genuine mulch, such as 50 mm of pine bark or another lignitic substance, to mimic the effects of forest litter or grass from the grasslands in keeping the soil surface cool, as the corm sits just beneath the soil surface. Unfortunately the compost we had available was composted chicken litter. This is primarily cellulosic and breaks down as the season progresses; the initial NPK levels may also have been too low. For farmers wishing to use organic fertilizer, it is suggested to consider some top dressing. Further field trials should address this issue.

3.5 References

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Appendix 3.1: Soil Analysis of terraces (shown just for first season; second season essentially the same).

#	Density (g ml ⁻¹)	P (mg L ⁻¹)	K (mg L ⁻¹)	Ca (mg L ⁻¹)	Mg (mg L ⁻¹)	Exch. Acidity (mol L ⁻¹)	Total cations (mol L ⁻¹)	pH (KCL)	Zn (mg L ⁻¹)	Mn (mg L ⁻¹)	Cu (mg L ⁻¹)	NIRS clay (%)
1	1.00	10	326	2395	602	0.08	17.62	5.15	25.8	13	16.1	49
2	1.03	22	580	2148	571	0.04	16.94	5.68	41.7	17	13.6	58

Notes: Acid Saturation % was Zero for both samples. NIRS organic carbon % was not registered for either sample. Soil analysis provided by Cedara's Fertilizer Advisory Service, KZN Dept. of Agricultural and Environmental Affairs.

Appendix 3.2: Composted Chicken Litter Analysis

N (%)	Ca (%)	Mg (%)	K (%)	Na (%)	P (%)	Zn (mg L ⁻¹)	Cu (mg L ⁻¹)	Mn (mg L ⁻¹)	B (mg L ⁻¹)
1.99	6.56	0.79	2.23	0.54	2.53	584	69	653	77

Data on 100% dry matter basis, courtesy of Eston Organics. Soil analysis provided by Cedara's Fertilizer Advisory Service, KZN Dept. of Agricultural and Environmental Affairs.

Appendix 3.3: Weather Data

Data for SAWS station [0239698 5] - PIETERMARITZBURG -29.6330 30.4000, 673 m

Mean Max and Min Temps, Mean and Daily Rainfall, 2005-2006										
Month	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug
Max	26,6	27	28,4	28,3	26	25,2	21,6	21,6	24,4	22,7
Min	16,1	15,4	18,8	19,4	14,9	13,5	7,5	4,8	6,3	7,9
30°C	9	10	13	9	5	0	0	0	0	0
Rainfall (mm)	71,4	102,2	185,6	54,8	98,6	109,2	68	1,4	0,4	52,2
Days of Rain	15	17	19	12	11	10	5	1,4	0,4	4
Daily Mean (mm)	2,38	3,30	5,99	1,96	3,18	3,64	2,19	0,05	0,01	1,68

Mean Max and Min Temps, Mean and Daily Rainfall, 2006-2007										
Month	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug
Max	25,6	27,1	29,07	30,66	27,54	26,11	26,59	22,63	23,71	25,19
Min	15,1	16,5	17,57	18,56	16,65	14,53	8,13	6,37	5,34	7,81
30+C	6	8	11	13	9	8	10	0	0	3
Rainfall (mm)	101	177,2	69,8	38	192,8	24,6	7,4	60,6	0	14
Days of Rain	17	19	10	5	15	11	1	3	0	3
Daily Mean (mm)	3,37	5,72	2,33	1,36	6,22	0,82	0,24	2,02	0	0,45

(Weather Data courtesy of the South African Weather Service)

Appendix 3.4: ANOVA test results

2005-2006, Corm Size by Spacing-Compost, 2-way (4x4) ANOVA of Harvest Biomass

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Corm Size	10.3799	3	3.10869	16.87	0.0000
Spacing-Compost	2.3169	3	0.77229	3.77	0.0201
Cormsize x Spacing-compost	1.0463	9	0.11626	0.57	0.8315
Error	6.5612	32			
Total	20.3043	47			

2005-2006, Compost & Spacing x Corm Size, 2-way (4x4) ANOVA of Total Corms per block

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Corm Size	27164.2	3	9054.74	47.7	0.0000
Spacing-Compost	1103.2	3	367.74	1.94	0.1434
Cormsize x Spacing-compost	2176.7	9	241.85	1.27	0.2885
Error	6074.7	32	189.83		
Total	36518.8	47			

2005-2006, Compost & Spacing x Corm Size, 2-way (4x4) ANOVA of Survival % (angular transformation)

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Corm Size	0.0357	3	0.0119	41.74	0.0000
Spacing-Compost	0.0013	3	0.00043	1.52	0.227
Cormsize x Spacing-compost	0.0032	9	0.00036	1.25	0.3017
Error	0.00912	32	0.00029		
Total	0.04933	47			

2005-2006, Compost Only, One-way ANOVA of Harvested Biomass

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Compost	0.17873	2	0.08937	2.48	0.1532
Error	0.24207	7	0.03601		
Total	0.4308	9			

2005-2006, Compost Only, One-way ANOVA of Total Corms/Block

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Compost	282.017	2	141.008	1.58	0.2712
Error	624.083	7	89.155		
Total	906.1	9			

2005-2006, Compost Only, One-way ANOVA of Survival % (angular transformation)

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Compost	0.0019	2	0.00095	0.83	0.4737
Error	0.00798	7	0.00114		
Total	0.00988	9			

2006-2007, Spacing Only, One-way ANOVA of Harvested Biomass

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Spacing	0.77262	5	0.15452	0.66	0.6613
Error	4.00497	17	0.23559		
Total	4.77758	22			

2006-2007, Spacing Only, One-way ANOVA of Total Corms/Block

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Spacing	205.62	5	41.1239	0.48	0.7883
Error	1465.25	17	86.1912		
Total	1670.87	22			

2006-2007, Spacing Only, One-way ANOVA of Survival % (angular transformation)

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Spacing	0.15755	5	0.03151	0.33	0.8857
Error	1.60673	17	0.09451		
Total	1.76428	22			

2006-2007, Compost Only, One-way ANOVA of Harvested Biomass

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Compost07	1.50816	3	0.50272	1.51	0.2851
Error	2.66793	8	0.33349		
Total	4.17609	11			

2006-2007, Compost Only, One-way ANOVA of Total Corms/Block

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Compost07	865.667	3	288.556	2.56	0.1284
Error	903.333	8	112.917		
Total	1769	11			

2006-2007, Compost Only, One-way ANOVA of Survival % (angular transformation)

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Compost07	0.00205	3	0.00068	1.42	0.3061
Error	0.00385	8	0.00048		
Total	0.0059	11			

Chapter 4: Effects of *Trichoderma* Treatment on Growth of *Siphonochilus aethiopicus* (Schweif.) B.L. Burt.

4.1. Introduction

Beneficial microbes have proven to be effective enhancers of crop production, both by providing improved pathogen protection and by increasing crop growth through multiple modes of action such as improved root size and length (Krauss et al 2004). As a preliminary investigation of whether biocontrol agents might improve *S. aethiopicus* productivity, crop trials were conducted for one season with the biocontrol fungus Eco-77 (*Trichoderma harzianum* Strain B77), and for two seasons with the biocontrol fungus Eco-T (*Trichoderma harzianum* Strain kd)⁴ (Hadar et al 1979, Schirmbock et al 1994).

The fungus in Eco-77 was originally isolated from a grape vine in Stellenbosch. It colonizes the first millimeter of plant tissue, and then consumes other pathogens attempting colonization. Registered in South Africa for control of *Eutypa lata* (Pers.) Tul. and C. Tul, the biocontrol fungus also protects against *Botrytis cinerea* Pers.:Fr. and *Sclerotinia sclerotiorum* (Lib.) de Bary, and is aggressively pathogenic to *Fusarium* sp. (M. Laing personal communication). *Siphonochilus* corms are often colonized by an as-yet unidentified fungus post-harvest and while in storage. Silverglen Nursery uses a common commercial fungicide (Benlate®)⁵ to treat the corms. This trial tested the effectiveness of using Eco-77 to colonize the split surfaces of the bulbs and prevent fungal growth.

Spores of the fungus in Eco-T (*Trichoderma harzianum* Strain kd) germinate and colonize the soil in the immediate root zone, surviving on plant-root exudates. Its anti-pathogen effectiveness results from stimulating plant immune responses, from outcompeting and displacing pathogenic microorganisms, and from synthesis of root-zone pathogen growth inhibitors (Elad et al 1980). Electron micrographs show *T. harzianum* strands coiling round, penetrating, and then consuming pathogenic fungal strands (Plant Health Products 2011). Among the several chitinolytic enzymes, endochitinase and chitobiosidase have been identified as used by *Trichoderma* to

⁴ Plant Health Products, Nottingham Road, South Africa; *Trichoderma harzianum* Strain Eco-77, and Strain Eco-T.

⁵ E.I. du Pont de Nemours and Company, Wilmington, Delaware, U.S.A.

dissolve the cell walls of target pathogenic fungi (Lorito et al 1993), as well as beta -1,3-glucanase and chitinase (Elad et al 1982). *Trichoderma* strains can improve drought resistance by increasing lateral root growth, and can decrease the requirement for supplemental nitrogen by dissolving insoluble minerals from the soil, and converting ammonium to nitrate so that ammonium is absorbed into the root; H⁺ dissolves mineral salts around roots and release P and Si, providing an acid effect at microscopic levels next to the roots. (Plant Health Products 2011, and M. Laing, personal communication). Our trials tested whether the treatment of *S. aethiopicus* corms with Eco-T either before, at, or after planting, improved productivity.

4.2. Materials and Methods

Eco-77 (*T. harzianum* Strain B77) and Eco-T (*T. harzianum* Strain kd) were supplied courtesy of Plant Health Products (Pty) Ltd. Eco-77 was applied at the recommended rate of 1 g per 2 litres water, dipping the corms and allowing them to air dry. The three treatments were control (no treatment), Eco-77 application, and fungicide application (Rovral® Flo).⁶ Eco-T was applied at the recommended rate of 3 level teaspoons per 9 liters of water. We used four reps of four treatments in complete randomized blocks, each with 9 corms per plot at 30 cm spacing, and 30 cm between blocks: Treatment 1: untreated (control); Treatment 2: Eco-T applied to corms (standard rate) at planting; Treatment 3: Eco-T applied to corms as drench (standard rate), one time, at planting. Treatment 4: Eco-T applied to corms as drench (standard rate), one time at planting, and a second time one month later (standard rate). In the 2006-2007 season we increased the number of corms from 9 to 15 per plot, again at 30 cm spacing and 30 cm between blocks, and eliminated the Eco-T application to corms prior to planting. We used 3 reps each of a) Control plots (no treatment), b) Standard Rate Drench at Planting (SDRP), and c) Drench at Planting plus Standard Rate Drench 4 weeks later (SDRP3+).

Crop trials were conducted over two seasons on a private farm just northeast of Pietermaritzburg, KwaZulu-Natal, at approximately 675 m altitude, lat-29.6330, long 30.4000. *Siphonochilus aethiopicus* corms were purchased from Silverglen Medicinal Plant Nursery, Chatsworth, Durban. The 1500 partially pre-split corms had had the

⁶ Bayer Crop Science AG, Monheim am Rhein, Germany.

tuberous roots removed by the Silverglen staff, and had been pre-treated with Benlate®⁷ fungicide after splitting. We split them further prior to planting, yielding 2850 plantable corms. After splitting, corms were separated and laid out to dry in the Ukulinga Research Farm (UKZN) Seed Sorting House. Fresh tissue sometimes exposed by splitting suberized within one day. Corms were dried at room temperature on tables for several weeks, creating an informal storage-drying test effect for germination, then re-bagged in brown paper bags and stored in metal lockers at room temperature. Corms were then removed from storage and sorted by size and number, yielding results as in the Table 4.1. Some additional miniscule corms (cormels) deemed too small for field planting were potted and tended in the farm’s nursery (courtesy of David Moon).

Table 4.1: 2005-2006 Planting, Bulb Counts and Sizes post-split

Corm Sizes	Number of Corms	Weight (g)	Height (mm)	Base Diameter (mm)
Large (L)	410	29.64g	52.37	44.10
Med Lg (ML)	990	19.82g	38.48	37.78
Med Small (MS)	610	9.95g	29.65	27.93
Small (S) (cormels)	840	2.28g	12.38	12.6
TOTAL	2850			

In 2005-2006 the Eco-T corms, along with all other corms except those in the Eco-77 trial, were treated with Eco-77 shortly prior to planting, but this was done after suberization of tissue exposed by the second splitting. In 2006-2007, calluses were allowed to form over tissue exposed by splitting, without treating the bulbs with Eco-77 or with the fungicide. The normal planting date for *S. aethiopicus* would be September, but for various logistical reasons planting was delayed until November. By the third week of November 2005 all the trials had been hand-planted, so this timing was repeated for the 2006-2007 trials. For the 2006-2007 season we planted a roughly even distribution of larger and smaller corms in each block for all the trials, so corm size variations were not separately recorded.

In all the trials plants were grown on level terraces in full sun, though a small amount of shade reached the plants in the late afternoon from nearby trees. Fertilizer was organic

⁷ E.I. du Pont de Nemours and Company, Wilmington, Delaware, U.S.A.

composted chicken litter supplied courtesy of Eston Organics⁸ (Appendix 4.1). Plants were irrigated as necessary during the growing season, depending on rains and resultant field moisture. Irrigation was approximately 30 mm per week of water to supplement irregular rainfall (Appendix 4.2).

Field preparation was by manually turning the soil with hoes and forks. The level terraces had previously been limed, and treated the previous season with 2:3:2 (28) fertilizer for growing cabbages. The P levels were low, 10-22 mg l⁻¹ (Appendix 4.3). Resident Guinea fowl controlled cutworm and snails.

Tables of Means were calculated and created in Excel. Statistical analysis was performed using MATLAB (version 7.9.0, The Mathworks, Inc., Natick, MA, USA). For the 2005-2006 Eco-77 trial and for the Eco-T trial, one-way ANOVAs were run for total corms/block, harvested biomass, and survival percentage (angular transformation). For the 2006-2007 Eco-T trial, one way ANOVAs were performed again for total corms/block, harvested biomass, and survival percentage (angular transformation). An additional Tukey's least significant difference T-test (LSD) was performed for total harvested corms for the Eco-77 2005-2006 trial.

4.2.1. Harvesting

Plots were watered before harvesting to soften the soil. Traditional healers had reported that harvesting when yellowing leaves were still visible above the soil produces corms with the highest medicinal value. In retrospect, it would have been easier to harvest at that time (in June) when the leaves were still visible, rather than later when all the leaves had died off and fallen away, as the lack of surface leaves made it more difficult to ensure one had successfully harvested all the corms present. A simple field washing system was used to remove soil from harvested corms.

4.3. Results

Mean corm weight was calculated post-splitting and cleaning, and these mean individual corm weights after both seasons closely paralleled the original weights from our purchased Silverglen material (Table 4.2). This indicated that none of the treatments had any significant effect on corm size, suggesting that corm size and weight may be

⁸ 1 Nutty Isles Farm 904/9 Umlaas (MR 21/1) Rd, Camperdown, South Africa.

genetically predetermined. However, these results are not definitive, given our late planting date each season. Our late planting date appeared to have diminished the total number of daughter corms per initial planted corms. There were only 3-4 new corms per planted corm. This contrasts with the notes from other farmers who have grown *S. aethiopicus* and reported 6-9 daughter corms per planted corm (D. Mitchell, P. Cele, Silverglen staff, personal communication 2003-2006).

Table 4.2: 2005-2006 Eco-77 Trial Harvest, Table of Means

Treatment	Small Corms	Large Corms	Total Corms	G block ⁻¹	No. of Surviving Plants	G corm ⁻¹	Survival %	Net Gain in Corms
Control	11,5	13	24,5	520	5,75	20,02	64%	15,5
Eco-77 Drench	17,8	14,5	32,3	840	8,25	24,72	92%	23,3
Fungicide	9,75	16,5	26,25	640	7,75	23,29	86%	17,3

A one-way ANOVA test for Total Corms was significant ($F(2,9)=4.73$, $p=0.0394$) (Appendix 4.4). Tukey's LSD analysis revealed that the Eco77 Drench at Planting produced a Total Corm per Block Count significantly different than that of the Fungicide, but neither was significantly different than the Control (Table 4.3).

