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ALLELOPATHIC POTENTIAL OF *CONYZA BONARIENSIS*

By

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Submitted in partial fulfillment of the requirements for the degree

M Inst Agrar Agronomy

in the Department of Plant Production and Soil Science, Faculty of Natural and Agricultural Sciences,

University of Pretoria

Supervisor: Prof. C.F. Reinhardt

Co-supervisor: Dr. N.J. Taylor

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DECLARATION

I, the undersigned, hereby declare that the dissertation submitted herewith for the degree M Inst Agrar Agronomy to the University of Pretoria, contains my own independent work and has not been submitted for any degree at any other university.

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Matseleng W. Malatji

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ABSTRACT

Conyza bonariensis, flaxleaf fleabane, is a major weed threat on cultivated and non-cultivated lands, gardens, roadsides and waste places. The weed in South Africa is believed to have originated from South America, and the first herbarium sample is from a plant collected in May 1895 at Franschhoek. Adding to its problem status is the recent discovery that certain *C. bonariensis* biotypes in South Africa and other parts of the world are resistant to the herbicide glyphosate, and in certain cases to both glyphosate and paraquat. Despite its invasiveness and ability to compete severely with crops, the mechanisms of interference (= allelopathy + competition) employed by *C. bonariensis* are poorly understood and have not yet been thoroughly investigated. There is a need to expand on the knowledge of interference mechanisms of *C. bonariensis* in order to better understand its success as a weed, and to improve on knowledge for the successful management of this weed. In the present study, allelopathic potential of *C. bonariensis* was assessed, first by means of germination bioassays, followed by investigation employing hydroponics, leachate, and replacement series experiments. In a laboratory bioassay, the plant's leaves and

roots were extracted using two solvents, water and hexane, to which seeds of the test (acceptor) species lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum*) were exposed in order to determine where the strongest allelopathic potential resides. Moreover, differential potency of crude extracts prepared with the two solvents (polar and non-polar) would at least provide some evidence on the nature of putative allelochemicals involved. Germination bioassays revealed that leaves harboured the strongest allelopathic potential (potency). Water extracts (infusions) caused greater growth inhibition of the test species than hexane extracts. Osmolalities of the water infusions were tested and found not to be inhibitory to germination and early seedling development of lettuce. Following on the germination bioassays, a hydroponic experiment was set up in a greenhouse in order to investigate whether *C. bonariensis* possesses and releases chemicals with allelopathic potential through its roots. Lettuce top and root growth was significantly reduced by all three populations of *C. bonariensis* (one from Pretoria; two from the Western Cape). No significant differences were observed in the degree of growth inhibition caused by the three weed populations on the growth of lettuce, except in the case of root dry mass results where the Hatfield population caused more damage (85% growth reduction). The leachate experiment was then performed to determine if leachate from *C. bonariensis* affected the growth of test species exposed to different leachate concentrations. Although there was no growth inhibition observed for both lettuce and tomato in this experiment, growth stimulation of tomato roots was observed at the highest leachate concentration (100%). Finally, in an attempt to simulate the allelopathic potential of *C. bonariensis* in a natural field situation, a replacement series experiment was conducted to determine the relative interference of *Conyza bonariensis* in relation to lettuce and tomato. Dry mass results showed that there was no growth inhibition of both crop species. RYT was > 1 at all weed: crop combinations, which implies that both crop species and *C. bonariensis* were less affected by interspecific interactions than in their respective monocultures. It is suggested that the results of this study can attributed to methodology and growth media. The results of this study represent the first step in showing that allelopathic potential *C. bonariensis* may contribute to the success of this weed as an invasive weed species and that this weed should not be allowed to attain significant biomass on crop field. Further research should include field trials that will yield a better understanding of the practical relevance of the allelopathic

potential of *C. bonariensis*. Finally, crop producers and weed management practitioners should recognize that this important weed has the ability to interfere with the growth and development of a crop through two mechanisms, competition plus allelopathy.

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LIST OF ABBREVIATIONS

ANOVA : Analysis of variance

gL^{-1} : grams per litre

LSD : Least significant difference

$mOsm\ kg^{-1}$: milliOsmol per kilogram

PEG : polyethylene glycol

RY : relative yield

RYT : relative yield total

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INTRODUCTION

The invasion of newly colonised areas by alien species is a problem of great significance globally. Apart from displacing the indigenous plants, these plants are able to survive, reproduce and spread at alarming rates. The comprehension of survival mechanisms utilized by such species is an imperative process before implementing control strategies. *Conyza* spp among many other invasive alien species have become major weed pests in South Africa (Bromilow, 2010) and other parts of the world (Heap, 2012). Although the first record of the *Conyza* spp in the country was over a century ago, they seem to have become more troublesome in recent years.

While all plant species compete to survive, invasive species appear to have specific traits or a combination of these traits, which allow them to out compete native species (Kolar and Lodge, 2001).Facilitation is the mechanism that some species use to change their environment through chemical or physical manipulation of biotic and abiotic factors, usually to make conditions unfavourable for other species which compete with them. Allelopathy is an example of a chemical facilitative mechanism (Hierro and Callaway, 2003). Among the weed species reported worldwide a considerable number reportedly possess allelopathic potential. In allelopathic interactions there is production and release of chemical substances by certain plants aimed at inhibiting the growth and development of neighbouring species. They are released into the environment by root exudation, leaching from aboveground parts, and volatilisation and/or by decomposition of plant material, and can be present in several parts of plants including roots, rhizomes, leaves, stems, pollen, seeds and flowers. Several papers have suggested allelopathy as an alternative to weed management (Macias, 1995; An *et al*., 1998; Inderjit and Keating, 1999). Options such as using allelochemicals as herbicides, and improving the allelopathic activity of crops through breeding strategies or by genetic engineering have been explored (Macias, 1995; Chou, 1999).

In South Africa there are three main species of *Conyza* namely *Conyza canadensis*, *Conyza bonariensis*, *Conyza sumatrensis*, commonly known as Canadian fleabane,

flax-leaf fleabane, and tall fleabane respectively. Of the three, *C. bonariensis* and *C. sumatrensis* seem to have a wide distribution in the country. Previously the biology and ecology of *Conyza* have been the main focus of studies, which entailed studies on population dynamics, seed production, emergence and distribution. Other studies on the weed focused on the resistance of *C. bonariensis* to herbicides, and it being the first broadleaf weed documented as resistant to glyphosate (Shrestha and Hembree, 2005; Heap, 2006; Weaver, 2001). *Conyza* spp have succeeded as well-equipped competitors in a range of habitats and ecosystems. With the exception of *C. canadensis* and *C. sumatrensis*, little attention has been given to the competitive advantages that aid *Conyza* spp in survival to persist in new environments and foreign lands.

The main aim of the study was to determine if *C. bonariensis* in South Africa possess allelopathic potential, specifically the ability to suppress crop growth, through the release of allelochemicals from the roots. The hypothesis is thus that *C. bonariensis* produces compounds with allelopathic potential that affect the growth of surrounding plants, thereby gaining a competitive advantage.

Specific objectives were the following:

- To verify whether *C. bonariensis* has different impacts on seed germination and seedling growth of a test species;
- To evaluate the influence of different plant parts of *C. bonariensis* on seed germination and seedling growth of the test species;
- To investigate whether *C. bonariensis* possess chemicals with allelopathic potential by growing it together with test species in a nutrient solution and using plant growth as measure of effect;
- To verify if different biotypes of *C. bonariensis* would have the same effect on the growth of the same test species;
- To determine test plant responses to different concentrations of root leachate collected from *C. bonariensis* plants;
- To assess the interference of *C. bonariensis* with growth of the test species by increasing *C. bonariensis* plant density, and thus the concentration of compounds with allelopathic potential in the growth medium.

CHAPTER 1
LITERATURE REVIEW

1.1 Invasive alien plants

A great number of plant species have the ability to grow in conditions that are similar but also quite different from those in their native habitats. Consequently, many plants are currently in places where they never existed before. The term invasive species refers to non-indigenous species that affect the habitats they invade environmentally, ecologically, and economically (Kolar and Lodge, 2001). These types of plants are able to survive, reproduce and spread unaided sometimes at alarming rates across the landscape. Their impact on agriculture is considered to be significantly greater in developing than in developed countries (Perrings, 2005).

In a review on the impact and management of invasive plants in Africa, Witt (2010) states that, the impact of invasive alien species on the continent, especially introduced weeds, is significant because more than 80% of the population are small-scale farmers who are dependent on natural resources for their survival. In countries such as Angola, Zambia, Malawi, Uganda, Mozambique, Ethiopia, and Sudan the agricultural labour force is about 80% of the total labour force, and hand weeding accounts for up to 60% of pre-harvest labour (Webb and Conroy, 1995; Witt, 2010). In South Africa it has been documented that thousands of plant species have been brought into the country for a range of purposes, such as crop species for timber and firewood, as garden ornamentals, for stabilizing sand dunes as barriers and hedge plants (Van Wilgen *et al*., 2001). An estimated 750 tree species and around 8000 shrubby succulent and herbaceous species have been introduced to South Africa, with 161 species regarded as seriously invasive. Suggestions that 750 000 ha of invaded land should be cleared annually, if the battle against invasive plants is to be won within 20 years, have been made (MacDonald *et al*., 2003). However, this 20-year effort would come at a projected cost of R5.5 billion (Le Maitre *et al*., 2000).

While all plant species compete to survive, invasive species appear to have specific traits or a combination of these traits, which allow them to out-compete native species (Kolar and Lodge, 2001). An introduced species might become invasive if it

can out-compete native species for resources such as nutrients, light, physical space, and water. Invasive species might be able to use resources unavailable to native species, such as deep water accessed by a long taproot, or an ability to live on previously uninhabitable soil types. These species have evolved under great competition and predation, and the new environment allows them to proliferate quickly (Stohlgren *et al*., 1999). Perfect examples of successful invader alien plants are: *Parthenium hysterophorus* which is present in Kenya, Uganda, Tanzania, South Africa, Mozambique, and Swaziland, and is currently considered to be the most important weed in both croplands and grazing areas by 90% of farmers in the lowlands of Ethiopia (Tamado and Millberg, 2000), with sorghum yields being reduced by 97% in experimental fields with high densities of parthenium (Tamado *et al*., 2002). The impact of parthenium has also been well documented in Australia and India (Evans, 1997). *Conyza* spp are another invader group of weeds that were introduced into South Africa about a century ago from South and North America. They now cause problems in cultivated and non-cultivated lands, gardens, roadsides and waste places (Ciba-Geigy, 1985).

Many exotic plant species competitively exclude and eliminate their neighbours in invaded “recipient” communities, but coexist in relative harmony with neighbours in species-diverse systems in their native habitat (Hierro and Callaway, 2003). Researchers have suggested that this is due to the existence of empty niches in recipient communities, rapid genetic changes in invader populations in response to selection pressure in the novel environment, and special adaptation to human disturbance by invaders (Mack *et al*., 2000; Sakai *et al*., 2001).

The primary theory for the unusual success of invasive plants is that they have escaped the natural enemies that hold them in check, thus freeing them to utilize their full competitive potential – the “natural enemies hypothesis” (Darwin, 1859; Williams, 1954; Elton, 1958; Gillet, 1962). The hypothesis has been tested around the world by releasing hundreds of types of biocontrol agents, but the majority of them have been ineffective (Maron and Vila, 2001). This indicated that an inquiry about mechanisms for the general success of many exotic invasive plants was essential for gaining control over invading species.

Several mechanisms have been proposed to explain invasive plant species success within introduced areas compared to their natural ranges. More recently, allelopathy has been suggested as a potentially important mechanism of plant invasion success, particularly when the invaders produce evolutionarily novel chemicals (Hierro and Callaway, 2003; Inderjit *et al*., 2008).

1.2 Allelopathy: A background

In their communities plants will interact either positively or negatively. However, it is more common that neighbouring plants will interact in a negative manner, whereby the emergence and growth of one or more engaged in the interaction, is inhibited. This adverse effect of a neighbouring plant in an association is termed interference (Muller, 1969; Foy and Inderjit, 2001). Plant interference is generally explained by two phenomena, resource competition and allelopathy. Competition implies limitation of resources such as light, water, space, and nutrients, and allelopathy can be defined as all effects of plants on neighbouring plants through the release of chemical compounds into the environment (Rice, 1984).

In nature it is particularly difficult to separate allelopathic interference from resource competition because there are many factors interacting simultaneously (Weston and Duke, 2003). Proof of allelopathy involves isolating compounds and demonstrating that a toxic effect on other plant species is the main function of the compound and that when the other interactions such as resource limitations are alleviated, the allelopathic effect persists (Williamson, 1990). Under controlled conditions, factors in competition may be separated, and it is possible to prove that chemical interactions are either totally or partially responsible for the interference observed. In devising laboratory and greenhouse studies, efforts have been made to assure that the biological activities obtained are indeed due to the extracellular toxins by the donor plants (Qasem and Foy, 2001). For example, Belz *et al*. (2009) conducted a study to investigate whether or not the plant metabolite parthenin is sufficiently persistent, phytotoxic, and bioavailable in soils to cause an allelopathic effect that makes it attributable to the invasive success of the weed *P. hysterophorus*. In this study, parthenin was found to be quickly degraded without any evident accumulation to toxic levels over time and therefore; the hypothesis that parthenin contributes to the invasiveness of *P. hysterophorus* was rejected.

Allelopathy has been suggested as a mechanism for the success of invasive plants by establishing a virtual monoculture and may contribute to the ability of particular exotic species to become dominant in invaded plant communities (Hierro and Callaway, 2003). It is expected to be an important mechanism in the plant invasion process because the lack of co-evolved tolerance of resistant vegetation to chemicals produced by the invader, which allows the newly arrived species to dominate natural plant communities.

1.2.1 Brief definition and history

The concept of allelopathy has been cited in literature for over 2000 years (Weston and Duke, 2003). Theophrastus (372 to 285 BC), a disciple of Aristotle, speculated that there might be chemical interactions between weeds and plants but provided little evidence to substantiate this claim. De Candolle (1932), a pioneer in allelopathy research of weeds on crop plants, concluded that exudates of certain weed species injured specific crop plants. His research stimulated interest in the chemical ecology of plants, but it was Molish (1937) who coined the term allelopathy, derived from the Greek words *allelon* (of each other) and *pathos* (to suffer).Rice (1984) defines allelopathy as any direct or indirect effect by one plant, including micro-organisms, on another through the production of chemical compounds that escape into the environment and subsequently influence the growth and development of neighbouring plants.

Over the last three decades, there has been an increase in publications on allelopathy and a considerable amount of literature is available that implicates allelopathy as an important form of plant interference. The term is today generally accepted to cover both inhibitory and stimulatory effects of one plant on another plant (Qasem and Foy, 2001).

In 1996 the International Allelopathy Society defined allelopathy as follows: “Any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects” (Torres *et al*., 1996).Ten years ago, 240 weed species were reported to have

inhibitory action on crop plants alone. Major progress in the science has recently occurred and the phenomenon is of worldwide importance (Qasem and Foy, 2001).

1.2.2 Interactions of allelochemicals

Allelochemicals cause germination and growth inhibition, and influence a wide variety of metabolic processes. These substances can be isolated from plant tissues. Allelochemicals can be found in numerous parts of a plant such as roots, rhizomes, leaves, stems, pollen, seed, and flowers, and are usually products of secondary plant metabolism (Rice, 1984).

The most important allelochemicals include alkaloids, terpenoids, flavonoids, steroids, tannins, and phenolic compounds (Whittaker and Feeny, 1971; Mandava, 1985; Shakaut *et al*., 2003). Phenolic compounds are reported to constitute the principal allelopathic agents in weeds and other allelopathic plants. Often their function in the plant is unknown but some allelochemicals are reported to have structural functions e.g., as intermediates of lignification or play a role in general defence against pathogens (Niemeyer, 1988; Corcuera, 1993; Einhellig, 1995). Allelochemicals are released into the environment by root exudation, leaching from aboveground parts, and volatilisation and/or by decomposition of plant material (Rice, 1984), and their ability to persist in soil is determined by sorption, fixation, leaching and chemical or microbial degradation (Inderjit, 1998). The degree of phytotoxicity depends on residue persistence and the extent of dissipation in the soil environment.

According to Inderjit and Weiner (2001) allelochemical effects in the field could be due to four possibilities: (i) direct harmful effects of chemicals released from donor plants, (ii) degraded or transformed products of released chemicals, (iii) effect of released chemicals on physical, chemical and biological soil factors, and (iv) induction of release of biologically active chemicals by a third species. Since it is difficult to distinguish between these four possibilities, Inderjit and Weiner (2001) proposed that allelopathy be understood in its ecological context rather that based on direct plant-plant allelopathic interference.

Allelopathy is strongly coupled with other stresses of the crop environment including insect and disease, temperature extremes, light, nutrients and moisture variables, and herbicides, and is strongly influenced by habitat ecology (Inderjit and Keating, 1999).Environmental factors also have the ability to influence the production of allelochemicals and their effects. Plants growing in resource-limited environments exhibit higher tissue concentration of secondary compounds when compared to those growing under less stressful conditions. For example, Koeppe (1976) found that increased amounts of allelopathic substances were produced when plants grew in phosphorus-deficient soil.

Drought has been reported to have ability to increase the amount of allelopathic compounds in soil (Gershenzon, 1984).It has been shown that allelopathic activities are more pronounced when plant species grow underwater stress (Einhellig, 1987, 1989). Ardi (1986) found that the reduction of sweet corn *(Zea mays)* yield due to purple nutsedge *(Cyperus rotundus)* was most severe when the greatest water stress was imposed. Thus, growth inhibition of sweet corn may be due to the combined stress of direct water deficit and greater production of allelopathic substances in purple nutsedge under these conditions.

Chemicals released by plants including allelochemicals also play an important role in influencing ecological processes in plant communities through their effects on soil ecology (Wardle *et al*., 1998). Many secondary metabolites such as phenolics and terpenoids are known to form complexes with organic ions and influence accumulation of nutrients. Phenolics may affect phosphate availability by competing for anion absorption sites. They can bind to Al, Fe, and Mn, thus releasing phosphate otherwise bound to these cations (Appel, 1993).

Allelochemicals may also influence microbial ecology by their effects on soil microbes and plant pathogens. Population densities of soil-borne microorganisms are affected by soil enrichment with phenolic acids, ferullic, p-coumaric, and vanillic acids (Blum and Shafer, 1988). However, microbial degradation of allelochemicals may prevent them from reaching phytotoxic levels in natural soils (Schmidt and Ley, 1999). Soil is a very complex system and it affects both the quantitative and qualitative ability of allelochemicals and therefore allelopathic responses of the plant

(Inderjit *et al*., 1999). Inderjit and Weiner (2001) suggested that research on the influence of allelochemicals on different components of the soil ecosystem and their role in shaping community structure and composition is needed.

The study of allelopathy therefore has numerous aspects or dimensions, namely: ecology, plant physiology, microbiology, molecular biology, natural product chemistry and agriculture. Its application to agricultural production has been anticipated and researchers have found allelopathic plants that are now used as cover crops for sources of allelochemicals, and these compounds are serving as leads in the development of new herbicides (Hirai, 2003).

1.2.3 Allelopathy and agriculture

Weeds account for more than 1% of the total plant species on earth, but cause great damage by interfering with food production, health, economic stability and welfare (Qasem and Foy, 2001). They may be defined as plants with little economic value and possessing the potential to colonize disturbed habitats or those modified by human activities (Macias *et al*., 2004). Simply put, weeds are often plants that are uniquely adapted to a wide range of environmental conditions, and they did not acquire problem status until humans developed agriculture. Therefore, it is up to humans to find a solution to the problems weeds cause in agriculture.

Various researchers have referred to allelopathic agents as the future natural pesticides or nature's herbicides in action (Putnam, 1983; Rice, 1995). Qasem and Foy (2001), state that the limited work on mode of action of allelochemicals suggests that they affect a variety of sites and biochemical processes, many of which are familiar to those affected by synthetic herbicides. Allelochemicals are considered safer than synthetic chemicals because of their biodegradability.

Allelopathic crops, when used as cover crops, mulch, green manures, or grown in rotation, are helpful in reducing noxious weeds and plant pathogens (Khanh *et al*., 2005). Common examples of crops exhibiting allelopathy include, *Sorghum bicolor* (Putnam, 1983), *Triticum aestivum* (Kimber, 1973), *Oryza sativa* (Chou, 1995) and *Zea mays* (Yakle and Cruse, 1984).

Crop rotation is reported to have a greater effect on weed species and densities than tillage practices (Weston, 1996), and the practice simultaneously controls pests, enhances ecosystem diversity and improves crop productivity (Mamolos and Kalburtji, 2001). Japanese farmers use beans in spring, buckwheat in summer and then wheat in winter (Kahn *et al*., 2005). The beans are reported to help with soil nutrient enrichment, whilst buckwheat is known as a weed "killer" and can be used as green manure that contributes to soil nutrients. Therefore, buckwheat plants are incorporated in the soil to help reduce weeds and increase the yield of wheat.

Microorganisms can be considered as a source of new allelochemicals; hence their phytotoxic and pharmacologic properties have created growing interest (Macias *et al*., 2004). According to Khalid *et al*. (2002), microbially produced phytotoxins have more potential than some herbicides, because they are selective and, compared to using the actual pathogens, they are easy to formulate, less likely to spread diseases to non-target species, and their activity is less dependent on environmental conditions. This comparison may hold true for certain microbial toxins and synthetic herbicides, but mostly the latter are more selective in terms of controlling weeds without harming the crop, and they have better residual activity than most herbicides of biological origin.

Allelopathy, as a science, is rapidly growing and its significant role in nature is now fairly well acknowledged. However, more experimental evidence and a great deal of more intensive, precise investigation is still required (Qasem and Foy, 2001). With modern analytical technical methods (HPLC, GC-MS, IR, NMR, etc.), more allelochemicals are likely to be isolated to produce bioactive herbicides and pesticides (Khan *et al*., 2005).

1.2.4 Allelopathy and biodiversity

After direct habitat destruction, biological invasions have been viewed as the second largest global threat to diversity, given their effect on agriculture, forestry and human health (Wilcove *et al*., 1998; Walker and Steffen, 1999). It has been suggested by global reviews that the most harmful species transform ecosystems by utilising excessive amounts of resources (particularly water, light and oxygen), by adding resources (particularly nitrogen), by promoting or suppressing fire, by stabilizing sand movement and/or promoting erosion by accumulating litter or by accumulating

or redistributing salt (Richardson *et al*., 2000). According to Vitousek (1990), these changes possibly alter the flow, availability or quality of nutrient resources in biogeochemical cycles; they modify trophic resources within food webs; and they alter physical resources such as living space or habitat, sediment, light and water. Therefore, alien invaders are likely to act as 'ecosystem engineers' by rapidly changing disturbance regimes (Crooks, 2002).

The importance of plant diversity is due to its ability to provide insurance against large changes in ecosystem processes and manage efficiency of resource utilization (Inderjit and Foy, 2001). Reduction in genetic diversity of crops and wild plants is a direct consequence of loss in plant diversity (Solbrig, 1991). Through evolutionary processes both competition and allelopathy play important roles in regulating the species diversity in a plant community (Inderjit and Foy, 2001). Allelopathic compounds have been shown to play important roles in determining plant diversity, dominance, succession and climax of natural vegetation, and in the plant productivity of agroecosystems (Chou, 1999). By applying an excess of fertilizers, herbicides, fungicides, and nematacides, etc., modern agricultural practices can jeopardize the physical-chemical properties of the soil, and pollute the soil and water to the detriment of the global ecosystem (Chou, 1999). In order to achieve the goal of sustainable agriculture, extensive research is needed and has been done on plant breeding, soil fertility and tillage, crop protection, and cropping systems (Chou, 1999).

1.2.5 Assessing allelopathic potential

Bioassays, as a tool for assessing the biological activity of natural and synthetic chemicals, are defined as the assessment of the potency of a compound via the application-induced response to that compound (Webster, 1980; Govindarajulu, 1988). In allelopathy, bioassays are necessary in each step of the isolation, purification, and identification processes of active compounds (Rice, 1974). Bioassays are an important part of allelopathy studies that employ whole plants, plant parts or plant tissues. Bioassays have been successful in detecting the biological activity of several synthetic compounds and natural products (Inderjit and Nilsen, 2003).

Allelochemicals produced in nature are largely influenced by habitat ecology and environmental factors (Inderjit, 1996). Different mechanisms of interference may occur at the same time, therefore making it difficult to separate these mechanisms at the field level (Inderjit and Dakshini, 1995). Laboratory, greenhouse, and growth chamber bioassays provide controlled conditions which allow the researcher to have control over the interactions that take place in nature. Numerous bioassays have been proposed for testing allelopathy; however, there also has been criticism on them often providing little or no connection to plant interactions that occur in the field, because it is difficult for bioassay experiments to simulate natural field conditions (May and Ash, 1990). However, the presence of phytotoxic chemicals in a plant does at least imply allelopathic potential in a natural setting (Heisey, 1990). For definite proof of allelopathy, demonstration that the allelopathic compound is released into the environment at a concentration high enough to cause allelopathic effects is essential (Inderjit and Keating**,** 1999). The use of various test plant species in bioassays can provide information on the phytotoxicity, selectivity or species sensitivity to allelochemicals (Hoagland and Williams, 2003). Specific molecular assays can also be performed on proven allelochemicals in order to elucidate modes-of-action (absorption, translocation and mechanism-of-action).

Hoagland and Williams (2003) state that bioassays have inherent limitations, such as: exhibition of large standard errors for means in dose-response curves compared to data from physicochemical methods, and the presence of interfering substances in non-purified extracts that may have greater effects in bioassays than in physicochemical analyses. They proposed that these limitations can be minimized by proper experimental designs, test material, test methodology, replication, and judicious selection of statistical analysis method. Furthermore, they pointed out that improved techniques such as HPLC, GC, mass spectrometry, NMR, immunological methods, etc., provide greater sensitivity/specificity and are more accurate than bioassays.

Among the many measures of phytotoxicity of allelochemicals, the inhibition (or stimulation) of seed germination, radicle elongation, and/or seedling growth in soil with surface debris or containing incorporated plant debris, have been the parameters of choice for most investigations (Leather and Einhellig, 1986). These

parameters are accepted as indirect measures of other physiological processes affected by chemical interaction. In this way, a wide range of effects are covered, and such bioassays serve to select compounds that can be evaluated in greenhouse and field studies (Macias *et al*., 2000).

1.2.5.1General plant bioassays

1.2.5.1a Germination bioassays

In general, extract bioassays are conducted in Petri dishes, by placing seeds of the receiver species on substrate (often filter paper) moistened with aqueous plant extracts of donor species (Wu *et al*., 1998). During extraction, care should be taken to ensure that seed germination is not delayed by the osmotic potential of the extract solution (Hoagland and Williams, 2003). The Petri dishes are placed in an incubator under controlled light and dark periods, and are regularly checked for their germination, usually up to seven days. Data generated is used to calculate percentage germination, which is often used for validating the existence of allelopathy in natural or in agro-ecosystems (Anjum and Bajwa, 2005). Rather, proof of allelopathy as a natural phenomenon requires a far more complex approach which should consider the production and exudation of allelochemicals by the donor species, the fate of the compounds in the environment in which they are released, as well as the uptake and growth responses of the acceptor species (Leather and Einhellig, 1988). Most studies on allelopathy in particular those based on bioassays, only achieve “proof of concept” by providing evidence that plants exhibit allelopathic potential.

