INVESTIGATION INTO THE MORTALITY OF POTTED *CHROMOLAENA ODORATA* (L.) R. M. KING & H. ROBINSON (ASTERACEAE)

By

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DEDICATION

This dissertation is dedicated to my family. Thank you for being my pillar of strength during the difficult times and for ensuring that I always strived for academic excellence; *but more especially thank you for your continuous support*, *encouragement and for always being there for me*. *This has given me the ability to venture forward despite the many challenges that I faced during the past few years of my academic career*.

ABSTRACT

The neotropical semi-woody perennial shrub *Chromolaena odorata* (L.) King and Robinson (Asteraceae: Eupatorieae) has become a serious invasive weed in the sub-tropical regions of southern Africa, resulting in the initiation of an insect biocontrol research programme at Cedara in the KwaZulu-Natal province. The programme has experienced difficulties in growing and maintaining healthy potted *C. odorata* plants for research, for almost ten years. In an attempt to identify possible causes of mortality, plants were grown on elevated grids in two trials; (winter-spring 2003) and (autumn-winter 2004) in Durban (subtropical region, within the natural range of *C. odorata*) and Cedara (temperate region, outside its natural range). Plants that established at all sites, in Trials 1 and 2, did not display any visual symptoms associated with mortality; however, results from Trial 1 indicated that plants grown in a tunnel at Cedara were physiologically stressed evidenced by lower transpiration rates despite higher vapour pressure deficits, compared with plants grown in a tunnel in Durban. Environmental stress was further indicated by the chlorophyll fluorescence ratio and a change in allocation of total non-structural carbohydrate (TNC) within Cedara-grown plants compared with Durban-grown plants. An attempt to relate mortality symptoms of unhealthy *C. odorata* plants with TNC content of the roots was unsuccessful but unhealthy plants had lower levels of N, P and K than healthy plants. Although mortality seem to be exacerbated by pathogenic fungi in saturated soil, infection was reduced by treating the soil with Previcur N and Benlate drench. Morphological adaptations of plants exposed to waterlogged soils include hypertrophied lenticels and new lateral roots; these were observed on the stem base of unhealthy *C. odorata* plants placed on the ground, that had reached a critical stage of die-back or had died back and re-sprouted. Examination of the root anatomy of *C. odorata* plants indicated an absence of cortical aerenchyma (interconnected system of air spaces), which may explain plant mortality in waterlogged pots wherein levels of oxygen in the soil may be reduced. *C. odorata* stock plants at Cedara have been placed on elevated grids since August 2004 and two years later plant mortality has been significantly reduced. Presently, the only feasible option is to grow potted *C. odorata* plants on elevated grids at Cedara because this allows for adequate drainage, aeration of soil and air-nipping of roots, and spacing plants on the grids also improves penetration of light.

PREFACE

The experimental work described in this dissertation was carried out in the Schools of Biological and Conservation Sciences, and Agriculture and Agribusiness, University of KwaZulu-Natal, (Durban and Pietermaritzburg respectively), as well as at the Agricultural Research Council-Plant Protection Research Institute (Cedara Weeds Laboratory), Cedara, from June 2003 to February 2007 under the supervision of Professors Norman W. Pammenter and John P. Bower.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others, it is duly acknowledged in the text.

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My family and friends do however, deserve the greatest thanks for their assistance, support and encouragement.

Writing a dissertation and completing a master's degree is similar to running a marathon; you have to be determined to achieve your goal……passing the FINISH line!

LIST OF ABBREVIATIONS

Term	Abbreviation	Units
absorbance	A	
carbon dioxide	CO_2	
centimetres	cm	cm
centimetres cubed	cm^3	cm^3
degrees Celsius	^oC	^oC
final volume	V	ml
fresh weight	W	g
fluorescence ratio	F_v/F_m	
grams	g	g
gram per gram	g/g	g/g
milligrams	mg	mg
milligrams/kilogram	mg/kg	mg/kg
millilitre	ml	ml
millimetre	mm	mm
micro moles	µmol	
nanometres	nm	nm
N:P:K	Nitrogen:Phosphorus: Potassium	
oxygen	O_2	
percent	%	
photosynthetically active radiation	PAR	µmol m^{-2} s^{-1}
relative humidity	RH	
saturation vapour pressure	SVP	e^o
specific leaf area	SLA	m^2 g^{-1}
stomatal conductance	g_s	
temperature	T	^oC
total non-structural carbohydrates	TNC	
vapour pressure deficit	VPD	kPa
volume per volume	v/v	v/v

CHAPTER 1 INTRODUCTION

Weeds are plants with no economic value, or whose value is unknown, and are generally pioneers of open and disturbed habitats (Hill, 1977; Myers & Bazely, 2003). There are many definitions of what constitutes a weed, but the simplest and most widely accepted one is "any plant growing where it is not wanted" (Hill, 1977; Barrett, 1982). A plant is also regarded as a weed "if in any specified geographic area, its population grows entirely or predominantly in situations markedly disturbed by man, without being deliberately cultivated" (Baker, 1965 *loc. cit*. Barrett, 1982). The main characteristic of a successful weed is its ability to out-compete surrounding vegetation for resources (light, nutrients, water and space) and its physiological attributes e.g. rapid root growth and reproductive output, which enable it to grow and spread rapidly, and hence become detrimental to crops, economically important plants and the natural environment (Hill, 1977; Liggitt, 1983). Indigenous weeds are plants that originate in a specific country whilst most of the problem weeds of the world are those that have been introduced either intentionally (for cultivation purposes) or accidentally into new habitats via human activity (Myers & Bazely, 2003).

The term 'exotic' or alien invasive weeds is applied to plants that have become naturalised (i.e. established and spreading) in the country of introduction without direct assistance from people, and have the potential to become wide-spread and invade undisturbed habitats or ecosystems (Cronk & Fuller, 1995; Richardson *et al*., 2000; Henderson, 2001). According to Henderson (2001), introduced plants that invade natural or semi-natural habitats are classified as environmental weeds (declared weeds) or invaders, while those that invade sites of severe human disturbance, i.e. waste places and cultivated lands, are classified as ruderal and agrestal weeds, respectively. There are several species of alien plants that do not spread aggressively and are not regarded as serious threats to their new environments, but those that do cause significant changes to the composition, structure or condition of natural ecosystems, and are referred to as "transformers" (Wells & Poynton *et al*., 1986; Richardson *et al*., 2000; Henderson, 2001). The impact of invasive species (both plants and animals) on ecosystems worldwide has become a global concern and biological invasions are considered the second

major threat to biodiversity after habitat destruction (Coblentz, 1990 *loc*. *cit*. Cronk & Fuller, 1995; Bright, 1998; Myers & Bazely, 2003).

There are three generally accepted theories which explain the success of invasive plants. The first is the 'enemy free' hypothesis which implies that in the absence of natural enemies or predators (insects and/or pathogens) in the new environment, growth and seed production of invaders are not restrained, resulting in an increase in population density (Hill, 1977). The second theory relates to 'ecological vacuum' or vacant niches in new or disturbed areas; invaders may be better suited to new habitats due to minimal or no environmental constraints and reduced competitive pressures from surrounding vegetation, resulting in efficient utilisation of natural resources and colonisation of the area. The third theory concerns the characteristics that enable invasive plants to rapidly invade and dominate suitable habitats (Hill, 1977).

Chromolaena odorata (L.) R. M. King and H. Robinson, pertinent to this dissertation, is a serious invader which takes advantage of disturbance (e.g. overgrazing, deforestation) by spreading rapidly and transforming natural habitats into unproductive areas (Liggitt, 1983). It also acts as a secondary invader in areas cleared of other invasive plants (e.g. *Lantana camara*), especially via seed germination (Erasmus, 1985; Erasmus, 1986; Witkowski, 2002). *C*. *odorata*, like other weeds, also has a phytochrome-mediated germination response which allows rapid colonisation of disturbed areas (Erasmus, 1985; Erasmus and van Staden, 1986).

1.1 The invasive weed, *Chromolaena odorata* (L.) R. M. King and H. Robinson

1.1.1 Origin and Distribution

The family Asteraceae (formerly Compositae) is one of the largest and highly-evolved plant families, consisting mainly of dicotyledonous herbs and shrubs, many of which are widespread weeds (Bennett & Rao, 1968; Wells & Balsinhas *et al*., 1986; McFadyen, 1988; McFadyen, 1991; Cronk & Fuller, 1995). *Chromolaena odorata* has a wide natural distribution in tropical and sub-tropical areas of the Americas, extending from southern Florida in United States of America through to the Caribbean Islands (West Indies), Amazonian and southern Brazil, Paraguay and north-west Argentina (King & Robinson, 1970; McFadyen, 1991; Barreto &

Evans, 1996). In its native habitat it is not a serious weed, but a pioneer species and is found mainly in disturbed areas (Cruttwell, 1972). *C. odorata* was originally referred to as *Eupatorium odoratum* prior to being transferred to the revised sub-genus *Chromolaena* which is part of the Tribe Eupatorieae (King & Robinson, 1970). The genus *Chromolaena* comprises of approximately 129 weed species of which *C. odorata* has become the most invasive in the Old World humid tropics and sub-tropics (King & Robinson, 1970; Holm *et al*., 1977). Hence *C. odorata* has been rated as one of the world's worst weeds and has been listed as one of the world's 100 worst invasive species by the World Conservation Union (IUCN), Invasive Species Specialist Group (ISSG), due to the serious impact that it has on agriculture, plantations, pastures and conservation areas in many parts of Asia, Africa and the Pacific (Holm *et al*., 1977; McFadyen, 1989; IUCN- ISSG, 2000; Wilson, 2005).

1.1.2 Growth characteristics

The wide distribution of *C. odorata* in the neotropics and the Old World can be attributed to its plasticity and ability to survive in a variety of habitats. It can grow in several soil types because it can tolerate a broad pH range, but prefers well-drained sites and dies back in waterlogged and or saline soils (Bennett & Rao, 1968; Pancho & Plucknett, 1971; Ismail *et al*., 1996). In waterlogged soil, *C. odorata* plants become susceptible to pathogenic fungi (root diseases); leaves yellow, stems blacken and die back. Plants grow well in nutrient-rich soil but when plants are cut-back in nutrient-poor soil, growth is affected (Cruttwell, 1972; McFadyen, 1991). *C. odorata* does not grow in full shade but in open and disturbed areas with full or partial light and within a temperature range of 20 to 37 ^{o}C, growth is rapid (Bennett & Rao, 1968; Yadav & Tripathi, 1981; Muniappan & Marutani, 1988; McFadyen, 1989).

A major advantage of perennial plants (e.g. *C. odorata*) and a key survival strategy is the total non-structural carbohydrates (TNC) reserves stored in the roots which enable plants to survive disturbance and winter (Kozlowski, 1992; Ikuenobe & Ayeni, 1998). The quantity of reserves influences the plant's ability to re-sprout successfully, (production of new tissue (growth) and repairing damaged tissue (maintenance)), enables the plant to survive competition from surrounding vegetation and allows rapid invasion (Hill, 1977; Kozlowski, 1992; Hartmann *et al*., 1997). TNC reserves also influences the plant's response to stress, which can affect plant

health. Carbohydrate metabolism is important for replenishment of reserves and for regulating energy available for plant tolerance to environmental stress (Kozlowski, 1992).

C. odorata has a vigorous growth rate due to efficient allocation of resources (Gautier, 1992) and produces a large quantity of viable wind-dispersed seeds annually, resulting in 'seed rain' (Blackmore, 1998). The architecture of the seeds (achenes) also allows for dispersal by water and the tiny spines of the seeds aid dispersal by sticking to clothing, animals and vehicles, thereby enabling long distance transportation (Hill, 1977; Erasmus, 1986; Blackmore, 1998; Henderson, 2002). Although *C. odorata* reproduces primarily by seeds, it can also reproduce vegetatively by producing adventitious roots when stems touch the ground, resulting in daughter plants (Gautier, 1993), and can coppice from stem bases after clearing (Liggitt, 1983; Henderson, 2002).

Chromolaena odorata has a profuse multi-stemmed branching habit which is due to removal or suppression of the apical shoot, resulting in dense infestations or thickets that smother indigenous vegetation (Pancho & Plucknet 1971; Sajise *et al*., 1974; Holm *et al*., 1977; Erasmus, 1986; McFadyen, 1989; Goodall & Erasmus, 1996). Growth form is further dependent on light availability. In grasslands or open areas, *C. odorata* plants are upright shrubs with semi-woody stems, 3-4 m or higher, whilst amongst trees it is a scrambler, reaching between 7-10 m in height (Holm *et al*., 1977; Erasmus, 1986). Its fibrous root system allows for efficient absorption of nutrients, thereby increasing its competitiveness (McFadyen, 1988; McFadyen, 1991). Allelopathy further enhances its competitive ability by suppressing or inhibiting growth of surrounding vegetation, enabling dominance of the area (Hill, 1977; Muniappan & Marutani, 1995; Ambika & Poornima, 2004; Gupta, 2005).

