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Allelopathic interference potential of the alien invader plant *Parthenium hysterophorus*

by

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Submitted in fulfillment of part of the requirements for the degree of MSc (Agric) Agronomy in the faculty of Natural and Agricultural Sciences University of Pretoria

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DECLARATION

I, Michael van der Laan, hereby declare that this dissertation for the degree MSc (Agric) Agronomy at the University of Pretoria is my own work and has never been submitted by myself at any other University. The research work reported is the result of my own investigation, except where acknowledged.

M VAN DER LAAN
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ABSTRACT

The alien invader plant *Parthenium hysterophorus* is a Category 1 weed in South Africa, where it poses a serious threat to indigenous vegetation in particular, and to biodiversity in general. In addition to its competitive ability, it is hypothesized that the successful invasiveness of *P. hysterophorus* is linked to the allelopathic potential of the plant. One compound in particular, parthenin, is alleged to play a major role in this allelopathic potential. Interference between *P. hysterophorus* and three indigenous grass species (*Eragrostis curvula, Panicum maximum, Digitaria eriantha*) was investigated on a site with a natural parthenium infestation at Skukuza, Kruger National Park. The trial was conducted over two growing seasons on exclosure plots which eliminated mammal herbivory. *P. maximum* displayed best overall performance and was eventually able to completely overwhelm *P. hysterophorus*. *Eragrostis curvula* and *D. eriantha* grew more favourably in the second season after becoming better established but were clearly not well adapted to the trial conditions. Although *P. maximum* was the supreme interferer, all grasses were able to significantly interfere with *P. hysterophorus* growth in the second season. The ability of *P. maximum* to interfere with *P. hysterophorus* growth so efficiently that it caused mortalities of the latter species, indicates that *P. maximum* exhibits high potential for use as an antagonistic species in an integrated control programme. An investigation on the production dynamics of parthenin in the leaves of *P. hysterophorus* indicated that

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high levels of this compound are produced and maintained in the plant up until senescence. The high resource allocation priority of the plant towards this secondary metabolite even in the final growth stages may indicate the use of residual allelopathy to inhibit or impede the recruitment of other species. Studies on the persistence of parthenin in soil revealed that parthenin is readily degraded in soil and that microbial degradation appears to play a predominant role. Significant differences between parthenin disappearance-time half-life (DT_{50}) values were observed in soils incubated at different temperatures and in soils with different textures. Exposure of the three grass species to pure parthenin showed that, in terms of their early development, the order of sensitivity of the grasses was: *Panicum maximum>Digitaria eriantha>Eragrostis curvula.* It may therefore prove challenging to establish *P. maximum* from seed in *P. hysterophorus* stands during the execution of an integrated control programme due to the sensitivity of this grass species to parthenin. From the research findings it appears possible that *P. hysterophorus* can inhibit or impede the recruitment of indigenous vegetation under natural conditions. At least one mechanism through which this alien species can exert its negative influence on other plant species is the production and release of parthenin.

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INTRODUCTION

From its native range in tropical America (Haseler, 1976), *Parthenium hysterophorus* has aggressively spread across the globe and is now listed as an invasive weed in many countries. It was first observed in India and Australia in the mid-1950's, where it has since become particularly problematic (Pandey & Dubey, 1991; Navie *et al*., 1996). Lack of adequate control measures has seen this weed continue to spread, having a detrimental effect on crop production, biodiversity, animal husbandry and human health (Navie *et al*., 1996). Although the weed was first recorded in South Africa over 100 years ago, it has only become troublesome in the last two decades (Henderson, undated). In South Africa, *P. hysterophorus* has been declared a "Category 1" weed which according to legislation implies that: 'These are prohibited plants that will no longer be tolerated, neither in rural nor urban areas, except with the written permission of the executive officer or in an approved biocontrol reserve. These plants may no longer be planted or propagated, and all trade in their seeds, cuttings or other propagative material is prohibited. They may not be transported or be allowed to disperse' (Conservation of Agricultural Resources Act, 1983; Act No 43 of 1983).

The plant characteristic of allelopathy – broadly defined as chemical interactions between plants – is believed to be an important attribute contributing to the successful spread of *P. hysterophorus* in non-native ranges. Scientists of diverse disciplines have conducted chemical studies and bioassays to better understand *P. hysterophorus* allelopathy (Kanchan & Jayachandra, 1979; Mersie & Singh, 1987, 1988; Adkins & Sowerby, 1996; Kraus, 2003; Reinhardt *et al*., 2004; Belz *et al*., 2006). However, our knowledge and understanding of the effect of allelochemicals from *P. hysterophorus* on other plant species under natural conditions could be regarded as juvenile. This is largely due to the complexity of allelopathy research, with 'myriad biological, chemical and physical factors' interacting at every step, from allelochemical production, transport to and receptivity of target species, to fate of the compound in the environment (Reinhardt *et al*., 1999). Inderjit & Weiner (2001) emphasize the importance of the effects of plant secondary metabolites on soil factors, such as soil ecology and nutrient availability on plant community structure.

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The purpose of this study was to promote understanding of the allelopathic potential of *P. hysterophorus*, and the reliance of this invader plant on allelopathic interference in displacing natural vegetation and/or preventing natural succession. Limited success in discovering adequate insect or pathogen biological control agents for controlling this weed necessitates the need to discover other means of control, for example through employment of antagonistic plant species. Plants that can adequately interfere with parthenium are also useful in restoring areas previously infested with this weed.

Collaboration between the University of Pretoria and the University of Hohenheim in Stuttgart, Germany, was first initiated in 2000, with Kruger National Park Scientific Services subsequently joining. The collaboration is particularly efficient as it allows for relevant field work to be conducted in South Africa, while first-rate allelochemistry studies can be conducted in Germany. To date, some findings by the team have been reported by Kraus (2003), Reinhardt *et al*. (2004), and Belz *et al*. (2006). In a continuation of research by the team, the objectives of the current study were to investigate: (a) interference between *P. hysterophorus* and indigenous grass species, (b) the production dynamics of parthenin during the life-cycle of *P. hysterophorus*, and, (c) the degradation of parthenin in soil. Aspect (a) involved a field trial in the Kruger National Park, and bioassays done under controlled conditions at the University of Pretoria. Aspects (b) and (c) were both conducted at the University of Hohenheim as part of a study visit.

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CHAPTER I – LITERATURE REVIEW

1.1 Alien invasive plants

An exponential increase in the movement of plant species across the world has been observed as a result of globalization. In some cases these species have become established in areas far from their native ranges, and under favourable conditions and in the absence of natural enemies have spread prolifically, often becoming a threat to biodiversity in these regions. Secondary effects on the structure and function of ecosystems can also be highly detrimental (Clout & De Poorter, 2005). Furthermore, these species can have adverse economic impacts by reducing crop yields or grazing land quality (Goslee *et al*., 2001) Recognizing this threat, the United Nations Convention on Biological Diversity calls on contracting parties [Article 8(h)] to 'prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats and species' (Clout & De Poorter, 2005).

The introduction of one or more natural enemies to biologically control an invasive species has been a successful strategy in some instances. According to Fowler *et al*. (2000), however, 'complete success of biocontrol, where no other control methods are required, accounts for approximately one-third of all successfully completed biological control programmes'. Other control measures are therefore often required for incorporation into an integrated control programme.

For South Africa, Nel *et al*. (2004) listed 117 major invaders – well-established species that already have a significant impact on natural and semi-natural ecosystems- and 84 emerging invaders – species with the attributes to potentially spread over the next few decades. According to Foxcroft & Richardson (2003), surveys revealed that by the end of 2001, 366 alien plant taxa were known to occur in the Kruger National Park. Invasive weeds present a very real threat in South Africa, and control measures have been unsatisfactory, with lack of resources being a major factor (Kluge & Erasmus, 1991; Goodall & Naudé, 1998).

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1.2 Allelopathy

1.2.1 Definition and brief history

Allelopathy involves direct and indirect chemical interactions between plants as well as micro-organisms and was first termed by Molisch, an Austrian plant physiologist, in 1937. The term is derived from the Greek words 'allelon'- meaning mutual - and 'pathos' - meaning harm or affection. Weston & Duke (2003) further define it 'as an important mechanism of plant interference mediated by the addition of plant-produced secondary products to the rhizosphere'. Chemical interactions between plants were recorded thousands of years ago. The effect of *Cicer arietinum* (chickpea) on other plants was recorded in 300 BC, and the effects of several harmful plants on croplands were mentioned by Pliny in 1 AD. Pliny also observed the effects of the walnut tree (*Juglans nigra* and *J. regia*), which is one of the most widely known examples of an allelopathic plant today.

A vast diversity of secondary compounds is produced by plants, from simple hydrocarbons to complex polycyclic aromatics (Weston & Duke, 2003). Effects of allelochemicals in the field, as summarized by Inderjit & Weiner (2001), can be due to (i) direct effects of allelochemicals from donor plants, (ii) effects of transformed or degraded products from released allelochemicals, (iii) effect of allelochemicals released on chemical, physical or biological soil factors, and (iv) chemical induction of release of allelochemicals by a third species. Although allelopathy has been extensively studied under controlled conditions and our knowledge of growth inhibition mechanisms and allelochemical modes of action has been greatly enhanced (Inderjit & Weston, 2000), less is known on the fate of allelochemicals in the environment and their effect on soil ecology. Inderjit & Weiner (2001) propose that vegetation behaviour can be better understood 'in terms of allelochemical interactions with soil ecological processes rather than the classical concept of direct plant-plant allelopathic interference'; and 'researchers have now started to appreciate the ecological importance of allelochemicals on the ecosystem-level processes' (Wardle *et al.*, 1998; Inderjit & Weiner, 2001). Allelopathic research has become inter-disciplinary, involving collaborative work by plant scientists, weed scientists, soil scientists, ecologists and others.

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1.2.2 Modes of action of allelochemicals

Allelochemicals are able to effect both the germination and growth of plants. This is achieved by influencing a wide variety of metabolic processes. The exact modes of action of these chemicals is often very difficult to determine with any certainty, and of the vast quantity of allelochemicals that have been identified, modes of action have only been ascertained for a very small number of these (Einhellig, 1995). There is no single established method for determining the mode of action for these chemicals (Einhellig, 1995). Observations made during dose-response experiments can often be used to narrow down the possibilities of the site of action (Vyvyan, 2002), but these observations should not be over-interpreted. The mitotic index can be measured to determine an allelochemical's effect on root cell division; and chlorophyll concentration, fluorescence and carbon dioxide exchange can all be used to determine the agent's effect on photosynthetic efficiency of a particular plant (Vyvyan, 2002). Conductivity measurements can be used to determine whether allelochemicals disrupt cell membranes, and can additionally be used to assess whether the mode of action is light dependent. Careful study of the molecule's structure and the use of structure-activity databases can be helpful in determining modes of action. Macías *et al*. (1992) reported that various spatial arrangements which the molecule can adopt play an important role in activity.

Plant processes which have been found to be influenced by allelochemicals that have so far been identified include: mineral uptake, cell division and elongation, action of plant growth regulators, respiration, photosynthesis, stomatal opening, protein synthesis, haemoglobin synthesis, lipid and organic acid metabolism, membrane permeability and action of certain enzymes (Retig *et al*., 1972; Rice, 1974; Harper & Balke, 1981).

1.2.3 Allelopathy and agriculture

Weeds can interfere with crop growth and reduce yields, deteriorate crop quality, clog waterways and cause health problems; with eradication costs being massive (Singh *et al*., 2003). An estimated 240 weeds have been reported to have allelopathic potential (Qasem & Foy, 2001), although many of these species have been tested with

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unrealistic bioassays (Inderjit & Keating, 1999). In turn, allelopathic crops that are able to chemically interfere with weed growth have also been identified, such as *Secale cereale* (rye), *Triticum aestivum* (wheat), *Sorghum bicolour* (sorghum), *Oryza sativa* (rice), and *Helianthus annuus* (sunflower). In addition to beneficial chemical interference of crops with weed growth, there is potential for the advantageous use of allelopathy for practices such as crop rotation, cover and smother crops and retention of crop residues (Singh *et al*., 2003). According to Duke *et al*. (2002), two approaches for improving the utilization of allelopathy in crops to increase weed suppression are possible: (i) to enhance the existing allelopathic potential of a particular crop, and (ii) to introduce allelopathic potential through the insertion of foreign genes encoding for allelochemicals. This can be achieved through employing conventional breeding techniques as well as genetic modification techniques. With increased environmental awareness and public pressures, less detrimental means of weed control are continually being sought. One such approach is to consider allelochemicals as new sources of herbicides. This approach may be beneficial as natural plant products have advantages over synthetic herbicides, including: (i) allelochemicals often possess complex structures and exhibit structural diversity, making them valuable lead compounds, (ii) the compounds have high molecular weight with little or no halogens or heavy atoms, (iii) allelochemicals have little environmental impact as they degrade rapidly in the environment, and (iv) allelochemicals have novel target sites very often different to those of synthetics (Dayan *et al*., 1999; Duke *et al*., 2002; Singh *et al*., 2003).

1.2.4 Allelopathy and biodiversity

The end result of invasive plant spread is often a massive loss of biodiversity. Maintaining diversity is important as it enhances resource utilization efficiency (Foy & Inderjit, 2001), acts as a buffer against large ecosystem shifts, and maintains highly valued crop and wild plant genetic diversity (Chou, 1999). Allelopathy may play an important role in plant community structure and researchers have begun to recognize the ecological significance of allelochemicals on ecosystem-level processes (Wardle *et al*., 1998). Allelopathic potential may be an important attribute of certain successful invader plant species in displacing natural vegetation, and according to Hardin (1960),

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may be an explanation for the ability of invasive weeds to endure beyond early stages of secondary succession.

1.3 *Parthenium hysterophorus*

Parthenium hysterophorus L. belongs to the Heliantheae tribe, a member of the Asteraceae family. Within the Parthenium genus there are 15 species all of which are native to the Americas (Navie *et al*., 1996). *P. hysterophorus* specifically originates from tropical America from the areas surrounding the Gulf of Mexico (Haseler, 1976). The recent appearance of *P. hysterophorus* in many parts of the world has resulted in several common names for the plant, including: parthenium and Demoina weed (South Africa), carrot weed and congress weed (India), ragweed parthenium (USA), and parthenium weed (Australia).

