ALLELOPATHIC POTENTIAL OF THE ALIEN INVADER WEED *CAMPULOCLINIUM MACROCEPHALUM* (Less) D.C.

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Campuloclinium macrocephalum, Reitvlei Nature Reserve, Pretoria 2005

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Declaration

I, Gemma Michelle Dixon, hereby declare that this dissertation for the degree M Inst Agrar Agronomy at the Department of Plant Production and Soil Science from the University of Pretoria is my own work and has never been submitted by myself at any other university.

The research work reported is the result of my own investigation, except where acknowledged.

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ABSTRACT

It has been suggested that the Category 1 weed, *Campuloclinium macrocephalum* (Less) D.C has allelopathic potential, which would, at least partially, explain its apparent success as an alien plant in South Africa.

Studies were done on the plant's root, stems and leaves to determine where the strongest allelopathic potential can be found. Once it was determined that the leaves held the strongest potential, bioassay studies were conducted on lettuce (*Lactuca sativa*), *Eragrostis tef*, *Eragrostis curvula* and *Panicum maximum* with positive results found for *C. macrocephalum's* allelopathic potential.

Electron microscopy was performed to determine whether allelopathic substances originate and/or are stored on the surfaces of the leaf. Positive results proved that there are possible sources of allelochemicals on both adaxial and abaxial surfaces of young and mature leaves. A dipping experiment involving dichloromethane then followed to determine the solubility of the contents of the glands found on the leaf surfaces.

It can be deduced from results of all of the experiments performed that *C. macrocephalum* is potentially allelopathic to dicotyledonous species and to grasses. Structures found on the leaves of the plant could possibly contain the allelochemicals used by the plant to ensure its successful invasive growth habits in South Africa. The allelopathic effects that this weed will have on desirable species should be considered within the broader context of its ability to interfere with those species. In this regard its competitive ability should also be studied. *Campuloclinium macrocephalum* is fast invading susceptible areas of South Africa; if continuous research on control and eradication of this plant is not carried out soon, the country could suffer grave economic losses.

INTRODUCTION

Due to globalisation, invasive alien species are becoming a threat to many countries' natural biodiversity, therefore, studies on the strategies employed by these species for displacement of other species is a necessity to control them and to keep natural biodiversity high.

The alien invader weed *Campuloclinium macrocephalum* (Less) D.C., more commonly known as the pompom weed, is an emerging Category 1 weed in South Africa. According to the Conservation of Agricultural Resources Act, 1983; Act No. 43 of 1983, a Category 1 weed implies: "These are prohibited plants that will no longer be tolerated, neither in rural nor urban areas, except with the written permission of the executive officer or in an approved biocontrol reserve. These plants may no longer be planted or propagated, and all trade in their seeds, cuttings or other propogative material is prohibited. They may not be transported or be allowed to disperse." *Campuloclinium macrocephalum* is becoming a major problem in South Africa as it invades savannahs, roadsides, grasslands and open woodlands leaving detrimental effects to the country's plant biodiversity in its wake. This Category 1 weed needs to be brought under control before it causes irreversible economic losses to South Africa's agricultural and planted grasslands.

Little literature is available on this alien invader plant, and research of the plants morphology, growth habits and allelopathic potential is required to determine the best methodology to control the weed.

Apart from competition between plants for natural resources, plants also interact among themselves and with other organisms by chemical means known as allelopathy. Allelopathy is defined as the introduction of a substance into the environment, which may have positive or negative effects on the surrounding plant community (Macías, Castellano, Oliva, Cross and Torres, 1997). A study of the allelopathic potential of *C. macrocephalum* needs to be made to determine the plant's invasive ability and impact on South African plant species. Allelochemicals (also representative of secondary metabolites)

can be found in several plant parts and are released in a number of ways including root exudation, leaching from stems and leaves, and during plant decomposition (Vyvyan, 2002; Eljarret and Barcellò, 2001). These released chemicals may directly or indirectly influence the surrounding plant community (Inderjit and Weiner, 2001). Allelochemical production is apparently related to plant stress; also, reactions to allelochemicals may be more severe when plants are under stress (Gupta, 2005). It has been suggested that allelochemicals could be used as natural herbicides and/or pesticides (Vyvyan, 2002). Planting allelopathic crops could be a direct way to combat weeds. Allelochemicals produced from mulching can suppress weed emergence (Singh, Batish and Kohli, 2003).

The purpose of this study is to promote the understanding of *C. macrocephalum's* allelopathic potential and its reliance on allelopathic interference for displacing natural plant biodiversity and/or succession in South African plant communities. Little research on determining adequate control measures for this alien invader necessitates the need to obtain more knowledge about the nature of this invasive plant specie.

The first aspect of this study was to determine the allelopathic potential of *C. macrocephalum*, specifically its ability to suppress early growth and development of the test species *Latuca sativa* (lettuce, cultivar Great Lakes). All major plant parts were used to determine the main site of allelopathic activity. Osmotic potential of all infusions were to be eliminated for proper allelopathic potential to be determined. Other test species such as: *Eragrostis curvula, Eragrostis tef* and *Panicum maximum* represent some of the grass species which inhabit South Africa's natural grasslands and savannahs which are being invaded by *C. macrocephalum*.

A great variety of structures may be found on the plant surface of a number of species. These structures may vary in size, shape, number of cells, location and function (Werker, 2000). The final aspect of study will be to determine the location of sites of possible allelochemical storage on or in the plant. This investigation will help to gain knowledge of *C. macrocephalum's* anatomy and

possible source of the plants allelopathic potential. This information will assist in decisions of what herbicide to use to control this plant.

CHAPTER 1
LITERATURE REVIEW

1.1 *Campuloclinium macrocephalum* (Less) D.C.

1.1.1 Introduction

Campuloclinium macrocephalum, more commonly known in South Africa as pompom weed, is an emerging weed in the country. It is currently invading roadsides, grasslands, open woodlands and savannahs where it has detrimental effects on the growth and well-being of the natural vegetation and animal life.

South Africa has declared *C. macrocephalum* a Category 1 weed according to the Conservation of Agriculture Act, Act 43 of 1983, and Amended in March 2001. In terms of Regulation 15A, Category 1 weeds are prohibited plants that will no longer be tolerated in rural and urban areas in South Africa. The only circumstances under which these plants are allowed to be grown are when written permission is obtained from the executive officer, or if they are grown in an approved bio-control reserve. No planting or propagation of Category 1 weeds is allowed. All trade in plant seeds and/or propagative material is prohibited, and Category 1 weeds may not be transported or allowed to be dispersed (Klein, 2002).

Campuloclinium macrocephalum originated in the Tropical Americas, in Argentina and Honduras, as well as in Mexico. The plant was brought to South Africa as an ornamental for gardens and nurseries. Its first sighting in South Africa was in Johannesburg in 1962 (Henderson, Goodall and Klein, 2003).

There are six prominent characteristics of *C. macrocephalum* that cause it to be problematic (Klein, 2002):

- Prolific seed production.
- The plant can establish itself in disturbed and denuded areas.
- The dense rosette formation of leaves at the base of stems prevents native plant germination and growth.

- Rhizomes can spread laterally under native vegetation, increasing growth of *C. macrocephalum* and diminishing the native species in the area.
- Rhizomes store nutrients which help to increase the recovery rate after winter damage to above-ground parts of the plant.
- Nodes on the rhizomes can give rise to flowering stalks, thus increasing efficacy of the plant's overall growth.

Campuloclinium macrocephalum is fast becoming a major problem in significant tracts of vegetation in South Africa. Measures need to be taken to control this plant so that it does not take over and destroy the natural biodiversity of the country. Drastic planning, strategies and regulations should be put in place to prevent this weed from becoming an even greater menace than is currently the case.

Due to scant scientific literature found on this plant, more research needs to be conducted to assess how to best control the plant, be it with herbicides or by natural measures.

1.1.2 Botanical description of *Campuloclinium macrocephalum*

1.1.2.1 Taxonomy

Campuloclinium macrocephalum (Pompom weed) was formerly known as *Eupatorium macrocephalum* (Triffid weed).

Division:	Magnoliophyta magnoliophytina
Class:	Magnoliatae Asterdae Asteranae
Order:	Asterales
Family:	Asteraceae Dumort
Genus:	Eupatorium
Species:	*Eupatorium macrocephalum*

(www.biologie.uni-ulm.de/systax/browse/index.html, 2006)

The following description of *C. macrocephalum*, unless otherwise stated, has been adapted from Henderson (2001).

Campuloclinium macrocephalum is an erect perennial herb; the stems are green to purplish in colour, can grow up to 1.3 m tall and will die back annually to a root crown. The roots contain rhizomes and are thickened and tuber-like. The stems and leaves are covered with rough, bristly hairs. The leaves are light green in colour, lanceolate elliptic with serrated margins and up to 80 mm long x 20 mm wide. The leaves become smaller and more spaced out towards the top of the stem. Flowers are fluffy and pink in colour. Flowers are surrounded by purple bracts. Flowers occur in compact terminal heads that are 15 mm long x 25 mm wide. Flowering occurs from December to March. The fruits are brown, one-seeded achenes that are approximately 5 mm long and ringed with a tuft of bristles.

1.1.2.2 Distinguishing characteristics of the tribe Eupatorieae

Unless otherwise stated, the following information has been taken from Retief (2002). The tribe Eupatorieae is distinguished from all other tribes by these characteristics:

- Style branches with conspicuous pappilose appendages;
- Capitula discoid;
- Florets are bisexual;
- Florets are white, blue, mauve, purplish pink (as in the case of *C. macrocephalum*) or purple in colour;
- Corolla with five (four) relatively short, broad apical lobes;
- Mature achenes (cypsellas) are black in colour.

1.1.2.3 Morphological characteristics of taxonomic importance

The morphological characteristics stated here have been taken from Retief, 2002.
Habit: Members of the Eupatorieae family are herbs or shrubs, erect, twining or scrambling plants. *C. macrocephalum* is a perennial herb, usually suffrutescent.
Leaf: The leaves are sessile or with short petioles and the blades are more or less ovate.
Florets: The bract is covered with sessile glands and simple multicellular hairs. The receptacle is hemispherical to conical.
Pappus: Pappi of Eupatorieae plants display various features that can be used to distinguish the species. *Campuloclinium, Chromolaena, Mikania* and *Stomatanthes* are characterized by capillary, scabrid, barbellate bristles.
Achene: Achenes are black with a carbonized layer in the achene wall. Achenes are three to five angled glandular trichomes in *C. macrocephalum*.

1.1.3 Distribution and habitat

1.1.3.1 Current and predicted infestation

The earliest record of the plant in Johannesburg, Gauteng, was in 1962 (Henderson *et al.*, 2003). *Campuloclinium macrocephalum* grows well in disturbed areas such as roadsides. From roadsides it moves into grasslands, open wetlands and savannah, gradually overtaking the natural vegetation it invades. The plant's invasion is most prominent in parts of Gauteng province, with areas of northern KwaZulu-Natal and western Mpumalanga also being infected (Figure 1.1). Predicted invasion areas are Limpopo province, Mpumalanga, KwaZulu-Natal, Eastern Cape and the Free State (Henderson 2001, Henderson *et al.*, 2003)

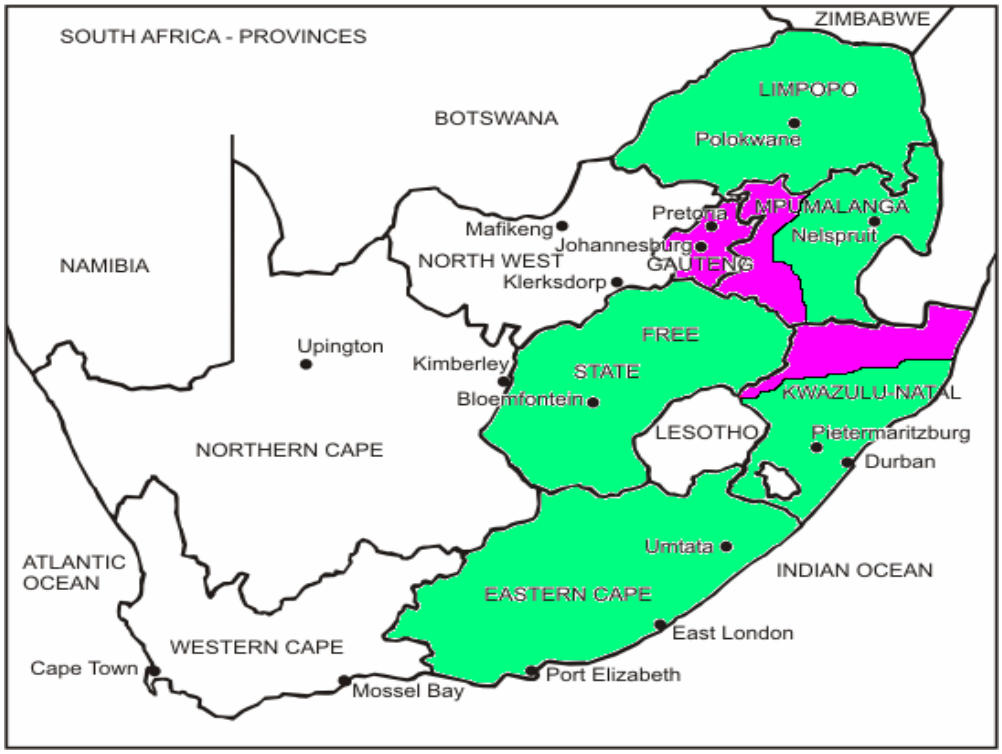


Figure 1.1 Map of current and predicted infestation of *Campuloclinium macrocephalum* in South Africa, where purple areas show current infestation and green areas show predicted infestation of the plant
(Adapted from http://www.afrilux.co.za/quickies/South_Africa.htm)

Based on observations made during personal travels since October 2005, *C. macrocephalum* infestation has been noted in the following places:

KwaZulu-Natal: Areas adjacent to the N3 toll route and R103 from Gauteng to Durban is apparently free from *C. macrocephalum*. There have been sightings of the weed in the Hilton area of Pietermaritzburg, but the population there has apparently been exterminated. The only visible population in KZN now is one on the "Midlands Meander" (road R103) in the Lidgerton area.

Mpumalanga: *Campuloclinium macrocephalum* can be seen along the side of the N4 highway from Witbank to Middleburg. The weed was also spotted on the R36 from Badplaas to Barberton. The numbers dwindled to nothing outside of Barberton traveling northbound to Malelane. There have been no sightings of the plant in areas surrounding or inside the Kruger National Park. Observations on this

area were made in February 2006. By December 2006 populations of the plant had spread to White River, which will pose a threat to the Kruger National Park as its further spread will probably be in that direction.

Gauteng: The Tshwane and Johannesburg metropoles are heavily infested with *C. macrocephalum*. The East Rand of Johannesburg (roadside of the R21/R24 from Pretoria to Boksburg and N12 towards Witbank) have also provided some sightings. Springs is apparently free of *C. macrocephalum* plants. These observations were made from November 2005 to March 2006. From The Fourways area (north of Johannesburg) on the N1 south to the Vaal River there were no sightings of pompoms. Going southwest on the N14 from Pretoria to Krugersdorp there have been sightings up to the Valhalla area, beyond which the plants seem to dwindle away in number. No *C. macrocephalum* plants have been recorded in the Krugersdorp district.

1.1.4 Control

Control and management of the plant should aim at maintaining a healthy and productive state of the invaded natural vegetation, as this will probably contribute to limiting *C. macrocephalum* incursions. A combination of methods may be required to uphold this statement. These may include conventional control methods combined with agro-pastoral practices such as mowing, burning and minimum tillage with grass over-seeding (Henderson *et al.*, 2003).

1.1.4.1 Chemical control

At the moment there is only one registered herbicide (Trade name: Brush-Off with the active ingredient metsulfuron-methyl) to control *C. macrocephalum,* but chemicals with the active ingredients 2,4-D and picloram have given positive results in controlling the plant (Personal Communication, July 2006).[1]

The recommended rate of Brush-Off is 25 g of granules per 100 L of water (Neser,

[1] Personal Communications from group discussions at the 20th South African Weed Science Society Congress – July 2006.

2007). By spraying only the leaves of *C. macrocephalum* to the point where they are shining but not dripping, will minimize damage to desirable plants. The herbicide should not be sprayed in windy conditions, when temperatures exceed 28 °C, or when there is dew on the leaves or when rains are likely to occur within two hours of spraying. Annual follow-up spraying is essential due to about 20% of the weed population surviving as seeds in the soil or as plants that recover from not receiving enough herbicide.

1.1.4.2 Mechanical control

Mechanical control of the plant can be effective if uprooting and burning is done before the flowering season – this prevents dispersal of seeds (Klein 2002). Gloves should be worn at all times while handling the plant as it can cause skin irritation. Removing the flowerhead stops seed dispersal, but vegetative reproduction will still occur. Thus in all mechanical control methods, follow up actions must be taken.

1.1.4.3 Biological control

The use of host-specific natural enemies in the form of biological control to reduce a population of *C. macrocephalum* has not yet been implemented. The planning stages for this process are still underway and it will probably take several years before any agents may be certified to be released (Henderson *et al.*, 2003).

Although no registered biological control agent has been released for *C. macrocephalum,* it has been noted to have been negatively affected by a rust fungus in some areas of the country (Neser, 2007). This rust fungus has been noted to be related to, but not the same as, two *Puccinia* species known to occur on *C. macrocephalum* plants in South America.

Visible symptoms of the fungus are yellowing of leaves near the stem base, followed by the protrusion of small brown pustules (protruding lesions) surrounded by a small pale area. Heavily infected leaves will die back and drop off the plant; the fungus will also weaken roots. Weakened plants and young seedlings around

sick plants may not survive the winter and/or possible competition effects of surrounding grasses in undisturbed veld (Neser, 2007).

1.2 Alien invasive plants

Globalization is increasing at an astounding rate. With expansion of the trade-based global economy and increased international travel, the introduction of exotic plant species into many countries is on the rise. These exotic species are called alien invader plants. Invader plants are those that can successfully inhabit and spread to new habitats without human intervention (Inderjit, 2004). Perfect examples of alien invader plants are: ragweed parthenium (*Parthenium hysterophorus*) which entered into India accidentally and is now one of the country's most noxious weeds (Singh *et al.,* 2003). Pompom weed (*Campuloclinium macrocephalum*) is another invader, which was introduced to South Africa as an ornamental plant and is currently rated a Category 1 weed in the country (Henderson *et al.*, 2003).

Invasive alien species are a great threat to the biological diversity of the world, thus the study of these invasions is a necessity to keep natural biodiversity high. Biological invasions in the form of alien species are now the second leading factor, after habitat destruction, in biodiversity loss and species endangerment (US Congress, 1993).

There are many advantages to studying alien invasive plants – these include the observation of genetic and ecological processes in real time, rather than observing them by the patterns they generate. Invasions by alien species provide insight into the large-scale and long-term processes in ecology, evolution and biogeography (Clout and de Poorter, 2005). They further contend that the impacts which alien invasive plants have on the biodiversity and ecosystem functioning of an area can be more complex than most impacts of agricultural weeds. Thus an international approach needs to be applied to this global phenomenon.

1.3 The study of allelopathy

Plant interference can be considered the total adverse effects one plant has on another specific plant in the community, and comprises both competition and allelopathy (Foy and Inderjit, 2001).

Whereas competition involves the disproportionate removal by plants of a growth factor (e.g. nutrients, light or water) from the environment, thereby causing detrimental effects on weaker competitors in the community; allelopathy involves the introduction of a chemical substance into the environment, which may have positive or negative effects on the plant community (Chon, Jang, Kim, Kim, Boo and Kim, 2005; Macias, Galindo, Molinillo and Cutler, 2004; Weidenheimer, 1996). Plants may interfere with their neighbours through direct resource competition or chemical competition, or by indirect use of chemicals to attract herbivores or insects that affect surrounding plants. These various mechanisms of interference may occur simultaneously, thus making it difficult to differentiate them experimentally (Weidenhamer, Hartnett and Romeo, 1989). There could be as many as 240 weed species that may interfere with plant communities through allelopathic mechanisms at any one time (Singh *et al*., 2003). It has been stated that the mere fact that dense colonies of pure stands of perennials occur, in itself implies allelopathy (Chou, Waller and Reinhardt, 1999).

Since competition is generally described as a process whereby plants compete for utilization of limiting resources, and allelopathy involves the secretion of chemicals into the surrounding environment, it becomes extremely difficult to separate the two interference mechanisms in the field (Weston and Duke, 2003). Although, knowledge of the molecular basis of allelopathy in plants will provide possibilities for breeding of crop cultivars that are more competitive against weeds, the usefulness of such applications will depend on the identification of allelopathic compounds, or related genes and their effects on the surrounding environment (Inderjit, 2004).

1.3.1 Definition and brief history

Molisch was the first to introduce the term “allelopathy” in 1937 (Weston and Duke, 2003). The word “allelopathy” is derived from the Greek words “Allen” meaning mutual and “Pathos” meaning harm, in other words the injurious effect of one plant on another. The term “allelochemical” is used to describe the chemicals produced to cause such interactions among plants and microbes (Gupta, 2005).

Allelopathy is a form of interference competition that can be defined as involving chemicals, generally toxic organic compounds, released from higher plants, which influence development, germination, establishment, growth, survival or fecundity of one or more other plant species in close proximity to the donor species. Allelopathy is generally an undirectional process (Inderjit, 2004; Van Andel, 2005).

The International Allelopathy Society prefers to define allelopathy as “any process involving secondary metabolites (allelochemicals) produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including the positive and negative effects” (Macías *et al*., 1997). The allelochemicals causing the effect may be directly phytotoxic or indirectly phytotoxic through mediation of the soil environment (Inderjit, 2004).

The concept of allelopathy has been cited in literature for over 2000 years (Weston and Duke, 2003). Ancient writings described growth of crops that “rob the soil of its nutrients” and “sicken the soil.” In 300 BC Theophrastus recorded observations of allelopathy. It wasn’t until 1832 that the first experiments on allelopathy were performed by DeCandolle. Interest in allelopathy only revived in the 20th century after the development of suitable techniques for extraction, bioassay and chemical isolation and identification.

1.3.2 Plant-plant interactions involving allelochemicals

Allelochemicals are found in several parts of plants and may be released into the surrounding environment in a number of ways. These include exudation from roots, leaching from stems and leaves (during precipitation) and from decomposing plant matter (Vyvyan, 2002; Eljarrat and Barcelò, 2001). The chemicals released may directly or indirectly influence community and vegetation structure (Inderjit and Weiner, 2001).

The effects of allelochemicals in the field could be due to a number of factors, according to Inderjit and Weiner (2001):

- Direct harmful effects of chemicals released from donor plants;
- Degraded or transformed products of released chemicals;
- Effect of released chemicals on physical, chemical or biological soil factors;
- Induction of release of biologically active chemicals by a third species.

Chemicals released by plants can affect abiotic and biotic components of the ecosystem (Inderjit and Weiner, 2001). Many chemical classes that display allelopathic activity include tannins, cyogenic glycosides, flavonoids and phenolic acids. However, the most allelopathic compounds all come from the same chemical family – the benzoxazinones. Rice (1984) and Monaco, Weller and Ashton (2002) classified allelopathic agents into 14 chemical categories. These are secondary compounds produced by the plants and are associated with the shikimic acid and acetate pathways. Most of the isolated allelochemicals have one or more rings with complicated structures.

Allelochemical production can be related to plant stress. Plant stresses seldom occur alone in nature, this is also the case with allelochemical stress – environmental factors affect allelochemical production, transformation and efficacy (Gupta, 2005). Reactions to allelochemicals may be more severe when plants are under stress. Allelochemical production will also increase under stressed conditions – for example, Inderjit and Weiner (2001) detected an increase in phenolic acid content in *Helianthus annuus* when nutrient stress was increased.

They also found that the addition of fertilisers, in some cases resulted in elimination of allelochemical inhibition. Depression of leaf water potential is a good indicator of allelochemical stress from frulic and p-coumaric acids (Gupta, 2005).

Microbial ecology in soil may be influenced by allelochemicals through their effects on soil microbes and plant pathogens (Einheillig, 1995). But microbial activity may also prevent allelochemicals from reaching phytotoxic levels in the soil ecosystem (Schmidt and Ley, 1999). Inderjit and Weiner (2001) state that soil ecological processes cause quantitative and qualitative variation in chemical activity in soil environments, but much more research needs to be done before we can truly understand the interactions of soil microbial ecology and allelochemical phytotoxicity. It has been suggested that allelochemicals in the soil do not necessarily need to be absorbed by plant roots, but rather that contact with roots of target species is sufficient to bring about growth inhibition in that plant (Foy and Inderjit, 2001).