1.1.3 Spread to the Old World Tropics and Sub-tropics

Chromolaena odorata was first introduced as an ornamental plant into Serampore Botanical Gardens in Calcutta, India in the 1840s (McFadyen, 1989), and to Singapore around the 1920s via seeds embedded in ballasts of cargo ships sailing from the West Indies (Biswas, 1934 *loc. cit*. Bennett & Rao, 1968). *C. odorata* populations subsequently increased and spread to other parts of India and south-east Asia (McFadyen, 1989). By the 1940s, it was a serious weed in

Sri Lanka (McFadyen, 1989) and by the 1960s it was a major weed in Mauritius and Indo-China (Bennett & Rao, 1968). During the 1960s and 1970s it spread to the Philippines (Pancho & Plucknett, 1971), southern China (Wu *et al*., *loc*. *cit*. McFadyen, 1989) and by the late 1980s it was already present in Timor (McFadyen, 1989) and islands of the Pacific region (Muniappan & Marutani, 1988; McFadyen, 1989). Its most recent invasion was Australia in 1994 (Waterhouse, 1994).

C. *odorata* was accidentally introduced to west Africa (Nigeria) through contaminated seeds of the forest tree, *Gmelina arborea* Roxb. from Sri Lanka in the late 1930s (Odukwe, 1965 *loc*. *cit*. Akobundu & Ekeleme, 1996) and intentionally as a cover crop in coffee and pepper plantations in Cameroon and Central African Republic (Delabarre, 1977 *loc*. *cit*. Lanaud *et al*., 1991). It spread rapidly to other parts of Africa during the 1960s (Hoevers & M′ boob, 1996) and now occurs in west, east and central Africa (Gautier, 1992).

The introduction into South Africa seemed to have been directly from the neotropics and not from west Africa, as displayed by the different morphological characteristics of the South African form or 'biotype' (i.e. genotype) (white flowers, glabrous stems and leaves and a distinct odour when leaves are crushed) (Zachariades *et al*., 1999) compared with the Asian or African form, characterised by lilac to pink flowers, hairy stems and leaves and a distinct odour when leaves are crushed (Land Protection, 2006). In addition, stem rooting does not seem to occur in the South African form of *C*. *odorata* (Erasmus, 1985; C Zachariades, 2006, pers. comm.[1]). Two populations of *C*. *odorata* which flower a month apart co-exist in Australia (Scott *et al*., 1998), further reflecting the great variability in characteristics of *C*. *odorata* in the neotropics. In 2001, Dr H. Robinson (Smithsonian Institute) confirmed that the species invading South Africa was definitely *Chromolaena odorata* and that it originated from the West Indies (C Zachariades, 2002, pers. comm.[1]). Recent evidence confirmed that the South African biotype of *C*. *odorata* most likely originated from islands of the Greater Antilles (Jamaica, Puerto Rico and Cuba) and possibly from a few northern Caribbean Islands (Zachariades *et al*., 2004). *C*. *odorata* is commonly known as Siam weed in various parts of

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the Old World, whilst in South Africa it is called triffid weed, paraffin weed or simply, chromolaena.

1.2 *Chromolaena odorata* in South Africa

Approximately 7 % of the declared weeds in South Africa are asteraceous species, and six of these (Tribe Eupatorieae) are amongst the most invasive weeds in southern Africa, with *Chromolaena odorata* being the worst invader (Henderson, 2002). According to the Conservation of Agricultural Resources Act, 1983 (Act no. 43 of 1983) (CARA), amended in March 2001, it is regarded as the second worst weed (category 1) after black wattle (*Acacia mearnsii*) (Henderson, 2001). *C. odorata* is a major threat to biodiversity conservation in South Africa (Macdonald, 1983), but unlike most other alien invasive weeds in South Africa, it does not utilise large quantities of water (Zachariades & Goodall, 2002). It suppresses germination, growth and regeneration of indigenous vegetation and reduces species diversity (Liggitt, 1983; Goodall, 2000).

Populations of the Nile crocodile and black rhinoceros in different conservation areas on the north coast of the KwaZulu-Natal (KZN) province have already been threatened by *C. odorata*. Incubation temperature of crocodile eggs determines the gender of hatchlings, and females are produced in nesting sites that are shaded by *C. odorata* plants; hence there was great concern that if the invasion continued, a skew towards a female-biased sex ratio and possibly egg mortality would result. In addition, the fibrous roots of the plants create unsuitable nesting sites and further limit the availability of suitable sites (Leslie & Spotila, 2001). *C. odorata* also affects the endangered black rhinoceros populations by replacing thicket vegetation used for browsing and protection with dense infestations (Goodall, 2000). In addition to conservation areas, it also impacts negatively on pastoral and crop agriculture and forestry (especially young plantations) in the subtropical regions of southern Africa (Zachariades *et al*., 1999).

1.2.1 Introduction and Distribution

Chromolaena odorata was first recorded as naturalised in 1947 at Ndwedwe, near Durban, in the KZN province (Egberink & Pickworth, 1969; Hilliard, 1977) and the most commonly cited

theory of its introduction was via Durban harbour in packing material contaminated with chromolaena seed, during World War II (Pickworth, 1976). However, several other possibilities relating to its introduction were recently suggested (Zachariades *et al*., 2004). The density of *C. odorata* populations and the distance between infested sites in KZN during the 1940s (C. Zachariades, unpubl. data), as well as records of Jamaican *C. odorata* plants grown in Cape Town Botanical Gardens around the 1850s (Wells & Poynton *et al*., 1986), indicate that it may have been in KZN prior to the 1940s (Zachariades *et al*., 2004). During the period 1944 to 1979 *C. odorata* infested the eastern subtropical regions of KZN (Fig. 1.1), extending from Mkuze on the north coast to Port Shepstone on the south coast (Egberink & Pickworth, 1969; Pickworth 1976).

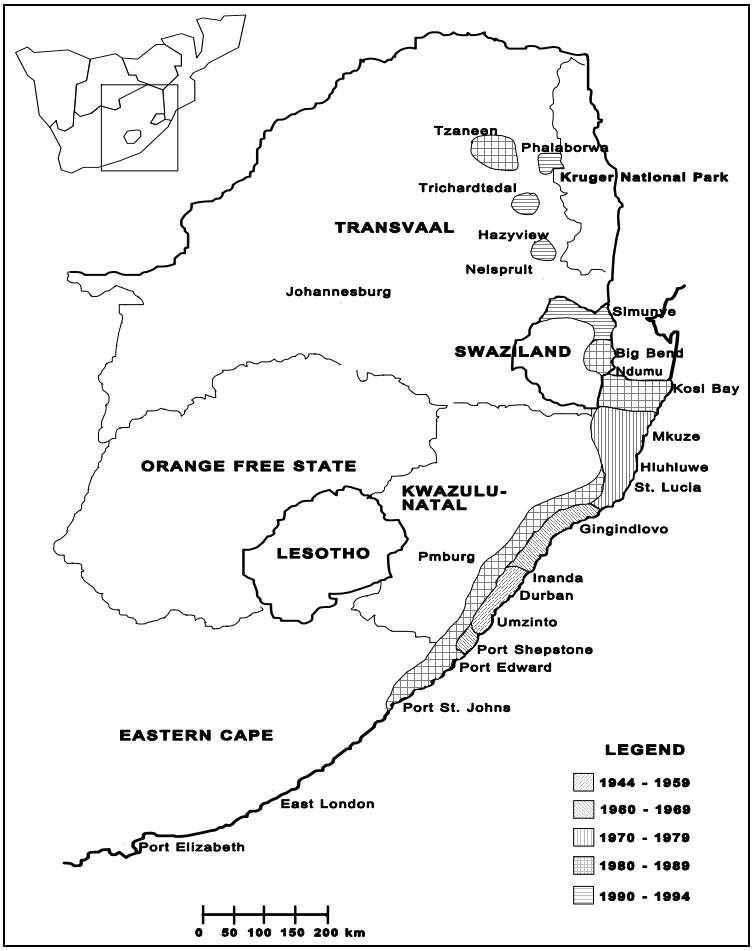

Figure 1.1: Spread of *Chromolaena odorata* in South Africa between 1944 and 1994 (after Goodall & Erasmus, 1996).

During the 1980s *C. odorata* spread to the Eastern Cape province, inland to Pietermaritzburg (near KZN midlands) (Liggitt, 1983), and to Limpopo province and a neighbouring country, Swaziland, in the lowveld (Goodall & Erasmus, 1996). It also invaded Mozambique (Goodall & Erasmus, 1996; Zachariades & Goodall, 2002). By 1994 it was present in Mpumalanga and also the Kruger National Park (Goodall & Erasmus, 1996; Foxcroft & Martin, 2002). Infestations increased in density and *C. odorata* continued to spread in the lowveld from the mid 1990s onwards (Fig. 1.2) (Henderson, 2002). The presence of *C. odorata* in Zimbabwe seems to be from West Africa (C Zachariades, 2006, pers. comm.[1]).

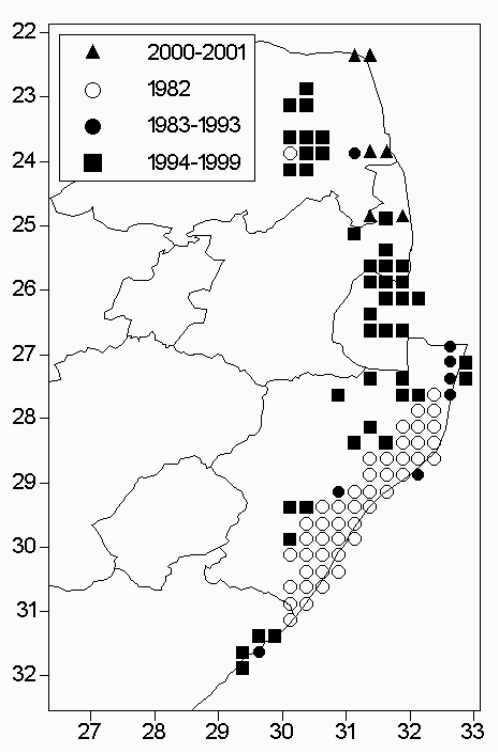

Figure 1.2: Distribution of *Chromolaena odorata* between 1994 and 2001 (SAPIA database: after Henderson, 2002).

Spread of *C. odorata* inland (beyond Pietermaritzburg into the KZN midlands region) seem to be limited by the occurrence of high levels of frost (Fig. 1.3) and to a lesser extent, low

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rainfall; as evident by its invasion of (i) forests and (ii) grasslands and arid bushvelds (annual rainfall: (i) 1500 mm and (ii) 500 mm or less) (Goodall & Erasmus, 1996). It survives in dry areas by defoliation and some stem die-back, but re-growth occurs rapidly after rain (Liggitt, 1983). *C. odorata* does not occur at altitudes higher than 1000 m (McFadyen, 1988), which may further explain its absence in the KZN midlands.

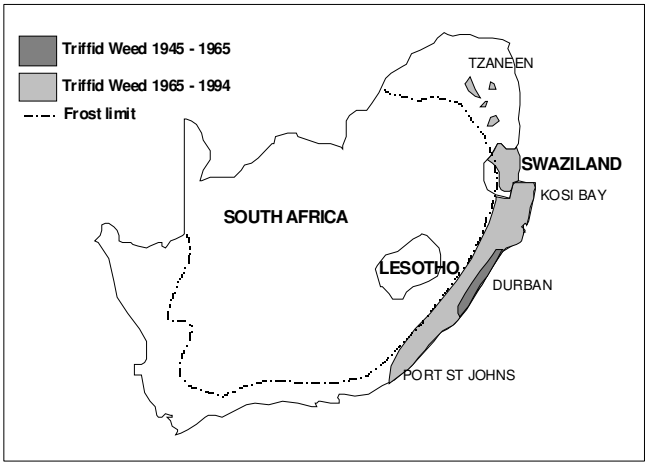

Figure 1.3: Climatic limitations to inland spread
(after Goodall & Erasmus, 1996).

1.2.2 Management and Control

To control *C. odorata* populations in the field it is essential to suppress plant growth and reduce seed and vegetative reproduction, thereby preventing further spread into other areas. Three methods of control, viz. mechanical or physical (uprooting and slashing), chemical (foliar and stump herbicides) and cultural (prescribed fire) have proved effective for controlling *C. odorata* populations in the short-term, but are labour-intensive, expensive and not feasible over a long period considering the high growth rates and large areas invaded by *C. odorata*. Biological control (biocontrol) is therefore recognised as the only long-term cost-effective and sustainable method of controlling *C. odorata* and is a critical component of an integrated control strategy for *C. odorata*, having its effect by reducing its growth and reproductive output (Goodall & Erasmus, 1996; Zachariades *et al*., 1999; Henderson, 2002). Since *C. odorata* is not a beneficial plant in South Africa and closely-related to only few

asteraceous plants, biocontrol can be implemented and all plant organs can be targeted (Zachariades *et al*., 1999; Henderson, 2002). Classical biocontrol involves the use of natural enemies, i.e. herbivorous insects, mites and pathogens from the native country of the weed to attack the vegetative or reproductive parts of invasive plants in the country of introduction. These host-specific biocontrol agents are required to reduce the density and spread of invasive plants to manageable levels, where other control methods can be implemented if necessary (Samways, 1981; McFadyen, 1998; Zimmermann *et al*., 2004).