1.3.1 Botanical description

Unless otherwise stated, the following description was obtained from Navie *et al*. (1996) and personal observation. Parthenium is an upright, herbaceous plant often displaying prolific branching. It displays highly vigorous growth in suitable climates and can reach a height of two metres. Following emergence the plant has two hairless cotyledons with short petioles. A rosette is formed by the young plant with dark green leaves that are up to 20cm in length and 4-8 cm broad. The leaves are pale green in colour and lobed. Leaves borne higher on the stem are smaller and narrower than the basal leaves. Leaves are borne alternatively on the stem. The stems and upper and lower leaves are covered in trichomes, including uniseriate macrohairs, uniseriate trichomes, monoiliform trichomes, capitate-sessile trichomes and capitate-stalked trichomes (Reinhardt *et al*., 2004). The stem is longitudinally grooved and the plant has a deep tap root system. Capitula are 3-5 mm in diameter and formed by many flower heads which are formed by five fertile ray florets and about 40 male disc florets and are white in colour. The first capitula are formed in the terminal leaf axil of the plant, after which the capitula are borne successively down the stem on lateral branches. Williams & Groves (1980) noted that temperature is a factor controlling the vegetative growth period before flowering and that no specific day-length was required for flowering. The cypsela has two sterile florets which adhere as 'wings'

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and is commonly termed as an achene. Achenes are 2-3 mm in length and 2 mm wide. The two sterile florets act as air sacs and assist with seed dispersal. The achene is flattened and narrows towards the base and is crowned by a pappus of orbicular scales. The seed is grey to black, flattened and spatulate in shape. Navie *et al*. (1998) reported that 73.7% of seeds remained viable after being buried for two years and estimated the half-life of the seeds to be about six years. Reports on seed dormancy have been contradictory but the germination of fresh seed has been observed. Although fresh parthenium seed has been noted to germinate immediately, the achene complex is known to contain germination inhibiting autotoxins (Picman & Picman, 1984; Reinhardt *et al*., 2004). Joshi (1991a) suggests that this imposed dormancy is removed through the natural course of weathering. Gupta & Chanda (1991) calculated that 9600 pollen grains per staminate flower were released and Lewis *et al*. (1988) observed that pollen is not transported over great distances but tends to remain airborne in substantial quantities around the plant source.

1.3.2 Distribution and habitat

From its natural occurrence in tropical America, parthenium has spread beyond its natural range in the Americas (Navie *et al*., 1996) and to many parts of the world, often becoming an invasive threat. Its spread has often been the result of the movement of military machinery and via contaminated produce and crop seed, and the plant has successfully become established in moderate and warm climates all over the world. Amongst others, *P. hysterophorus* has been reported in the following countries: South Africa, Bangladesh, Madagascar, Kenya, Mozambique, Ethiopia, Mauritius, Rodriguez, the Seychelles, Israel, India, Nepal, China, Vietnam, Taiwan, many South Pacific Islands, and India and Australia, where it may be having the greatest impact (Navie *et al*., 1996). In South Africa, although observed in the area formerly known as Natal as early as the 1880's, parthenium only became notorious in the 1980's (Henderson, undated), and its spread is believed to be linked to the cyclone Demoina which moved across the eastern coast of the country in 1986.

P. hysterophorus is quick to invade disturbed areas such as along roadsides and railways, cleared areas and croplands, and mismanaged rangelands. From these areas it often establishes a foothold for progressive, peripheral invasion, often at the

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expense of natural vegetation. McFadyen (1992) reported high incidence of the weed in areas that are regularly flooded, because grass cover is killed as a result of the submersion, leaving the weed with no competition. Parthenium is known to grow on a wide range of soil types and over a wide variety of different climates. Experiments and field observations conducted by Tamado *et al*. (2002b) suggested that the germination of *P. hysterophorus* was not affected by a variety of climatic conditions, although the seeds did have a high moisture requirement. Several cohorts of parthenium seedlings have been observed to emerge in a single growing season and plants can complete their life-cycle in a shorter period of time in less favourable conditions. An optimum day/night temperature regime of 33/22ºC for biomass production was determined by Williams & Groves (1980).

1.3.3 *P. hysterophorus* allelopathy

1.3.3.1 Allellochemistry

Broadly defined, allelopathy is the chemical interaction between plants. An *et al*. (1993) define the allelopathic characteristic of an allelochemical as the biological property of the allelochemical as opposed to its physical or chemical properties. In parthenium, phenolics and sesquiterpene lactones have been identified as the two major groups of allelochemicals.

Over 3000 sesquiterpene structures are known in nature (Harborne, 1999), and these structures are often associated with specialized secretary structures, such as glandular trichomes (Jordon-Thaden & Louda, 2003). Numerous sesquiterpene lactones have been isolated and identified in *P. hysterophorus*, including parthenin (Herz & Watanabe, 1959), coronopilin (Picman *et al*., 1980), damsin (Mabry, 1973), dihydroisoparthein and hysterin (Romo de Vivar *et al*., 1966), hymenin (Rodriguez, 1977), tetraneurin A (Picman & Towers, 1982) and others. Sequiterpene lactones that have thus far been discovered in nature have a wide variety of chemical structures, matched with a diversity of biological activities (Picman, 1986). Sesquiterpene lactones are known for their anti-inflammatory, analgesic, anticancer, cytotoxic, anti-malarial, anti-bacteria and anti-fungal properties (Picman, 1986; Lomniczi de Upton

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et al., 1999). Picman & Towers (1982) classified parthenium plants growing on several different continents in seven types according to sesquiterpene lactone content:

Type I	: Parthenin, coronopolin and tetraneurin A
Type II	: Parthenin, coronopolin
Type III	: Coronopolin
Type IV	: Hymenin, coronopolin and dihydrohymenin
Type V	: Hymenin, coronopolin and hysterin
Type VI	: Hymenin and hysterin
Type VII	: Hymenin

Plants growing in South Africa were classified by Picman & Towers (1982) into the 'parthenin race' – plants containing parthenin, coronopolin and tetraneurin A. Rodriguez (1977) suggested that differences in secondary metabolite content may be a response to different environmental factors. Lomniczi de Upton *et al*. (1999) observed that the nature of the secondary metabolites in plants growing at the same location do not differ, only the percentages of these secondary lactones differ. De la Feunte *et al*. (2000) found differences in sesquiterpene lactone chemistry according to habitat in Argentina and Lomniczi de Upton *et al.* (1999) noted correlations between sesquiterpene lactone content and altitude.

Of these sesquiterpene lactones, parthenin is reported to be the most important and biologically active compound. Parthenin has been implicated for its phytotoxicity on a vast range of target species, autotoxicity (Picman & Picman, 1984; Kumari & Kohli, 1987), allergic reactions such as allergic eczematous contact dermatitis (Lewis *et al*., 1991; McFadyen, 1995), and live-stock poisoning (Narasimhan *et al*., 1984). The allelopathic potential of parthenium leaf extracts as well as pure parthenin has been reported in abundance (Pandey, 1994, 1996; Batish *et al*., 1997, 2002a, 2002b; Datta & Saxena, 2001; Belz *et al*., 2006). Parthenin has been observed to be released through leaching as well as through the decomposition of plant residual matter. The overall contribution of parthenin to the allelopathy of *P. hysterophorus* is still vague. Working with parthenium leaf extracts and comparable concentrations of pure parthenin in germination bioassays, Belz *et al*. (2006) observed that pure parthenin

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contributed between 16 and 100% of the relative potency of leaf extracts, and was highly dependent on the concentration of parthenin within extracts.

As mentioned, phenolics also constitute an important role in *P. hysterophorus* allelopathy. Caffeic, vanillic, p-coumaric, chlorogenic and ferulic acids have been identified in the plant (Kanchan & Jayachandra, 1980b). Phytotoxic effects of these phenolics have been investigated in numerous cases (Kanchan & Jayachandra, 1980b; Patterson, 1981; Williams & Hoagland, 1982; Mersie & Singh, 1988). According to Blum *et al*. (1999), phenolics are the most potent inhibitors among the water-soluble allelochemicals and can also affect nutrient availability through interference with decomposition, mineralization and humification (Van Andel, 2005).

1.3.3.2 Allelopathic effects

The allelopathic potential of *P. hysterophorus* is believed to play an important role in the ability of the plant to displace natural vegetation and interrupt natural succession. An abundance of literature exists on investigations into the allelopathic effects of leachates from various plant parts, as well as for compounds isolated from *P. hysterophorus,* on a plethora of test species. Phytotoxic effects of leachates or pure compounds from *P. hysterophorus* have been observed on important crops such as *Cicer arietinum* (chickpea), *Raphanus sativus* (radish), *Triticum aestivum* (wheat), *Zea mays* (maize), *Glycine max* (soybean), *Phaseolus vulgaris* (bean), *Lycopersicon esculentum* (tomato) (Kanchan & Jayachandra, 1979; Mersie & Singh, 1987, 1988; Batish *et al*., 2002a); aquatic plants such as *Salvinia molesta* (salvinia) and *Eichhornia crassipes* (water hyacinth) (Pandey *et al*., 1993; Pandey, 1994), grass species such as *Cenchrus ciliaris* (buffel grass), *Eragrostis curvula* (weeping love grass), *Eragrostis tef* (tef) and *Echinochloa crus-galli* (Adkins & Sowerby, 1996, Belz *et al*., 2006) and many other species including weeds species.

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1.3.4 Importance of *P. hysterophorus*

1.3.4.1 Detrimental impacts

Impact on human health

The sesquiterpene lactone, parthenin, can cause allergic eczematous contact dermatitis in those who have continual contact with the weed, and hundreds of cases have been reported in India where it has been an epidemic (Subba Rao *et al*., 1977; Towers, 1981). Parthenium pollen has been observed to cause allergic rhinitis (hayfever) and allergic bronchitis (asthma) in humans (Navie *et al*., 1996).

Impact on rangelands and croplands

P. hysterophorus is a highly efficient interferer and can cause substantial yield losses. Yield losses of up to 40% were reported in India (Khosla & Sobti, 1981) and *P. hysterophorus* has been reported to negatively effect crop production in the Caribbean, Australia, Kenya, Ethiopia (up to 97% yield loss) (Tamado *et al*., 2002a), South Africa and most likely many other countries which it has invaded. Nath (1988) reported losses of forage production in grasslands by up to 90%. The weed is especially quick to infest mismanaged rangelands, and is particularly troublesome in Queensland, Australia, where by 1991 it was estimated to cover 170 000 km^2, which amounts to 10% of the entire state (Chippendale & Panetta, 1994). Due to the high seed production of *P. hysterophorus*, the marketing of produce such as grain can be adversely affected due to contamination risks.

Impact on livestock

P. hysterophorus can affect animal health and productivity and milk and meat quality. Although animals usually avoid the weed, it poses serious health hazards to the animals, and animals have been observed to eat vast quantities when dense stands do occur (Navie *et al*., 1996; Evans, 1997).

Impact on biodiversity

P. hysterophorus is notorious for its aggressive interference with other plant species and is often able to form pure, dense stands at the expense of the natural vegetation of the areas it has invaded. Total habitat alterations have been reported in grasslands,

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open woodlands, riverbanks and floodplains in Australia by McFadyen (1992) and Chippendale & Panetta (1994). Invasions of national wildlife parks in India (Evans, 1997) and South Africa pose a serious threat.

1.3.4.2 Beneficial attributes

South American Indians have been observed to use a boiled root decoction to cure dysentery (Uphof, 1959), and parthenin has been reported to be active against neuralgia and certain types of rheumatism (Dominguez & Sierra, 1970). It is applied externally on skin disorders and taken orally for a variety of ailments in the Carribean and central America, and even used as a flea-repellent for dogs and other animals in Jamaica (Dominguez & Sierra, 1970; Morton, 1981). The weed has also been reported as a good source of potash and oxalic acid, as well as a source of easily extractable protein for stockfeeds (Navie *et al*., 1996). Other promising properties of the sesquiterpene lactones, especially parthenin, such as anti-tumor activity, toxicity to insects, fungi and plants have high potential for future exploitation.

1.3.5 Control of *P. hysterophorus*

Attributes of high growth vigour, strong reproductive and regenerative potential, tolerance to many herbicides, and lack of effective bio-control agents makes the control of *P. hysterophorus* infestations very challenging. For these reasons, areas that are susceptible to *P. hysterophorus* infestation should receive special attention and management practices should focus on preventing the spread of *P. hysterophorus* as this is the most effective method of control. Furthermore, the tendency for *P. hysterophorus* to invade disturbed areas such as roadsides and old dumpsites often makes *P. hysterophorus* infestations uneconomical to control. The potential threat these infestations pose as propagule sources for further invasions should however not be underestimated. Preventive measures include: ensuring that *P. hysterophorus* seed is not introduced into an area via contaminated feed, pasture/crop seed, stock, machinery, vehicles or by any other means. Maintaining ‘healthy, robust, diverse, competitive’ pastures will increase resistance of the pastures to *P. hysterophorus* infestations (Parthenium Action Group, 2000). The land owner/manager must be aware of any isolated outbreaks and take immediate, suitable action before the

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situation worsens (Parthenium Action Group, 2000). Mechanical, chemical, and biological control methods are discussed below. The integrated use of these different practices often achieves the best results.

1.3.5.1 Mechanical control

Manual removal of *P. hysterophorus* is often not cost-effective and therefore used on a limited basis. Hand-pulling should ensure the removal of the entire crown to prevent regeneration from remaining lateral shoots. Protective clothing should be worn to prevent the possibility of allergic reaction (Gupta & Sharma, 1977). Slashing of *P. hysterophorus* is often not effective due to the plant's regenerative potential. Slashing may also stimulate denser branching and shorten the vegetative phase. Tillage, mowing or slashing should be performed before seed-set to reduce seedbank levels, since these practices can aid in the spread of achenes (Gupta & Sharma, 1977). Although burning has been successful in some instances, it is not generally accepted as a control practice as it may increase the vulnerability of the land to infestations by damaging native pastures, and because *P. hysterophorus* apparently does not burn well (Parthenium Action Group, 2000).