Table 4.3: Eco77 2005-2006 Total harvested corms, LSD Table (* marks significance)

Comparisons	Lower bound	Tukey's LSD	Upper Bound
Ctr x Eco77	-25.6912	-12.8125	0.0662
Ctr x Fng	-8.9412	3.9375	16.8162
Eco77 x Fng	3.8713	16.7500*	29.6287

One-way ANOVA tests (with transformations) for other measured parameters (Harvest Biomass, Survival Percentage) were not statistically significant (Appendix 4.4), yet one can see a clear trend for greater productivity with the Eco-77 drench before planting when compared with the fungicide and control treatments (Table 4.3): 840 g block⁻¹ for Eco-77, vs. 640 g block⁻¹ for Fungicide and 520 g block⁻¹ for control, and survivability: 92% for Eco-77 drench, vs. 86% for Fungicide, and 64% for control. Also noticeable was the lack of *Erwinia* infection on the Eco-77 trial plots. With the exception of large corms,

all the other production measures showed improvement with Eco-77 as compared with Fungicide or Control (Fig 4.1).

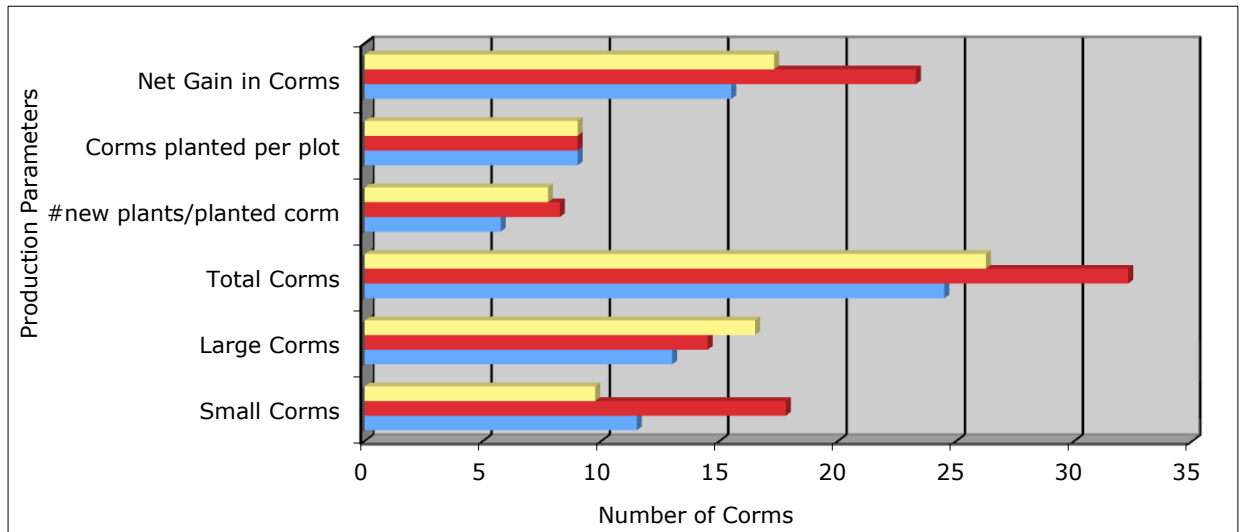


Figure 4.1: Production Parameters of the Eco-77 Trial; Red = Eco-77 treatment; Yellow = Fungicide; Blue = Control.

The results from the 2005-2006 Eco-T trial were inconclusive. In many measures the control was as good as or outperformed any of the treatments. One-way ANOVA tests (and transformations) for effects of Eco-T showed no significant differences of treatments for the measured parameters (Appendix 4.4).

Table 4.4: Table of Means, 2005-2006 Eco-T Trial Harvest (9 corms planted per block)

Treatment	Total Small Corms block ⁻¹	Total Large Corms block ⁻¹	Total Corms block ⁻¹	Total g block ⁻¹	No. Surviving Plants	g corm ⁻¹	Survival %	% Gain in corms
Control	10	13,5	23,8	410	5	18	55,56	264
Eco-T on bulb	7	9,5	16,5	280	4,75	17	52,78	183
Eco-T on bulb and 1x drench	6,8	10	16,8	300	4,5	18	50,00	187
Eco-T on bulb and 2x drench	7,5	13	20,5	380	6	19	66,67	228

We repeated the Eco-T trial in the 2006-2007 season, eliminating the direct treatment of the corms prior to planting. Instead we drenched at planting, and for one treatment then again after 4 weeks (Figs 4.2 and 4.3). Again, results failed to reach significance in

the one way ANOVA for harvested biomass, total corms/block, or survival percentage (Appendix 4.4). Although again not statistically significant, the trend suggests that a drench of Eco-T at planting may have some effect in enhancing productivity both in terms of total number of harvestable corms (and therefore planting material for the following season), and in total biomass. The second drench appears detrimental and is therefore not recommended.

Table 4.5: Eco-T Harvest 2006-2007 Table of Means

Treatment	No. Small Corms	No. Lg Corms	Total Corms	g plot ⁻¹	No. of Plants	Survival %	kg corm ⁻¹
None	14,67	4	18,67	150	8	89	0,080
D	29,33	2	31,33	164	9,67	107	0,052
D+	20	1,33	21,33	148	6,67	74	0,069

Notes: 0 = Control; D = Drench at planting; D+ = Drench at Planting and 4 weeks.

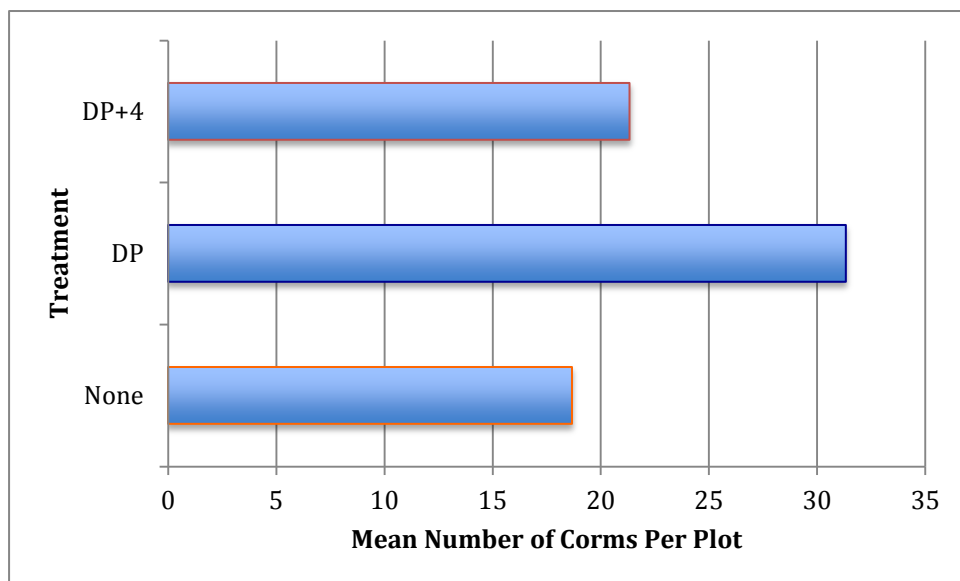


Figure 4.2: Eco-T Corms Per Plot at Harvest x Treatment. DP: drench at planting, DP+4, drench at planting +4 weeks; None: Control.

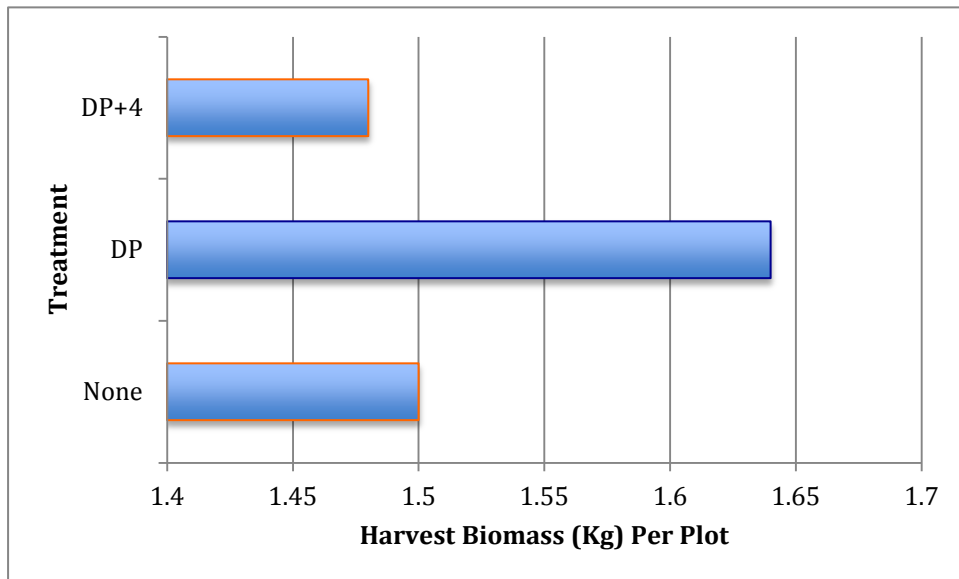


Figure 4.3: Eco-T Harvest Biomass (Kg) Per Plot x Treatment. . DP: drench at planting, DP+4, drench at planting +4 weeks; None: Control.

4.4. Discussion

For all of the data sets, we ran an analysis of variance (ANOVA, including with standard transformations). Of these tests, only the Eco 77 Trial Total Corms measure showed a significant difference between the higher value for Eco 77 treated plots compared to the Fungicide. Neither treatment was significantly different than the Control plots, so no reliable conclusions can be drawn. In all the other ANOVAS of Eco 77 and EcoT treatments and measures, none of the treatments produced statistically different results. However, examination of the means suggests the following trends: Eco-77 appeared to improve survivability and biomass production as compared with the Fungicide treatment and the Control treatment. This suggests it may have some effects on reducing root pathogens and increasing nutrient uptake. In the Eco-77 trial, both the Fungicide and Eco-77 treatments exhibited a positive response to treatment as compared with the Control. The corms in storage developed a fungus, and this fungus may inhibit growth of the corms. Further research is needed. The small number of corms available for the trial was a significant limiting factor (primarily due to cost). The ideal number of corms per plot would be 35-40, and with 3-4 reps per treatment. Additionally, one would ideally be able to plant corms in September, and harvest in June, which was not possible for this trial.

The Eco-T trials were also hampered by a limited number of corms available, the lack of uniform sized corms, and the delayed planting and harvesting times at each season. The Eco-T trial in the first season gave inconclusive results. The results in the second season suggest the possibility of some stunting of plant growth and development from overdosing with Eco-T. Neumann (2005) showed that high levels of Eco-T do cause toxic levels of ammonium uptake by plants under warm conditions. Our preliminary results appear to confirm his findings, suggesting that *S. aethiopicus* may also be sensitive to ammonium toxicity, and that farmers should use a preponderance of nitrate nitrogen fertilizers in summer. This should be tested more carefully with a larger trial, comparing two or three types of nitrogen fertilizer.

One notable result of the Eco-T trial in the 2006-2007 season was that the total grams per block were much higher than that of adjacent crop trials (Shade, Fertilizer, Spacing, Compost) (Table 4.6), even though all trials were planted with 15 corms per block, and all with the exception of the spacing trial were at 30 cm between corms. *T. harzianum* has been shown to contribute to control of *Rhizoctonia solani* damping-off (Yobo et al 2011), suggesting that investigation of *S. aethiopicus* susceptibility to damping-off may be advisable.

Table 4.6: 2006-2007 Inter-trial comparison

Trial	Mean Grams/Block	% of Planted Corms surviving
Fert	1085,5	68,62%
Shade	790,35	66,30%
Eco-T	1538,89	54,00%
Spacing	770	51,17%
Compost	1430	73,77%

However, if we look closely at the data from the 2006-2007 Eco-T trial (Table 4.7), we also see that the control plots showed higher grams/block at harvest as well.

Table 4.7: 2006-2007 Eco-T trial

Treatment	g/block	Treatment	g/block	Treatment	g/block
0	1800	D	2120	D+	2440
0	1700	D	1650	D+	840
0	1000	D	1140	D+	1160

It may simply have been that the soil in the area of the terraces used for the Eco-T trial had more nutrients remaining from the prior season's crops. Unfortunately we could not perform individual soil analyses for each trial plot. However, the result does suggest that it is possible to gain much higher productivity than what we showed in our crop trials.

One final point is that in both the Eco-77 and Eco-T trials, as with the Compost-spacing-corm size trial, few of the plants showed the same degree of chlorosis as the plants in the fertilizer trial during the high heat of the first growing season. Whether this was due to the mulching effect of the compost, was simply a field effect, or had something to do with a plant-strengthening effect of the biocontrol agents would have to be determined in further research trials. All of the plants with the compost, instead of the fertilizer, emerged earlier than the fertilized plants.

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Appendix 4.1: Soil Analysis of terraces (shown just for first season; second season essentially the same).

#	Density (g ml ⁻¹)	P (mg L ⁻¹)	K (mg L ⁻¹)	Ca (mg L ⁻¹)	Mg (mg L ⁻¹)	Exch. Acidity (mol L ⁻¹)	Total cations (mol L ⁻¹)	pH (KCL)	Zn (mg L ⁻¹)	Mn (mg L ⁻¹)	Cu (mg L ⁻¹)	NIRS clay (%)
1	1.00	10	326	2395	602	0.08	17.62	5.15	25.8	13	16.1	49
2	1.03	22	580	2148	571	0.04	16.94	5.68	41.7	17	13.6	58

Notes: Acid Saturation % was Zero for both samples. NIRS organic carbon % was not registered for either sample. Soil analysis provided by Cedara's Fertilizer Advisory Service, KZN Dept. of Agricultural and Environmental Affairs.

Appendix 4.2: Composted Chicken Litter Analysis

N	Ca	Mg	K	Na	P	Zn	Cu	Mn	B
(%)	(%)	(%)	(%)	(%)	(%)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)
1.99	6.56	0.79	2.23	0.54	2.53	584	69	653	77

Data on 100% dry matter basis, courtesy of Eston Organics. Soil analysis provided by Cedara's Fertilizer Advisory Service, KZN Dept. of Agricultural and Environmental Affairs.