1.3 Biological control of *Chromolaena odorata*

An insect-based *C. odorata* biocontrol programme was initiated in 1988 (Kluge, 1991), as part of the Weeds Division within the Agricultural Research Council-Plant Protection Research Institute (ARC-PPRI) at Cedara (29°32'S ; 30°17'E). Biocontrol research involves culturing biocontrol agents and setting up host-specificity tests and hence requires a large quantity of healthy potted *C. odorata* plants annually; grown in small (18 cm) and medium (26 cm) pots in order to fit comfortably into cages. In order to supplement the stock culture, *C. odorata* plants were propagated at Cedara from rooted saplings that were collected from the field at the coast in Durban. Over the past 10 years the biocontrol programme has experienced difficulties in growing and maintaining healthy potted *C. odorata* plants, resulting in high mortality rates, thereby placing a constraint on research. One possibility for this is that Cedara is a temperate region situated at an altitude of 1076 m, is prone to frost and lies outside the invasive range of *C. odorata*.

1.3.1 Plant growth problems encountered

During spring 1998 mortality symptoms (yellowing and wilting of leaves, leaf die-back followed by stem die-back) were first observed when plants were moved to the newly built shade-house for protection against hail storms and high temperatures during summer at Cedara. Although some plants re-sprouted (Figs. 1.4, 1.5), none recovered fully to attain normal growth (Figs. 1.6, 1.7). From hereon plants which displayed mortality symptoms will be categorised as 'unhealthy plants' and those without these symptoms will be categorised as 'healthy plants'. Plants also died back when moved to the growth tunnel during winter to protect them from low temperatures and frost. Several plants that were placed on the ground in

the shade-house and tunnel rooted into the ground resulting in damage to new roots when picked up for use. Although several species of indigenous weed, crop and ornamental species (closely related to Asteraceae) were grown at Cedara, only one species (*Vernonia angulifolia*) displayed similar symptoms to *C. odorata* at that time.

Figure 1.4: Unhealthy plant (died back and re-sprouted).

Figure 1.5: Unhealthy plant roots (brown-black, sparse, no root hairs).

Figure 1.6: Healthy plant.

Figure 1.7: Healthy plant roots (cream, prolific branching, profusion of root hairs).

1.3.2 Attempted solutions towards growing healthy potted plants (1998-2002)

A heater was installed in the tunnel at Cedara to provide suitable temperatures for plant growth during winter; a fertigation system (dosatron) was installed in the tunnel and shade-house which allowed control over water and nutrient supply via drippers. An overhead fogger system was installed in the shade-house to cool foliage when air temperatures reached 32 ^{o}C or higher and a plastic dome was erected over part of the shade-house to prevent *C. odorata* plants from being over-watered during rain; however, this reduced the light intensity further. Gravel was initially placed on the ground in the shade-house and tunnel to inhibit root growth out of pots and to improve aeration but this was unsuitable and gravel in the tunnel was later replaced with a black woven plastic to allow adequate drainage for potted plants, but this too was unsuccessful. The growing medium (i.e. commercial potting mix), fertilising and watering regimes were also changed, but the quality of plants did not improve.

In 2002 a commercial nursery was contracted to grow potted *C. odorata* plants in Durban, an environment which is suitable for its growth and plants were delivered to Cedara monthly;

however, plants continued to die back. Results from a preliminary pathological test conducted on unhealthy *C. odorata* stock plants indicated that pathogen activity was a secondary cause of mortality and further indicated that the primary problem was possibly physiological and not pathological. Although much research has been done on *C. odorata* world-wide, limited information is available on its growth and physiology in different habitats, especially in South Africa. Hence, as a final attempt it was imperative to investigate the relationship of environmental factors to mortality of potted *C. odorata* plants at Cedara.

1.4 Study objectives

The objective of this study was to try to determine the cause of mortality of potted *C. odorata* plants at Cedara and to make recommendations to reduce this. From previous observations, it was hypothesised that *C. odorata* plants grown at Cedara were more stressed than those grown in Durban, which is within the invasive range, perhaps due to high vapour pressure deficits (VPD) and/or low temperatures especially during winter. This, coupled with reduced light intensity, especially in the tunnel and shade-house micro-habitats, may limit the plant's ability to accumulate the necessary quantity of total non-structural carbohydrate (TNC) required to supplement depleted reserves and overcome stress. In accordance with this, potted plants were grown at Cedara (temperate region) and in Durban (subtropical region) and various aspects of plant growth and physiology were measured and related to environmental conditions, particularly VPD and temperature. Physiological measurements included transpiration, chlorophyll fluorescence (F_v/F_m ratio), chlorophyll and anthocyanin contents, and plant growth analysis. The F_v/F_m ratio indicates levels of stress in a plant by assessing the potential photochemical efficiency of photosystem (PS) II. Additionally, as non-structural carbohydrates are important reserves enabling plants to survive and recover from stress, TNCs were also measured. Leaf nutrient analysis was also included in the study because leaves are the main site in which symptoms relating to plant stress are displayed, and also reflects the nutrient availability in the soil. Nutrient deficiencies can result in leaf yellowing (chlorosis), which is an early visual symptom of stress and is associated with a decline in concentration of chlorophyll (Webb & Fletcher, 1996; Manetas *et al*., 1998). Additional trials were setup to determine if there were other factors causing plant mortality. These included a plant growth trial setup during autumn-winter to investigate seasonal differences; a spacing and drainage

trial to investigate the effects of air-nipping of roots, drainage and spacing on plant growth; the effects of using fertilisers with higher N:P:K on plant growth, as well as the effects of fungicide application to control soil pathogens. TNC analysis was also conducted on a group of plants which displayed varying degrees of mortality symptoms based on visual assessment. The stem base and root anatomy of plants displaying mortality symptoms, and the redox potential of soil in which they were rooted, were also investigated.

CHAPTER 2 MATERIALS AND METHODS

2.1 GENERAL TECHNIQUES

2.1.1 Plant propagation and study sites

Chromolaena odorata plants (4 to 6 mm stem diameters), were collected from sun and semi-shade areas around the University of KwaZulu-Natal (UKZN), Howard College campus in Durban ($29^{o}87$'S ; $30^{o}98$'E). Plants were defoliated, cut-back above the third node and planted in medium-sized plastic pots containing a commercial potting mix from Burgess Nursery, Durban. Pots measuring 26 cm in diameter, 21 cm deep, with five 2 cm diameter drainage holes were filled to a height of 19 cm to account for reduction of the potting mix due to compaction.

Plants were divided into three groups of 25 (arranged in 5 by 5 rows) and left to establish on elevated grids (2 m x 1 m x 0.85 m) in (i) the polycarbonate clad tunnel (green-house) in Durban, (ii) the polyethylene clad heated tunnel at Cedara, set at a minimum temperature of (15 ^{o}C); both tunnels were supplied with wet-wall cooling. Plants in the tunnels were fertilised every two months using Burgess [5:1:5 (33)] slow-release fertiliser (10 g of granules per pot). The third group of plants was left to establish under natural conditions in Durban, on black woven plastic on the ground and rotated at regular intervals to prevent root growth from the pots into the ground. Plants growing in the Durban tunnel were watered by an automatic watering system (set up permanently in the tunnel), whilst at the other two sites plants were watered daily using a hose-pipe.

In a second trial, two groups of 25 plants (propagated as above) were left to establish on elevated grids in shade-houses at Cedara and in Durban and the third group (25 plants) under natural conditions in Durban. All plants were watered daily at 8:30 a.m. and 2:30 p.m. with an automatic watering system (Gardena Water Computer Profi C 1060) and fertilised monthly. Different fertilising and watering regimes were used in Trial 2 to investigate the effects of different regimes on growth of *C. odorata*. One-fifth of the unharvested plants from the first trial was moved to the shade-house in Durban and Cedara during summer. Plants from both

trials were then moved to the tunnels in Durban and Cedara around the end of May 2004 and back to the shade-houses in Durban and Cedara in early November 2004 to be consistent with the set up at Cedara for winter and summer periods respectively.

2.1.2 Plant maintenance

Plants in all Trials 1 and 2 were hand-weeded as necessary, checked for pests (aphids, spider mites, mealybugs, powdery mildew) and treated with appropriate insecticides. Plants that were not harvested for growth analysis at the end of trial were maintained to 30 cm height by pruning plants at six month intervals. During winter, buds were nipped off from these plants to prevent flowering and to ensure that more carbohydrate reserves were allocated to vegetative growth. *C. odorata* stock plants at Cedara are maintained similarly.

2.1.3 Transpiration measurements and vapour pressure deficit (VPD)

Transpiration was determined by weighing potted plants 3 times per day between 10 a.m. and 4 p.m. for two consecutive days, every fortnight, for three months. Pots were placed in plastic bags (62 cm by 35 cm) and secured with elastic bands at the base of the plant to prevent soil evaporation. The plants remained in plastic bags for the two-day period of data collection and were not watered. Comparisons were made between plants grown in the tunnels at Cedara and in Durban from August to October 2003.

A Supco DLTH data logger was placed at each site to log air temperature and relative humidity data daily at 10 minute intervals and data were downloaded monthly from June to November 2003. The average air temperature (T) and average relative humidity (RH) data were used to estimate average monthly vapour pressure deficit (VPD). VPD is saturation vapour pressure minus actual vapour pressure and is calculated as (SVP@T – SVP@T*RH/100), (SVP is the saturation vapour pressure at that particular temperature). Light intensity was measured at 11 a.m. and 2 p.m. from August to November 2003 using an ESR-1 light meter.

2.1.4 Chlorophyll fluorescence measurements

Measurements were taken using a Plant Efficiency Analyser (Hansatech, UK), in the morning on all plants at each site, after five months of growth (November 2003). Four uppermost young (fully-expanded) and four mature uniform leaves, i.e. the 3rd or 4th, and 5th or 6th pair, respectively, were selected and portions of the upper leaf surface (4 mm diameter area) were initially dark-adapted for 30 minutes using light-exclusion leaf clips; care was taken to avoid the mid-rib. After illumination at an intensity of 1500 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) for a period of 1 second, the F_v/F_m ratio was determined.

2.1.5 Pigment composition

Leaves used for chlorophyll fluorescence measurements were removed from the plants the next day; chlorophyll was extracted from the mature leaves (fresh weight) and anthocyanins from the young leaves (fresh weight). Extractions and quantitation were conducted according to Arnon (1949) for chlorophyll and Mancinelli *et al*. (1975) for anthocyanins.

Chlorophyll was extracted in 80 % (v/v) acetone and absorbances were read at wavelengths of 663 nm and 645 nm to determine chlorophyll contents. Total chlorophyll was calculated as $(20.2 * A_{645} + 8.02 * A_{663}) * V/(1000 * W)$ [A is the absorbance of solution at wavelength indicated by subscript, V is final volume of the chlorophyll extract (ml) and W is fresh weight of tissue extracted (g)]. Anthocyanins were extracted in 80 % (v/v) acidified methanol (MeOH:water:HCl) at (79:20:1) and absorbances were read at wavelengths 657 nm and 530 nm to determine anthocyanin contents. Total anthocyanins were calculated as $A_{530} – 1/3 * A_{657}$.

2.1.6 Harvesting, sorting and drying

Plants were cut at the soil surface and separated into leaves and stems. Dead leaves or necrotic tissue on green leaves was also removed and all fresh material was then weighed. The total leaf area per plant was measured for each plant using CI 251-auto leaf area meter. Roots were cut from the main stem at the point of discolouration and were carefully washed free of potting mix and pieces of pine-bark stuck between the roots were carefully removed. Roots were then patted dry with absorbent paper. Plant material was oven-dried to a constant weight at 80 ^{o}C

for 72 hours and weighed thereafter. Samples were then placed into labelled brown paper bags and transported to Cedara to be milled.

2.1.7 Biomass accumulation and growth analysis

Plant height was measured from the base of the plant (at the soil surface) to the tip of its tallest apical shoot. Stem diameter was measured and total number of stems grown was recorded. Leaf weight ratio (LWR), stem weight ratio (SWR) and root weight ratio (RWR) were expressed as percentages: [(dry weight of organ per total dry weight per plant) * 100]. This was representative of biomass (dry weight) partitioning or proportional allocation. One uppermost fully expanded leaf was randomly selected per plant per site for specific leaf area (SLA) measurements. SLA (cm^2 g^{-1}), i.e. leaf area per unit leaf biomass and root:shoot ratio were also calculated.