1.3.5.2 Chemical control

Selective herbicides can be used to control *P. hysterophorus* under most situations and several herbicides are registered for this purpose. As with mechanical control, chemical control of *P. hysterophorus* is often uneconomical in the short-term. Due to the high fecundity of *P. hysterophorus* newly emerged seedlings are often quick to appear after the successful control of mature plants. To a certain extent residual herbicides can solve this problem (Navie *et al*., 1996). Herbicides should be applied before seed set for most effective control and treated areas should be monitored for several seasons for any re-emergences. 2,4-D, picloram, dicamba, diuron, bromacil, karbutilate and atrazine (amongst many others) applied in high volume sprays can all be used for *P. hysterophorus* control (Navie *et al*., 1996). Parsons & Cuthbertson (1992) suggest spraying a mixture of atrazine and 2,4-D, with 2,4-D killing existing plants and atrazine having the residual activity to prevent re-emergences. Atrazine was recommended in Australia as the cheapest effective chemical for suitable long-

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term *P. hysterophorus* control, particularly along roadsides (Anon., 1978), while in India diquat, 2,4-D, linuron and bromacil provided quick and effective control (Gupta & Sharma, 1977).

1.3.5.3 Biological control

Abundant literature exists on the various natural enemies of *P. hysterophorus* that have been screened and/or introduced with varying degrees of success. Biological control would most likely offer the best and most effective solution to the *P. hysterophorus* weed problem (Haseler, 1976), but to date biological control of *P. hysterophorus* has only achieved limited control in Australia and India (McFadyen, 1992) and elsewhere in the world. Species that have successfully been introduced in Queensland, Australia include: *Zygogramma bicolorata*, a leaf-defoliating beetle; *Listronotus setosipennis*, a seed-feeding weevil; *Puccinia abrupta* var *partheniicola*, a winter rust; *Epiblema strenuana*, a stem-galling moth; *Conotrachelus* spp., a stem-galling weevil; *Platphalonidia mystica*, a stem-boring moth; *Carmenta nr ithacae*, a root-boring moth; and *Puccinia melampodii*, a summer rust (Parthenium Action Group, 2000). Many of the biological control agents' efficacy has been restrained by unsuitable climatic conditions. So far no immediate short term successes have been achieved in the biological control of *P. hysterophorus* and Evans (1997) describes the biological control programme in Australia as a 'costly failure'. The 'Parthenium Action Group' (2000) suggests the use of various biological control agents in combination for best results in reducing the competitive ability of *P. hysterophorus* and restoring the natural balance. Evans (1997) states that the long-term solution lies in releasing a number of agents that will attack as many plant organs as possible and so gradually reduce weed vigour over time.

In South Africa a parthenium biological control programme was started by the Agricultural Research Council Plant Protection Research Institute (ARC-PPRI) in 2003. A rust fungus, namely, *Puccinia melampodii* Dietel & Holw., and three insect species, namely *Zygogramma bicolorata* Pallister, *Epiblema strenuana* Walker and *Listronotus setosipennis* have been prioritised for the biocontrol programme. (Strathie *et al*., 2005).

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In addition to the use of arthropods and pathogenic micro-organisms, the use of antagonistic plants appears to be a plausible method of biological control. One such biological control plant which has been identified is *Cassia uniflora* Mill., which has been shown to suppress parthenium growth, reduce seed production and dissemination, and phenolic leachates from *C. uniflora* have been demonstrated to inhibit parthenium seed germination significantly and also reduce seedling vigour (Joshi, 1991b). Joshi observed *C. uniflora* replacing *P. hysterophorus* through a centrifugal mode of expansion and states that complete replacement can occur on a site within three to five years.

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CHAPTER II – INTERFERENCE POTENTIAL OF THE ALIEN INVADER PLANT *PARTHENIUM HYSTEROPHORUS* WITH THREE INDIGENOUS GRASS SPECIES IN THE KRUGER NATIONAL PARK

2.1 Introduction

From Central America, *Parthenium hysterophorus* has successfully invaded many parts of the world, often becoming a menace in disturbed areas, farmlands and natural biomes. Part of the ability of *P. hysterophorus* to successfully invade areas is attributed to its wide scope of ecological adaptation (Hedge & Patil, 1982), and different and challenging environments may lead to the expression of potentially beneficial genetic traits (Agrawal, 2001), some of which may promote invasiveness.

Parthenium competes strongly for soil moisture and nutrients and has been shown to be an efficient interferer with crop growth (Tamado *et al*., 2002a). Khosola & Sobti (1981) reported a yield decline of 40% for agricultural crops in India, and Nath (1988) reported that the weed can reduce forage production in grasslands by up to 90%. Parthenium has been observed to cause substantial yield loss in *Helianthus annuus* L. (sunflower) and *Sorghum bicolour* (sorghum) in Queensland, Australia (Parsons & Cuthbertson, 1992), in sorghum (Tamado *et al*., 2002a) and *Eragrostis tef* (tef) (Tefera, 2002) in Ethiopia, and is reported to be one of the most important weeds in *Coffea arabica* (coffee) in Kenya (Njoroge, 1986). In South Africa, *P. hysterophorus* is a 'major nuisance' in *Saccharum* spp. (sugarcane) and *Musa* spp. (banana) orchards (Bromilow, 2001). *P. hysterophorus* is a highly prolific seed producer right up to senescence and one plant is reported to potentially produce between 15 000 and 25 000 seeds (Haseler, 1976; Joshi, 1991b). *P. hysterophorus* seeds are capable of germination as soon as they have been released from the parent plant, although 'seeds may be induced into a state of conditional physiological dormancy by the ambient environmental conditions' (Navie *et al*. 1996). In India, Pandey & Dubey (1989) observed *P. hysterophorus* seedlings in three successive cohorts in a single season, with seedling density and survival to maturity declining with successive cohorts.

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It is widely believed that allelopathy also plays an important role in the invasiveness of *P. hysterophorus*. Allelochemicals have been identified in all *P. hysterophorus* plant parts and several sesquiterpene lactones and phenolics have been identified and implicated as the principal allelochemicals in *P. hysterophorus* (Picman & Picman, 1984; Swaminathan *et al*., 1990; Reinhardt *et al*., 2004). Release of these allelochemicals from the plant into the environment can be achieved through leaching from above- or below-ground plant parts or through the decomposition of plant residues. *P. hysterophorus* potentially uses all these mechanisms to release allelochemicals into the environment. Ridenour & Callaway (2001) point out that root mediated allelopathy would depend on factors such as plant densities, root distributions, root densities and microbial activity; and that the mobility of compounds in the soil may be less due to buffering or immobilization. Phenolics can interfere with plant growth directly by interfering with metabolic processes, affecting root symbionts, and by affecting site quality through interference with decomposition, mineralization and humification (Van Andel, 2005). In grasses, *P. hysterophorus* extracts have been demonstrated to be phytotoxic to *Eragrostis tef* (Tefera, 2002; Belz *et al*. 2006), and pure parthenin was phytotoxic to *E. curvula* and *Echinochloa crus-galli* (Belz *et al*., 2006).

Few studies have been conducted regarding the interference of *P. hysterophorus* with other plant species. Joshi (1991b) studied the interference effects of *Cassia uniflora* on *P. hysterophorus* and found that *C. uniflora* seedlings could suppress *P. hysterophorus* weed seedlings. *C. uniflora* is a short-lived shrub believed to also have allelopathic potential. Joshi (1991b) further observed that *P. hysterophorus* height dropped from 1.75 m to 0.9 m when exposed to interference from *C. uniflora*. A reduction in plant dry mass and number of inflorescences produced was also noticed when compared to a nearby stand of pure *P. hysterophorus*. Five years following the introduction to a site infested with *P. hysterophorus*, Joshi (1991b) reported an 84% reduction in the population of mature *P. hysterophorus* plants.

Since the first appearance of *P. hysterophorus* in southern Africa, it has spread at a steady, alarming rate and occurs in the warmer regions of South Africa, Zimbabwe, Mozambique and Swaziland (Henderson, undated; Bromilow, 2001). In the Kruger National Park, it is possible that at least one of the introductions of *P. hysterophorus*

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occurred when propagules entered the reserve via service vehicles. Another source of infestation is the former dumpsite adjacent to Skukuza rest camp in the reserve – the site chosen for the field trial reported on in this chapter.

Interactions between plants are often the result of complex combinations of specific mechanisms (Welden & Slauson, 1986; Callaway *et al*., 1991; Chapin *et al*., 1994), and although the fundamentals of competition and allelopathy are generally understood as isolated mechanisms, less is known about the relative contribution of these two mechanisms in overall interference interactions between plant species (Ridenour & Callaway, 2001). Ecologists have identified the importance of defining the individual effects more precisely (Ridenour & Callaway, 2001), but difficulty in separating the effects experimentally has hampered better understanding (Fuerst & Putnam, 1983). The objectives of the current study were to investigate the interference of *P. hysterophorus* with three indigenous grass species under naturally occurring conditions. Keeping the grass density constant while varying the *P. hysterophorus* density may help to assess the importance of the weed's density on plant interactions. The use of three different grass species serves to screen for one or more species that can adequately interfere with *P. hysterophorus* growth, and potentially be used as an antagonistic species in an integrated control programme.

2.2 Materials and methods

2.2.1 2003/2004 growing season

A field trial was established on an old dumpsite which has been invaded by *P. hysterophorus* near Skukuza in the Kruger National Park (Lat: -24.9800 Lon: 31.6000 Height 263 m). The dumping of general refuse at the site had ceased around twelve years earlier, since when the site was used for the dumping of garden refuse only until the commencement of the trial when this too was stopped. The trial site was cleared of vegetation and debris and a total of 36 plots, each measuring 4 m^2, were demarcated in a completely randomized design.

Following failure to establish the grasses *in situ* from seed in December 2003, *E. curvula*, *P. maximum* and *D. eriantha* seedlings were raised in seedling trays in the

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University of Pretoria glasshouses. Several seeds were sown into each tray cell to form a tuft consisting of several seedlings. Once the seedlings had attained a height of between four and seven centimetres, each species was transplanted into field plots at equal densities (16 tufts m^{-2}). Tufts were planted across from each other along dripper lines that spanned across the plots at 500 mm intervals. All three of the grasses chosen for the trial are indigenous to South Africa. Unless indicated otherwise, the following descriptions of the grasses are taken from the 'KYNOCH PASTURE HANDBOOK' (2004):

Eragrostis curvula (Weeping love grass): A tufted highly variable species which is a summer growing, perennial grass. Stem length varies from 600 mm to 1200 mm, and stems can be either slender or robust, growing upright or sideways. Leaves can be as long as 600 mm and 10 mm wide. Grass often droops (weeps) when it gets older. The inflorescence is an open panicle with many spikelets capable of bearing many seeds. It is the most cultivated grass on dryland in South Africa, preferring sandy soil and growing best in areas receiving more than 650 mm rainfall per annum. The growing season for *E. curvula* is from September to April. *E. curvula* is often observed in disturbed areas, especially on well drained, fertile soils and has been used for erosion control (Gibbs Russel *et al*., 1991; Van Oudtshoorn, 2002).

Panicum maximum (Guinea grass): A tufted, perennial grass which reaches a height of 1000 to 2000 mm. The grass has slender stems and is particularly leafy, with broad, highly palatable leaves. *P. maximum* prefers damp places with fertile soils (Van Oudtshoorn, 2002), often occurring under trees and in shrubs and bushes. The grass is well adapted to a variety of soil types but does not perform well on very sandy soils or on heavily structured soils. It can withstand frost, does well with a minimum of 500 mm rainfall and is suited to tropical and sub-tropical areas. Guinea grass forms a high density of roots in the upper soil layers, which may explain its quick reaction to even the lightest rains.

Digitaria eriantha (Smuts finger grass): A tufted, perennial grass with branched stalks which can attain a height of up to 1200 mm. Six to ten finger-shaped clusters of 70-130 mm long are developed on the inflorescence. The base of the leaf sheaf is hairy while the leaf blades are almost hairless. Leaves grow to about 600 mm long and 13 mm wide. The grass grows in a variety of conditions and thrives in areas with a rainfall higher than 500 mm per annum. It can be established on an extensive scale and has proved itself on a large number of low and medium potential soils. *D.*

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eriantha has been used to improve conditions by direct sowing into the veld (Van Oudtshoorn, 2002).

P. hysterophorus seedlings growing in the immediate vicinity of the field trial were transplanted into the plots at 5 or 7.5 plants m^{-2} densities. *P. hysterophorus* plants were planted between grass tufts along the dripper lines for the 5 plants m^{-2} density, and additional plants were planted in rows between the dripper lines for the 7.5 plants m^{-2} density. Plots with zero *P. hysterophorus* served as control. The trial was fully established on 18 January 2004 (Figure 2.1). A wire fence was erected around the perimeter of the trial to prevent interference from any wild animals, such as grazers, in the experiment. A gravitational drip-irrigation system was installed in an attempt to reduce any negative impacts of the unreliable rainfall characteristic for the area.

Figure 2.1 Trial site on day of establishment in 2003/2004 growth season

After eleven weeks (7 April) eight representative grass tufts were harvested from each plot and the dry mass determined. Final harvesting for the 2003/2004 season took place after eighteen weeks (27 May) when eight previously unharvested grass tufts were harvested from each plot, and six representative *P. hysterophorus* plants were harvested from the plots containing the weed. Harvesting of the re-growth from the first set of harvested tufts occurred after fifteen weeks (8 May) and again after another eighteen weeks (27 May). At the final harvest any plants that were not harvested for

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dry mass determination were cut down to ground level. Data were expressed on a per plant dry mass basis for the grasses and *P. hysterophorus*. As grass data were not normally distributed, the logarithm of grass dry mass accumulation expressed as percentage of control was analyzed using SAS®. *P. hysterophorus* dry mass accumulation was analyzed without transformation. A general linear model (GLM) of ANOVA was used and least significance differences (LSD) at $P \leq 0.05$ was used to separate means when significant differences did occur.

2.2.2 2004/2005 growing season

The field trial was re-established for a second growth season on 22 February 2005. The trial plan was modified to include parthenium controls plots containing only parthenium plants at 5 and 7.5 plants m^{-2} densities. Some of the *E. curvula* and *D. eriantha* plots on which some of the plants died naturally were converted for this purpose. Several of the grass tufts removed from these plots where used to replace grass tufts on other plots of the same species where mortalities had occurred. The only grass species not requiring replacement of plants that died was *P. maximum*. Parthenium plants had to be re-established by transplanting seedlings from outside the fenced area into the plots. Only one harvest took place during the 2005 growth season, 14 weeks after planting (30 May). The fresh mass of samples was measured in the field and representative samples from each species were oven-dried at 60ºC and weighed in order to determine the moisture percentage, enabling fresh mass to dry mass data conversion for all the samples. For grass dry mass accumulation, percentages of control were logarithmically transformed (as distribution was not normal) and analyzed using SAS®. Parthenium dry mass accumulation data were analyzed without transformation. A general linear model (GLM) of ANOVA was used and least significance differences (LSD) at $P \leq 0.05$ was used to detect significant differences between treatment means.