1.2.5.1b Plant growth bioassays

Bioassays that assess plant growth for a significant period of a plant’s life cycle are not used as often as bioassays that run for short periods (days rather than weeks), but they all aid in contributing to understanding of the overall effect allelochemicals have on plant growth (Hoagland and Williams, 2003). Preparation or collection of foliar and root exudates (leachate), followed by growth bioassays and quantification of allelochemicals in the various media, are commonly used techniques to study the release of allelochemicals by donor species and their biological effects on acceptor species. In addition, allelochemicals should be collected with the least disruption of the normal mode of release. In some cases plants are grown hydroponically in water

or in a porous medium (sand or soil), which is amended with allelochemicals or extracts. Since the plants are not grown in sterile conditions, metabolism of the compounds or conversion of compounds to a nonactive or even a more active state is always a possibility (Inderjit and Nilsen, 2003).

1.3 *Conyza* species

Conyza bonariensis, Conyza sumatrensis and *Conyza canadensis* commonly known as flax-leaf fleabane, tall fleabane and Canadian fleabane respectively, belong to the sunflower (Asteraceae) family. About 7% of the species listed as declared invaders and weeds in South Africa belong to the Asteraceae family (Henderson, 2001).*C. bonariensis*, *C. sumatrensis* and *C. canadensis* are closely related species, and therefore they do not differ much in their morphology during early growth stages. Characteristics that set the three species apart are mainly detail related to leaf morphology of mature plants, the flowers and seed.

1.3.1 Botanical description

1.3.1.1 Taxonomy

Division		Magnoliophyta (Flowering Plants)
Class		Magnoliopsida (Dicotyledons)
Subclass		Asteridae
Order		Asterales
Family		Asteraceae (Aster family)
Genus		Conyza Less (horseweed)
Species	*Conyza bonariensis*	(L.)Conquist (Flax-Leaf fleabane)

Conyza sumatrensis (L.)Conquist
(Tall fleabane)
Conyza canadensis (L.)Conquist
(Canadian fleabane)

1.3.1.2 Biology and ecology

C. sumatrensis (Figure 1.1) is native to South America, and is a nearly unbranched semi-woody, annual plant that grows to more than 2 m in height, with a sturdy taproot, and stems with short, dense green hairs (Botha, 2001). Side branches only

occur on the upper third of the plant and are never longer than the main stem. The leaves are simple, usually alternate, with pointed tips, and narrow gradually to the base. Leaves are approximately 10 cm long and 1.5 cm wide. Flowers are white, in heads of up to 4mm in diameter that occur in large, terminal plumes. The fruits are straw-coloured, flattened and short-haired. The pappus consists of persistent hairs and is up to 4 mm long (Ivens *et al*., 1978; Botha, 2001).

Figure 1.1 *Conyza sumatrensis* (from: *Weeds of Crops and Gardens in Southern Africa,* Ciba-Geigy (1985))

Also originally from South America, *C. bonariensis* (Figure1.2) is an erect, multibranched, semi-woody plant that grows up to 1.2 m high, with a taproot system (Botha, 2001). The stems are short-haired, greenish and finely corrugated. The side branches are always longer than the main stem. Leaves are narrow, crinkled, greyish in colour, slightly toothed around the edges, up to 10 cm long and 1cm wide. The flowers have small yellowish heads at the ends of the branched stem at the upper part of the plant. Fruits are straw-coloured, up to 1.5 mm long, flattened and

sparsely haired. The pappus consists of persistent hairs and is up to 5 mm long (Botha, 2001; Shrestha and Hembree, 2005).

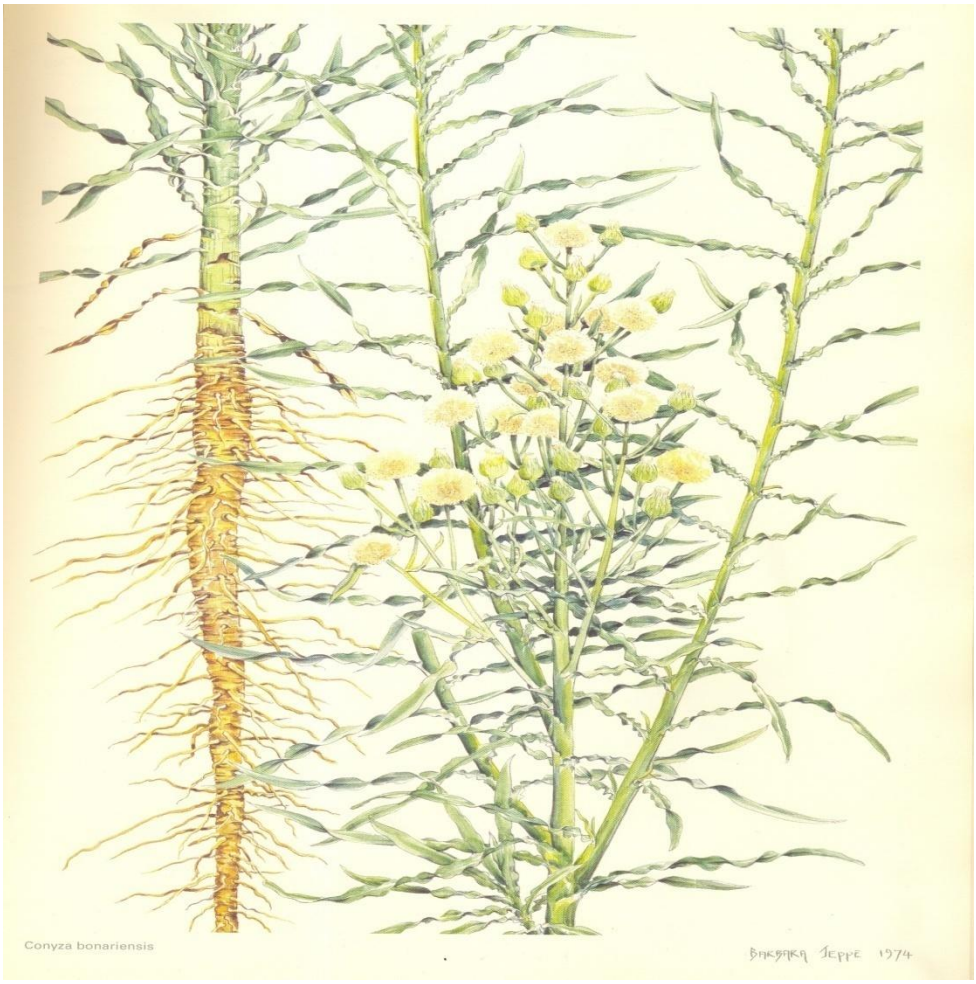

Figure 1.2 *Conyza bonariensis* (from: *Weeds of Crops and Gardens in Southern Africa,* Ciba-Geigy (1985))

C. canadensis (Figure 1.3) believed to be of North American origin, is an erect, semi-woody, winter or summer annual plant, with a short taproot. The stems are 1.8 m high, nearly smooth or bristly hairy, unbranched at the base, branched near the top, with many small flower heads. Stem leaves are alternate, numerous and crowded on the stem, often appearing opposite or even whorled, lanceolate to linear, with nearly entire margins; upper stem leaves only 5 mm wide (Weaver, 2001). Pale green to yellowish colour is given as a characteristic of this species. Flowers are 5 mm in diameter with white or slightly pink ray flowers and yellow disk flowers (Frankton and Mulligan, 1987; Alex, 1992). The fruits are straw-coloured, flattened and short-

haired. The pappus consists of persistent hairs and is up to2-4 mm long (Holm et al., 1997).

Figure 1.3 *Conyza canadensis* (from: *Weeds of Crops and Gardens in Southern Africa,* Ciba-Geigy (1985))

Conyza spp are prolific seed producers. *C bonariensis* reportedly produces about 375561 seeds per plant (Kempen and Graf, 1981). Seeds are dispersed within one or two capitula, for which time to maturity depends on the climate (Thebaud *et al*., 1996). Primary dispersal of *Conyza* seeds is via wind. There is no long seed dormancy in *Conyza*, with viability estimated as 1 to 2 years in the field (Weaver, 2001).

The optimum temperature regime for germination is 10°C minimum and 25°Cmaximum (Zinzolker *et al*., 1985). *Conyza* spp are small-seeded. Seeds only

emerge from (or near) the soil surface. For this reason the occurrence of *Conyza* is more common in zero or reduced till systems where the majority of seed remain on or close to the soil surface, and where increased stubble cover keeps the soil surface wet for longer (Wu and Walker, 2004). Although very limited emergence occurs in mid-winter, young autumn or early winter seedlings actively grow during winter despite cold and dry conditions (Weaver, 2001). Even where there does not seem to be much growth aboveground, root growth progresses. The building of a root system during winter provides sufficient food reserves for rapid growth during the following spring.

Due to difficulty in identification and confusion of these three species of *Conyza,* particularly because they grow in mixed populations allowing the occurrence of intermediate forms, hybridization has been speculated by some researchers (Thebaud and Abbott, 1995; Anzalone, 1964; Melzer, 1996). The differences of the three species have been explained as follows (Milovic, 2004):

- *C. canadensis* differs from *C. bonariensis* and *C. sumatrensis* by having a shorter, nearly glabrous involucre (3-4 mm long), only 25-40 female florets per capitula and having marginal florets with short (up to 1 mm) but well developed ligula.
- *C. sumatrensis* differs from closely related species *C. bonariensis* mostly by the marginal florets in the capitula. In the species *C. sumatrensis* marginal female florets are zygomorphic, while in *C. bonariensis* all the florets are actinomorphic. *C. sumatrensis* is otherwise much taller, branched out only in the upper part of the stem, lateral branches generally not overtopping the main axis and the inflorescence is rhombic in outline (Weaver, 2001)
- *C. sumatrensis* also has a greater number of leaves which are bigger, wider and with ramified lateral veins (Pignatti, 1982; Poldini and Kaligari, 2000; Sida, 2002). *C. sumatrensis* is recognisable particularly by its often well-developed winter rosettes (Anzalone, 1964, Poldini and Kaligari, 2000).

The genus *Conyza* represents one of the foremost examples of intercontinental plant invasions from the New World to the Old World. *C. sumatrensis* and another species of the genus are considered the most widespread species throughout the world

(Thebaud and Abbott, 1995). According to Hao *et al*. (2009), its invasiveness was always underestimated because of the difficulty in distinguishing the species from *Conyza bonariensis* in the field.

Widderick and Wu (2009) suggested the following as factors that make *Conyza* species major weeds.

- *Conyza* spp are major weeds of fallow land. These species competes for soil water and nutrients in both crop and fallow phases.
- *Conyza* spp are difficult to control with herbicides. Inconsistent control is often obtained with herbicide treatments, especially once plants in the rosette growth stage exceed a diameter of 30 mm. Where fleabane becomes a problem in fallows, weed control costs can increase by up to 80% due to the difficulty of controlling it.
- *Conyza* spp are capable of developing herbicide resistance.
- *C. bonariensis* flower throughout the year.The pappus on the seed enables it to be dispersed long distances by wind.

1.3.2 Distribution and habitat of *Conyza* spp in South Africa

In South Africa the first record of *C. sumatrensis* (formerly known as *Conyza albida)* was in 1896 from the Cape Peninsula. Other records of the plant include: White River district 1965, Orange Free State 45 km SE of Kimberly 1969, Potchefstroom 1974, Transvaal Naboomspruit 1977, 10km North of Hazyview 1989, Cape Town Ronderbosch 1989(Danin, 1990). These recordings indicate that the plant has invaded most, if not all the provinces in the country. Records of this species in neighbouring countries include Zimbabwe (1957), Namibia (1989), and Mozambique (1958). *C. sumatrensis* is alleged to be more competitive than the other species of *Conyza* (Thebaud *et al*., 1996). Case and Crawley (2000) stated that this species prefers highly disturbed areas and has a capacity to establish in a native ecosystem. In France, *C. sumatrensis* has been reported in gardens, vineyard, and in old fields (Case and Crawley, 2000). While in the Mediterranean, it typically grows and persists in old fields whose ages range from 20 to 30 years of abandonment (Thebaud *et al*., 1996). In England, *C. sumatrensis* has only been found in urban habitats, such as in concrete paving, gravel car parks and building sites (Wuizell,

1994). In West Africa it has been found near the edge of forests or in clearings as a weed in perennial crops (Ivens *et al*., 1978).

According to De Wet (2005) the first report of the occurrence of *C. bonariensis* in South Africa was made in May 1895 in Franschoek. Most recent reports of *Conyza* species in the country have been on *C. bonariensis*, particularly in the Western Cape. One such report is by Fourie and Raath (2009) who assessed the effect of organic and integrated soil cultivation practices on the weed population in a vineyard situated in the Paarl wine district. They found that of all the weeds present *C. bonariensis* filled the niche in this crop the most effectively and became the dominant species. In January 2003 a report of herbicide resistance in South Africa was made when resistance occurred in *C. bonariensis* in the Breede Valley, South Africa (Heap, 2005). The following year it was listed as one of the major weeds in South Africa that are well established and have substantial impact on natural ecosystems. In this survey it was also declared a riparian weed, which means that the number of times it occurred in riparian ecosystem exceeds that of landscapes in this particular survey (Nel *et al*., 2004). *C. bonariensis* has been documented as a host of insect pests which attack crop plants in New Zealand and South Africa, where the weed hosted mealiebug species, and in the Hex river valley where it was host to *Tetranychus urticae*, which is a spider mite that attacks deciduous fruit, and causes chlorotic spots (Fourie, 1996).

In a 1966 weeds survey on common weeds in South Africa conducted by Henderson and Anderson, *C. canadensis* was reported to be distributed in the Transvaal, Swaziland, Natal, Orange Free State, Basutholand, Northern Cape and Western Cape. Information on the occurrence of *C canadensis* in South Africa in recent years is scarce, although it has been reported to predominantly occur in the northern and eastern parts of the Western Cape (De Wet, 2005). Globally, *C. canadensis* is a weed of more than 40 crops (Holm *et al*., 1997). The list includes: fruit orchards, vineyards, field crops such as maize, soybean and cotton, particularly where conservation tillage or no-till systems are used, hay crops, pastures and rangeland (Kapusta, 1979; Buhler, 1992; Wiese *et al*., 1995;Leroux *et al*. 1996).*C. canadensis* has also been implicated in serving as a host to insect pests, such as the tarnished plant bug (*Lygus lineolaris)* and the alfalfa plant bug (*Adelphocoris lineolatus)*.

1.3.3 Interference and allelopathic potential of *Conyza* species

Allelochemicals released from plants often play a vital role in influencing the vegetational composition and population structure of a site (Shaukat *et al*., 2003).Intraspecific and interspecific competition of *Conyza* species has been explored. Thebaud *et al*. (1996) reported that the ability to absorb and utilise both water and nutrient resources within a competitive environment was greater in *Conyza sumatrensis* than in *Conyza canadensis*. Since this group of weeds has often been seen to form dense, almost pure stands and can tolerate a variety of habitats and environmental conditions (Economou *et al.*, 2002), it is reasonable for researchers to suspect allelopathy could be involved in the suppression of other plants in the vicinity. However, very limited literature exists on investigations into the allelopathic effects of leachates of different plant parts, as well as for compounds isolated from *Conyza* species. Phytotoxic effects of aqueous extracts of *C. canadensis* and *C. sumatrensis* have been observed on important crops such as tomato (*Lycopersicon esculentum*), wheat (*Triticum aestivum*), maize (*Zea mays*), millet (*Pennisetum americanum*), radish (*Raphanus sativus*), mungbean (*Vigna radiata*) and oats (*Avena sativa*) (Economou *et al*., 2002; Shaukat *et al*., 2003; Travalos *et al*., 2007).

1.3.4 Control measures

1.3.4.1 Mechanical control

For effective control of *Conyza* it is better to treat when small, at its early growth stages when it is actively growing, but before stem elongation. Hand-pulling after stem elongation is effective in light soils, but on heavier soils a hand-hoe is required to prevent the plant breaking and regrowing from the base. Planting of perennials to increase ground cover and the shading effect will help in reducing reinfestation (Wu, 2004). Soil tillage can completely control *Conyza* species without the use of herbicides but the former practice is not always practical, especially in minimum- and zero-tillage systems. Mowing has been reported to have the tendency to stimulate additional branching from the crown and only delays seed production. It also hardens the plants and makes control with post-emergence herbicides difficult.

1.3.4.2 Biological control

In annual crop systems, biological control offers hardly any options for weed control because of the requirement for absolute host-specificity in biocontrol agents, and the weed spectrum on crop fields is mostly diverse. Very little information is available on biocontrol options for *Conyza* species. However, the bacterium *Pseudomonas syringae pv tagetis* has been reported to affect these weeds, but this potential biocontrol agent has not yet been developed on a large scale (Charudattan, 2001).

1.3.4.3 Chemical control

The two most commonly used herbicides for the control of *Conyza* species are paraquat and glyphosate (De Wet, 2005).However, many other herbicides were listed in the 2012 Croplife (South Africa) Herbicide Module (Table 1.1).

Table 1.1 Herbicides registered to control *Conyza* species (Croplife South Africa, 2012)

Weed Species	Active ingredient	Recommended Rate	Trade name	Crops
Conyza bonariensis **Flax-leaf fleabane** ***Kleinskraalhans***	2,4-D/Dicamba	280/80 gL^{-1}	Trooper SL	Grass pastures; Lawns; Maize; Sugarcane; Turf; Wheat
	Bromoxynil/Ioxynil	200/200 gL^{-1}	Voloxytril 400 EC	Sugarcane
	Carfentrazone-Ethyl	400 gKg^{-1}	Aurora 40 WG	Almonds; Aloes; Apples; Avocadoes; Bananas; Barley; Citrus; Coffee; Granadilla; Grapes; Guavas; Hops; Kiwi; Litchi; Macadamias; Mangoes; Nectarines; Olives; Papaya; Papaya; Peaches; Pears; Pecans; Plums & Prunes; Tea; Wheat
	Dicamba	700 gKg^{-1}	Dominator	Grain sorghum; Wheat
	Diuron	800 gKg^{-1}	Karmex	Citrus; Pineapples; Sugarcane
	Diuron/Paraquat	300/100 gL^{-1}	Volmuron	Bananas; Citrus; Papaya; Sugarcane
Conyza canadensis **Canadian fleabane** ***Kanadese skraalhans***	Acetochlor/Ametryn	450/250 gL^{-1}	Acetamet 700 SC	Sugarcane
	Ametryn	500 gL^{-1}	Ametryn 500 SC	Bananas; Pineapples; Sugarcane
	Glufosinate-Ammonium	200 gL^{-1}	Basta	Almonds; Aloes; Apples; Avocadoes; Bananas;

				Barley; Citrus; Coffee; Granadilla; Grapes; Guavas; Hops; Kiwi; Litchi; Macadamias; Mangoes; Nectarines; Olives; Papaya; Papaya; Peaches; Pears; Pecans; Plums & Prunes
	Glyphosate	500 gKg^{-1}	Kilo WSG	Afforestation; Firebreaks
	Metribuzin	480 gL^{-1}	Metribuzin 480	Asparagus; Lucerne; Leguminous pastures; Potatoes; Sugarcane; Tomatoes
	Simazine	500 gL^{-1}	Simazine	Apples; Asparagus; Canola; Citrus; Grapes; Pears
	Tebuthiuron	50 gKg^{-1}	Spike 50 GR	Sisal
Conyza sumatrensis **Tall fleabane** ***Vaalskraalhans***	2,4-D/Dicamba	280/80 gL^{-1}	Trooper SL	Grass pastures; Lawns; Maize; Sugarcane; Turf; Wheat
	Atrazine/Sulcotrione	300/125 gL^{-1}	Caravelle	Maize; Sweetcorn
	Glyphosate	500 gKg^{-1}	Kalash 700 WSG	Most Agricultural Situations
	Hexazinone	750 gKg^{-1}	Velpar DF	Afforestation; Sugarcane
	Simazine	500 gL^{-1}	Simazol SC	Apples; Asparagus; Canola; Citrus; Grapes; Pears
	Tebuthiuron	50 gKg^{-1}	Spike 50 GR	Sisal

1.3.4.4 Herbicide resistance

Herbicide resistance can be defined as the inherent ability of a weed to survive a rate of herbicide which would normally result in effective control (WSSA, 1998).Most cases of herbicide resistance have occurred in situations where the same herbicides (or herbicides with the same mode of action) have been used repeatedly over a period of years(De Wet, 2005). Herbicide resistance can result from any inherited trait, which allows plant to survive herbicide applications. This could be due to biochemical or physiological changes, morphological alterations that affect herbicide uptake or interception or phonological changes, such as changes in germination patterns. Herbicide resistance is generally thought to occur within weed populations as a consequence of the intense selective pressure exerted by lack of diversity in weed management practices (Gressel and Segel, 1978).

In South Africa herbicide resistance was reported for the first time two decades ago in the Western Cape, when Cairns and Laubscher (1986) reported resistance of wild

oats (*Avena fatua*) to diclofop-methyl (De Wet, 2005). Pieterse (2010) states that following this report, other reports of herbicide resistance in grass species increased dramatically. Botes and Van Biljon (1993) showed multiple resistance of ryegrass (*L. rigidum*) to ACCase and ALS inhibitors (Heap, 2009). These findings were confirmed five years later (Smit and De Villiers, 1998; Smit*et al*., 1999).The occurrence of resistance in smooth pigweed (*Amaranthus hybridus*) to triazine in 1993 reported by Botes and Van Biljon was the first record of herbicide resistance in broadleaved weeds in South Africa (Heap, 2009; Pieterse, 2010). In recent years wild radish has shown indications of resistance to chlorsulfuron, and several other ALS inhibitors (Smit and Cairns, 2001; Heap, 2009; Pieterse, 2010).

Herbicide resistance has evolved within *Conyza* populations in several countries (VanGessel, 2001). Weed resistance to paraquat and glyphosate have been reported in *Conyza bonariensis* and *Conyza canadensis*. Paraquat is a foliage-active bipyridylium herbicide, which exerts its phytotoxic effect by catalyzing electron transfer from PSI to molecular oxygen-generating superoxide anion radicals and other active oxygen species. These phytotoxic oxygen species cause lipid peroxidation and membrane damage (Racz*et al.*, 2000). Glyphosate [N-(phosphonomethyl) glycine] is a non-selective, broad spectrum, systemic, post-emergence herbicide. This herbicide kills weeds by metabolic disruptions in the plant (Franz *et al*., 1997). It inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) which is essential for biosynthesis of certain aromatic amino acids (Mueller *et al*., 2003).

A paraquat resistant biotype of *Conyza* originated in the Tahrir irrigation area in Egypt (Shaaltieland and Gressel, 1986). An intensive paraquat spraying program was undertaken in vine and citrus plantations in 1970 and difficulties in controlling this weed were first observed in the mid-1970s (Fuerst *et al*., 1985). The exact site and mechanism of paraquat binding to sequester the herbicide remains to be determined, but Fuerst *et al.* (1985) proposed that it is primarily due to exclusion of the herbicide from the site ofaction in the chloroplast, resulting from rapid sequestration viaan unknown mechanism.

The first reported cases of glyphosate-resistant *C. bonariensis* were in South African orchards and vineyards in January 2003(Figure 1.4).Reports were received from the Breede Valley (about 100km north east of Cape Town) of glyphosate failing to control *C. bonariensis* at registered dosage rates (Heap, 2005; Heap, 2009).Other cases that followed were in 2004 and 2005 in Spanish and Brazilian orchards (Heap, 2007). The glyphosate resistance mechanism in *C. bonariensis* is still unknown to date, as no literature is available on this topic (Dinelli *et al.*, 2008). According to Pieterse (2010), a biotype of *C. canadensis* from the Limpopo province that was resistant to paraquat was also recorded (Dr PJ Pieterse,Department of Agronomy, University of Stellenbosch: unpublished results).

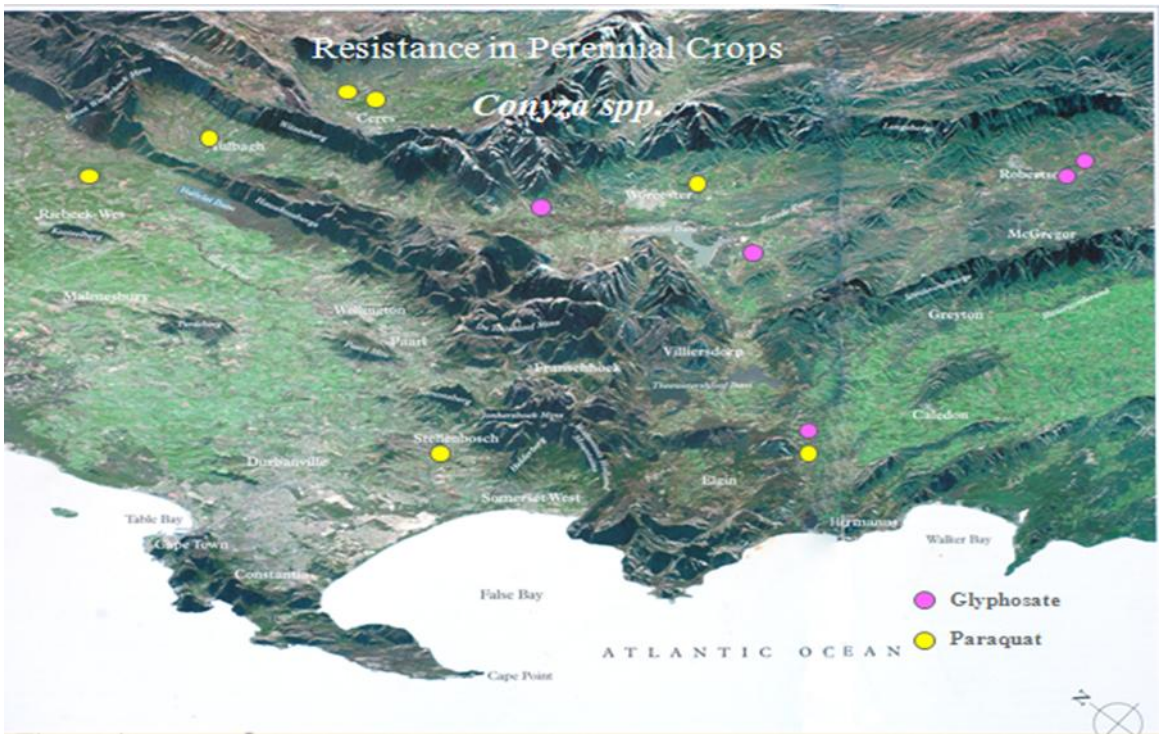

Figure 1.4 A map showing the distribution of glyphosate and paraquat resistant *C. bonariensis* in the Western Cape, South Africa (Prof. A Cairns, unpublished; De Wet, 2005)

A biotype of *C. sumatrensis* resistant to imazapyr was discovered on a farm in the province of Seville, Spain, on land that had been continuously treated with this herbicide (Osuna and De Prado, 2003). Imazapyr is a non-selective herbicide belonging to the imidazolinone family, used for the control of a broad range of weeds including annual and perennial grasses and broad-leaved species. The mode of

action of imazapyr is the inhibition of acetoacetate synthase, the first common enzyme in the biosynthesis of the branched-chain amino acids valine, leucine and isoleucine (Saari and Mauvais, 1996).