Appendix 4.3: Weather Data

Data for SAWS station [0239698 5] - PIETERMARITZBURG -29.6330 30.4000, 673 m

Mean Max and Min Temps, Mean and Daily Rainfall, 2005-2006										
Month	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug
Max	26,6	27	28,4	28,3	26	25,2	21,6	21,6	24,4	22,7
Min	16,1	15,4	18,8	19,4	14,9	13,5	7,5	4,8	6,3	7,9
30°C	9	10	13	9	5	0	0	0	0	0
Rainfall (mm)	71,4	102,2	185,6	54,8	98,6	109,2	68	1,4	0,4	52,2
Days of Rain	15	17	19	12	11	10	5	1,4	0,4	4
Daily Mean (mm)	2,38	3,30	5,99	1,96	3,18	3,64	2,19	0,05	0,01	1,68

Mean Max and Min Temps, Mean and Daily Rainfall, 2006-2007										
Month	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug
Max	25,6	27,1	29,07	30,66	27,54	26,11	26,59	22,63	23,71	25,19
Min	15,1	16,5	17,57	18,56	16,65	14,53	8,13	6,37	5,34	7,81
30+C	6	8	11	13	9	8	10	0	0	3
Rainfall (mm)	101	177,2	69,8	38	192,8	24,6	7,4	60,6	0	14
Days of Rain	17	19	10	5	15	11	1	3	0	3
Daily Mean (mm)	3,37	5,72	2,33	1,36	6,22	0,82	0,24	2,02	0	0,45

(Weather Data courtesy of the South African Weather Service)

Appendix 4.4: ANOVA Test Results

2005-2006 Eco-77, One Way ANOVA for Total Corms/Block

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Eco77	613.64	2	306.818	4.73	0.0394
Error	583.41	9	64.823		
Total	1197.04	11			

2005-2006 Eco-77, One Way ANOVA for Harvested Biomass

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Eco77	0.20672	2	0.10336	0.74	0.5056
Error	1.26315	9	0.14035		
Total	1.46987	11			

2005-2006 Eco-77, One Way ANOVA for Survival % (angular transformation)

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Eco77	0.00174	2	0.00087	1.25	0.3307
Error	0.00625	9	0.00069		
Total	0.00799	11			

2005-2006 Eco-T, One-way ANOVA for Harvested Biomass

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
EcoT	0.11502	3	0.03834	0.61	0.6239
Error	0.75977	12	0.06331		
Total	0.87479	15			

2005-2006 Eco-T, One-way ANOVA for Survival % (angular transformation)

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
EcoT	0.00064	3	0.00021	0.42	0.7439
Error	0.00617	12	0.00051		
Total	0.00682	15			

2005-2006 Eco-T, One-way ANOVA for Total Corms/Block

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
EcoT	142.25	3	47.4167	0.62	0.6161
Error	919.50	12	76.625		
Total	1061.75	15			

2006-2007 Eco-T, One-way ANOVA for Harvested Biomass

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
EcoT drench	0.04362	2	0.02181	0.06	0.9451
Error	2.29407	6	0.38234		
Total	2.33769	8			

2006-2007 Eco-T, One-way ANOVA for Total Corms/Block

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
EcoT drench	267.56	2	133.778	1.05	0.4079
Error	768	6	128		
Total	1035.56	8			

2006-2007 Eco-T, One-way ANOVA for Survival % (angular transformation)

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
EcoT drench	0.00166	2	0.00083	1.15	0.3773
Error	0.00432	6	0.00072		
Total	0.00597	8			

Chapter 5: Effects of Various Shade Densities and Colours on the Growth of *Siphonochilus aethiopicus* (Schweif.) B.L. Burt.

5.1. Introduction

Photosynthesis is active between 400-700 nm of visible light, while phytochrome and photomorphogenic activity is controlled by far-red/red (730 nm/660 nm) and blue (400-500 nm) wavelengths (Mcmahon et al. 1990). Plants also respond to ultraviolet (280-400 nm) and far-red (700-800 nm) (Young et al. 1994). Photoreceptive chlorophyll captures radiant energy and combines it with CO₂ and water to produce O₂ and assimilate carbon to synthesize carbohydrates for plant cell requirements (Young et al. 1994). The photomorphogenic red and far-red receptor phytochrome (655-665 nm and 725-735nm) interact with blue-light sensing cryptochromes to regulate photomorphogenic and photoperiodic responses such as stem elongation, plant height and flowering time, with higher amounts of far-red resulting in shorter plants (Kubota et al 2000, Rajapakse et al. 1998). The blue-light sensing phototropins regulate phototropism, chloroplast relocation and stomatal opening (Eckardt 2003).

Chlorophyll absorbs red light almost completely, while about 2/3rd of the higher energy blue light is absorbed, and about 1/3rd must be shed as heat. Red shade-cloth eliminates ultraviolet and blue light, and for plants that may be heat sensitive, eliminating the blue light might assist growth. White (TiO₂) shade cloth is an efficient reflector of the full light spectrum. Black shade-cloth collects the light and converts it to infrared heat; so it shades, but it does not reduce the heat. White and pearl/grey shade-cloth reflect the light, reducing the light and heat reaching the plants (Laing, pers. comm.; manufacturer's website www.polysack.com, accessed 14 June, 2009).

Some plants require shade, and respond to excess light by producing reactive intermediates that can cause photo-oxidative damage, inhibit photosynthesis, and reduce growth (Li et al. 2009). Ethnobotanical information from Zulu traditional healers on the native habitat of *Siphonochilus aethiopicus* (Cele, Dlamini, pers. co mm. 2004, 2006), published and field reports indicate natural growth in sub-canopy and forest edge habitats (Crouch et al. 2000, Hyde and Wursten 2008). One year of a full-sun crop trial in Pietermaritzburg showed the plant suffering tip-burn, transient chlorosis, and

susceptibility to *Erwinia* during periods of high daytime temperature and sun. These symptoms suggested that *S. aethiopicus* may perform better in shade or semi-shaded growth conditions. A small field trial was designed to test the effects of six different grades of shade-cloth against a control in full sun. Photosensitive netting spectrally modifies and scatters incoming sunlight, improving light distribution to the inner canopy and increasing radiation use efficiency (Shahak et al. 2004, 2008), and reduces sunburn (Smit 2007).

5.2. Material and Methods

The shade trial consisted of a set of complete randomized blocks, with plots of 1.5 m x 600 mm, 15 corms each at 30 cm spacing, and 2 replications. Treatments were: Control (full sun), 40% White (TiO₂, 23% shade), 40% Grey (28-30% shade), Light Black (40% shade), Medium Black (50% shade), Dark Black (80% shade) and Red (40% shade). ChromatiNet® shade-cloth, manufactured by Polysack (www.polysack.com), was supplied courtesy of Keith Hartley, Filmflex (Pty) Ltd. (Pinetown, KZN). Shadecloth was stretched over and attached to temporary 1.5 m high structures made from bent reinforcing bar wired together and inserted into the soil along the borders of the blocks; cloth covered the top and all four sides of the block, leaving a 250 mm open space along the ground to allow for air flow, and to permit access for weeding, measurements, etc. Planting of the corms was finished in the 3rd week of November 2006, and shadecloth was installed on January 19, 2007, after the shoots had emerged.

Crop trials were conducted on a private farm northeast of Pietermaritzburg, KwaZulu-Natal, at approximately 675 m altitude, lat. 29.6330, long. 30.4000. *Siphonochilus aethiopicus* corms/rhizomes purchased from Silverglen Medicinal Plant Nursery, Chatsworth, Durban. These were multiplied during a first year of crop trials. Planting in the first season of other crop trials (2005-2006) was completed only by the third week of November; this timing was then repeated for 2006-2007, and for consistency with the other trials, the shade trial was also planted in the third week of November 2006. In the 2006-2007 season we planted a balance of larger and smaller corms in each plot.

Plants were irrigated as necessary during the growing season, depending on rains and resultant field moisture. Irrigation was approximately 30 mm of water per week to supplement irregular rainfall (Appendix 5.1).

Field preparation was by manually turning the soil with forks. The level terrace had previously been limed, and treated in the previous season with 2:3:2 (28) fertilizer for growing cabbages. The P levels were low 10-22 mg l⁻¹ (Appendix 5.2). Resident Guinea fowl controlled cutworm and snails. Plots were watered before harvesting to soften the soil. Traditional healers had reported that harvesting when yellowing leaves were still visible above the soil produces corms with the highest medicinal value. In retrospect, it would have been easier to harvest at that time (in June) than later when all the leaves had died off and fallen away, as the lack of surface leaves made it more difficult to ensure one had successfully harvested all the corms present. Simple field-washing by hand was used to remove soil from corms.

Tables of means were calculated and tables and figures created in Excel. Statistical analysis was performed using MATLAB (version 7.9.0, The Mathworks Inc., Natick, MA, USA). One-way ANOVAs of shade trial results were performed for harvested biomass, total corms/block, and survival percentage (angular transformation).

5.3. Results

Plants growing under the shade-cloth developed much longer leaves (2-3 times the normal length, though precise measurements were not taken), and much taller plants, than either the Control plot plants or the plants in the adjacent trials growing in full sun. In some instances the plants toppled over as the elongated false stem was not strong enough to support the plant; far-red light leads to stem elongation, etiolation, and weaker plants (Poole et al. 1992). A comparison of all the trial blocks was done in March and April of 2007. The average height of shaded plants was 20-30% greater than non-shaded plants in adjacent trials in March and April. The max height of the shaded plants was also about 30% greater than the max height of neighboring plants. Nonetheless Shade plants did not develop any more leaves than the non-shaded plants, suggesting that the number of leaves per plant is genetically preprogrammed. Table 5.1

summarizes the mean values of harvest measures, while Table 5.2 provides the raw data. Specific comparative measures are illustrated in the figures.

Table 5.1: Shade Trial 2006-2007, Table of Means

Treatment	No. of small corms	No. of Lg. corms	Total No. of corms	Mass of corms (g)	No. of Plants	Survival %	Mass per corm and roots
1. Control (full sun)	13,5	1	14,5	327,5	8,5	57%	39
2. 40% White (TiO ₂) (23% shade)	32	6,5	38,5	920	11,5	77%	8
3. 40% Grey (28-30% shade)	25	4,5	27,5	945	12	80%	79
4. Light Black (40%)	26,5	1	27,3	970	13	87%	75
5. Medium Black (50%)	28	2	30	955	9	60%	106
6. Dark Black (80%)	14,5	4,5	19	595	6,5	43%	92
7. Red (40%)	33	2	35	820	9	60%	91

Table 5.2: 2006-2007 Shade Trial, Raw Data

Treatment	Plot	Small corms	Lg. Corms	Total Corms	Kg/bloc k Weight	Wt (g) Per Corm and Roots	Mean corms per surviving plant	No. of Plants	Survival %
Control	EE010	30	4	34	0,77	22,65	2,83	12	80,00
Control	EE014	6	0	6	0,07	11,67	0,60	10	66,67
Control	EE015	12	0	12	0,34	28,33	1,33	9	60,00
Control	EE016	6	0	6	0,13	21,67	2,00	3	20,00
40% White (TiO ₂ , 23% shade)	EE004	27	5	32	0,72	22,50	3,56	9	60,00
40% White (TiO ₂ , 23% shade)	EE012	37	8	45	1,12	24,89	3,21	14	93,33
40% Grey (28-30% shade)	EE005	17	3	20	0,82	41,00	1,67	12	80,00
40% Grey (28-30% shade)	EE008	29	6	35	1,07	30,57	2,92	12	80,00
Light Black (40%)	EE002	38	2	40	1,17	29,25	5,00	8	53,33
Light Black (40%)	EE011	26	2	28	0,72	25,71	1,87	15	100,00
Medium Black (50%)	EE003	15	8	23	0,82	35,65	2,88	8	53,33
Medium Black (50%)	EE007	27	0	27	1,22	45,19	2,45	11	73,33
Dark Black (80%)	EE001	14	1	15	0,37	24,67	3,00	5	33,33
Dark Black (80%)	EE009	18	2	20	0,74	37,00	2,00	10	66,67

Red (40%)	EE006	30	4	34	0,82	24,12	4,25	8	53,33
Red (40%)	EE013	36	0	36	0,82	22,78	3,60	10	66,67

Measuring total small corms at harvest, the 40% red and 40% white performed the best as a shade for *S. aethiopicus*. Plants in the full sun or with 80% black shade performed the worst (Fig 5.1).

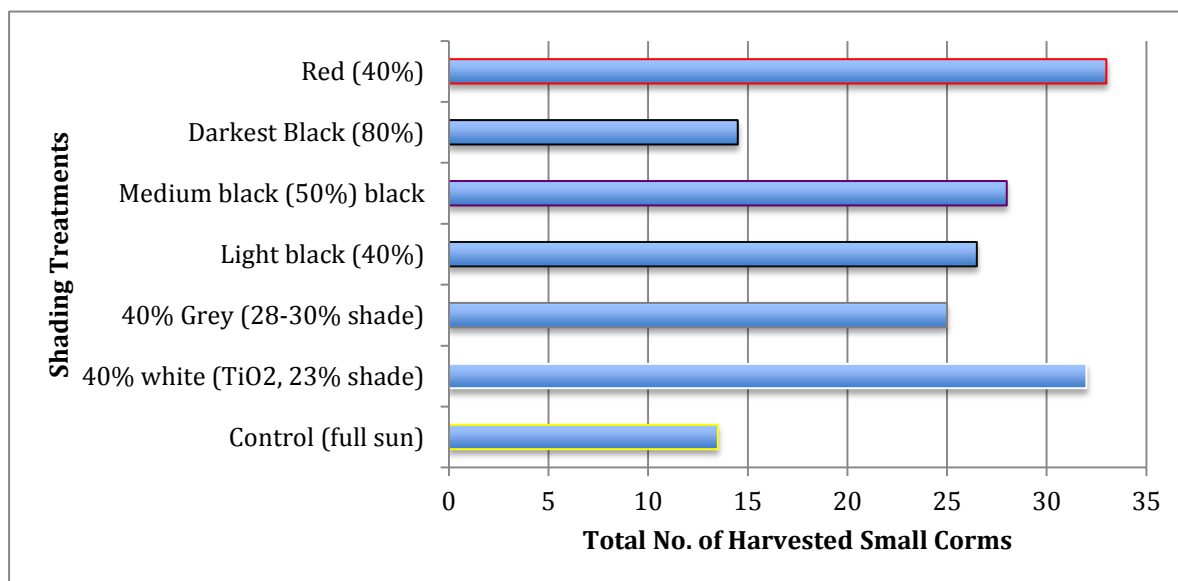


Figure 5.1: Total No. of Harvested Small Corms x Shade Treatment

Similar results were recorded for total number of harvested large corms (Figure 5.2).

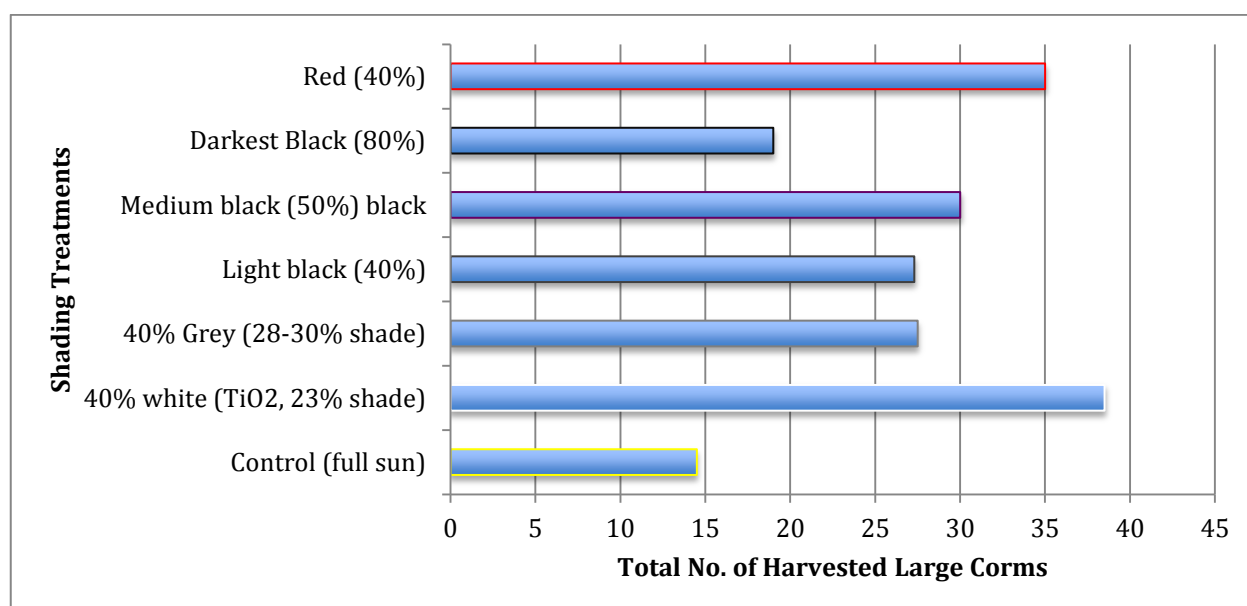


Figure 5.2: Total No. of Harvested Large Corms x Shade Treatment

Total biomass at harvest, summed across small and large corms and tuberous roots showed different results, with the 50% black, 40% black, 40% grey and 40% white performing the best, while 40% red was no longer optimal (Fig 5.3).