2.3 Results and discussion

2.3.1 2003/2004 growth season

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2.3.1.1 First harvest (7 April 2004)

From the first harvest it was clear that *P. maximum* performed the most favourably, with *E. curvula* and *D. eriantha* both growing very poorly (Figure 2.2). Although the latter two grass species did manage to become established, their relatively slow growth rates showed that these species were not adapted to the local environmental conditions. It is known that pH preferences for *E. curvula* are in the region of 5.4 (H_2O) [measured at 4.4 (KCl)], and 5.5 (H_2O) [measured at 4.5 (KCl)] for *D. eriantha* (Kynoch Pasture Handbook, 2004). The mean pH of two soil samples taken from the trial site in March 2004 was 7.7 (H_2O) (see Appendix for complete soil analysis results), suggesting that the soil was too alkaline for favourable growth of these two species. *P. maximum* was more suited for this alkaline soil with a pH preference of 5.5 – 7.5 (H_2O) [measured at 4.5 – 6.5 (KCl)] (Kynoch Pasture Handbook, 2004), thus reaffirming the importance of pH in grass performance. High temperatures and other environmental factors in Skukuza may also have influenced grass performance. Although an irrigation system was utilized during the growing season, *P. maximum* is known to tolerate a wider range of moisture regimes than the other two grass species (Agricol Product Guide, undated. Agricol, Eagle Street, Brackenfell).

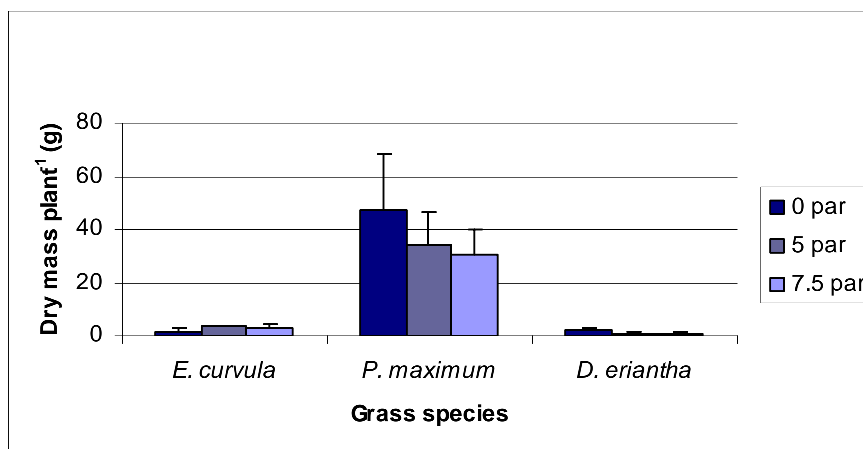

Figure 2.2 Grass dry mass accumulation over a period of 11 weeks on plots with 0, 5 or 7.5 parthenium plants m^{-2}

For percentage of control data, only the main species effect was significant, with *E. curvula* performing significantly better than *P. maximum* and *D. eriantha* in the presence of *P. hysterophorus* (Table 2.1). No significant differences between *P.*

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maximum and *D. eriantha* occurred. However, the poor performance and extremely low growth rate of *E. curvula* and *D. eriantha* makes these results of limited practical relevance. Perhaps one reason for the increased growth rate of *E. curvula* on treatment plots could be the uptake of low levels of allelochemicals from *P. hysterophorus* plants resulting in growth stimulation as observed under controlled conditions for all three grass species (see CHAPTER V – 5.3) and for *E. curvula* as observed by Belz *et al*. (2006).

Table 2.1 Grass dry mass accumulation over a period of 11 weeks expressed as percentage of control (Appendix 2.1)

	Grass species Dry mass percentage of control [%]		
P. hysterophorus density	*E. curvula*	*P. maximum*	*D. eriantha*
5 plants m^{-2}	211.4	72.7	55.7
7.5 plants m^{-2}	162.2	64.2	46.6
Mean	187.1a	68.4b	51.1b
LSDspp= 61.499			

Means followed by different letters differ significantly (LSD t –test, P=0.05)

2.3.1.2 Grass re-growth harvest (8 & 27 May 2004)

Although at this stage conditions were beginning to become less favourable for plant growth, *P. maximum* still had a much higher growth rate than the other two grass species; confirming that *P. maximum* has the best inherent adaptation for the site conditions (Figure 2.3). No significant differences for the main or interaction effects were observed for percentage of control dry mass data for the first re-growth harvest (Appendix 2.2).

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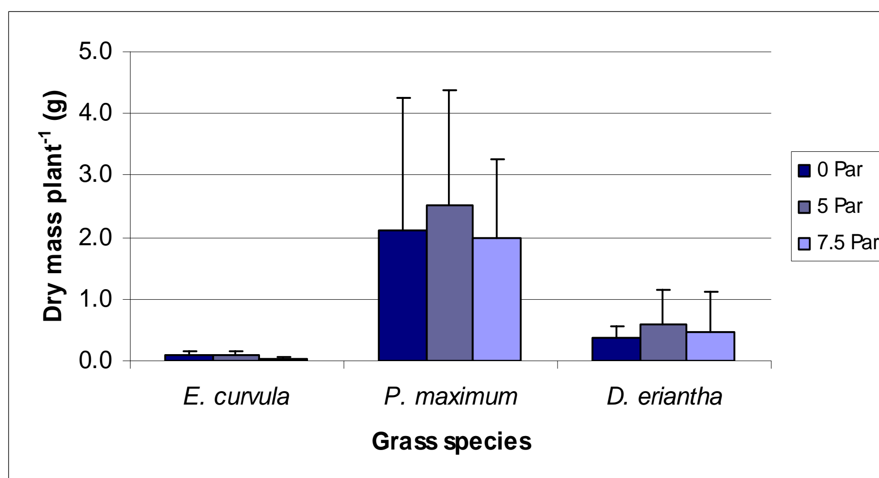

Figure 2.3 Grass re-growth dry mass accumulation over a period of 4 weeks on plots with 0, 5 or 7.5 parthenium plants m^{-2}

Grass re-growth was harvested for a second time three weeks later. By this time growth of *E. curvula* and *D. eriantha* had ceased almost completely on all plots. *P. maximum,* however, continued to grow. Mean percentage of control values for *P. maximum* showed a lower dry mass accumulation yield on plots with the higher parthenium density. Results were not significantly different however.

2.3.1.3 Final harvest (27 May 2004)

Grass data

Once again, harvesting of previously unharvested grass tufts which were allowed to grow for the entire duration of the field trial's growing season and determination of the dry mass accumulation of these plants showed very similar trends to previous data, with *P. maximum* performing by far the best of the three grass species (Figure 2.4).

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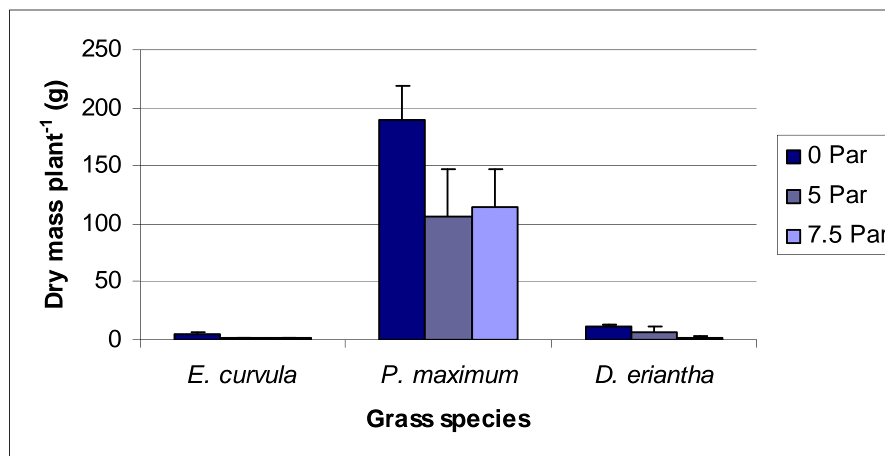

Figure 2.4 Grass dry mass accumulation over a period of 19 weeks on plots with 0, 5 or 7.5 parthenium plants m^{-2}

Analysis of dry mass accumulation expressed as percentage of control revealed that none of the main effects (species or parthenium density) were significant. At the P<0.075 significance level, however, the interaction effect was found to be significant (Table 2.2). Significant growth differences between the two parthenium densities were only observed for *D. eriantha*.

Table 2.2 Grass dry mass accumulation over a period of 19 weeks expressed as percentage of control (Appendix 2.3)

	Grass species Dry mass percentage of control [%]		
P. hysterophorus density	*E. curvula*	*P. maximum*	*D. eriantha*
5 plants/ m^2	28.8ab	56.4a	58a
7.5 plants/ m^2	37.7ab	60.2a	17.8b
LSD spp*par = 36.555			

Means followed by different letters differ significantly (LSD t –test, P=0.075)

Parthenium data

Per plant dry mass data for six representative parthenium plants indicated that only the main species effect was significant (Table 2.3). *P. maximum* was the most effective grass species regarding intereference with parthenium growth and

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significantly reduced the dry mass accumulation of the weed. Parthenium plants were further observed to be shorter and produced less seed relative to parthenium plants growing together with *E. curvula* and *D. eriantha*. *E. curvula* and *D. eriantha* did not perform well enough under the trial conditions to interfere significantly with parthenium growth. On *E. curvula* and *D. eriantha* plots parthenium yield on a per plant basis was higher on the plots with the lower parthenium density (5 plants m^{-2}) than on the plots with the higher weed density (7.5 plants m^{-2}), while the opposite occurred on *P. maximum* plots. Since *P. maximum* performed relatively better than the other two grass species it can be speculated that on the *E. curvula* and *D. eriantha* plots intra-species (parthenium-parthenium) interference dominated, while on the *P. maximum* plots inter-species (*P. maximum* – parthenium) interference was dominant.

Table 2.3 Parthenium dry mass accumulation over a period of 19 weeks (Appendix 2.4)

	Mean per plant parthenium dry mass (g)		
Grass species	5 plants m^{-2}	7.5 plants m^{-2}	Mean
E. curvula	62.9	46.5	54.7a
D. eriantha	49.6	43.9	46.8a
P. maximum	16.8	24.1	20.5b
LSDspp = 13.173			

Means followed by different letters differ significantly (LSD t –test, P=0.05)

1.3.2 2004/2005 growing season

Grass data

Similar to the previous season, *P. maximum* far outperformed the other two grass species in terms of growth (Figure 2.5), reaffirming that *P. maximum* is best suited to the environmental conditions of the trial site. *D. eriantha*, and to a lesser extent *E. curvula,* showed a noteworthy increase in growth rate for the 2004/2005 season, with aboveground dry mass increases on control plots of 406.8% and 233%, respectively from the 2003/2004 season. It can therefore be concluded that these species eventually became better adapted to the environmental conditions. In contrast, *P. maximum* showed a 26.8% reduction in dry mass accumulation from the 2003/2004 to

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the 2004/2005 growth season. This may be attributed to a shorter growth season and/or less favourable environmental conditions.

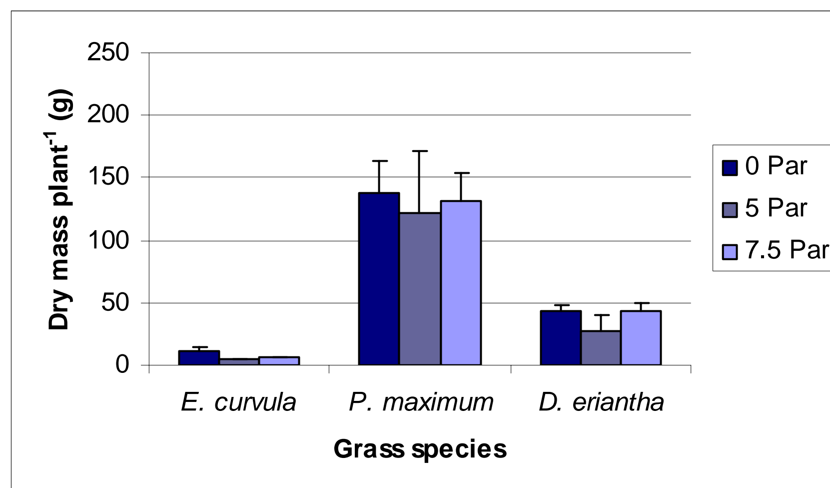

Figure 2.5 Grass dry mass accumulation over a period of 14 weeks on plots with 0, 5 and 7.5 parthenium plants m^{-2}

For percentage of control data, no significant differences were observed for the interaction effect. The main species effect was found to be significant ($P \leq 0.05$), however. Across the two parthenium densities, *P. maximum* was found to perform significantly better than *E. curvula* (Table 2.4).

Table 2.4 Grass dry mass accumulation over a period of 14 weeks expressed as percentage of control (Appendix 2.5)

	Grass species Dry mass percentage of control [%]		
Parthenium density	*E. curvula*	*P. maximum*	*D. eriantha*
5 plants m^{-2}	41.8	88.1	63.5
7.5 plants m^{-2}	59.6	95.3	98.5
Mean	50.7a	91.7b	81.0ab
LSDspp = 32.262			

Means followed by different letters differ significantly (LSD t –test, P=0.05)

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Parthenium data

As expected, *P. maximum* again proved to be most effective in interfering with parthenium growth, with lowest parthenium yields occurring on *P. maximum* plots. Once again parthenium plants were observed to be smaller and produce less seed compared to plants growing on plots without *P. maximum*, and a large number of parthenium mortalities were observed on *P. maximum* plots. Analysis of parthenium dry mass data revealed that the interaction effect was highly significant (Table 2.5). *D. eriantha,* and to a lesser extent *E. curvula,* were only able to significantly interfere with parthenium growth at the 5 plants m^{-2} density. Parthenium per plant dry mass yield was observed to be higher at the lower weed density (5 plants m^{-2}) on all plots except on *P. maximum* plots. A similar trend was observed in the previous growth season (see 2.3.1.3).