It has been suggested by many sources (Eljarrat and Barcelò, 2001; Vyvyan, 2002; Weston and Duke, 2003) that allelochemicals could be used as natural herbicides and/or pesticides. This would be a great possibility once sufficient knowledge of the exact functioning of the allelochemical compounds and their natural cycle through the environment has been obtained. Decomposition of plant residues is a time-determined process, thus affecting phytotoxicity of an allelochemical. Residue phytotoxicity will decline with an increase in decomposition time (An, Johnson and Lovett, 2002). Progress is being made because the first natural herbicide produced, Bialophos, the precursor of the herbicide glufosinate, is on the market today with no adverse reports associated with it (Reinhardt, Khalil and Bezuidenhout, 1999).

1.3.3 Allelopathy and agriculture

Weeds are generally defined as unwanted plants growing in a certain location. These weeds compete with agricultural crops for resources (such as nutrients, water and light); they lower crop yields, which leads to financial losses for the farmer, and can contaminate the field with their seeds extending the weed problem to future growing seasons (Vyvyan, 2002; Singh *et al.*, 2003). Management of these weeds is crucial for the maintenance of agro-ecosystems; allelopathy of certain plants and crops could help to maintain a sustainable cropping system from the viewpoint of keeping weeds under control.

Planting crops that may have an allelopathic effect on other plants (by producing allelochemicals) is a direct way of using allelopathy to combat weeds. Crop selection must be made wisely so as to ensure that the allelochemicals produced do not harm the following crop to be planted (Reinhardt *et al.*, 1999). An example of a crop used for its allelopathic ability is *Sorghum* species in which the allelochemical sorgoleone is found (Singh *et al.*, 2003). Furthermore, the allelochemicals produced by some weeds may be extracted and purified to be used in the same manner as synthetic herbicides. Examples of phytotoxic chemicals are found in the weed *Parthenium hysterophorus* from which parthenin is obtained (Belz, Duke and Hurle, 2005), and also *Artemisia* species from which we obtain artemisin (Duke, Paul, Elsohly, Sturts and Duke, 1994).

By manipulating allelopathic rotational crops one can provide an effective means for weed control (Chou *et al.*, 1999). For example, a study was done using the crop rotation soybean – corn – wheat under a reduced or no-till system (Singh *et al.*, 2003). By using this rotation a significant decrease was found in the invasion and population of giant foxtail (*Setaria faberii* Herrm.) A significant reduction in herbicide input was also obtained.

There are many other ways in which allelopathy can help to increase the sustainability of agro-ecosystems. Allelochemicals produced from organic mulch can help to suppress the emergence of weed seeds (Singh *et al.*, 2003). Studies have indicated that small-seeded crops and weeds are more susceptible to

allelochemicals – this information can be used when determining what crop to plant the year following allelochemical release by a plant or crop species (Liebman and Mohler, 2001).

Microbes play an important part in the breakdown and activation of some allelochemicals and so should not be forgotten when considering allelochemical production in the agro-ecosystem (Singh *et al.*, 2003). These organisms have a profound effect on allelopathic interactions in and above the soil by altering and/or transforming and/or decomposing the quantitative and qualitative nature of the chemicals produced by the plants.

The agricultural potential and synthetic challenges found in allelopathic products produced by natural sources are now becoming the centre of attention of synthetic chemists in the hope that natural herbicides may be the future for weed control (Vyvyan, 2002).

1.3.4 Allelopathy and biodiversity

Serious problems associated with weed management today are: the herbicide residues left in the environment, resistance of plants towards herbicides, artificial shifts in plant populations brought on by weed management practices and the reduction in plant biodiversity in many areas. Conservation of managed and natural ecosystems is under increasing pressure to become sustainable in their productivity and biodiversity. Plants and plant matter play an important role in supporting ecological processes and attaining sustainable objectives (Reinhardt *et al.*, 1999).

When compared to crop plants, weeds are often characterized by their hastened growth cycles (Weston and Duke, 2003). They have the ability to grow rapidly from germination to seed production, which is generally prolific. Some weeds also have the ability to reproduce vegetatively (as is the case with *C. macrocephalum*), thereby enhancing their chances of survival. All of these assets help weeds in invasive adaptation.

Plant diversity is important as it provides insurance against large changes in ecosystem processes and maintains efficiency of resource utilisation. Plant diversity has a major role in competition, interference, symbiosis and absorption of nutrients. When an area becomes invaded by alien species it loses its natural balance, which may reduce functioning of the ecosystem (Foy and Inderjit, 2001).

Resource competition and allelopathy have the ability to operate simultaneously thus influencing community structure of an ecosystem (Inderjit and Del Moral, 1997). Allelochemicals may influence a number of ecosystem processes (Wardle, Nillson, Gallet and Zackrisson, 1998). The direct effects of allelochemicals on surrounding plants can be affected by abiotic and biotic factors (Inderjit and Weiner, 2001).

Crop production should concern ecologists as it involves intentional destruction of the natural succession and biodiversity of an area. Herbicides have been the major factor in causing vegetation shifts and new weed problems (Zimdahl, 1993). During natural succession many pioneer plants produce allelochemicals toxic to themselves; this explains their relative short persistence in an area.

From the above information we are left to ponder the question: Are allelopathic interactions ecologically more important in natural communities where overall plant densities are lower due to environmental and/or other constraints? (Weidenhamer *et al*., 1989).

1.4 Assessing allelopathic potential

1.4.1 Introduction to bioassays

Bioassays are necessary and useful aids in the study of allelopathy and the evaluation of the activity of plant extracts during the purification and identification of allelochemicals (Hougland and Brandsaeter, 1996).

Hoagland and Williams (2003) state that bioassays using plants and/or plant tissues, have been successful in detecting the biological activity of numerous synthetic compounds and natural products (allelopathic/allelochemical activity) and that due to the difficulty of separating competitive from allelopathic interactions under field conditions, allelopathic studies have been based heavily upon biological assays conducted under laboratory or controlled conditions. A major advantage of the bioassay using multiple plant species is that it can provide information on the phytotoxic selectivity or species sensitivity to allelochemicals (Macías, Galindo, Molinillo and Cutler, 2004). These phytochemicals present in the substrate need not be extracted for assessment, thus relieving problems associated with chemical extraction (Reinhardt *et al*., 1999). The discovery and characterisation of some of the major classes of plant hormones can be linked to the use of bioassays (Hoagland and Williams, 2003).

Generally there are two types of measurements used for testing the biological activity of a plant's allelopathic compounds: for example photosynthesis inhibition or measurements of a plant growth parameter namely seed germination or root dry weight (Hougland and Brandsaeter, 1996).

Bioassays have limitations. Listed below are items that should be taken into consideration when performing the bioassay technique. "Bioassays exhibit large response curves compared to data from physicochemical methods. Log-linear concentration response curves do not allow assessment of concentration differences of allelochemicals. Interfering substances in non purified extracts may have greater effects in bioassays than in physicochemical analyses" (Macías *et al*., 2004). Another point to take note of is that improved techniques and/or

instrumentation such as improved isolation, separation and detection techniques (HPLC, GC, MS, NMR, immunological methods, etc.) have provided greater sensitivity, and such techniques are less variable than bioassays. However, limitations can be minimised if proper experimental design, test material (and treatment thereof), test parameters (seed germination and hypocotyl elongation are just two examples), replication, and statistical analysis are chosen (Hougland and Brandsaeter, 1996; Macías *et al*., 2004).

Since many allelochemicals have been shown to have relatively weak phytotoxicity (especially compared to herbicides), bioassays that have been developed for detecting and quantifying the measurement of Plant Growth Regulator (PGR) activity may be useful in allelopathy (Macías *et al.*, 2004).

1.4.2 The bioassay technique

Seeds of acceptor plant species are often placed on filter paper discs in Petri dishes and exposed to various concentrations of extracted plant material (Macías *et al.*, 2004). This is then followed by incubation at a predetermined temperature. Germination is determined at set times, usually in dark conditions. These conditions allow more rapid stem elongation and thus will increase bioassay sensitivity.

Allelochemicals are released from donor plants via exudation, leaching or decomposition and decay of plant tissues (Vyvyan, 2002; Eljarrat and Barcelò, 2001). These compounds can enter or affect another plant directly by uptake of the chemical or indirectly by effects of the allelochemical on the soil microorganisms that are either plant growth stimulators or are pathogenic (Belz, Duke and Hurle, 2005). In nature, allelopathic compounds may act on or be acted upon by many living organisms before an allelopathic reaction can be measured. The interactions between soil microorganisms and plant roots are complex, this makes proving an allelopathic action or reaction a challenging prospect. The development and use of innovative bioassays will be needed to prove these phenomena (Macías *et al.*, 2004).

1.4.2.1 Plant material used

The collection and bioassay of allelochemicals assumed to be found in roots and leaves of a specific plant species play an important role in the determination of that plant's allelopathic potential (Reinhardt *et al.*, 1999). Roshchina and Roshchina (1993) state that the highest content of inhibitors is usually present in the leaves of a plant.

1.4.2.2 Choice of test species

Belz and Hurle (2004) pre-screened many small seeded receiver species for use in bioassays according to parameters such as sensitivity of species, reliability of growth and ease to grow. *Lactuca sativa* rated among the top three species to use as receiver species in *P. hysterophorus* bioassays. After evaluation of several monocot and dicot species, lettuce was chosen by Macías, Castellano and Mollinillo (2000) as the most desirable test species for allelopathic bioassays.

Lettuce is an annual plant that forms part of the Compositae (Asteraceae) family (Chon *et al*., 2005), the same family as *C. macrocephalum*. From an ecological viewpoint, receiver species in bioassays should be naturally related to the allelopathic donor plant (Belz and Hurle, 2004; Romeo, 2000). Many weeds are known to interfere with lettuce, viz. barnyard grass (*Echinochloa colonum*), common purslane (*Portulaca oleracea*), smooth pigweed (*Amaranthus hybridus*), sheperd's purse (*Capsella bursa-pastoris*) and common lambsquarters (*Chenopodium album*). Lettuce is also often described as being sensitive to certain allelochemicals (Belz and Hurle, 2004). Thus it can be determined that lettuce is a good candidate to perform as a receiver species in bioassays.

1.4.3 Concluding remarks on bioassays

Hougland and Brandsaeter (1996) stated that bioassay methods, test species, climatic conditions, osmotic potential and the interactions between these factors clearly influence the sensitivity and results of bioassays. The involvement of factors confounding the effects on germination and growth may lead to misinterpretation of results.

1.5 Leaf anatomy and allelopathic potential

1.5.1 Introduction

Plants produce many secondary compounds that may have diverse biological activities and serve in functions such as pharmaceuticals, neutriceuticals, natural pesticides, flavourings, and fragrances or even for non-food or fibre purposes (Duke, Canel, Rimando, Tellez, Duke and Paul 2000). To maximise their interactions with the outside world these secondary plant compounds are often produced and/or stored on or near the plant surfaces, many being contained in specialised cells called glandular trichomes - where these products can be contained in concentrated form for maximum effect when sequestered. This storage method will help to avoid autotoxicity in the plant.

A great variety of structures may be found on organs of a number of plant species. These projections may vary in size, shape, number of cells, location on the plant, function and many more aspects (Werker, 2000). It is believed that the highest content of phytotoxic compounds in plants can be found in the leaves. These plant-inhibiting substances may be found in specialised structures inside or on top of the leaves (Roshchina and Roshchina, 1993). Secondary metabolites may be concentrated in trichomes, glandular hairs, stinging hairs or in the upper layer of the epidermis itself (Wink, 1999).

Trichomes may be defined as multicellular or unicellular appendages that originate only from epidermal cells. They develop outwards on the surfaces of plant organs.

None of the many methods of their classification is wholly satisfactory (Werker, 2000). Each method has its own drawbacks, overlaps and exceptions. There are two major distinctions between trichomes – that is, are they glandular or non-glandular? Non-glandular trichomes are distinguished by their morphology. The compounds that they secrete, accumulate and/or absorb primarily distinguish glandular trichomes. Glandular trichomes may also differ in their mode of production, in their structure and in their location (similar glands but on vegetative or reproductive organs of the plant).

When looking at the structure of trichomes, the cell walls of non-glandular trichomes may consist of a primary cell wall only or of a thick secondary wall as well (Werker, 2000). This secondary wall may be evenly or unevenly thickened. All or just parts of the cell wall may be impregnated by such substances as lignin, cutin or suberin to name but a few. The cuticle of non-glandular trichomes may acquire different thicknesses. Its outer surface may vary in texture and shape. The lateral walls of glandular trichomes may become cutinised or suberised or both, these walls also act as caspari strips in preventing apoplastic backflow of secreted substances. Glandular trichome's surfaces may be smooth or exhibit micro-orgmentation, if this orgmentation shows high diversity it will cause the glands to appear macropillate, warty, reticulate or striate.

1.5.2 Trichome development

Trichome development commences at an early stage in leaf development, more often than not, commencing prior to stomatal development and before leaf primordium can be distinguished (Werker, 2000). Three phases of development have been noted for glandular trichomes, these being: presecretory, secretory and post-secretory phases (Ascenão and Paris, 1987; Werker, 2000). Glandular hairs may be produced as long as cell division occurs. This explains the phenomenon of densely populated glandular hairs on the surfaces of young leaves and the gradual wider spread of these glandular hairs on maturing leaves (Werker, 2000).

The life span of a trichome will be determined by its function (Werker, 2000). Some glandular and non-glandular trichomes begin their functioning at very early stages

in their development and senesce before the organ on which they are situated has reached maturity. There are yet others which will only begin to function when the organ is mature. Glandular trichome cells will senesce when their secretion ends. Non-glandular trichomes may remain and serve a function on an organ well past their death. For example, the trichomes will remain on leaves as protection from herbivores, or on seeds and fruit aiding in their dispersal.

1.5.3 Trichome functions

The functions of glandular and non-glandular trichomes are either guessed or are completely unknown (Werker, 2000). It is assumed that the functions of glandular trichomes are due to their morphology, situation on an organ and their direction or orientation on that organ. Trichomes that are similar structurally may secrete substances for different uses. For example, mucilage trichomes on a plant's seeds may have different functions to those same trichomes on the leaves of that same plant.

There are a number of functions suggested for trichomes on the leaves of plants. Here are two that could relate to functions of the trichomes found on *Campuloclinium macrocephalum* (Less D.C) leaves.

i) The plant may need protection from external factors such as herbivores, pathogens, extreme temperatures, excessive water loss or allelopathy against other plants.

ii) Trichomes on leaves may be used for the secretion of salts. These salt-secreting trichomes, known as "hydathode-trichomes," consist of living cells that actively secrete mineral solutions to the plant. Salt-secreting glandular trichomes may differ in morphology, in the salts that they secrete and in the manner in which they secrete these salts (Werker, 2000).

1.5.4 Trichomes as sites of storage

A plant species that has glandular trichomes on its biology will generally produce large amounts of bioactive secondary products (Duke *et al.*, 2000). These multicellular trichome glands are normally the primary site of production of many of these secondary compounds. However in some cases, such as in tobacco and cotton, the trichomes have been clearly stated to be purely for storage only. Vacuoles of glandular trichomes also have the ability to store these interesting compounds. An example of this phenomenon is in the trichomes of certain *Hypericum* species where the vacuoles are peltate in superficial appearance but have no subcuticular space filled with secretory products.

1.5.5 Trichomes as sites for biosynthesis

The trichomes of some plant species appear to have the ability to photosynthesise, whereas others clearly do not (Duke *et al.*, 2000). Those trichomes that lack this ability rely on the underlying tissues as a carbon source. In some species, both cells with chloroplasts and cells with non-photosynthetic plastids can be found in one trichome.

1.5.6 Trichome products of commercial interest

Phenolic compounds produced in the Shikimate pathway are common constituents of some secretory glands. Many of these compounds found have interesting biological activities, an example being their role in plant defence mechanisms (Duke *et al.*, 2000). These products could prove useful in later development of herbicides and other useful products to protect important crops.

It was found by Kanchan and Jayachandra (1980) that growth inhibitors found in soft, fine trichomes of *Parthenium hysterophorus* leaves had an allelopathic growth inhibitory effect on 10-day old wheat (*Triticum aestivum* L.) seedlings. The root and shoot growth of lettuce (*Lactuca sativa* L. var. nigra), tomato (*Lycopersicon esculentum* Mill), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) seedlings have been negatively affected by several sesquiterpene lactones found

in the glandular trichomes of sunflower leaves (*Helianthus annuus* L.) ev. VYP® (Macías, Torres, Molinillo, Varela and Castellano, 1996). However, significant relationships between the number of glandular trichomes on leaves and/or the release and/or the activity of phytotoxic allelochemicals may not necessarily exist (Nilsson, Gallet and Wallstedt, 1998).

Trichomes are diverse in shape, size, structure, location, capability and function (Werker, 2000). Further studies of the chemical contents of trichomes and their role in the plant's chemical ecology will help gain better understanding of natural plant protection methods and lead to the possible discovery of natural compounds used for biological protection (Duke *et al.*, 2000).

1.6 Potential allelopathic effect of *Campuloclinium macrocephalum* on African grasses

1.6.1 Introduction

Biological invasions of alien species are of growing concern worldwide, with the general perception that a large number of invasions are responsible for plant community change and even species extinctions (Willis and Burks, 2006).

Many plant species have the ability to move due to their highly evolved mechanisms of dispersal, for example, using wind and water currents to travel great distances. Migrating animals can disperse plant seeds across the earth. Over the centuries increased human travel and globalization have also contributed to alien species invasions (Heady and Child, 1994). Invasive alien plant species are a major threat to biological diversity on a global scale; this necessitates international cooperation to solve the problem of species invasion (Clout and de Poorter, 2005).

A species can be classified as native or exotic according to its location in its specific area of evolutionary origin or whether human activity is responsible for its current distribution (Willis and Burks, 2006). An undesirable plant or one that

detracts from the objective use of a land is called a “weed” or “noxious weed.” Noxious may also refer to those plants that are extremely prolific, invasive, competitive, harmful, destructive or difficult to control, and restricted by law (Heady and Child, 1994). Heady and Child, (1994) also state that the United States Department of Agriculture observed that the occupation of land by weeds and brush in the 1960’s resulted in plant poisoning, physical injury, and increased costs of management, estimated at US$ 250 000 000 annually on western United States rangelands, and then a US$ 340 000 000 loss in 1989 in seventeen western United States due to invasions of poisonous plants and noxious weeds.

Biological invasion by alien species is now considered to be the leading factor (after habitat destruction) in biodiversity loss and species endangerment (Clout and de Poorter, 2005). Little is understood about the ways in which the presence of alien plants might alter the disturbance regime and influence the structure and functioning of an ecosystem (Crawley, 2005). The disturbance process plays an important role in initiating and altering successional pathways. Disturbance creates safe sites or eliminates site availability for plants; it also influences the timing of resource availability (Sheley, Jacobs and Svejcar, 2005). A hypothesis on the success of alien species is based upon how their new range differs from their native range in nutrient availability, insects and diseases, less competitive environments and competing plants that are more susceptible to the chemicals that the invasive plants produce (Blumenthal, 2005). It is suggested by Blumenthal (2005) that resource and enemy release may interact to cause invasion. Not only are enemies missing in an alien invasive plant’s new range but also the absence of those natural enemies is correlated with the invasiveness of that plant. The increased availability of nutrients in a plant’s new range adds to its invasive ability.

Biological invasions are complex, some regions and communities are more prone to invasions than others, and some species are better at invading. (Willis and Burks, 2006) Immigrating organisms may change their new found territory but disturbance and competition still occur (Heady and Child, 1994). The problems caused by invasive species are a direct result of their success in colonising new habitats. Understanding why these plants are so successful is essential to controlling their distribution (Blumenthal, 2005).

As human populations increase, their need for food increases, which increases the demand on productivity of each hectare of agricultural land. This increase in production needs to be done without degrading the natural resources of the country (Oucamp, 2000). Combining the current status of South African resources with the ever-increasing human population's demand for protein, there seems to be no alternative but to plan for an increase in the size of South Africa's herds and flocks. In this plan, the means to preserve South Africa's world renound rich grasslands must be included.

Campuloclinium macrocephalum is currently invading the grasslands of the Gauteng province in South Africa (Henderson, 2001). This is becoming a problem for farmers as the plant is taking up valuable space needed for palatable grasses to be used by grazing animals.

Campuloclinium macrocephalum has put great investment into its underground rootstock and tubers - the annual visible shoots and leaves only account for 30% of the plant's total biomass (Henderson *et al*., 2003). During winter the plant is protected from fires and frost due to its living parts being safely underground. During summer droughts *C. macrocephalum* can revert to a dormant state by transferring nutrients from the above ground parts to the roots. This plant has evolved many strategies to survive and propagate in South African grassland and savannah ecosystems.

Henderson *et al.* (2003) state that preliminary observations in Gauteng show that *C. macrocephalum* is adapted to a wide range of growing conditions; is able to establish itself on a number of soil types and its ability to establish on disturbed areas, for example roadsides, and abandoned fields and open savannahs are equal. In bottomlands *C. macrocephalum* can be seen growing in combination with another alien invasive plant, *Verbena bonariensis* L. The common names for *V. bonariensis* are purpletop vervain, purpletop, tall verbena, clustertop vervain, South American verbena and pretty verbena.

In an attempt to try to determine whether the invasion of *C. macrocephalum* is due to allelopathic potential or competition, three important grasses used in South African farming systems were used as test species in a bioassay approach. This bioassay was also used to determine the effect, if any; *C. macrocephalum* had on these three grass species' growth and development that would hinder them in a pompom infested situation (see Chapter 3 for experiment). The three grasses used were: *Eragrostis curvula*, *Eragrostis tef*, and *Panicum maximum.*

1.6.2 Background on grasses to be bioassayed

1.6.2.1 *Eragrostis curvula*

Eragrostis curvula, also known as weeping love grass, prefers well-drained soils and can be found in many disturbed areas (van Oudtshoorn, 1999). This easy to establish grass is the most cultivated grass in South Africa growing best in areas where the rainfall is more than 650 mm per annum. *Eragrostis curvula* dies back when frost occurs but regrowth starts early in the spring (Dickenson, Hyam, Breytenbach, Metcalf, Basson, Scheepers, Plint, Smith, Smith, van Vuuren, Viljoen, Archibald and Els, 2004).

Dickenson *et al.* (2004) describe *E. curvula* as an indigenous, tufted grass, and summer growing perennial. This species is highly variable with either relatively short stems (up to 60 mm) or with longer stems (up to 1200 mm).These stems can be either robust or slender and may grow upright or sideways. Leaves are concentrated at the base of the stem and can grow up to 600 mm long by 10 mm wide or be narrower with a courser feel. Some cultivars such as Ermelo have green leaves, while others such as Witbank and Kromdraai have blue/green leaves. Mature plants are inclined to droop. The inflorescence is a panicle which is open at maturity; it is highly branched with a number of spikelets, which are each capable of bearing a number of seeds. This grass originated from South and East Africa, but can today be found in many other tropical and subtropical countries (van Oudtshoorn, 1999).

This grass is the most important under cultivation on the Highveld of South Africa, it can also be found as a pasture grass in other parts of the world (van Oudtshoorn, 1999). Early regrowth of the grass in the spring is of great value for grazing animals as it provides green pastures several weeks before other summer growing grasses begin to shoot (Dickenson *et al.*, 2004). If it is not cut too late in the season, the grass will make good hay. *Eragrostis curvula* can be sown alone, or in a mixture with other grasses to stabilise exposed soil, e.g., alongside new roads, against dam walls and in other places where grass cover is needed (van Oudtshoorn, 1999). *Eragrostis curvula* is used as a fodder crop in many tropical and subtropical countries (van Oudtshoorn, 1999).

1.6.2.2 *Eragrostis tef*

This annual grass grows in the summer season (Dickenson *et al.*, 2004). It is able to establish and grow in disturbed places in most types of soil (van Oudtshoorn, 1999). *Eragrostis tef* is suited to a number of soils and can be sown on any soil that is not waterlogged. This crop is usually sown under dry land conditions in areas with an annual rainfall as low as 400 mm (Dickenson *et al.,* 2004).

Eragrostis tef originated from North East Africa. Tropical areas (including South Africa) were introduced to this seed from Ethiopia (van Oudtshoorn, 1999). The stems of this grass can reach heights between 200 and 900 mm. This grass has slender stems and fine leaves. The leaves can grow to 300 mm long by 4 mm wide (Dickenson *et al.*, 2004).

Eragrostis tef is often planted in cultivated lands and/or along new roadsides to prevent soil erosion (van Oudtshoorn, 1999). This annually cultivated pasture is particularly suitable for hay and fodder especially for horses. In Ethiopia, *E. tef* is cultivated on a large scale for its seeds, which are now used with great success (van Oudtshoorn, 1999; Dickenson *et al.*, 2004). More than half of Ethiopia's grain fodder originates from teff production. Teff has recently become a regular grass used to resow exposed ground (van Oudtshoorn, 1999) and is often used as a cover crop to prevent soil erosion in fallow lands (Dickenson *et al.,* 2004).