2.1.8 TNC analysis

All oven-dried plant material was milled as soon as possible to prevent moisture gain. The woody stems and root material from the six plants from each site were ground separately using a Retsch Impeller-type cutting mill fitted with a 1 mm screen. The ground plant material was homogenised to fine powder form with a Kenwood coffee grinder. The softer leaf material was also homogenised to fine powder using the coffee grinder. Milled samples were stored in plastic vials (4 cm by 6 cm) and later analysed for total non-structural carbohydrates using a modification of the method described by Rasmussen and Henry (1990). Six plants per site were used for TNC analysis.

The reagents used in the analysis were prepared and kept in a fridge at (4 ^{o}C). Sub-samples weighing 0.05 g were then taken from milled material of each organ (replicated three times) and added to 10 cm^3 test-tubes placed in labelled racks. 5 ml 80 % v/v ethanol was added and soluble sugars (reducing sugars: glucose and fructose and non-reducing sugars: sucrose) were extracted for 30 minutes in a water bath set at 80 ^{o}C and centrifuged in a Hermle 2510 refrigerated centrifuge for 10 minutes (Part I). The insoluble pellet was stored in a freezer at (-20 ^{o}C) for subsequent starch digestion. Starch is one of the main storage carbohydrates containing two polysaccharides: amylose and amylopectin. The enzyme-hydrolysis method

using termamyl and amyloglucosidase was used to hydrolyse starch to glucose equivalents in a water bath set at 90 ^{o}C for 30 minutes and 60 ^{o}C for 20 hours respectively, and centrifuged for 10 minutes (Part II). 100 µl of the supernatants from Part I and II of the extraction were initially transferred to test-tubes, made up to 5 ml using glucose oxidase colour solution and incubated in a water bath for 15 minutes at 40 ^{o}C. After cooling these were transferred to 10 mm plastic cuvettes and absorbances were read at 505 nm in a spectrophometer (Anthelie Advance), using a glucose solution as a calibration standard. Concentration of the supernatants from Parts I and II was obtained from the standard glucose curve.

2.1.9 Leaf nutrients

30 to 40 mature healthy leaves (5th to 7th pairs) of the same physiological age were collected around the end of November 2003 from fertigated healthy (n = 2) and unhealthy (n = 2) *C. odorata* stock plants at Cedara, to obtain a fresh weight of $\pm$ 500 g. This was sent in sealed labelled plastic bags to the Plant Laboratory at KwaZulu-Natal Department of Agriculture, Cedara, for nutrient analysis.

2.1.10 Structure of *C. odorata* stems and roots

Stem bases of a group of 16 stock plants (eight healthy and eight unhealthy) were checked for hypertrophied lenticels and lateral (secondary) roots. Hand cross-sections (0.5 to 1 micron) of six fresh lateral roots per plant (from eight healthy and eight unhealthy plants) were prepared for light microscopy using 1 % Toluidene Blue as a stain.

2.1.11 Statistical analyses

Analysis of Variance (One way ANOVA) was used to compare more than two treatments, whilst an independent samples (un-paired) t-test was used to compare between two treatments. Data from plants grown within a site were pooled and compared across sites. Two-tailed probabilities were used to test for significance, at p = 0.05 level. Data were checked for normality using a Kolmogorov-Smirnov (K-S) goodness of fit test and differences between the sites were tested for 95 % confidence levels using Tukey's test. Data were analysed using SPSS version 11.5 (Zar, 1999). Data presented in most figures represent the means $\pm$ S.E. of pooled values of plants per site.

2.2 EXPERIMENTS CONDUCTED

2.2.1 Trial 1 (winter-spring)

Trial 1 ran between June to November 2003 in growth tunnels at Cedara (Cedara-Tn) and in Durban (Durban-Tn) and under natural conditions in Durban (Durban-Op). The amount of water transpired per plant was determined by lysimetry and this was related to the vapour pressure deficit of the micro-habitat within which plants were kept. Transpiration was not expressed per unit leaf area because leaf area could not be measured non-destructively at the time of measurement. F_v/F_m was measured on the 3rd or 4th, and 5th or 6th pair of leaves from plants at all sites; the 3rd or 4th pair (young) leaves and 5th or 6th pair (mature) leaves were removed for anthocyanin and chlorophyll extraction, respectively. Six plants from each site were randomly selected and harvested for TNC analysis. Plants that were not harvested were allowed to grow for a further 12 months in order to determine the effects of plant age and pruning on growth and development.

2.2.2 Trial 2 (autumn-winter)

Trial 2 ran between March to August 2004 in shade-houses at Cedara and in Durban, and under natural conditions in Durban. The purpose of this trial was to visually compare plants grown during the cooler period (autumn-winter) with plants grown in Trial 1 during (winter-spring), for symptoms associated with mortality. Plants in this trial were also allowed to grow for a further 12 months in order to determine the effects of plant age and pruning on growth and development.

2.2.3 Fungicide trial and fertiliser treatments

Sixteen healthy plants of the same physiological age were selected by visual assessment and divided into two groups. Plants in Group 1 were watered with a hose-pipe and fertilised on a monthly basis with Burgess [5:1:5 (33)] similar to Trials 1 and 2, whilst plants in Group 2 were drip irrigated with Hortichem (water-soluble, quick release) [3:1:3 (38)], as applied to stock plants via the dosatron. All plants were equally spaced and placed on the ground in the tunnel for the duration of the trial (July to October 2004). A 10 L fungicide solution was prepared by adding 12 ml Previcur N and 10 g Benlate to tap water. 200 ml of the solution was added to four plants from both groups (1a and 2a), every three weeks whilst the other four

(1b and 2b) remained untreated (control). Previcur N and Benlate solution is a systemic fungicide that treats most common root diseases. At the end of the trial, two plants from each group (treatment and control) were sent to the Plant Pathology Laboratory at KwaZulu-Natal Department of Agriculture, Cedara for pathogen analysis. The purpose of this trial was to re-evaluate the role of pathogens in mortality of stock plants at Cedara using fertilisers with different N:P:K ratios. Percent die back of stems was recorded and quality of plants within groups was visually compared.

2.2.4 Spacing and drainage trial

This trial was set up around the end of July 2004 in the tunnel at Cedara. Twenty healthy plants grown in medium-sized pots were selected by visual assessment from plants grown at Burgess Nursery. Five plants were placed next to each other and another five, 25 cm apart (Fig. 2.1) in Group 1 (elevated grid) and Group 2 (ground). This trial was set up to determine (i) the effects of spacing on growth of established plants from the stock culture and (ii) the effects of drainage, aeration and air-nipping of roots on plant growth. During this eight month trial, plants were moved between the tunnel and the shade-house, and at termination plants were visually assessed for mortality symptoms and plant height was measured.

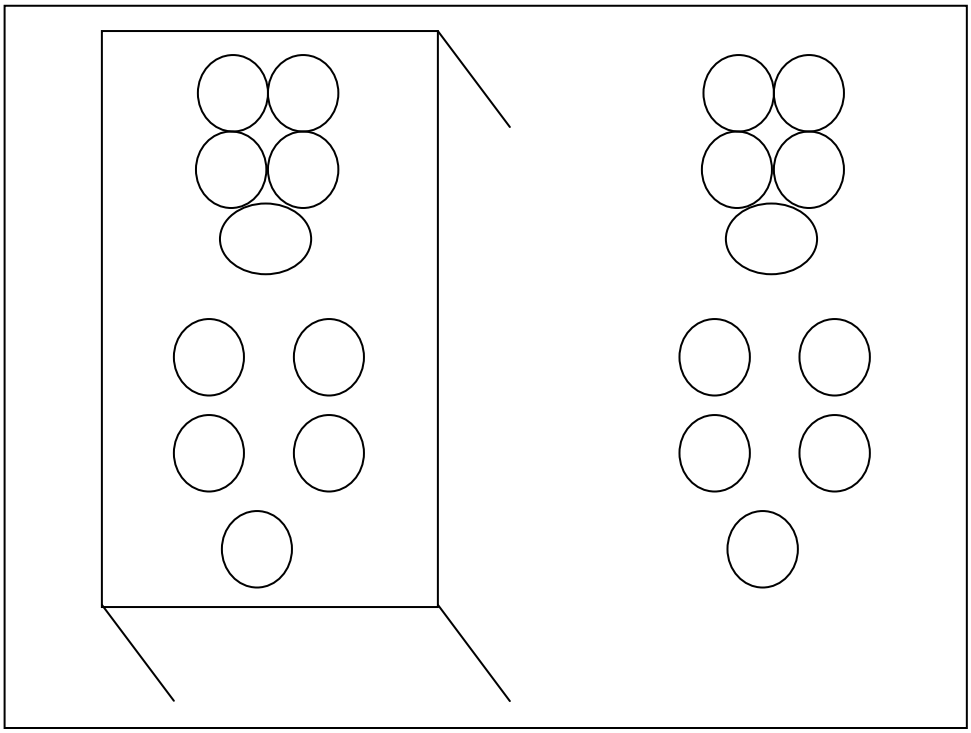

Figure 2.1: Spacing and drainage trial layout.

2.2.5 TNC analysis of unhealthy *C. odorata* stock plants

Plants used for TNC analysis were grown at Burgess Nursery for ± four months prior to being sent to Cedara where they were kept on elevated grids under natural conditions for ± 45 days during summer, due to limited space in the shade-house. Six small (18 cm) potted plants, ranging from most healthy to least healthy based on visual assessment were randomly selected during January 2005 and assigned numbers 1 to 6, respectively. Since plants could not be placed into categories, no replication was done. Plants were harvested as in Trial 1, oven-dried at 80 ^{o}C to a constant weight, milled and sent to the Feed Laboratory at KwaZulu-Natal Department of Agriculture, Cedara for TNC analysis.

2.2.6 Redox potential of the soil of *C. odorata* stock plants

Three healthy and three unhealthy small (18 cm) potted plants (based on visual assessment) were selected for redox measurements. 10 g of soil (three replicates) were taken from the area closest to the roots, saturated with 25 ml distilled water and stirred briefly to minimise oxidation and the redox (reduction-oxidation) potential (E_h) was measured at standard temperature (23 ^{o}C).

CHAPTER 3 RESULTS

3.1 Trial 1 (winter-spring)

Plants that established on elevated grids in growth tunnels at Cedara (Cedara-Tn) and in Durban (Durban-Tn), and on the ground (Durban-Op) appeared healthy and did not display chlorosis or any other visual symptoms associated with mortality for the duration of the trial. Plants that established at all three sites survived, even after the growth period was extended for a further 12 months, indicating that *C. odorata* plants can be grown at Cedara and that plant age, pruning or seasonal effects were not contributing factors to mortality.

3.1.1 Growth parameters (Total biomass, Root/Shoot Ratio and Specific Leaf Area)

Variation among plants with regard to total plant biomass (Fig. 3.1) and specific leaf area (SLA) (Fig. 3.2) was high and results from a One-Way ANOVA showed no significant differences in total plant biomass ($p = 0.248$) and SLA ($p = 0.693$) among the sites. However, there was a significant difference in root/shoot ratio (R/S) ($p = 0.0001$) of plants grown at the three sites (Fig. 3.3). Although R/S may have been influenced by light, the differences in R/S are big, but the differences in light intensity are small (average: 62 % and 60 % of light intensity in the open outside Durban-Tn and Cedara-Tn, respectively).

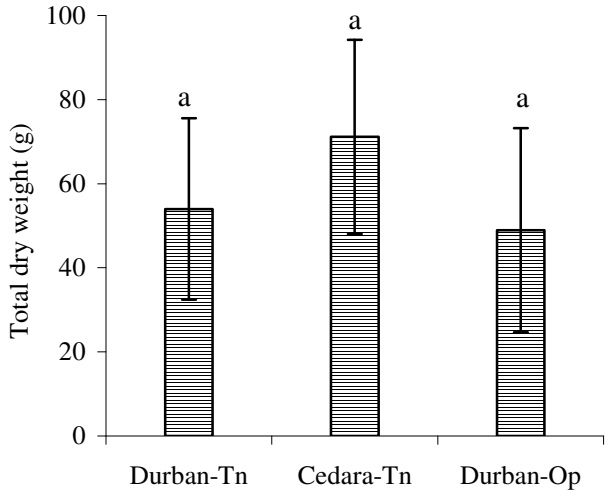

Figure 3.1: Total biomass of *C. odorata* plants grown in the tunnel at Cedara and in Durban (same letters indicate no significant difference and bars represent mean $\pm$ 1 S.E.).