Table 2.5 Parthenium dry mass accumulation over a period of 19 weeks (Appendix 2.6)

	Plant species Dry mass accumulation (g $plant^{-1}$)			
Parthenium density	*P. hysterophorus*	*E. curvula*	*P. maximum*	*D.eriantha*
5 plants m^{-2}	32.3a	22.0b	0.23e	9.7cd
7.5 plants m^{-2}	14.5bc	12.2c	3.2de	7.2cde
LSDspp*par = 8.1024				

Means followed by different letters differ significantly (LSD t –test, P=0.05)

Buckley *et al.* (2004) mention that ‘for successful [invasive plant] control, it may be necessary to change disturbance regimes or the succession trajectory of the community by creating favourable establishment opportunities for native competitors and unfavourable opportunities for weed regeneration’. It is important to mention that antagonistic species should be selected according to environment compatibility in addition to interference potential with the invader plant.

Significant differences for grass dry mass accumulation between the 5 and 7.5 parthenium plants m^{-2} were not always observed. No general statements can therefore

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be made on the effect of parthenium density. Cousens (1991) points out, that at low weed densities no significant differences most likely mean that the differences are too small to be detected because of variability. In hindsight it was observed that the selected weed densities employed in the field experiment were low relative to parthenium densities of as many as 96 mature plants m^{-2} which have since become established in the area. Relatively higher yields for both grasses and parthenium plants growing on the same plot in certain cases (= replications) may indicate that some plots within the trial were more favourable for plant growth and this may have contributed to experimental error, and hence, have made significant differences less detectable.

As *P. maximum* is a highly palatable grass, under natural conditions we can most likely expect a high grazing pressure on the grass which may influence its interference potential with parthenium. *P. maximum* is known not to tolerate intensive, frequent grazing (Fair, 1989). To the best of our knowledge, parthenium is not eaten by any herbivores. In the first growth season, all species were transferred into the trial as seedlings. It is not certain how the grasses would have performed in this parthenium infested area if seedlings had to develop from seed sown *in situ*. Allelochemicals from parthenium have been observed to inhibit germination and to stunt seedling growth of a wide variety of species. This must be considered and further investigated if the use of an antagonistic species in a biological control programme is considered.

2.4 Conclusions

P. maximum dominated with regard to overall performance in terms of dry mass accumulation as well as with suppression of parthenium growth. *D. eriantha* performed better than *E. curvula* but both of these species preformed poorly in comparison with *P. maximum*. The better performance of *P. maximum* is attributed to better adaptation to the environment conditions, probably especially due to soil pH and soil texture. *E. curvula* and *D. eriantha* performed better in the second growth season, indicating better adaptation to the environmental conditions after a longer establishment period. The suppression of parthenium growth, and even parthenium seedling mortality on *P. maximum* plots, together with good seed production by the grass when co-existing with parthenium, indicate that this species shows high

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potential for use as an antagonistic species in a biological weed control programme. There is also the possibility that *P. maximum* has an allelopathic effect on parthenium. Further research is required to progress our understanding of the interference mechanisms between parthenium and *P. maximum*.

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CHAPTER III – PRODUCTION DYNAMICS OF PARTHENIN IN THE LEAVES OF *PARTHENIUM HYSTEROPHORUS*

3.1 Introduction

Parthenin, a sequiterpene lactone, is believed to play a major role in the allelopathy of *P. hysterophorus*, and it may play a role in the displacement of naturally occurring vegetation for the weed to become established in an area. On the molecular level, sesquiterpene lactone biosynthesis is regulated at the transcriptional level, and these compounds generally originate from the mevalonic acid pathway (Duke & Inderjit, 2003). It has been suggested that all terpenes originate from the common precursor, isopentenyl diphosphate (Fonseca *et al*., 2005). In addition to its phytotoxic properties, parthenin is also known for its allergenic, anti-feedant and anti-microbial properties. Parthenin has been reported to be located in various plant parts with especially high concentrations occurring in trichomes on the leaves (Kanchan, 1975; Towers *et al*., 1992; McFadyen, 1995, Reinhardt *et al*., 2004). Four types of glandular and non-glandular trichomes occurring on the leaves and achene-complex were described by Rodriguez *et al*. (1975) who identified parthenin and ambrosin in external chloroform washings of flowers and leaves. Reinhardt *et al*. (2004) determined that one trichome type in particular, the capitate-sessile trichome, contained virtually 100% parthenin. Reinhardt *et al*. (2004) further quantified the amount of parthenin present in one capitate-sessile gland at 0.3 µg parthenin per gland and suggested that these trichomes are the main source of parthenin that is released from the plant. Futhermore, they proposed that extrapolation of per plant parthenin amounts to field-scale production makes it plausible that parthenin can contribute significantly to the ability of *P. hysterophorus* to displace other species.

Allelochemical production in living plants is apparently affected by biotic and abiotic factors (Dakshini *et al*., 1999), which in turn affect a plant's allelopathic potential (Hedin, 1990; Lovett & Hoult, 1995; Einhellig, 1995). Periodic peaks in allelochemical production have been reported, especially in response to biotic factors (Woodhead, 1981; Baldwin, 1989). The production of secondary metabolites is determined by a plant's genetic make-up in combination with environmental factors

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(An *et al*., 2003). Stressful environmental conditions, such as abnormal radiation, mineral deficiencies, water deficits, temperature extremes, and pathogen/predator attack can induce increased allelochemical production in plants (An *et al*., 2003). This can be beneficial in several ways. Phenolics are considered to protect plants from UV radiation (McClure, 1975), and allelochemicals may advantage the producer under stressful conditions that result in resource competition (Kuo *et al*., 1989), plus these compounds can protect against pathogens and herbivores (Picman *et al*., 1981; Datta & Saxena, 2001). The decrease with age of allelochemical concentrations in living plants has been well documented in several instances (An *et al*., 2003), but there are exceptions (Woodhead, 1981). Chou (1999) suggested that allelochemicals possibly perform an autotoxic role in order to regulate population levels according to growth conditions and resource availability.

An obvious advantage for attaining and maintaining dominance in a plant community would be sustained production of allelochemicals at high levels throughout the life cycle of a plant. Increased production towards the end of a life cycle could point to a strategy of reliance on allelopathic residues for suppressing the germination and establishment of other, or even the same, species. Considering the location of parthenin in *P. hysterophorus* (Reinhardt *et al*., 2004) it is most likely that parthenin is released either through leaching off leaves and/or in the process of leaf decomposition. The combined process of parthenin production, release mechanism(s), and its persistence in the environment will determine its own contribution to the overall allelopathic effect of *P. hysterophorus*. However, growth responses of acceptor plants will not be determined only by the parthenin effect, but also by that of other allelochemicals produced and released by *P. hysterophorus*. The relative contribution of the various allelochemicals associated with P. hysterophorus to its allelopathic influence is still not fully understood (Belz *et al*., 2006), but clearly there is much evidence to suggest that parthenin plays a major role.

Little is known about the production and release of parthenin during the growth stages of *P. hysterophorus*. Earlier, Belz *et al*. (2006) observed variability in the amounts of parthenin extracted from the leaves of the same plants harvested at different stages, and speculated that these differences may be age-dependent. The aim of the current study was to investigate parthenin production dynamics by determining parthenin

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concentrations in parthenium leaf material at different phenological stages of the plant. This will contribute to further illumination of the role of parthenin in *P. hysterophorus* allelopathy.

3.2 Materials and methods

3.2.1 Cultivation and harvesting of *P. hysterophorus* plants

P. hysterophorus plants were cultivated under greenhouse conditions (13/11 h, 22/18 ºC, 300 µE/m^2s) at the University of Hohenheim. Plants were grown from seed collected at an infested site in Kruger National Park, South Africa. Seeds were pre-germinated in vermiculite and 25 days later seedlings were transplanted into pots (15 x 15 x 20 cm) filled with a 1:3 (v/v) mixture of humus soil (Humusoil, Floragard, Germany) and sand as growth medium. Watering was done as required with tap water and fertilizer was applied once weekly [1 ml L^{-1} Wuxal® Super (Fa. Aglukon Spezialdünger, Germany)]. Plants were harvested at different phenological stages from the 4-leaf stage until senescence. Parameters measured at each harvesting included: total number of leaves, fresh and dry mass of leaves, fresh mass of entire plant, and plant height (from base to tip of uppermost leaf). Fresh leaf material was frozen at -20ºC immediately after harvesting for chemical analysis of parthenin.

3.2.2 Chemical analysis

3.2.2.1 Sample preparation

Frozen samples were defrosted and diced into sections of 1 cm^2. As the moisture percentage of leaf material harvested at different stages would vary, a portion of the leaf material was used to measure the dry weight and determine moisture percentage of the sample. Depending on the amount of leaf material available for analysis, 0.4 - 12 g of leaf fresh weight was analyzed per replicate. A mixture of acetonitrile:water [1:1 (v/v); ACN:H_20] was added to the leaf material at a concentration of 0.1 g ml^{-1}. The chopped leaf material together with ACN:H_20 was homogenized for three minutes at 20 000 rpm with an Ultra Turrax blender (Janke & Kunkel Ltd.,

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Germany). The homogenate was filtered, centrifuged (10 min; 20 000 rpm), and a 1 ml aliquot was transferred to a glass vial for chemical analysis.

3.2.2.2 Preparation of pure parthenin standard

Preparative high-performance liquid chromatography (HPLC) was used to obtain parthenin as HPLC standards [as described by Belz *et al*., (2006)]. Fresh leaf material from *P. hysterophorus* plants was dipped for ten seconds in tert-butyl methyl ether (250 mg FM ml^{-1} TBME). Organic leaf extracts were filtered over anhydrous sodium sulphate (Na_2SO_4) and the extract concentrated with a rotary evaporator (40ºC, 250 mbar). The oily, green residue obtained was re-dissolved in 1:1 (v/v) ACN:H_2O and fractionated by preparative HPLC (Varian model chromatograph) with UV detection (Varian UV-VIS detector model 345; detection wavelength 225/254 nm). A Grom Nucleosil 120 C-4 column [250 mm by 16 mm (5 µm), Grom, Germany] was used, and eluted with a gradient of 20% ACN and 80% Na_2HPO_4-buffer (1 mM, pH 3, 10% ACN) for 0-20 min, 100% ACN for 20-26 min, then re-equilibrated to starting conditions (6 ml min^{-1} flow rate). Injection volume was 100 µl. Parthenin was identified in the fraction ranging from 9.1 – 10.3 minutes. Standard purity was verified by HPLC-DAD and results confirmed by HPLC-ESI-MS.

3.2.2.3 Quantification of leaf parthenin content

HPLC analysis (Waters model chromatograph) with DAD detection (photodiode array detector, Waters 991) for determination of parthenin in leaves was done according to Belz *et al*. (2005). A Synergi polar C-18 reversed phase column [250 mm by 4.6 mm (4 µm), Phenomenex, Germany; 35°C column oven temperature] was used, and eluted with a gradient of 5% ACN and 95% Na_2HPO_4-buffer (1 mM, pH 2.4, 10% ACN) for 0-8 min (0.65 ml min^{-1} flow rate), 30% ACN and 70% Na_2HPO_4-buffer for 8-26 min (0.7 ml min^{-1} flow rate), 100% ACN for 26-29 min (0.7 ml min^{-1} flow rate), 100% ACN for 29-31 min (0.7 ml min^{-1} flow rate), then re-equilibrated to starting conditions. Injection volume was 50 µl. Parthenin was identified and quantified at 220 nm. Retention time was 26.07 ± 0.02 min. Quantitative analysis was done by external calibration curves.

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3.2.2.4 Calculation of parthenin concentration

A clear peak was observed on the HPLC chromatogram at 17.25 min and was identified as parthenin. Pure parthenin standards with known concentrations were used to obtain a parthenin concentration versus peak area calibration line (Figure 3.1). Parthenin concentration in the samples could then be calculated using the computer generated equation for this line.

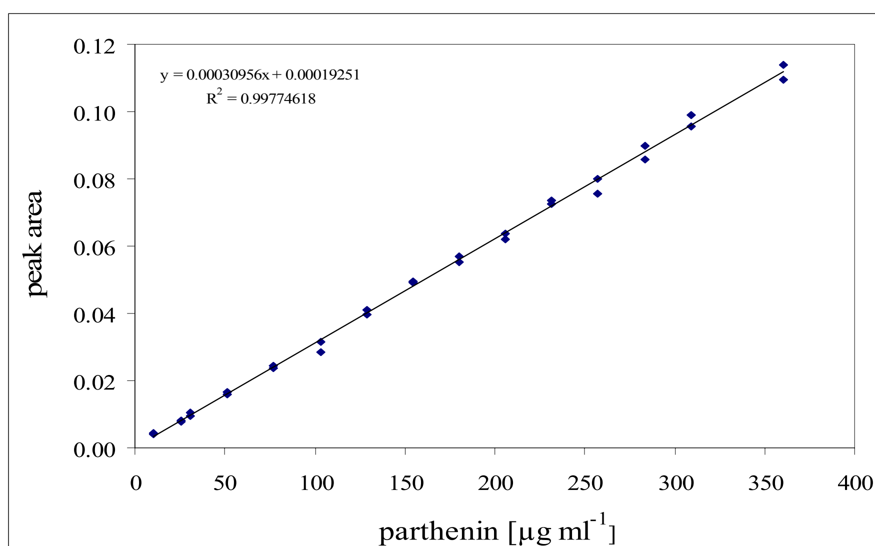

Figure 3.1 Parthenin concentration versus peak area calibration line

Parthenin concentration of the leaf extracts was calculated using the following equation:

$$\frac{x\ \mu g/ml\ ([Sample])\ x\ \ z\ ml\ (ml\ of\ extract)}{g\ (initial\ weight)} = \mu g\ parthenin/g\ initial\ weight$$

3.2.3 Statistical analysis

Parthenin concentrations in extracts prepared from leaves of plants harvested at the same growth stages were analyzed using SAS® to detect significant differences. Data were analyzed after a logarithm transformation to achieve normal distribution of the data. A general linear model (GLM) of ANOVA was used and significant differences between means were determined using Tukey's studentised range test at $P \leq 0.05$.

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3.3 Results and discussion

Mean leaf moisture percentage was observed to decrease with plant age (Table 3.1).