1.6.2.3 *Panicum maximum*

Panicum maximum is also known as guinea grass and prefers areas of shade under trees and shrubs (Dickenson *et al.*, 2004). *Panicum maximum* can be found in bushveld areas where it is known as Bushveld Buffalo Grass (Dickenson *et al.*, 2004). This grass grows well in damp conditions with fertile soils (van Oudtshoorn, 1999). Guinea grass is adapted to growing in a variety of soils but does not fair well in very sandy soils or heavily structured soils such as an Arcadia soil type. This grass can survive frosty conditions and can grow in areas with a minimum rainfall of 500 mm per annum; it is well suited to tropical and subtropical areas (Dickenson *et al.*, 2004).

Panicum maximum is an indigenous, tufted perennial grass that can reach up to 1.2 m in height. This is a leafy grass having broad leaves and slender stems. It is a sweet and palatable grass (Dickenson *et al.*, 2004). Guinea grass originated in Africa, but today is found in virtually all tropical parts of the world (van Oudtshoorn, 1999).

It has been stated by Dickenson *et al.* (2004) that the palatable *P. maximum* is the most valuable of the grazing grasses in its areas of distribution. A sign of a good veld is when this grass appears in abundance. Guinea grass is well used to make hay. This grass is a weed when found growing in areas under cultivation, especially sugarcane fields (van Oudtshoorn, 1999).

The study on *C. macrocephalum* took place in the Gauteng province of South Africa. This area, from north to south is 71% grassland and 29% savannah (www.grasslands.org, 2008). The whole province has a semi-arid climate (www.sanbi.org, 2008). Of Gauteng's eight grassland vegetation types, two are critically endangered. The most important issue in grassland degradation in this area is the loss of plant cover associated with the change in composition of the plant species. We know that Gauteng's Brackenveld veldtype is heavily invaded by *C. macrocephalum* (Henderson, 2001; www.grasslands.org, 2008), thus by studying the weed's growth habits and morphology we could be able to prevent the loss of any more of this province's precious grasslands.

1.7 Conclusion

As an invading species and Category 1 weed in South Africa, *Campuloclinium macrocephalum* is posing a great threat to the natural resources of the country. There is reason to believe that the plant possesses allelopathic potential, which could be the cause behind its invasive ability. Allelochemicals found in the plant could be stored in glands and/or occurring on trichomes of the leaves of the plant. Bioassays and Scanning Electron Microscopy (SEM) of the plant will help to determine the possible allelopathic functioning of the weed.

CHAPTER 2

ASSESSMENT OF THE ALLELOPATHIC POTENTIAL OF *CAMPULOCLINIUM MACROCEPHALUM* USING BIOASSAYS

2.1 Introduction

Campuloclinium macrocephalum is a declared Category 1 weed in South Africa (Klein, 2002). This status could at least partly be due to the plant's high competitive ability and/or its allelopathic potential. The contribution of allelopathy to this species' ability to interfere with the growth and development of other plants can be assessed by using bioassays designed to determine the allelopathic potential of this alien invader. Bioassays are necessary and useful aids in the study of allelopathy and the evaluation of the activity of plant extracts during the purification and identification of allelochemicals (Hougland and Brandsaeter, 1996). Hoagland and Williams (2003) state that bioassays using plants and/or plant tissues have been successful in detecting the biological activity of numerous synthetic compounds and natural products (allelopathic/allelochemical activity). Due to the difficulty of separating competitive from allelopathic interactions under field conditions, allelopathic studies have been based heavily upon biological assays conducted under laboratory or controlled conditions.

Generally there are two types of measurements used for testing the biological activity of allelopathic compounds: measurements of physiological processes, for example photosynthesis, or plant growth parameters such as seed germination and/or plant and root mass (Hougland and Brandsaeter, 1996). Since many allelochemicals have been shown to have relatively weak phytotoxicity, especially compared to herbicides, bioassays that have been developed for detecting and quantifying the measurement of Plant Growth Regulator (PGR) activity may be useful in the study of allelopathic pathways (Macías *et al.*, 2004).

By performing bioassays on *C. macrocephalum* plant parts, the weed's allelopathic potential can be determined in order to give insight into this plant's invasive ability. The aim of the experiments presented here was to determine the allelopathic potential of *C. macrocephalum* and determine which plant part played the most

important role in this regard.

2.2 Materials and methods

2.2.1 General bioassay technique

Seeds of acceptor plant species are often placed on filter paper discs in Petri dishes and exposed to various concentrations of extracted plant material (Macías *et al.*, 2004). This is followed by incubation at a predetermined temperature. Germination is determined at set times, usually in dark conditions. These conditions allow more rapid stem elongation and thus will increase bioassay sensitivity.

2.2.1.1 Plant material used

The type, amount and location of allelochemicals may play an important role in the determination of that plant's allelopathic potential (Reinhardt *et al.*, 1999). Plant material used in all preliminary experiments for this particular project included root, stem and leaf material from *C. macrocephalum*. Once it was determined that the main site of allelochemical concentration was in the leaves, only leaves were used in subsequent bioassay experiments. Roschina and Roschina (1993) state that the highest content of inhibitors is usually present in the leaves of a plant.

2.2.1.2 Procedures for preparing aqueous extracts from plant material

Using crushed (homogenized) plant material as substrate for making infusions for testing in bioassays is generally not advised, therefore, intact, frozen (or fresh) individual plant parts were used. The roots, stems and leaves of *C. macrocephalum* were soaked separately in distilled water for 24 hours before being diluted into specific concentrations for use in the bioassay experiments. Due to the fact that the plant material was frozen before being soaked in distilled water means that possible rupture of cell membranes and the consequent leaking out of cell contents could have occurred. It is conceivable that freezing of test material could cause more allelochemicals to be released from the plant material than would have occurred if fresh, unfrozen material were used.

2.2.1.3 Choice of test species

Lettuce (*Lactuca sativa*) was chosen by Macías *et al*. (2000) from amongst several dicots and monocots as the most desirable target species for allelopathy bioassays. Thus lettuce was used as the acceptor species in all preliminary bioassays performed in this study. Once it was confirmed that the growth and germination of lettuce was inhibited by the *C. macrocephalum* infusions it was then used as a control in bioassays involving other plant species (see Chapter 3). Other acceptor species used in experiments reported on in chapters to follow occur naturally in areas where *C. macrocephalum* is currently invading. Grasses indigenous to Africa were used, namely *Eragrostis curvula, Eragrostis tef,* and *Panicum maximum*. From amongst the crop species, lettuce (cv. Great Lakes) was selected.

2.2.1.4 Germination assessment

Seeds of all acceptor species were treated alike. Germination of all seeds was determined at set times in order that no discrimination could be made between acceptor species as to length of time needed to germinate. Seeds were considered to have germinated if their radicles were at least 1 mm in length. Seeds were tested prior to being bioassayed for viability to ensure optimum germination rates.

2.2.1.5 Sterilization procedures

In order to ensure the exclusion of microbial contamination the following precautions were taken: (a) commercial seeds pre-treated with fungicide were used, (b) sterilized Petri dishes; sealed boxes of filter paper and distilled water were used in experiments. All bioassays were performed under aseptic conditions in the tissue culture laboratory on the Hatfield Experimental Farm of the University of Pretoria.

2.2.2 Preliminary experiment: Exclusion of osmotic interference

According to Macías *et al.* (2004): "When testing extracted plant material, care should be taken to ensure that seed germination is not delayed by the osmotic potential of the extract solution." Few researchers take the osmotic potential of test solutions into account when reporting on allelopathic potential of plant species. Extreme osmotic potentials of test solutions in bioassays inhibit germination and growth of many plant species (Hougland and Brandsaeter, 1996). Therefore it is important to know the osmotic potential of extract solutions tested using the bioassay technique.

Polyethylene glycol (PEG-6000) has been used by Trotel-Aziz, Niogret and Larher (2004) and Oliviera, Ferreira and Borghetti (2004) for testing plant responses to osmolarities of substrates. PEG-6000 was used for the same purpose in the present study. An osmotic range was prepared by dissolving different amounts of PEG-6000 in distilled water. It has previously been determined what concentrations of PEG would give the best osmotic range for bioassay studies. These concentrations are as follows: 12.5, 25, 50 and 75 g PEG-6000 per litre of water.

Mature *C. macrocephalum* plants were collected from outside of the Valhalla air force base, Thswane, in March of 2005. The plant parts were then separated and kept frozen until their use in the bioassay experiments. Roots (150 g) were put into a beaker containing 1000 ml water; stirred and left to stand for 24 hours – this comprised the aqueous infusion used for the root aspect of the experiment. The same procedure was then carried out for the stems and leaves of the plant. After 24 hours the infusions were diluted into concentrations of 25% (25 ml infusion added to 75 ml water to make up 100 ml), 50% (50 ml infusion added to 50 ml water), 75% (75 ml infusion added to 25 ml water), and 100% (100 ml undiluted infusion). A control of 100 ml distilled water was included.

Osmolarity was measured using a Hermann Roebling digital micro-osmometer measuring freezing point depression. The Hermann Roebling micro-osmometer measures the freezing point of aqueous solutions. The freezing point reduction of a solution below that of pure water is a direct measure of the osmotic concentration of that solution. For example, pure water freezes at 0 °C, with 0 osmolality (measured

in Osmol/kg). An aqueous solution with osmolality measuring 1 Osmol/kg water will thus freeze at a lower temperature, namely at -1.858 °C. At room temperature a sample of 100 µl will be pipetted into a sample vessel, which is placed onto the measuring head, which is then placed into a cooling aperture beneath its guide rod. The solution will now begin to cool. The digital display on the machine will show decreasing values of temperature. When the temperature level of the cooled solution reaches minus 1000 °C (shown on the digital display of the machine) a cold needle is inserted into the sample to initiate ice formation. The temperature then rises until freezing point is reached, thus giving us the value used for osmolality. Since osmolality is directly related to freezing point reduction, the digital display of the machine will display milliOsmol, and not °C.

2.2.3 Determining the allelopathic potential of *Campuloclinium macrocephalum* using bioassays

Mature *C. macrocephalum* plants were collected from outside of the Valhalla air force base, Thswane, in March of 2005. The different plant parts were then separated into leaves, stems, and roots and kept frozen until used in the bioassay experiments. Roots (150 g) were put into a beaker containing 1000 ml water; stirred and left to stand for 24 hours – this comprised the aqueous infusion used for the root aspect of the experiment. The same procedure was then carried out for the stems and leaves of the plant.

After 24 hours the infusions were diluted into concentrations of 25% (25 ml infusion added to 75 ml water to make up 100 ml), 50%, 75%, and 100% (undiluted infusion). A control of 100 ml distilled water was included. Ten lettuce seeds were placed on filter paper in each Petri dish. Five ml of infusion was poured onto the filter paper. Five replications of each concentration for root, stem and leaf infusions were prepared. The Petri dishes were then sealed with parafilm, placed in black plastic bags and stored in a growth chamber at 26 °C for eight days. The germination percentage, radicle and shoot length of each plant were measured on day eight.

2.3 Results and discussion

2.3.1 Results for preliminary experiment: Exclusion of osmotic interference

The following results were found for increasing osmolalities of PEG-6000 and concentrations of *C. macrocephalum* infusions.

Table 2.1 Effect of PEG-6000 solutions of increasing osmolality on germination and mean root lengths of lettuce seedlings (adapted from Bothma, 2004)

PEG-6000 concentration (g/l)	Osmolality (mOsm/kg)	Percentage germination	Root length
0	0	98a	15.40b
12.5	3	97a	17.40ab
25	8	100a	17.20ab
50	24	98a	17.74a
75	53	96a	18.69a

Means in each column followed by different letters are significantly different according to Tukey's Studentised Range test.

Table 2.2 Effect of *Campuloclinium macrocephalum* infusions of increasing osmolality on germination and mean root lengths of lettuce seedlings

C. macrocephalum Infusion concentration (% concentration)	Osmolality (mOsm/kg)	Percentage germination	Root length (mm)
0	0	98a	15.5b
25	0.026	100a	17.5ab
50	0.043	96a	10.9ab
75	0.059	50a	5.61a
100	0.092	0b	0.04c

Means in each column followed by different letters are significantly different according to Tukey's Studentised Range test.

Based on data in Table 2.1, increasing osmolality did not affect root growth or seed germination adversely within the range of 3 to 53 mOsm/kg. Because osmolalities of the aqueous infusions prepared from *C. macrocephalum* leaf material (Table 2.2) were far below those levels causing growth inhibition, it can be concluded that osmotic effects did not distort effects of any of the *C. macrocephalum* infusion concentrations that were tested in the bioassays performed.

Hougland and Brandsaeter (1996) state that bioassay methods, test species, climatic conditions, osmotic potential and the interactions between these factors clearly influence the sensitivity and results of bioassays. Factors that confound the effects of allelochemicals on germination and growth may lead to misinterpretation of results. Bioassays form an integral part of the experimental discovery process. The bioassays performed for this study were conducted in a controlled, aseptic environment that effectively excluded unwanted interferences from experiments.

2.3.2 Results for determining the allelopathic potential of *Campuloclinium macrocephalum* using bioassays

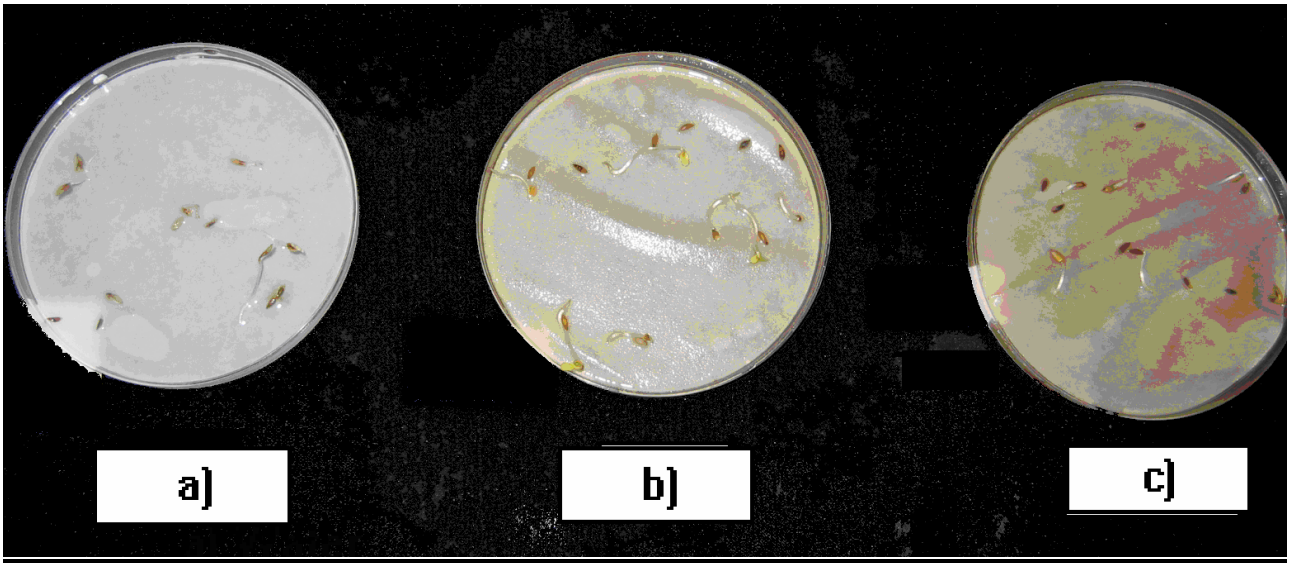


Figure 2.1a Effect of different concentrations of a leaf infusion of *C. macrocephalum* on germination and radicle development of lettuce: (a) Control (distilled water), (b) 25%, (c) 50%

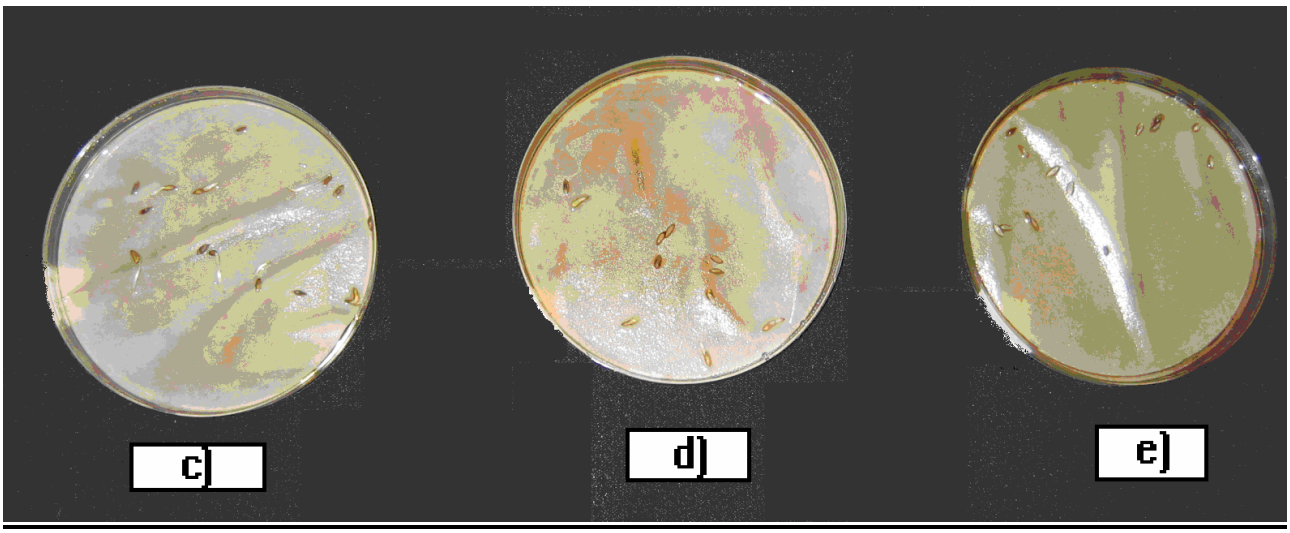


Figure 2.1b Effect of different concentrations of a leaf infusion of *C. macrocephalum* on germination and radicle development of lettuce: (c) 50%, (d) 75%, (e) 100%

Figures 2.1a and 2.1b show photographs of the lettuce bioassays, taken after eight days in the growth chamber at 26 ºC. The control shows up clearer than the rest of the Petri dishes as it involved distilled water which did not stain the filter paper. The discolouration caused by the infusion solution increased with an increase in concentration of *C. macrocephalum* infusions. Lettuce seeds exposed to distilled

water germinated faster and seedlings developed more rapidly than at infusion concentrations of 50% and higher. Seeds exposed to the 25% infusion concentration showed a greater growth rate than those of the control treatment. This phenomenon, where a stimulatory or beneficial effect is seen at low concentrations and inhibition at high concentrations of an infusion, is termed hormesis (Belz, Duke and Hurle, 2005). Mersie and Singh (1987) state that there is strong correlation between extract concentration and increased toxicity to the test species, the toxicity of plant part extracts was also concentration dependant.

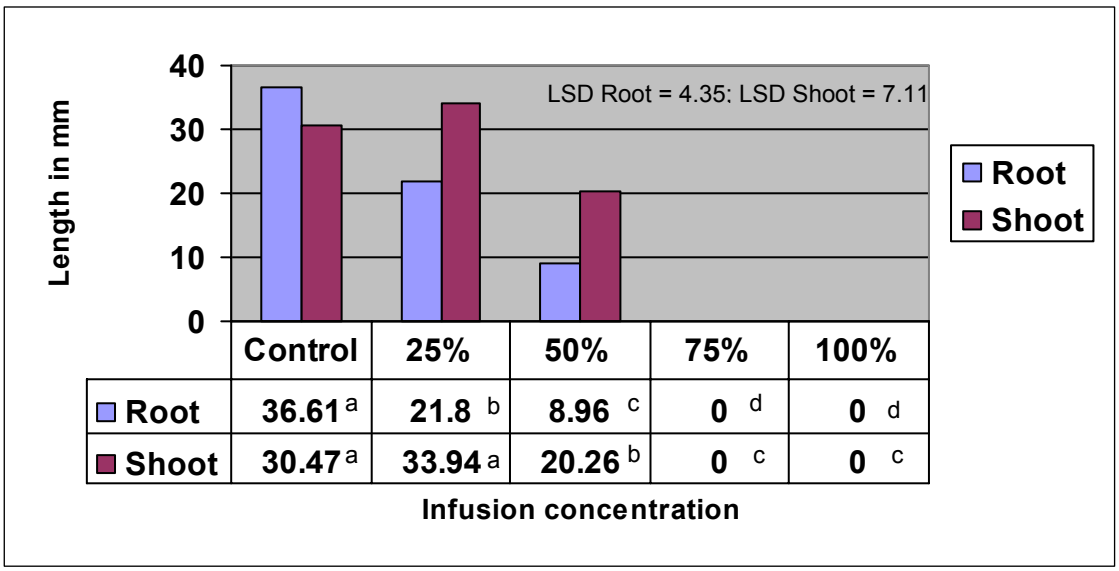


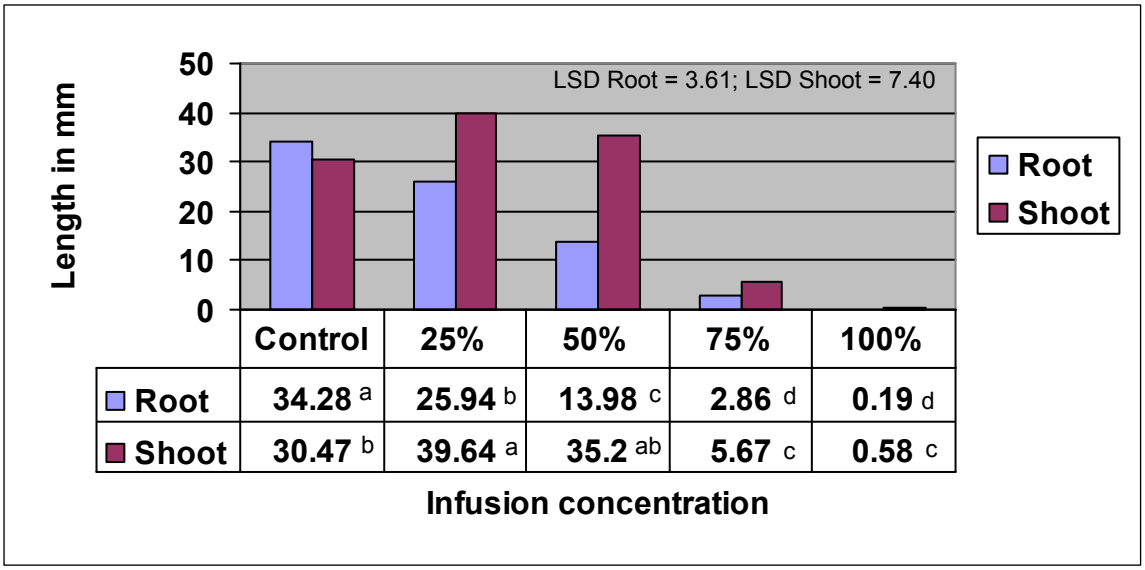
Figure 2.2 Germination bioassay of *Lactuca sativa* exposed to *C. macrocephalum* leaf infusions (ANOVA in Appendix A, Table A1 and A2)

Means followed by the same letters are not significantly different.

From the above graph it can be seen that *C. macrocephalum* does have allelopathic potential. Studies by Kohli, Rani, Singh and Kumars (1996), using similar methods of bioassay showed that the aqueous leachates of fresh leaves of *Parthenium hysterophorus*, another alien invader from the Asteraceae family, exerted phytotoxic impact on the germination parameters of pulses, vegetables and forages. Kohli *et al.* (1996) further state that some of the seeds exposed to the above mentioned experiment, especially those belonging to the pulses totally failed to germinate; this statement can also be made for the seeds exposed to the high concentrations of *C. macrocephalum* infusion. Mersie and Singh (1987) state that root inhibition increased with an increase in concentration of the extract used during leachate experiments with *P. Hysterophorus*. The same effect is evident in results

for *C. macrocephalum* (Figures 2.2 – 2.5). Thus results from the bioassays using leaf extracts of *C. macrocephalum* concur with experiments of other researchers on other alien invaders in terms of confirming the allelopathic potential of this alien invader. Similar results for leaf infusion bioassays were found for *P. hysterophorus* by Picman and Picman (1984) showing that the leaves contained the highest amount of autotoxin throughout the plant.

The stimulation of growth at low concentrations *viz.* 25% infusion and inhibition at high infusion concentrations *i.e.* from 75% is a phenomenon called hormesis (Belz *et al.*, 2005). It can also be seen that the lettuce shoots were less affected (or less sensitive) than the roots. Complete lack of germination of seeds of test species at 100% infusion concentration was found in bioassays on *P. hysterophorus* (Kohli *et al.*, 1996), this result can also be found in *C. macrocephalum* bioassays. It should also be noted that the rate of germination represented as seed vigour was seen to be further reduced in bioassays of both *P. hysterophorus* (Kohli *et al.*, 1996), and in this study, *C. macrocephalum*; germination rate was considerably reduced in seeds which otherwise exhibited 100% germination under controlled conditions.