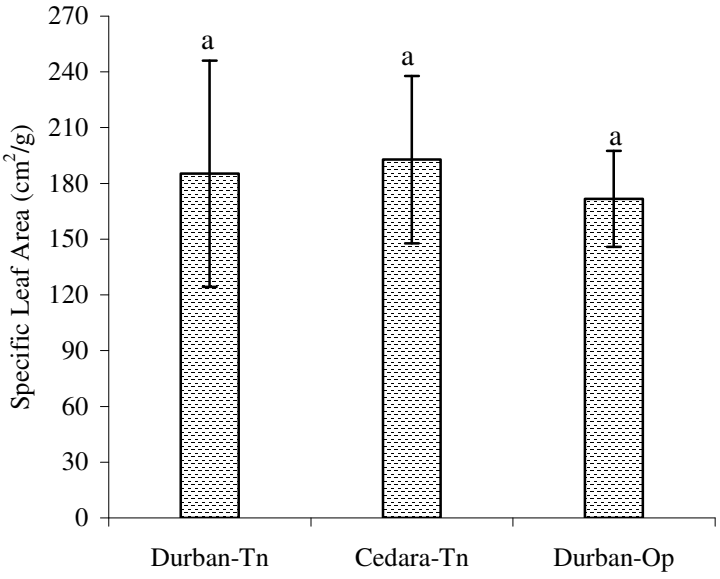

Figure 3.2: Specific Leaf Area of plants grown in the tunnel at Cedara and in Durban (same letters indicate no significant difference and bars represent mean ± 1 S.E.).

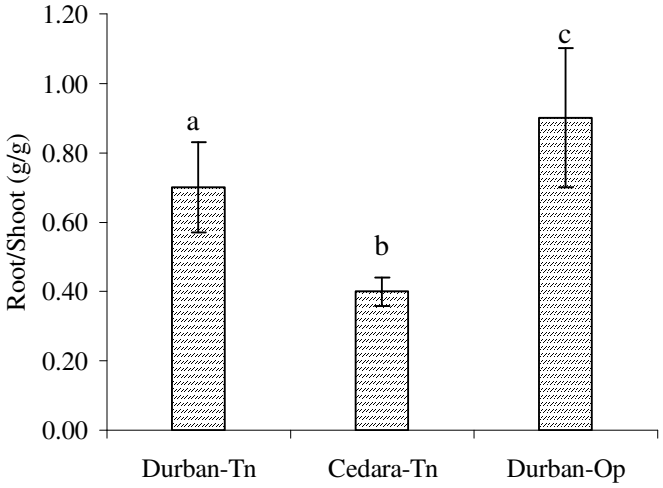

Figure 3.3: Root/Shoot ratio of plants grown in the tunnel at Cedara and in Durban (different letters indicate a significant difference and bars represent mean ± 1 S.E.).

3.1.2 Fluorescence ratio (F_v/F_m) and Pigment composition

There was a significant difference in values of F_v/F_m of leaves of plants grown in Cedara-Tn (Fig. 3.4) compared with plants grown in Durban (One-Way ANOVA; p = 0.001), indicating lower potential photochemical efficiency of PS II in leaves of Cedara-grown plants. This suggests that these plants were experiencing some form of environmental stress. There was a marginally significant difference (p = 0.06) in total anthocyanin contents of young leaves and a significant difference in total chlorophyll content of mature leaves (p = 0.0001) of plants grown in Cedara-Tn compared with those grown at the Durban sites (Fig. 3.5).

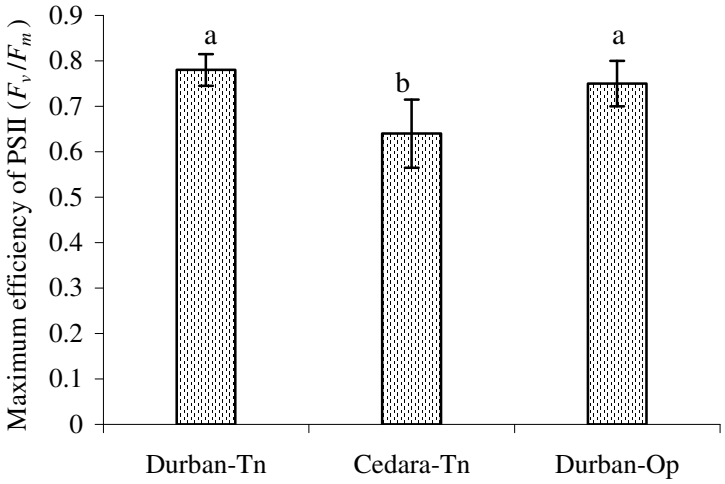

Figure 3.4: Measure of the potential quantum efficiency of plants grown in the tunnel at Cedara and in Durban (different letters indicate a significant difference and bars represent mean $\pm$ 1 S.E.).

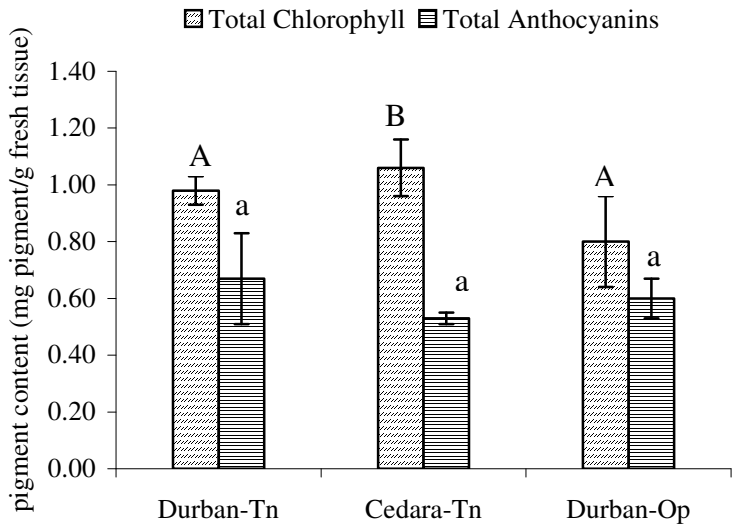

Figure 3.5: Pigment content of leaves of plants grown in the tunnel at Cedara and in Durban (different letters for the same pigment indicate a significant difference and bars represent mean $\pm$ 1 S.E.).

3.1.3 Transpiration rates and Vapour Pressure Deficit (VPD)

Transpiration rates are driven mainly by VPD and regulated by stomata and under conditions of high VPD, plants are expected to have high transpiration rates. Plants grown in Cedara-Tn had lower transpiration rates, calculated per pot, than those grown in Durban-Tn (Fig. 3.6) despite being exposed to high VPDs (Fig. 3.7). Results from an un-paired t-test indicated that there were no significant differences in transpiration rates between plants grown in Durban-Tn and Cedara-Tn for August ($p = 0.357$) and September ($p = 0.778$), but there was a significant difference in October ($p = 0.042$). The lower rate in the Cedara-Tn plants suggests that the stomatal conductance (g_s) was reduced in these plants, to reduce water loss under these conditions; this could indicate a water stress. Furthermore, at the end of the trial plants grown in Cedara-Tn had a total leaf area in excess of three times that of plants grown in Durban-Tn (Table 3.1). If this differential was maintained throughout the growth period, then the transpiration per pot in Cedara-Tn would be expected to be three times that per pot in Durban-Tn. This, however was not the case; transpiration per pot was similar at the two sites, suggesting that transpiration per unit leaf area was lower in Cedara-Tn than in Durban-Tn,

supporting the suggestion of reduced stomatal conductance in plants grown in Cedara-Tn, indicating some stress.

Table 3.1: Leaf area of harvested *C. odorata* plants from Trial 1.

Site	Leaf Area (cm^2)
Durban-Tn	1157
Cedara-Tn	3768

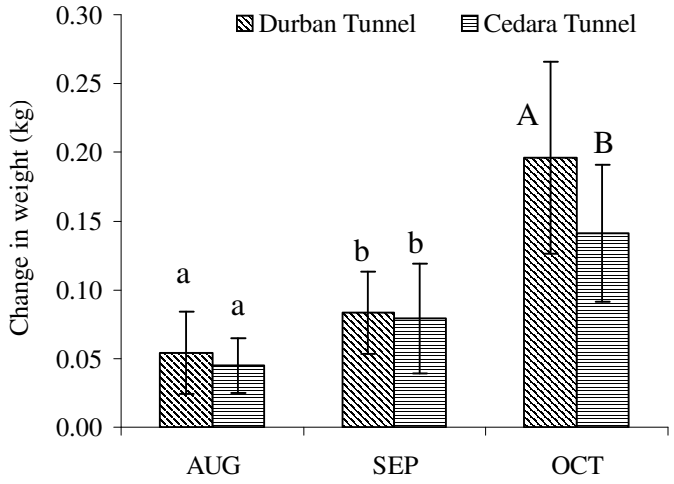

Figure 3.6: Water transpired from plants grown in the tunnels at Cedara and in Durban between August-October 2003 (different letters within a month indicate a significant difference and bars represent mean $\pm$ 1 S.E.).

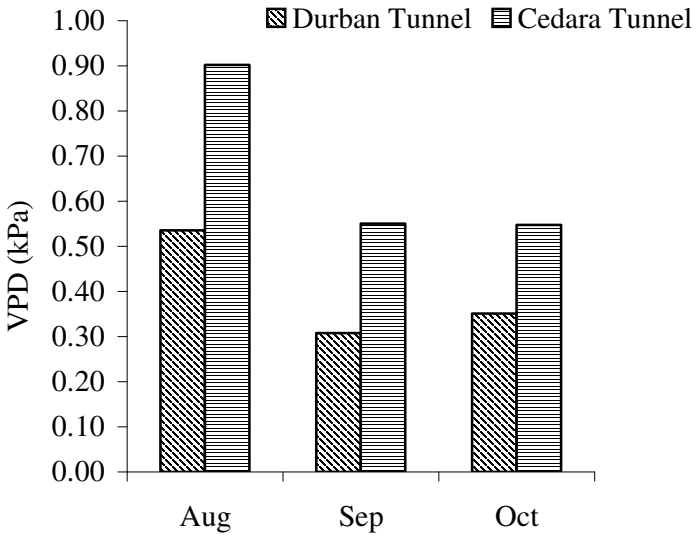

Figure 3.7: Average vapour pressure deficit (VPD) in the growth tunnels between August-October 2003.

3.1.4 Total non-structural carbohydrate (TNC) reserves

Plants grown in Cedara-Tn partitioned less TNC to the roots, and to a lesser extent, the stems, compared with plants grown in Durban (Fig. 3.8). This may have been a consequence of change in relative growth rates of the plant parts due to stress, (higher leaf growth of plants grown in Cedara-Tn compared with plants grown in Durban), despite the fact that averaged across the whole plant, TNC concentrations (Fig. 3.9) are the same. Results from a One-Way ANOVA indicated significant differences in TNC reserves allocated to the leaves ($p = 0.0001$) and roots ($p = 0.0001$) of plants grown in Cedara-Tn and the Durban sites; however there were no significant differences in the concentration of TNC ($p = 0.733$) within plants grown at all sites and in TNC reserves allocated to the stems ($p = 0.035$).

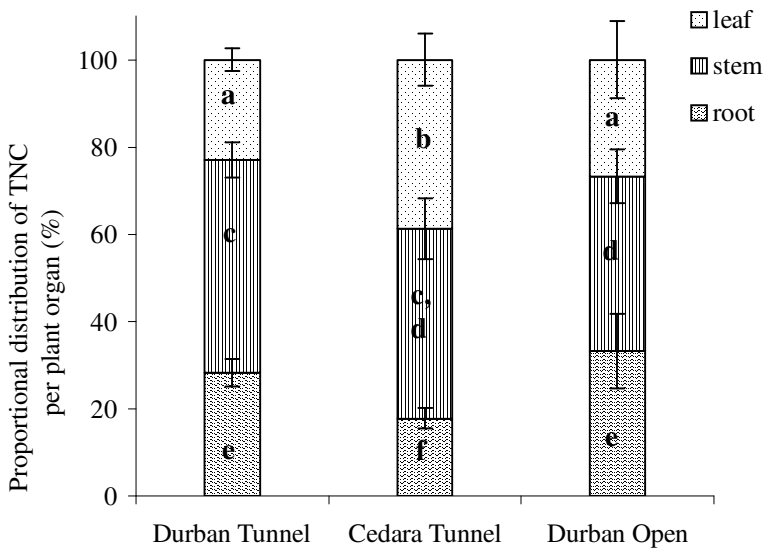

Figure 3.8: Partitioning of TNC reserves per plant organ (different letters for the same plant organ indicate a significant difference and bars represent mean $\pm$ 1 S.E.).