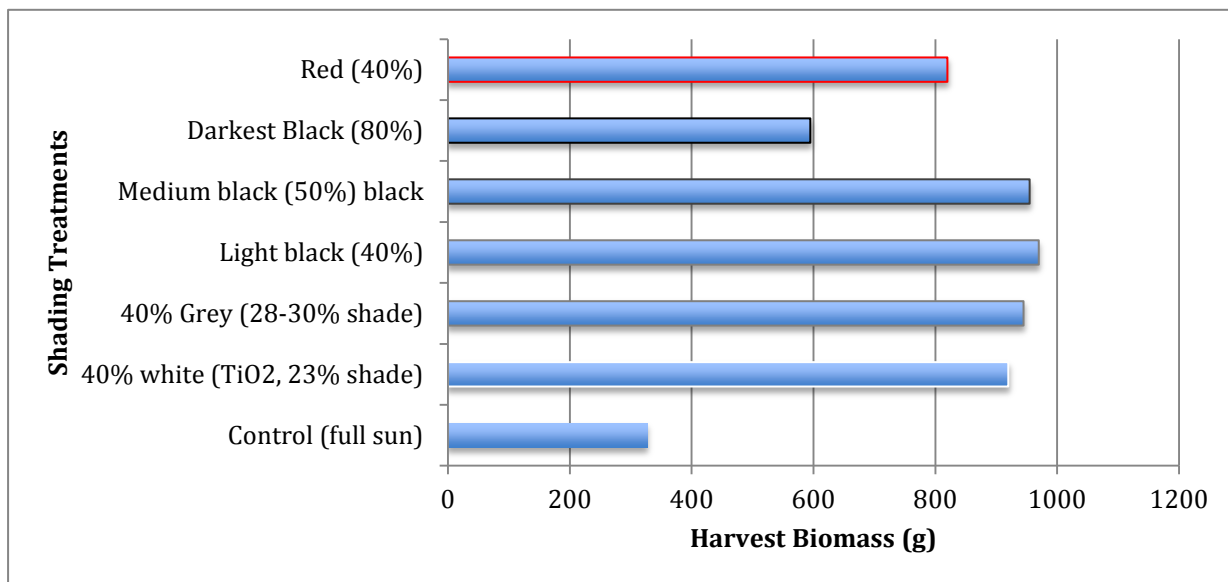


Figure 5.3: Total Harvest Biomass (g) per block x Shade Treatment

In terms of survival percentage, 40% black was by far the best treatment, and 80% black was the worst (Fig 5.4).

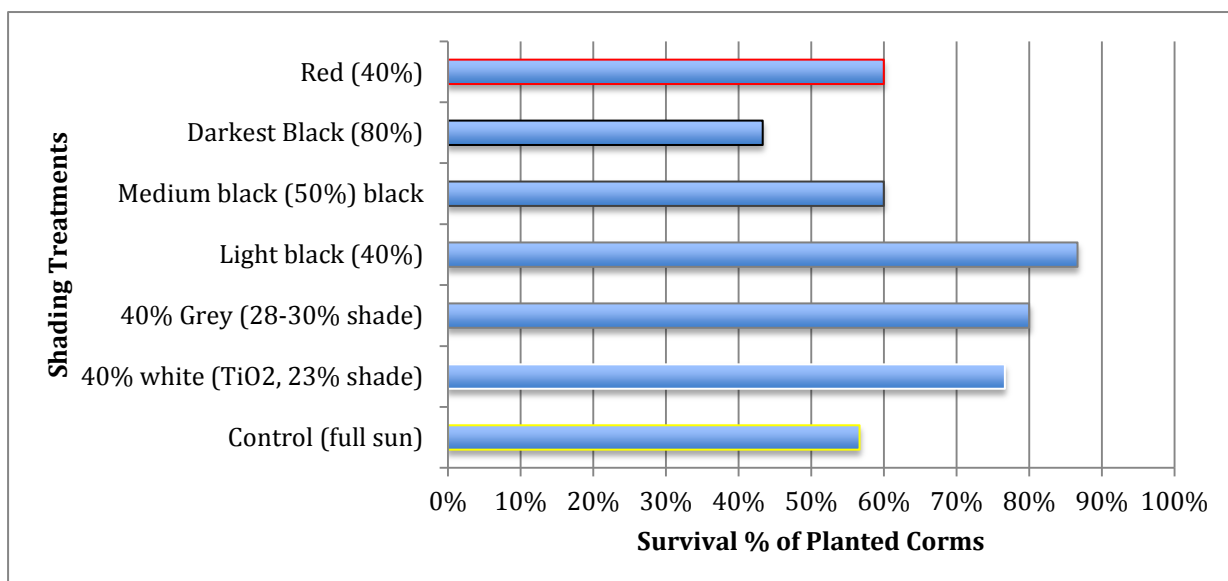


Figure 5.4: Shade Trial, Planted Corm Survival % x Shade Treatment

5.4. Discussion

ANOVA tests of the data did not identify statistically significant differences, even with standard transformations (Appendix 5.3). However, the data do show the following trends. The Control plots, i.e. plants exposed to full sun, and the Darkest Black shade-cloth plants both performed noticeably worse than the other treatments, suggesting strongly that full sun or overly dark growing conditions are to be avoided. Within the range of the shade-cloth other than the Darkest Black, the lack of statistical significance makes definitive conclusions impossible. Larger trials with more corms per plot, more reps, and 2-3 years' study would be required to determine optimum shade levels. Light black shade at 40% for instance had 100% survival in one plot, 53% in the other plot, so the mean measures may give a false impression due to the small overall trial size and the lack of sufficient corms for 3-4 reps of 35 corms each.

During the period of intense heat and sunlight, there was some transient chlorosis to the leaves of the plants in the 40% White (TiO₂, 23% shade) shade plots, but not in the other plots.

The impressive extra height and leaf length of the shade plants as compared with other trials appears to have been simply a response to the increase in Red/Far Red, as it did not produce any noticeable comparative benefit in total biomass per block or survivability (Table 5.3).

Table 5.3: 2006-2007 Inter-trial comparison

Trial	Mean g block ⁻¹	% of Planted Corms
		surviving
Fert	1085,50	68,62
Shade	790,35	66,30
Eco-T	1538,89	54,00
Spacing	770,00	51,17
Compost	1430,00	73,77

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(<http://www.controlledenvironments.org/Light1994Conf/index.htm>).

Appendix 5.1: Weather Data

Data for SAWS station [0239698 5] - PIETERMARITZBURG -29.6330 30.4000, 673 m

Mean Max and Min Temps, Mean and Daily Rainfall, 2006-2007										
Month	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug
Max	25,6	27,1	29,07	30,66	27,54	26,11	26,59	22,63	23,71	25,19
Min	15,1	16,5	17,57	18,56	16,65	14,53	8,13	6,37	5,34	7,81
30+C	6	8	11	13	9	8	10	0	0	3
Rainfall (mm)	101	177,2	69,8	38	192,8	24,6	7,4	60,6	0	14
Days of Rainfall	17	19	10	5	15	11	1	3	0	3
Daily Mean (mm)	3,37	5,72	2,33	1,36	6,22	0,82	0,24	2,02	0	0,45

Weather Data courtesy of the South African Weather Service.

Appendix 5.2: Soil Analysis of terraces.

#	Density (g ml ⁻¹)	P (mg L ⁻¹)	K (mg L ⁻¹)	Ca (mg L ⁻¹)	Mg (mg L ⁻¹)	Exch. Acidity (mol L ⁻¹)	Total cations (mol L ⁻¹)	pH (KCL)	Zn (mg L ⁻¹)	Mn (mg L ⁻¹)	Cu (mg L ⁻¹)	NIRS clay (%)
1	1.00	10	326	2395	602	0.08	17.62	5.15	25.8	13	16.1	49
2	1.03	22	580	2148	571	0.04	16.94	5.68	41.7	17	13.6	58

Notes: Acid Saturation % was Zero for both samples. NIRS organic carbon % was not registered for either sample. Soil analysis provided by Cedara's Fertilizer Advisory Service, KZN Dept. of Agricultural and Environmental Affairs.

Appendix 5.3: ANOVA Test results

2006-2007, Shade Trial, One-way ANOVA of harvested biomass

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Shade	2.25068	6	0.19178	2.61	0.0951
Error	0.66222	9	0.07358		
Total	1.18129	15			

2006-2007, Shade Trial, One-way ANOVA of Survival % (angular transformation)

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Shade	0.00179	6	0.0003	0.59	0.7292
Error	0.00451	9	0.0005		
Total	0.0063	15			

2006-2007, Shade Trial, One-way ANOVA of Total Corms/Block

Source	Sum Sq.	d.f.	Mean Sq.	F.	Prob > F
Shade	1281.94	6	213.656	2.34	0.1216
Error	822.5	9	91.389		
Total	2104.44	15			

Chapter 6: Effects of Macronutrients on Growth of *Siphonochilus aethiopicus* (Schweif.) B.L. Burt.

6.1. Introduction

Nitrogen, Phosphorous, and Potassium (NPK) are referred to as macronutrients as they are required in large quantities for plant growth. Species-specific optimal NPK levels typically increase productivity. *Gladiolus* sp (L.) responded positively to increasing levels of NPK fertilizer, with maximum corms and cormels per plant at $N_{50}P_{25}K_{25}$ g m⁻² (Sharma and Singh 2011), while *Colchicum heirosolymitanum* Feinbr. and *Colchicum tunicatum* Feinbr. showed highest corm yield at NPK fertilization of 50:75:50 kg ha⁻¹ and 75:100:75 kg ha⁻¹ respectively (Al-Fayyad et al 2004).

6.1.1. Nitrogen

Nitrogen is a key atom in nucleic acid and protein structure, and essential to all enzymatic activity. Plants absorb usable nitrogen from the soil solution in several ways. Plants stimulate root-zone microbial activity through root-exudate organic and amino acids and carbohydrates, and nematodes and amoebae consume bacteria and secrete root-absorbable excess N (Aerts and Chapin 2000). Root-zone *Nitrosome* and *Nitrobacter* microbes mediate nitrification, the conversion of organic nitrogen sources to NH₄⁺ or NO₃⁻ (Serrano 2005). Most plants also have ectomycorrhizal fungal symbionts in the root zone, which can directly take up and make available to plants organic N (amino acids and peptides) with N-mobilizing enzymes (Lilleskove et al 2002). Within the plant NH₄⁺ is enzymatically assimilated in plastids and in the cytosol into the amino acids glutamine (Gln) and glutamate (Glu), which are the primary amino group donors for biosynthesis of proteins, nucleic acids, polyamines and chlorophylls, and many secondary metabolites (Plaxton and Podesta 2006). One study of rice plants found that > 90% of the nitrogen in nitrate fertilizer applied to the roots was translocated in the sieve tubes as amino acids (Hayashi et al 1997). Nitrate reduction and oxidative synthesis both produce nitric oxide (NO) which regulates tyrosine nitration and S-nitrosylation of cytoskeletal proteins (Forhlich and Durner 2011; Yemets et al 2011).

6.1.2. Phosphorous

Phosphate makes up about 2% of plant dry weight, and is after N the second most limiting plant growth macronutrient. About 20-80% of soil P is in organic form, with the remainder inorganic, and immobile P is released into the soil solution by microbes and then moves by diffusion. Plants have specialized transporters at the root/soil interface for Pi (phosphate) uptake, and direct P (phosphorous) absorption is also facilitated by mycorrhiza (Schachtman et al 1998). Protein kinases and phosphatases catalyze reversible protein phosphorylation, a basic step in almost all aspects of cell physiology, metabolism and growth. The protein kinases are the largest known protein family (Plaxton and Podesta 2006). Phosphate is a key component of the nucleic acids DNA and RNA, and is found at higher concentrations in growing plant tissue due to high protein synthesis levels in ribosomal RNA. It is also part of biomembranes in phospholipids. Phosphate esters and diphosphates provide the cellular metabolic energy which is derived from photosynthesis, aerobic respiration and glycolysis. Phosphates are taken up at physiological pH mainly as H_2PO_4^- , becoming a simple phosphate ester or attaching to another phosphate in the ATP cycle by an energy-rich phosphate bond (Marschner 2011). The ATP cycle itself occurs in the mitochondria, and P enters the mitochondria via co-transporters and specific exchangers (Plaxton and Podesta 2006).

6.1.3. Potassium

Potassium (K^+), the most abundant cation in higher plant cells, with concentrations in cytosole, liquid parts and vacuoles ranging from 50-150 mM (Aerts and Chapin 2000). It is a mobile ion, which moves through the soil via bulk flow and diffusion (Schachtman et al 1998), and is essential for membrane transport in plants, facilitating many plant physiological processes, such as efficient use of soil moisture (Aerts and Chapin 2000) and complex auxin cross-linking for cell elongation (Christian et al 2006). Many critical enzymes, such as starch synthase and membrane-bound proton pumping ATPases, are stimulated by or dependent upon K^+ , which along with other univalent cations induces protein conformational changes (Marschner 2011). Higher concentrations of K^+ are required for protein synthesis (Marschner 2011), and decreased leaf potassium content has been shown to directly downregulate photosynthesis rate in a variety of plants (Terry and Ulrich 1973; O'Toole et al 1980; Jin et al 2011).

6.1.4. Motivation for this study

In order to establish a baseline of NPK requirements for optimal field growth of *Siphonochilus aethiopicus*, we conducted randomized block experiments with multiple NPK factorials.

6.2. Materials and Methods

Single macronutrients and their interactions were tested in a randomized block design: N (Ammonium Nitrate), P (Super Phosphate), K (Potassium Chloride), NxP, NxK, PxK, and NxPxK. (Appendix 6.1). The number of corms available and required permitted only 2 reps of the 64 treatments in 2005-2006, resulting in 128 blocks with 9 corms per block, with each block = 2.52 m². The trial was repeated in 2006-2007 season with 3 reps of each treatment, resulting in 192 blocks with 15 corms per block, with each block = 3.15 m². Fertilizer amounts per block were adjusted accordingly in 2006-2007 to keep the same application rates as 2005-2006.

Various methods of fertilizer application were considered, in the end the granulated version of the fertilizer was used for ease of mixing and measuring and preserving in prepared plot packets prior to application at planting. Fertilizer levels were determined in consultation with Cedara Agricultural College's Soil Science section (courtesy of Alan Manson) set at levels 0, 1, 2, and 3. Granulated Double Super P, 19.6%, LAN 28% and KCl 50% were purchased from Omnia Fertilizer in Cato Ridge. Simple conversion calculations were performed, resulting in converted gram weights per each trial plot (Appendix 6.2).

Crop trials were conducted on a private farm northeast of Pietermaritzburg, KwaZulu-Natal, at approximately 675 m altitude, lat. 29.6330, long. 30.4000 *Siphonochilus aethiopicus* corms were purchased from Silverglen Medicinal Plant Nursery, Chatsworth, Durban. These were multiplied during a first year of crop trials. Planting in 2005-2006 was completed only by the third week of November; this timing was then repeated for 2006-2007, and for consistency with the other trials, the fertilizer trial was also planted in the third week of November 2006. In the 2006-2007 season we planted a balance of larger and smaller corms in each plot.

Plants were irrigated as necessary during the growing season, depending on rains and resultant field moisture. Irrigation was approximately 30 mm of water per week to supplement irregular rainfall (Appendix 6.3).

Field preparation was by manually turning the soil with forks. The level terrace had previously been limed, and treated in the previous season received with 2:3:2 (28) fertilizer for growing cabbages. The P levels were low, 10-22 mg l⁻¹ (Appendix 6.3). Resident Guinea fowl controlled cutworm and snails. Plots were watered before harvesting to soften the soil. Traditional healers had reported that harvesting when yellowing leaves were still visible above the soil produces corms with the highest medicinal value. In retrospect, it would have been easier to harvest at that time (in June) than later when all the leaves had died off and fallen away, as the lack of surface leaves made it more difficult to ensure one had successfully harvested all the corms present. Simple field-washing by hand was used to remove soil from corms.