Table 3.1 Mean leaf moisture percentages at different growth stages

Growth stage	Mean water content [% of FM]
10-51	86.1 ± 4.2
41-60	81.9 ± 4.6
70	64.8 ± 7.6
80	20.3 ± 0

Growth stages: 10-51: Beginning of leaf development to flowering in all leaf axils; 41-60: Flower buds formed in all axils to fruit development; 70: Ripening/maturity of fruit and seeds; 80: Senescence.

An increase in parthenin concentration with plant age was observed (Figure 3.2), with highest levels occurring in the final three growth stages for both fresh and dry mass, as well as for overall parthenin content in all leaf material.

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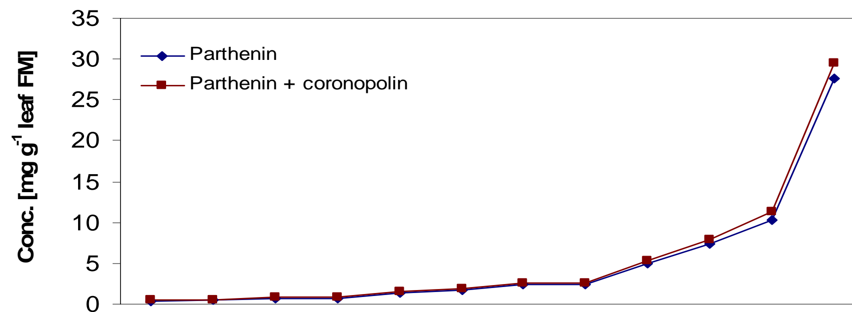
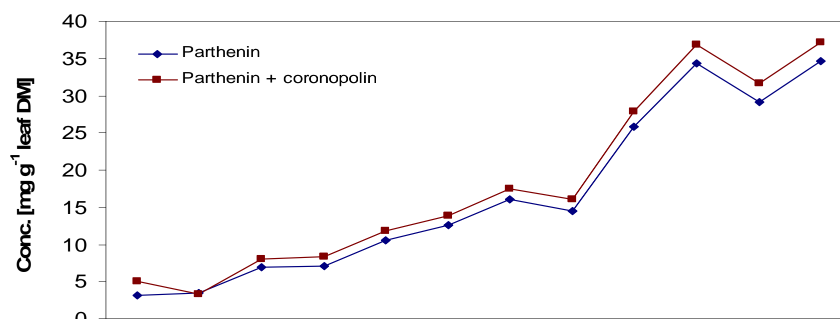
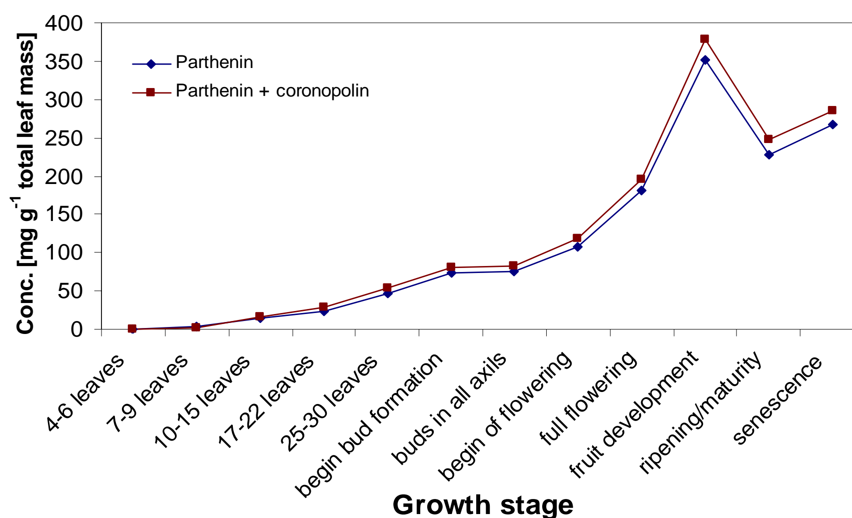


(a)



(b)



(c)

Figure 3.2 Concentrations of parthenin as well as parthenin and coronopolin in leaf fresh (a) and dry (b) material at different growth stages of the plant according to the BBCH code; and (c) total parthenin content in plant leaf material at the different growth stages

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Highly significant differences were observed for parthenin concentration at different phenological stages (Table 3.2). Youngest leaves produced the least parthenin, and oldest leaves the most. Highest parthenin concentrations occurred in the final three growth stages under the experimental growth conditions. The parthenin analogue, coronopolin, which is considered to be biologically inactive, was also analysed and found to follow closely the production trend of the former over the entire life-cycle of the plant (Figure 3.2).

Table 3.2 Parthenin concentrations in leaf dry mass of plants at different growth stages (Appendix 3.1)

Growth stage	Mean water content [% of FM]	Total number of leaves	Total FM of leaves [g]	Total DM of leaves [g]	FM of entire plant [g]	Plant height [cm]	Parthenin [mg g^{-1} leaf DM]
4-6 leaves	88.6±1.6	5.4	1.3	0.2	1.4	8.3	2.94 d
7-9 leaves	86.0±4.3	7.6	4.2	0.6	4.7	13.1	3.41 d
10-15 leaves	89.7±1.1	11.8	16.8	1.7	19.9	22.5	6.58 dc
17-22 leaves	89.1±1.2	19.0	26.6	2.9	31.6	26.8	6.97 dc
25-30 leaves	87.2±2.6	27.8	35.8	4.6	43.6	27.4	11.10 bc
begin of bud formation	86.5±3.6	27.6	40.5	5.5	53.2	35.6	12.59 abc
buds in all axils	84.9±3.4	40.7	31.6	4.8	50.5	48.0	16.13 abc
begin of flowering	84.8±4.3	44.8	45.0	6.8	67.4	50.3	14.53 abc
full flowering	80.5±3.5	77.3	38.4	7.5	86.0	83.0	25.85 ab
fruit development	77.0±2.6	146.3	48.6	11.2	148.1	124.3	34.33 a
ripening/maturity	64.8±7.5	164.5	23.1	8.1	115.4	112.5	29.15 ab
senescence	20.3±0.0	77.0	9.7	7.7	85.9	125.5	34.7 a

Means followed by different letters differ significantly (Tukey –test, P=0.05)

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Reinhardt *et al*. (2004) reported a parthenin concentration of 14.5 mg g^{-1} dry mass in leaves of flowering plants cultivated in a greenhouse at the University of Hohenheim. Parthenium leaf material harvested from flowering plants in Kruger National Park in December 2004 yielded a parthenin concentration of 16.73 ± 1.76 mg g^{-1} dry mass (Belz *et al*., unpublished). These values correspond with the findings of the present experiment for plants at the bud formation to beginning of flowering growth stages. Kraus (2003) noted that trichome density decreased with leaf expansion and leaf age, and this correlated with higher parthenin concentrations in juvenile leaves. However, when parthenin content of leaf homogenate was analysed, higher parthenin concentrations was found in older leaves. Secondary metabolite chemical concentrations have been found to differ between younger and older leaves (Koeppe *et al*., 1970; Harrison, 1982). Differences in parthenin content between older and younger leaves of the same plant were not considered in this experiment. Under the conditions that prevailed in the present experiment, parthenin did not decrease with plant age as has been observed for numerous other allelochemicals (Koeppe *et al*., 1970; Woodhead & Bernays, 1978; Weston *et al*., 1989; Wolfson & Murdock, 1990).

It may be considered logical that if allelochemicals play a role in plant defence it might mean that the concentration of these allelochemicals could decrease with plant age. (An *et al*., 2003). A build-up of allelochemicals with age may, however, be important if a plant utilizes residual allelopathy in its interference strategy. Such a strategy would be aimed at avoiding or limiting the recruitment of other, or even the same, species.

High levels of parthenin have also been reported in the flowers and achenes of parthenium (Rodriguez *et al*., 1975; Picman *et al*., 1979). Reinhardt *et al*. (2004) measured parthenin concentrations in the flowers and achenes at 3.7 mg g^{-1} and 4.4 mg g^{-1}, respectively. Parthenin concentrations in achenes from plants grown in the Univeristy of Hohenheim glasshouses and from plants growing in the Kruger National Park were measured at 9.63 mg g^{-1} and 28.46 mg g^{-1}, respectively. These additional sources of parthenin will boost the potential quantity of parthenin that could be released into the environment. At senescence, plants were calculated to contain a final parthenin content of 267.19 mg. Over the life cycle of *P. hysterophorus*, a single plant can therefore introduce > 267.19 mg into the environment in a single growing season.

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The exact mechanism(s) of parthenin release from the plant is still speculative. Rodriguez *et al*. (1975) reported an abundance of trichomes in dry plant parts that had been disseminated by the wind. Kanchan & Jayachandra (1980a, b) observed that the trichomes can easily become detached from dry parts of parthenium and observed leaching from live vegetative parts. Although it is difficult to predict how much parthenin would be released from the plants under natural conditions, it is clear that the plant does retain high levels of parthenin right until the end of its life-cycle. In addition to the role of parthenin in allelopathy, parthenin may also play an important role in herbivore and pathogen defence. Maintaining high parthenin levels in the plant until after flowering may therefore be of huge benefit to the plant.

Duke *et al*. (2000) points out that 'few systematic studies exist of how cultural methods and the environment affect the production of trichome-borne compounds'. Kimura *et al*. (2000) reported changes in the metabolite level of trichomes in response to environmental changes. Generally, it was observed that allelochemical production increased under stressful conditions for donor plants (Niemeyer, 1988; Putnam, 1988). Fonseca *et al*. (2005) observed changes in the levels of a sesquiterpene lactone, parthenolide (PRT) levels in feverfew (*Tanacetum parthenium* L.). PRT levels varied on a daily basis, and increased in plants recovering from water stress. Perhaps even higher levels of parthenin than those found in the present study can be expected in plants growing in natural environments, for example, in the Kruger National Park, where plants are subjected to a range of severe stresses such as intermittent droughts and fire.

Under the trial conditions, parthenin was not observed to decrease with plant age as has been observed to be the case for numerous other allelochemicals studied (Koeppe *et al*., 1970b; Woodhead & Bernays, 1978; Weston *et al*.,1989; Wolfson & Murdock, 1990). It can not be assumed, however, that greenhouse conditions are comparable to natural conditions and knowledge of the influence of precipitation, wind and other factors on parthenin release is lacking.

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3.4 Conclusions

The increase of leaf parthenin concentrations with plant age, and attainment of highest parthenin concentrations in the final three growth stages, indicate a high resource allocation priority of the plant towards this secondary metabolite. This may be indicative of the importance of this compound in the well-being of the plant through allelopahtic interactions, pathogen and/or herbivore defence, or in multiple roles. Weidenhamer stated (1996) 'Quantification of allelochemical release rates in the environment and the demonstration that concentrations are sufficient to inhibit growth are key steps in validating a hypothesis of allelopathic interference'. Further research in this direction should study the influence of abiotic and biotic factors on parthenin production, and the modes of parthenin release from the plant.

(Note: The findings presented in this chapter have since been published: Reinhardt *et al.*, 2006).

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CHAPTER IV – PERSISTENCE OF PARTHENIN IN SOIL

4.1 Introduction

Parthenin has been identified as one of the major allelochemicals in *Parthenium hysterophorus* and the phytotoxicity of this compound has been investigated on a variety of test species (Datta & Saxena, 2001; Batish *et al*., 2002b; Belz *et al*., 2006). Although parthenin has been found in all parthenium plant parts it occurs most abundantly in trichomes on the surfaces of the leaves (Rodriguez *et al*., 1975, Kanchan, 1975; Reinhardt *et al*., 2004). Reinhardt *et al*. (2004) observed a parthenin concentration of 24.3 mg g^{-1} in the capitate-sessile trichomes (virtually 100% of trichome contents) occurring on leaves. Individual trichome parthenin content was measured at 0.3 µg. When plant residues decompose they can release secondary metabolites that are phytotoxic on other plant species (An *et al*., 2002). In CHAPTER III it was observed that at senescence, parthenium plants grown under controlled conditions have total parthenin content in leaves of 267.1 mg $plant^{-1}$, with smaller amounts from the achenes and other plant parts potentially adding to this volume. It was concluded that a parthenin amount of more than 267 mg would therefore potentially be available for release into the environment by a single plant in a growing season.

Although there is an abundance of literature on allelopathy, few reports have addressed the fate of allelochemicals in the soil environment (Cheng, 1992). Thompson (1985) emphasized the importance of understanding the effects of soil and microbial flora on allelochemical activity in the natural environment. In turn it can be expected that secondary compounds released from plants will also influence microbial ecology, as well as resource competition, nutrient dynamics, mycorrhizae and abiotic factors (Wardle *et al*., 1998). Once a chemical enters the soil a number of interacting processes may take place, some of which may transform or degrade the allelochemical. These are influenced by the nature of the compound, organisms present, soil properties (mineral and organic matter contents, particle size distribution, pH, ion exchange characteristics, oxidation state) and environmental factors (Cheng, 1992). These abiotic and biotic soil factors can influence and limit the quality and

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quantity of alleochemical required to cause plant injury (Inderjit, 2001). Therefore, the accumulation of chemicals at phytotoxic levels and their fate and persistence in soil are important determining factors in plant interactions (Inderjit, 2001).

The main objective of this study was to investigate the persistence of parthenin in soil.

4.2 Preliminary experiments

4.2.1 Preliminary experiment 1: Extraction of parthenin from compost soil

4.2.1.1 Materials and methods

Biologically active soil [hereafter referred to as compost soil (CS)] was obtained from the University of Hohenheim store. The equivalent of 50 g of dry soil was added to glass jars. Deionized water together with parthenin dissolved in acetone was added to each soil sample to achieve a parthenin concentration of 10 µg g^{-1} (10 µl acetone g^{-1}) in the soil and a water-holding capacity (WHC) of 40%. The soil was then stirred thoroughly with a spatula to ensure an even distribution of parthenin within the soil. Loose-fitting glass lids which allowed air circulation were placed on each jar and jars were kept at 20°C in darkness. Sampling was done after one hour incubation time to determine the recovery rate, and thereafter daily for one week. Samples were frozen at -20ºC until extraction and analysis. One soil sample was sterilized by autoclaving at 120ºC for two hours and then air-dried, treated with parthenin and sampled after 14 days.