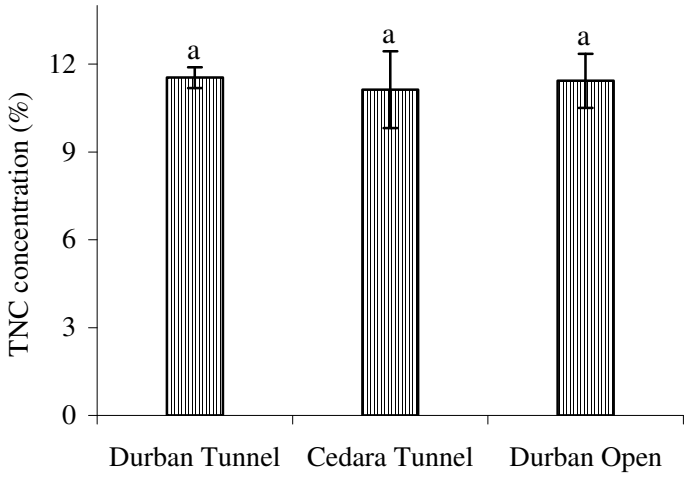

Figure 3.9: Concentration of total non-structural carbohydrate (TNC) of plants grown at all sites (same letters indicate no significant difference and bars represent mean $\pm$ 1 S.E.).

Although it was hypothesised that unhealthy stock plants at Cedara are intolerant of stress because of low root TNC reserves, this was unclear from the results of the TNC analysis in Trial 1. A possible reason is that plants grown in Cedara-Tn were healthy and although less TNC reserves were allocated to the plant roots, the quantity of TNC may have been sufficient

to supplement depleted reserves and overcome stress, thereby preventing die-back. A separate group of unhealthy *C. odorata* stock plants were selected to re-evaluate the above hypothesis.

3.2 TNC content and partitioning of reserves to roots of unhealthy stock plants

The results from the TNC analysis conducted on the group of unhealthy stock plants also did not support the above hypothesis. Additionally, there was no direct correlation between plant health (based on visual symptoms (Fig 3.10)) and TNC allocated to the roots (Fig. 3.11) of plants 1 to 6. A possible reason for this is that these plants were grown in Durban and kept at Cedara for a short period only, hence plant growth and storage of reserves was determined by the Durban environment and not the Cedara environment. These plants may have been able to alleviate stressful conditions at Cedara due to the availability of TNC reserves in the roots, which may have been reduced if these plants were grown at Cedara. The TNC concentration across the whole plant (Fig. 3.12) indicated that two of the three unhealthy plants had lower TNC concentrations compared with the healthy plants.

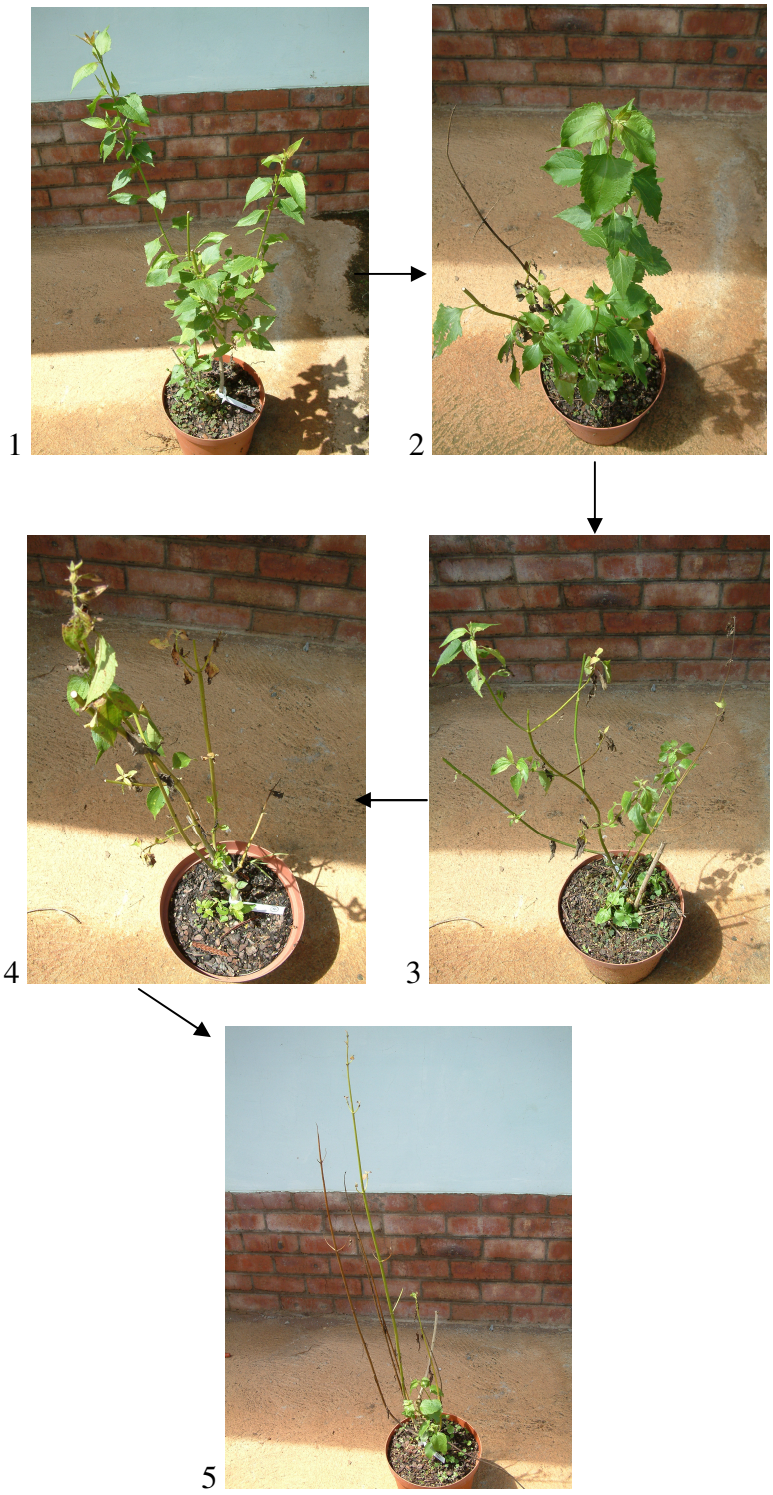

Fig 3.10: Degree of health in *C. odorata* plants displaying mortality symptoms. Plants 1 to 5 represent most to least healthy plants. Plants 3 and 4 show symptoms of yellowing, wilting (plant 4), die-back and stunted growth after re-sprouting (plant 5).

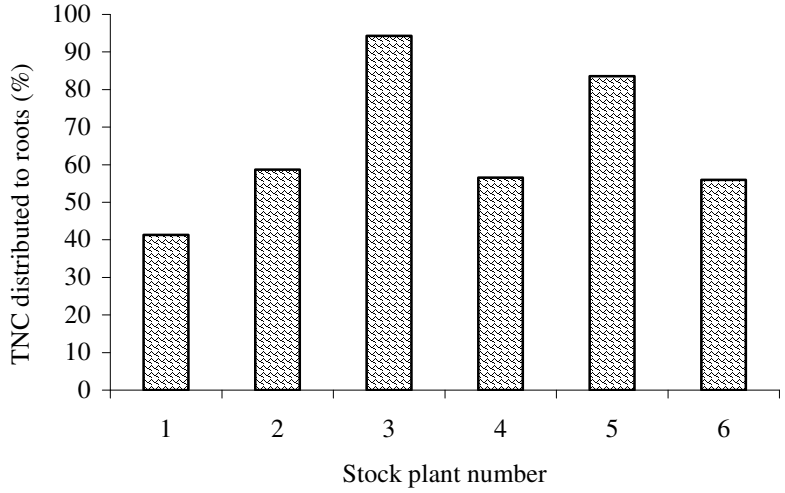

Figure 3.11: TNC allocated to roots of *C. odorata* stock plants at Cedara. Plants 1 to 6 are in order of decreasing health as per visual assessment.

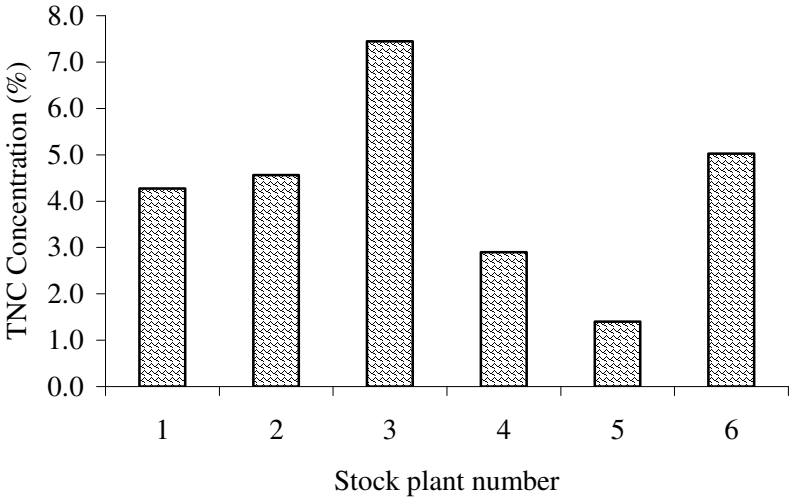

Figure 3.12: TNC concentration of unhealthy *C. odorata* stock plants at Cedara. Plants 1 to 6 are in order of decreasing health as per visual assessment.

3.3 Leaf nutrient analysis of healthy versus unhealthy *C. odorata* stock plants

There was a reduction in all macronutrients (Table 3.2), especially phosphorus (P) and potassium (K) in the unhealthy *C. odorata* stock plants, which also had approximately 40 % less manganese (Mn) than healthy plants. Anthocyanin pigments were also observed on leaves of unhealthy stock plants and this may be attributable to P deficiency. P was 30 % lower in leaves of unhealthy plants compared with healthy plants, indicating that the unhealthy plants were experiencing stress.

Table 3.2: Leaf nutrient content of *C. odorata* stock plants at Cedara.

Site	N %	P %	K %	Mn (mg/Kg)
Healthy	2.61 $\pm$ 0.18	1.30 $\pm$ 0.12	2.10 $\pm$ 0.76	459 $\pm$ 120
Unhealthy	2.28 $\pm$ 0.15	0.36 $\pm$ 0.10	1.44 $\pm$ 0.59	197 $\pm$ 86.27

3.4 Trial 2 (autumn-winter)

Trial 2 was run only to confirm observations of Trial 1 and since no die-back occurred under experimental conditions, no measurements were taken on Trial 2. Plants that established on elevated grids in Trial 2 in shade-houses at Cedara and Durban remained healthy during the trial and also after the growth period was extended for a further 12 months. Results from this trial also indicated that plant age, pruning and movement between the tunnel and shade-house micro-habitats (seasonal effects) were not contributing factors to plant mortality.

3.5 Fungicide trial (pathogen activity) and fertiliser treatments

Results from the fungicide trial indicated that pathogens (*Pythium*, *Fusarium* and *Trichoderma*) were present in the soil of healthy and unhealthy stock plants and the roots of healthy plants. These three pathogens together with *Rhizoctonia* were found in the roots of unhealthy stock plants. The pathogens were not identified to species level. The difference between healthy and unhealthy plants may have been related to the quantity of pathogens, but this was unknown because the pathogens were not quantified. Roots and soil are always exposed to micro-organisms, however, these may become pathogenic in soil that remains wet and becomes waterlogged due to inadequate drainage which reduces soil aeration, increases pathogen activity and affects growth of new roots. Plant mortality tends to be exacerbated by

secondary infection of roots under these conditions. Application of Previcur N and Benlate fungicide enables plant roots to function properly by becoming more resistant to attack by pathogens and hence enable plants to grow normally. Plants were visually compared at the end of the trial and amongst the treated and untreated groups, Burgess (5:1:5) slow-release seems to be more suitable for growth of potted *Chromolaena odorata* than Hortichem (3:1:3) quick-release (Table 3.3).

Table 3.3: Number of plants lost to mortality and proportion of plant die-back per fertiliser treatment (four plants in each group).

Group number	Fertiliser ratio	Fungicide applied?	Number of plants that died-back	Proportion of leaf & stem die-back
1a	5:1:5	Yes (treated)	0	0 %
1b	5:1:5	No (control)	2 (partial die-back)	2 at 25 %
2a	3:1:3	Yes (treated)	4 (partial die-back)	3 at 35 % 1 at 45 %
2b	3:1:3	No (control)	2 (complete die-back) 1 (partial die-back)	2 at 100 % 1 at 45 %

Growth, development and survival of *C. odorata* plants grown on elevated grids in Trials 1 and 2 at Cedara seem to have been influenced by adequate drainage and spacing, hence a spacing and drainage trial was set up to investigate this further. Results were related to growth of stock plants at Cedara which were placed on the ground and generally overcrowded due to limited space in the tunnel and shade-house.