According to anecdotal evidence, there is currently uncertainty about which *Conyza* spp occur where in South Africa. A certain school of thought believes that *C. canadensis* might not occur in the Western Cape. As mentioned above Pieterse tested a *C. canadensis* from Limpopo Province, which is about 2000 km removed from the Western Cape. This begs the question, what is the real situation with regards the distribution of *Conyza* spp in South Africa.

CHAPTER 2

ALLELOPATHIC INFLUENCE OF *CONYZA BONARIENSIS* ON LETTUCE AND TOMATO SEED GERMINATION AND EARLY SEEDLING DEVELOPMENT

2.1 Introduction

Conyza spp are annual, herbaceous, invasive weeds of the Asteraceae family. Worldwide, three *Conyza* spp (*Conyza bonariensis*, *Conyza canadensis, and Conyza sumatrensis*) are noxious weeds in many crops (Everett, 1990; Weaver, 2001; Milovic, 2004). In South Africa, these three species are well known weeds and were first noticed about a century ago, with infestations of one or more species in every province (Danin, 1990; Botha, 2001). *C. bonariensis* is a weed of cultivated and non-cultivated lands, gardens, roadsides and waste places (Ciba-Geigy, 1985; Botha, 2001). Adding to its problem status is the recent discovery that certain *C. bonariensis* biotypes in South Africa and other parts of the world are resistant to the herbicide glyphosate, and in certain cases to both glyphosate and paraquat (Pieterse, 2010). Despite its invasiveness and ability to compete severely with crops, little is known about the mechanisms of interference employed by *C. bonariensis*.

The phenomenon of allelopathy is known to be one of two predominant forces in the development of plant communities and spatial patterns therein (Rice, 1984). Among the weed species reported globally, a considerable number are known to possess allelopathic potential (Ashraf and Sen, 1978; Shaukat *et al.*, 1983; Ahmed and Wardle, 1994). To date, very few studies have assessed the allelopathic potential of *C. bonariensis.* However, studies on a related species *C. canadensis*, identified three active enyne derivatives, (2Z,8Z)-matricaria acid methyl ester, (4Z,8Z)-matricarialactone, and (4Z)-lachnophyllum lactone (Queiroz *et al*., 2012). According to Queiroz *et al*. (2012), the three isolated acetylenes may be involved in the reported allelopathy of *C. canadensis*, and that since these compounds are found in other Asteraceae plants, they may play a role in allelopathic properties of different species in this family. In their study, it was proposed that (4Z)-lachnophyllum lactone showed most promise as a potential herbicide. In preliminary studies investigating the allelochemical characteristics of *C. sumatrensis* another closely related species

of *C. bonariensis,* it was found to have an inhibitory effect on oat germination and seedling growth (Shaukat *et al.*, 1983; Economou *et al.*, 2002).

Allelopathy can be seen as a problem, or if viewed positively, can serve as a weed management tool for sustainable agriculture (Weston and Duke, 2003; Ferreira and Reinhardt, 2010; Bezuidenhout *et al*., 2012). Because the potential of undesirable environmental contamination from herbicides is high in some instances, researchers have suggested the use of plant-produced secondary metabolites as natural pesticides in agriculture or as structural leads for new synthetic pesticides, which are environmentally safe and are equivalent in terms of efficacy and selectivity to the currently available synthetic herbicides (Putnam *et al*., 1983; Travalos *et al*., 2007; Queiroz *et al*., 2012).

When considering the allelopathic potential of plants, it is imperative to distinguish between the effects of competitive and chemical (allelopathy) interference (Fuerst and Putnam, 1983; Leather and Einhellig, 1986; Inderjit and Olofsdotter, 1998). Therefore, bioassays in allelopathy research should be designed to eliminate the effects of plant-plant competition. Laboratory bioassays allow researchers to eliminate possible alternative interferences through controlled experimental designs and manipulation of nearly all parameters, in order that investigators can vary complex field conditions one at a time in the search for mechanistic interactions.

C. bonariensis plants in South Africa are often observed to form dense, almost pure stands; therefore, it is conceivable that this species could employ both competition and allelopathy in the suppression of other plants in the surrounding area. Because little is known about the allelopathic nature, as compared to competition effects of this weed, experiments in the present study were designed to assess the allelopathic potential of *C. bonariensis*. The objectives of the investigation were to: (1) evaluate the effect of aqueous extracts of *C. bonariensis* on seed germination and seedling growth of two test species; and (2)to establish if the compounds responsible for germination and seedling growth response are polar or non-polar in nature by using two solvents with differing polarities to extract *C. bonariensis* plant tissue. Experiments in this bioassay approach were designed to minimise potential interfering factors, e.g., competition, osmotic effects and pathogenic organisms.

2.2 Materials and Methods

2.2.1 The bioassay technique

In general, extract bioassays are conducted in Petri dishes by placing seeds of receiver or test species on substrata (often filter paper) moistened with aqueous infusions or plant extracts of donor species (Wu *et al.,* 1998). The Petri dishes, which usually are placed in an incubator under controlled light and dark periods, are regularly checked for seed germination, and early seedling development, often for at least seven days. Data recorded are typically used to calculate percentage germination and to determine early seedling growth. Results are employed to make inferences on allelopathy in natural ecosystems or in agro-ecosystems.

2.2.1.1 *C. bonariensis* material used in the study

Mature plants of *C. bonariensis* were collected on the Hatfield Experimental Farm of the University of Pretoria. Test species were tomato and lettuce. According to Reinhardt *et al.* (1999), the type, amount and location of allelochemicals may play an important role in the determination of a plant's allelopathic potential. Leaf and root material of *C.bonariensis* collected at the pre-flowering stage were used in all experiments. The highest content of inhibitors (allelochemicals) is reportedly usually present in the leaves of a plant (Roshchina and Roshchina, 1993). It has also been observed that phytotoxic activity of upper leaves and inflorescence of related species *C. sumatrensis* is significantly higher than in other tissues studied, e.g., in stems (Economou *et al.*, 2002). Therefore, it was assumed that the leaf material used in these studies was probably the richest in potential inhibitors.

2.2.1.2 Preparation of crude extracts

After sampling, the plant material was frozen immediately and then freeze-dried prior to extraction. Allelopathic bioassays with ground and frozen plant material have received a great deal of criticism, for the reason that grinding results in the release of certain compounds, which may not be released under natural circumstances. It is possible that the extraction procedure may cause qualitative and quantitative changes in the phytochemical profile of the plant material. We considered the freeze-drying process and subsequent non-drastic extraction as practical for demonstration of allelopathic potential.

For germination bioassay, leaf and root extracts were prepared by extracting 50 g of *C. bonariensis* leaves or roots with 350 ml of two solvents: pure water (polar) and hexane (non-polar). Plant material was extracted separately, and not consecutively, with the two solvents. Plant-solvent mixtures were stirred, covered with aluminium foil and placed in the dark for 24 h at room temperature. Extract solutions were filtered through Whatman No.1 filter paper and diluted with the respective solvents to give a concentration range of 25, 50, 75, and 100% (v/v). The control treatment was distilled water. Aliquots of 5 ml of each of the extract solutions were added to filter paper in Petri dishes. For the hexane treatments the solvent was allowed to evaporate off the filter paper before 5 ml distilled water was added to the Petri dishes, each containing 10 seeds of either lettuce or tomato that had been sterilized beforehand in 1% sodium hypochlorite. Each treatment was replicated ten times. Petri dishes were sealed with Para-film-® and stored in a growth chamber at 25ºC (12h/12h light/dark) for seven days. Seed germination was recorded every day, and root (radicle) and shoot length were measured on day 7 only.

2.2.1.3 Choice of test species

Lettuce and tomato were the chosen test species. Many similar bioassay studies have used lettuce and tomato as test species because of their known germination and growth behaviours. Most often lettuce (*Lactuca sativa* L.) is used to simulate plant response to allelochemicals because of its fast germination and high sensitivity (Rasmussen and Einhellig, 1979; Leather, 1983; Yu and Matsui, 1994; Macias *et al.*, 2000). It is used extensively in allelopathy studies and allows comparison of bioassay results for many different compounds.

2.2.1.4 Germination and seedling development assessments

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 and to develop. Seeds were considered to have germinated if their radicles had emerged and 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 Sterilisation procedures

To ensure the exclusion of microbial contamination that have the potential to cloud the results the following measures were introduced:

- Distilled water was autoclavedat121 ºC for 30 minutes.
- Sterilised Petri dishes and filter paper from sealed boxes were used in the experiments.
- All experiments were conducted in a laminar flow cabinet, where aseptic conditions were maintained by swabbing surfaces and instruments with 70% ethanol and through flaming.
- Fungicide-coated seeds of lettuce variety Great Lakes and tomato variety Moneymaker were surface-disinfected by soaking them in 1% commercial bleach for twenty minutes. The seeds were then rinsed three times with sterilized distilled water and air-dried under laminar flow.

2.2.2 Exclusion of osmotic potential effects on germination and growth

It is often assumed that the response of seed or seedlings to plant extracts is due entirely to allelopathy, however, the possibility exists that the extracts may also exert negative osmosis effects on the test species (Bell, 1974), and some investigators have assessed the relative importance of osmotic influence and allelopathic potential of plant extracts on seed germination and early seedling development (Stowe, 1979; Bothma, 2002; Dixon, 2008). Osmotic effects are well known to induce stress responses in plants, primarily by causing dehydration of plant material (Slayter, 1967).In the case of seed exposed to water that contain dissolved materials, high osmotic potential (low water potential) could limit or prevent water imbibition by the seeds required for germination.

2.2.2.1 Measuring principle in determining osmotic potential

In this study the Herman Roebling digital micro-osmometer was used. The principle of its operation is that freezing point depression below that of pure water is a direct measure of the osmotic concentration of an aqueous solution. Pure water freezes at 0°C, whereas an aqueous solution with an osmolality of 1 $Osmolkg^{-1}$water freezes at −1,858°C. The sample starts off at room temperature. It is pipetted into a sample tube, which is placed onto the measuring head. The measuring head is pushed

beneath its guide rod, thus inserting the sample tube into the cone shaped cooling aperture. Now the sample begins to be cooled. The digital display will show decreasing values. Once zero is reached, increasing negative values will be displayed. At a certain stage of supercooling (when the digital display reads −70°C), a cooled needle is inserted manually to initiate ice formation. The temperature will begin to rise until the freezing point is reached. The point of disparity thus achieved is the value. The digital display of the machine will display milliOsmol and not °C, because osmolality is directly related to freezing point reduction.

2.2.2.2 Use of polyethylene glycol in studying osmotic potential effects

As mentioned above**,** the aim of this experiment was partly to demonstrate that osmotic effects could cloud allelopathic effects in bioassays employing seed germination and early seedling development as parameters for allelopathic effects. The same procedure was not followed in the case of tomato test species because the aim was to demonstrate the principle that cognisance ought to be taken of osmotic potential in bioassays of this nature, and for this purpose lettuce served as test case.

Polyethylene glycol (PEG−6000) is commonly used for testing plant responses to osmolalities of substrates. It affects seed germination only by altering the osmolality of water such that any effect observed on the germinating seed is a result of osmotic potential of the solution. An osmotic range was prepared by dissolving different amounts of PEG−6000 in distilled water. It has been previously determined that concentrations of 12.5, 25, 50, and 75 gL^{-1}water of PEG would give the best osmotic range for bioassay studies (Hoagland and Brandsaeter, 1996). However, in this study 100 and 125 g of PEG-6000 were included because osmolality recorded for the aqueous weed extracts exceeded that provided by PEG-6000 concentrations ranging from 12.5 to 75 gL^{-1}.

Therefore, in order to exclude negative osmosis as a possible cause of lettuce seed germination inhibition, osmolalities of *C. bonariensis* aqueous extracts were measured in a preliminary experiment using the Herman Roebling digital micro-osmometer. This was done only in the case of the lettuce aqueous extracts since the hexane extracts are not water soluble.

2.2.3 Statistical analysis

Analysis of variance (ANOVA) was done using the statistical program SAS 9.2 (2002). A completely randomised design was used in all experiments. Analysis of variance was used to test for differences between treatments. Radicle length data for lettuce exposed to extract solutions were subjected to rank transformation, otherwise the shoot and radicle data were acceptably normal with homogenous treatment variances. In the case of germination percentages, angular transformation was used to stabilise variances. Treatment means were separated using Tukey's studentised range test least significant difference (LSD) at the 5% level of significance.

2.3 Results and Discussion

2.3.1 Effect of osmotic potential on lettuce seed germination and early seedling development

When considering the influence of osmotic potential of PEG-6000 solutions on lettuce germination, experiments showed that no significant inhibition of germination occurred at any of the osmolalities created with PEG-6000 (Table. 2.1). Osmotic potential did not interfere with radicle growth of lettuce up to and including 50 mOsmkg^{-1}, the second highest osmolality tested. However, shoot length seems to have been more sensitive, as significant inhibition occurred at the lowest osmolality tested (24 mOsm kg^{-1}).

Table 2.1 The effect of PEG-6000 solutions of increasing osmolality on germination and radicle and shoot lengths of lettuce seedlings

PEG-6000 concentration gL^{-1}	Osmolality (mOsm kg^{-1})	Percentage germination	Radicle length (mm)	Shoot length (mm)
0	0	100a	53.7a	18.5a
50	24	98a	51.5a	14.5b
75	50	97a	50.1ab	7.1c
100	96	98a	43.2b	6.9c
125	147	96a	34.4c	5.6c

Means in each column followed by different letters are significantly different according to Tukey's Studentised Range test LSD (P< 0.05).

Based on the above findings, the significant reduction in germination of lettuce seed that occurred as a result of exposure to *C.bonariensis* leaf aqueous extracts, was attributable to a possible allelopathic effect and not to osmotic potential effects (Table 2.2). Radicle length was significantly inhibited by an infusion concentration of 72 mOsm kg^{-1}, which probably is due to possible allelopathic effects and osmotic effects, whereas the significant reduction in shoot length that occurred from 110 mOsm kg^{-1} is probably due to a combination of allelopathic and osmotic effects. Although germination was not significantly affected by osmotic effects, radicle and shoot growth were at least partly influenced by osmotic potential.

Table 2.2 Effect of *C. bonariensis* leaf infusions of increasing osmolality on germination and mean radicle and length of lettuce seedlings

Extract concentration (%)	Osmolality (mOsm kg^{-1})	Percentage germination	Radicle length (mm)	Shoot length (mm)
0	0	97a	24.5a	13.8a
25	36	97a	20.8a	11.8ab
50	72	86a	12.5b	8.3ab
75	110	62b	6.0c	3.8c
100	138	56b	5.3c	5.5 bc

Means in each column followed by different letters are significantly different according to Tukey's Studentised Range test LSD (P< 0.05).

Based on the data in (Table 2.1) increasing osmolality of PEG-6000 did not affect radicle growth adversely within the range of 24 to 96 mOsm kg^{-1}. Therefore, it is possible that at the highest two osmolality (96 and 147mOsm kg^{-1}), osmotic effects may have interfered with radicle growth. As the osmolalities of the aqueous infusions, prepared from *C. bonariensis* leaf material (Table 2.2), up to 36mOsm kg^{-1} were below the limit for growth inhibition in the PEG-6000 experiments, it can be concluded that, apart from the three osmolalities (72,110 and 138mOsm kg^{-1}) osmotic effects did not play a role in the inhibitory effects of *C.bonariensis* infusions on seed germination and seedling growth. Considering that growth inhibition of lettuce shoots occurred at 24 mOsm kg^{-1} in the PEG-6000 experiments (Table 2.1), it is highly probable that osmotic inhibition may have been a contributing factor from 36 mOsm kg^{-1} to 138 mOsm kg^{-1} in the shoot growth inhibition caused by *C.bonariensis* aqueous leaf extracts, as illustrated in Table 2.2.

2.3.2 Effect of aqueous extracts of *Conyza bonariensis* on germination and seedling growth of two test species

2.3.2.1Effects of aqueous extracts on lettuce

Germination: Significant inhibition of seed germination was observed at the 75% concentration for only the weed leaf infusion, and at 100% concentration for both the leaf and root infusions. At 100% concentration the allelopathic effect of root extracts was significantly higher than that of the leaves (Figure 2.1). As osmotic effects on lettuce seed germination can be excluded, based on findings presented in Tables 2.1 and 2.2, germination inhibition observed here can be ascribed to possible allelopathic effects.

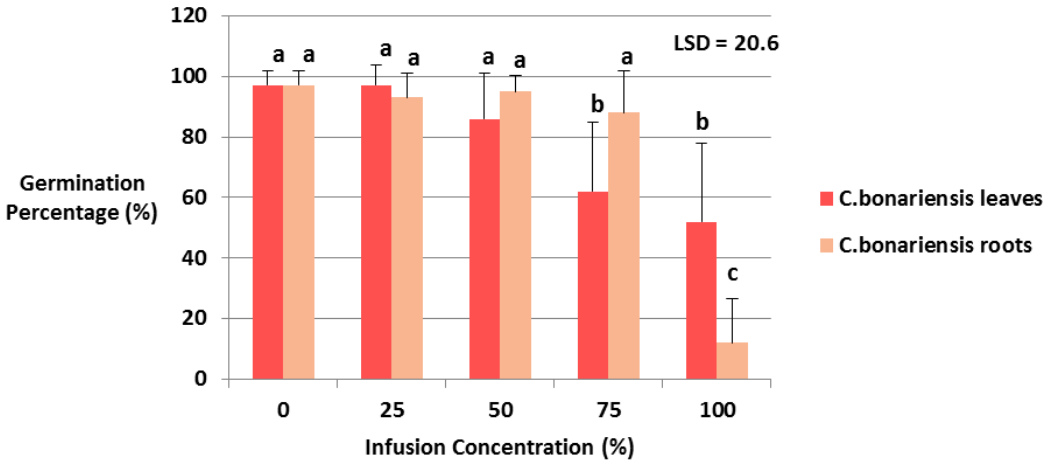

Figure 2.1 Effect of aqueous leaf and root extracts of *C. bonariensis* on seed germination of lettuce (Means with same letters do not differ significantly at P=0.05; Comparisons were made across plant part and infusion concentrations; ANOVA presented in Appendix A, Table A1)

Radicle growth: Figure 2.2shows that both leaf and root infusions of *C. bonariensis* significantly inhibited radicle growth of lettuce from the 25% infusion concentrations onwards. Overall, there is not a clear difference in the intensity of effects between extracts of leaf and root material of *C. bonariensis*. It is important to note that the osmotic potential results (Table 2.2) implicate that osmotic potential is playing a role in plant responses from the 50% infusion concentration and higher.

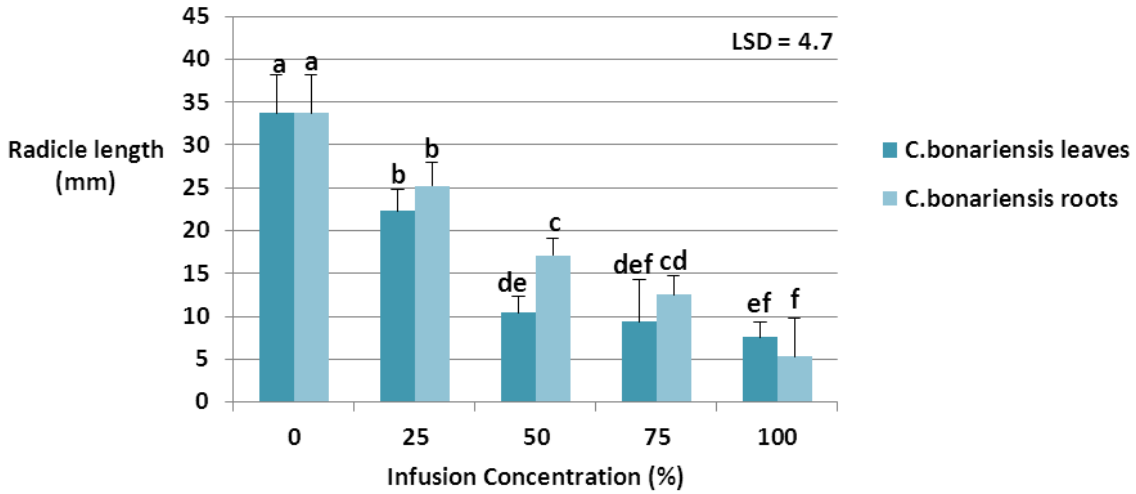

Figure 2.2 Effect of aqueous leaf and root extracts of *C. bonariensis* on root (radicle) growth of lettuce (Means with same letters do not differ significantly at P=0.05; Comparisons were made across plant part and infusion concentrations; ANOVA presented in Appendix A, Table A2)

It needs to be emphasised that the perceived role of solution osmotic potential does not mean that allelopathy had no role at 75 and 100% infusion concentrations (Figure 2.2). The degree of inhibition tended to increase with increasing infusion concentration, but was not significant in all instances. At 50% infusion concentration only, the leaf infusion had a significantly greater effect on root growth reduction compared to the control than the root infusion. Studies by Economou *et al*. (2002), using similar methods of bioassay, showed an aqueous extract of dried aerial parts of *C.sumatrensis*, another cosmopolitan species occurring in South Africa, to have an inhibitory effect on the germination and seedling growth of oat (*Avena sativa*). Oat radicle elongation was reduced with increasing extract concentration. Similar results were reported from leachate experiments with *Parthenium hysterophorus*, a weed also belonging to the Asteraceae family (Mersie and Singh, 1987). Therefore, results from the bioassays using leaf extract of *C. bonariensis* agree with work done by other researchers on Asteraceae species in relation to allelopathic potential.

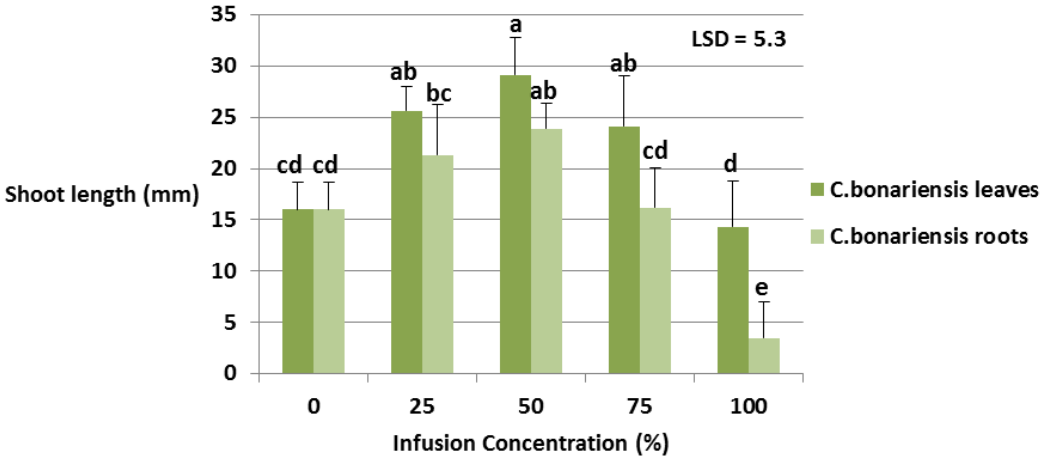

Figure 2.3 Effect of aqueous leaf and root extracts of *C. bonariensis* on shoot growth of lettuce (Means with same letters do not differ significantly at P=0.05; Comparisons were made across plant part and infusion concentrations; ANOVA presented in Appendix A, Table A3)

Shoot growth: In Figure 2.3 there is a trend for apparent growth stimulation between 25 and 75% infusion concentrations for both leaf and root extracts. At all the leaf infusion concentrations, lettuce shoots were significantly longer than those of the control and the 100% infusion concentration. This stimulatory effect was significant for the root infusions at 25 and 50%. However, at 100% concentration the root extract of the weed significantly reduced shoot growth of lettuce compared to the control and all the other infusion concentrations. According to Belz *et al.* (2005) some allelochemicals, which are toxic at high concentrations, can have a stimulatory effect on one or several traits in a plant when applied at low concentrations. This phenomenon is called hormesis.Belz *et al.* (2007) reported a significant hormesis effect for *Eragrostis curvula*, with growth stimulation occurring at low parthenin concentrations, and inhibition at higher doses. However it would take a far more detailed experiment to prove this theory here. The significant inhibitory effect on shoot growth of lettuce observed at 100% root infusion may at least partly be attributed to osmotic potential considering the results presented in Table 2.2.

2.3.2.2 Effects of aqueous extracts on tomato

Germination: Germination of tomato seed was significantly reduced by the leaf aqueous extracts of *C. bonariensis* at 50% and higher infusion concentrations (Figure 2.4). At 50 and 75% concentrations the leaf extract had a significantly greater inhibitory effect than the root extract. At 100% infusion concentration tomato seed exposed to the root extract did not germinate at all. A complete lack of germination for tomato at 75% and 100% by shoot extracts of *C. canadensis* was also observed by Shaukat *et al.* (2003).

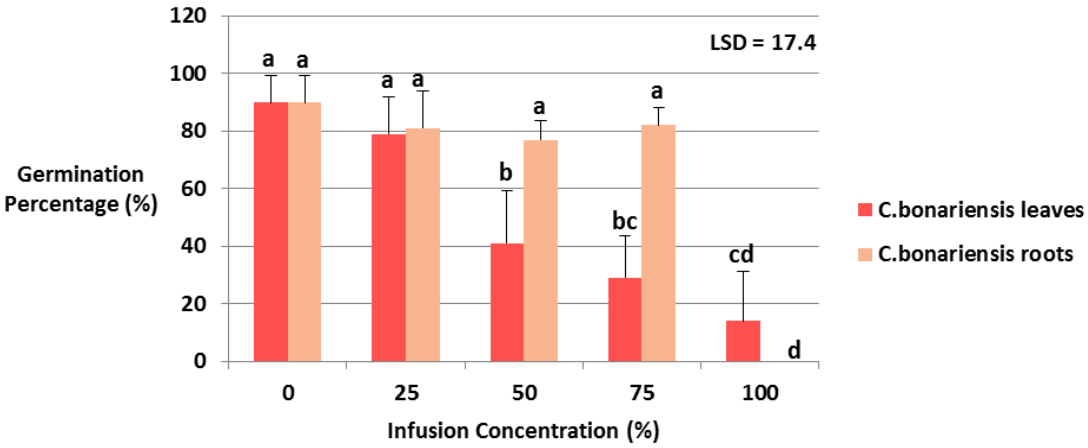

Figure 2.4 Effect of aqueous leaf and root extracts of *C. bonariensis* on seed germination of tomato (Means with same letters do not differ significantly at P=0.05; Comparisons were made across plant part and infusion concentrations; ANOVA presented in Appendix A, Table A4)

Radicle growth: The length of tomato radicles exposed to foliar and root extracts of *C.bonariensis* were significantly reduced, and inhibition tended to increase with increasing infusion concentration (Figure 2.5). At each of the 25%, 50% and 75% concentrations, the leaf extract showed significantly greater inhibition than the root extract. However, at 100% infusion concentrations there were no differences in radicle length between the two extracts, largely as a result of very limited germination. As in the present study, results showing that leaves contained the

highest amount of phytotoxic substances were found by Picman and Picman (1984) in *P. hysterophorus*.