In 2005-2006 all corms were treated with Eco-77⁹ to guard against post-split fungal infection of the wounds, but this was done after suberization, about a week prior to planting. In 2006-2007, calluses were allowed to form without treating the bulbs with Eco-77 or with fungicide. Normal planting date for *S. aethiopicus* would be September, but for various logistical reasons planting was delayed until November. By the third week of November 2005 all the trials had been hand-planted, so this timing was repeated for the 2006-2007 trials.

Tables of means were calculated and constructed with related figures in Excel. Statistical analysis was conducted using MATLAB (version 7.9.0, The Mathworks, Inc., Natick, MA, USA). ANOVAs were conducted with raw data and standard transformations (natural log, square root, and cube root) for harvested biomass, total corms, and survival percentage, examining the NxPxK interactions collectively and in pairs. A bootstrap resampling analysis (n=100,000) was also conducted to provide estimates of mean harvested biomass per treatment (with confidence interval set to 95%), using corrected means readjusted with replicate mean added back in.

⁹ Plant Health Products, Nottingham Road, South Africa; *Trichoderma harzianum* Strain Eco-77.

6.3. Results

Although we repeated the 2005-2006 fertilizer trial with the same factorials of fertilizer levels in 2006-2007, the use of 15 corms per block in 2006-2007 instead of the 9 per block from 2005-2006 makes combining the data sets problematic, so we have analyzed each year separately. We present tables of means for both 2005-2006 and 2006-2007, but Figures only for 2006-2007, because 2006-2007 had 3 reps and the larger number of corms (15) per plot. Averaging across the means of all the treatments for 2005-2006, including the two control plots, we find the following results on a per block basis, planted with 9 corms: 7,3 small corms, 9,64 large, 17 total, 320,75g per block total harvested biomass, 21,42g per corm and roots, 3,16 average corms produced per planted corm, 63,78% survival rate, and a net gain in corms of 7,9 above the 9 planted (Table 6.1). For 2006-2007 the average of the means across the treatments was 22,45 small, 6,71 large, 29,12 total corms, 14,18 net gain in corms above the 15 planted, 1085,50 g per block, 68.62% survival, 35,3 average mean grams of corm and roots, and 2,8 averages corms per planted corm (Table 6.2).

Table 6.1: 2005-2006 Fertilizer Trial Results, Table of Means

Treatment	Small Corms	Large Corms	Total Corms	Grams/block	No. Surviving Plant Clumps	g/corm and roots	Survival %	Net Gain in corms	% gain in corms
N0,P0,K0	12	9	21	450	8,5	20	94,44	12	133,33
N0,P0,K1	21,5	16,5	38	570	12	15,1	127,78*	29	322,22
N0,P0,K2	10,5	10,5	21	370	6	17,1	66,67	12	133,33
N0,P0,K3	6	7,5	13,5	200	7,5	14,8	83,33	4,5	50,00
N0,P1,K0	9	7,5	16,5	300	5	17,8	55,56	7,5	83,33
N0,P1,K1	2,5	4,5	7	140	2,5	19,4	27,78	-2	-22,22
N0,P1,K2	6	7,5	13,5	270	4,5	19,3	50	4,5	50,00
N0,P1,K3	6,5	11	17,5	390	6	25,2	66,67	8,5	94,44
N0,P2,K0	8	18	26	590	8	225	88,89	17	188,89
N0,P2,K1	9	15	24	520	7	212	77,78	15	166,67
N0,P2,K2	6,5	6,5	13	250	5	19,6	55,56	4	44,44
N0,P2,K3	8	11	19	265	6	15,02	66,67	9,5	105,56
N0,P3,K0	8,5	7,5	16	310	4	20,2	44,44	7	77,78
N0,P3,K1	6,5	9	15,5	280	4	17,8	44,44	6,5	72,22
N0,P3,K2	6	12,5	18,5	300	4	20,3	44,44	9,5	105,56
N0,P3,K3	3,5	5	8,5	620	2,5	134,2	38,89	-0,5	-5,56
N1,P0,K0	2,5	4,5	7	170	3,5	23,3	38,89	-2	-22,22
N1,P0,K1	7	10	17	350	4,5	21,6	50	8	88,89

N1,P0,K2	3	7	10	590	4	92,7	44,44	1	11,11
N1,P0,K3	4,5	10,5	15	200	5	13,4	55,56	6	66,67
N1,P1,K0	3,5	10,5	14	250	3,5	17,7	38,89	5	55,56
N1,P1,K1	15	17	32	650	12	20,3	127,78*	23	255,56
N1,P1,K2	7	7,5	14,5	270	5	18,6	55,56	5,5	61,11
N1,P1,K3	6	4,5	10,5	130	3,5	18	38,89	1,5	16,67
N1,P2,K0	4	3	7	100	4	14,7	44,44	-2	-22,22
N1,P2,K1	4	11,5	15,5	360	5	21,6	55,56	6,5	72,22
N1,P2,K2	6,5	12	18,5	410	7	22,6	77,78	9,5	105,56
N1,P2,K3	4	7,5	11,5	210	2,5	17,6	27,78	2,5	27,78
N1,P3,K0	13,5	7	20,5	400	6	18,7	66,67	11,5	127,78
N1,P3,K1	11	14,5	25,5	560	8,5	21,6	94,44	16,5	183,33
N1,P3,K2	7,67	7	14,67	200	5,7	14,5	62,96	5,67	62,96
N1,P3,K3	10	12,5	22,5	340	10	15,2	111,11	13,5	150,00
N1,P0,K0	1	2,5	3,5	420	1,5	189	16,67	-5,5	61,11
N1,P0,K1	11	10	21	330	8,5	14,5	94,44	12	133,33
N1,P0,K2	4	3	7	110	2,5	14,8	27,78	-2	-22,22
N1,P0,K3	7,5	9	16,5	320	3	18,8	33,33	7,5	83,33
N1,P2,K0	7	17	24	360	6,5	14,8	72,22	15	166,67
N1,P1,K1	9	15	24	600	6,5	25,2	72,22	15	166,67
N1,P1,K2	10,5	16,5	27	560	11	21,3	116,67	18	200,00
N1,P1,K3	6,5	4	10,5	240	3,5	21,9	38,89	1,5	16,67
N1,P2,K0	4	8	12	190	3,5	14,5	38,89	3	33,33
N1,P2,K1	11,5	14	25,5	640	5	21,5	55,56	16,5	183,33
N1,P2,K2	8,5	12	20,5	360	7,5	16,6	83,33	11,5	127,78
N1,P2,K3	17,5	13,5	31	560	6	18,1	66,67	22	244,44
N1,P3,K0	3,5	9	12,5	220	3,5	18	38,89	3,5	38,89
N1,P3,K1	7,25	7	14,25	250	6,5	15,4	72,22	5,25	56,33
N1,P3,K2	9,5	12,5	22	360	6,5	15,6	72,22	13	144,44
N1,P3,K3	9	10	19	270	5	13,2	55,56	10	111,11
N3,P0,K0	11	12,5	23,5	400	6	16,2	66,67	14,5	161,11
N3,P0,K1	10	18	28	770	12	27,5	133,33*	19	211,11
N3,P0,K2	8	5	13	230	5,5	18,8	61,11	4	44,44
N3,P0,K3	3,5	8,5	12	190	4,5	15,7	50	3	33,33
N3,P1,K0	6	8	14	230	6,5	16	72,22	5	55,56
N3,P1,K1	3	6	9	170	3,5	20,7	38,89	0	0
N3,P1,K2	2,5	10	12,5	230	5,5	18,5	61,11	3,5	38,89
N3,P1,K3	7,67	9	16,67	260	5,3	16,9	59,26	7,67	85,19
N3,P2,K0	3,5	4,5	8	170	3,5	20,4	38,89	-1	-11,11
N3,P2,K1	3	6	9	80	4	9	44,44	0	0
N3,P2,K2	3	8	11	230	5	21	55,56	2	22,22
N3,P2,K3	3	5,5	8,5	180	2,5	31,2	27,78	-0,5	-5,56
N3,P3,K0	6	14	20	410	10	19,5	111,11*	11	122,22
N3,P3,K1			0	0		0			
N3,P3,K2	7	8,6	15,6	280	7	17,4	77,78	6,5	72,22
N3,P3,K3	10	13	23	350	8	14,9	88,89	14	155,56

Note: N3,P3,K1 was not planted. * Survival above 100% was recorded for some plots due to some daughter corms reaching full size at harvest.

Table 6.2: 2006-2007 Fertilizer Trial, Table of Means

Treatment	Small corms	Large corms	Total corms	Grams biomass/ block	No. Survi- ving plants	Survival %	g/corm and roots	No. corms/ Surviving per Plant clump
N0,P0,K0	25,33	5,67	31,00	996,67	12,00	80,00	32,17	2,58
N0,P0,K1	29,33	4,00	33,33	1253,33	13,00	86,67	36,80	2,56
N0,P0,K2	15,67	7,33	23,00	893,33	9,67	64,44	37,88	2,38
N0,P0,K3	31,33	5,00	36,33	1356,67	14,00	93,33	38,44	2,60
N0,P1,K0	13,00	2,00	15,00	596,67	8,00	53,33	32,95	1,88
N0,P1,K1	20,33	9,67	30,00	1073,33	10,67	71,11	36,93	2,81
N0,P1,K2	25,33	9,00	34,33	1453,33	12,33	82,22	43,50	2,78
N0,P1,K3	22,00	4,00	26,00	786,67	8,67	57,78	35,59	3,00
N0,P2,K0	30,67	4,33	35,00	1150,00	12,67	84,44	32,17	2,76
N0,P2,K1	18,33	2,33	20,67	603,33	9,33	62,22	27,64	2,21
N0,P2,K2	19,67	5,00	24,67	970,00	8,67	57,78	38,42	2,85
N0,P2,K3	21,33	8,33	29,67	1120,00	11,00	73,33	36,13	2,70
N0,P3,K0	15,00	6,33	21,33	1086,67	9,33	62,22	44,48	2,29
N0,P3,K1	30,67	5,33	36,00	1096,67	11,33	75,56	29,28	3,18
N0,P3,K2	27,00	10,33	37,33	1333,33	11,33	75,56	35,94	3,29
N0,P3,K3	16,67	9,33	26,00	973,33	11,33	75,56	36,58	2,29
N1,P0,K0	15,33	9,00	24,33	986,67	8,33	55,56	37,86	2,92
N1,P0,K1	25,33	8,33	33,67	1366,67	10,00	66,67	36,54	3,37
N1,P0,K2	27,67	8,67	36,33	1543,33	13,00	86,67	43,38	2,79
N1,P0,K3	29,00	11,67	40,67	1890,00	12,00	80,00	42,26	3,39
N1,P1,K0	24,67	8,67	33,33	1473,33	8,67	57,78	43,17	3,85
N1,P1,K1	24,33	2,67	27,00	1036,67	10,00	66,67	44,02	2,70
N1,P1,K2	24,67	12,67	37,33	1813,33	11,67	77,78	45,41	3,20
N1,P1,K3	16,00	7,33	23,33	983,33	9,67	64,44	40,77	2,41
N1,P2,K0	10,67	2,67	13,33	453,33	7,00	46,67	23,10	1,90
N1,P2,K1	31,33	12,67	44,00	1960,00	14,33	95,56	39,71	3,07
N1,P2,K2	21,33	3,33	24,67	840,00	9,33	62,22	31,17	2,64
N1,P2,K3	22,67	9,33	32,00	1283,33	9,67	64,44	37,36	3,31
N1,P3,K0	15,33	10,00	25,33	1193,33	11,00	73,33	49,39	2,30
N1,P3,K1	28,67	4,33	33,00	1186,67	11,00	73,33	36,52	3,00
N1,P3,K2	22,00	3,67	25,67	866,67	8,67	57,78	30,51	2,96
N1,P3,K3	24,00	4,33	28,33	1003,33	11,00	73,33	34,12	2,58
N1,P0,K0	22,00	11,00	33,00	1120,00	10,67	71,11	25,90	3,09
N1,P0,K1	20,00	11,00	31,00	1116,67	8,67	57,78	33,47	3,58
N1,P0,K2	23,00	13,33	36,33	1713,33	11,33	75,56	45,51	3,21
N1,P0,K3	29,00	15,00	44,00	1146,67	8,33	55,56	25,27	5,28
N1,P1,K0	24,33	6,00	30,33	926,67	9,00	60,00	28,67	3,37
N1,P1,K1	18,00	5,33	23,33	293,33	9,00	60,00	12,79	2,59
N1,P1,K2	27,33	7,67	35,00	1326,67	11,67	77,78	37,22	3,00
N1,P1,K3	23,33	12,00	35,33	1633,33	12,00	80,00	44,52	2,94
N1,P2,K0	18,67	1,67	21,00	653,33	9,67	64,44	29,10	2,17
N1,P2,K1	25,33	6,00	31,33	1330,00	12,67	84,44	46,44	2,47
N1,P2,K2	21,67	9,33	31,00	1083,33	11,00	73,33	35,47	2,82
N1,P2,K3	27,33	6,00	33,33	1076,67	11,00	73,33	30,95	3,03
N1,P3,K0	22,67	4,33	27,00	893,33	8,00	53,33	30,58	3,38

N1,P3,K1	28,67	1,67	30,33	950,00	12,00	80,00	30,29	2,53
N1,P3,K2	25,67	12,33	38,00	1413,33	10,67	71,11	35,50	3,56
N1,P3,K3	16,00	3,67	19,67	986,67	8,33	55,56	47,86	2,36
N3,P0,K0	20,67	2,67	23,33	966,67	11,67	77,78	39,73	2,00
N3,P0,K1	15,33	2,00	17,33	610,00	9,67	64,44	31,34	1,79
N3,P0,K2	24,67	14,33	39,00	1593,33	12,00	80,00	41,48	3,25
N3,P0,K3	29,00	5,33	34,33	1330,00	11,33	75,56	38,93	3,03
N3,P1,K0	18,67	1,00	19,67	586,67	8,33	55,56	27,87	2,36
N3,P1,K1	23,00	7,00	30,00	1130,00	10,67	71,11	32,87	2,81
N3,P1,K2	16,00	7,00	23,00	1146,67	8,67	57,78	45,51	2,65
N3,P1,K3	24,00	7,67	31,67	1160,00	9,67	64,44	36,11	3,28
N3,P2,K0	21,00	6,33	27,33	1043,33	9,00	60,00	38,39	3,04
N3,P2,K1	30,33	7,33	37,67	1005,00	10,75	71,67	32,73	3,50
N3,P2,K2	24,67	3,00	27,67	1196,67	10,67	71,11	35,94	2,59
N3,P2,K3	18,00	1,00	19,00	540,00	7,00	46,67	24,37	2,71
N3,P3,K0	13,33	5,00	18,33	310,00	8,67	57,78	20,19	2,12
N3,P3,K1	24,33	8,33	32,67	966,67	11,33	75,56	29,60	2,88
N3,P3,K2	21,67	6,33	28,00	986,67	9,67	64,44	33,93	2,90
N3,P3,K3	14,67	1,67	16,33	613,33	7,00	46,67	22,83	2,33

In Figure 6.1 we have plotted the top performers, i.e. the treatments that produced total biomass per block > 1 kg. We have also ordered the factorials of each of the two macronutrients when the third is held at zero (data not shown, see section 6.3.1.).