	Control	25%	50%	75%	100%
Root	34.28 a	25.94 b	13.98 c	2.86 d	0.19 d
Shoot	30.47 b	39.64 a	35.2 ab	5.67 c	0.58 c

Figure 2.3 Germination bioassay of *Lactuca sativa* exposed to *C. macrocephalum* stem infusions (ANOVA in Appendix A, Table A3 and A4)

Means followed by the same letters are not significantly different.

The stem material of *C. macrocephalum* has less allelopathic potential than the leaves of the plant, this also holds true for *P. hysterophorus* extracts (Picman and Picman, 1984). The stems of *C. macrocephalum* did however have some

allelopathic potential, although this material was not considered for further bioassay work in this study.

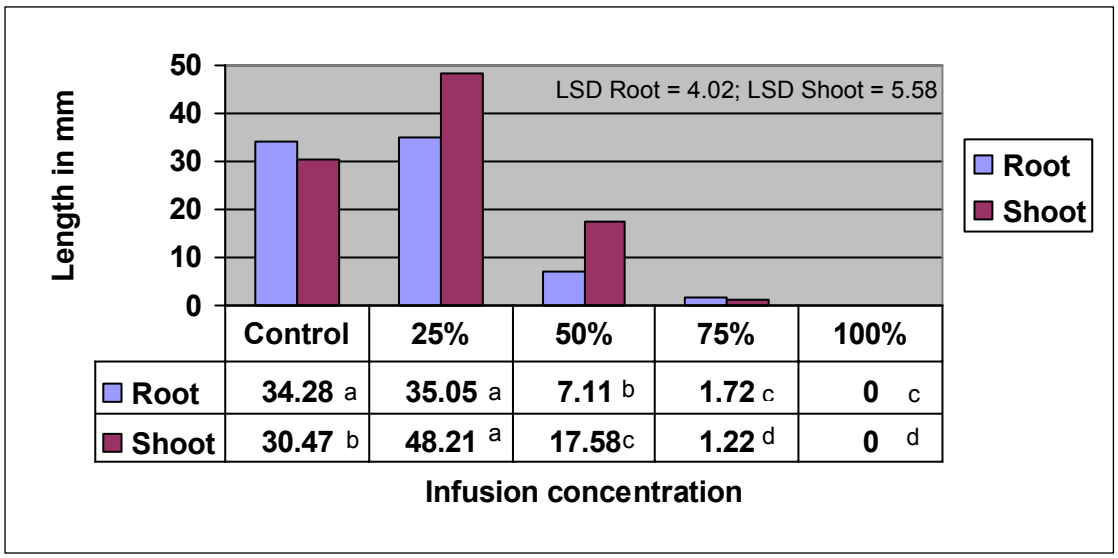


Figure 2.4 Germination bioassay of *Lactuca sativa* exposed to *C. macrocephalum* root infusions (ANOVA in Appendix A, Table A5 and A6)

Means followed by the same letters are not significantly different.

The roots of *C. macrocephalum* did have allelopathic potential. Figure 2.4 shows the same apparent hormetic effect – stimulation at low concentrations of infusions and inhibition at high concentrations (Belz *et al*., 2005) – as seen with leaf and stem infusions. The roots however did not have as high a potential as the leaves. These results can be compared with those of Kanchan and Jayachandra (1979) who state that the inhibitory nature of the root exudate of *P. hysterophorus* was confirmed under sterile conditions by its effect on wheat (*Triticum aestivum* var. UP 301) seedling growth.

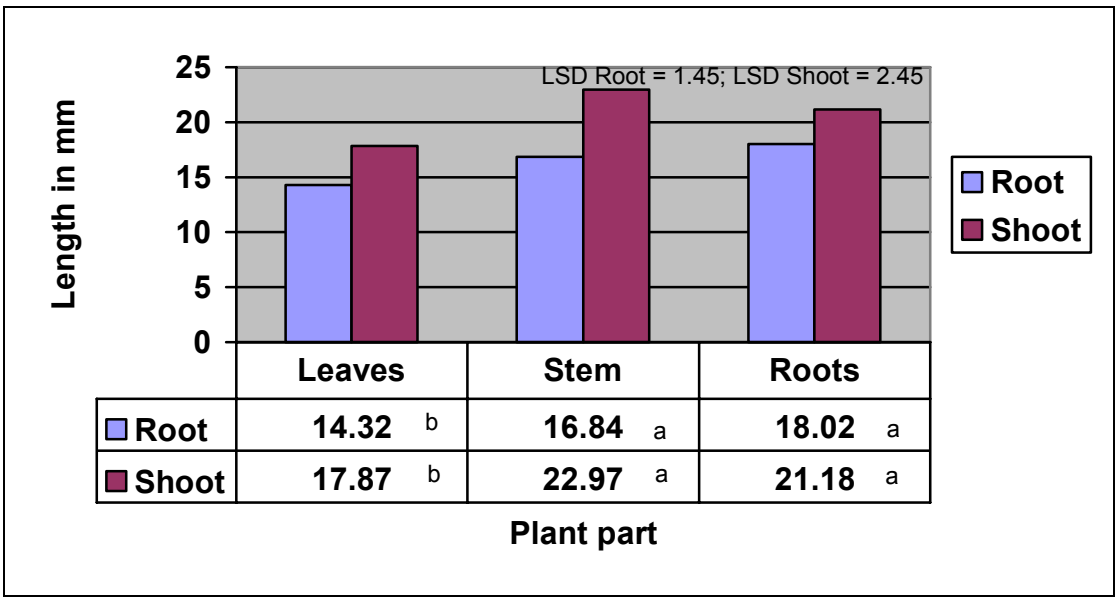


Figure 2.5 Comparison of allelopathic potential of *C. macrocephalum* plant parts (ANOVA in Appendix A, Table A7 and A8)

Means followed by the same letters are not significantly different.

Data in Figure 2.5 show that the leaves of *C. macrocephalum* had a significantly higher allelopathic potential than both the roots and stems of the plant.

In similar laboratory experiments performed by Adetayo, Lawal, Alabi and Owolade (2005) it was found that the close relative to *C. macrocephalum* – Siam weed (*Chromolaena odorata*) also has allelopathic potential. In their experiments the weed inhibited seed germination of other weeds and of crops but stimulated the growth of certain other crops. They reported 14 and 8% reduction on cowpea and soybean germination, respectively, when compared to untreated controls. By comparing the findings from experiments on *C. odorata* and *P. hysterophorus* to those from this study on *C. macrocephalum* the allelopathic potential of the latter weed can be clearly confirmed.

2.4 Conclusion

Results suggest that *C. macrocephalum* does have significant allelopathic potential. Of the plant parts investigated, stems had the least allelopathic potential – but still showed inhibitory effects on lettuce root and shoot growth. Root infusions showed inhibition of lettuce root and shoot growth, indicating that the roots of *C. macrocephalum* did have allelopathic potential, although it was lower than the effects from leaves which had the highest allelopathic potential of all plant parts tested. The root infusions may have had lower allelopathic potential than the leaves in this bioassay experiment but this could be different for roots excreting allelochemicals into the soil under natural conditions, thus it should be considered that their allelochemical contribution may have been under-expressed in the laboratory bioassay.

Leaf, stem and root infusions of *C. macrocephalum* showed stimulation of growth of lettuce leaves at low concentrations, *viz.* 25% concentration, and inhibition of growth of lettuce roots and shoots occurred from 50% concentration. This phenomenon is called hormesis (Belz *et al.,* 2005) and has been recorded in a number of allelopathy studies.

The results from this experiment concur with those of Mersie and Singh (1987) who state that the biological activity of water extracts from allelopathic weeds was directly related to the extract concentrations.

CHAPTER 3

POTENTIAL ALLELOPATHIC EFFECT OF *CAMPULOCLINIUM MACROCEPHALUM* ON AFRICAN GRASSES

3.1 Introduction

There are many advantages to studying species invasions, including the ecological and genetic processes that can be observed in real time rather than from the patterns they generate (Crawley, 2005). Biological invasion by alien species is now considered to be the leading factor (after habitat destruction) in biodiversity loss and species endangerment (Clout and de Poorter, 2005). Little is understood about the ways in which the presence of alien plants might alter the disturbance regime and influence the structure and functioning of an ecosystem (Crawley, 2005). The disturbance process plays an important role in initiating and altering successional pathways. Disturbance creates safe sites or eliminates site availability for indigenous plant growth; it also influences the timing of resource availability (Sheley *et al*., 2005). A hypothesis on the success of alien plant species is based upon how their new habitat differs from their native habitat in nutrient availability, insects and diseases, less competitive environments and competing plants that are more susceptible to the chemicals that the invasive plants produce (Blumenthal, 2005). It is suggested by Blumenthal (2005) that an increase in resource availability and decrease in enemy occurrence may interact to cause invasion. Not only are enemies missing in an alien invasive plant's new habitat but also the absence of those natural enemies is correlated with the invasiveness of that plant. The increased availability of nutrients in a plant's new range adds to its invasive ability.

Biological invasions are complex, some regions and communities are more prone to invasions than others, and some species are better invaders (Willis and Burks, 2006). Immigrating organisms may change their receiving systems (or habitats) but disturbance and competition will still occur (Heady and Child, 1994). The problems caused by invasive species are a direct result of their success in colonizing new habitats. Understanding why these plants are so successful is essential to controlling their invasion into new areas (Blumenthal, 2005).

Campuloclinium macrocephalum (pompom) is currently invading the grasslands of the Gauteng, Mpumalanga and KwaZulu-Natal provinces in South Africa (Henderson *et al*., 2003). This is becoming a problem for farmers as the plant is colonizing areas where palatable grasses occur, which can be used by grazing production animals. *Campuloclinium macrocephalum* has a very well developed underground rootstock and tubers, thus the visible shoots and leaves only account for about 30% of the plant's total biomass. During winter the plant can survive fires and frost due to its living parts being safely underground. During summer droughts, *C. macrocephalum* can revert to a dormant state by transferring nutrients from the shoots to the roots. This plant has evolved many strategies to survive and propagate in South African grassland and savannah ecosystems (Henderson *et al*., 2003).

According to Henderson *et al.* (2003), preliminary observations in Gauteng show that *C. macrocephalum* is adapted to a wide range of growing conditions and is able to establish itself on a number of soil types. It has the ability to establish on disturbed areas, for example roadsides, abandoned fields and open savannahs. In low lying areas (bottomlands), *C. macrocephalum* can be seen growing in combination with another alien invasive plant, *Verbena bonariensis,* commonly known as purpletop vervain or South American verbena.

In order to determine whether the invasion of *C. macrocephalum* is at least partly due to its allelopathic potential, the susceptibility of three common grasses found in natural grasslands, savannahs and as pastures for animal production systems, was assessed. A bioassay experiment was done to determine if allelochemicals produced by *C. macrocephalum* could affect the three grass species' growth and development to such an extent that their survival in a pompom infested field would be jeopardized. The three grasses used were: *Eragrostis curvula* (perennial), *Eragrostis tef* (annual), and *Panicum maximum* (perennial).

3.2 Materials and methods

In preparation for bioassay, 150 g of *C. macrocephalum* leaves were soaked per 1000 ml distilled water. The *C. macrocephalum* material was frozen after collection in the field and had been stored in this state for three months before use. Frozen *C. macrocephalum* leaves were put into a beaker containing 1000 ml distilled water, stirred, covered with aluminium foil and placed in a dark cupboard. The beaker was then left to stand for 24 hours at room temperature – this composed the 100% aqueous infusion used in the experiment. After this 24 hour period the infusion solution was filtered out and diluted into five different infusion concentrations: 25% (25 ml solution added to 75 ml water to make up 100 ml), 50% (50 ml infusion added to 50 ml water), 75% (75 ml infusion added to 25 ml water), and 100% (100 ml undiluted infusion). Distilled water (100 ml) was included as a control treatment.

The grass seeds were then exposed to the different aqueous solutions in Petri dishes. Ten seeds of each of *Eragrostis curvula, Eragrostis tef* and *Panicum maximum* were used in each Petri dish. Five ml of infusion solution was poured onto the single layer Whatman No. 1 filter paper onto which the seeds were placed in each Petri dish. Five replications of each concentration were performed for each grass species. The Petri dishes were then sealed with parafilm and stored in a dark growth chamber at a constant 26 °C for eight days. At the end of this incubation period the germination percentage, root and shoot length of each plant were measured.

3.3 Results and discussion

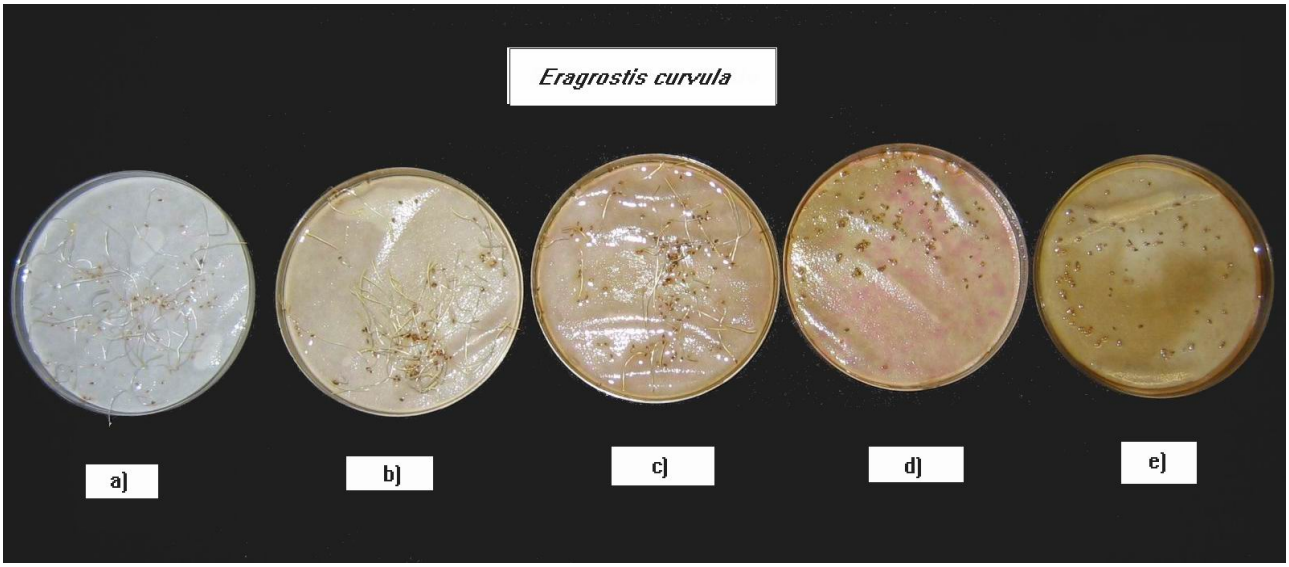
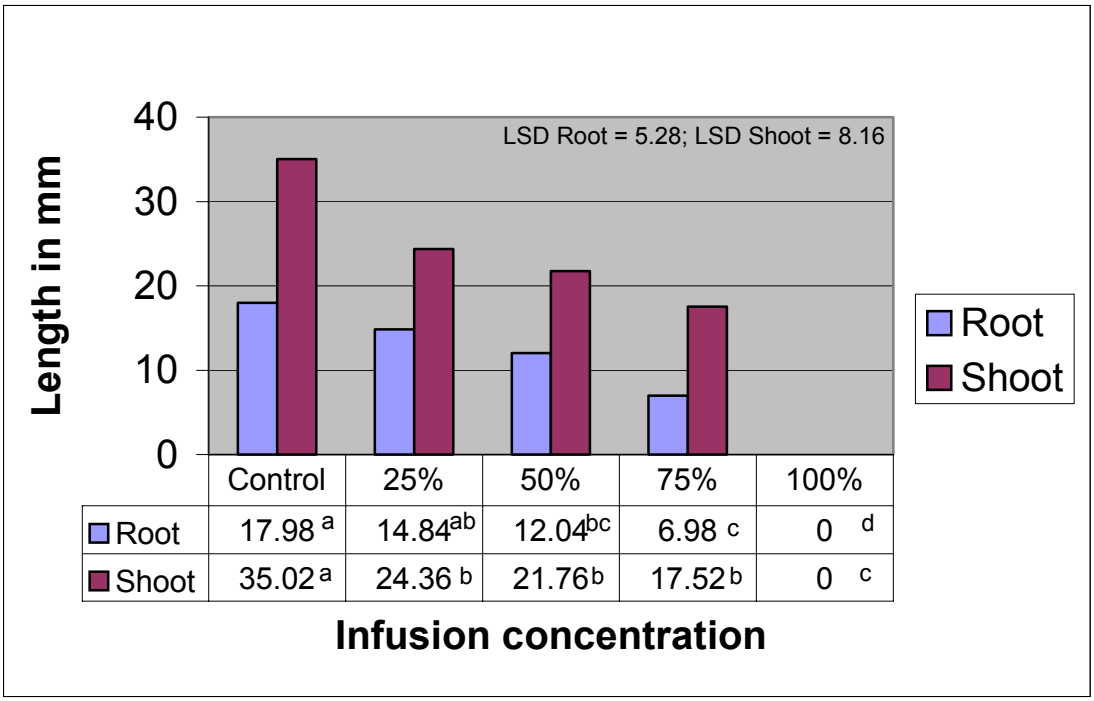


Figure 3.1 Effect on growth of *Eragrostis curvula* seedlings grown from seed exposed to different *C. macrocephalum* infusion concentrations, from left to right, (a) Control (distilled water), (b) 25%, (c) 50%, (d) 75%, (e) 100%



	Control	25%	50%	75%	100%
Root	17.98 a	14.84 ab	12.04 bc	6.98 c	0 d
Shoot	35.02 a	24.36 b	21.76 b	17.52 b	0 c

Figure 3.2 Effect of *C. macrocephalum* infusions on root and shoot growth of *Eragrostis curvula* seedlings after seeds were incubated for eight days (ANOVA in Appendix B, Table B1 and B2)

Means followed by the same letters are not significantly different.

Data for early root and shoot development for *E. curvula* indicated that a significant reduction in growth of roots first occurred at 50% infusion concentration and for shoots at 25% (Figure 3.2). Germination did not occur at 100% infusion concentration. Pure parthenin as well as crude extracts prepared from *Parthenium hysterophorus*, also of the Asteraceae family, have been found to inhibit the germination and growth of a variety of plants including pasture grasses, cereals, vegetables, other weeds and even tree species (Nath, 1981; Mersie and Singh, 1987; Swaminathan, 1990; Reinhardt, van der Laan, Belz, Hurle and Foxcroft, 2006). The sesquiterpine lactone, parthenin, is a major allelochemical compound that occurs at near 100% concentration in capitate-sessile glands on the leaf surfaces of *P. hysterophorus* (Reinhardt *et al*., 2004). This evidence helps to prove allelopathic potential for *C. macrocephalum*.

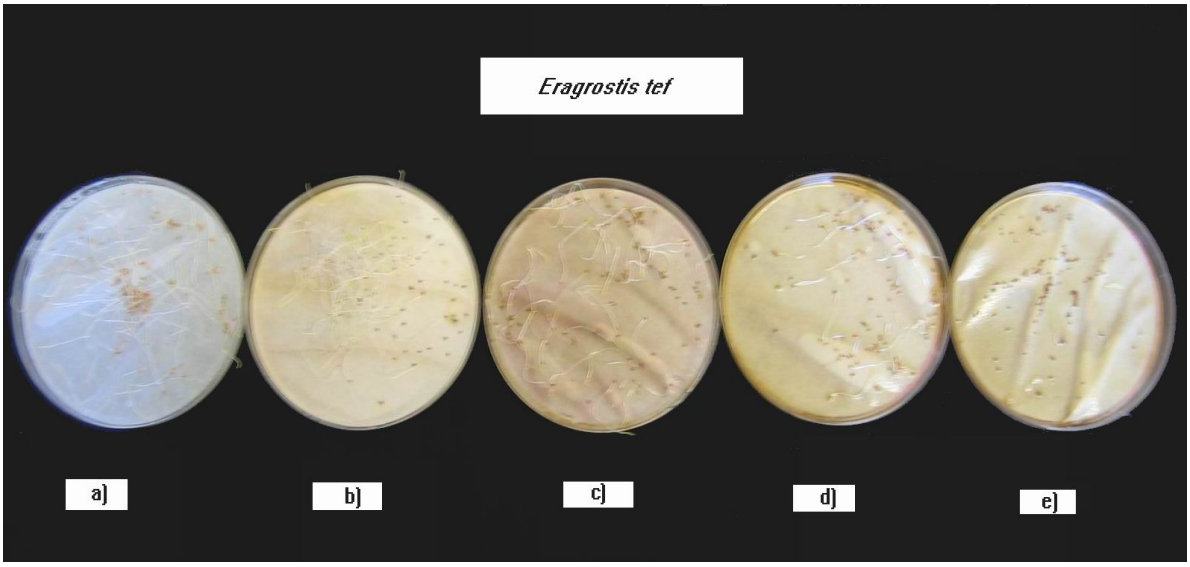
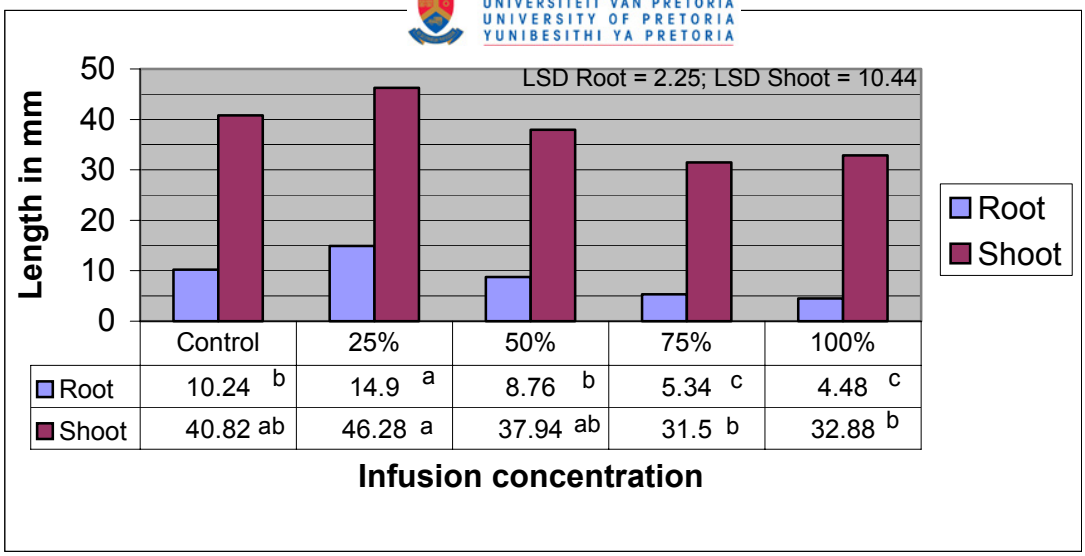


Figure 3.3 Effect on growth of *Eragrostis tef* seedlings grown from seed exposed to different *C. macrocephalum* infusion concentrations, from left to right, (a) Control (distilled water), (b) 25%, (c) 50%, (d) 75%, (e) 100%



	Control	25%	50%	75%	100%
Root	10.24 b	14.9 a	8.76 b	5.34 c	4.48 c
Shoot	40.82 ab	46.28 a	37.94 ab	31.5 b	32.88 b

Figure 3.4 Effect of *C. macrocephalum* leaf infusions on root and shoot growth of *Eragrostis tef* seedlings after seeds were incubated for eight days (ANOVA in Appendix B, Table B3 and B4)

Means followed by the same letters are not significantly different

From Figure 3.4 it can be seen that *Eragrostis tef* roots were generally more sensitive to *C. macrocephalum* infusions than the shoots. This difference in susceptibility was pronounced at 75% and 100% infusion concentrations. Compared to the control treatment, significant reduction in root growth first occurred at 75% concentration, but for shoot growth not even the 100% concentration decreased growth significantly. The difference in susceptibility between roots and shoots was also found by Mersie and Singh (1987) in experiments on *P. hysterophorus* where root inhibition increased with an increase in concentration of the water extract used. There appeared to be stimulation of growth at the 25% concentration in the case of roots of this grass. Grass root growth was inhibited from 50% infusion concentration and higher. This phenomenon where there is stimulation at low concentrations and reduction of growth at high concentrations of the same plant extract or of pure allelochemicals is known as hormesis (Belz *et al*., 2005).