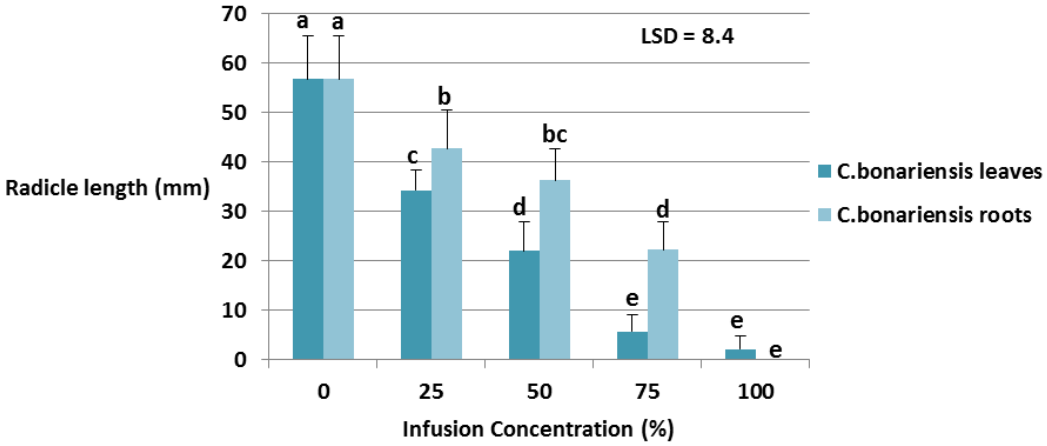

Figure 2.5 Effect of aqueous leaf and root extracts of *C. bonariensis* on root growth (radicle) of tomato (Means with same letters do not differ significantly at P=0.05; Comparisons were made across plant part and infusion concentrations; ANOVA presented in Appendix A, Table A5)

Shoot growth: For tomato shoots (Figure 2.6), there was a similar trend of growth stimulation as in lettuce shoots between 25% and 50% infusion concentrations of the leaf extract. However, this stimulation was only statistically significant at 25%.As stated previously; this effect could be due to hormesis, which was not investigated further in this study. At 75% infusion concentration, the leaf extract completely inhibited shoot growth of tomato and the root extract significantly reduced growth compared to the control. The 100% infusions of both plant extracts completely inhibited shoot growth of tomato.

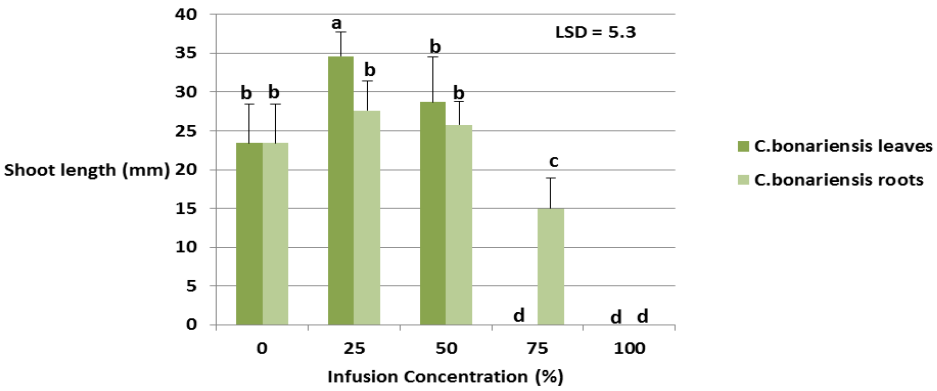

Figure 2.6 Effect of aqueous leaf and root extracts of *C. bonariensis* on shoot growth of tomato (Means with same letters do not differ significantly at P=0.05; Comparisons were made across plant part and infusion concentrations; ANOVA presented in Appendix A, Table A6)

2.3.3 Effects of hexane extracts of leaves and roots of *C. bonariensis* on germination and early seedling growth of two test species

2.3.3.1Effects of hexane leaf and root extracts on lettuce

Germination: Inhibition of lettuce seed germination by leaf and root hexane extracts of *C. bonariensis* occurred only at the 75 and 100% infusion concentrations (Figure 2.7). At 75% infusion concentration the roots inhibited germination relative to the control, whilst at the 100% infusion concentration the observed inhibition effect was as a result of the leaf extract.

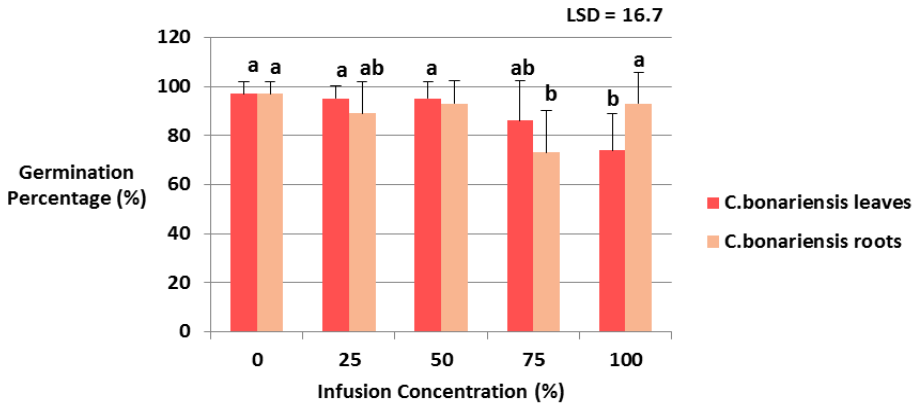

Figure 2.7 Effect of hexane leaf and root extracts of *C. bonariensis* on root growth of lettuce (Means with same letters do not differ significantly at P=0.05; Comparisons

were made across plant part and infusion concentrations ANOVA presented Appendix A, Table A7)

Radicle growth: The hexane extract of *C. bonariensis* leaves significantly reduced lettuce radicle growth from the 25% infusion concentration onwards as compared to the control, with the 100% infusion concentration causing the greatest inhibition (Figure 2.8). The degree of inhibition was not always significant from one infusion concentration to the next. Significant radicle reduction was only observed at 25% and 75% infusion concentrations for the root extract. As in the aqueous extract experiment, the leaves of *C.bonariensis* exhibited a higher phytotoxic effect than the roots.

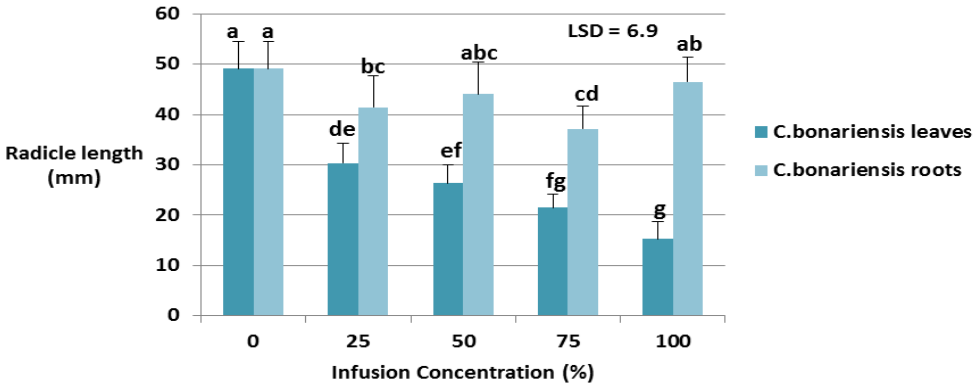

Figure 2.8 Effect of hexane leaf and root extracts of *C. bonariensis* on root (radicle) growth of lettuce (Means with same letters do not differ significantly at P=0.05 Comparisons were made across plant part and infusion concentrations ANOVA presented in Appendix A, Table A8)

Shoot growth: Unlike the aqueous extracts, the hexane extracts of *C.bonariensis* did not stimulate the growth of lettuce shoots at any concentration (Figure 2.9). Shoot growth of lettuce was significantly reduced by the hexane leaf extract of *C. bonariensis* from50% infusion concentration onwards. The root extract at all concentrations significantly reduced shoot growth relative to the control, with the highest reduction at 100% infusion concentration.

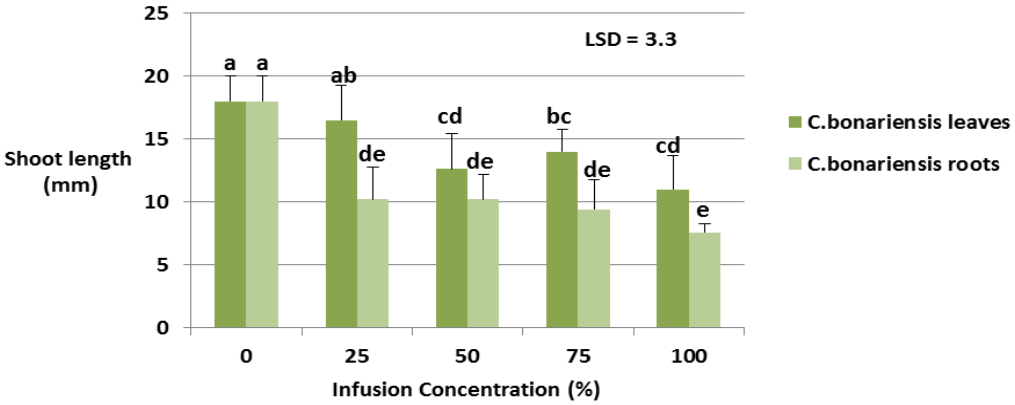

Figure 2.9 Effect of hexane leaf and root extracts of *C. bonariensis* on shoot growth of lettuce (Means with same letters do not differ significantly at P=0.05; Comparisons were made across plant part and infusion concentrations; ANOVA presented in Appendix A, Table A9)

2.3.3.2 Effects of hexane leaf and root extracts on tomato

Germination: Inhibitory effects on tomato seed germination by hexane extracts of *C. bonariensis* leaves only occurred at 100% concentration. However, germination was significantly inhibited by hexane extracts of *C. bonariensis* roots from the 50% concentration onwards when compared to the control (Figure 2.10). The greatest inhibition of tomato seed germination occurred at 100% concentration of the root extract.

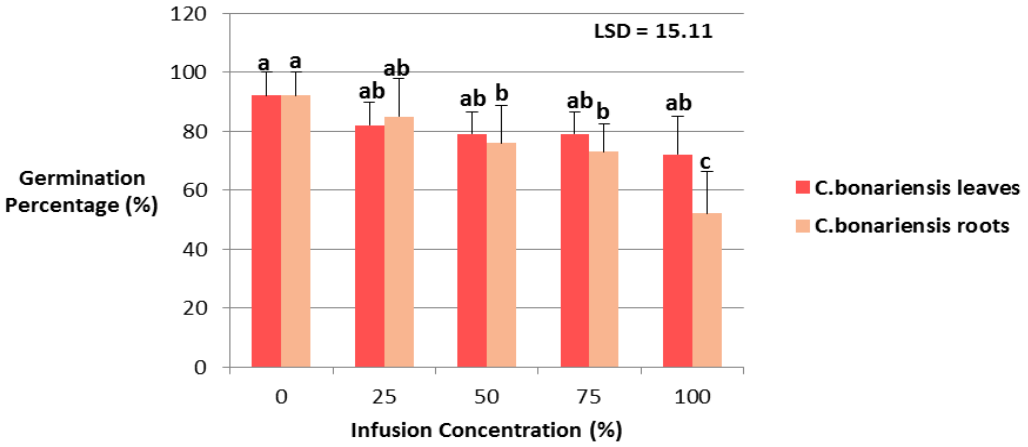

Figure 2.10 Effect of hexane leaf and root extracts of *C. bonariensis* on seed germination of tomato (Means with same letters do not differ significantly at P=0.05; Comparisons were made across plant part and infusion concentrations; ANOVA presented in Appendix A, Table A10)

Radicle growth: Both the root and leaf extracts of *C.bonariensis* significantly inhibited radicle growth of tomato from the 25% concentration onwards (Figure 2.11) when compared to the control. At 75% concentration, the degree to which the leaf extract inhibited shoot growth was significantly higher than that of the root extract.

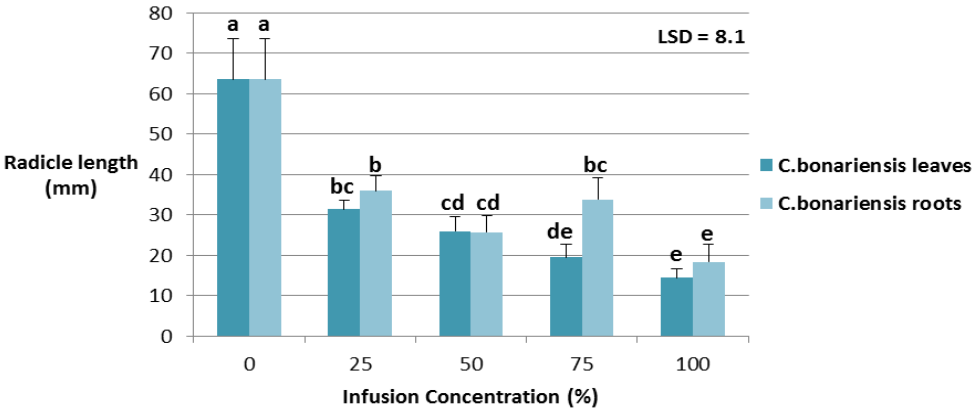

Figure 2.11 Effect of hexane leaf and root extracts of *C. bonariensis* on root (radicle) growth of tomato (Means with same letters do not differ significantly at P=0.05; Comparisons were made across plant part and infusion concentrations; ANOVA presented in Appendix A, Table A11)

Shoot growth: The reduction of tomato shoot growth by *C. bonariensis* hexane leaf extract was significant only at 100% infusion concentration (Figure 2.12). For the root extract, compared to the control, significant inhibition was already observed at the 25% concentration.

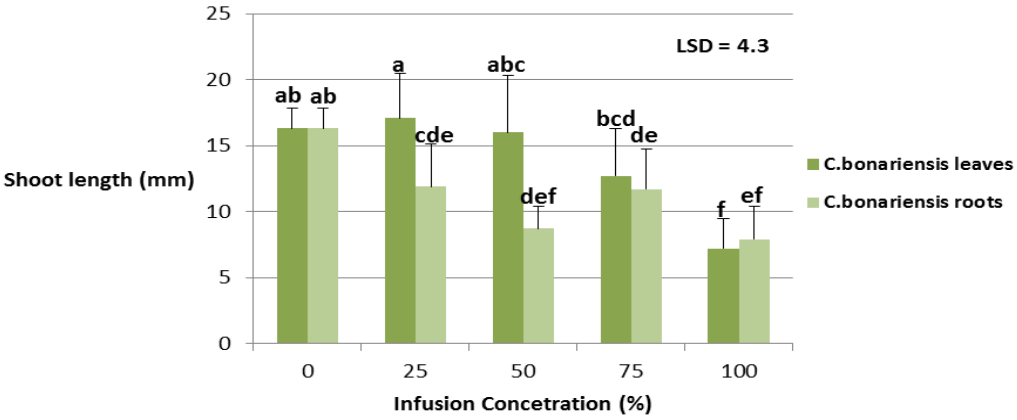

Figure 2.12 Effect of hexane leaf and root extracts of *C. bonariensis* on shoot growth of tomato (Means with same letters do not differ significantly at P=0.05; Comparisons were made across plant part and infusion concentrations; ANOVA presented in Appendix A, Table A12)

2.4 Conclusions

Results from the germination and initial seedling growth studies suggest that *C.bonariensis* contains phytotoxic allelochemicals that can inhibit, or at least retard, the germination and early seedling development of crop species. Based on the evidence from the germination studies water extracts appeared to contain different inhibitory substances (allelochemicals) to the hexane extracts, which in some cases were inhibitory to germination and subsequent growth. Allelochemicals contained in the leaves of the weed appear to be more potent than those in roots. 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 the contribution of allelochemicals contained in roots may have been underestimated in the laboratory bioassay. However, if the results from these experiments depict what happens in the field, the practical consequence of inhibitory compounds present in the leaves is that incorporation of *C. bonariensis* foliage into the crop seedbed may impede

germination and early seedling development of the two crop species tested. This means that the weed should not be allowed to attain significant biomass on crop fields at any stage, irrespective of whether the crop is present or not. Competition for growth resources is therefore not the only plant-to-plant interference mechanism which *C. bonariensis* possesses, and hence, there is an additional imperative for controlling this weed.

CHAPTER 3

ASSESSMENT OF THE ALLELOPATHIC POTENTIAL OF *CONYZA BONARIENSIS* ROOT EXUDATES

3.1 Introduction

Plant roots have several functions in plant growth and development, including: anchorage, provision of nutrients and water, and production of exudates with growth regulatory properties. For the purpose of this discussion, compounds with growth regulatory properties will be referred to as allelochemical compounds. Root activity is confined to the rhizosphere, where roots affect soil structure, aeration and biological activity as they are the major source of organic inputs, and are also responsible for depletion of large supplies of inorganic compounds (Bertin *et al.,* 2003). Exudates of roots are often released in large quantities into the soil rhizosphere from living root hairs or fibrous root systems. Root-specific metabolites are released that have critical ecological impacts on soil macro- and micro-biota, amongst other plants of the same or different species. Through the exudation of a wide variety of compounds, roots influence the soil microbial community in their immediate vicinity, imparts resistance to pests, support beneficial symbioses, alter the chemical and physical properties of the soil, and inhibit the growth of competing plant species (Takahashi, 1984).

Root exudates represent one of the largest direct inputs of plant-produced chemicals into the rhizosphere, and therefore, root exudates also likely represent the largest source of allelochemical inputs into the soil environment (Bertin *et al.,* 2003). Roots also have the potential to influence the two mechanisms of interference, *viz*. competition and allelopathy. For a number of plant species, root exudates play a direct role in plant-plant interactions through phytotoxins (allelochemicals) involved in mediating chemical interference, i.e., allelopathy.

Allelochemicals, the organic compounds involved in the phenomenon of allelopathy, are likely released from live plants and residual plant matter in great chemical diversity and at different concentrations into the environment by root exudation, leaching from aboveground parts, volatilisation and/or by decomposition of plant

material (Rice, 1984; Inderjit and Dakshini 1995; Gibson, 2002; Pisula and Meiners 2010). The ability of allelochemicals to persist in soil is determined by sorption to soil colloids, leaching and chemical or microbial degradation (Inderjit, 1998). Allelochemicals can be highly selective, in that they can influence the growth of only one organism, or they can exhibit broad activity in influencing the growth of many species. Their synthesis and exudation, along with increased overall root exudate production, is typically enhanced by stress conditions that the plant encounters, such as extreme temperature, drought and UV exposure (Pramanik *et al*., 2000; Inderjit and Weston, 2003).

Various allelochemicals in root exudates can affect metabolite production, photosynthesis, respiration, membrane transport, germination, root growth, shoot growth, and cell mortality in susceptible plants (Weir *et al*., 2004). These effects on plant physiology, growth, and survival may in turn influence plant and soil community composition and dynamics. Allelopathic root exudates can mediate negative plant-plant interactions only if present at sufficient concentrations to affect plant growth and survival. Preparation of foliar leachate and root exudates followed by growth bioassays and quantification of allelochemicals in the medium are commonly used techniques to study release of allelochemicals in the growth medium of plants (Inderjit and Callaway, 2003).

In the present study the following research questions were addressed: (a) do *Conyza bonariensis* roots release chemicals with allelopathic potential that are capable of influencing the growth of neighbouring plants?; and (b) does the inhibitory effect of *C. bonariensis* root exudates depend on the concentration of the toxins exuded by the roots and released into the growth medium? A hydroponic culture system was used to investigate whether *C. bonariensis* possesses and releases, through its roots, chemicals with allelopathic potential by growing it together with test species in a nutrient solution, and using plant growth as measure of effect. To answer the second question, an experiment was done to determine if leachate from *C. bonariensis* affected the growth of test species exposed to different leachate concentrations.

3.2 Materials and Methods

3.2.1 Hydroponic experiments

3.2.1.1 *C. bonariensis* (Pretoria population)

The experiment was a completely randomized design. *C. bonariensis* plants were collected at the rosette stage on the University of Pretoria's Hatfield experimental farm, and were grown hydroponically together with lettuce seedlings (Figure 3.1). This population will be referred to as the Hatfield population in the document. Lettuce seedlings, in the two-leaf stage, were obtained from Die Tuinhoekie nursery, and transplanted to the pots. Lettuce had been chosen previously in similar studies because it is generally considered an allelochemical-sensitive species (Meyer *et al.*, 2007). The three treatments were: (i) one *C. bonariensis* plant placed in the middle and two lettuce seedlings on either side; (ii) one lettuce plant per pot, and (iii) one *C. bonariensis* plant per pot. Treatments (ii) and (iii) served as the crop and weed control treatment. Treatments were repeated 10 times and in total there were 30 pots. Initially all pots contained 1100 ml of Hoagland's nutrient solution and every second day the water lost via transpiration and evaporation was replaced with nutrient solution to the level of the original volume. The nutrient solution in pots was replaced every seven days in order to avoid discrepancies in nutrient supply between treatments.

Figure 3.1 Hydroponic system used to study the effect of allelochemicals released by the roots of *C. bonariensis* plants on lettuce seedlings

Air was bubbled through the nutrient solution by an airline entering through a separate hole in the styro-foam lid of the pots (Figure 3.1). This experiment was set up in a glasshouse with natural light and temperature range of 18 to 28 ºC. Electroconductivity and pH were measured every day to make sure no changes were occurring in the nutrient solution.

When considering the allelopathic potential of plants, it is essential to distinguish between the effects of competition and allelopathy (Fuerst and Putnam, 1983; Leather and Einhellig, 1986; Inderjit and Olofsdotter, 1998). Thus, bioassays in allelopathy research should be designed to eliminate the effects of competitive interference from the experimental system. In the present study, the possibility that effects on plant growth might be the result of interference by competition was eliminated by supplying all the plants with the same amount of nutrient solution and light, hence a hydroponic system was chosen. The trial ran for four weeks, and in the fifth week all plants were harvested and fresh and dry mass of shoots (above-ground parts) and roots were measured.

3.2.1.2*C. bonariensis* (two Western Cape populations)

This experiment was repeated with two biotypes of *C. bonariensis* collected in the Western Cape at two different locations, namely: Naboomsrivier in the Breede River valley, and Willow Creek Boerdery in Heatlievale (Figure 3.2). One of the two populations was suspected to be resistant to the herbicide glyphosate, and the other susceptible.

Figure 3.2 Hydroponic system in which one *C. bonariensis* from either Naboomsrivier or Willow Creek Boerdery were grown with two lettuce seedlings; *C. bonariensis* and lettuce plants grown on their own served as control

Data from the two experiments were compared in order to establish differences in the allelopathic effects of the three provenances of *C. bonariensis*. Although all three provenances were identified as *C. bonariensis*, there were clear morphological differences between plants of the Pretoria and Western Cape populations, in particular with regard to leaf shape and size (Figures 3.1 and 3.2).

All data were subjected to analysis of variance (ANOVA) using the statistical programme SAS 9.2 (2002), and mean separation was done with the least significant difference test of Tukey at P=0.05.

3.2.2 Leachate experiment

The experiment was a completely randomized. *C. bonariensis* plants were transplanted when at the rosette stage from a crop field on the Hatfield experimental farm of the University of Pretoria, and grown to maturity in the glasshouse in pure quartz sand medium at a density of one plant per pot. Two seedlings of either lettuce or tomato were transplanted into separate pots also containing quartz sand (Figure

3.3). For each test species there were 25 pots. Every morning 300 ml of nutrient solution was added to the 15 pots with *C. bonariensis* plants, and the leachate collected at the base of the pots (Figure 3.4). The collected leachate was combined from all 25 pots immediately after watering and a dilution series of 0% (pure nutrient solution serving as control), 25%, 50%, 75%, and 100% (undiluted leachate) was prepared. Test plants were treated with 200 ml of each leachate concentration in the series every second day. Harvesting of the trial was done four weeks after treatment commenced and dry mass of shoots and roots were measured.

Data were subjected to analysis of variance and mean separation was done with the least significant difference test of Tukey at P=0.05. Analysis of variance (ANOVA) was done using the statistical programme SAS 9.2 (2002).

Figure 3.3 Test species that were used in the *C. bonariensis* leachate experiment: lettuce seedlings (left); tomato seedlings (right)

Figure 3.4 Leachate experiment for the assessment of allelopathic effects of *C.bonariensis*; Mitscherlich pots with *C. bonariensis* plants (donor plants) were supplied with pans at bottom for leachate collection

3.3 Results and Discussion

3.3.1 Effect of root exudates released by *C. bonariensis* plants on growth of lettuce plants in hydroponic system

3.3.1.1 Hatfield *C. bonariensis* population

Fresh mass: There were significant differences in shoot and root mass between lettuce grown alone (control) and lettuce grown with *C. bonariensis* from Hatfield (Figure 3.5). On average there was 83% growth reduction in the roots of lettuce by the weed treatment, and 65% growth reduction in the case of lettuce shoots.