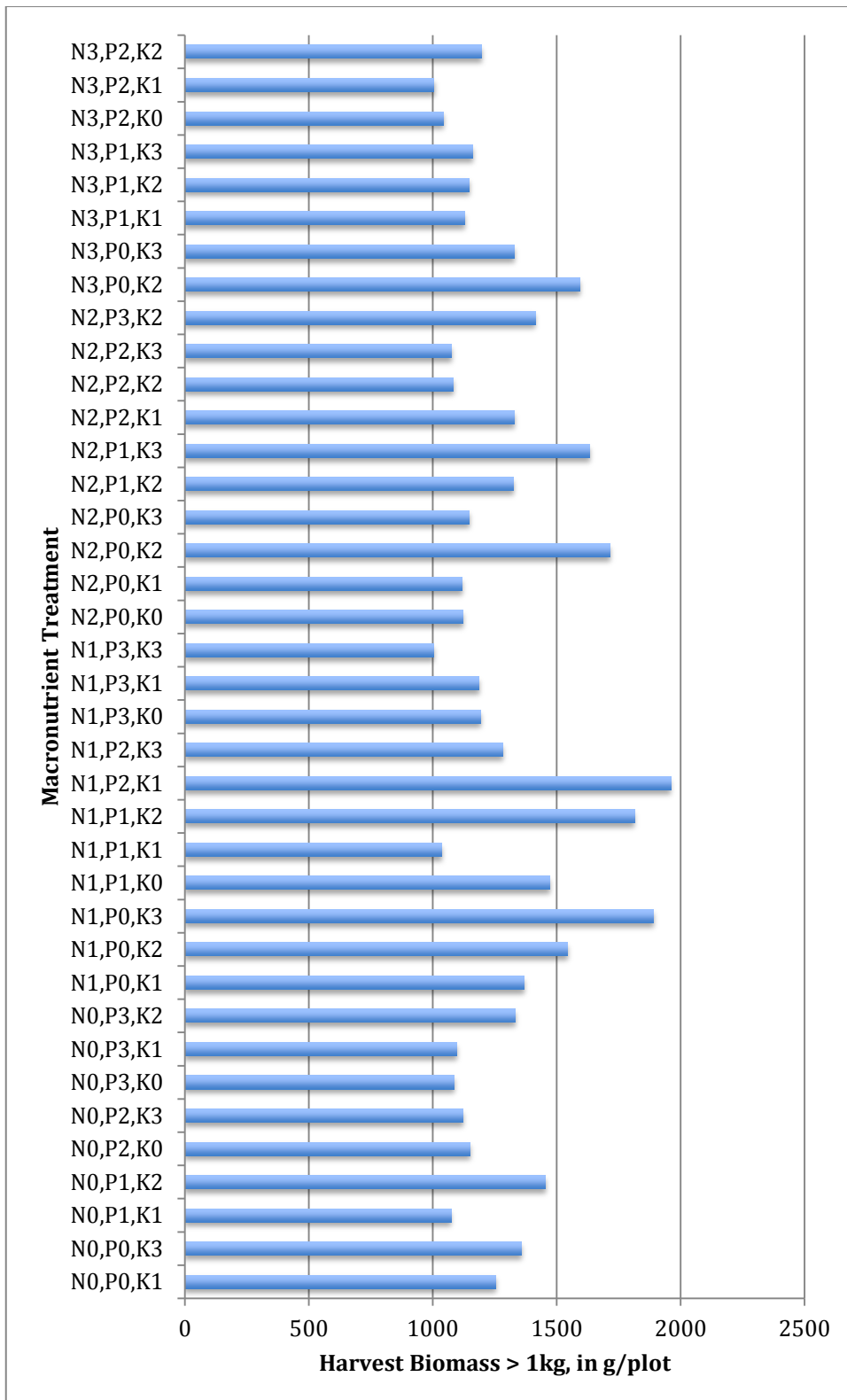


Figure 6.1: 2006-2007 Fertilizer Trial, Harvest Biomass > 1 kg x Macronutrient Treatment

6.3.1. Initial ANOVA Test

From a graph of just the top performers in 2006-2007 (Figure 6.1), it was very difficult to pick up any distinct trends. Indexing for N levels (Figure not shown) for the top performers in terms of total biomass at harvest, we see that 3 of the top 4 had N1, with the highest performer in the entire trial N1,P2,K1. Indexing the results for P levels (Figure not shown), the highest biomass levels tend to be in the lower levels of P, though P2 in combination with K1 and N1 gave the best result. Indexing the results for K levels (Figure not shown), the data show the three top performers are at K1, K2 and K3. Looking at all the combinations that approached or surpassed 1500 g, though, shows a larger percentage in K2 and K3.

We ran ANOVA tests with the raw data and the standard transformations (natural log, square root, and cube root). Almost all the ANOVA tests failed significance thresholds for main effects and interaction effects (2005-2006, harvested biomass, N: $F(3,54) = 0.78$, $p = 0.51$; P: $F(3,54) = 0.32$, $p = 0.82$; K: $F(3,54) = 0.79$, $p = 0.51$. 2006-2007, harvested biomass, N: $F(3,54) = 3.54$, $p = 0.11$; P: $F(3,54) = 1.44$, $p = 0.24$; K: $F(3,54) = 2.08$, $p = 0.11$. See Appendix 5 for full ANOVA test tables). The one exception was the NxP interaction in 2005-2006, which was significant for biomass ($F(8,65) = 2.33$, $p = 0.0289$), total corms ($F(8,65) = 4.17$, $p = 0.0004$), and survival percentage ($F(8,65) = 3.28$, $p = 0.0033$). Another nearly significant result was the NxPxK interaction for total corms, $F(26,65) = 1.53$, $p = 0.0864$, which probably reflects a real result, but a high experimental error value. This is an expected result since in most crops it is the NxPxK interaction that is the most important, and reflects the needs of plants for a balanced access to all three macronutrients. The result shows that the land used for the field trials was deficient in residual N, P and K, and hence there was a response to all three macronutrients.

6.3.2. Bootstrap Resampling Analysis

Using the mean corrected values, we then ran a bootstrap resampling analysis ($n = 100,000$) to give us estimates of the means of harvested biomass and 95% confidence intervals (CI) on the total biomass harvested per treatment, depending on the levels of N, P, and K. The results are plotted in Figs 6.2-7, giving the highest performing N levels for each year against all combinations of K and P, the highest performing P levels for both

years against all levels of N and K, and the highest performing K levels in both years for all levels of N and P. Results were obtained using the corrected means, which were then readjusted (adding back in the replicate mean) to give actual production estimates. The bootstrap analysis gives us the best performance of N, P and K, and the trend indicate what would be the optimal combination of these macronutrients. We show the plot of the bootstrap estimates of the mean corrected values (x axis) of kg plot⁻¹ harvested. Y-axis = arbitrary values. The accompanying charts illustrate the confidence intervals for each corrected mean estimate.

For the N levels, although the differences between the means are not statistically significant, both the graph of the bootstrap values (Fig 6.2a) and the bar graph (Fig 6.2b) show the trend that N2 was the best performer.

N Levels, 2005-2006

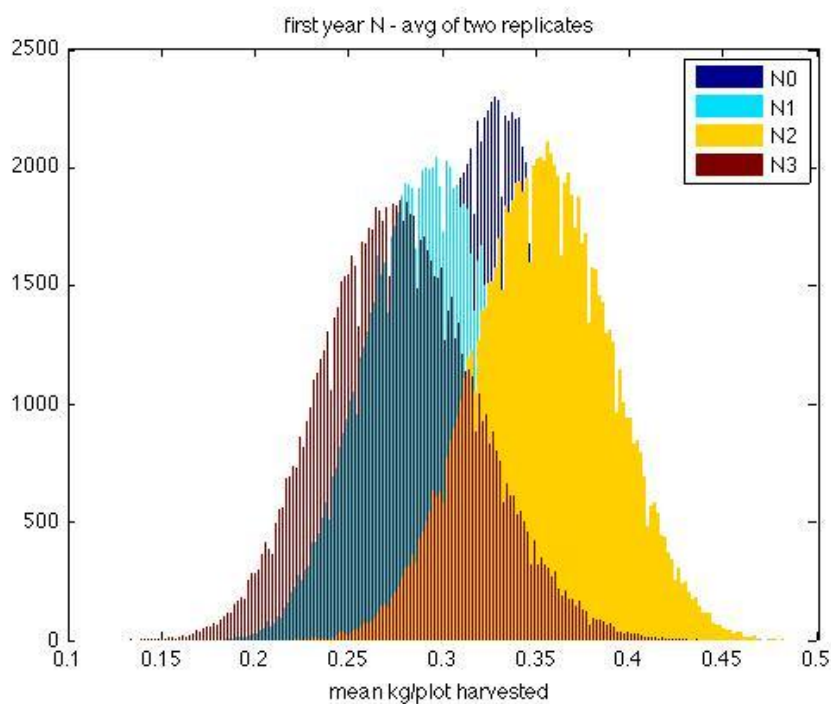


Figure 6.2a: Bootstrap Plot of N Levels for 2005-2006 (y-axis = arbitrary values) x mean Harvest biomass (kg/plot).

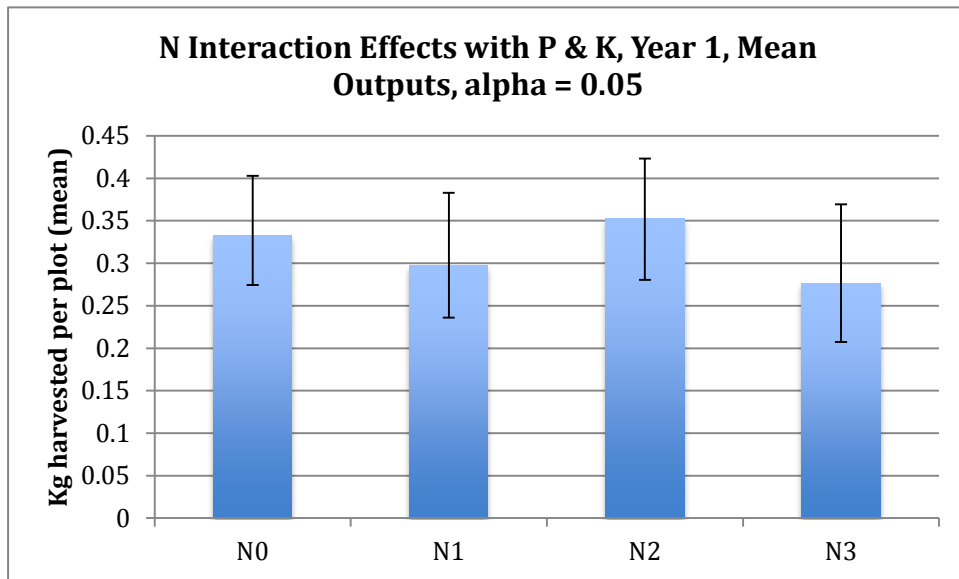


Figure 6.2b: Chart shows corrected mean values of harvested biomass for each level of N, for all levels of P and K, with Confidence Intervals plotted as error bars.

For the P levels, although mean differences are not statistically significant, the bootstrap values (Fig 6.3a) and the bar graph (Fig 6.3b) show the trend that P0 gave the best performance, particularly in comparison to P3.

P Levels, 2005-2006

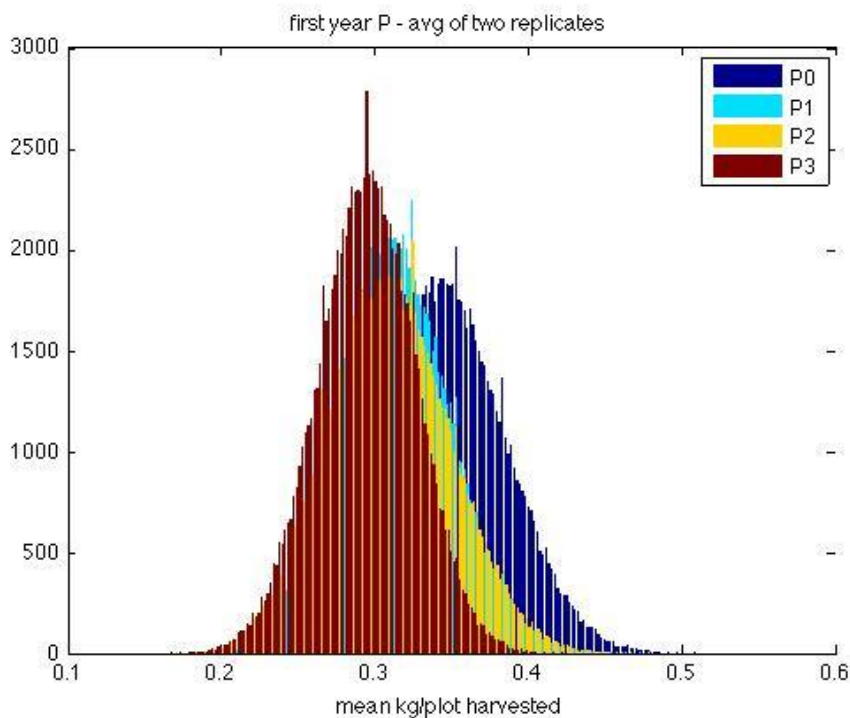


Figure 6.3a: Bootstrap Plot of P Levels for 2005-2006 (y-axis = arbitrary values) x mean Harvest biomass (kg plot⁻¹).

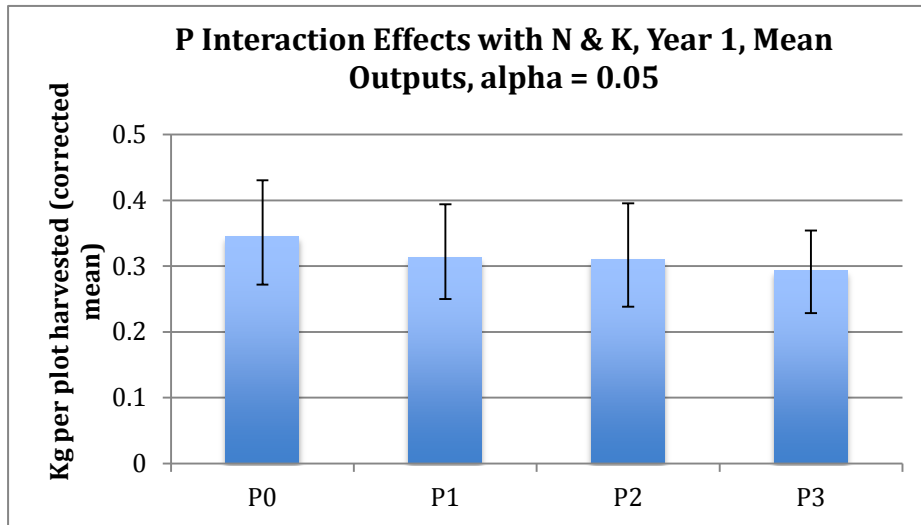


Figure 6.3b: Corrected means of harvested biomass for each level of P calculated against all levels of N and K, with Confidence Intervals plotted as error bars.

K Levels, 2005-2006

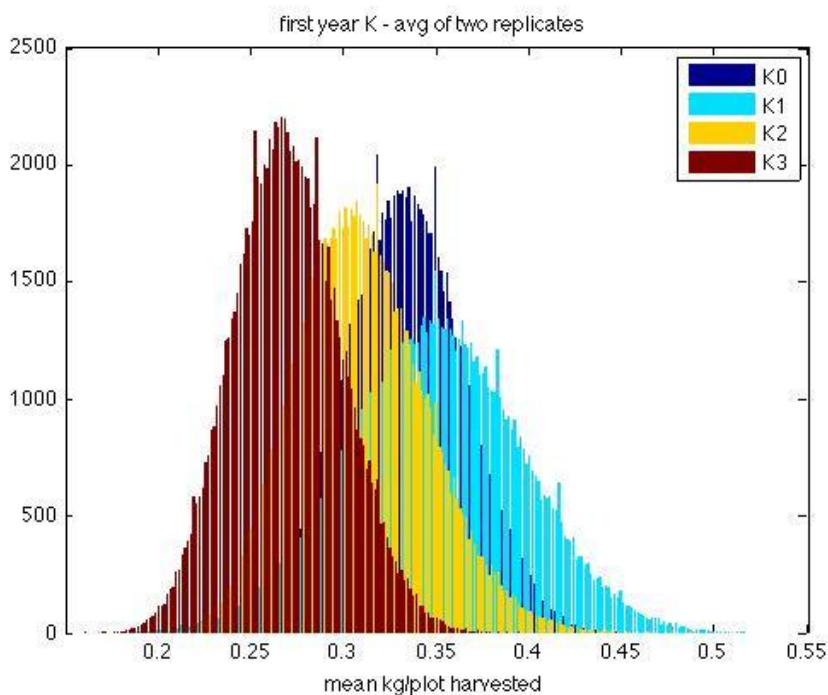


Figure 4a: Bootstrap Plot of K Levels for 2005-2006 (y-axis = arbitrary values) x mean Harvest biomass (kg/plot).