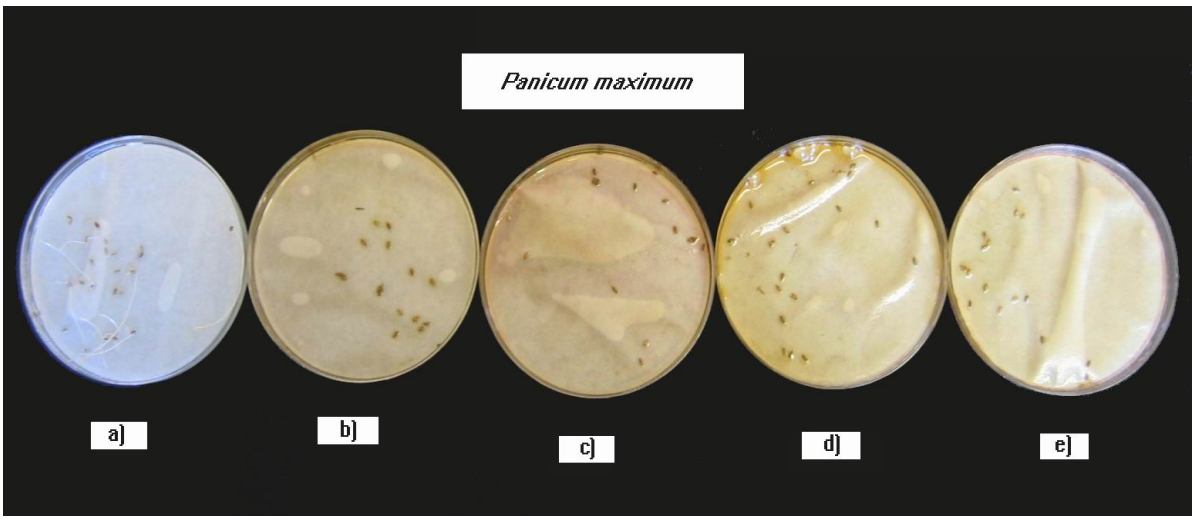
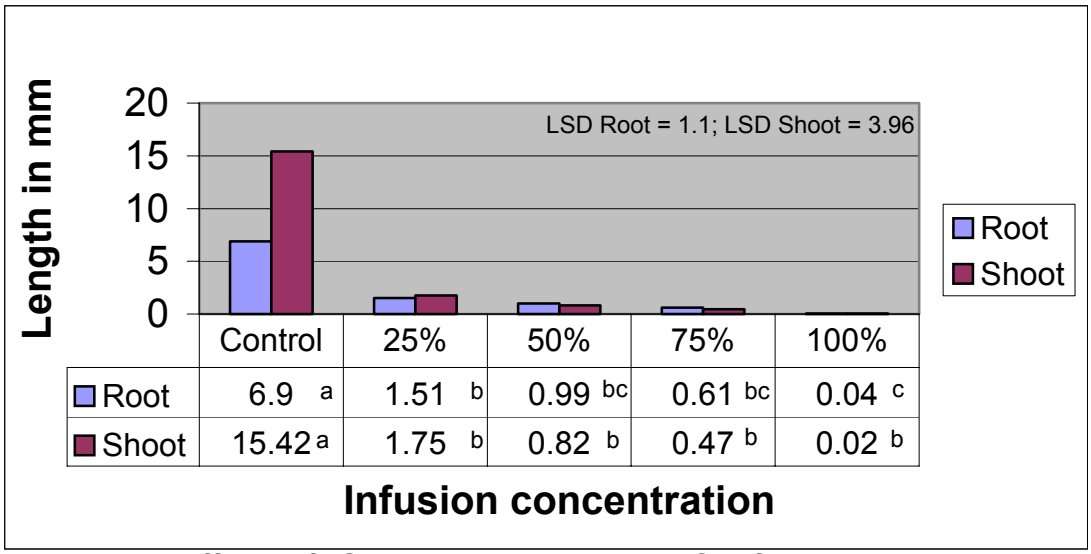


Figure 3.5 Effect on growth of *Panicum maximum* seeds grown in *C. macrocephalum* infusion concentrations, from left to right, (a) Control (distilled water), (b) 25%, (c) 50%, (d) 75%, (e) 100%



	Control	25%	50%	75%	100%
Root	6.9 a	1.51 b	0.99 bc	0.61 bc	0.04 c
Shoot	15.42 a	1.75 b	0.82 b	0.47 b	0.02 b

Figure 3.6 Effect of *C. macrocephalum* leaf infusions on root and shoot growth of *Panicum maximum* seedlings after seeds were incubated for eight days (ANOVA in Appendix B, Table B5 and B6)

Means followed by the same letters are not significantly different.

Panicum maximum was more sensitive to *C. macrocephalum* infusions than the other two grass species (Figures 3.4, 3.5, 3.6). Both root and shoot growth of *P. maximum* were already significantly inhibited by the 25% infusion concentration. Shoot growth of this grass species was especially sensitive. At 100% concentration of infusion the germination percentage was effectively zero. The same effect occurred in experiments on *P. hysterophorus* where at high concentrations of extract no germination of test species occured (Kohli *et al*., 1996). *Parthenium*

hysterophorus leachates where concentrations (63 to 250 mg leaf material per ml) significantly inhibited germination of liverseed grass (*Urochloa panicoides* Beauv.), foxtail buffalo grass (*Cenchrus ciliaris* L.) and climbing buckwheat (*Polygonum convolvulus* L.) (Adkins and Sowberry, 1996).

Mersie and Singh (1987) demonstrated species-specific allelopathy found in barley (*Hordeum vulgare* L.) because its inhibitory action varied with test species. Species-specific allelopathy was also demonstrated for *C. macrocephalum* in the present study where *E. tef* was less affected (and even grew well in the 100% concentration of infusions) than *E. curvula,* and where *P. maximum* was extremely sensitive across the range of *C. macrocephalum* infusions. Van der Laan, Reinhardt, Belz, Truter, Foxcroft and Hurle (2008) found that the root length and germination percentage of *P. maximum* were more sensitive than the same parameters for *E. curvula* when exposed to *P. hysterophorus* infusions in a laboratory bioassay.

Mersie and Singh (1987) state that the discharge of allelochemicals into the environment may occur in a number of ways, such as: by the exudation of volatile chemicals from living plant parts; leaching of water-soluble toxins from above-ground plant parts possibly after rain as well as from below-ground parts; and finally from the release of toxins from decaying plant matter. All of these methods of allelochemical release could be possible where *C. macrocephalum* is currently invading, thus promoting the survival of the plant in its new habitat. The higher the density of the weed the greater its allelopathic effect could be. Several weed species such as Johnson grass (*Sorghum halepense*), purple sage (*Salvia leucophylla*) and California sagebrush (*Artemisia californica*) that form dense stands are reported to exert an allelopathic influence on their surrounding flora (Kanchan and Jayachandra 1979).

3.4 Conclusion

All three grass species assayed were negatively affected by the allelochemicals contained in the *C. macrocephalum* leaf infusions. *Panicum maximum* was the most sensitive grass while *E. tef* was the least sensitive, with indications of hormesis occurring in the latter case due to growth stimulation at 25% infusion concentration. As was found by Reinhardt *et al.* (2006) for *P. hysterophorus*, the possible

implications for the field situations where *C. macrocephalum* is invading are that some grasses are likely to be more sensitive than others, and then at the very vulnerable growth stage of establishment of seedlings. Irrespective of how vigorous or robust a grass species may be during its lifetime, failure to establish well or even to establish at all, will be the key determining factor of the sensitivity of the grass population towards invasion by *C. macrocephalum*. This weed has very prominent root reserves that enable quick regeneration and growth in the spring (Henderson, 2001) – this characteristic will be an advantage in competition of the weed with grasses. With both competition and allelopathy in its arsenal the weed is likely to interfere strongly with both grass establishment from seed and with regrowth from dormant tufts. Further work on the allelopathic properties and effects of *C. macrocephalum* are required; the susceptibility of grasses need to be ascertained not only at seed germination and early seedling development growth stages, but also for regrowth from dormant tufts in the case of perennial species.

CHAPTER 4
LINK BETWEEN ALLELOPATHIC POTENTIAL AND THE CAPITATE-SESSILE GLANDS AND TRICHOMES ON LEAF SURFACES OF *CAMPULOCLINIUM MACROCEPHALUM* (LESS) D. C.

4.1 Scanning electron microscopy study of leaf surfaces

4.1.1 Introduction

Plants produce many secondary compounds that may have diverse biological activities and economic value in functions such as pharmaceuticals, neutriceuticals, natural pesticides, flavourings, and fragrances or even for non-food or fibre purposes (Duke *et al.*, 2000). To maximise their interactions with the outside world these secondary plant compounds are often produced and/or stored on or near the plant surfaces, many being contained in specialised cells called glandular trichomes - where these products can be contained in concentrated form for maximum effect when sequestered. This storage method will help to avoid autotoxicity in the plant.

A great variety of structures may be found on organs of a number of plant species. These projections may vary in size, shape, number of cells, location on the plant, function and many more aspects (Werker, 2000). It is believed that the highest content of phytotoxic compounds in plants can be found in the leaves. These plant-inhibiting substances may be found in specialised structures inside or on top of the leaves (Roshchina and Roshchina, 1993). Secondary metabolites may be concentrated in trichomes, glandular hairs, stinging hairs or in the upper layer of the epidermis itself (Wink, 1999).

Trichomes may be defined as multicellular or unicellular appendages that originate only from epidermal cells. They develop outwards on the surfaces of plant organs. None of the many methods of their classification is wholly satisfactory. Each method has its own drawbacks, overlaps and exceptions (Werker, 2000).

There are two major distinctions between trichomes – that is, are they glandular or non-glandular? Non-glandular trichomes are distinguished by their morphology. The

compounds that they secrete, accumulate and/or absorb primarily distinguish glandular trichomes. Glandular trichomes may also differ in their mode of production, in their structure and in their location (similar glands but on vegetative or reproductive organs of the plant) (Werker, 2000).

When looking at the structure of trichomes, the cell walls of non-glandular trichomes may consist of a primary cell wall only or of a thick secondary wall as well (Werker, 2000). This secondary wall may be evenly or unevenly thickened. All or just parts of the cell wall may be impregnated by such substances as lignin, cutin or suberin to name but a few (Duke *et al.*, 2000). The cuticle of non-glandular trichomes may acquire different thicknesses. Its outer surface may vary in texture and shape (Werker, 2000). The lateral walls of glandular trichomes may become cutinised or suberised or both. These walls also act as caspari strips in preventing apoplastic backflow of secreted substances. Glandular trichome surfaces may be smooth or exhibit micro-orgmentation and if this orgmentation shows high diversity it will cause the glands to appear macropillate, warty, reticulate or striate (Werker, 2000).

Trichomes are diverse in shape, size, structure, location, capability and function (Werker, 2000). Further studies of the chemical contents of trichomes and their role in the plant's chemical ecology will help in gaining better understanding of natural plant protection methods and lead to the possible discovery of natural compounds used for biological protection (Duke *et al.*, 2000).

In this chapter the results of a study of the leaf anatomy of *Campuloclinium macrocephalum* (Less) D. C. are presented in an attempt to relate specific trichomes and/or glands to its allelopathic potential detected in previous bioassay experiments (see previous chapters). Therefore, the primary focus was on identifying trichomes and/or glands on the leaf surfaces that could be linked to the release of phytotoxic allelopathic substances.

4.1.2 Materials and methods

Plant material

Leaves of *Campuloclinium macrocephalum* were collected from plants at anthesis at the Swartkops Air force Base in Valhalla, Pretoria. Leaves of two sizes were selected – mature leaves that were about 60 mm in length, and young leaves about 10 mm long. Plant material was collected on 10 March 2006 and kept frozen until it was freeze-dried just before use in the electron microscopy experiment. After freeze-drying, the plant material was stored in a fridge.

Scanning electron microscopy (SEM)

Leaf sections of approximately 3 x 5 mm were excised from the middle of the laminas between the mid-rib and leaf margin and then mounted on an aluminium stub. Two sections were made from each lamina – the abaxial and adaxial sides were mounted for both the mature and young leaves. After mounting, the stub with the leaf surfaces exposed was coated in gold using a SEM Autoclaving unit E5200. The coating process was performed six times. Colloidal carbon was placed on the edges of the leaves on top of the gold coating. Colloidal carbon was used as a glue and for its conductive properties to conduct excess electrons away from the areas to be examined. Observations and photographs were made on a JEOL JSM 840 scanning electron microscope.

4.1.3 Results and discussion

Numerous capitate-sessile trichomes and sitting glands were found on both the adaxial and abaxial leaf surfaces (Figures 4.1-4.6). These glands may or may not be the site for storage of allelopathic compounds or secondary metabolites produced by the plant. Sessile glands and trichomes were found on both the young and mature leaf surfaces (Figures 4.1-4.4).

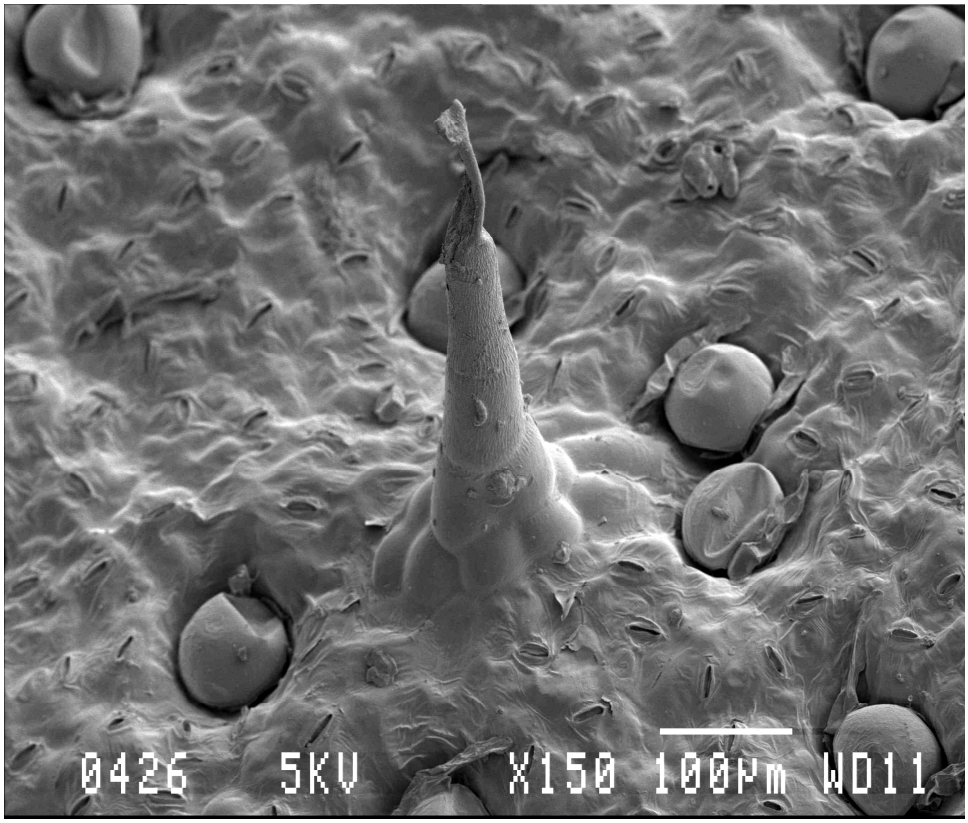


Figure 4.1 Trichome and capitate-sessile glands on the abaxial surface of mature leaves of *C. macrocephalum*

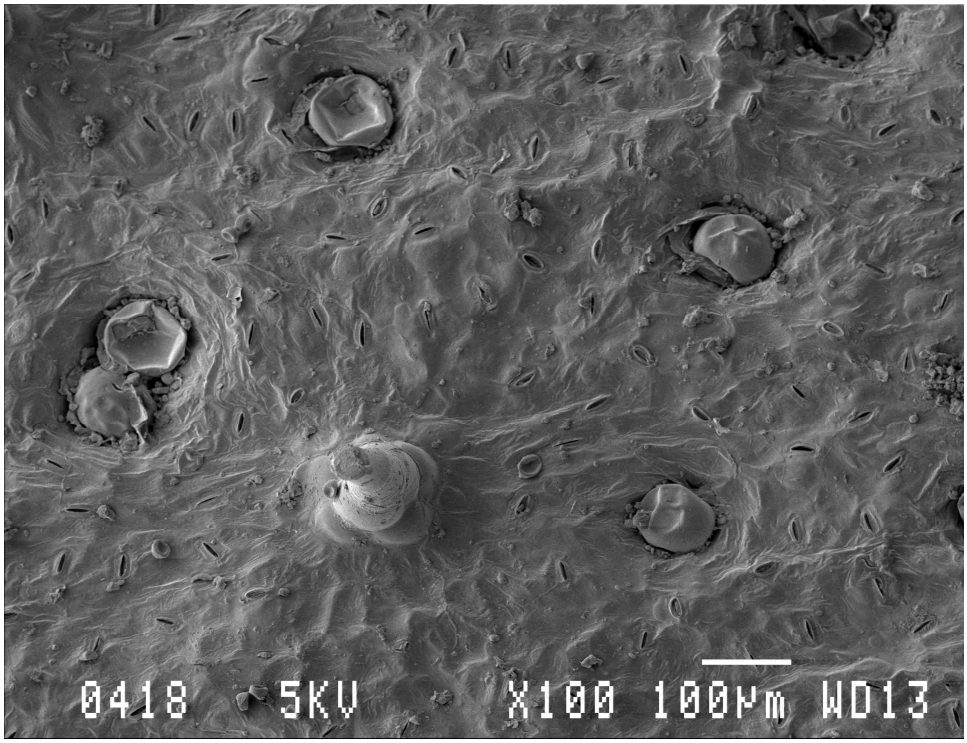


Figure 4.2 Trichome and capitate-sessile glands on the adaxial surface of mature leaves of *C. macrocephalum*

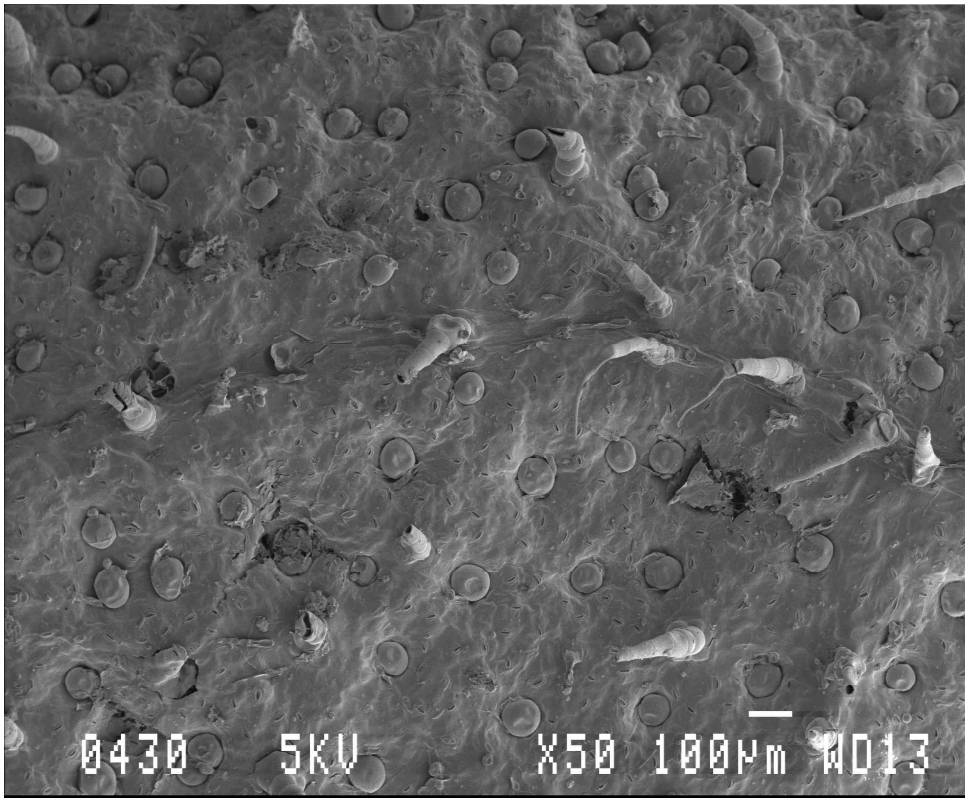


Figure 4.3 Trichomes and capitate-sessile glands on the abaxial surface of young leaves of *C. macrocephalum*

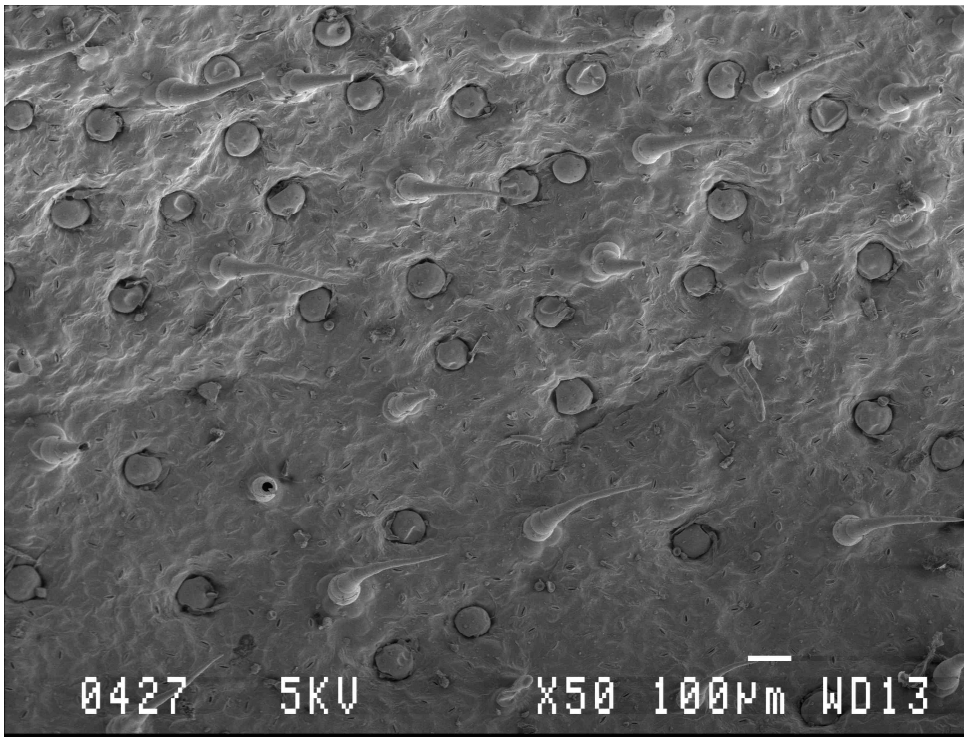


Figure 4.4 Trichomes and capitate-sessile glands on the adaxial surface of young leaves of *C. macrocephalum*

As can be seen from Figures 4.1, 4.2, 4.3 and 4.4 there appear to be trichomes and capitate-sessile glands on both the adaxial and abaxial surfaces of the young and mature leaves of *C. macrocephalum*. This trait is also seen in another member of the Asteraceae family, *Parthenium hysterophorus* (Reinhardt *et al*., 2004). In the present study it was determined that there is a thick cuticle that could include a wax layer over the leaf surfaces (on both young and old leaves) of *C. macrocephalum* (Personal communication, Professor P. J. Robbertse, University of Pretoria). The leaves are amphistomatic (have stomata on both abaxial and adaxial surfaces of the leaves). The sessile glands seem to be located between the cuticle and epidermis layer of the leaf. The thickness of the cuticle could mean that the release of possible allelochemicals could be prolonged in this way. This could also have an effect on residue release from the plant after death. The thick cuticle could also pose a barrier to herbicide absorption by the plant. Wagner (1991) states that storage of possible secondary metabolites (allelochemicals) occurs in the space between gland cell walls and the cuticle outside the plant body.