Extraction technique

Deionized water was added to the soil to obtain a final volume of 15 ml water in the sample. Acetone was pre-warmed to 40ºC and 85 ml was then added to each sample after which the samples were subjected to four minutes of ultra sound followed by 30 minutes of shaking extraction on a mechanical shaker at 200 rpm. A 30 minute sedimentation period was allowed following shaking. The supernatant of each sample was filtered over two spoons of both Na_2SO_4 and quartz sand into Erlenmeyer flasks. A 50 ml aliquot was then removed and added to a separating funnel, followed by the

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addition of 50 ml H_20 and a small amount of NaCl. A liquid-liquid extraction was conducted with 50 ml TBME using a two minute shaking period. The TBME phase was filtered over Na_2SO_4/quartz sand into a round-bottomed flask and a second liquid-liquid extraction was repeated in the same way as described above. Supernatants were pooled and concentrated in a rotary vacuum evaporator followed by vacuum centrifugation at 40ºC until a volume of less than 250 µl was obtained. Acetonitrile was added to take the total volume up to 500 µl and samples were subsequently centrifuged at 28 000 rpm for 20 minutes at 4ºC. Finally, samples were transferred to glass vials and subjected to HPLC analysis.

Quantification of parthenin

HPLC analysis for the determination of the parthenin concentration was done using the method described in CHAPTER III (see 3.2.2.2 - 3.2.2.4).

4.2.1.2 Results and discussion

Parthenin was extractable from the soil, and the concentration of parthenin in the samples could be detected without any interference from other compounds in the soil. The CS soil was therefore judged suitable for use in further degradation experiments. However, a recovery rate of 70% was decided to be inadequate to allow for an accurate study of parthenin at very low concentrations and, therefore, the extraction technique needed to be improved.

From this preliminary experiment it could be determined that parthenin degraded relatively quickly in the soil, with a half-life (DT_{50}) value of less than three days when applied at a concentration of 10 µg g^{-1} (Figure 4.1). By day 14 the parthenin concentration in the soil was measured at 0.14 µg g^{-1}. After 14 days the sample which had been initially sterilized had a considerably higher parthenin concentration than the non-sterilized sample, and it was decided to include a sterilized treatment in the main degradation experiment.

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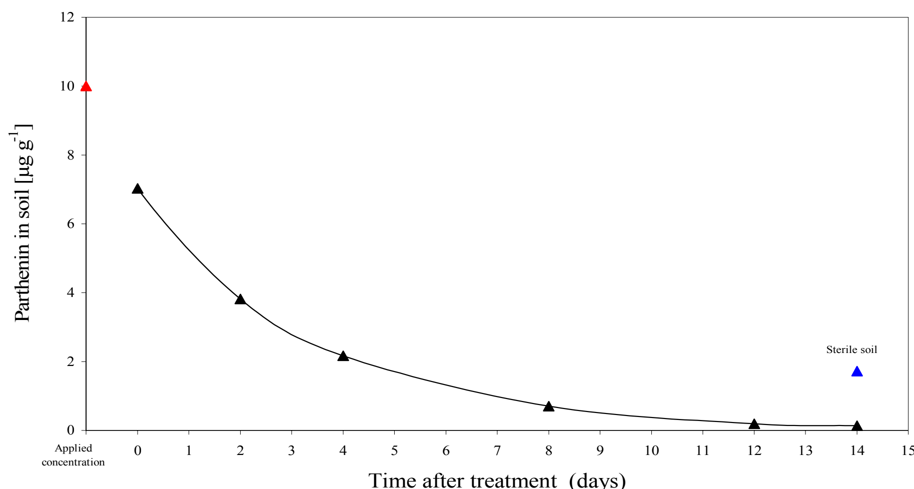

Figure 4.1 Disappearance of parthenin at 20°C in darkness over a period of 14 days added at an original concentration of 10 µg g-1 in sterilized (▲) and non-sterilized (▲) soil

4.2.2 Preliminary experiment 2: Extraction of parthenin from three different soil types

4.2.2.1 Materials and methods

Three different standard soils types, labelled 2.1, 3A and 5M were obtained from the ‘Landwirtschaftliche Untersuchungs- und Forschungsanstalt – Speyer’ (LUFA – Germany). Properties for the soils are presented in Table 4.1.

Table 4.1 Properties for the different soil types provided by LUFA and the compost soil (CS) provided by the University of Hohenheim

Soil	Org C in %	pH value (0.01 M $CaCl_2$)	CEC (mval 100 g^{-1})	Soil type (USDA)	Water-holding capacity (g 100 g^{-1})
2.1	1.21±0.27	6.1 ± 1.0	7 ± 1	Sand	34.7 ± 5.0
5M	1.56 ± 0.3	7.1 ± 0.3	13 ± 2	Sandy loam	42.1 ± 1.8
3A	2.2 ± 0.1	7.1 ± 0.1	19 ± 5	Loam	49.4 ± 5.5
CS	5.12	6.9	23.2	Very loamy sand	54.7

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The experiment was conducted as described in 4.2.1.1. Two replicates for each soil were used and the recovery rate of parthenin was calculated after one hour incubation time using the same technique as described in 4.2.1.1. Untreated soils were subjected to the same analysis in order to determine whether any other compounds present in the soil would interfere with parthenin detection by HPLC.

4.2.2.2 Results and discussion

Parthenin was successfully extracted and detected in all three of the soils. Recovery rates for the three soils are presented in Table 4.2. It was therefore decided that all three soils, in addition to the CS soil could be used for the main degradation experiment. Recovery rates varied between the soils and were less than desired (64.6±3.6%) which necessitated an improvement in extraction technique.

Table 4.2 Recovery rates of parthenin from three different soil types

Soil type	Recovery Rate [%]
2.1	59.2
5M	69.9
3A	64.7

4.2.3 Preliminary experiment 3: Evaluation of different extraction techniques for obtaining the highest recovery rate

4.2.3.1 Introduction

After previous experiments yielded less than desirable parthenin recovery rates it was decided to conduct and compare five different extraction techniques to maximize the recovery rate of parthenin from soil.

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4.2.3.2 Materials and methods

For each sample, 50 g of dry compost soil was added to glass jars. A volume of 15 ml H_20 containing 500 µl parthenin in acetone was then added to the soil and mixed thoroughly with a spatula to attain homogenization. Three replicates were measured for each of the five methods. An incubation period of one hour was allowed before different extraction methods as described below were utilized.

Method 1 and 2: Acetone was warmed to 40ºC and 85 ml was added to each soil sample. Samples were then shaken for 30 minutes on a mechanical shaker at 200 rpm and allowed to sediment for a further 30 minutes. The supernatant from each sample was filtered over Na_2SO_4 and quartz sand. The aliquot was transferred to a separating funnel and 50 ml H_20, a small amount of NaCl, and 50 ml TBME added and a liquid-liquid extraction with a two minute shaking period conducted. The TBME phase was then transferred to a round-bottom flask while another 50 ml of TBME was added to the water phase and a second liquid-liquid extraction conducted. The TBME phases were pooled and then concentrated in a rotary vacuum evaporator and transferred to calibrated test tubes and vacuum centrifuged until a final volume of less then 250 µl was obtained.

Method 1: acetonitrile (ACN) added to attain a final volume of 500 µl.
Method 2: ACN:H_2O added to attain a final volume of 2000 µl.

In both methods samples were centrifuged for 20 minutes at 28 000 rpm before transferring 500 µl to glass vials for HPLC analysis.

Method 3 and 4**:** 85 ml of extraction solvent [Method 3: acetone; Method 4: acetone:TBME 1:1 (v/v)] was added to each soil sample and samples were shaken for 30 minutes on a mechanical shaker at 200 rpm. After shaking, 15 minutes of sedimentation was allowed before the supernatant was filtered over Na_2SO_4/quartz sand. Aliquots of 40 ml were pipetted into round-bottom flasks and the aliquots were concentrated in a rotary vacuum evaporator. Concentrated samples were then transferred to graduated centrifuge tubes. Additional TBME was used to remove any remaining residues of the sample from the walls of the round-bottomed flasks.

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Samples were then vacuum-centrifuged at 40ºC to obtain a final volume of less than 600 µl. Deionized water was added to take samples to 600 µl, and 400 µl ACN added to obtain a final volume of 1000 µl. The samples were then centrifuged and 500 µl was transferred to glass vials for HPLC analysis.

Method 5: 85 ml acetone (at 40ºC) was added to the soil, followed by 30 minutes of shaking extraction at 200 rpm and 15 minutes of sedimentation. The supernatant was then filtered over NA_2SO_4/quartz sand. The soil remaining in the glass jar together with any remaining acetone was transferred to a 50 ml test tube and centrifuged at 4000 rpm for 20 minutes. The initial filtrate together with the supernatant from the centrifugation process was then conveyed to the rotary evaporator followed by vacuum centrifugation until <600 µl of solution was left. This was then taken up to 600 µl with deionized water and 400 µl ACN added to obtain a final volume of 1000 µl. The sample was then centrifuged and 500 µl was transferred to HPLC vials for analysis.

4.2.3.3 Results and discussion

Recovery rates (Table 4.3) varied considerably between the different methods tested. Through Methods 3 and 4 the highest recovery rates were achieved, and it was decided to use Method 4 (acetone:TBME as extracting solvent) in the main degradation experiment.

Table 4.3 Mean recovery rates for parthenin from 'CS' soil using different extraction techniques

Method used	Recovery rate [%]
Method 1	51.6
Method 2	80.8
Method 3	106
Method 4	97.9
Method 5	67.2

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4.2.4 Preliminary experiment 4: Determination of the consistency of recovery rates

4.2.4.1 Introduction

In order to obtain useful and consistent data the reliability of the extraction technique and consistency of recovery rates were investigated.

4.2.4.2 Materials and methods

For each of the four soil types given in Table 4.1, the equivalent of 50 g dry soil was added to glass jars and deionized water together with parthenin dissolved in acetone was added to obtain 40% WHC and 10 µg g^{-1} parthenin concentration in the soil. Extraction Method 4 (see 4.2.3.2) was used to extract parthenin from the soil and to assess consistency and reliability of the recovery rates.

4.2.4.3 Results and discussion

Mean recovery rate and standard deviation across the four replicates for the four soils is presented in Table 4.4. Recovery rates were judged to be sufficiently consistent and reliable.

Table 4.4 Mean parthenin recovery rates with standard deviations for the four soil types

Soil type	Mean recovery rate [%]
2.1	95.8 ± 2.8
5M	105.2 ± 6.1
3A	96.6 ± 4.5
CS	109.2 ± 2.9

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4.2.5 Preliminary experiment 5: Persistence of pure parthenin at different concentrations in soil

4.2.5.1 Introduction

The phytotoxicity of herbicides in the soil is correlated with the concentration of the herbicide in the soil water but not with amount of herbicide per entire soil mass (Kobayashi *et al*., 1994; Kobayashi *et al*., 1996). Ito *et al*. (1998) observed that the amount of dehydromatricaria ester (DME) adsorbed to the soil solids depended on the concentrations applied. The objective of this experiment was to determine the persistence of parthenin applied at three different concentrations (in magnitudes of ten) to study the effect of concentration and to determine at which concentration the main degradation experiment should be conducted.

4.2.5.2 Materials and methods

Fifty grams of soil was placed into each glass jar and deionized water was added to achieve a 40% WHC. Aliquots of a stock solution of parthenin in acetone (10 mg ml^{-1}) were added to the soil to obtain parthenin concentrations of 100, 10 and 1 µg g^{-1} respectively. An additional treatment was prepared at the 100 µg g^{-1} concentration, using soil that had been sterilized by autoclaving for two hours at 120ºC and then left to air-dry. Samples were kept in the dark at a constant temperature of 20ºC. Sampling occurred after one hour incubation and then regularly over a one week period.

4.2.5.3 Results and discussion

Parthenin proved to degrade slower when applied at 100 µg g^{-1} than at 10 and 1 µg g^{-1} (Figure 4.2). Chemicals have often been observed to degrade slower in soil when present at higher concentrations, as has also been noted for allelochemicals by Fomsgaard *et al.* (2004) and Weidenhamer & Romeo (2004). Ito *et al.* (1998) observed that the higher the DME concentration in the soil, the longer the DME concentration was maintained in the soil water. Parthenin applied at 1 and 10 µg g^{-1} degraded at a similar rate initially, having a similar DT_{50} value, but after four days degradation rate in soils to which 1 µg g^{-1} parthenin was much faster than at 10 µg g^{-1}

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(Figure 4.2). Parthenin at 100 µg g^{-1} also began degrading rapidly after four days, prior to which, very little degradation had taken place. In the initially sterilized soil to which parthenin had been added at a concentration of 100 µg g^{-1} no degradation was evident within the seven day period examined.

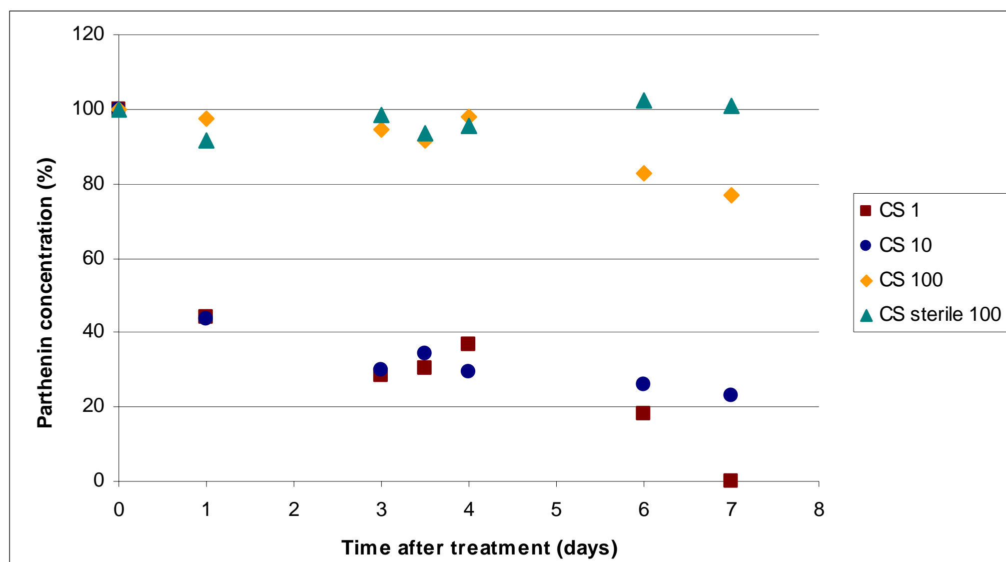

Figure 4.2 Disappearance of parthenin at 20°C in darkness over a period of seven days added at an original concentration of 1, 10 and 100 µg g^{-1} to non-sterilized soil and in sterilized soil at 100 µg g^{-1}

4.3 Main experiment

4.3.1 Introduction

Based on results of the preliminary experiments described above it was decided to use a parthenin concentration of 10 µg g^{-1} in the soil for the main experiment and a sampling period of 22 days.