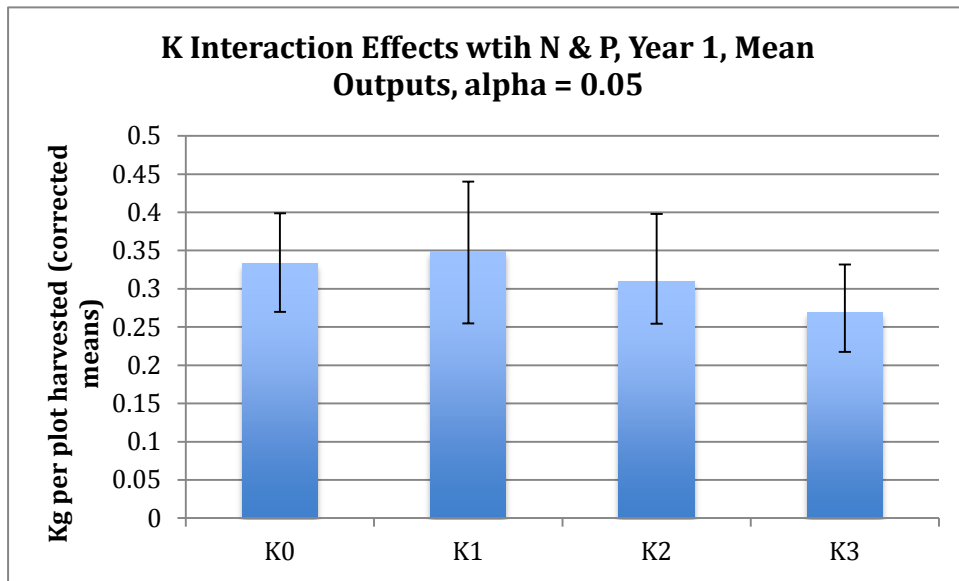


Figure 4b; Chart shows corrected mean values of harvested material for each level of K calculated against all levels of N and P, with Confidence Intervals plotted as error bars.

Although the differences between the means are not statistically significant, both the graph of the bootstrap values and the bar show the trend that K0 and K1 provided better results than K2 or K3.

We treat the 2006-2007 results separately because the plots contained 15 plants, rather than the 9 used in 2005-2006, and the 2006-2007 fertilizer amounts per plot were adjusted accordingly.

For N in 2006-2007, although the differences between the means are not statistically significant, both the graph of the bootstrap values (Fig 6.5a) and the bar graph (Fig 6.5b) show the trend that N1 and N2 gave the best performance, tending to confirm the finding of N2 as top performer in 2005-2006.

N Levels 2006-2007

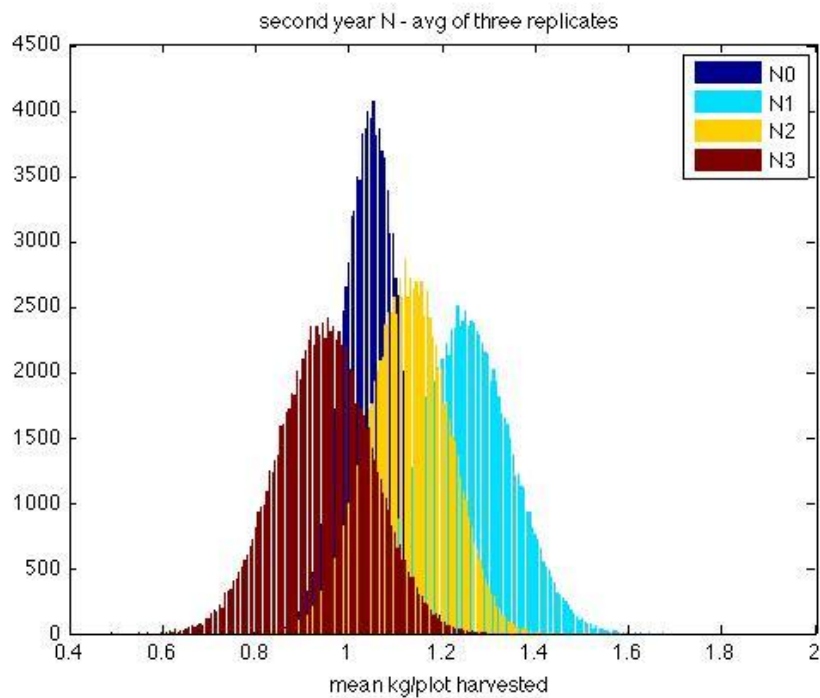


Figure 6.5a: Bootstrap Plot of N Levels for 2006-2007 (y-axis = arbitrary values) x mean Harvest biomass (kg/plot).

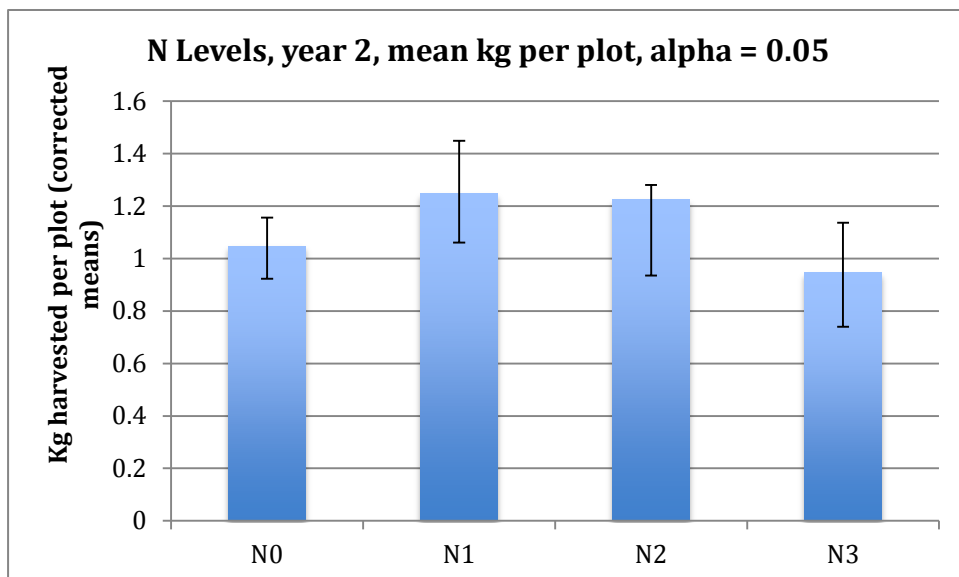


Figure 6.5b: Chart shows corrected mean values of harvested material for each level of N, for all levels of P and K, with Confidence Intervals plotted as error bars.

For P in 2006-2007, although the differences between the means are not statistically significant, both the graph of the bootstrap values (Fig 6.6a) and the bar graph (Fig 6.6b) show the trend that P0 gave the best performance, tending to confirm the finding from 2005-2006, with P3 again giving the worst performance.

P Levels, 2006-2007

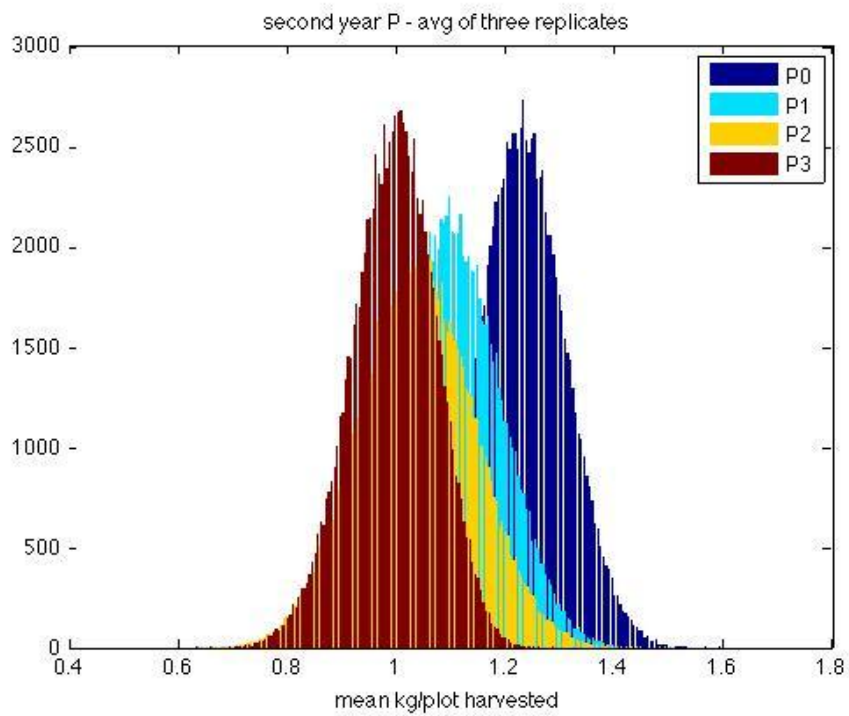


Figure 6.6a: Bootstrap Plot of P Levels for 2006-2007 (y-axis = arbitrary values) x mean Harvest biomass (kg/plot).

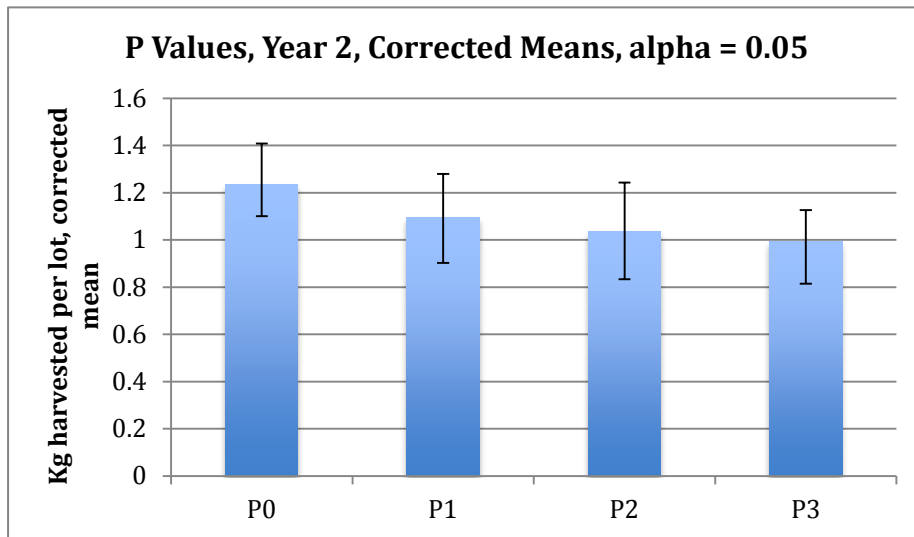


Figure 6.6b: Chart shows corrected mean values of harvested material for each level of N, for all levels of P and K, with Confidence Intervals plotted as error bars.

For K in 2006-2007, although the differences between the means are not statistically significant (except between K2 and K0), both the graph of the bootstrap values (Fig 6.7a) and the bar graph (Fig 6.7b) show the trend that K2 provided the best results, differing from 2005-2006.

K Levels, 2006-2007

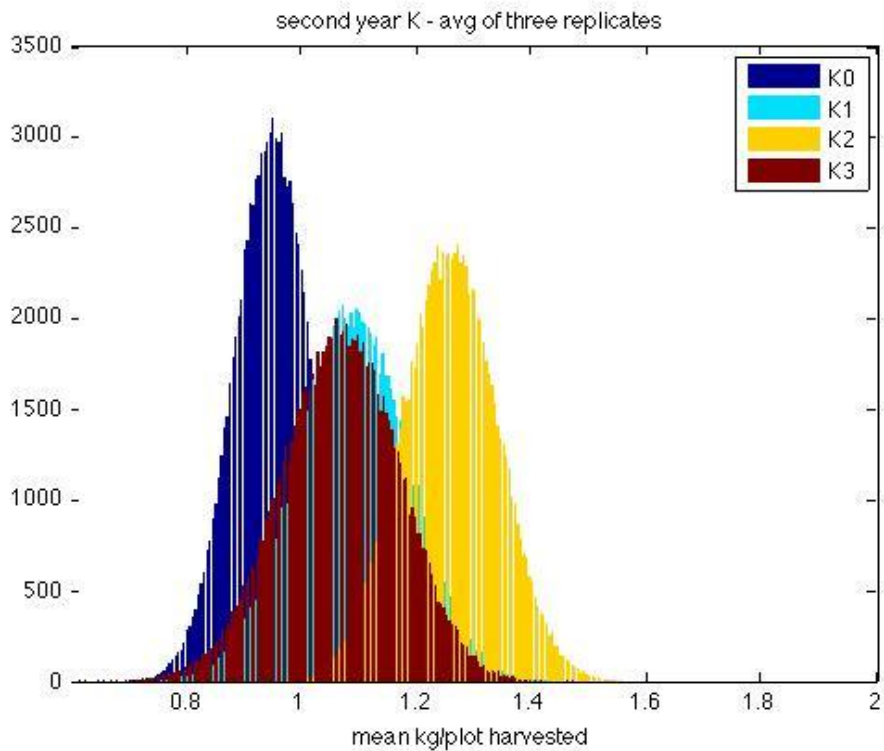


Figure 7a: Bootstrap Plot of K Levels for 2006-2007 (y-axis = arbitrary values) x mean Harvest biomass (kg/plot).

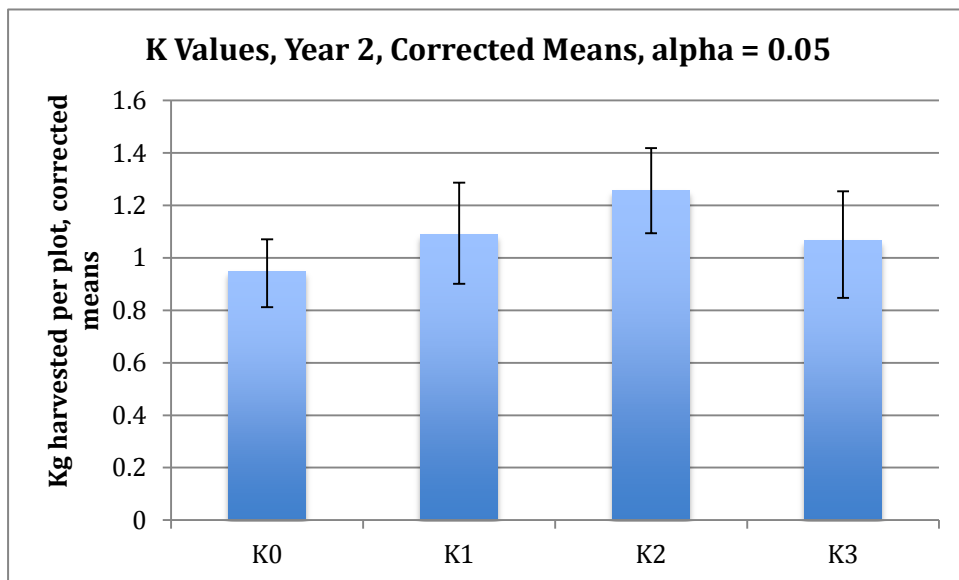


Figure 7b: Chart shows corrected mean values of harvested material for each level of K calculated against all levels of N and P, with Confidence Intervals plotted as error bars.

6.4. Discussion

The statistically significant positive interaction effect between N and P in 2005-2006 was a normal and expected result: most plants need a combination of NPK to make protein and to grow well. The lack of significant N or P main effects, but NxP significance shows that *S. aethiopicus* needs them both at the same time, and getting one or the other fertilizer by itself is not very helpful to the plant. The combination of N+P is needed to to make proteins, and these soils were very short of N and P.