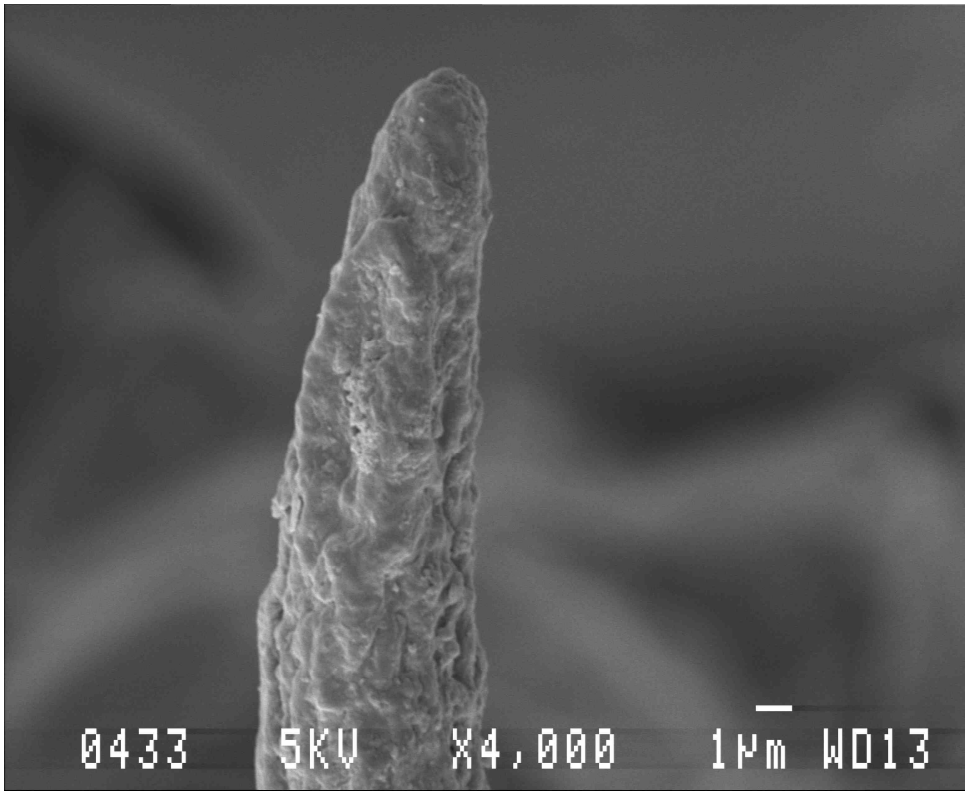


Figure 4.5 Tip of a trichome on the abaxial leaf surface of *C. macrocephalum*

From Figure 4.5 it can be seen that the tips of the capitate-sessile trichomes are quite large – a size of 1 μm as compared to the size of a razor blade tip which is 2 μm. This could explain the skin irritation that develops when the plant is handled without protective clothing. A similar skin irritation or contact dermatitis was found in *P. hysterophorus* plants handled without protective gear, after such discovery and consequent experiments it was concluded that the allelochemical parthenin is present in different plant parts, particularly the trichomes on leaves and stems (Reinhardt *et al*., 2004), this could possibly be the case in *C. macrocephalum* too, but further research is needed to confirm this.

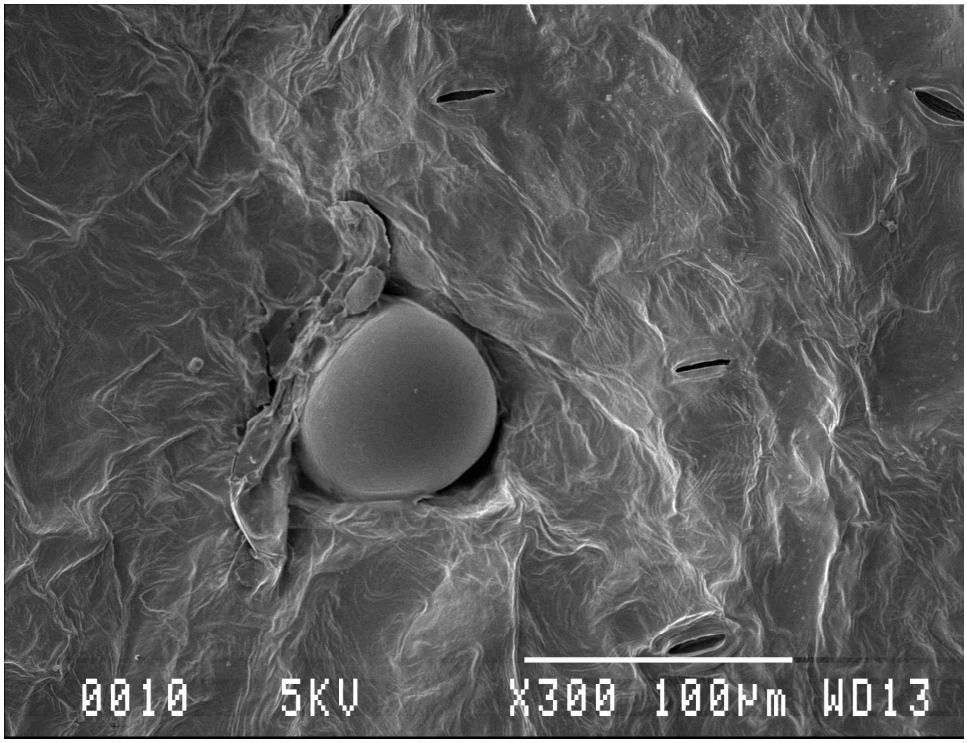


Figure 4.6 Sessile gland and stomata on adaxial leaf surface of *C. macrocephalum*

In Figure 4.6 it is clear that the capitate-sessile glands that sit between the cuticle and epidermis are much larger than the stomata on the leaf surface of *C. macrocephalum*. This could be due to the contents held by the glands – as seen in *P. hysterophorus* – the glandular capitate-sessile glands are the source of the allelochemical parthenin (Reinhardt *et al*., 2004). The production and storage of allelochemicals in these capitate-sessile glands and trichomes could be a mechanism to prevent autotoxicity within the plant, in this way preventing

allelochemical interference with important processes within the plant. This has been seen in *P. hysterophorus* where parthenin and coronopilin have been found to be restricted mostly to trichomes (Picman and Picman, 1984). An advantage to producing and storing secondary plant metabolites in trichomes on the leaves could lie in protection against herbivorous attack and diseases – since these will be the first plant parts to come in contact with the foe (Picman and Picman, 1984).

In a study by Retief (2002) on the tribe Eupatorieae with focus on *Chromolaena odorata* eradication in South Africa, certain plant parts of *C. odorata* and *C. macrocephalum* were compared. The bracts and achenes of *C. macrocephalum* were found to contain capitate-sessile glands. No electron microscopy work was reported on the leaves of *C. macrocephalum* in that study. Capitate-sessile glands may be common on above-ground plant parts of *C. macrocephalum*. Further study will have to be made to determine the distribution and contents of these glands and their functioning with regards to protection and survival of the plant.

4.1.4 Conclusion

It is clear from the photographs obtained from SEM that the plant species *C. macrocephalum* possesses trichomes and capitate-sessile glands on the surface of its leaves; a characteristic similar to that of its relative *P. hysterophorus* (Reinhardt *et al*., 2004). These glands could be sites of storage and release of possible allelochemicals produced by the plant. Further research will be required to determine the true contents and functioning of the glands of the weed. Such research could provide insight into the invasive ability of this noxious weed, especially as regards the role of allelochemicals produced by it.

4.2 Assessment of the contribution of substances on the leaf surfaces of *C. macrocephalum* towards its allelopathic potential

4.2.1 Introduction

Roschina and Roschina (1993) state that the highest content of phytotoxic compounds in plants can be found in the leaves and that these plant-inhibiting substances may be contained in specialised structures. These specialised cells may have various functions, such as secretion. The secretory cells occur on the external surfaces of many plants in the form of trichomes (Fahn, 2000). Secondary metabolites are often concentrated in trichomes or glandular hairs, stinging hairs or in the epidermis itself (Wink, 1999). Specialised secretory glandular trichomes produce secondary metabolites (allelochemicals) that may be stored or volatised from the leaf surface. Storage of these metabolites may occur between the gland cell walls and the cuticle outside the plant body (Wagner, 1991).

Secondary metabolites are considered to be allelopathic by depressing the seed germination of other plant species and thereby increasing the competitiveness of the plant containing the metabolites in its trichomes (Roschina and Roschina, 1993). Kanchan and Jayachandra (1980) found growth-inhibiting metabolites contained in soft, fine trichomes of *Parthenium hysterophorus* L. (a relative of *Campuloclinium macrocephalum*). These metabolites caused allelopathic growth-inhibition of ten day old wheat (*Triticum aestivum* L.) seedlings. Macías *et al.* (1996) states that sesquiterpene lactones found in glandular trichomes on the leaves of sunflower (*Helianthus annuus* L.) caused allelopathic growth-inhibition in roots and shoots of the test species lettuce (*Lactuca sativa* cultivar: Nigra), tomato (*Lycopersicon esculentum* Mill.), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) There has also been a description by Roschina and Roschina (1993) of the secretion of alkaloids by glandular hairs of two Solanaceous species, *viz*. potato (*Solanum tuberosum* L.) and tobacco (*Nicotiana tobacum* L.). The potential that secreting trichomes (such as those from the above-mentioned species and possibly including *C. macrocephalum*) accumulate allelochemicals is highly significant and under optimal conditions, secreted substances can reach levels of up to 10 to 30% of a plant's dry weight (Kelsey and Reynolds, 1984).

The brief dipping of leaves in an organic solvent is a rapid method to determine the yield of secretions found on the leaf surfaces of a plant (Wagner, 1991). Duke *et al.* (1994) describes the importance of a study exploring the phytotoxic activity of a leaf wash of fresh intact foliage with water or an organic solvent. The use of an organic solvent in allelochemical extraction is effective due to the minimisation of possible reactions with degrading enzymes. Such a leaf wash would include most, if not all, the substances present on the leaf surfaces. This will enable the determination of whether allelochemicals produced by a specific plant are indeed located in structures such as glandular trichomes on the leaf surface or if they are secreted onto the leaf surface by specialised secretory cells. This method is widely used in tobacco to recover exudates simply and cleanly by submersion of the plant in a non-invasive solvent (Wagner, 1991).

In the bioassay reported on here, the contribution of secondary metabolites located on the leaf surfaces of *C. macrocephalum* to the suppressed germination and development of seedlings of *L. sativa* (cultivar: Great Lakes) was examined in order to determine a possible contribution of allelochemicals exclusively located on the leaf surface to *C. macrocephalum*'s allelopathic potential. Furthermore, scanning electron microscopy (SEM) sections of dipped leaves were prepared to investigate the leaf surface before and after dipping in the organic solvent in order to detect possible changes to the trichomes.

4.2.2 Materials and methods

Campuloclinium macrocephalum seeds, collected in 2005 from Valhalla in Thswane, were planted on 31 October 2006. The seeds were planted in 3 kg potted soil taken from the University of Pretoria's Hatfield Experimental Farm. The seedlings were thinned on 17 December 2006 to two plants per pot and further cultivated in the glasshouse with a mean temperature of about 20 °C and a relative humidity of about 35%. The plants were watered with tap water daily and received a complete nutrient solution three times per week. On 27 February 2007, leaves from the cultivated plants were picked randomly to perform the dipping test. Leaves used were all of similar size – approximately 80 mm in length. The dipping experiment took place at

the Phytotron D laboratory on the University of Pretoria's Hatfield Experimental Farm.

Leaf-dipping experiments

Bioassays for determining the effect of the metabolites exclusively located on the leaf surface of *C. macrocephalum* on germination and seedling development of *L. sativa* were done to determine the possible contribution of those allelochemicals to the allelopathic potential of the weed. Different concentrations of the organic solvent were used because of the unknown structural strength of the glands and trichomes reported on in Chapter 4.1.

Bioassay 1: Five second dipping in dichloromethane

Ten leaves, weighing 15.06 g, were picked from mature *C. macrocephalum* plants. The leaf surface area of the leaves was measured with a LI-3100 Area Meter and found to be 388.46 cm^2 (which should be multiplied by two for a total leaf surface area as trichomes and capitate-sessile glands are found in approximately equal numbers on both the abaxial and adaxial surfaces of the leaves). These leaves were subsequently dipped in 100 ml dichloromethane (this relates to aqueous infusion concentrations from previous chapters – 150 g leaf material in 1000 ml water) for five seconds. Each leaf was dipped separately making sure that the leaf petiole was not included in the dip. This solution was regarded as full strength (100%). Dichloromethane was then used to make up concentrations of 25%, 50%, and 75%. Pure dichloromethane was used as a control treatment. The bioassay was conducted in glass Petri dishes (90 mm diameter) lined with one layer of Whatman No. 1 filter paper and wetted with 5 ml of the respective test solutions. The dichloromethane was then allowed to evaporate in a laminar flow cabinet. Once the filter paper had dried, 5 ml of distilled water and ten *L. sativa* seeds (cultivar Great Lakes) were added to the Petri dishes. After sealing the Petri dishes with parafilm, they were placed in the dark in a growth chamber at ± 26 °C for eight days. For each extract concentration five replications were used. After eight days the root length (≥1mm), shoot length (≥1mm) and germination percentage were determined.

Bioassay 2: Ten second dipping in dichloromethane

Ten intact *C. macrocephalum* leaves (15.19 g; Leaf surface area 407.10 cm^2) were collected and subsequently dipped in 100 ml dichloromethane for 10 seconds. Each leaf was dipped separately. Care was taken not to include the petiole of the leaves when dipping in the solvent. This solution was regarded as full strength extract (0.15 g/ml i.e. 100%). Dichloromethane was then used to prepare dilutions (25%, 50%, 75%) and for the control treatment. For the control treatment dichloromethane only was used. Bioassays were conducted in glass Petri dishes (90 mm diameter) lined with one layer of Whatman No. 1 filter paper and wetted with 5 ml of the respective test solution. The dichloromethane was allowed to evaporate in a laminar flow cabinet. After the filter paper had dried 5 ml of distilled water and ten *L. sativa* seeds (cultivar Great Lakes) were added. Petri dishes were sealed with parafilm and stored in a dark growth chamber at ±26 °C. For each extract concentration five replications were performed. After eight days root length (≥1 mm), shoot length (≥1 mm) and germination percentage were determined.

Bioassay 3: Ten second dipping in dichloromethane, followed by 24 hours in distilled water

The same process as described above was then repeated with 10 leaves (16.01 g, leaf surface area 422.26 cm^2) that were dipped in dichloromethane for 10 seconds and then soaked in distilled water for 24 hours.

Bioassay 4: 24 hour soaking of leaves in distilled water

This is the control or standard bioassay as allelopathic potential for *C. macrocephalum* has been found based on this method previously (Chapter 2). Ten intact *C. macrocephalum* leaves (15.03 g; leaf surface area 371.39 cm^2) were placed in a beaker of distilled water (100.20 ml), stirred, covered and left in a dark place for 24 hours. After 24 hours the infusion was diluted into concentrations of 25%, 50%, 75%, and 100% (undiluted infusion). A control of 100 ml distilled water was included. Ten lettuce seeds were placed on filter paper in each Petri dish. Five ml of infusion was poured onto the filter paper. Five replications of each

concentration were prepared. The Petri dishes were then sealed with parafilm, and stored in a dark growth chamber at ±26 °C for eight days. The germination percentage, root and shoot length of each plant were measured on day eight.

Only seedlings with a root or shoot length of more than 1 mm were measured. Seeds that failed to develop a root or shoot to this extent were considered not germinated, and therefore, for them root and shoot length were regarded as zero. For statistical analysis a general linear model (GLM) of ANOVA was used. Significant differences between treatment means was assessed with Tukey`s Studentised range test using least significant differences (LSD) at $P<0.005$.

Scanning electron microscopy

Scanning electron microscopy (SEM) was performed in conjunction with each bioassay. Thus SEM was done on leaf sections of leaves that were:

1. Dipped in dichloromethane for five seconds
2. Dipped in dichloromethane for ten seconds
3. Dipped in dichloromethane for ten seconds, then soaked in distilled water for 24 hours
4. Soaked in distilled water for 24 hours
5. Fresh, undipped leaves were used as a SEM control comparison.

After their use in the bioassay experiment the intact leaves from each bioassay were frozen until their use in the SEM section of the experiment when they were freeze-dried and then taken to the lab for SEM work to begin. For scanning electron microscopy (SEM) leaf sections of approximately 3 x 5 mm were excised from the middle of the laminas between the mid-rib and leaf margin. After mounting, the stub with the leaf surfaces exposed was coated in gold using a SEM Autoclaving unit E5200. The coating process was performed six times. Colloidal carbon was placed on the edges of the leaves on top of the gold coating. Colloidal carbon was used as a glue and for its conductive properties to conduct excess electrons away from the areas to be examined. Observations and photographs were made using a JEOL JSM 840 scanning electron microscope.

4.2.3 Results and discussion

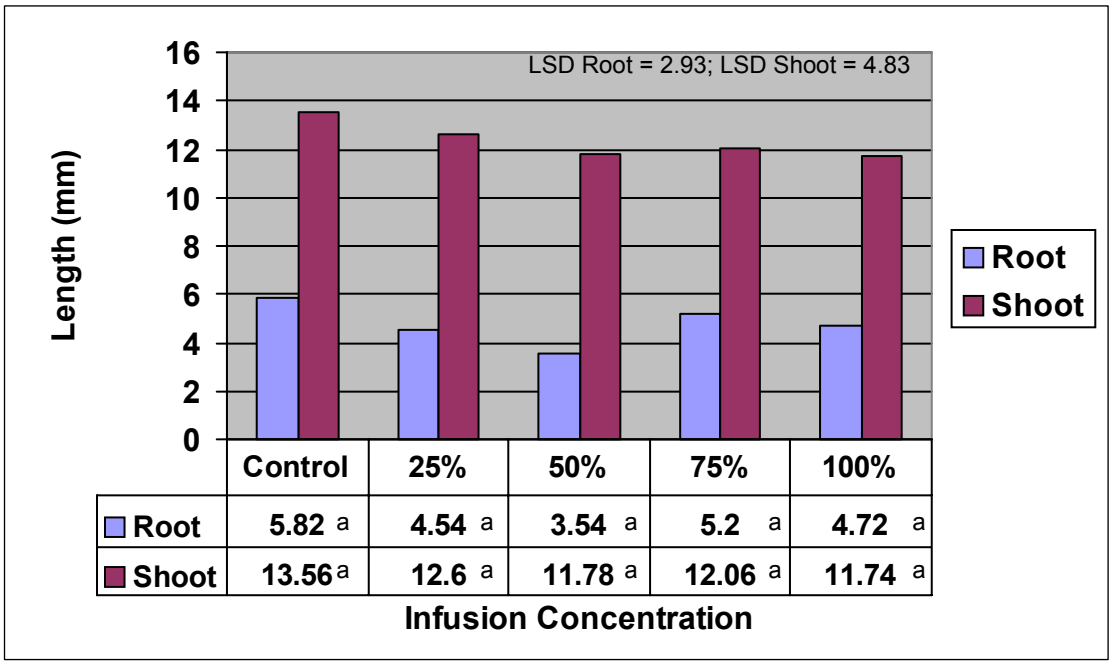


Figure 4.7 Root and shoot length of lettuce seedlings exposed to different dichloromethane solutions prepared by dipping *C. macrocephalum* leaves for five seconds – Bioassay 1 (ANOVA in Appendix C, Table C1 and C2)

Means followed by the same letters are not significantly different.

Bioassay 1

The results for this bioassay did not show any specific growth tendency, e.g., hormesis or growth inhibition as reported by Belz and Hurle (2004). This does not correspond with findings by Kraus (2003) from similar experiments on *Parthenium hysterophorus*, where high biological activity was found in similar bioassays where leaves were dipped in the organic solvent, dichloromethane.

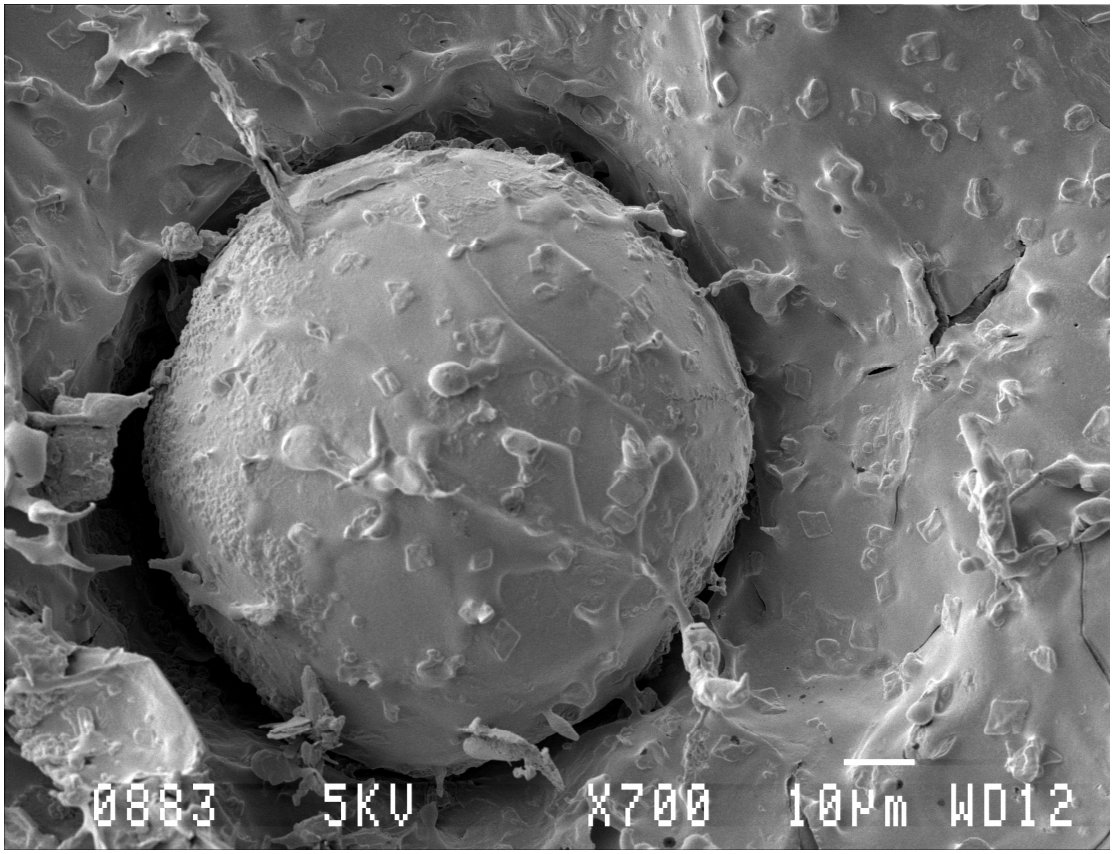
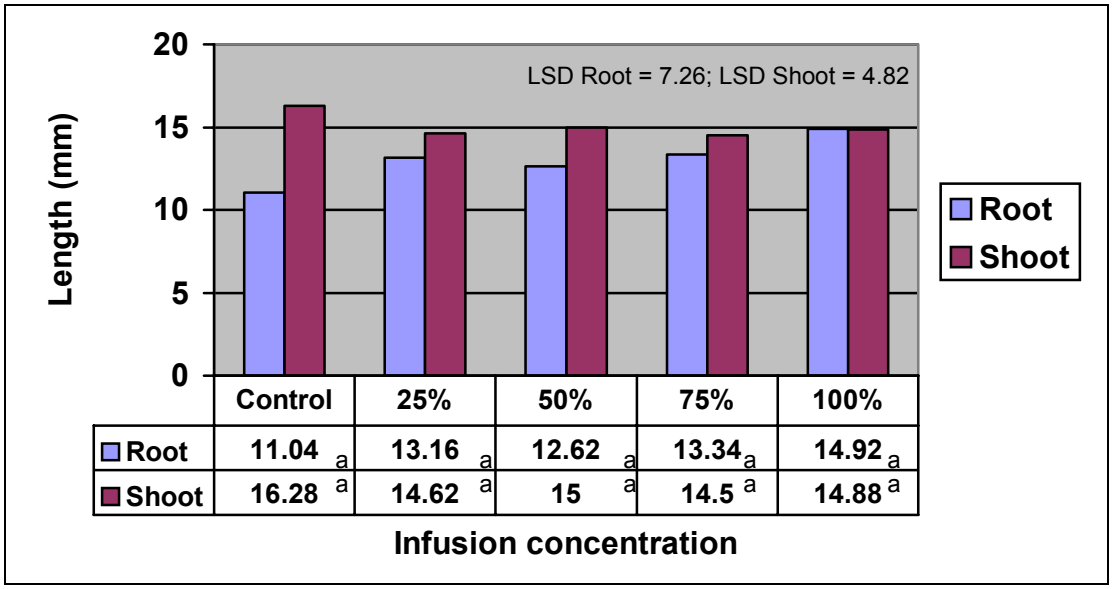


Figure 4.8 Effect of dichloromethane on *C. macrocephalum* leaf after dipping for five seconds

The above scanning electron micrograph shows that very little damage was caused by the dichloromethane to the capitate-sessile gland and cutilcle of the leaf after dipping for five seconds. The surface of the leaf is no longer smooth as was seen in section 4.1. In a leaf washing (dipping) experiment on *P. hysterophorus* reported by Reinhardt *et al*. (2004) leaves dipped in TBME yielded up to 13.4 mg/g of the allelochemical parthenin whereas the aqueous extract of leaves gave only 1.3 mg/g. They reported that capitate-sessile glands appeared deflated after dipping. This was not the case with *C. macrocephalum* as can be seen in Figures 4.7 and 4.8 where the dichloromethane had little to no effect on the leaf surfaces and glands, which perhaps explains why no allelochemicals were apparently released into the dichloromethane after five seconds.