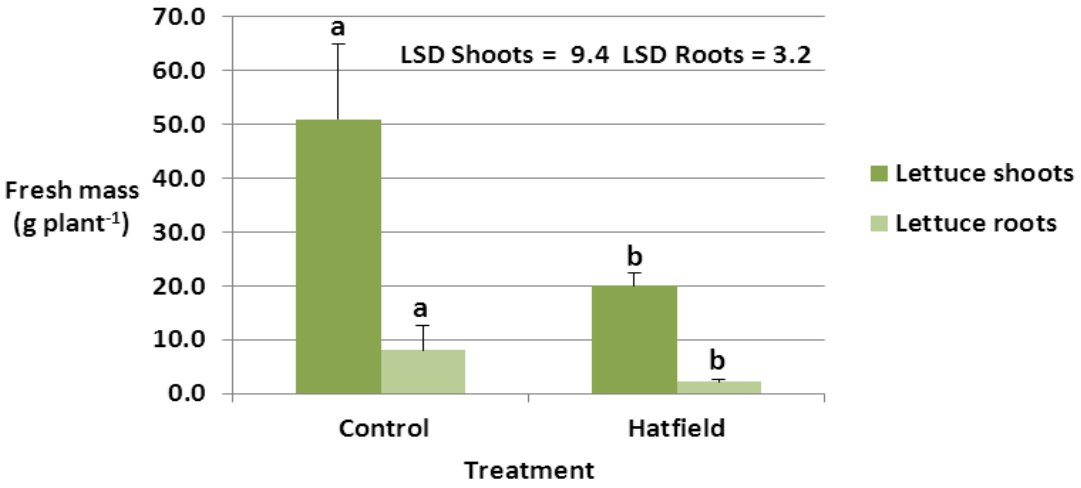

Figure 3.5 Shoot and root fresh mass of test species lettuce grown hydroponically with *C. bonariensis* plants collected on the Hatfield experimental farm (Means with same letters do not differ significantly at P=0.05; ANOVA presented in Appendix B, Tables B1 and B2)

Figure3.6 Root growth variation between the roots of lettuce grown alone (left) and lettuce grown with *C.bonariensis* (right)

Figure 3.7 Root and shoot mass comparison between plants representing the controls of *C. bonariensis* and lettuce (left side of ruler), and plants from the weed-crop combination treatment (right side of ruler)

C. bonariensis plants grown with lettuce did not differ much from those grown alone; except for the chlorosis in the leaves and the slight reduction in shoot mass (Figure 3.7). This is an indication that even though there was no drastic reduction in plant mass of *C. bonariensis* plants, competition for nutrients probably took place and/or lettuce had an allelopathic effect on the weed. In a study by Chon *et al*. (2005), to determine lettuce allelopathic effects on seed germination and early seedling growth of several plant species, results suggested that extracts or residues from lettuce plants had potent allelopathic activity and that the activity differed depending on cultivar, extract or fraction.

Dry mass: Shoot and root dry mass of lettuce grown with *C.bonariensis* from the Hatfield experimental farm was significantly reduced compared to the control (Figure 3.8). There was a 61% and 85% reduction in leaf and root mass respectively.

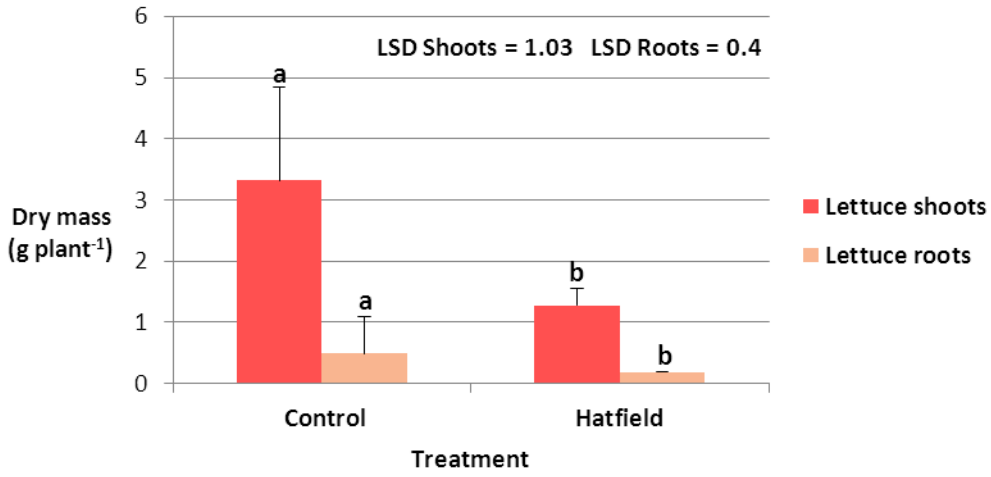

Figure 3.8 Shoot and root dry mass of test species lettuce grown hydroponically with *C. bonariensis* plants collected on the Hatfield experimental farm (Means with same letters do not differ significantly at P=0.05; ANOVA presented in Appendix B, Tables B3 and B4)

This type of experiment has been previously been used to demonstrate potential allelopathic effects. A study by Irons and Burnside (1982) revealed that sorghum plants grown in nutrient solution in which sunflowers were previously grown were significantly shorter and their fresh and dry mass less than for those grown in fresh

nutrient solution. Jose and Gillespie (1998) investigated the effects of juglone (active agent causing growth inhibition found in black walnut) on the growth and physiology of hydroponically grown corn (*Zea mays* L.) and soybean (*Glycine max* L. Merr.). They found that soybean was more sensitive to juglone than corn, and that root relative growth was the most inhibited variable for both species, with reductions of 86.5 and 99% observed in corn and soybean, respectively. As far as we were able to ascertain, the allelopathic potential of *C. bonariensis* or any closely related species, have thus far not been demonstrated using hydroponic experiments.

3.3.1.2 Western Cape *C. bonariensis* populations

Fresh mass: Significant inhibition of lettuce shoot and root growth occurred when this species was grown together with *C. bonariensis* sourced at two locations in the Western Cape (Figure 3.9). The degree to which lettuce shoots and roots were reduced by the two biotypes did not differ significantly. For lettuce grown with *C. bonariensis* from Naboomsrivier there was a 71% and 64% reduction in shoot and root mass, respectively. Lettuce grown with *C. bonariensis* from Willow Creek Boerdery showed a 59% and 67% reduction in mass for shoots and roots, respectively.

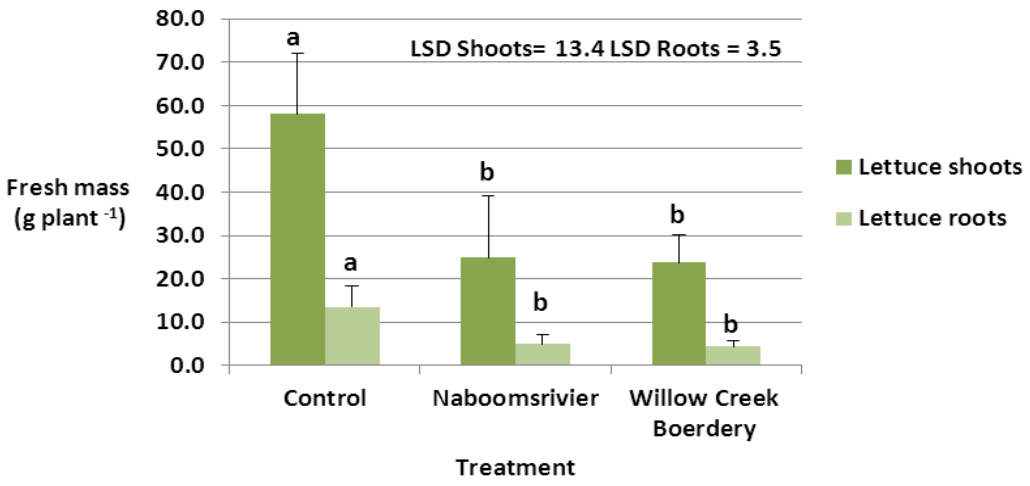

Figure 3.9 Shoot and root fresh mass of test species lettuce grown hydroponically with two Western Cape provenances of *C. bonariensis* (Means with same letters do not differ significantly at P=0.05; ANOVA presented in Appendix B, Tables B5 and B6)

Dry mass: Shoot and root dry mass of lettuce grown with *C. bonariensis* from the Western Cape showed significant reductions compared to the control (Figure 3.10). There were a 53% and 49% reduction in shoot and root growth, respectively, for lettuce grown with *C. bonariensis* from Naboomsrivier. Lettuce grown *with C. bonariensis* from Willow Creek Boerdery showed a 49% and 65% reduction in shoot and root growth, respectively. Similarly, for fresh mass data, there were no significant differences in the inhibitory effects of the two Western Cape populations.

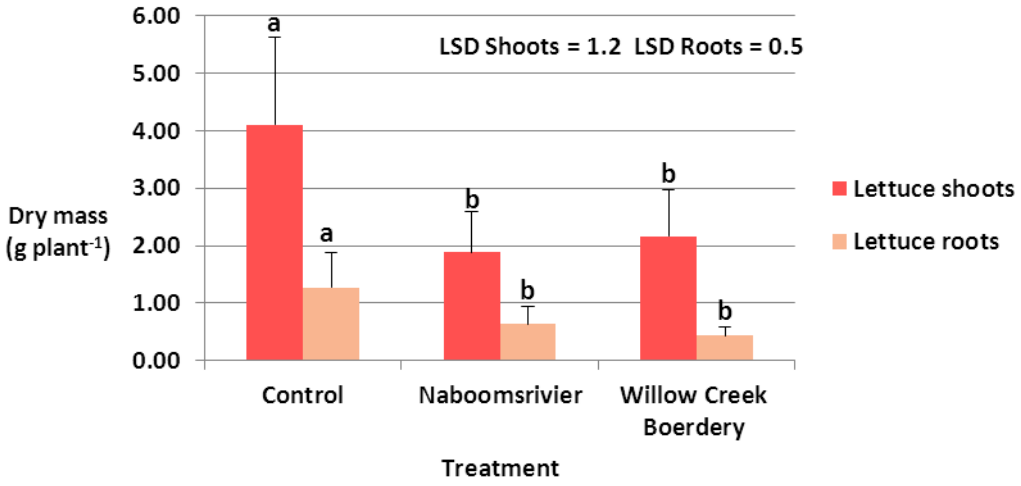

Figure 3.10 Shoot and root dry mass of test species lettuce grown hydroponically with two Western Cape provenances of *C. bonariensis*(Means with same letters do not differ significantly at P=0.05; ANOVA presented in Appendix B, Tables B7 and B8)

Although the findings reported in Chapter 2 pointed to leaves of *C. bonariensis* being a more important source of allelochemicals than the roots, those results were obtained at the earliest stages of test species development. Moreover, the donor plants in Chapter 2 were not alive as was the case in this experiment, and allelochemicals were obtained in an unnatural way, i.e., through either aqueous infusion or extraction with an organic solvent. Therefore, it is conceivable that different types of allelochemicals and concentrations were involved in the present study that involved live donor plants that could actively exude allelochemicals into the growth medium of acceptor species.

3.3.2 Effect of *C. bonariensis* leachate on the growth of two test species

3.3.2.1 Effect of Pretoria *C. bonariensis* leachate on the growth of lettuce

Fresh mass: There was no significant growth reduction in lettuce shoots and roots caused by *C. bonariensis* leachate at all concentrations tested (Figure 3.11). This implies that the growth of lettuce was not affected by increasing leachate concentrations.

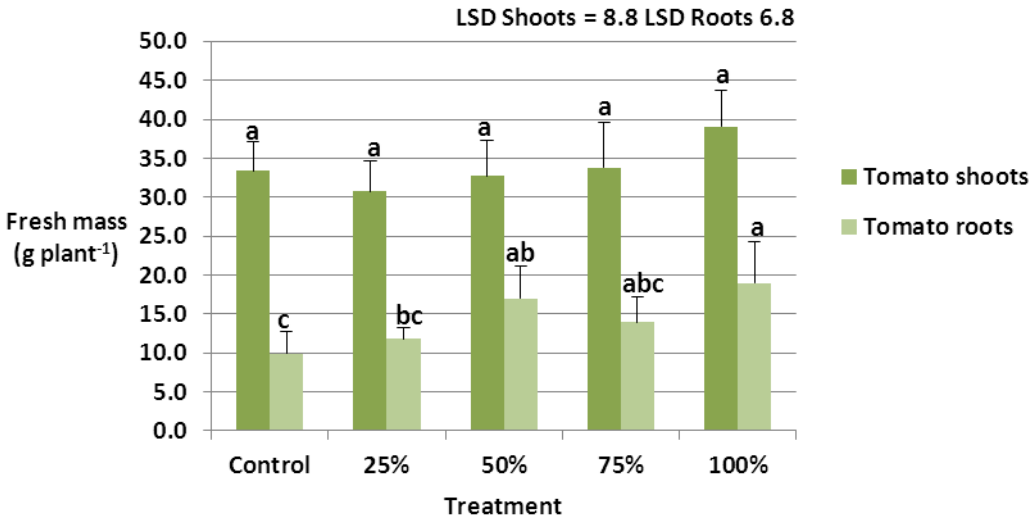

Figure3.11 Shoot and root fresh mass of lettuce that was exposed to *C.bonariensis* leachate concentrations ranging from 0 to 100% (Means with same letters do not differ significantly at P=0.05; ANOVA presented in Appendix B, Tables B9 and B10

Dry mass: Dry mass of lettuce shoots and roots exposed to leachate collected from *C. bonariensis* were not significantly reduced (Figure 3.12). Although there was a trend for apparent growth stimulation for roots of lettuce at all leachate concentrations relative to the control, these differences were not significant.

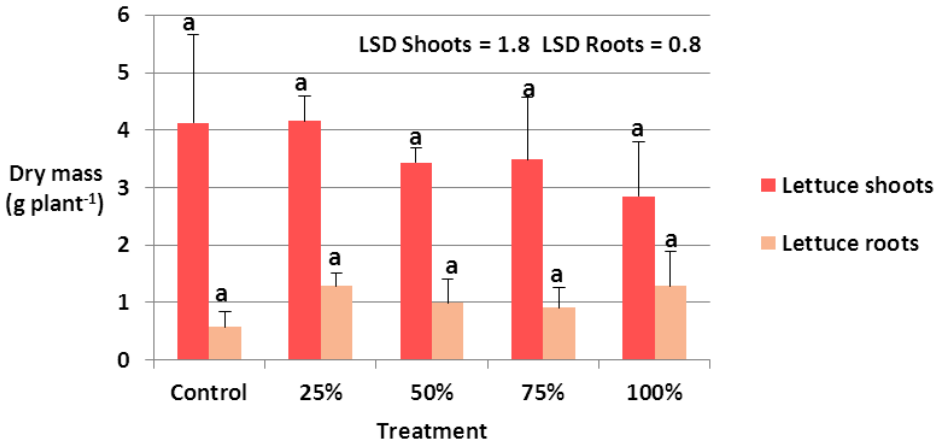

Figure 3.12 Shoot and root dry mass of lettuce plants exposed *C. bonariensis* leachate concentrations ranging from 0 to 100% (Means with same letters do not differ significantly at P=0.05; ANOVA presented in Appendix B, Tables B11 and B12)

3.3.2.1 Effect of Pretoria *C. bonariensis* leachates on the growth of tomato

Fresh mass: As in the lettuce experiment, tomato plants treated with *C. bonariensis* leachate showed no significant growth reduction in the fresh mass of shoots. However, significant stimulation of root growth was apparent at 50 and 100% leachate concentrations (Figure 3.13 and 3.14).

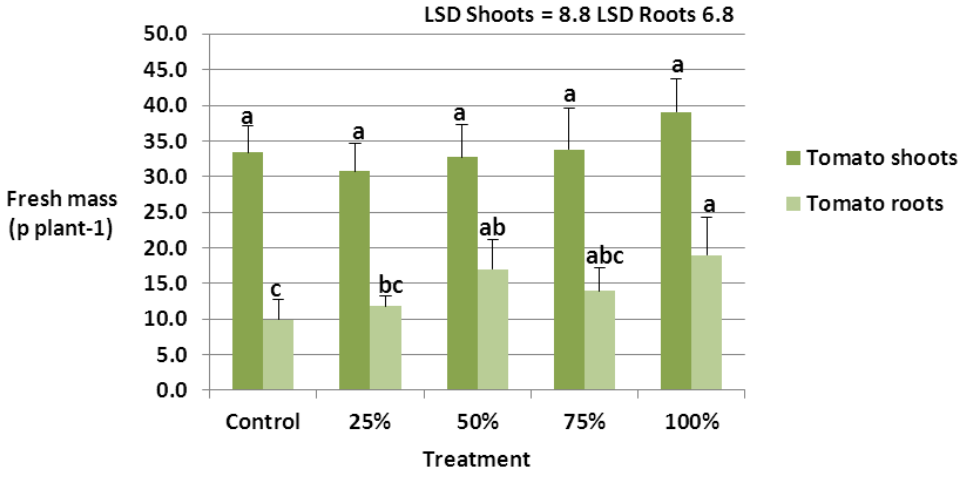

Figure 3.13 Shoot and root fresh mass of tomato plants exposed to different C. bonariensis leachate concentrations ranging from 0 to 100% (Means with same letters do not differ significantly at P=0.05; ANOVA presented in Appendix B, Tables B13 and B4)

Figure 3.14 A: Roots of tomato grown in pure nutrient solution; **B**: roots of plants treated with 100% *C. bonariensis* leachate concentration

Dry mass: Dry mass data for tomato showed that significant stimulation of tomato root growth occurred only at 100% leachate concentration when compared to the control (Fig 3.14B and 3.15). For both fresh and dry mass the trend for apparent stimulation of tomato shoots at 100% leachate concentration was not statistically significant when compared to the control.

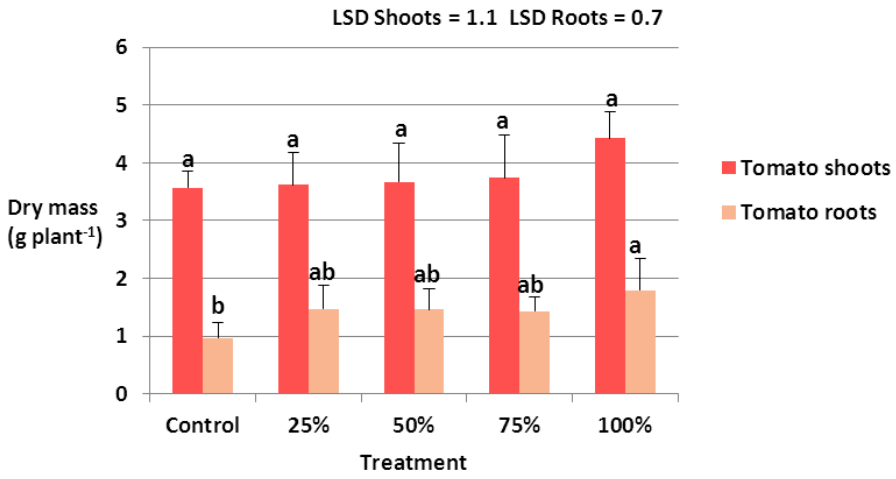

Fig 3.15 Shoot tops and root dry mass of tomato plants exposed to a range of *C. bonariensis* leachate concentrations ranging from 0 to 100% (Means with same letters do not differ significantly at P=0.05; ANOVA presented in Appendix B, Tables B15 and B16)

The inability of C. bonariensis leachate to reduce lettuce and tomato plant growth could be due to two reasons; firstly, insufficient accumulation of putative allelopathic compounds given the methodology and secondly, the duration of the experiment. To elaborate on the first reason, it is important to note that the growth medium used in this experiment was pure sand for both donor and test species. Although plants can grow in pure sand, the latter does not have adsorptive capacity to bind water; therefore, the retention of allelochemicals in the growth medium would likely also have been negligible in this experiment, and hence, considerable allelochemical loss through leaching likely took place. To support this notion of decreased concentration of allelochemicals, for dry mass, apparent stimulation of tomato plant growth was significant only at 100% leachate concentration instead of the lower concentrations as in the germination bioassays (Chapter 2). This growth stimulation is an indication that C. bonariensis plants did in fact produce and release putative allelopathic compounds, however, the concentrations were not high enough in the pots of the receiver plants to cause plant growth inhibition, since almost half of the 200ml applied to pots leached every morning. The second explanation for these results is the duration of the experiment. Due to the nature of the growth media used in the experiments, results suggest that there was a need for the experiment to be conducted for a longer period in order to allow for putative allelopathic compounds to accumulate to higher (toxic) concentrations in the pots and plants. Therefore terminating the trial after four weeks was perhaps premature. In addition to the duration of the experiment, the leaching of the allelochemicals was probably done at too short intervals. It might have been beneficial to implement leaching treatments on donor pots on a weekly basis in order for the allelochemical concentration in the donor plant pots to accumulate. In a leachate pot experiment by Viard-Cretat et al. (2009) to investigate whether the release of allelochemicals by the dominant tussock grass (*Festuca paniculata*) is responsible for its dominance by inhibiting growth of neighbour grasses in subalpine grasslands, plant species were given enough time (1 year) to exert a chemical influence on the soil medium

3.4 Conclusion

Results from the hydroponic experiment indicate that *C. bonariensis* roots contain and release growth inhibitors that are capable of reducing the growth of lettuce. Although *C. bonariensis* from Hatfield and those from the Western Cape differ

morphologically, the degree of phytotoxicity on lettuce plant growth did not vary, except in the case of root dry mass where the Hatfield population caused significantly greater reduction in lettuce root growth than the other two populations. While allelopathy seemed to have played a major role in the results of this experiment, one must not rule out the possibility of competition. For example, even though the feeding solution was balanced and changed every week it is still possible that certain micro- or macro elements might have not become limiting during each week. It is also possible that C. bonariensis could have been a better competitor for light due to its growth form. The leachate experiment demonstrated that, as with many other weed species, the allelopathic potential of *C. bonariensis* varies with plant species exposed to the potential allelochemicals and amount of allelochemicals present. Effects of *C. bonariensis* leachate on tomato plant growth confirmed that allelopathy as an interference mechanism is not only harmful (inhibitory) but apparently can also be beneficial (stimulatory). The observed stimulatory effects of *C. bonariensis* at certain leachate concentrations should be investigated further. Although the scope of this study precluded chemical identification of allelochemicals involved in the responses of test species, modern molecular and biotechnological tools allow for in-depth studies on allelopathy, the role of root exudates in allelopathy, and the linking of such plant attributes with the interfering/invasive ability of particular plants in both agro- and natural ecosystems.

CHAPTER 4

REPLACEMENT SERIES APPROACH FOR DETERMINING THE RELATIVE INTERFERENCE OF *CONYZA BONARIENSIS* IN RELATION TO LETTUCE AND TOMATO

4.1 Introduction

Allelopathy is better demonstrated through experiments in which a toxic product is shown to be released from the putative aggressor, and arrives at the putative victim in functional concentrations under reasonably natural conditions (Blum, 1995). The plant with allelopathic potential is referred to as the "donor plant," while the plant in the surrounding area affected by the allelopathic compounds from the donor plant is referred to as the "receiver plant." Donor and receiver plants can affect each other through allelopathy and competition (Muller, 1969).

The relative density between donor and receiver species is believed to be an important factor in the degree of expression of allelopathy and this has been suggested as a method to distinguish between allelopathy and resource competition. Weidenhamer *et al*. (1989) were among some of the first scientists to demonstrate that allelopathic interference and resource competition can be distinguished experimentally by the density-dependent nature of phytotoxic effects, which cause deviations from predicted yield-density relationships. For monocultures, phytotoxicity decreases as plant density increases as a result of the dilution of the available toxin among many plants at high densities, such that each receives a sub-lethal dose. As the observation of growth reductions at low but not at high densities is inconsistent with a hypothesis of resource competition, such results constitute strong evidence for the presence of an inhibitor in soil. An experimental design that demonstrated allelopathic interference in mixed cultures of two species would be more broadly applicable (Wu *et al*., 2002).

Previously researchers have used two different experimental designs, additive and replacement series, to study the interactive behaviour of components in mixed stands. In additive series (e.g., Donald 1958), various densities of a second species

supplement a constant density of an indicator species. Studies using additive series have normally demonstrated that increasing densities of the second species depress the yield of the indicator species. In replacement series (e.g., de Wit 1960), a constant total density of plants is used and the planting density of one species is proportionately decreased as the planting density of the second species is increased.

In ecology, replacement series have been used to explore many issues, including species coexistence, exclusion, co-adaptation, niche differentiation, abundance, distribution, productivity and diversity (e.g., Aberg *et al*., 1943; Black, 1958). In agriculture and forestry, replacement series have regularly been used in studies of weed-crop associations, and they are the common setting for evaluating yield advantages in intercrops (Jolliffe, 2000). Experiments that use multiple densities make it possible to compare monoculture stands, and allow for the determination of the relative extent of intra-and interspecific competition between the species (Jolliffe *et al*., 1984; Santos *et al*., 1997).

Relative yield total (RYT) and relative yield (RY) are commonly used variables to calculate the yield of a species in the mixture as a proportion of its yield in monoculture and thus measures interspecific and intraspecific competition (Santos *et al*., 1997; Hector, 2006). An RYT less than one implies that mutual antagonism is occurring (Harper, 1977) or that not all the resources available to plants are being used. One explanation for this non-use may be that plants may inhibit the growth of each other through allelopathy (Putnam and Tang, 1986). The objective of this study was to assess the allelopathy of *C. bonariensis* in relation to that of lettuce and tomato by increasing *C. bonariensis* plant density, and thus increasing the concentration of putative compounds with allelopathic potential in the growth medium.

4.2 Materials and Methods

Replacement series experiments were conducted in a greenhouse at the Hatfield experimental farm. The experimental design was completely randomized (Figure 4.1). *C. bonariensis* plants were collected at the rosette stage on the Hatfield experimental farm, and were grown in pots (20 cm height x 20 cm diameter) in 4 kg sterilized field soil (sandy-loam) together with lettuce(*Lactuca sativa*) and tomato (*Lycopersicon esculentum*) seedlings. Lettuce and tomato seedlings were obtained from Die Tuinhoekie nursery, and the seedlings were in the 2-leaf stage when transplanted to the pots. Treatments consisted of combinations of six proportions of *C. bonariensis* and lettuce and combinations of six proportions of *C. bonariensis* and tomato. The experiment was laid out in replacement series as outlined by Radosevich *et al*., 1996. Each treatment was replicated five times.

The treatment combinations were arranged in two sets, as follows:

1. 5 *C. bonariensis*+ 0 lettuce (*C. bonariensis* monoculture)
2. 4 *C. bonariensis*+ 1 lettuce
3. 3 *C. bonariensis*+ 2 lettuce
4. 2 *C. bonariensis*+ 3 lettuce
5. 1 *C. bonariensis*+ 4 lettuce
6. 0 *C. bonariensis*+ 5 lettuce (lettuce monoculture)

1. 5 *C. bonariensis*+ 0 tomato (*C. bonariensis* monoculture)
2. 4 *C. bonariensis*+ 1 tomato
3. 3 *C. bonariensis*+ 2 tomato
4. 2 *C. bonariensis*+ 3 tomato
5. 1 *C. bonariensis*+ 4 tomato
6. 0 *C. bonariensis*+ 5 tomato (tomato monoculture)

Pots were surface-watered with 200 ml of Hoagland's nutrient solution every second day throughout the experiment with the purpose of excluding competition for water and nutrients. The experiment was set up in a glasshouse with natural light and temperature range of 18 to 28 ºC. Harvesting of the trial was done four weeks after treatment commenced and dry mass of shoots (all above ground parts) and roots were measured. To obtain dry mass, plants were divided into shoot and root and put to dry in a forced air circulation incubator at 60ºC for a period of 72 hours. Then weighing was conducted; mean dry mass corresponded to the sum of shoot dry mass plus root dry mass ratio in each proportion. Data were subjected to analysis of variance and mean separation was done with the least significant difference test of Tukey at P=0.05. Analysis of variance (ANOVA) was done using the statistical programme SAS 9.2 (2002).