S. aethiopicus needs N,P and K at roughly 1:1:1 ratios. The fact that the NxK, PxK and NxPxK interactions were not significant suggest that there was a relatively high background level of K in the soil already, or supplied by compost but not measured. Although we were not able to combine the results from 2005-2006 and 2006-2007, the data as reanalyzed in the Bootstrap resampling analysis, while not defining statistically significant differences between treatments, did show clear trends. In both 2005-2006 and 2006-2007, N2 or N1 gave the best performance, N3 the worst. In both years P0 gave best performance, P3 worst. For K, K1 was best in 2005-2006, K2 in 2006-2007, but in both years K3 was worst. These trends suggest that an optimization strategy of macronutrients for *S. aethiopicus* should focus on mid-range N1 or N2, i.e. between 40-80 kg ha⁻¹, low P, and mid-range K, between 100-200 kg ha⁻¹, and one should avoid both very low N or K, avoid very high doses of N or K, and probably not need to add P. Returning to our original fertilizer chart, we highlight in yellow/black the optimal choices (Table 6.3).

Table 6.3: Suggestions for Optimization of NPK Levels for *S. aethiopicus*

Level	Field Rate (kg ha ⁻¹)	Level	Field Rate (kg ha ⁻¹)	Level	Field Rate (kg ha ⁻¹)
N0	0	P0	0	K0	0
N1	40	P1	60	K1	100
N1	80	P2	120	K2	200
N3	120	P3	200	K3	400

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Appendix 6.1: Treatment Table for Seasons 1 and 2, NPK Trial

Treat- ment#	N0	Treat- ment#	N1	Treat- ment#	N1	Treat- ment#	N3
1	P0, K0	17	P0, K0	33	P0, K0	49	P0, K0
2	P1, K0	18	P1, K0	34	P1, K0	50	P1, K0
3	P2, K0	19	P2, K0	35	P2, K0	51	P2, K0
4	P3, K0	20	P3, K0	36	P3, K0	52	P3, K0
5	P0, K1	21	P0, K1	37	P0, K1	53	P0, K1
6	P1, K1	22	P1, K1	38	P1, K1	54	P1, K1

7	P2, K1	23	P2, K1	39	P2, K1	55	P2, K1
8	P3, K1	24	P3, K1	40	P3, K1	56	P3, K1
9	P0, K2	25	P0, K2	41	P0, K2	57	P0, K2
10	P1, K2	26	P1, K2	42	P1, K2	58	P1, K2
11	P2, K2	27	P2, K2	43	P2, K2	59	P2, K2
12	P3, K2	28	P3, K2	44	P3, K2	60	P3, K2
13	P0, K3	29	P0, K3	45	P0, K3	61	P0, K3
14	P1, K3	30	P1, K3	46	P1, K3	62	P1, K3
15	P2, K3	31	P2, K3	47	P2, K3	63	P2, K3
16	P3, K3	32	P3, K3	48	P3, K3	64	P3, K3

Appendix 6.2: 2005-2006 Fertilizer calculations, 2005-2006 season, 9 plants/block

Level	Field Rate (kg ha ⁻¹)	Grams /block	Level	Field Rate (kg ha ⁻¹)	Grams /block	Level	Field Rate (kg ha ⁻¹)	Grams /block
N0	0	0	P0	0	0	K0	0	0
N1	40	36	P1	60	77.11	K1	100	50.4
N1	80	72	P2	120	154.22	K2	200	100.8
N3	120	144	P3	200	257	K3	400	201.6

Appendix 6.3: Weather Data

Data for SAWS station [0239698 5] - PIETERMARITZBURG -29.6330 30.4000, 673 m

Mean Max and Min Temps, Mean and Daily Rainfall, 2006-2007										
Month	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug
Max	25,6	27,1	29,07	30,66	27,54	26,11	26,59	22,63	23,71	25,19
Min	15,1	16,5	17,57	18,56	16,65	14,53	8,13	6,37	5,34	7,81
30+C	6	8	11	13	9	8	10	0	0	3
Rainfall (mm)	101	177,2	69,8	38	192,8	24,6	7,4	60,6	0	14
Days of Rainfall	17	19	10	5	15	11	1	3	0	3
Daily Mean (mm)	3,37	5,72	2,33	1,36	6,22	0,82	0,24	2,02	0	0,45

Weather Data courtesy of the South African Weather Service.

Appendix 6.4: Soil Analysis of terraces.

#	Density (g ml ⁻¹)	P (mg L ⁻¹)	K (mg L ⁻¹)	Ca (mg L ⁻¹)	Mg (mg L ⁻¹)	Exch. Acidity (mol L ⁻¹)	Total cations (mol L ⁻¹)	pH (KCL)	Zn (mg L ⁻¹)	Mn (mg L ⁻¹)	Cu (mg L ⁻¹)	NIRS clay (%)
1	1.00	10	326	2395	602	0.08	17.62	5.15	25.8	13	16.1	49

2	1.03	22	580	2148	571	0.04	16.94	5.68	41.7	17	13.6	58
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Notes: Acid Saturation % was Zero for both samples. NIRS organic carbon % was not registered for either sample. Soil analysis provided by Cedara's Fertilizer Advisory Service, KZN Dept. of Agricultural and Environmental Affairs.

Appendix 6.5: NPK ANOVA Test Results

2006-2007, NPK Trial, three-way ANOVA (4x4x4) of harvested biomass (note: trial had one extra block, hence the df of 192, instead of 191).

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob > F
N	2.2622	3	0.75405	1.62	0.1878
P	2.2122	3	0.73738	1.58	0.1962
K	3.324	3	1.10799	2.38	0.725
NxP	1.6044	9	0.17827	0.38	0.9414
NxK	1.2632	9	0.14036	0.3	0.9731
PxK	1.752	9	0.19467	0.42	0.9234
NxPxK	11.6432	27	0.43123	0.93	
Error	60.0914	129	0.46527		
Total	83.5617	192			

2006-2007, NPK Trial, three-way ANOVA (4x4x4) of Total Corms/Block

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob > F
N	636	3	212.004	1.13	0.3395
P	803.3	3	285.238	1.52	0.2124
K	1304.5	3	434.817	2.32	0.0786
NxP	600.4	9	66.711	0.36	0.9537
NxK	420.6	9	44.729	0.25	0.9862
PxK	1127.90	9	125.319	0.67	0.7366
NxPxK	4704.10	27	174.225	0.93	0.5711
Error	31045.10	183	169.645		
Total	33797	192			

2006-2007, NPK Trial, three-way ANOVA (4x4x4) of Survival % (asin)

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob > F
N	0.00147	3	0.00049	0.98	0.4023
P	0.00099	3	0.00033	0.66	0.5773
K	0.00252	3	0.00084	1.68	0.1737
NxP	0.00163	9	0.00018	0.36	0.951
NxK	0.00268	9	0.0003	0.6	0.7981
PxK	0.00262	9	0.00029	0.58	0.8904
NxPxK	0.01236	27	0.00046	0.92	0.5857
Error	0.06434	129	0.0005		
Total	0.08875	192			

2005-2006, NPK Trial, three-way ANOVA (4x4x4) of harvested biomass

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob > F
N	0.4325	2	0.21627	0.62	0.5386
P	0.064	2	0.03199	0.09	0.9118
K	0.2057	2	0.10284	0.3	0.744
NxP	6.451	8	0.80638	2.33	0.0289
NxK	3.0687	8	0.38358	1.11	0.3695
PxK	3.1697	8	0.39622	1.14	0.3462
NxPxK	12.755	26	0.49058	1.42	0.1297
Error	22.4992	65			
Total	51.6432	127			

2005-2006, NPK Trial, three-way ANOVA (4x4x4) of Total Corms/Block

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob > F
N	95.54	2	47.768	0.89	0.415
P	3.55	2	1.774	0.03	0.9675
K	7.71	2	3.854	0.07	0.9307
NxP	178964	8	223.705	4.17	0.0004
NxK	477.77	8	59.721	1.11	0.3654
PxK	508.82	8	63.602	1.19	0.3207
NxPxK	2126.55	26	81.791	1.53	0.0864
Error	3483.08§	65	53.586		
Total	9447.55	127			

2005-2006, NPK Trial, three-way ANOVA (4x4x4) of Survival % (asin)

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob > F
N	0.00038	2	0.00019	0.19	0.8303
P	0.00117	2	0.00058	0.57	0.5694
K	0.00061	2	0.0003	0.29	0.7462
NxP	0.027	8	0.00337	3.28	0.0033
NxK	0.00833	8	0.00104	1.01	0.4358
PxK	0.01035	8	0.00129	1.26	0.2813
NxPxK	0.02643	26	0.00102	0.99	0.4956
Error	0.06687	65	0.00103		
Total	0.15437	127			

Thesis Overview

This study was undertaken to learn how to optimize production parameters for field-cropping *Siphonochilus aethiopicus* (Schweif.) B.L. Burt., a wild-harvested medicinal plant whose popularity has led to local extinction in KwaZulu-Natal and increasingly threatened status in other areas of South Africa and bordering countries. Many researchers (discussed in Chapter 1) have raised the subject of the need for medicinal plant cultivation as the most effective strategy for long-term conservation in the face of depletion of wild stocks, but these exhortations have not generally led to development of practical agronomic information for field-cropping the threatened, wild-harvested medicinal plants.

The studies undertaken for this thesis have overturned a number of previously reported claims, and extensive literature review revealed that much is known about *S. aethiopicus*. Using DNA markers, Kress et al (2002) clarified the plant's taxonomy as a basal lineage and sole member of the Africa-restricted Siphonochiloideae, with 12 (not 15) members, contradicting Van Wyk's (2008) claim that no comprehensive studies on the genus *Siphonochilus* had been conducted, and Larsen's (2005) report that the Siphonochiloideae contain 15 members, when in fact the number is 12. Reliable citations for *S. aethiopicus*'s African distribution showed it grows natively in Benin, Ethiopia, northern Ghana, Malawi-Mozambique, Nigeria, Niger, Swaziland, Tanzania, and Zimbabwe, in addition to South Africa, contradicting reports by some (e.g. Holzapfel et al 2002) that it is restricted to southern Africa. In South Africa the plant is clearly endangered, Red Listed by South African National Biodiversity Institute and the National Biodiversity Act, scarce in Limpopo, Gauteng, Mpumalanga and Swaziland, and long-since extinct in the wild in KwaZulu-Natal.

As discussed in Chapter 2 of this thesis, careful field observations during two years of field trials, botanical comparisons, and communication with researchers in other countries revealed that *S. aethiopicus* grows from a corm, not a rhizome. Chapter 2 contains numerous other original observations on plant characteristics and growth behavior. Among these we note that Gordon-Gray et al (1989) and Smith (1998) reported between 4 and 8 leaves developing on the unbranched false stem up to 60 cm

tall during or after flowering. Our field observations during two years of crop trials showed the majority of plants consistently produced higher numbers of leaves, closer to the ratios originally described by Medley-Wood and Franks (1911a), and that these higher leaf numbers (11-15) remain constant even when plant height increases 20-30% under shade. The Gordon-Gray et al (1989) height observations were largely confirmed for open-field grown plants without shade. Nichols (1989) reported that emerging leaves only continue to grow and expand once flowering is completed in mid-December, and other researchers have uncritically repeated his view (see for example Crouch et al 2000:122). Our field observations during the two years of crop trials showed rather different behavior: in both years of trials the leaves of all plants grew and expanded continuously from the first emergence of the shoots until the time of senescence in early winter. On at least one occasion a perfectly formed flower emerged in March in Pietermaritzburg from an otherwise nearly full-grown plant. Nichols (1989) also cited the dormancy period as "June to November." In our trials we found that though dormancy did begin in June, the plants sprouted in September in the Pietermaritzburg area, suggesting that the dormancy period is more precisely June to September. We also found that the plant seems to be genetically preprogrammed to shoot in September, and that plant shoots are generally of uniform length, giving a good guide for depth of corm planting. Corm size also appears to be genetically preprogrammed, as corm size at harvest was generally uniform across all treatments in all trials.

Cropping Findings

The principle objective of our study was to learn about field-cropping parameters for *S. aethiopicus*. We will discuss the cropping findings in more detail below, in the context of each trial, but some key considerations to emerge from our trials were that *S. aethiopicus* appears to be susceptible to cutworm at emergence, and to chlorosis and *Erwinia* when grown in high heat conditions without shading, and shows a strong growth height response to shading that nonetheless appears to produce little change in harvestable biomass. Even under open-field conditions without any appreciable shade, *S. aethiopicus* exhibited a remarkable resistance to common crop pests, with the exception of cutworm at emergence, and an ability to withstand severe hailstorms.

Compost-Spacing-Corm Size

This trial produced clear trends for better field survival of medium large and large corms over medium small and small corms. The larger ~30 cm spacing and higher levels of composted chicken litter showed trends for greater corm number and harvest biomass, confirming in part Masevhe's (2004) finding of optimal yield at ~30 cm spacing. Our trials were too small to give any clear idea of optimal compost levels, and both seasons' production was restricted by late planting date; nonetheless it appears that a mid-season top dressing would appear to be useful, as originally suggested by Crouch and Symmonds (2002). Preprogrammed shooting behavior in September strongly suggests farmers do not want to miss an early September planting, at least in KwaZulu-Natal. Retention of tuberous roots at harvest and for replanting can also be recommended, as they appear to store water and nutrients for overwintering in natural conditions. Use of a genuine mulch seems to be indicated, both for weed suppression and keeping the soil surface temperatures lower to mimic the plant's natural habitat in lowland forests and grasslands. Details of methods developed for field harvesting and storage are given in Chapter 2.

Biocontrol Agents

Our *Trichoderma spp* trials were inconclusive, again because of small size. PHP's Eco-77 appears to improve survivability and biomass production as compared with Fungicide and Control treatments. Eco-T results were less clear, though surprisingly the small Eco-T trial outperformed adjacent trials in Harvest Biomass (not significant) in 2006-2007, indicating further investigation may prove fruitful.

Shade Trials

Ethnobotanical information from Zulu traditional healers on the native habitat of *Siphonochilus aethiopicus* (Cele, Dlamini, pers. comm. 2004, 2006), and both published and field reports indicate the plant naturally grows under native sub-canopy and forest edge habitats (Crouch et al. 2000, Hyde and Wursten 2008). We have already mentioned the likely benefits of a good mulch. The effectiveness of shade cloth, a more expensive option, was not clear from our trials. One clear negative trend was that excessive shading with 80% shade and growing in full sun both appeared to inhibit growth. A dramatic response of the plant to other shade conditions, by substantially lengthening

its false stem, in the end did not produce more or larger corms. It might be helpful to repeat a shade trial with mid-level shade cloth planting in September, with a larger number of plots and corms.

NPK Factorial Trials

Our largest trial in both 2005-2006 and 2006-2007 was the macronutrient interaction trials using NPK factorials. Though the ANOVA tests failed to show significant differences, with the exception of the N x P interaction in 2005-2006, a Bootstrapping resampling test indicated strong trends suggesting that N levels of 40-80 kg ha⁻¹ combined with K levels of 100-200 kg ha⁻¹, and low levels of P, might give the best results in terms of biomass at harvest. Certainly these trials indicate that larger scale field trials, with more reps, could clarify these trends for practical farming production.

Overall Conclusions

Cultivation trials were conducted in response to a need to develop sensible cropping strategies for endangered medicinal plants. Father Jacob Gerstner wrote in 1946 of “the lamentable process of extinction” of medicinal plants from overharvesting, and recommended as the solution cultivation, “taken up by state nurseries run on scientific lines” (Cunningham 1988). Gordon-Gray et al (1989) recommended that commercial cultivation would be necessary to maintain supply and keep prices reasonable.

The combination of crop trials conducted in this study, combined with careful field observations and extensive literature review helped to demonstrate the viability of these methods as a prototype tool for exploring the domestication of wild medicinal plants as a conservation measure. By going carefully through a range of studies and reports, discussing with traditional healers, market makers, and a variety of scientists, and then designing and carefully implementing controlled field trials over at least two seasons, it is possible to begin to lay the groundwork for developing sensible cropping guidelines which can be applied as practical conservation strategies for currently wild-harvested and endangered medicinal plant species.

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