	Control	25%	50%	75%	100%
Root	11.04 a	13.16 a	12.62 a	13.34 a	14.92 a
Shoot	16.28 a	14.62 a	15 a	14.5 a	14.88 a

Figure 4.9 Root and shoot length of lettuce seedlings exposed to different dichloromethane solutions prepared by dipping *C. macrocephalum* leaves for ten seconds – Bioassay 2 (ANOVA in Appendix C, Table C3 and C4)

Means followed by the same letters are not significantly different

Bioassay 2

No clear tendency for either hormesis or growth-inhibition was observed (Figure 4.9). No significant differences were found in Bioassay 2.

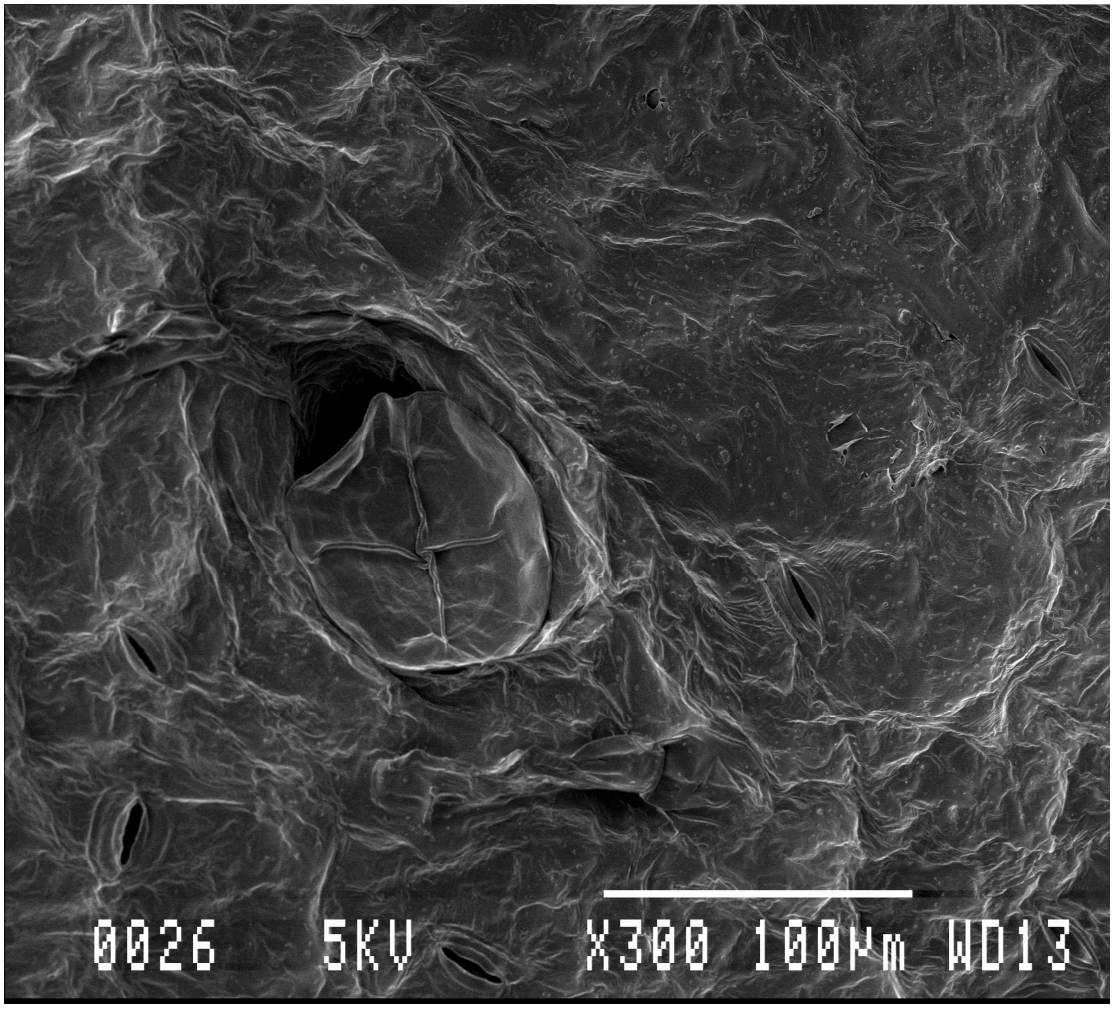


Figure 4.10 Effect of dichloromethane on *C. macrocephalum* leaf after dipping for ten seconds

Figure 4.10 shows the effect of dichloromethane on the *C. macrocephalum* leaf after ten seconds. The surface seems to look the same as the surface of the leaf after dipping for five seconds. There is still very little damage to the leaf surface, although the capitate sessile gland does seem to have collapsed. Duke *et al*. (1994) found that dipping leaves of *Atrimisia annua* into chloroform for a few seconds removed the contents of the peltate glands without causing any structural damage other than collapsing the cuticle covering these glands. Reinhardt *et al*. (2004) found similar effects (collapsed glands) in a study on *P. hysterophorus* when the contents of capitate sessile glands were removed using dichloromethane. In the experiment by Reinhardt *et al*. (2004), besides collapsed capitate-sessile glands, SEM showed a slit in the cell wall of capitate-sessile glands on *P. hysterophorus* leaves with other trichomes appearing relatively unaffected. Kraus (2003) observed high biological

activity towards test species in bioassays using these extracts. This was not the case with *C. macrocephalum*. The graphs in Figures 4.7 and 4.9 show no specific growth tendencies, specifically hormesis – an important indicator of allelopathic potential of a plant (Belz and Hurle, 2004). Thus it is assumed that allelochemicals are either not found on the leaf surfaces of *C. macrocephalum* or they must be insoluble in the organic solvent dichloromethane.

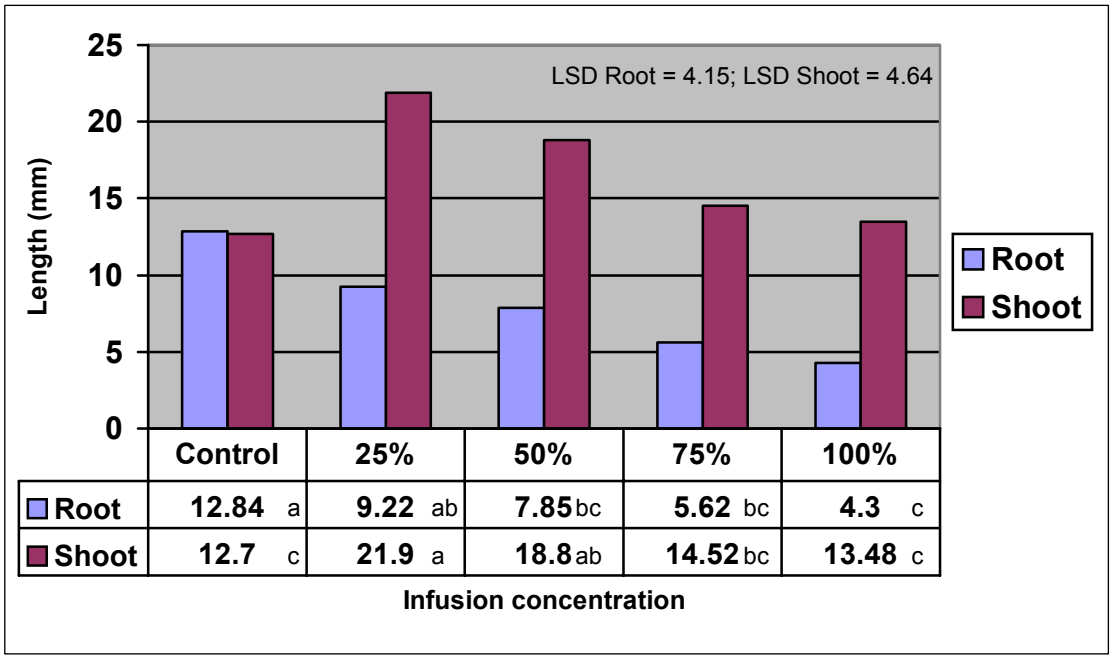


Figure 4.11 Root and shoot length of lettuce seedlings exposed to different solutions prepared by dipping *C. macrocephalum* leaves in dichloromethane for ten seconds and then soaking in distilled water for 24 hours – Bioassay 3 (ANOVA in Appendix C, Table C5 and C6)

Means followed by the same letters are not significantly different

Bioassay 3

From the above graph it can be seen that the lettuce shoots showed a typical hormesis response to the aqueous infusion prepared after leaves were dipped in dichloromethane. The roots however showed a progressive decrease in growth with increase in infusion concentration. This finding points to water-soluble allelochemicals being responsible for the allelopathic potential displayed by *C. macrocephalum.*

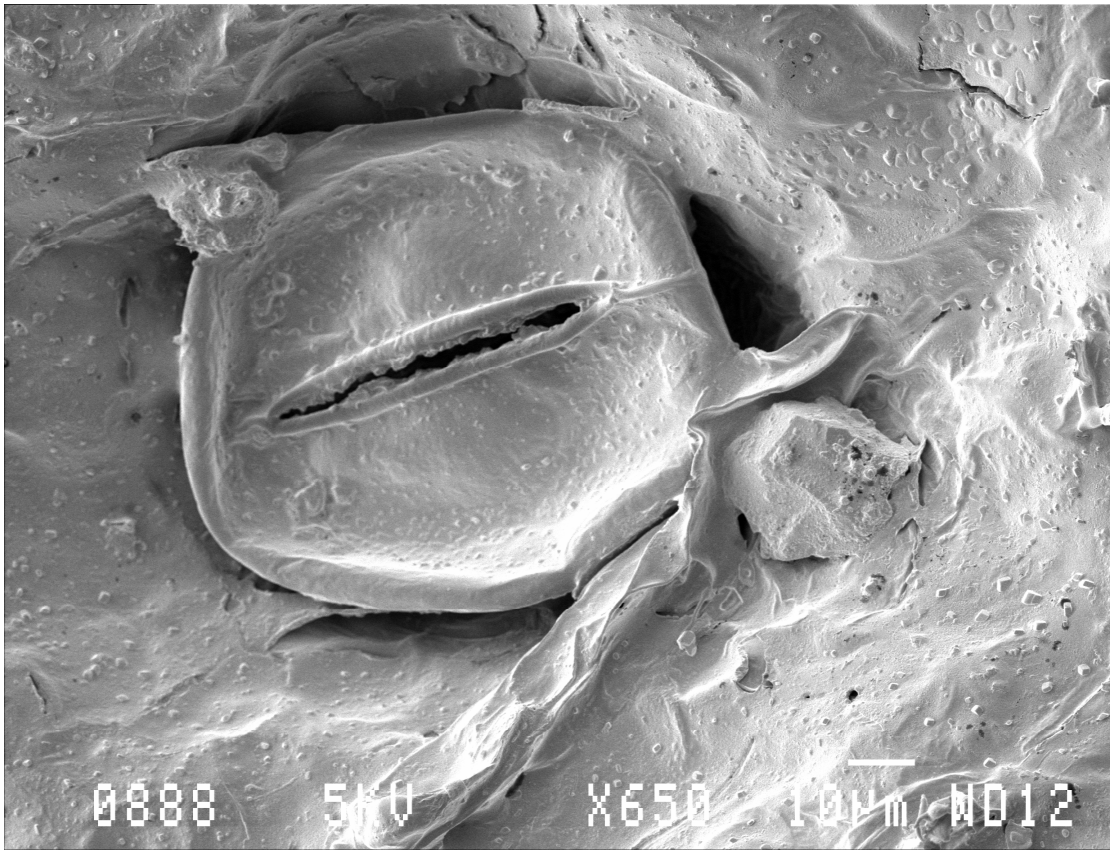


Figure 4.12 Effect on *C. macrocephalum* leaf after dipping in dichloromethane for ten seconds, and then soaking in distilled water for 24 hours

The effect of dipping the *C. macrocephalum* leaf for ten seconds and then soaking it for 24 hours in distilled water can be seen in Figure 4.12 – the capitate-sessile gland appears to consist of two cells and when treated this way a schizogenesis split results (Personal communication, Professor P. J. Robbertse, University of Pretoria). Further work is needed to confirm structure and function of the glands. In appearance this gland resembles the capitate-sessile glands which Reinhardt *et al.* (2004) found to contain virtually pure parthenin. This could possibly be the site of release of the allelochemicals. This effect where the organic solvent split the cuticle covering the capitate sessile trichome was also seen in experiments by Duke *et al*. (1994) on *A. annua* and by Reinhardt *et al*. (2004) on *P. hysterophorus*.

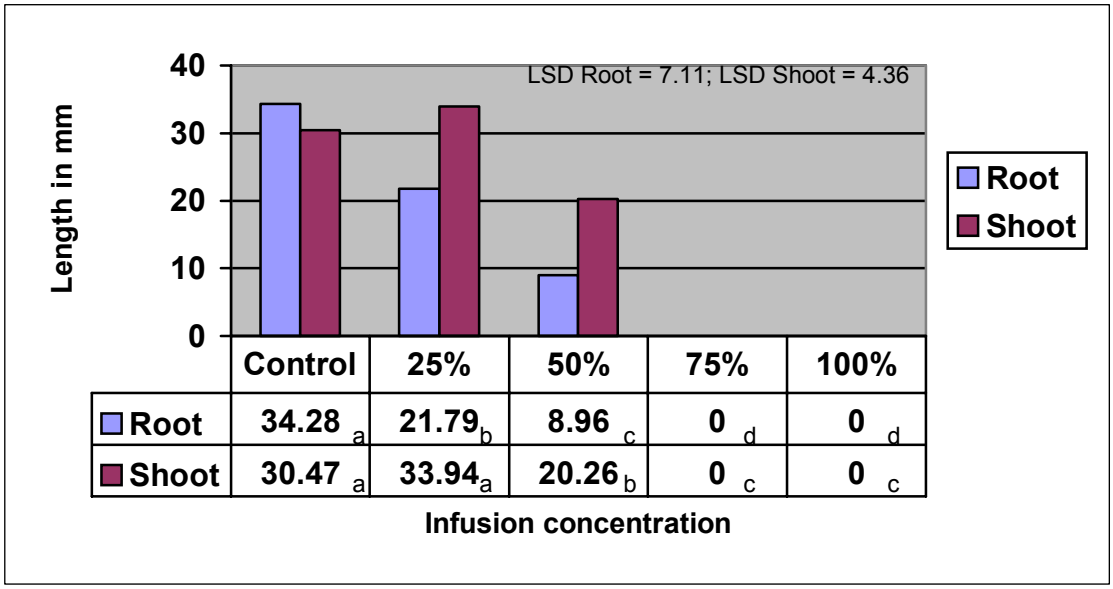


Figure 4.13 Root and shoot length of lettuce seedlings exposed to different infusion concentrations of *C. macrocephalum* after soaking leaves in distilled water for 24 hours – Bioassay 4 (ANOVA in Appendix C, Table C7 and C8)

Means followed by the same letters are not significantly different

Bioassay 4

A clear hormesis effect occurred here. There was stimulation at low concentrations and inhibition at high concentrations in accordance with findings by Belz and Hurle (2004). Lettuce shoot growth was much greater than the root growth at all concentrations, thus suggesting that the roots were more sensitive to *C. macrocephalum* allelochemicals. Results suggest that *C. macrocephalum* has allelopathic potential that is due to water-soluble allelochemicals that are located on leaf surfaces and/or in leaf tissue.

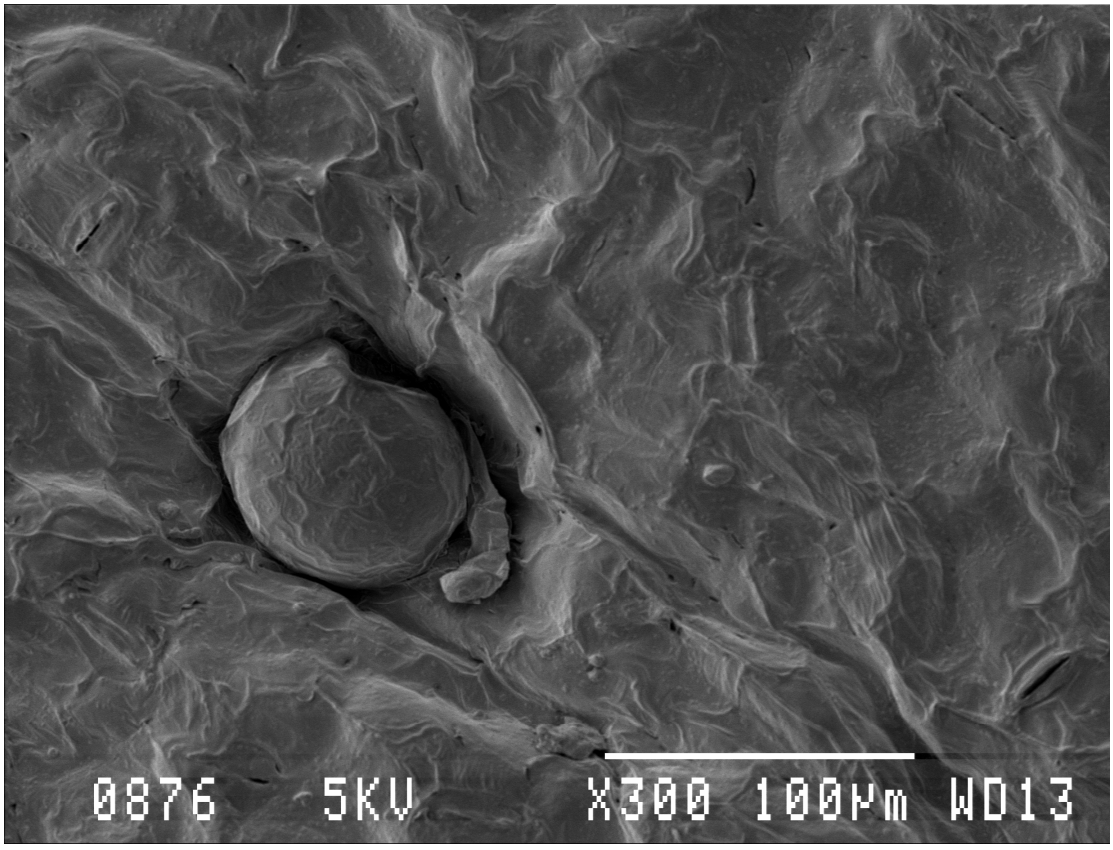


Figure 4.14 Effect of water soaking on *C. macrocephalum* leaf after 24 hours

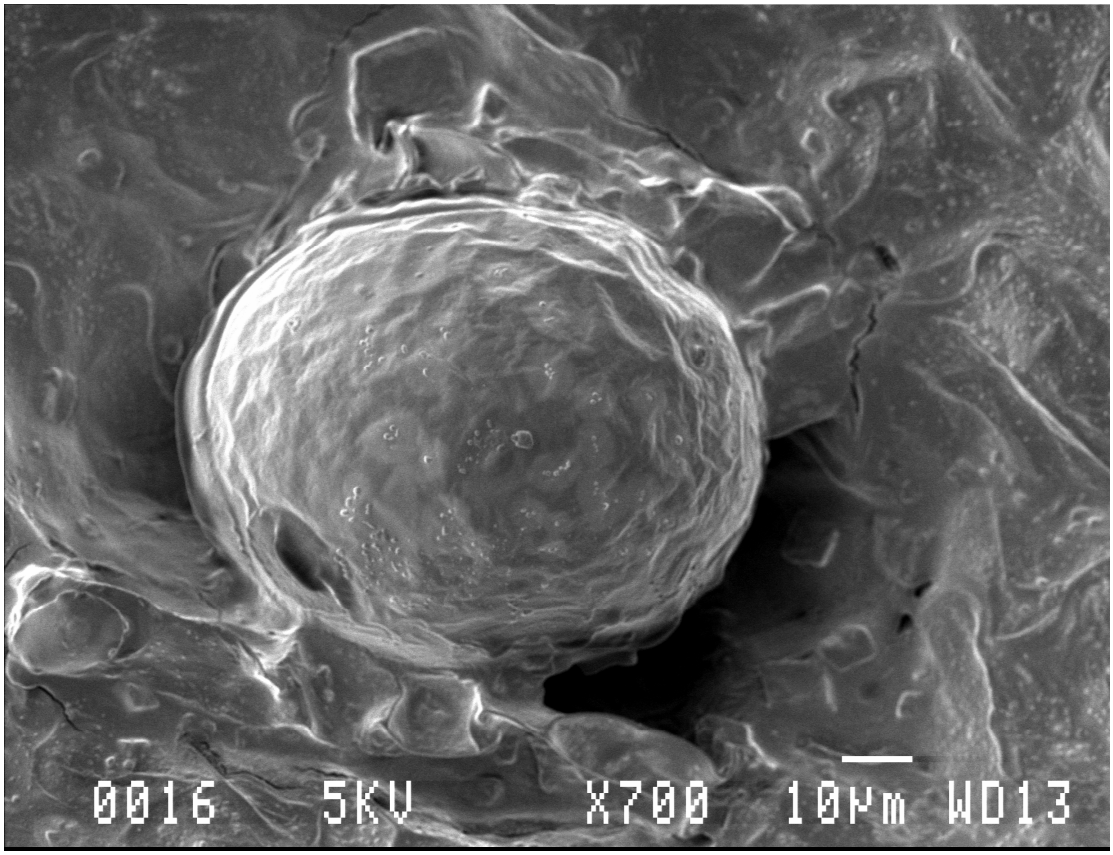


Figure 4.15 Fresh *C. macrocephalum* leaf neither soaked in distilled water nor dipped in dichloromethane

The effect of water soaking for 24 hours showed little damage to the leaf surface as can be seen in Figure 4.14. Figure 4.15 shows a leaf that was neither soaked in water nor dichloromethane. The sitting gland looks complete with absolutely no damage appearing on the surface of the leaf. Results of experiments on *P. hysterophorus* (Reinhardt *et al*., 2004) showed that aqueous extracts of leaves yielded less parthenin (mg/g) than when dipped in the organic solvent TBME. Extraction for a 48 hour period only yielded slightly more parthenin compared to a 24 hour extraction period. Also the water solubility of parthenin is fairly low. As can be seen from the results presented above, allelochemicals present on the leaf surfaces or in the leaf tissue of *C. macrocephalum* have a higher water solubility than organic solvent solubility, making the findings of this experiment different to those of Reinhardt *et al*. (2004) and Duke *et al*. (1994). Picman and Picman (1984) suggest that in *P. hysterophorus* the water soluble plant metabolites play important roles not only in allelopathy and protection against predators and disease but also as autotoxins in population regulation and the timing of the germination process. Further research will have to prove the same for *C. macrocephalum* but it could likely be the same for both species. In nature, rain, fog, dew and mist can facilitate movement of water-soluble secondary metabolites from the plant to the immediate environment where they may persist to negatively affect surrounding vegetation (Kohli *et al*., 1996).

4.2.4 Conclusion

From the above results it can be determined that the allelochemicals causing allelopathic potential of *C. macrocephalum* are probably water-soluble (polar compounds). If these chemicals were non-polar, the graphs in Figures 4.7, 4.9 and 4.11 would have shown a typical hormesis curve like the one shown in Figure 4.13. Thus it can be stated that the organic solvent dichloromethane does not extract allelochemicals of *C. macrocephalum*. If dichloromethane is an organic solvent that does not extract the allelochemicals possessed by *C. macrocephalum*, there must be another reason behind the allelopathic potential of the weed. The water-soluble allelochemicals appear to be contained either on leaf surfaces and/or in leaf tissue. Further studies should be conducted on the glands, trichomes and leaf tissue of the plant to determine the true source and nature of its allelopathic potential.

CHAPTER 5
GENERAL DISCUSSION AND CONCLUSIONS

Campuloclinium macrocephalum is a declared Category 1 weed in South Africa according to the Conservation of Agriculture Act, Act 43 of 1983 and Amended in March of 2001. The weed originated in the tropical Americas but was first brought to South Africa as an ornamental in 1962 (Henderson *et al.*, 2003). This weed is currently invading roadsides, grasslands, open woodlands and savannahs in the following provinces: Gauteng, parts of northern Kwa-Zulu Natal and western Mpumalanga. At its rapid rate of spread it is expected to invade most of Kwa-Zulu Natal, Mpumalanga, Free State and the Eastern Cape in the near future (Henderson, 2001; Henderson *et al.*, 2003). With few methods of control available to contain the plant, its spread could pose great threat to South Africa's natural and agricultural vegetation, thus reducing the country's biodiversity.