3.6 Spacing and drainage trial

Visual comparison of plant growth in this trial indicated that plants placed (apart) on elevated grids were taller and more branched with no die-back, whilst those placed (close) together on grids were shorter and less branched, and one plant died-back (Table 3.4). Plants placed on the ground (apart) were tall and branched (only one plant died back), whilst those placed (close) were tall and less branched (two plants died back). Growth of *C. odorata* plants placed (close)

together seem to be affected by mutual shading which is a consequence of overcrowding and growth of potted plants placed on the ground is further affected by inadequate drainage.

Table 3.4: Maximum stem length and number of plants lost to mortality (five plants in each group).

Place	Spacing	Plant height (cm)	Number of plants that died	Proportion of leaf & stem die-back
Elevated grids	Apart	145	0	0 %
	Close	65	1	100 %
Ground	Apart	155	1	100 %
	Close	150	2	100 %

The effects of drainage (aeration of soil and airnipping of roots) on plant growth is further indicated by Fig. 3.13, which shows a considerable reduction in total number of stock plants lost to mortality at Cedara between August 2004 and August 2006. The first bar represents the number of plants lost to mortality during August 2003-2004, when potted plants were mainly placed on the ground. *C. odorata* stock plants were placed on newly manufactured elevated grids (3 m x 0.72 m x 0.26 m) since August 2004 and by August 2006 the number of plants lost to mortality had been significantly reduced. Fig. 3.13 gives absolute data (number of deaths) of 600 plants (the number of plants that had died-back and the number of plants that were healthy was recorded on a monthly basis for a group of 50 plants over a twelve month period). This indicates that the high mortality of potted *C. odorata* plants seems to be associated with inadequate drainage when the soil of plants placed on the ground becomes saturated with water (waterlogged), especially during cool weather at Cedara, when evaporative demands on the plants, and consequently transpiration, are reduced.

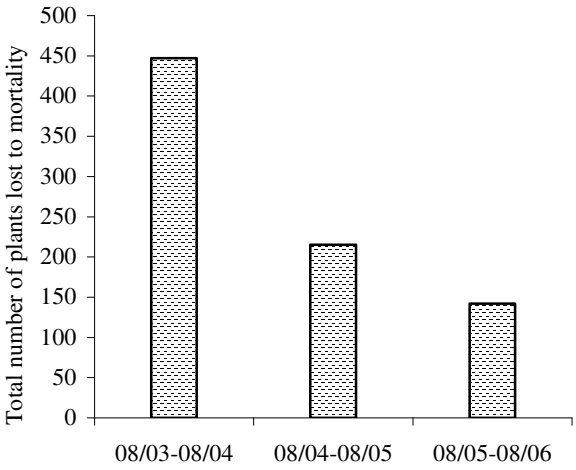

Figure 3.13: Reduction in mortality rate of small and medium potted *C. odorata* plants at Cedara (from stock plant maintenance schedule).

Potted *C. odorata* plants grown on the ground under natural conditions (Durban-Op) in Trials 1 and 2 remained healthy and were unaffected by inadequate drainage. The environmental conditions in Durban may have been more suitable for growth of *C. odorata* plants than the Cedara environment (tunnel or shade-house). It seems that a combination of environmental factors are influencing growth of plants at Cedara and with any additional stresses, e.g. inadequate drainage, plants may be unable to alleviate this stress as efficiently as they would, if grown in a more favourable environment, i.e. Durban.

3.7 Stem structure and root anatomy

Lateral (secondary) roots and hypertrophied lenticels (Fig. 3.14 a) were observed to be more developed mainly on the stem bases of unhealthy *Chromolaena odorata* plants that had reached a critical stage of die-back or had died back and re-sprouted, compared to the healthy plants. New lateral roots were easily distinguished from the main root system because these were larger in diameter, cream in colour and unbranched (Fig. 3.14 b). Aerenchyma is an interconnected system of air spaces in the cortex which develops in plant roots exposed to oxygen deficient soil. The microscopical study of *C. odorata* lateral roots indicated that although the cortical cells had a cubic packing arrangement i.e. each cell having four near neighbours resulting in a concave quadrangulus intercellular air space (Fig. 3.15), aerenchyma

was absent. The inability of roots of *C. odorata* to form aerenchyma under waterlogged conditions may explain the mortality of potted stock plants.

Figure 3.14 a, b: Stem bases of unhealthy *C. odorata* plants.

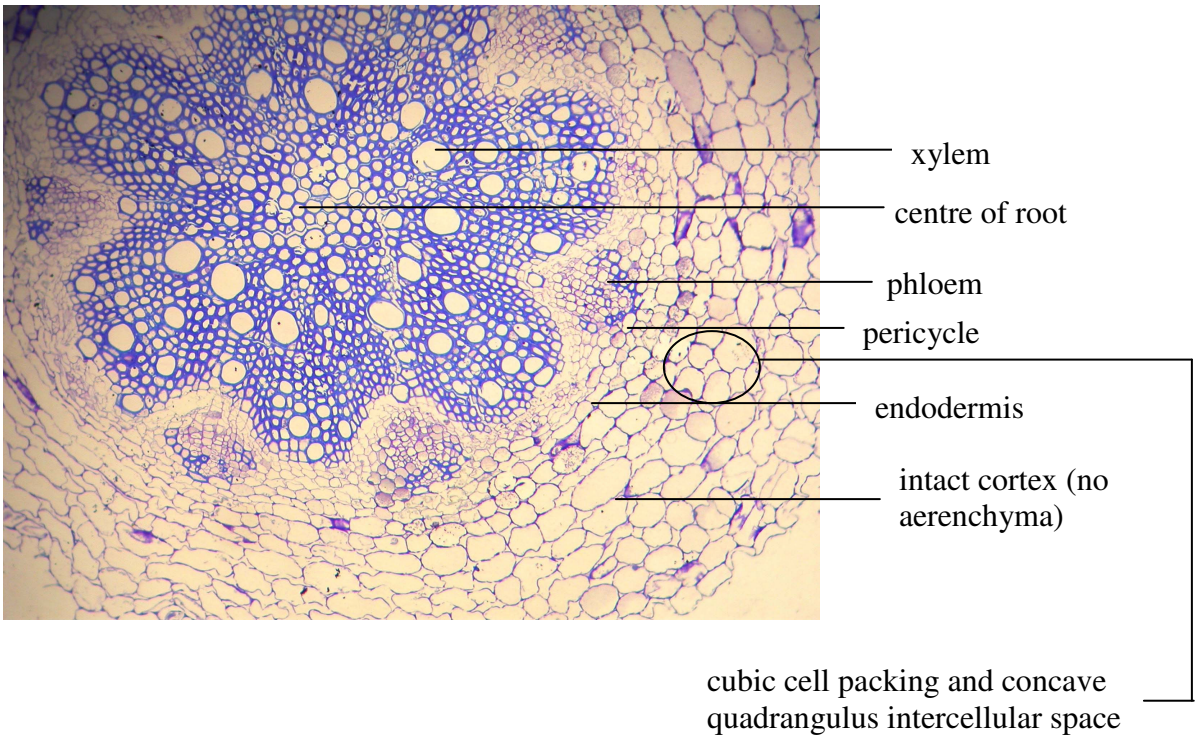

Figure 3.15: Cross section of unhealthy *Chromolaena odorata* lateral root (x100).

Although waterlogging seems to be the causative factor in mortality of potted *Chromolaena odorata* plants at Cedara, as a final step, it was important to investigate the intensity of waterlogging in relation to mortality, hence redox measurements were conducted on a small group of *C. odorata* plants at Cedara. Due to time constraints, an indepth investigation was not conducted.

3.8 Intensity of waterlogging and redox potential of the soil of *C. odorata* stock plants

The intensity of waterlogging relates to the redox potential of the soil which is an important factor for plant growth. The redox potential (Table 3.5) of soil of unhealthy and healthy *Chromolaena odorata* stock plants indicated that E_h of both groups of plants were greater than 400 mV. According to Fig. 3.16, a redox potential of below +350 mV is indicative of the absence of oxygen (O_2) from the soil, so the soil of both unhealthy and healthy plants was still aerobic; however, results from an un-paired t-test indicated a marginally significant difference ($p = 0.057$) in redox potentials of soils of unhealthy plants (427 mV) and healthy plants (438 mV). Although the redox potentials in Table 3.5 seem to indicate saturated soil, this may become short-term waterlogged if drainage continues to be affected. O_2 could become displaced from the soil and redox potentials would decline. The mortality symptoms (leaf chlorosis, leaf wilting followed by leaf and stem die-back) of unhealthy *C. odorata* plants seems to be similar to the response of plants in O_2 deficient conditions, which occurs in soil that remains wet and becomes waterlogged with time.

Table 3.5: Redox potential (E_h) of soil of healthy versus unhealthy *C. odorata* stock plants.

Plant number	E_h (mV) of	
	Unhealthy plants	Healthy plants
1	434 $\pm$ 9	439 $\pm$ 5
2	427 $\pm$ 10	435 $\pm$ 2
3	421 $\pm$ 8	441 $\pm$ 7
Averages	427 $\pm$ 7	438 $\pm$ 3

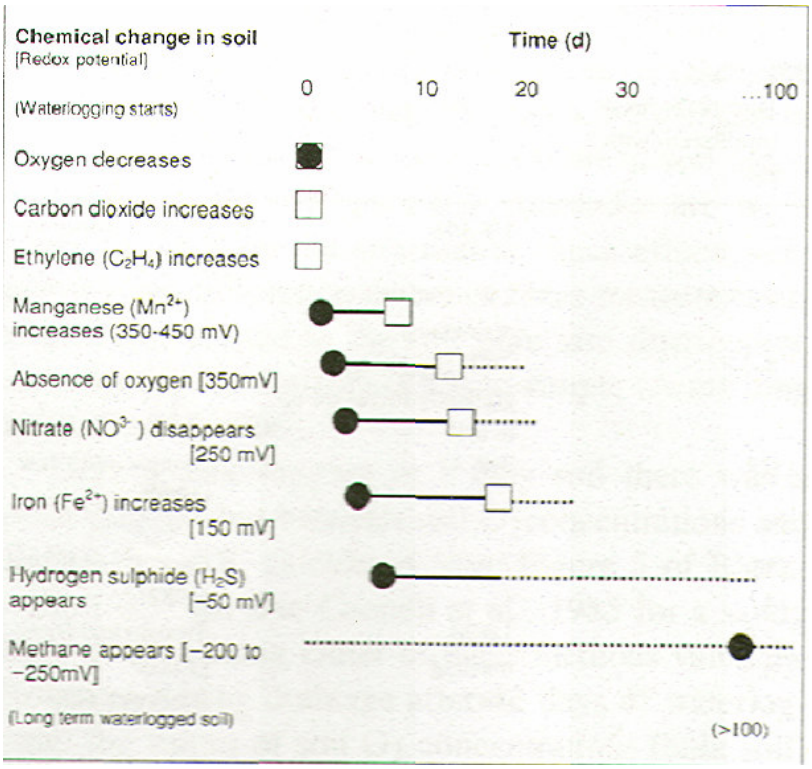

Figure 3.16: Changes in soil chemistry during waterlogging (after Setter & Waters, 2003).

CHAPTER 4 DISCUSSION

4.1 Influence of the environment on growth of potted *Chromolaena odorata* plants

Cedara is not the most suitable place to grow *C. odorata* and hence one of the greatest challenges for the *Chromolaena odorata* biocontrol programme for the past ten years has been the difficulty in growing and maintaining healthy, non-stressed potted *C. odorata* plants for research. In order to determine the effects of the environment on growth and development of potted *C. odorata* and possibly the cause of mortality, plants were grown at Cedara (one site) and in Durban (two sites). Plants that established at all three sites in Trial 1 during the winter-spring period (2003) survived, remained healthy and did not display any visual symptoms associated with mortality. Plants that established during the (cooler) autumn-winter period (2004) in Trial 2 (set up to confirm observations of Trial 1), also did not display any visual symptoms associated with mortality. However, results indicated that plants grown at Cedara were consistently under environmental stress compared with those grown in Durban.

4.1.1 Transpiration rates, VPD and TNC reserves

Under conditions of low VPD, transpiration rates are reduced but when VPD increases stomatal closure may occur in plants in order to prevent high rates of transpiration (Hartmann *et al*., 1997; Kozlowski & Pallardy, 2002). However, this is dependent on other factors e.g. available soil water, root size relative to leaf area and also on how big the increase in VPD is. Plants with small root systems have lower rates of respiration, water and nutrient absorption and ultimately reduced transpiration rates (Patterson, 1995), presumably due to lowered stomatal conductance. Plants in the tunnel at Cedara were exposed to high vapour pressure deficits but had low transpiration rates, which seems to be a consequence of reduced stomatal conductance (not measured in this study). Reduced stomatal conductance is in turn a consequence of reduced ability to absorb water, as a result of reduced allocation of TNC to the roots. Hence plants grown in the tunnel at Cedara were unable to meet the high evaporative demand of the environment resulting in stress. This stress was also indicated by the lower fluorescence ratio (F_v/F_m) of plants grown at Cedara compared with the plants grown in Durban.