Figure 4.1 A replacement series experiment to investigate the effect of different densities of *C. bonariensis* on plant growth of lettuce

Relative yield (RY) and relative yield total (RYT) (Radosevich, 1988) were calculated as:

$$Relative\ yield = \frac{Yield\ in\ mixture}{Yield\ in\ pure\ stand}$$

$$Relative\ yield\ total = Relative\ yield\ of\ crop + Relative\ yield\ of\ weed$$

The relative yield of both the crop and the weed can be calculated as summed to give the relative yield totals (RYT). The RYT can be used to describe the mutual interaction that occurs between the species:

1. RYT = 1: this situation implies that each species is making the same demands for "space" as the other; they are "mutually exclusive" or complementary.
2. RYT > 1: this situation suggests that one or both of the species are less affected by interspecific interactions than could be predicted from their monoculture responses; it suggests that they are: (a) making different demands on the same resources; (b) occupy different niches in time or space; or (c) exhibit some sort of symbiotic relationship.
3. RYT < 1: this situation occurs when one or both species are more negatively affected by interspecific competition than would be expected from their pure stand responses and indicates mutual antagonism. Possible mechanisms that could explain this interaction are: (a) the action of allelopathic compounds produced by one or both species, or (b) the loss of the pure stand effect in the mixture.

Traditionally, the RYT concept applies to competition studies only. In our approach we attempted to eliminate competition in order to make findings that are only applicable to allelopathic effects.

4.3 Results and Discussion

4.3.1 Dry mass

Lettuce: Results for dry mass of lettuce grown at different proportions with *C. bonariensis* show that there were no significant effects on the growth of the crop species at all proportions. Even though there is a trend for apparent growth stimulation of *C. bonariensis* at proportion 3 lettuce: 2 *Conyza*, it is not statistically significant when compared to the control.

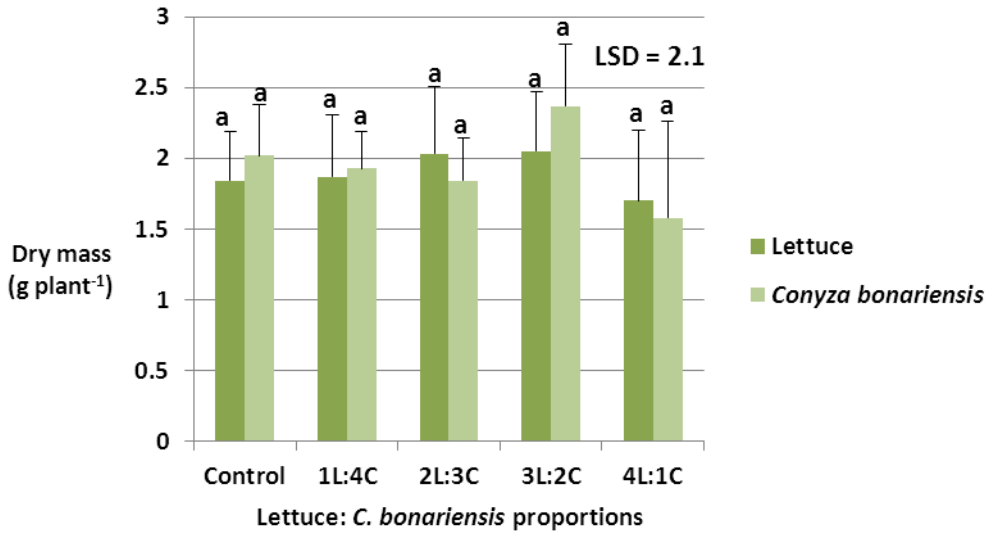

Figure 4.2 Dry mass of *C. bonariensis* and lettuce grown together in a replacement series at different proportions (Means with same letters do not differ significantly at P=0.05; ANOVA presented in Appendix C, Table C1)

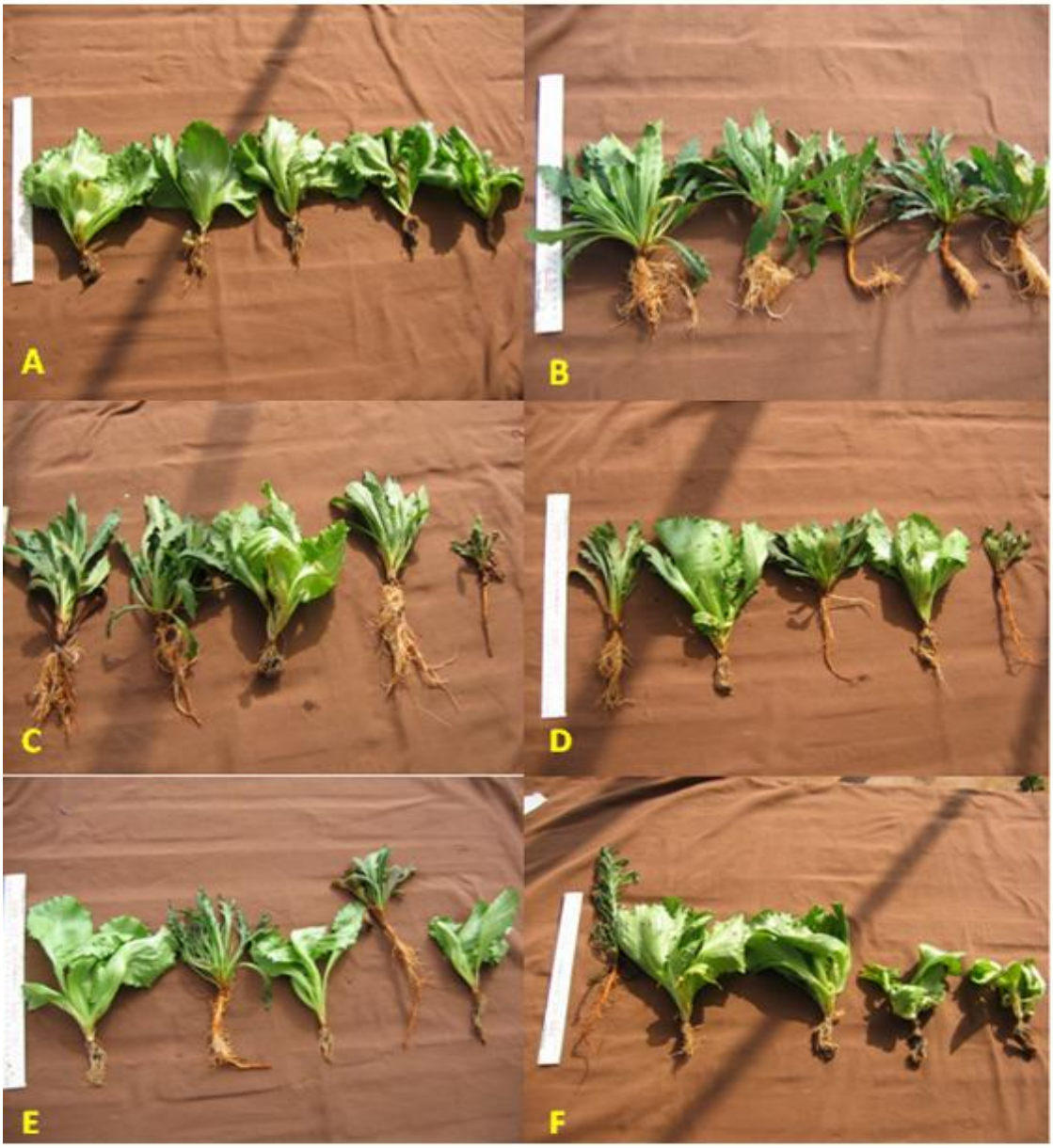

Figure 4.3 Root and shoot growth comparison between *C. bonariensis* and *L. sativa* from the replacement series. **A**: 5 lettuce + 0 *C. bonariensis*; **B**: 0 lettuce + 5 *C. bonariensis*; **C**: 4 *C. bonariensis* +1 lettuce; **D**: 3 *C. bonariensis* +2 lettuce; **E**: 2 *C. bonariensis* + 3 lettuce; **F**: 1 *C. bonariensis* + 4 lettuce

Allelopathy is usually interspecific; but if the donor and the recipient belong to same species it becomes intraspecific allelopathy and the term used is autotoxicity. Therefore, autotoxicity occurs when a plant releases toxic chemical substances into the environment that inhibit germination and growth of the same plant species (Miller, 1996). According to Reinhardt *et al*. (1999) it is probable that autotoxicity may have a confounding influence when the growth of species grown together is

compared. It can be seen from Figure 4.3 A and B that there may have autotoxicity when lettuce and *C. bonariensis* where grown separately in the controls. Autotoxicity has been reported for Asteraceae weeds such as *Amaranthus palmerii*, *Helianthus occidentalis, Parthenium hysterophorus* and *Plantago lanceolate* (Curtis and Cottam, 1950; Newman and Rovira, 1975; Kumari and Kohli, 1987). It is also possible that lettuce and C. bonariensis in the combinations mentioned above were involved in intra-species competition. Although competition for water and nutrients were eliminated by regularly adding a nutrient solution, competition for light and space could have still taken place.

Tomato: As with lettuce, dry mass for tomato plants grown with *C. bonariensis* showed no significant differences when compared to the control. The trend for apparent growth stimulation of *C. bonariensis* at all combinations is statistically not significant.

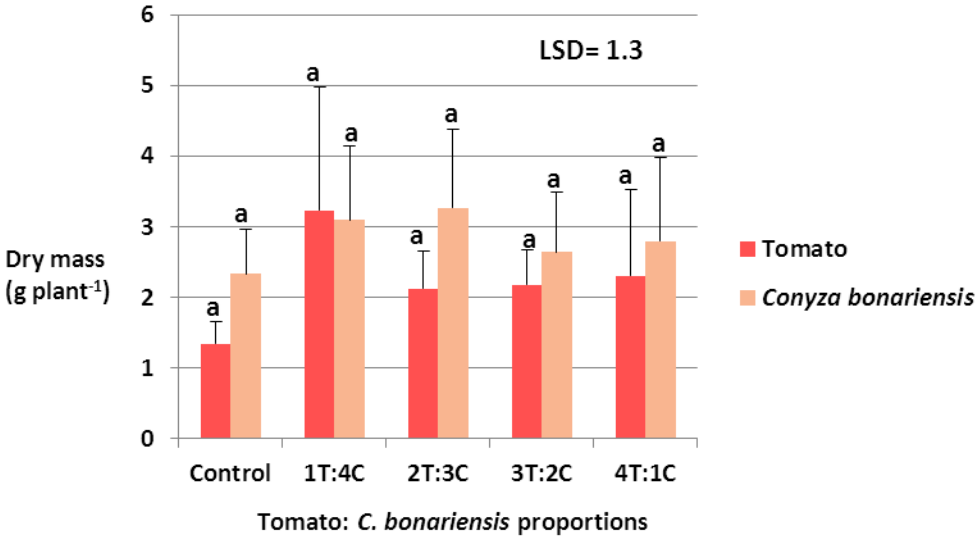

Figure 4.4 Dry mass of *Conyza bonariensis* and tomato grown together in a replacement series at different proportions (Means with same letters do not differ significantly at P=0.05; ANOVA presented in Appendix C, Table C2)

Figure 4.5 Root and shoot growth comparison between *C. bonariensis* and tomato from the replacement series. **A**: 5 tomato + 0 *C. bonariensis*; **B**: 5 *C.bonariensis* + 0 tomato; **C**: 4 *C. bonariensis* + 1 tomato; **D**: 3 *C. bonariensis* + 2 tomato; **E**: 2 *C. bonariensis* + 3 tomato; **F**: 1 *C. bonariensis* + 4 tomato

Tomato and *C. bonariensis* plants in Figure 4.5 A and B suggest that there may have been autotoxicity involved in the controls of both the donor and test species. *C. bonariensis* in Figure 4.5 C and D exhibit apparent autotoxicity with some plants being smaller than others, just as in the lettuce experiment.

When comparing the tomato plants in Figure 4.5 F (1 *C. bonariensis* + 4 tomato*)* to those in other plant density combinations of tomato and *C. bonariensis*, there is apparent growth stimulation in tomato. This could have been due to the single *C. bonariensis* plant in that particular mixture producing such low concentration of allelochemical(s) that the effect was stimulatory instead of inhibitory. The same response was observed in Chapter 2, Figure 2.6.In a replacement series study by Santos *et al*. (1997), in which tomato plants were grown with yellow and purple nutsedge, results showed that tomato dry weight per plant increased and dry weight per plant of nutsedge decreased as their relative proportions decreased in mixture.

4.3.2 Relative yield

Lettuce: At all the combinations of lettuce and *C. bonariensis* the relative yield of *C. bonariensis* was slightly greater than that of lettuce. Up to 3 lettuce: 2 *C. bonariensis*, RYT was increasing with increasing lettuce number which means the less *C. bonariensis* the more RYT. At 4 Lettuce: 1 *C. bonariensis* combination, RYT decreased and relative yield of lettuce was < 1, suggesting that there was an action of phytotoxins produced by one or both species. This effect was probably due to lettuce autotoxicity, considering that there was more lettuce plant in the pot.

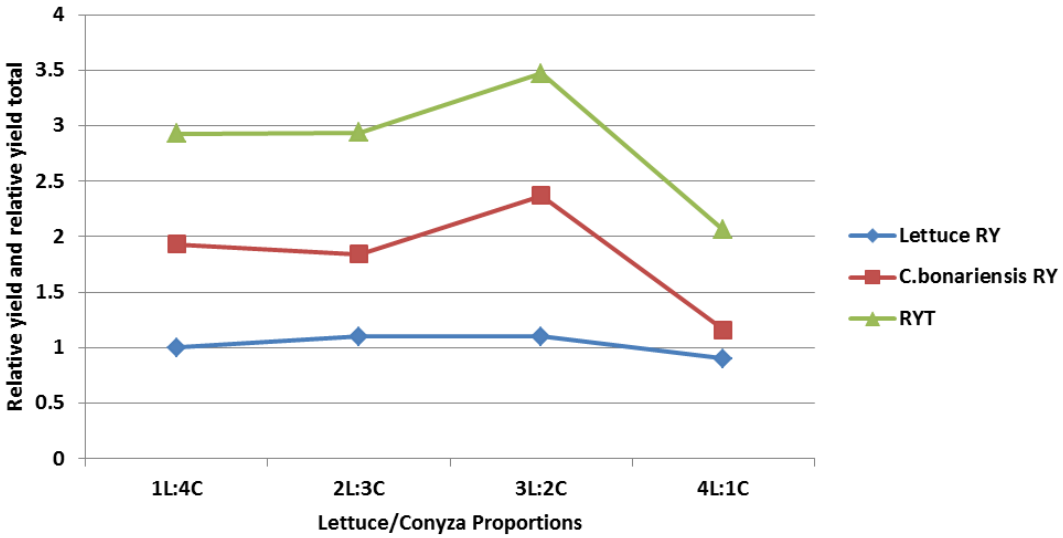

Figure 4.6 Relative yields (RY) of lettuce and C. *bonariensis* and relative yield total (RYT) four weeks after transplanting under different densities and proportions

Tomato: For all the combinations of tomato and *C. bonariensis,* the RY of tomato was greater than that of *C. bonariensis*. According to Bianchi *et al*. (2006), generally, replacement experiments demonstrate that the crop is more competitive than the weed species, since the effect of the weeds in crops is not due to their higher competitive ability, but to the degree of infestation. However, their research did not consider allelopathy. RYT in all the combinations was > 1 which probably implies that both species were less affected by interspecific interactions than in their respective monocultures.

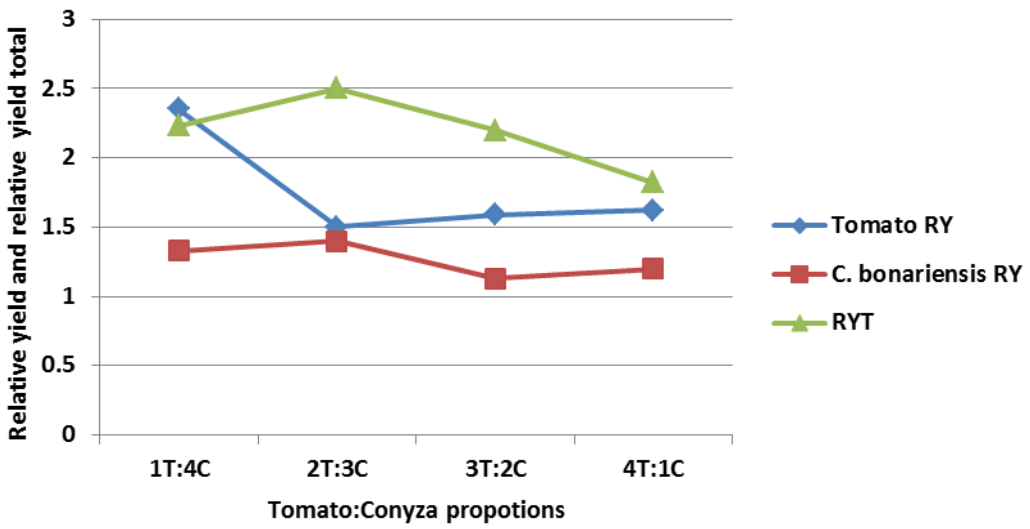

Figure 4.7 Relative yields (RY) of tomato and C. *bonariensis* and relative yield total (RYT) four weeks after transplanting under different densities and proportions

4.4 Conclusions

According to the results of this study lettuce and tomato possess phytotoxic ability equivalent to that of *C. bonariensis* in relation to total dry mass, given that there were no significant differences in plant mass between the various combinations. It is important to consider that the inhibition of lettuce and tomato germination assigned to allelochemicals produced by *C. bonariensis* and growth observed in previous studies (Chapter 2 and 3) is not ostensible in the results of this study because of methodology, choice of growth media and competition.

The methodology in this chapter may have attempted to eliminate competition resulting from nutrient and water stress; however, allelopathy in soil is a complicated

phenomenon that is governed not only by the physiochemical properties but also by the soil organic matter and microorganisms. It has been suggested that the concentration of an allelochemical in soil water is a dominant factor directly determining the phytotoxic activity in the soil (Kobayashi, 2004), and that the concentration is controlled by soil factors that affect the behavior of adsorption, desorption and degradation in soil. Therefore, it is probable that since the allelochemicals in the plant extract and hydroponic experiments were in water or similar media, and therefore not affected by soil factors, the concentration of allelochemicals was therefore not affected and reached the receiver plants in relatively high doses. Poor correspondences between bioassays and field studies have often been found. Belz *et al*. (2009) demonstrated that although much research has been done to study the allelopathic potential of *P. hysterophorus*, its invasiveness could not be attributed to the plant metabolite parthenin, when its persistence and phytotoxicity in soil was studied.

Growth stage of receiver plants is also one of many factors that affect phytotoxicity of allelochemicals. Allelopathy is usually more pronounced at seed germination and early seedling development stages. Plants used in this study were more matured than seed/seedlings used in the seed germination bioassay, thus making them less susceptible to the putative allelochemicals. Finally, in the germination bioassay it was found that the leaves of *C. bonariensis* contained more phototoxic compounds when compared to the roots, but in the present study receiver plants were only exposed to compounds released by roots. In conclusion, although *C. bonariensis* exhibited statistically significant and often dramatic phytotoxicity on lettuce and tomato in previous experiments, this study reveals that there may be other aspects connected to the allelopathic potential of *C.bonariensis* in the field.

Finally, the third aspect to consider in the almost non-existent allelopathic effect of C. bonariensis on the crop species is competition for light. Setting up an experiment in a greenhouse with natural light hardly eliminates competition if the leaves of the plants start to grow over one another; particularly in the case of lettuce where large leaves may compete for space and space and light.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

Much research on *Conyza bonariensis* has been mainly directed towards its resistance to herbicides, while literature concerning its interference mechanisms and possible allelopathic interference with crop species is extremely limited. The weed originates from South America and was first reported to occur in South Africa in May 1895 in Franschoek (De Wet, 2005). This weed is currently invading cultivated and non-cultivated lands, gardens, roadsides and waste places, with infestations of one or more species in every province (Danin, 1990; Botha, 2001). Due to the importance of this weed, it would be of great value to know and understand the mechanisms by which *C. bonariensis* interferes with crops. This investigation on the allelopathic potential of *C. bonariensis* was done to evaluate whether this alien invader has the capacity to interfere with other species in this way.

5.1 Allelopathic influence of *Conyza bonariensis* on lettuce and tomato seed germination and early seedling development

The study presented in Chapter 2 was aimed at investigating the allelopathic effects of aqueous extracts of *C. bonariensis* on seed germination and seedling growth of two test species; and to verify whether the compounds influencing germination were polar or non-polar in nature, and if they have different impacts on seed germination and seedling growth of the test species. This study revealed the presence of allelopathic substances in leaves and roots of *C. bonariensis*. Germination and early seedling growth of both lettuce and tomato were inhibited by aqueous infusions and by hexane extracts. The possible confounding effects of osmotic potential of extracts were negated. From the results obtained it is clear that putative allelochemicals contained in *C. bonariensis* leaves are more potent than those in the roots. Since compounds extracted by hexane would not be water-soluble, the lower potency observed with these extracts could have been due to poor or zero absorption by seed/seedlings or the fact that the inhibitory compounds present in *C. bonariensis* are polar in nature .

5.2 Assessment of the allelopathic potential of *Conyza bonariensis* root exudates

Albeit laboratory bioassays are valuable when investigating the allelopathic potential of plant extracts, they are not sufficient in concluding the presence of allelopathic compounds under natural conditions. The use of plant extracts is often criticized because it is too far removed from what occurs in nature. This experiment was conducted with two main objectives: the first was to investigate whether *C. bonariensis* contains and releases, through its roots, chemicals with allelopathic potential by growing it together with test species in a nutrient solution, and using plant growth as a measure of effect. The second objective was to determine if leachate from *C. bonariensis* affected the growth of test species exposed to different leachate concentrations. In the hydroponic experiment three populations of *C. bonariensis* were used, one from Pretoria and two from the Western Cape. Lettuce shoot and root growth was significantly reduced by all three populations of *C. bonariensis*. Generally, there were no significant differences in the degree of inhibition caused by the three biotypes on the growth of lettuce, except in the case of root dry mass results, where the Pretoria population caused significantly greater reduction in lettuce root growth than the other two populations. These findings suggest that *C. bonariensis* produces and releases allelochemicals into the environment from its roots, at least. In the leachate experiment there was no growth inhibition observed for both test species. However, there was apparent growth stimulation of tomato roots at the highest concentration. This stimulatory effect of *C. bonariensis* leachate should be investigated further, and if such findings would lend support, the weed or extracts prepared from it could conceivably be used as a growth stimulator on responsive crops.

5.3Replacement series approach for determining the relative interference of *Conyza bonariensis* in relation to lettuce and tomato

In order to hamper plant growth, allelochemicals must accumulate and persist at phytotoxic levels in soil (Jilani *et al*., 2008). Replacement series experiments in Chapter 4 were conducted under greenhouse conditions to assess the allelopathy of *Conyza bonariensis* in relation to that of lettuce and tomato by increasing *C. bonariensis* plant density, thus increasing the concentration of putative compounds with allelopathic potential in the growth medium. These experiments represented an

environment that is closer to natural field conditions than the bioassay approach. Results for dry mass of lettuce and tomato grown at different proportions with *C. bonariensis* showed that there were no significant effects on the growth of the crop species at all proportions. Furthermore, except for the proportion 4:1 lettuce: *C. bonariensis*, RYT was > 1 at all the combinations, which probably implies that both crop species and *C. bonariensis* were less affected by interspecific interactions than in their respective monocultures. The difference in results obtained in Chapter 4 in relation to those in Chapter 2 and 3 are attributed to methodology and growth media. Since plants in Chapter 4 were grown in natural soil, it is highly probable that, unlike in the plant extract and hydroponic experiments where water media were used, allelochemicals were not absorbed by receiver plants in lethal dosages. Growth stage of receiver plant and plant organ of donor plant are the other two factors suspected to have restricted the phytotoxicity of allelochemicals in this experiment. In the preceding bioassay studies, the acceptor species were in the seed/seedling growth stages when it was concluded that the leaves of *C. bonariensis* contained allelochemicals of higher potency than the roots. We propose that allelochemicals in the present experiment were either adsorbed on soil colloids and/or were metabolized by soil microorganisms. This theory, however, needs to be substantiated with further investigations.

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5.4 Conclusions and recommendations

Current findings suggest that mature *C. bonariensis* plants can detrimentally affect the germination and early seedling growth of other plants, e.g., the crop, and that the relative maturity of crop and weed determines the nature and intensity of the allelopathic interaction. For example, if crop seed are sown into an environment where there are either live *C. bonariensis* plants or dead weed material that was either incorporated into the soil or present on the soil surface, it would constitute a risk of allelopathic effect on the crop. It is suggested that this investigation provides strong evidence that *C. bonariensis* has significant allelopathic potential, as shown by inhibition of seed germination and early seedling development of lettuce and tomato. *C. bonariensis* from the Western Cape and Gauteng provinces (populations separated by more than 1,000 km) showed this potential. The highest allelopathic potential (potency) may be found in the leaves of the plant, with lower potency occurring in the roots. A possible explanation for this difference in potency between

plant organs could be found in the ecological strategy of this weed. Perhaps this weed species relies on allelopathy exerted by its above-ground material, which logically could become relevant in the case of high infestation levels. For *C. bonariensis* to have a direct phytotoxic effect on other plants its allelochemicals must be available in the soil for plant uptake at sufficiently high concentrations.

Further research should be performed to identify the active compounds involved in the allelopathy of *C. bonariensis* and their fate and persistence in soil. Shoots and roots of tomato were significantly stimulated in the germination bioassay and leachate experiment, respectively. In the replacement series, although not significant, there was apparent growth stimulation of tomato plants when grown with a single *C.bonariensis* plant, which corresponds with growth stimulation of tomato shoots at low extract concentrations in the germination bioassay. The apparent stimulatory effects observed can be attributed to the phenomenon of hormesis, where a particular allelochemical can stimulate plant growth at sub-lethal concentrations, and inhibit growth at higher concentrations. In order to verify the practical relevance of knowledge contributed by this study, future work should also involve field experiments. However, the study of allelopathy in field trials will pose further challenges, the main one being the need to separate competition and allelopathy. Until such time as further research yields better understanding of the practical relevance of the allelopathic potential of *C. bonariensis*, crop producers and weed management practitioners should recognize that this important weed has the ability to interfere with the growth and development of a crop through two mechanisms, competition plus allelopathy. In essence, what this boils down to is that weeds should not be allowed to attain telling numbers and/or mass on crop fields, and hence, weeds must be controlled at an early growth stage, in accordance with recommendations appearing on herbicide product labels.

SUMMARY

ALLELOPATHIC POTENTIAL OF *CONYZA BONARIENSIS*

Conyza bonariensis is widespread in the world, and has become a common weed of cultivated and non-cultivated lands, gardens, roadsides and waste places in South Africa over the past century. Since then the weed has not only naturalized itself in many parts of the country but has spread in an alarming rate, and exploded into aggressive herbicide resistant populations. Despite the importance of this weed in agroecosystems little is known about the mechanisms by which this weed competes with crops. The possibility that *C. bonariensis* populations in South Africa might possess allelopathic compounds was researched in this study.