Allelopathy along with competition is a form of interference (Foy and Inderjit, 2001). The positive and/or negative effects of possible allelopathic substances produced by *C. macrocephalum* were studied to determine the plant's allelopathic potential. Allelopathy research applies the use of bioassays in the isolation and identification of possible allelochemicals (Leather and Einhellig, 1998). Measuring germination and growth parameters can quantify the allelopathic potential of a certain plant (Lovett, Ryuntyu and Liu, 1989). These parameters indirectly measure other physiological processes affected by chemical interactions. Root, stem and leaf infusions as well as leaf surface content were assessed to determine the allelopathic potential of *C. macrocephalum* on specific test species, namely *Lactuca sativa, Eragrostis curvula, Eragrostis tef* and *Panicum maximum. Lactuca sativa* has proved to be a good test species due to its fast and uniform germination rate and homogenous growth. Bioassays were performed in Petri dishes, which made them quick and easy, producing consistent results.

In the above-mentioned bioassays it could be seen that possible allelochemicals contained in *C. macrocephalum* were capable of negatively affecting the growth of *L. sativa*. This was specifically seen in the radicle growth. The allochemicals

released by the plant could have inhibited growth by preventing cell division in the root, or it could be possible that cell elongation was affected by *C. macrocephalum*'s allelochemicals. It has been found that many phytotoxins and sequiterpenes can inhibit gibberelin and indol-acetic acid induced growth (Duke *et al*., 2000). Allelochemicals are also known to be able to form structural analogues to phytohormones (Tomoszewski and Thiman, 1966). Therefore it could be possible that allelochemicals from *C. macrocephalum* use this method of intervention to inhibit growth of an acceptor species such as the lettuce and selected grass species studied in this dissertation.

Biological invasion by alien species is a leading factor in biodiversity loss and species extinction (Clout and de Poorter, 2005). When looking at this statement in terms of *C. macrocephalum* invasion in South Africa it can be seen that prominent economic grass species and their habitats could be seriously affected by the weed. Results obtained from bioassays show that leaf infusions from *C. macrocephalum* give a negative growth response when applied to three grass species *viz. Eragrostis curvula*, *Eragrostis tef* and *Panicum maximum*. *Eragrostis tef* was the most resilient grass tested showing stimulation at low concentrations of *C. macrocephalum* leaf infusions, but was less inhibited than the other grasses at high concentrations. *Eragrostis curvula* showed zero germination at 100% concentration of *C. macrocephalum* leaf infusion, but was still stimulated at 25% concentration infusion. *Panicum maximum* was found to be the most sensitive grass tested in this series of bioassays showing little growth at all leaf infusion concentrations when compared to the well-grown control. From the results obtained it is clear that *C. macrocephalum* leaf infusions affect the root growth of three prominent grasses. When applied to a grassland situation this could mean that *C. macrocephalum* could affect the roots of grasses in the field depleting their winter reserves and slowing their growth in the spring. With *C. macrocephalum's* extensive root system and the probable inhibition of grass root growth, *C. macrocephalum* will easily take over open grassland causing great economic and livestock food losses for the country.

After closer inspection of the leaf surface of *C. macrocephalum*, sitting glands and trichomes were discovered and determined to be possible sites of allelochemical

storage in the plant. The sitting glands are embedded in the plant's thick waxy cuticle, while the trichomes protrude out from it. This cuticle could be a form of protection against herbicide activity. The trichomes on the leaf surfaces could be the cause of skin irritations observed when handling the plant. Bioassays using dichloromethane were performed to determine the solubility of the substances contained in the glands on the leaf surfaces. Results concurred that these substances are poorly soluble in organic solvents but dissolve well in water. This could mean that herbicides used on the plant need a wetting agent to get the chemical into the plant system. There could also be allelopathic repercussions once the plant has died and decaying matter in a grass field becomes wet in rainy seasons.

Further research should be performed to determine the active compounds found in the sitting glands and trichomes found on *C. macrocephalum*. Studies need also to be done on the plant's invasive ability throughout South Africa. Control measures need to be taken before this alien invader takes over precious economic land in the country.

In conclusion, *C. macrocephalum* does have allelopathic potential. The highest potential may be found in the leaves of the plant, with lower intensity of potential occurring in the roots and the stems. The lower potential in the roots could be due to leaching of the allelopathic substances from the organ into the ground. Capitate-sessile glands and trichomes found on the leaf surfaces may be the main source of allelopathic potential in the plant.

SUMMARY

Campuloclinium macrocephalum (Less D.C), more commonly known as pompom weed, is a declared Category 1 weed in South Africa. Its alarming rate of invasion through grasslands, savannahs and along roadsides could be partly due to its allelopathic potential.

Allelopathy is a form of interference competition that can be defined as chemicals, generally toxic organic compounds, released from higher plants, which influence development, germination, establishment, growth, survival or fecundity of one or more other plant species in close proximity to the donor species. Allelopathy is generally an undirectional process (Inderjit, 2004; van Andel, 2005).

Due to lack of knowledge about the plant and the fact that *C. macrocephalum* is invading some of South Africa's grasslands at an alarming rate and possibly reducing the grazing capacity thereof, a study of the plant's allelopathic potential was undertaken to evaluate the allelopathic threat the plant holds.

Preliminary experiments were performed to determine the sites of allelochemicals in the weed. *Lactuca sativa* (cv. Great Lakes) was used as the acceptor species in all preliminary experiments. Germination percentage, root and shoot growth were all inhibited when exposed to aqueous solutions of roots, stems and leaves of the weed. Evaluation of the results obtained determined that the main site of allelochemicals in *C. macrocephalum* was in the leaves.

Bioassays were performed under controlled conditions to assess the early growth responses (seed germination, root and shoot growth) of *Lactuca sativa*, *Eragrostis curvula*, *Eragrostis tef* and *Panicum maximum* in the presence of aqueous extracts prepared from leaves of *C. macrocephalum* plants. All species tested exhibited reduced root development. The grass species *E. curvula* also showed seed germination inhibition. These results show that the weed could negatively affect the establishment of desirable grass species.

The test species *Lactuca sativa* exhibited significant reduction in germination percentage and root and shoot length when treated with aqueous leaf extracts of *C. macrocephalum*. The toxicity of plant extracts was concentration-dependent thus an increase in inhibitory activity of the extracts was observed with increasing concentrations of said extracts. Morphological abnormalities such as retarded root growth and necrotic root tips were evident in all bioassays.

Results from bioassays using grass species as acceptor species showed that *C. macrocephalum* has negative impacts on the germination percentage and radicle and shoot growth of the grasses. While all grasses tested were negatively affected, *E. tef* was the least affected when compared to *P. maximum* which was highly affected at all concentrations of aqueous solutions of *C. macrocephalum* leaves. The germination rate of *E. curvula* was highly affected by *C. macrocephalum* leaf infusions.

Scanning Electron Microscopy was used to determine the appearance of the leaf surface of the pompom weed. It was found that the leaves of *C. macrocephalum* contain capitate-sessile glands and trichomes that are embedded into a thick cuticle on the surface of the leaves. Dichloromethane was then used in a dipping experiment to determine the solubility of the glands and their contents. It was found in this experiment that the glands on the leaves of *C. macrocephalum* are not organically solvent, as the dichloromethane did not significantly affect them.

Findings strongly suggest that *C. macrocephalum* possesses allelopathic potential. The possible main site of allelochemicals contained by the plant can be found on the leaves. *Lactuca sativa, E. curvula, E. tef* and *P. maximum* were all negatively affected by the secondary metabolites of *C. macrocephalum*. This weed is fast invading the susceptible grassland areas of South Africa; if more research is not done soon on how to control and eradicate the plant the country could suffer grave economic losses.

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APPENDIX A: Chapter 2

Bioassays to determine *Campuloclinium macrocephalum*'s allelopathic potential.

Table A1. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* shoot growth exposed to *C. macrocephalum* leaf infusions (Figure 2.2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6202.151466	1550.537867	93.18	<.0001
Error	24	399.346120	16.639422		
Corrected Total	28	6601.497586			

R^2	C.V	Root MSE	Shoot Mean
0.939507	22.82589	4.079145	17.87069

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	6202.151466	1550.537867	93.18	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	6202.151466	1550.537867	93.18	<.0001

Tukey's Studentised Range (HSD) Test for lettuce shoots in *C. macrocephalum* leaf infusions

Tukey Grouping	Mean	N	Treatment
A	33.940	6	25%
A			
A	30.470	7	Control (0%)
B	20.264	5	50%
C	0.000	6	100%
C			
C	0.000	5	75%

Table A2. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* root growth exposed to *C. macrocephalum* leaf infusions (Figure 2.2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5525.916891	1381.479223	221.53	<.0001
Error	24	149.663853	6.235994		
Corrected Total	28	5675.580745			

R^2	C.V	Root MSE	Root Mean
0.973630	17.43685	2.497197	14.32138

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	5525.916891	1381.479223	221.53	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	5525.916891	1381.479223	221.53	<.0001

Tukey's Studentised Range (HSD) Test for lettuce roots in *C. macrocephalum* leaf infusions

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	34.280	7	Control (0%)
B	21.797	6	25%
C	8.916	5	50%
D	0.000	6	100%
D			
D	0.000	5	75%

Table A3. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* shoot growth exposed to *C. macrocephalum* stem infusions (Figure 2.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6575.279227	1643.819807	99.45	<.0001
Error	22	363.644440	16.529293		
Corrected Total	26	6938.923667			

R^2	C.V	Root MSE	Shoot Mean
0.947594	17.69543	4.065623	22.97556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	6575.279227	1643.819807	99.45	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	6575.279227	1643.819807	99.45	<.0001

Tukey's Studentised Range (HSD) Test for lettuce shoots in *C. macrocephalum* stem infusions

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	39.644	5	25%
A			
B A	35.520	5	50%
B			
B	30.470	7	Control (0%)
C	5.670	5	75%
C			
C	0.576	5	100%

Table A4. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* root growth exposed to *C. macrocephalum* stem infusions (Figure 2.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4947.337074	1236.834269	315.02	<.0001
Error	22	86.377600	3.926255		
Corrected Total	26	5033.714674			

R^2	C.V	Root MSE	Root Mean
0.982840	11.76313	1.981478	16.84481

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	4947.337074	1236.834269	315.02	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	4947.337074	1236.834269	315.02	<.0001

Tukey's Studentised Range (HSD) Test for lettuce roots in *C. macrocephalum* stem infusions

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	34.280	7	Control (0%)
B	25.940	5	25%
C	13.980	5	50%
D	2.860	5	75%
D D	0.190	5	100%

Table A5. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* shoot growth exposed to *C. macrocephalum* root infusions (Figure 2.4)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	8145.533535	2036.383384	244.77	<.0001
Error	20	166.394969	8.319748		
Corrected Total	24	8311.928504			

R^2	C.V	Root MSE	Shoot Mean
0.979981	13.61708	2.884397	21.18220

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	8145.533535	2036.383384	244.77	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	8145.533535	2036.383384	244.77	<.0001

Tukey's Studentised Range (HSD) Test for lettuce shoots in *C. macrocephalum* root infusions

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	48.210	5	25%
B	30.470	7	Control (0%)
C	17.584	4	50%
D	1.220	4	75%
D			
D	0.000	5	100%

Table A6. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* root growth exposed to *C. macrocephalum* root infusions (Figure 2.4)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6463.135061	1615.783765	372.90	<.0001
Error	20	86.659689	4.332984		
Corrected Total	24	6549.794750			

R^2	C. V	Root MSE	Root Mean
0.986769	11.55023	2.081582	18.02200

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	6463.135061	1615.783765	372.90	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	6463.135061	1615.783765	372.90	<.0001

Tukey's Studentised Range (HSD) Test for lettuce roots in *C. macrocephalum* root infusions

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	35.051	5	25%
A			
A	34.280	7	Control (0%)
B	7.114	4	50%
C	1.720	4	75%
C			
C	0.000	5	100%

Table A7. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* shoot growth exposed to *C. macrocephalum* leaf, stem and root infusions (Figure 2.5)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	21299.82864	1521.41633	108.04	<.0001
Error	66	929.38553	14.08160		
Corrected Total	80	22229.21417			

R^2	C.V	Root MSE	Shoot Mean
0.958191	18.22121	3.752546	20.59438

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	19767.93342	4941.98336	350.95	<.0001
Parts	2	363.92074	181.96037	12.92	<.0001
Parts*Treatment	8	1167.97448	145.99681	10.37	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	19856.69793	4964.17448	352.53	<.0001
Parts	2	406.95580	203.47790	14.45	<.0001
Parts*Treatment	8	1167.97448	145.99681	10.37	<.0001

Tukey's Studentised Range (HSD) Test for lettuce shoots comparison of parts

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Parts - Treatment
A	22.976	27	stem
A			
A	21.182	25	root
B	17.871	29	leaves

Table A8. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* root growth exposed to *C. macrocephalum* leaf, stem and root infusions (Figure 2.5)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	17132.06683	1223.71906	250.28	<.0001
Error	66	322.70114	4.88941		
Corrected Total	80	17454.76797			

R^2	C.V	Root MSE	Root Mean
0.981512	13.56175	2.211201	16.30469

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	16502.18253	4125.54563	843.77	<.0001
parts	2	120.49861	60.24930	12.32	<.0001
parts*Treatment	8	509.38569	63.67321	13.02	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	16572.73915	4143.18479	847.38	<.0001
parts	2	117.88042	58.94021	12.05	<.0001
parts*Treatment	8	509.38569	63.67321	13.02	<.0001

Tukey's Studentised Range (HSD) Test for lettuce roots comparison of parts

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Parts - Treatment
A	18.0220	25	root
A			
A	16.8448	27	stem
B	14.3214	29	leaves

APPENDIX B: Chapter 3

The potential allelopathic effect of *Campuloclinium macrocephalum* on African grasses.

Table B1. Abbreviated ANOVA table for germination bioassays of *Eragrostis curvula* shoot growth exposed to *C. macrocephalum* leaf infusions (Figure 3.2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3267.494400	816.873600	43.96	<.0001
Error	20	371.680000	18.584000		
Corrected Total	24	3639.174400			

R^2	C.V	Root MSE	shoot Mean
0.897867	21.84734	4.310916	19.73200

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	3267.494400	816.873600	43.96	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	3267.494400	816.873600	43.96	<.0001

Tukey's Studentised Range (HSD) Test for *Eragrostis curvula* shoot growth in *C. macrocephalum* infusions.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	35.020	5	Control (0%)
B	24.360	5	25%
B			
B	21.760	5	50%
B			
B	17.520	5	75%
C	0.000	5	100%

Table B2. Abbreviated ANOVA table for germination bioassays of *Eragrostis curvula* root growth exposed to *C. macrocephalum* leaf infusions (Figure 3.2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	998.554400	249.638600	32.04	<.0001
Error	20	155.840000	7.792000		
Corrected Total	24	1154.394400			

R^2	C.V	Root MSE	Root Mean
0.865003	26.92337	2.791415	10.36800

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	998.5544000	249.6386000	32.04	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	998.5544000	249.6386000	32.04	<.0001

Tukey's Studentised Range (HSD) Test for *Eragrostis curvula* root growth in *C. macrocephalum* infusions.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	17.980	5	Control (0%)
A			
B A	14.840	5	25%
B			
B C	12.040	5	50%
C			
C	6.980	5	75%
D	0.000	5	100%

Table B3. Abbreviated ANOVA table for germination bioassays of *Eragrostis tef* shoot growth exposed to *C. macrocephalum* leaf infusions (Figure3.4)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	724.557600	181.139400	2.89	0.0486
Error	20	1252.836000	62.641800		
Corrected Total	24	1977.393600			

R^2	C.V	Root MSE	Shoots Mean
0.366421	20.89182	7.914657	37.88400

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	724.5576000	181.1394000	2.89	0.0486

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	724.5576000	181.1394000	2.89	0.0486

Tukey's Studentised Range (HSD) Test for *Eragrostis tef* shoot growth in *C. macrocephalum* infusions.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	46.280	5	25%
A			
A	40.820	5	Control (0%)
A			
A	37.940	5	50%
A			
A	32.880	5	100%
A	31.500	5	75%

Table B4. Abbreviated ANOVA table for germination bioassays of *Eragrostis tef* root growth exposed to *C. macrocephalum* leaf infusions (Figure 3.4)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	349.5176000	87.3794000	30.15	<.0001
Error	20	57.9640000	2.8982000		
Corrected Total	24	407.4816000			

R^2	C.V	Root MSE	Roots Mean
0.857751	19.46947	1.702410	8.744000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	349.5176000	87.3794000	30.15	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	349.5176000	87.3794000	30.15	<.0001

Tukey's Studentised Range (HSD) Test for *Eragrostis tef* root growth in *C. macrocephalum* infusions.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	14.900	5	25%
B	10.240	5	Control (0%)
B			
B	8.760	5	50%
C	5.340	5	75%
C			
C	4.480	5	100%

Table B5. Abbreviated ANOVA table for germination bioassays of *Panicum maximum* shoot growth exposed to *C. macrocephalum* leaf infusions (Figure 3.6)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	867.152600	216.788150	23.95	<.0001
Error	20	181.032000	9.051600		
Corrected Total	24	1048.184600			

R^2	C.V	Root MSE	Shoots Mean
0.827290	81.40118	3.008588	3.696000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	867.1526000	216.7881500	23.95	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	867.1526000	216.7881500	23.95	<.0001

Tukey's Studentised Range (HSD) Test for *Panicum maximum* shoot growth in *C. macrocephalum* infusions.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	15.420	5	Control (0%)
B	1.750	5	25%
B			
B	0.820	5	75%
B			
B	0.470	5	50%
B			
B	0.020	5	100%

Table B6. Abbreviated ANOVA table for germination bioassays of *Panicum maximum* root growth exposed to *C. macrocephalum* leaf infusions (Figure 3.6)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	94.5970000	23.6492500	34.00	<.0001
Error	20	13.9130000	0.6956500		
Corrected Total	24	108.5100000			

R^2	C.V	Root MSE	Roots Mean
0.871781	48.21135	0.834056	1.730000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	94.59700000	23.64925000	34.00	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	94.59700000	23.64925000	34.00	<.0001

Tukey's Studentised Range (HSD) Test for *Panicum maximum* root growth in *C. macrocephalum* infusions.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	5.5000	5	Control (0%)
B	1.5100	5	25%
B			
B	0.9900	5	50%
B			
B	0.6100	5	75%
B			
B	0.0400	5	100%

APPENDIX C: Chapter 4

Contribution of substances on the leaf surfaces of *Campuloclinium macrocephalum* towards its allelopathic potential, data on dichloromethane dipping experiment.

Table C1. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* shoot growth exposed to *C. macrocephalum* leaf infusions dipped in dichloromethane for five seconds (Figure 4.7)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	11.5384000	2.8846000	0.44	0.7764
Error	20	130.3440000	6.5172000		
Corrected Total	24	141.8824000			

R^2	C.V	Root MSE	Shoot Mean
0.081324	20.67445	2.552881	12.34800

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	11.53840000	2.88460000	0.44	0.7764

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	11.53840000	2.88460000	0.44	0.7764

Tukey's Studentised Range (HSD) Test for lettuce shoot growth in *C. macrocephalum* infusions dipped in dichloromethane for 5 seconds.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	13.560	5	Control (0%)
A			
A	12.600	5	25%
A			
A	12.060	5	75%
A			
A	11.780	5	50%
A			
A	11.740	5	100%

Table C2. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* root growth exposed to *C. macrocephalum* leaf infusions dipped in dichloromethane for five seconds (Figure 4.7)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	14.27760000	3.56940000	1.49	0.2430
Error	20	47.94000000	2.39700000		
Corrected Total	24	62.21760000			

R^2	C.V	Root MSE	Root Mean
0.229478	32.49842	1.548225	4.764000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	14.27760000	3.56940000	1.49	0.2430

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	14.27760000	3.56940000	1.49	0.2430

Tukey's Studentised Range (HSD) Test for lettuce root growth in *C. macrocephalum* infusions dipped in dichloromethane for 5 seconds.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	5.8200	5	Control (0%)
A			
A	5.2000	5	75%
A			
A	4.7200	5	100%
A			
A	4.5400	5	25%
A			
A	3.5400	5	50%

Table C3. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* shoot growth exposed to *C. macrocephalum* leaf infusions dipped in dichloromethane for 10 seconds (Figure 4.9)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	10.1576000	2.5394000	0.39	0.8148
Error	20	130.9440000	6.5472000		
Corrected Total	24	141.1016000			

R^2	C.V	Root MSE	Shoot Mean
0.071988	16.99488	2.558750	15.05600

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	10.15760000	2.53940000	0.39	0.8148

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	10.15760000	2.53940000	0.39	0.8148

Tukey's Studentised Range (HSD) Test for lettuce shoot growth in *C. macrocephalum* infusions dipped in dichloromethane for 10 seconds.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	16.280	5	Control (0%)
A			
A	15.000	5	50%
A			
A	14.880	5	100%
A			
A	14.620	5	25%
A			
A	14.500	5	75%

Table C4. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* root growth exposed to *C. macrocephalum* leaf infusions dipped in dichloromethane for 10 seconds (Figure 4.9)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	39.0616000	9.7654000	0.66	0.6257
Error	20	295.1120000	14.7556000		
Corrected Total	24	334.1736000			

R^2	C.V	Root MSE	Root Mean
0.116890	29.51215	3.841302	13.01600

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	39.06160000	9.76540000	0.66	0.6257

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	39.06160000	9.76540000	0.66	0.6257

Tukey's Studentised Range (HSD) Test for lettuce root growth in *C. macrocephalum* infusions dipped in dichloromethane for 10 seconds.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	14.920	5	100%
A			
A	13.340	5	75%
A			
A	13.160	5	25%
A			
A	12.620	5	50%
A			
A	11.040	5	Control (0%)

Table C5. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* shoot growth exposed to *C. macrocephalum* leaf infusions dipped in dichloromethane for 10 seconds and water soaked for 24-hours (Figure 4.11)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	308.4440000	77.1110000	12.84	<.0001
Error	20	120.1160000	6.0058000		
Corrected Total	24	428.5600000			

R^2	C.V	Root MSE	Shoot Mean
0.719722	15.05328	2.450673	16.28000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	308.4440000	77.1110000	12.84	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	308.4440000	77.1110000	12.84	<.0001

Tukey's Studentised Range (HSD) Test for lettuce shoot growth in *C. macrocephalum* infusions dipped in dichloromethane for 10 seconds and then water soaked for 24-hours.
Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	21.900	5	25%
A			
B A	18.800	5	50%
B			
B C	14.520	5	75%
C			
C	13.480	5	100%
C			
C	12.700	5	Control (0%)

Table C6. Abbreviated ANOVA table for germination bioassays of *Lactuca sativa* root growth exposed to *C. macrocephalum* leaf infusions dipped in dichloromethane for 10 seconds and water soaked for 24-hours (Figure 4.11)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	221.4144000	55.3536000	11.52	<.0001
Error	20	96.0800000	4.8040000		
Corrected Total	24	317.4944000			

R^2	C.V	Root MSE	Root Mean
0.697380	27.50757	2.191803	7.968000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	221.4144000	55.3536000	11.52	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	221.4144000	55.3536000	11.52	<.0001

Tukey's Studentised Range (HSD) Test for lettuce root growth in *C. macrocephalum* infusions dipped in dichloromethane for 10 seconds and then water for 24-hours.

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	12.840	5	Control (0%)
A			
B A	9.220	5	25%
B			
B C	7.860	5	50%
B C			
B C	5.620	5	75%
C			
C	4.300	5	100%

Table C7. Abbreviated ANOVA table for shoot length of lettuce seedlings exposed to different infusion concentrations of *C. macrocephalum* after soaking leaves in distilled water for 24 hours (Figure 4.13)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6202.151466	1550.537867	93.18	<.0001
Error	24	399.346120	16.639422		
Corrected Total	28	6601.497586			

R^2	C.V	Root MSE	Shoot Mean
0.939507	22.82589	4.079145	17.87069

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	6202.151466	1550.537867	93.18	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	6202.151466	1550.537867	93.18	<.0001

Tukey's Studentised Range (HSD) Test for lettuce shoots in *C. macrocephalum* leaf infusions

Tukey Grouping	Mean	N	Treatment
A	33.940	6	25%
A			
A	30.470	7	Control (0%)
B	20.264	5	50%
C	0.000	6	100%
C			
C	0.000	5	75%

Table C8. Abbreviated ANOVA table for root length of lettuce seedlings exposed to different infusion concentrations of *C. macrocephalum* after soaking leaves in distilled water for 24 hours (Figure 4.13)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5525.916891	1381.479223	221.53	<.0001
Error	24	149.663853	6.235994		
Corrected Total	28	5675.580745			

R^2	C.V	Root MSE	Root Mean
0.973630	17.43685	2.497197	14.32138

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	4	5525.916891	1381.479223	221.53	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	5525.916891	1381.479223	221.53	<.0001

Tukey's Studentised Range (HSD) Test for lettuce roots in *C. macrocephalum* leaf infusions

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	Treatment
A	34.280	7	Control (0%)
B	21.797	6	25%
C	8.916	5	50%
D	0.000	6	100%
D D	0.000	5	75%