This stress may consequently limit the ability of the plants to utilise photosynthates (Joseph & Yelenosky, 1991), resulting in a change in relative growth rates of the plant parts; leaf growth was more than three times higher in Cedara-grown plants compared with Durban-grown plants and this is also indicated by the low root/shoot ratio of Cedara-grown plants. This is attributable to an accumulation of carbohydrates in the leaves and limited distribution to the roots of plants grown in the tunnel at Cedara compared with plants grown in Durban, thereby reducing the availability of carbohydrates for production of new tissue, maintenance of reserves and tolerance of stress in Cedara-grown plants. Plants grown in Trials 1 and 2 may have had sufficient TNC reserves to alleviate effects of environmental stress in the tunnel and shade-house micro-habitats at Cedara, and this may have allowed their survival. Although the attempt to relate quantity of root TNC reserves with mortality symptoms of unhealthy *C. odorata* stock plants was unsuccessful, the TNC concentration across the whole plant was lower in two of the three unhealthy plants compared with the three healthy plants.

4.1.2 Effects of spacing and drainage on plant growth

When resources for growth are limited in a particular environment or under certain environmental conditions, plants, being phenotypically plastic, can adapt by adjustments of their morphology and physiology in order to increase the ability for acquisition of a particular resource (Patterson, 1995; Ryser and Eek, 2000). Plants placed (close to each other) on the ground in the spacing and drainage trial grew taller than those placed (close to each other) on elevated grids but both groups of plants were less branched than those placed (apart) on elevated grids and on the ground. Plants growing close together may have shaded each other while spacing may have allowed the other two groups of plants to produce more lateral branches as a result of improved light penetration through the canopy. According to Mummigatti *et al*. (1998), *C. odorata* plants that are exposed to less light develop slender and tall stems due to competition for light. Spacing *C. odorata* plants on elevated grids at Cedara is important because this prevents mutual shading of plants, which is density-dependent.

Among the two groups of plants placed on elevated grids at Cedara, one plant from the group of five placed (close to each other) died back whilst none died back from the group of five plants placed (apart). Additionally, of the two groups of plants placed on the ground, two

plants from the group of five placed (close to each other) together died back, whilst only one plant died back from the group of five placed (apart). Although this was a small group of *C. odorata* plants, drainage and to a lesser extent, spacing seem to have influenced growth and development (improving plant health), and this may be attributable to lack of mortality of plants grown in Trials 1 and 2.

Drainage would appear to be important for plant growth at Cedara as, over the past two years, since stock plants have been placed on elevated grids, the mortality rate has been significantly reduced. Growing potted *C. odorata* plants on elevated grids at Cedara allows for adequate drainage, which in turn improves aeration of the soil and roots are air-nipped. Decreased plant mortality may also be attributed to use of plants grown in Durban which may have adequate TNC reserves to withstand environmental stress at Cedara. However, even if these 'Durban-grown' stock plants are placed on the ground at Cedara for a period of time, an additional stress (oxygen deficiency) in the soil as a result of inadequate drainage (waterlogging) may cause die-back.

4.2 Effects of waterlogging on plant growth

C. odorata, like most rapidly growing plants, require sufficient O_2 in the soil for growth and development. Roots utilise oxygen (O_2) from the soil for aerobic respiration which provides energy to roots for metabolic processes (e.g. nutrient absorption and carbohydrate metabolism, i.e. replenishing TNC reserves) but when soil becomes saturated with water, the transfer of O_2 from air into the soil is blocked. O_2 deficiency results from an imbalance of slow diffusion of gases through water-filled pores which is 10 000 times slower than through air-filled pores (Armstrong, 1979), and the potential rate of O_2 consumption (respiration) by roots and aerobic micro-organisms, which is also dependent on soil temperature (Drew & Lynch, 1980; Jackson & Drew, 1984; Setter & Belford, 1990; Visser *et al*., 2003). The lack of O_2 in the soil affects plant growth and development (Setter & Belford, 1990).

The resultant anaerobic soil conditions and pathogens result in the formation of toxic substances (CO_2, ethylene, H_2S) in the saturated soil (Mengel, 1982; Kozlowski, 1984; Webb & Fletcher, 1996). Low O_2 concentrations reduce energy levels within roots that can be

utilised for active uptake of ions and also lowers the availability of some nutrients e.g. Fe, P and Mn (Levitt, 1980; Kozlowski & Pallardy, 1984; Gupta, 2005). The inability to supply adequate quantity of nutrients and water for plant growth, due to decreased root hydraulic conductance, results in leaf dehydration (wilted leaves) and subsequently stomatal closure in waterlogged plants (Jackson & Hall 1987; Jackson *et al*., 2003). Leaf wilting was one of the symptoms observed in unhealthy *C. odorata* plants.

Stomatal closure leads to low stomatal conductance (g_s) affecting CO_2 assimilation rates, and low root metabolism influencing hydraulic conductivity and TNC translocation (or source/sink relationships) resulting in reduced root growth and eventually root mortality due to carbohydrate starvation (Kramer & Kozlowski, 1979; Jackson & Drew, 1984; Kozlowski, 1984; Kozlowski, 1997; Malik *et al*., 2001; Smethurst & Shabala, 2003). Pathogenic micro-organisms also affect malfunctioning of roots and contribute to root and subsequently plant mortality. Since stock plants grown at Cedara are under frequent stress, the ability to resist attack by pathogens may be reduced. O_2 deficiency in waterlogged soil prevents TNC reserves from being completely oxidised, resulting in an accumulation of intermediate compounds. Hence the amount of energy released is reduced and may become inadequate to support plant growth, resulting in stunted plants (Kozlowski, 1991; Malik *et al*., 2001; Smethurst & Shabala, 2003) and this may explain why *C. odorata* stock plants do not attain normal growth once plants have re-sprouted after initial die-back.

4.2.1 Visual symptoms of mortality and redox potential of the soil of *C. odorata* plants

In waterlogging stress, symptoms appear on the leaves only after the roots have been affected and damaged (Kozlowski, 1984; Vartapetian & Jackson, 1997). In their study Smethurst & Shabala (2003) working on the effects of waterlogging on lucerne (*Medicago sativa*) observed chlorosis and wilting of leaves and eventually plant die-back, which are symptoms of plants grown in waterlogged soil (Treshow, 1970); although other stresses are likely to produce similar symptoms, these mortality symptoms were also displayed on unhealthy stock plants at

Cedara, as well as *C. odorata* plants growing in waterlogged soil in the field in Cannonbrae, along the south coast of KZN (C Zachariades, 2005, pers. comm.[1]).

Results from nutrient analysis showed that leaves of unhealthy *Chromolaena odorata* stock plants at Cedara had lower N, P and K levels compared with the healthy stock plants. Smethurst & Shabala (2003) observed reddening of stems of plants grown under waterlogged conditions, due to phosphorus deficiency (deficiency was inferred from symptoms), but in unhealthy *C. odorata* plants, phosphorus deficiency was mainly observed as red pigmentation on the leaves. When mobile elements such as nitrogen become limiting, the elements are translocated from old to young leaves and symptoms of chlorosis appear in older leaves whilst chlorosis of young leaves is a consequence of a deficiency of an immobile element, such as iron or calcium. Nitrogen and iron are components of chlorophyll molecule and a deficiency of either reduces photosynthesis and causes chlorosis (Kramer & Kozlowski, 1960). Nitrification generates available nitrate for plants and is dependent on O_2 (Blom *et al*., 1994) but in waterlogged soil, nitrogen becomes deficient after it has been converted into nitrite, a leachable and phytotoxic form (Vartapetian & Jackson, 1997).

With time waterlogged soil gradually loses some or most of its O_2, with a concomitant increase and accumulation of phytotoxins. Chemical changes depend on the intensity of waterlogging and are associated with the reduction and oxidation (redox) status of the soil environment (Setter & Waters, 2003). Although redox potentials indicated that the soil of unhealthy and healthy plants was aerobic at the time of measurement, statistical analysis of redox potentials indicated that there was a marginally significant difference between the two groups of plants. According to Handreck & Black (1994) waterlogged soil has a foul odour due to H_2S, and algal growth is observed on the soil surface; algal growth was observed on the surface of the soil supporting unhealthy *C. odorata* stock plants that were dying back. H_2S is produced at redox potential of – 50 mV (Setter & Waters, 2003) and although the measured values were not close to this number, nevertheless there was an odour associated with very wet soils.

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4.2.2 Morphological adaptations to waterlogging

Plants in waterlogged soil maintain metabolic processes in order to survive, but in order to continue growing, plants tend to develop aerenchyma in roots for facilitation of gas diffusion (Armstrong, 1979; Blom *et al*., 1994; Jackson & Armstrong, 1999). Aerenchyma is an interconnected system of gas-filled spaces (lacunae) which provides an internal aeration system for movement of gases from shoots to roots, thereby increasing root porosity (air space content) (Justin & Armstrong, 1987; Drew *et al*., 2000). This may allow survival and growth of roots of plants growing in oxygen-deficient soils (Setter & Belford, 1990; Bacanamwo & Purcell, 1999; Setter & Waters, 2003; Visser *et al*., 2003).

Roots that tolerate waterlogging have different physiological characteristics which enable growth and survival during periods of anaerobiosis with less injury than occurs in sensitive root systems (Kramer & Kozlowski, 1960). Anaerobic conditions mainly affect growth of waterlogging-sensitive or -intolerant plants (Kozlowski, 1984). Plants of some species e.g. wheat are waterlogging-intolerant but develop aerenchyma and survive in waterlogged soil, but for a short period only, and eventually do die-back (Voesenek *et al*., 1999). Flood-tolerant plants develop aerenchyma in new adventitious or lateral (secondary) roots, which also compensates physiologically for decay of original roots under anaerobiosis (Justin & Armstrong, 1987; Kozlowski, 2002). Hypertrophied lenticels develop on the below-ground stem (stem base) to allow gaseous exchange between internal tissues and the atmosphere thereby increasing O_2 supply to plant roots when pore spaces in the soil becomes saturated with water (Kozlowski, 1997; Bacanamwo & Purcell, 1999).

No hypertrophied lenticels were observed in *Chromolaena odorata* plants in Trials 1 or 2, however, these were observed mainly in unhealthy *C. odorata* stock plants, indicating reduced O_2 levels in the soil. Although *C. odorata* developed this morphological adaptation, there was an absence of aerenchyma in the root cortex which indicates that *C. odorata* is not genetically programmed to produce this adaptation to aid survival in waterlogged soil. Lack of aerenchyma and low rates of internal O_2 diffusion restrict root growth in anaerobic soil and tend to contribute to plant mortality of *Brassica napus*, a waterlogging-intolerant species (Voesenek *et al*., 1999) and this may also explain mortality of *C. odorata* plants in

waterlogged soil. According to Justin & Armstrong (1987) several species belonging to the Asteraceae (Compositae) and few other species are unable to develop aerenchyma and hence *C. odorata* can be classified as waterlogging-intolerant.

4.3 Conclusion and Recommendations

Plants grown in Durban were exposed to a less stressful environment than those grown in the micro-habitats at Cedara, which may explain the survival of plants grown on the ground under natural conditions in Durban. However, rotation of potted plants to prevent rooting into the ground is time consuming and growing potted *C. odorata* plants on elevated grids in Durban is a better option because roots get air-nipped and soil aeration is increased. Since *C. odorata* cannot survive and grow in waterlogged soil due to its inability to develop aerenchyma, growing potted plants on elevated grids allows for adequate drainage, thereby reducing saturation of the soil during times of over-watering when evaporative demands on the plants, and consequently transpiration, are reduced. Soil aeration is also improved and roots are air-nipped instead of growing into the ground, thereby limiting stress on the plants when these are transported between the tunnel and shade-house micro-habitats.

Although this set up has improved the general quality of stock plants at Cedara, a few plants are still being lost to mortality due to additional factors relating to plant maintenance (e.g. overcrowding due to limited space resulting in mutual shading, or wilted plants due to malfunctioning of irrigation drippers). Therefore it is recommended that the soil water content of potted *C. odorata* plants grown at Cedara be monitored closely. Probes can be placed into the soil in order to measure the soil water content or potential and linked to a system which regulates the quantity of water per pot in relation to plant size and to meet the evaporative demands. This may be the best long-term solution to prevent loss of *C. odorata* plants to mortality, although it is extremely expensive.

Presently the only feasible option is to grow potted *C. odorata* plants on elevated grids at Cedara in the different micro-habitats and to treat plants with Previcur N and benlate drench as required. Adequate plant maintenance and regular monitoring of the growth of potted *C. odorata* is essential towards ensuring a continuous supply of healthy plants for biological

control research. An alternative solution would be to grow potted *C. odorata* plants in Durban and to keep stock plants at Cedara for a short period only, but currently this is not feasible.

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