Initial investigations focussed on the allelopathic interference of *C. bonariensis* on lettuce and tomato seed germination and early seedling development under laboratory conditions. Crude extracts for preparation of test solutions were obtained using two solvents (water and hexane) to verify whether the compounds influencing germination were polar or non-polar in nature. To refine the bioassay technique attempts were made to eliminate, or at least reduce, possible confounding factors such as osmotic inhibition, pathogenic microorganisms and phytotoxic residues of organic solvents used for extraction. In all bioassays, germination percentage, root and shoot growth of both test species were inhibited following exposure to aqueous and hexane extracts of roots and leaves of the weed. Evaluation of the results obtained suggested that the leaves of *C. bonariensis* are the main site of allelochemicals. These results show that incorporation of live or dead material of the weed into the soil could negatively affect the establishment of crop species.

The second investigation was divided into two parts. Firstly, the ability of *C. bonariensis* to release putative allelochemicals through its roots was studied using three provenances of the weed (one from Pretoria in Hatfield, and two from the Western Cape), and the second study's was aimed to determine if leachate from *C. bonariensis* affected the growth of test species exposed to different leachate concentrations. For both experiments, plants were grown in a greenhouse on the Experimental Farm at the University of Pretoria. In the first experiment, in which the

weed and test species (lettuce) were grown together hydroponically, highly significant differences were observed in the growth of lettuce plants grown with *C. bonariensis* when compared to the control. This growth reduction in lettuce plants may indicate that even though *C. bonariensis* may have the highest content in its leaves, the roots may also release putative allelochemicals into the environment. Another interesting feature in this study was that the Hatfield population caused significantly greater reduction in lettuce root growth than the other two populations. In the second experiment growth inhibition was not observed for lettuce and tomato plants treated with *C. bonariensis* leachate supplied at different concentrations. However, there was apparent growth stimulation of tomato roots at the highest concentration.

In the third investigation a greenhouse replacement series experiment was conducted for determining the relative interference of *C. bonariensis* in relation to lettuce and tomato. The use of soil in this investigation was to narrow the gap between laboratory and field conditions. Results for dry mass of lettuce and tomato grown at different proportions with *C. bonariensis* showed that there were no significant effects on the growth of the crop species at all proportions. Relative yield calculations at all combination imply that both crop species and *C. bonariensis* were less affected by interspecific interactions than in their respective monocultures. The contrasting results obtained in this study were attributed to methodology.

The results of the above mentioned studies suggest that C*. bonariensis* possesses allelopathic potential and that the weed could have significant debilitating effects on agriculture and natural ecosystems. The possible main site of allelochemicals could be the leaves. Results reported here are from laboratory bioassays and pot experiments, further research should be extended to the field using more crop species for studying weed-crop interactions.

REFERENCES

ABERG, E., JOHNSON, I.J. & WILSIE, C.D., 1943. Associations between species of grasses and legumes. *Journal of the American Society of Agronomy*, 35, 357-369.

AHMED, M. & WARDLE, D. A., 1994. Allelopathic potential of vegetative and flowering *Senecio jacobaea* plants against associated pasture species. *Plant and Soil 164*, 61-68.

ALEX, J. F., 1992. Ontario Weeds. Ontario Ministry of Agriculture and Food Publication 505, Agdex 640, Toronto, ON, pp. 304.

AN, M., PRATLEY, J. E., HAIG. T., 1998. Allelopathy: from concept to reality. Proc. 9th Aust. Agron. Conf., Wagga Wagga, Australia, pp. 563-566.

ANJUM, T. & BAJWA, R., 2005. Importance of germination indices in interpretation of allelochemical effects. *International Journal of Agriculture & Biology* 7, 417-419.

ANZALONE, B., 1964: Un nuovo Erigeron nella Flora Italiana. *Annali di Botanica* 28, 25-39.

APPEL, H. M., 1993. Phenolics in ecological interactions: the importance of oxidation.*Journal of Chemical Ecology* 19, 1521-1552.

ARDI., 1986. Influence between sweet-corn (Zea mays L.) and purple nutsedge (Cyperus rotundus L.) at different irrigation levels. M.S. thesis, University of Hawaii, Honolulu.

ASHRAF, N. & SEH, D.N., 1978. Allelopathic potential of *Celosia argentea* in arid land crop fields. *Oecologia Plantarum* 13, 331-338.

BELL, D. T., 1974. The influence of osmotic pressure in tests for allelopathy. *Transactions of the Illinois State Academy of Science* 67,312-317.

BELZ, R.G., HURLE, K. & DUKE, S.O., 2005. Dose–response – a challenge for allelopathy? *Nonlinearity in Biology, Toxicology, and Medicine* 3, 173-211.

BELZ, R.G., REINHARDT, C.F., FOXCROFT, L.C. & HURLE, K., 2007. Residue allelopathy in *Parthenium hysterophorus* L. – does parthenin play a leading role? *Crop Protection* 26, 237–245.

BELZ, R.G., VAN DER LAAN, M., REINHARDT, C.F. & HURLE. K., 2009. Soil degradation of parthenin—Does it contradict the role of allelopathy in the invasive weed *Parthenium hysterophorus* L.? *Journal of Chemical Ecology* 35, 1137-1150.

BERTIN, C., YANG, X. & WESTON, L. A., 2003. The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil* 256**,** 67-83.

BEZUIDENHOUT, S. R., REINHARDT, C. F. & WHITWELL, M. I., 2012. Cover crops of oats, stooling rye and three annual ryegrass cultivars influence maize and *Cyperus esculentus* growth. *Weed Research* 52, 153-160.

BIANCHI, M. A.; FLECK, N. G.; FEDERIZZI, L. C., 2006. Características de plantas de soja que conferem habilidade competitiva com plantas daninhas. *Bragantia* 65 4, 623-632.

BLACK, J.N., 1958. Competition between plants of different initial seed sizes in swards of subterranean clover (*Trifolium subterraneum L*.) with particular referenceto leaf area and the light microclimate. *Australian Journal of Agricultural Research*, 9, 299-318.

BLUM, U., 1995. The value of model plant-microbe-soil systems forunderstanding processes associated with allelopathic interaction. In *Allelopathy: Organisms, Processes and Applications* (ed. Inderjit, K. M.M. Dakshini and F. A. Einhellig), pp. 127-131. American Chemical Society, Washington, DC.

BLUM, U. & SHAFER, S. R., 1988. Microbial populations and phenolic acids in soil.*Soil Biology and Biochemistry* 20, 793-800.

BOTHA, C., 2001. Common Weeds of Crops and Gardens in Southern Africa. Business Printing Center. Pretoria.

BOTHMA, A., 2002. Allelopathic potential of silverleaf nightshade. (*Solanum elaeagnifolium Cav.).* MSc Thesis. Department of Plant Production and Soil Science, University of Pretoria, Pretoria, South Africa

BROMILOW, C., 2010. Problem plants and Alien weeds of South Africa. Briza publications. South Africa

BUHLER, D. D., 1992. Population dynamics and control of annual weeds in corn (*Zea mays*) as influenced by tillage systems. *Weed Science* 40, 241-248.

CAIRNS, A.L.P. & LAUBSCHER, E.W., 1986. Differential tolerance of Western Cape wild oat to diclofop-methyl and mixtures containing diclofop-methyl. Final report, Department of Agronomy and Pastures, University of Stellenbosch, Stellenbosch.

CASE, C.M. & CRAWLEY, M.J., 2000. Effect of interspecific competition and herbivory on the recruitment of an invasive alien plant Conyza sumatrensis. *Biological Invasions* 2, 103-110.

CHARUDATTAN, R., 2001. Biological control of weeds by means of plant pathogens: Significance for integrated weed management in modern agro-ecology.*Biological Control*46, 229-260.

CHON, S., H. JANG, D. KIM, Y. KIM, H. BOO, & KIM, Y., 2005. Allelopathic potential in lettuce (*Lactuca sativa* L.) plants. *Scientia Horticulturae* 106,309-317.

CHOU, C.H., 1995. Allelopathy and sustainable agriculture. In: K. M. Inderjit, M. Dakshini, and F. A. Einhellig, eds. Processes and Applications. ACS symposium Series 582, pp. 211-223. American Chemical Society, Washington, DC.

CHOU, C.H., 1999. Roles of allelopathy in plant biodiversity and sustainable agriculture. *Critical Reviews in Plant Sciences* 18, 609-636.

CIBA-GEIGY., 1985. Weeds of crops and gardens in Southern Africa. Seal Publishing (Pty) Ltd, Johannesburg, South Africa.

CRONQUIST, A., 1976. *Conyza* Less. In: TUTIN et al. (eds.), Flora Europaea 4, Cambridge University Press, Cambridge.

CORCUERA, L.J., 1993. Biochemistry basis for the resistance of barley to aphids. Review article 78. *Phytochemistry* 50, 17-24.

CROOKS J.A., 2002. Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos* 97, 153-166.

CROPLIFE SOUTH AFRICA AGRICULTURAL REMEDIES DATABASE., 2012. Available at www.croplife.co.za

CURTIS, J. T. & COTTAM, G., 1950. Antibiotic and autotoxic effects in prairie sunflower. *Bulletin of the Torrey Botanical Club* 77, 187-191.

DANIN, A., 1990. New records of four synanthropic plants in southern Africa. *South African Journal of Botany* 56, 412-413.

DARWIN, C., 1859. On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life. John Murray, London.

DE CANDOLLE, M.A.P., 1832. *Physiologie Vegetale*. Tome III, pp. 1474-1475. BechetJeune, Lib, Fac. Med., Paris.

DE WET, H., 2005. Paraquat and glyphosate resistance in C*onyza bonariensis* in the Western Cape in the Republic of South Africa. MSc Agric thesis, University of Stellenbosch, Stellenbosch.

DE WIT, C. T., 1960. On Competition. *Verslagen van landbouwkundige Onderzoekingen* 66(8), 1-82.

DINELLI, G., MAROTTI. I., BONETTI, A., CATIZONE, P., URBANO, J.M. & BARNES, J., 2008. Physiological and molecular bases of glyphosate resistance in Conyza bonariensis biotypes from Spain. *Weed Research* 48, 257-265.

DIXON, G.M.,2008. Allelopathic potential of the alien invader plant Campuloclinium macrocephalum (Less) DC, M InstAgrar dissertation, University of Pretoria, Pretoria.

DONALD, C. M., 1958. The interaction of competition for light and for nutrients. *Australian Journal of Agricultural Research* 9, 421-432.

ECONOMOU, G., TZAKOU, G., GANI, A., YANNITSAROS, A. & BILALIS, D., 2002. Allelopathic Effect of *Conyza albida* on *Avena sativa* and *Spirodela polyrhiza. Journal of Agronomy and Crop Science* 188, 248-253.

EINHELLIG, F. A., 1987. Interactions among allelochemicals and other stress factors of the plant environment. Pp. 343-357 in G. R. Waller (ed.), Allelochemicals: role in agriculture and forestry. ACS Symposium Series 330, American Chemical Society, Washington, DC

EINHELING, F.A., 1989. Interactive effects of allelochemicals and environmental stress. Pp. 101-118 in C. H. Chou & G. R. Waller (eds.), Phytochemical ecology: allelochemicals, mycotoxins, and insect pheromones and allomones. Institute of Botany, Academia Sinica, Monograph Series No. 9, Talpei. 9 1996. Interactions involving allelopathy in cropping systems. *Agronomy Journal* 88, 886-893.

EINHELING, F.A., 1995. Mechanisms of action of allelochemicals in allelopathy. Allelopathy. Organisms, processes and applicants. ACS symposium series 582, pp. 96-116 American chemical Society, Washington, DC.

ELTON, C. S., 1958. The Ecology of Invasions by Animals and Plants. Metheun, London.

EVANS, H.C., 1997. Parthenium hysterophorus: a review of its weed status and the possibilities for its control. *Biocontrol News and Information* 18(3), 89-98.

EVERETT, J., 1990. Asteraceae/Conyza in ‘Flora of New South Wales’, ed. G.J. Harden. NSW University Press, Kensington.

FERREIRA, M. I. &REINHARDT C. F., 2010. Field Assessment of Crop Residues for Allelopathic Effects on Both Crops and Weeds. *Agronomy Journal*102 (6), 1593-1600.

FOURIE, J. & RAATH, P., 2009, Effect of organic and integrated soil cultivation practices on soil nutrient status and performance of a Sauvignon blanc vineyard situated in the Paarl wine district (Part 2): Grapevine performance. www.wynboer.co.za/recentarticles/200907soil-part2.php3

FOURIE, J.C., 1996. Uitkeemin en chemiese eheer van belangrike onkruidin in Wingerde van Suid-Africa. Nooigedaeth Perse Kaapstad.

FOY, C.L. & INDERJIT. 2001., Understanding the role of allelopathy in weed interference and declining plant diversity. *Weed Technology.* 15, 873–876.

FRANKTON, C. & MULLIGAN, G. A. 1987. Weeds of Canada (revised). Publication 948. Ministry of Supply and Services Canada. NC Press Limited, Toronto, pp.217.

FRANZ, J.E., MAO, M.K., SIKORSKI, J.A., 1997. Glyphosate's molecular mode of action. *In* Glyphosate: A Unique Global Herbicide. American Chemical Society, Washington, DC, pp. 521-642.

FUERST, E. P., NAKATANI, H. Y., DODGE A.D., PENNER, D. & ARTZEN. C. J., 1985. Paraquat resistance in Conyza. *Plant Physiology* 77, 984-989.

FUERST, E.R. & PUTNAM, A.R., 1983. Separating the competitive and allelopathic components of interference: Theoretical principles. *Journal of Chemical Ecology* 9, 937-944.

GERSHENZON, J., 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. *Recent Advances in Phytochemistry* 18, 273-320

GIBSON, D. J. 2002. Methods in Comparative Plant Population Ecology. Oxford University Press, Oxford, New York, NY, pp.344.

GILLET, J. B., 1962. Pest pressure, an underestimated factor in evolution. *In* Systematics Association Publication No. 4. *Taxonomy and Geography*, 37-46.

GRABANDT, K., 1985. Weeds of crops and garden in South Africa. Ciba-Geigy (Pty) Ltd, Johannesburg.

GOVINDARAJULU, Z., 1988. Statistical Techniques in Bioassays. Karger. New York.

GRESSEL, J. & SEGEL, L. A., 1978. The paucity of plants involving genetic resistance to herbicides: possible reasons and implications. *Journal of Theoretical Biology* 75, 349-371.

HAO, J.H., QIANG, SH., LIU, Q.Q. & CAO, F. 2009. Reproductive traits associated with invasiveness in *Conyza sumatrensis*. *Journal of Systematics and Evolution*47(3), 245-254.

HARPER, J. L., 1977.Population biology of plants. Aca-demic Press, London.

HEAP, I., 2005. International survey of herbicide resistant weeds. Available at: www.weedscience.org.

HEAP I., 2006 International Survey of Herbicide Resistant Plants. Available at: www.weedscience.org.

HEAP I., 2007 International Survey of Herbicide Resistant Plants. Available at: www.weedscience.org.

HEAP, I., 2009. International survey of herbicide resistant weeds. Available at: www.weedscience.org.

HEAP, I., 2012. International survey of herbicide resistant weeds. Available at: www.weedscience.org.

HECTOR, A., 2006. Overyielding and stable species coexistence.*New Phytologist,* 172, 1-3.

HIRAI, N., 2003. Application of Allelochemicals to Agriculture, *Biological Invasion Space* 17, 4-5.

HEISEY, R.M., 1990. Evidence for allelopathy by tree-of-heaven (*Ailanthus altissima*). *Journal of Chemical Ecology*16, 2039-2055.

HENDERSON, L., 2001. Alien weeds and invasive plants. Plant Protection Research Institute Handbook No. 12. Agricultural Research Council, Pretoria.

HIERRO, J. & CALLAWAY, R.M., 2003. Allelopathy and exotic plant invasion. *Plant and Soil* 256, 29-39

HOAGLAND, R. E. &BRANDSAETER, L.O., 1996. Experiments on bioassay sensitivity in the study of allelopathy.*Journal of Chemical Ecology* 22 (10), 1845-1859

HOAGLAND, R. E. & WILLIAMS, R. D., 2003. Bioassays: Useful tools for the study of allelopathy. In: Macías, F. A., Galindo, J. C. G., Mollinillo, J. M. G. & Cutler, H. G. Editors. Allelopathy: Chemistry and mode of action of allelochemicals. CRC Press. Boca Raton' pp. 315-351.

HOLM, L., DOLL, J., HOLM, E., PANCHO, J. & HERBERGER, J., 1997. World weeds: Natural histories and distribution. John Wiley &Sons, Inc., Toronto, ON, pp. 226-235.

INDERJIT, A.U., 1996. Plant Phenolics in Allelopathy. *The Botanical Review* 62(2), 186-202.

INDERJIT, A.U., 1998. Influence of *Pluchea lanceolata* (Asteraceae) on selected soil properties. *American Journal of Botany* 85, 64-69.

INDERJIT, A.U., ASAKAWA C. &DAKSHINI K.M., 1999. Allelopathic potential of *Vebesina encelioides* root leachate in soil. *Canadian Journal of Botany* 77, 1419-1424.

INDERJIT, A.U. &CALLAWAY, R.M., 2003. Experimental designs for the study of allelopathy. *Plant and Soil* 256, 1-11.

INDERJIT, A.U. &DAKSHINI, K.M.M., 1995. Allelopathic potential of the annual weed *Polygonum monospeliensis*, in crops in India. *Plant and Soil* 173, 251-256.

INDERJIT, A.U. &DAKSHINI, K.M.M., 1999. Bioassays for allelopathy; interactions of soil organic and inorganic constituents, pp. 35–44, in Inderjit, K. M. M. Dakshini, and C. L. Foy (eds.). Principlesand Procedures in Plant Ecology: Allelochemicals Interactions. CRC Press, Boca Raton,Florida.

INDERJIT, A.U. &DEL MORAL, R. 1997. Is separating resource competition from allelopathy realistic? *Botanical Review* 63, 221-230.

INDERJIT, A.U. & FOY, C. L., 2001. On the Significance of Field Studies in Allelopathy. *Weed Technology* 15, 792-797.

INDERJIT, A.U. &KEATING, K.I., 1999. Allelopathy: principles, procedures, processes, and promises for biological control. *Advance Agronomy* 67,141–231.

INDERJIT, A.U. &NILSEN, E.T., 2003. Bioassays and field studies for allelopathy in terrestrial plants: progress and problems. *Critical Reviews in Plant Sciences*22, 221-238.

INDERJIT, A.U. & OLOSFSDOTTER. M., 1998. Using and improving laboratory bioassays in rice allelopathy research. In: Olofsdotter M (ed) Allelopathy in Rice. International Rice Research Institute, Manila, pp. 45-55.

INDERJIT,A.U., SEASTEDT, T. R., CALLAWAY, R.M., POLLOCK, J. L., & KAUR, J., 2008. Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. *Biological Invasions* 10,875-890.

INDERJIT, A.U. & WEANER, J., 2001. Plant allelochemical interference or soil chemical ecology? *Perspectives in Plant Ecology, Evolution and Systematics* 41, 3-12.

INDERJIT, A.U. &WESTON, L. A., 2003 Root exudation: an overview. In *Root Ecology.* Eds. de Kroon and E JW Visser. Springer-Verlag,Heidelberg, Germany. (in press).

IRONS, S. M. & BURNSIDE, 0. C., 1982. Competitive and allelopathic effects of sunflower (Helianthus annuus). *Weed Science* 30, 372-377

IVENS, G., MOODY, M. & EGUJOBI, J.K., 1978. West African Weeds. Oxford University Press. Nigeria.

NIMBAL, C. I., PEDERSEN, J. F., YERKES, C. N., WESTON, L. A., & WELLER, S. C., 1996. Phytotoxicity and distribution of sorgoleone in grain sorghum germplasm. *Journal of Agricultural and Food Chemistry* 44, 1343–1347.

JILANI, G., MAHMOOD, S., CHAUDHRY, A., HASSAN, I & AKRAM, M., 2008. Allelochemicals: sources, toxicity and microbial transformation in soil—a review. *Annals of Microbiology* 58, 351-357.

JOLLIFFE, P. A., 2000. The replacement series. *Journal of Ecology* 88, 371-385.

JOLLIFFE, P.A., MINJAS, A.N. & RUNECKLES, V.C.,1984. A reinterpretation of yield relationships in replacement series experiments. Journal of Applied Ecology, 21, 227-243.

JOSE, S. & GILLESPIE A.R., 1998. Allelopathy in black walnut (*Juglans nigra* L.) alley cropping: I. Spatio-temporal variation in soil juglone in a black walnut – corn (*Zea mays* L.) alley cropping system in the midwestern US. *Plant and Soil* 203, 191–197

KAPUSTA, G., 1979. Seedbed tillage and herbicide influence on soybean (*Glycine max*) weed control and yield. *Weed Science*27, 520-526.

KOBAYASHI, K., 2004. Factors affecting phytotoxic activity of allelochemicals in soil. *Weed Biology and Management* 4, 1-7.

KOEPPE, E. D. E., SOUTHWICK, L. M. & BITTELI, L E., 1976. The relationship of tissue chlorogenic acid concentrations and leaching of phenolics from sunflowers grown under varying phosphate nutrient conditions. *Canadian Journal of Botany* 54, 593-599.

KEMPEN, H.M. & GRAF, J., 1981. Weed seed production. *Weed Science* 34, 78-81.

KHALID, S., AHMAD, T. & SHAD, R.A., 2002. Use of Allelopathy in Agriculture. *Asian Journal of Plant Science* 3, 292-297.

KHANH, T.D., CHUNG, M.I., XUNG, T.D. & TAWAT, S., 2005. The Exploitation of Crop Allelopathy in Sustainable Agricultural Production. *Journal of Agronomy & Crop Science* 191, 172-184.

KIMBER, R. W. W., 1973: Phytotoxicity from plant residues. III. The relative effect of toxins and nitrogen immobilization on germination and growth of weed. *Plant and Soil* 38, 543 - 555

KOLAR, C.S. & LODGE, D.M., 2001. Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* 16 (4), 199-204.

KUMARI, A. & KOHLI, R. K., 1987. Autotoxicity of ragweed parthenium (*Parthenium hysterophorus*). *Weed Science,* 35,629-632.

LEATHER, G.R. 1983. Sunflowers *(Helianthus annuus)* are allelopathic to weeds. *Weed Science*31, 37-42.

LEATHER, G. R. & EINHELLIG, F. A., 1986. Bioassay of naturally occurring allelochemicals for phytotoxicity. *Journal of Chemical Ecology* 14 (10), 1821-1844.

LE MAITRE, D.C., VERSFELD, D.B. & CHAPMAN, R.A., 2000. The Impact of Invading Alien Plants on Surface Water Resources in South Africa: A Preliminary Assessment. *Water SA* 26(3), 397-408.

LEROUX, G. D., BENOIT, D. L. & BANVILLE, S., 1996. Effect of crop rotations on weed control, *Bidens cernua* and *Erigeron canadensis* populations, and carrot yields in organic soils. *Crop Protection*15,171-178.

MACÍAS, F. A., 1995. Allelopathy in the search for Natural Herbicide Models. In Allelopathy: Organisms, Processes, and Applications, eds. Inderjit, K.M.M. Dakshini, and F.A. Einhellig. ACS Symposium Series 582. Washington, DC: American Chemical Society, pp. 310-329.

MACIAS, F. A., CASTELLANO, D. & MOLLINILLO, J. M. G., 2000. Search for a standard phytotoxic bioassay for allelochemicals – Selection of a standard target species. *Journal of Agricultural Food Chemistry* 28, 2512-2521.

MACIAS, F. A., MOLINILLO, J. M. G., OLIVEROS-BASTIDAS, A., MARI´N, D., CHINCHILLA, D., 2004. Allelopathy. A natural strategy for weed control. *Communications in Agricultural and Applied Biological Sciences69*, 13-23.

MACDONALD, I.A.W., REASER, J.K., BRIGHT, C., NEVILLE, L.E., HOWARD, G.W., MURPHY, S.J., &PRESTON, G (eds) 2003. Invasive Alien Species in Southern Africa: National Reports and Directory of Resources. Cape Town: Global Invasive Species Programme.

MACK, R. N., SIMBERLOFF, D., LONSDALE, W. M., EVANS, H., CLOUT, M & BAZZAZ F A 2000 Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10, 689-710.

MAMOLOS, A. P. & KALBURTJI, K. L., 2001. Significance of allelopathy in crop rotation. *Journal of Crop Production* 4,197- 218.

MANDAVA, N.B., 1985. Chemistry and biology of allelopathic agents. In: The Chemistry of Allelopathy: Biochemical interactions among plants. A. C. Thompson (Ed), American Chemistry Society, Washington, pp. 33-54.

MARON, J. L., & VILÀ, M., 2001. When do herbivores affect plant invasion? Evidence from the natural enemies and biotic resistance hypotheses. *Oikos* 95, 361-373.

MAY, F.E. & ASH, J.E., 1990. An Assessment of the Allelopathic Potential of *Eucalyptus. Australian Journal of Botany* 38, 245-254.

MELZER, H., 1996. Neues zur Flora von Steiermark, XXXV. *Mitteilungen des Naturwissenschaftlichen Vereines für Steiermark* 126, 99-104.

MERSIE, W & SINGH, M., 1987. Allelopathic effect of parthenium (Parthenium hysterophorus L.) extract and residue on some agronomic crops and weeds. *Journal of Chemical Ecology*13, 1739-1747.

MEYER, J.J.M., VAN DER KOOY, F., JOUBERT, A., 2007. Identification of plumbagin epoxide as a germination inhibitory compound through a rapid bioassay on TLC. *South African Journal of Botany* 73, 654–656.

MILLER, D. A., 1996. Allelopathy in forage crop systems. *Agronomy Journal* 88(6), 854-859.

MILOVIC, M., 2004. Naturalised species from the genus *Conyza* Less. (Asteraceae) in Croatia. *Acta Botanica Croatica*63 (2), 147-170.

MOLISH, H., 1937. Der einflus eiener Pfanze auf die andere- Allelopathi, Gustav Fisher, Jena

MUELLER, T.C., MASSEY, J.H., HAYES, R.M., MAIN, C.L. & STEWARD, C.N.J., 2003. Shikimate accumulates in both glyphosate-sensitive and glyphosate-resistant horseweed (*Conyza canadensis* (L) Cronq.*Journal of Agricultural and Food Chemistry*51, 680-684.

MULLER, C.H., 1969. Allelopathy as factor in ecological processes. *Vegetatio* 18, 348-357.

NEL, J.L., RICHARDSON, D.M., ROUGET, M. T., MGIDI, N., MDZEKE, N., LE MAITRE, D.C., VAN WILGEN, B.W., SCHONEGEVEL, L., HENDERSON, L. & NESER, S., 2004. A proposed classification of invasive alien plantspecies in South Africa: towards prioritizing species and areas for management action. *South African Journal of Science*100, 53-60

NEWMAN, E. I. & ROVIRA, A. D., 1975. Allelopathy among some British grassland species. *Journal of Chemical Ecology* 63,727-737.

NIEMEYER, H.M., 1998. Hydroxamic acids (4-hydroxyp-4-benzoxazin-3- ones), defense mechanism in the *Gramineae*. *Phytochemistry* 27, 3349-3358

PERRINGS, C. 2005. Mitigation and adaptation strategies for the control of biological invasions. *Ecological Economics* 52 (3), 315-325.

PICMAN, J. & PICMAN, A. K., 1984: Autotoxicity in *Parthenium hysterophorus* and its possible role in control of germination. *Biochemical Systematics and Ecology*12, 287-297.

PIGNATTI, S., 1982: *Conyza* Less. In: Pignatti, S. (ed.), Flora d´Italia 3, Edagricole. Bologna.

PISULA, N. L. & MEINERS, S. J., 2010. Relative allelopathic potential of invasive plant species in a young disturbed woodland. *Journal of the Torrey Botanical Society* 137, 81-87.

PIETERSE, P.J., 2010. Herbicide resistance in weeds - a threat to effective chemical weed control in South Africa. South African Journal of Plant & Soil 27 (1), 1983-2008.

POLDINI, L.&KALIGARI, M., 2000: *Bidens pilosa* and *Conyza sumatrensis*, two new naturalised species in the flora of Slovenia. *Annales* 10, 77-80.

PRAMANIK, M. H. R., NAGAL, M., ASAO, M & MATSUI, Y., 2000 Effect of temperature and photoperiod on phytotoxic root exudates of cucumber *(Cucumis sativus)* in hydroponic culture. *Journal of Chemical Ecology*26, 1953-1967.

PUTNAM, A.R., 1983. Allelopathic chemicals: Nature's herbicide in action. *ChemicalAnd Engineering News* 61, 34-45.

PUTNAM, A.R., DEFRANK, J.,&BARNES, J.P., 1983. Exploitation of allelopathy for weed control in annual and perennial cropping systems. *Journal of Chemical Ecology* 9, 1001-1009.

PUTNAM, A.R. & TANG, C.S., 1986. The Science of Allelopathy. John Wiley and Sons, Inc. USA, pp. 317

QASEM, J. R & FOY, C. L., 2001. Weed Allelopathy, Its Ecological Impacts and Future Prospects. *Journal of Crop Production* 4, 43-119.

QUEIROZ, S.C.N., CANTRELL, C.L., DUKE, S.O., WEDGE, D.E., NANDULA, V.K., MORAES, R. M. & CERDEIRA, A. L., 2012. Bioassay-directed isolation and identification of phytotoxic and fungitoxic acetylenes from *Conyza canadensis*.*Journal of Agricultural and Food Chemistry* 60, 5893-5898.

OSUNA, M.D. & DE PRADO, R., 2002. Conyza albida: a new biotype with ALS inhibitor resistance. *Weed Research* 43, 221-226.

RACZ, I., LASZTITY, D., DARKO, HIDVEGI, E. & SZIGETI, Z., 2000. Paraquat resistance of horseweed (*Erigeron canadensis* L.) is not caused by polyamines. *Pesticide Biochemistry and Physiology*68, 1-10.

RASMUSSEN, J.A. & EINHELLIG, F.A. 1979. Inhibitory effects of combinations of three phenolicacids on grain sorghum germination. *Plant Science Letters*14, 69-74.

REINHARDT, C.F., KHALIL, S. & BEZUIDENHOUT, S., 1999. Bioassay techniques in assessing the allelopathic effect of weeds on crop and plantation species. Proceedings from the first world congress on allelopathy. Cadiz, Spain.

RICE, E. L., 1974. Allelopathy. Academic Press. New York.

RICE, E.L., 1984. Allelopathy. 2nd Edition. Academic Press. New York.

RICE, E.L., 1995. Biological Control of Weeds and Plant Diseases: Advances in Applied Allelopathy. Norman, OK: University of Oklahoma Press, pp. 439

RICHARDSON, D.M., PYSEK, P., REJMÁNEK, M., BARBOUR, M.G., PANETTA F.D. & WEST C.J., 2000. Naturalization and invasion of alien plants: concepts and definitions. *Diversity Distribution* 6, 93-107.

RADOSEVICH, S. R., 1988. Methods to study crop and weed interactions, pp. 121-143 in M. A. Aetieri and M. Z. Liebman, editors. Weed management in agroecosystems: ecological approaches. CRC Press, Boca Raton, Florida,USA.

RADOSEVICH, S. R., HOLT, J. & GHERSA, C. M., 1997. Weed Ecology, Implicationsfor Management. New York: J. Wiley. pp. 114-160.

ROSHCHINA, V. V & ROSHCHINA V. D., 1993. The excretory function of higher plants. Springer, Berlin Heidelberg New York, pp. 314.

SAARI, L.L. & MAUVAIS, C.J., 1996. Sulfonylurea herbicide resistant crops. In: Herbicide Resistant Crops (ed. SO DUKE), pp. 127-143. CRC Press, Boca Raton, FL, USA.

SAKAI, A. K., ALLENDORF, F. W., HOLT, J. S., LODGE, D. M., MOLOFSKY. J., WITH, K .A., BAUGHMAN, S., CABIN, R. J., COHEN, J. E., ELLSTRAND, N. C,, MC-CAULEY, D. E,, O'NEIL, P., PARKER, I. M., THOMPSON, J. N.& WELLER, S.G., 2001 The population biology of invasive species. *Annual Review of Ecology And Systematics* 32, 305-332.

SANTOS, B.M., BEWICK, T.T., STALL, W.M. & SHILLING, D.G., 1997. Competitive interactions of tomato (*Lycopersicon esculentum*) and nutsedges (*Cyperus* spp.). *Weed Science* 45 (2), 229-233.

SANTOS,B.M., DUSKY, J, A., STALL,W.M., SHILLING, D.G. & BEWICK, T.T., 1998.Phosphorus effects on competitive interactions of smooth pigweed (*Amaranthus hybridus*) and common purslane (*Portulaca oleracea*) with lettuce (*Lactuca sativa*). *Weed Science* 46 (3), 307-312.

SAS Institute Inc., 2002. SAS proprietary software version 9.1. Cary, NC: SAS Institute.

SCHMIDT, S. K. & LEY, R. E., 1999. Microbial competition and soil structure limit the expression of allelochemicals in nature, pp. 339–351, *in* Inderjit, K. M. M. Dakshini, and C. L. Foy (eds.). Principles and Practices in Plant Ecology: Allelochemical Interactions. CRC Press, Boca Raton, Florida.

SHAALTIEL. Y., GRESSEL. J.,1986.Multienzyme oxygen radical detoxifying system correlated with paraquat resistance in *Conyza bonariensis*. *Pesticide biochemistry and physiology* 26, 22-28.

SHAUKAT, S.S., KHAN, D., & ALI, S.T., 1983. Suppression of herbs by *Inula grantioides* Boiss, in Sind desert, Pakistan. *Pakistan Journal of Botany*15, 43-67.
SHAUKAT, S.S., MUIR, N. & SIDDIQUI, I.A., 2003. Allelopathic Responses of *Conyza canadensis* (L) Cronquist: A Cosmopolitan Weed. *Asian Journal of Plant Science* 2 (14), 1034-1039.

SHRESTHA, A., HEMBREE, K., 2005, Biology, Identification, Losses, and Control Options for Horseweed and Hairy Fleabane in Tree and Vine Crops in California's Southern San Joaquin Valley. Unpublished.

SIDA, O., 2002: *Conyza* Less. In: KUBAT, K., HROUDA, L., CHRTEK, J., KAPLAN, Z., KIRSCHNER, J., [TEPANEK, J. (eds.), Kli~ ke Kvetene ^eske republiky. Academia, Praha

SLAYTER, R. O., 1967 Plant-Water Relations. Acad. Press, New York, pp. 366.

SMIT, J.J. & CAIRNS, A.L.P., 2000. Resistance of little seeded canary grass *(Phalaris minor* Retz.) to ACC-ase inhibitors. *South African Journal of Plant & Soil* 17, 124-127.

SMIT, J.J. & DE VILLIERS, B.J., 1998. *Lolium* spp. resistance to ACC-ase inhibitors in wheat (*Triticum aestivum* L.) within the RSA: a preliminary study. *South African Journal of Plant & Soil* 15, 158-161.

SMIT, J.J., SMIT, H.A. & DE VILLIERS, B.L., 1999. Differential efficacy of tralkoxydim and diclofop-methyl on a suspected resistant ryegrass (*Lolium rigidum* Gaud.) biotype. *South African Journal of Plant & Soil* 16, 169-172.

SOLBRIG, O. T., 1991. *Biodiversity*. Scientific issues and collaborative research proposal. UNESCO, Paris

STOHLGREN, T.G., BINKLEY, D., CHONG, G.W., KALKHAN, M.A., SCHELL, L.D., BULL, K.A., OTSUKI, T.Y., NEWMAN, G., BASHKIN, M., SON,Y. 1999. Exotic plant species invade hot spots of native plant diversity. *Ecological Monographs* 69, 25-46.

STOWE, L. G., 1979. Allelopathy and its influence on the distribution of plants in an Illinois old-field.*Journal of Ecology* 67, 1065-1085.

TAKAHASHI, K. 1984. Replant failure problems in vegetables. *Res. Data Natl. Res, Inst. Vegetables*18, 87-99.

TAMADO, T. & MILBERG, P. 2000. Weed flora in arable fields of eastern Ethiopia with emphasis on the occurrence of *Parthenium hysterophorus*. *Weed Research* 40, 507-521.

TAMADO, T., OHLANDER, L. & MILBERG, P. 2002. Interference by the weed *Parthenium hysterophorus* L With grain sorghum: influence of weed density and duration of competition. *International Journal of Pest Management* 48(3), 183-188.

THEBAUD, C., ABBOTT, R.J., 1995. Characterization of invasive *Conyza* species (Asteraceae) in Europe: quantitative trait and isozyme analysis. *American Journal of Botany* 82, 360- 368.

THEBAUD, C., FINZI, A.C., AFFRE, L., DEBUSSCHE, M. & ESCARRE, J., 1996. Assessing why two introduced *Conyza* differ in their ability to invade Mediterranean old fields. *Ecology* 77, 791-804.

THEOPHRASTUS (ca. 300 B.C). 1916. *Enquiry into Plants and Minor Works on Odours and Weather Signs*. 2 Vols. Translated to English by A. Hort and W. Heinemann, London.

TORRES, A., OLIVIA, R.M., CASTELLANO, D. & CROSS, P., 1996. In: Proceedings of the First World Congress on Allelopathy: A Science of the Future. SAI (University Cadiz), Spring Cadiz, 278 pp.

TRAVALOS, I, S., ECONOMOU, G., KANATA, P.J. & TZAKOU, O., 2007. Aspects of the Allelopathic Potential of Horseweed (*Conyza albida*). *International Journal of Agricultural Research* 2(4), 397-401.

VIARD-CRETAT, F., GALLET, C., LEFEBVRE, M. & LAVOREL S., 2009. A leachate a day keeps the seedlings away: mowing and the inhibitory effects of *Festuca paniculata* in subalpine grasslands. *Annals of Botany* 103, 1271–1278.

VANGESSEL, M.J., 2001. Glyphosate-resistant horseweed from Delaware. *Weed Science*.49, 703-705.

VAN WILGEN, B.W., RICHARDSON, D.M., MARAIS, C., MAGADLELA, D., 2001. The economic consequences of alien plant invasions: examples of impacts and approaches to sustainable management in South Africa. *Environment, Development and Sustainability* 3, 145-168.

VITOUSEK, P.M., 1990. Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos* 57, 7-13.

WALKER, B.H. & STEFFEN,W.L., 1999. Interactive and integrated effects of global change on terrestrial ecosystems. In *The Terrestrial Biosphere and Global ChangeImplications for Natural and Managed Ecosystems. Synthesis volume,* eds B.Walker, W. Steffen, J. Canadell and J. Ingram, pp. 329-375. International Geosphere- Biosphere Program Book Series 4, Cambridge University Press, Cambridge.

WARDLE, D.A., NILSSON, M.C., GALLET, C. & ZACKRISSON, O., 1998. An ecosystem level perspective of allelopathy. Biological Reviews, 73, 305–319.

WEAVER, S.E., 2001. The biology of Canadian weeds, 115: *C. canadensis*. *Canadian Journal of Plant Science* 81, 867-75.

WEBB, M. & CONROY, C., 1995. The socio-economics of weed control on smallholder farms in Uganda.Proceedings of the Brighton Crop Protection Conference 1, 157-162.

WEBSTER, S., 1980. *New Collegate Dictionary*. Merrian, C. Co. Springfield.

WEED SCIENCE SOCIETY OF AMERICA (WSSA). 1998. *Herbicide Handbook* (Suppl.).Weed Science Society of America, Lawrence, Kansas.

WEIDENHAMER, J. D., HARTNETT, D. C. & ROMEO, J. T., 1989. Density dependent phytotoxicity: distinguishing resource competition and allelopathic interference in plants. *Journal of Applied Ecology* 26, 613-624.

WEIR, T.L, PARK S.W., VIVANCO, J.M., 2004. Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology* 7, 472-479.

WESTON, L. A., 1996. Distinguishing resource competition and chemical interference overcoming the methodological impasse. *Agronomy Journal* 88, 866-875.

WESTON, L.A. & DUKE, S.O., 2003 Weed and crop allelopathy. *Critical Review Plant Science* (in press).

WHITTAKER, R. H. &FEENY, P. P., 1971. Allelochemics: chemical interactions between species, *Science* 171, 757-770.

WIDDERICK. & WU., 2009. Fleabane. Department of Agriculture and Food, Government of Western Australia. www.agric.wa.gov.au/PC93457.html.

WIESE, A. F., SALISBURY, C. D., & BEAN, B. W., 1995., Downy brome (*Bromus tectorum*), jointed goatgrass (*Aegilops cylindrica*)and horseweed (*Conyza canadensis*) control in fallow. *Weed Technology*9, 249- 254.

WILLIAMS, J. R., 1954. The biological control of weeds. In Report of the Sixth Commonwealth Entomological Congress, London, pp. 95–98.

WILLIAMSON, G. B.,1990 Allelopathy, Koch's postulates, and the neck riddle. In: Grace JB, Tilman D (eds) Perspectives onplant competition. Academic Press, San Diego, Calif., pp143–162.

WILCOVE, D.S., ROTHSTEIN, D., DUBOW, J., PHILLIPS, A. & LOSOS E., 1998. Quantifying threats to imperilled species in the United States. *Biological Science* 48,607–615.

WITT, A., 2010. Impacts of invasive plants and their sustainable management in agro-ecosystems in Africa: a review. CABI Africa, NRB, 1102-1109.

WU, H., 2004. Fleabane biology and its control. *In Northern Herbicide Resistance Reporter*.

WU, H., & WALKER, S.R., 2004. Fleabane biology and control. Fleabane: workshop proceedings, eds S.R. Walker, M. Widderick and H. Wu, Toowoomba

WU, H., PRATLEY, J., LEMERLE, D., HAIG T. & VERBEEK, B. 1998. Differential allelopathic potential among wheat accessions to annual ryegrass, pp.567-71. *In: Proc. 9th Australian Agronomy Conference.,* Wagga Wagga*,* NSW,Australia.

WU, H., PRATLEY, J., LEMERLE, D., HAIG, T. & AM, N. Screening Methods for the Evaluation of Crop Allelopathic Potential. The Botanical Review 67 (3), 403-415.

WUIZELL, B., 1994. A history of Conyza in London. Botanical Society for *British Isles News* 65, 34-39.

YAKLE, G. A. & CRUSE, R. M., 1984: Effects of fresh and decomposing corn plant residue extracts on corn seedling development. *Soil Science Society of America Journal .* 48, 1143-1146.

YU, J. Q.&MATSUI, Y., 1994. Phytotoxic substances in the root exudates of *Cucumis sativus L. Journal of Chemical Ecology* 20, 21-31.

ZINZOLKER, A., KIGEL, J. & RUBIN, B., 1985. Effects of environmental factors on the germination and flowering of *Conyza albida*, *C. bonariensis* and *C. canadensis*. *Phytoparasitica* 13, 229-230.

APPENDIX A: Chapter 2

Bioassays to determine *Conyza bonariensis*' allelopathic potential.

Table A1. Abbreviated ANOVA table for germination bioassays of lettuce exposed to *C. bonariensis* leaf infusions (Figure 2.1)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	86811.00000			
Plantpart	1	169.00000	169.00000	0.83	0.3637
Concentration	4	55016.00000	13754.00000	67.83	<.0001
Plantpart*Concentration	4	13376.00000	3344.00000	16.49	<.0001
Error	90	18250.00000	202.77778		

Table A2. Abbreviated ANOVA table for germination bioassays of lettuce root (radicle) growth exposed to *C. bonariensis* leaf and root infusions (Figure 2.2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	10882.16000			
Plantpart	1	108.160000	108.160000	10.42	0.0017
Concentration	4	9606.560000	2401.640000	231.27	<.0001
Plantpart*Concentration	4	232.840000	58.210000	5.61	0.0004
Error	90	934.60000	10.38444		

Table A3. Abbreviated ANOVA table for germination bioassays of lettuce shoot growth exposed to *C. bonariensis* leaf and root infusions (Figure 2.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	6144.910000			
Plantpart	1	778.410000	778.410000	57.18	<.0001
Concentration	4	3828.660000	957.165000	70.31	<.0001
Plantpart*Concentration	4	312.540000	78.135000	5.74	0.0004
Error	90	1225.300000	13.614444		

Table A4. Abbreviated ANOVA table for germination bioassays of tomato exposed to *C. bonariensis* leaf and root infusions (Figure 2.4)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	116811.0000			
Plantpart	1	5929.00000	5929.00000	41.14	<.0001
Concentration	4	82316.00000	20579.00000	142.80	<.0001
Plantpart*Concentration	4	15596.00000	3899.00000	27.06	<.0001
Error	90	12970.0000	144.1111		

Table A5. Abbreviated ANOVA table for germination bioassays of tomato root (radicle) growth exposed to *C. bonariensis* leaf and root infusions (Figure 2.5)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	42952.11000			
Plantpart	1	1391.29000	1391.29000	41.89	<.0001
Concentration	4	37179.26000	9294.81500	279.84	<.0001
Plantpart*Concentration	4	1392.26000	348.06500	10.48	<.0001
Error	90	2989.30000	33.21444		

Table A6. Abbreviated ANOVA table for germination bioassays of tomato shoot growth exposed to *C. bonariensis* leaf and root infusions (Figure 2.6)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	17034.75000			
Plantpart	1	26.01000	26.01000	1.93	0.1683
Concentration	4	14409.40000	3602.35000	267.21	<.0001
Plantpart*Concentration	4	1386.04000	346.51000	25.70	<.0001
Error	90	1213.30000	13.48111		

Table A7. Abbreviated ANOVA table for germination bioassays of lettuce exposed to *C. bonariensis* hexane leaf and root extract (Figure 2.7)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	19136.00000			
Plantpart	1	4.000000	4.000000	0.03	0.8624
Concentration	4	4366.000000	1091.500000	8.24	<.0001
Plantpart*Concentration	4	2846.000000	711.500000	5.37	0.0006
Error	90	11920.00000	132.44444		

Table A8. Abbreviated ANOVA table for germination bioassays of lettuce roots (radicles) exposed to *C. bonariensis* hexane leaf and root extract (Figure 2.8)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	15260.75000			
Plantpart	1	5730.490000	5730.490000	247.02	<.0001
Concentration	4	4875.100000	1218.775000	52.54	<.0001
Plantpart*Concentration	4	2567.260000	641.815000	27.67	<.0001
Error	90	2087.90000	23.19889		

Table A9. Abbreviated ANOVA table for germination bioassays of lettuce shoot growth exposed to *C. bonariensis* hexane root extract (Figure 2.9)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	1868.510000			
Plantpart	1	272.250000	272.250000	53.86	<.0001
Concentration	4	1035.160000	258.790000	51.20	<.0001
Plantpart*Concentration	4	106.200000	26.550000	5.25	0.0008
Error	90	454.900000	5.054444		

Table A10. Abbreviated ANOVA table for germination bioassays of tomato exposed to *C. bonariensis* hexane leaf extract (Figure 2.10)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	19804.00000			
Plantpart	1	484.000000	484.000000	4.46	0.0374
Concentration	4	8494.000000	2123.500000	19.58	<.0001
Plantpart*Concentration	4	1066.000000	266.500000	2.46	0.0512
Error	90	9760.00000	108.44444		

Table A11. Abbreviated ANOVA table for germination bioassays of tomato root (radicle) growth exposed to *C. bonariensis* hexane leaf and root extract (Figure 2.11)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	30114.75000			
Plantpart	1	506.25000	506.25000	15.86	0.0001
Concentration	4	26037.70000	6509.42500	203.96	<.0001
Plantpart*Concentration	4	698.50000	174.62500	5.47	0.0005
Error	90	2872.30000	31.91444		

Table A12. Abbreviated ANOVA table for germination bioassays of tomato shoot growth exposed to *C. bonariensis* hexane root extract (Figure 2.12)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	99	2056.360000			
Plantpart	1	163.8400000	163.8400000	18.74	<.0001
Concentration	4	860.4600000	215.1150000	24.61	<.0001
Plantpart*Concentration	4	245.2600000	61.3150000	7.01	<.0001
Error	90	786.800000	8.742222		

APPENDIX B: Chapter 3

Assessment of the allelopathic potential of *Conyza bonariensis* root exudates

Table B1. Abbreviated ANOVA table for leaf fresh mass of lettuce grown hydroponically with *C. bonariensis* plants collected on Hatfield experimental farm (Figure 3.5)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	19	9037.877280			
Treatment	1	7239.773520	7239.773520	72.47	<.0001
Error	18	1798.103760	99.894653		

Table B2. Abbreviated ANOVA table for root fresh mass of lettuce grown hydroponically with *C. bonariensis* plants collected on Hatfield experimental farm (Figure 3.5)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	19	844.1960550			
Treatment	1	641.0516450	641.0516450	56.80	<.0001
Error	18	203.1444100	11.2858006		

Table B3. Abbreviated ANOVA table for leaf dry mass of lettuce grown hydroponically with *C. bonariensis* plants collected on Hatfield experimental farm (Figure 3.8)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	19	61.66678000			
Treatment	1	39.81842000	39.81842000	32.80	<.0001
Error	18	21.84836000	1.21379778		

Table B4. Abbreviated ANOVA table for root dry mass of lettuce grown hydroponically with *C. bonariensis* plants collected on Hatfield experimental farm (Figure 3.8)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	19	9.38125500			
Treatment	1	5.95140500	5.95140500	31.23	<.0001
Error	18	3.42985000	0.19054722		

Table B5. Abbreviated ANOVA table for leaf fresh mass of lettuce grown hydroponically with two Western Cape provenances of *C. bonariensis* (Figure 3.9)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	29	11584.02212			
Treatment	2	7614.978660	3807.489330	25.90	<.0001
Error	27	3969.04346	147.00161		

Table B6. Abbreviated ANOVA table for root fresh mass of lettuce grown hydroponically with two Western Cape provenances of *C. bonariensis*(Figure 3.9)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	29	788.7474667			
Treatment	2	525.9911267	262.9955633	27.02	<.0001
Error	27	262.7563400	9.7317163		

Table B7. Abbreviated ANOVA table for leaf dry mass of lettuce grown hydroponically with two Western Cape provenances of *C. bonariensis* (Figure 3.10)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	29	60.57773667			
Treatment	2	29.06312667	14.53156333	12.45	0.0001
Error	27	31.51461000	1.16720778		

Table B8. Abbreviated ANOVA table for root dry mass of lettuce grown hydroponically with two Western Cape provenances of *C. bonariensis* (Figure 3.10)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	29	8.26692417			
Treatment	2	3.83442167	1.91721083	11.68	0.0002
Error	27	4.43250250	0.16416676		

Table B9. Abbreviated ANOVA table for leaf fresh mass of lettuce that was exposed to *C. bonariensis* leachate (Figure 3.11)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	24	7166.788296			
Treatment	4	1803.072976	450.768244	1.68	0.1938
Error	20	5363.715320	268.185766		

Table B10. Abbreviated ANOVA table for root fresh mass of lettuce that was exposed to *C. bonariensis* leachate(Figure 3.11)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	24	120.2069440			
Treatment	4	48.10766400	12.02691600	3.34	0.0301
Error	20	72.0992800	3.6049640		

Table B11. Abbreviated ANOVA table for leaf dry mass of lettuce that was exposed to *C. bonariensis* leachate(Figure 3.12)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	24	24.75205600			
Treatment	4	5.93213600	1.48303400	1.58	0.2193
Error	20	18.81992000	0.94099600		

Table B12. Abbreviated ANOVA table for leaf dry mass of lettuce that was exposed to *C. bonariensis* leachate (Figure 3.12)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	24	4.91153400			
Treatment	4	1.70381400	0.42595350	2.66	0.0631
Error	20	3.20772000	0.16038600		

Table B13. Abbreviated ANOVA table for leaf fresh mass of tomato that was exposed to *C. bonariensis* leachate (Figure 3.13)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	24	623.0834000			
Treatment	4	192.2481200	48.0620300	2.23	0.1021
Error	20	430.8352800	21.5417640		

Table B14. Abbreviated ANOVA table for root fresh mass of tomatothat was exposed to *C. bonariensis* leachate (Figure 3.13)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	24	528.3116160			
Treatment	4	272.7044560	68.1761140	5.33	0.0043
Error	20	255.6071600	12.7803580		

Table B15. Abbreviated ANOVA table for leaf dry mass of tomato that was exposed to *C. bonariensis* leachate (Figure 3.15)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	24	8.89534400			
Treatment	4	2.48542400	0.62135600	1.94	0.1432
Error	20	6.40992000	0.32049600		

Table B15. Abbreviated ANOVA table for root dry mass of tomato that was exposed to *C. bonariensis* leachate (Figure 3.15)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Corrected Total	24	4.74825600			
Treatment	4	1.72725600	0.43181400	2.86	0.0504
Error	20	3.02100000	0.15105000		

APPENDIX C: Chapter 4

The role of allelopathy in *Conyza bonariensis* inhibition of lettuce and tomato

Table C1. Abbreviated ANOVA table for Dry mass of *C. bonariensis* and lettuce grown together in a replacement series at different proportions (Figure 4.4)

Source	DF	Squares	Mean Square	F Value	Pr > F
Corrected Total	49	19.45036800			
Species	1	0.01620000	0.01620000	0.04	0.8370
Combinations	4	3.18890800	0.79722700	2.11	0.0973
Species*Combinations	4	1.14182000	0.28545500	0.76	0.5601
Error	40	15.10344000	0.37758600		

Table C2. Abbreviated ANOVA table for Dry mass of *C. bonariensis* and tomato grown together in a replacement series at different proportions (Figure 4.5)

Source	DF	Squares	Mean Square	F Value	Pr > F
Corrected Total	49	54.97751808			
Species	1	4.18414592	4.18414592	4.26	0.0455
Combinations	4	9.03324768	2.25831192	2.30	0.0754
Species*Combinations	4	2.50015168	0.62503792	0.64	0.6393
Error	40	39.25997280	0.98149932		