4.3.2 Materials and methods

The WHC of the four soil types classified as: sand (labelled 2.1), sandy loam (5M), loam (3A) and compost soil (CS), was determined. For each of the soils, the equivalent of 50 g of dry soil was placed into glass jars and the correct volume of deionized water together with parthenin dissolved in acetone was added to achieve a

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WHC of 40% and a parthenin concentration of 10 µg g^{-1}. The soil was then thoroughly mixed with a spatula to achieve homogenization. Jars were closed with loose fitting glass lids which allowed for free air movement. Samples were placed in permanent darkness at a constant temperature of 20ºC. In addition to the above treatments, both sterilized and non-sterilized CS soils were incubated at 20, 25 and 30ºC in order to determine parthenin degradation in initially sterilized and non-sterilized soils at different temperatures. Sterilization was achieved through autoclaving the soil at 120ºC for two hours and then allowing the soils to air-dry. For each treatment a total of 15 samples were taken over 22 days with sampling frequency decreasing over time. Water was replenished every 3-4 days to maintain the soil moisture at 40% WHC. Any seedlings that germinated in the soil were immediately removed. Sampling was done by replacing the glass lid with a tight fitting plastic lid and freezing the sample at -20ºC until analyzed.

Parthenin extraction

Samples were removed from refrigeration and defrosted in a heat bath at 30ºC. All samples were at 40% WHC and an additional volume of deionized H_2O, depending on the soil type, was added to attain a final volume of 15 ml H_2O in the soil. A volume of 85 ml of 1:1 acetone:TMBE was added to each sample. Plastic lids lined with parafilm were placed over the jars and samples were shaken for 30 minutes on a mechanical shaker at 150 rpm. After shaking and 30 minutes of sedimentation the supernatant was filtered over Na_2SO_4/quartz sand. A 40 ml aliquot was then transferred to flat-bottomed flasks and the sample was concentrated in a rotary vacuum evaporator. The concentrated sample was transferred to graduated centrifuge test tubes. A small amount of TBME was used to rinse the flat-bottom flasks to ensure transferral of the entire sample to the centrifuge tubes. Samples were then vacuum-centrifuged at 30ºC for 20 minutes, and then at 45ºC with the cooling unit switched on until a volume of less than 600 µl was obtained. Deionized H_2O was added to obtain 600 µl, and then 400 µl ACN. Samples were centrifuged at 28 000 rpm for 20 minutes before transferral to glass vials for HPLC analysis.

Parthenin quantification

Parthenin concentration in the samples was determined using the method described in Chapter III (see 3.2.2.2 – 3.2.2.4). Nonlinear regression analysis was done using

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SPSS® regression models and degradation curves were compared using *F* test for lack-of-fit based on analysis of variances (P≤0.05).

4.3.3 Results and discussion

4.3.3.1 Parthenin degradation in different soil types

Parthenin was quickly degraded in all four soils tested under the particular experimental conditions used. The degradation curves for the soils tested were parallel indicating a similar degradation mechanism in all soils (Figure 4.3).

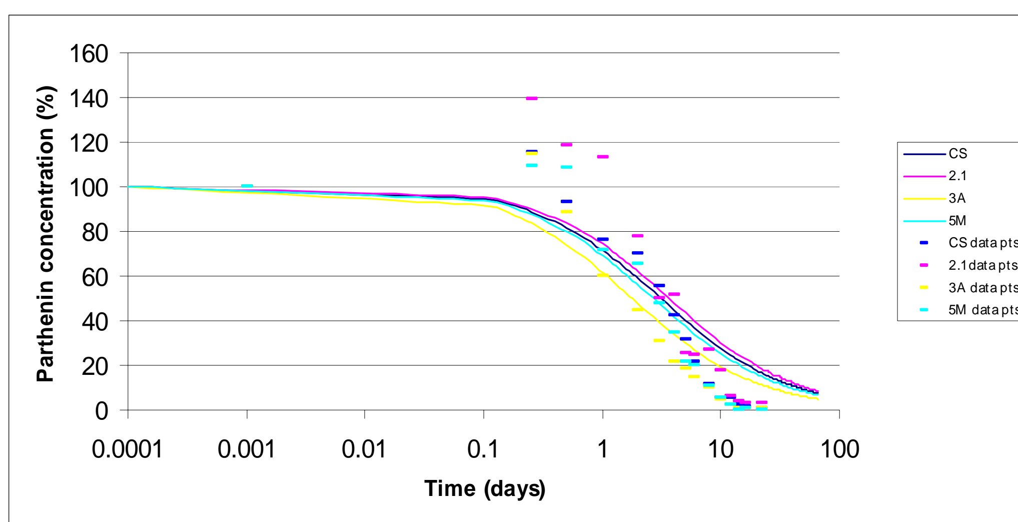

Figure 4.3 Disappearance of parthenin at 20°C in darkness added at an original concentration of 10 μg g^{-1} to four different soil types

DT_{50} values for the soils ranged from 1.78 to 3.64 days and differed significantly (Table 4.5). DT_{10} and DT_{90} values (also presented in Table 4.5) are representative of the time of degradation onset and the end of the degradation process, respectively.

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Table 4.5 Disappearance-time (DT) for 10, 50 and 90% degradation for the four different soils used in the experiment

Soil		DT_{10}	DT_{50} **(Days)**	DT_{90}
3A	(loam)	0.34	1.78a	27.24
5M	(sandy loam)	0.50	2.67ab	40.81
CS	(very loamy sand)	0.58	3.10b	47.32
2.1	(sand)	0.69	3.64b	55.58

Means followed by different letters differ significantly (F-test, P=0.05)

Correlation between soil characteristics and DT_{50} values were found to be negative and significant for WHC and soil cation exchange capacity, but not significant for pH and organic carbon content (Figure 4.4). PH values for the different soils were relatively close together which may be the reason for the non-significant correlation. Calvet *et al*. (1980) pointed out that for non-ionic herbicides, correlation between degradation and soil organic matter is not always very good across the range of 0 to 4% organic matter. This range includes most temperate arable soils and it is likely that the soils used in this study contained too little organic carbon for a significant correlation between DT_{50} values and organic carbon percentage. Soils with higher clay and organic matter contents generally have greater adsorptive power.

Although analyses were performed for the CS soil, it was not included in the correlation analysis due to the unnatural constitution of this “soil”.

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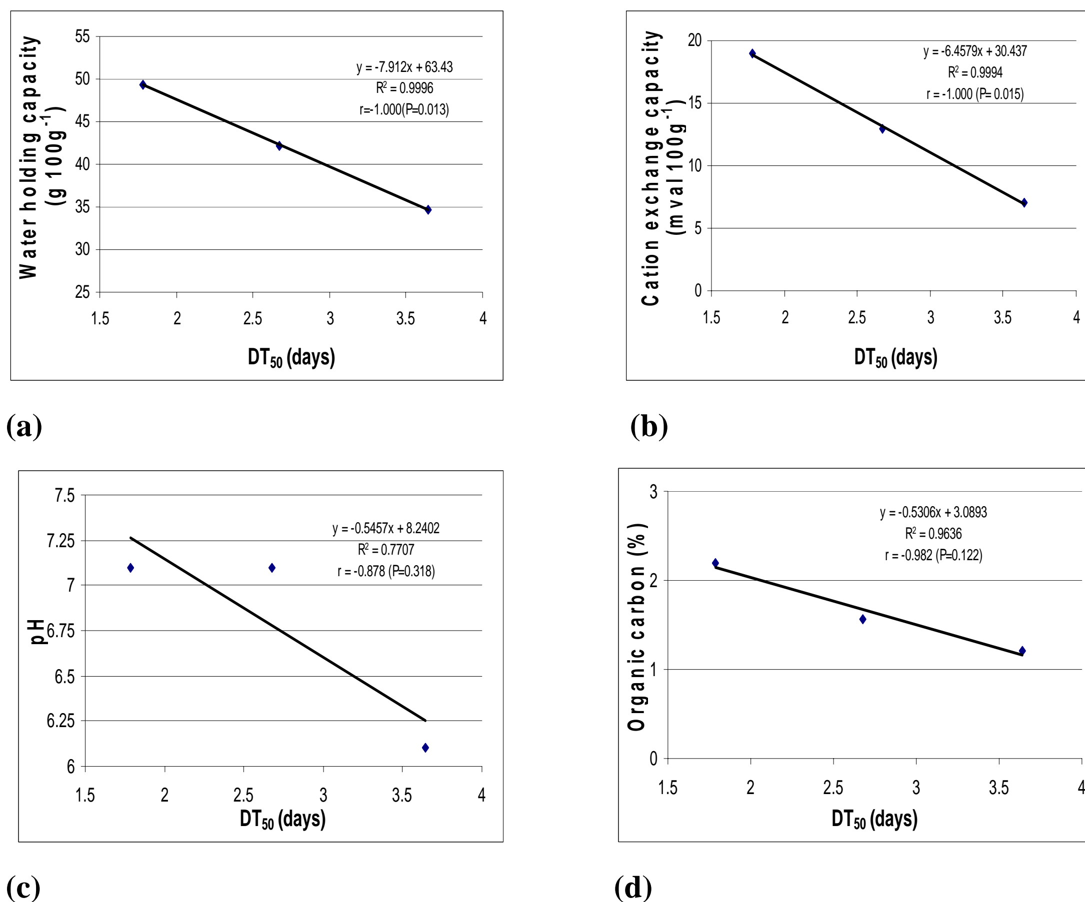

(a) **(b)**

(c) **(d)**

Figure 4.4 Correlation between DT_{50} value and (a) water holding capacity, (b) cation exchange capacity, (c) pH, and (d) organic carbon percentage for the degradation of parthenin in the 3A, 5M and 2.1 soils

4.3.3.2 Parthenin degradation in sterilized and non-sterilized compost soil at three different temperature regimes

Similar to parthenin degradation in different soil types, the sterilized and non-sterilized compost soil placed at different temperatures all had parallel curves but different DT_{50} values (Figure 4.5).

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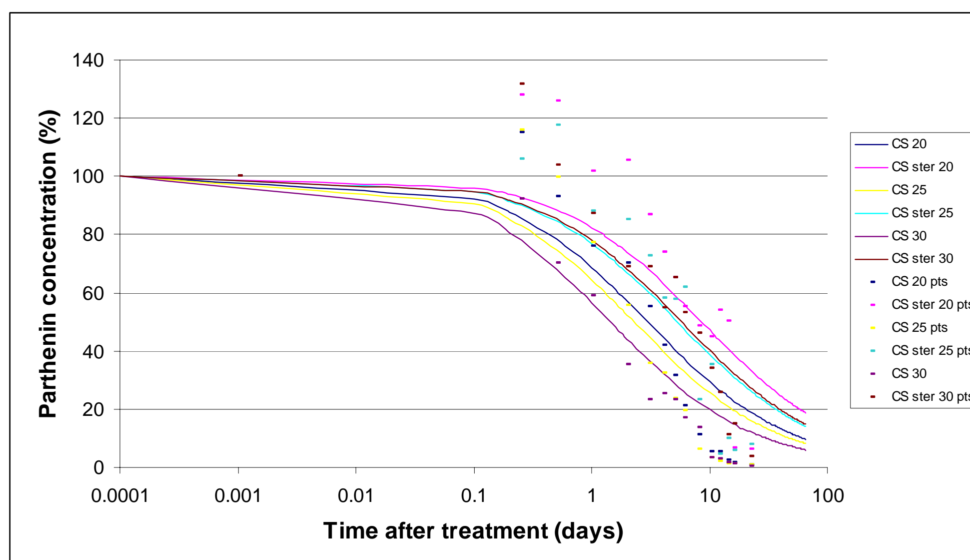

Figure 4.5 Disappearance of parthenin added at an original concentration of 10 µg g^{-1} in sterilized and non-sterilized compost soil (CS) incubated at temperature regimes of 20, 25 and 30ºC in darkness

From Figure 4.5 it is apparent that parthenin degraded faster in soils which were not sterilized than in soils which were autoclaved. Ito *et al*. (1998) also observed that the degradation of the allelochemical dehydromatricaria ester was slowed by autoclaving the soil. According to Grover (1988), chemicals are absorbed, degraded or leached in the soil. Picman (1987) concluded that when isoalantolactone, a sesquiterpene lactone, was added to soil at a concentration of 100 µg g^{-1}, microbial degradation was most likely responsible for the disappearance of this sesquiterpene from the soil. After 90 days isoalantolactone was not detected in the organic soil used and only traces could be detected in the mineral soil used. Picman (1987) suggested that the initial disappearance of the chemical compound from the soils, especially from the organic soil, was due to the compound forming 'bound residues' with humic material in the soil. Inderjit (2001) pointed out the need to evaluate other soil properties including electrical conductivity, inorganic ions, clay minerals and water content. As leaching and light degradation was not possible under the present experimental conditions, it seems plausible that microbial degradation was the predominant cause of the disappearance of parthenin from the soil. It is not entirely certain that all microbes capable of playing a role in degrading parthenin were neutralized during the autoclaving process. It can, however, be expected that microbe numbers were at least drastically reduced. As the sterilized soil was not kept under completely sterile

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conditions following autoclaving, it can be expected that microbial populations in the originally sterilized soils would increase in size and diversity over time.

In non-sterilized soils, parthenin degraded significantly quicker in soil kept at 30ºC compared to soils kept at 20 or 25ºC (Table 4.6). There was a trend for faster degradation in soil incubated at 25ºC than at 20ºC but this was not significant. This finding supports the hypothesis that microbial degradation played an important role in degradation as we would expect faster metabolism at higher temperatures. In sterilized soils, parthenin degraded significantly slower in soils incubated at 20ºC than in soils kept at 25 or 30ºC.

Table 4.6 Parthenin disappearance-time (DT) for 10, 50 and 90 % degradation in sterile and non-sterile compost soil (CS) placed at temperature regimes of 20, 25 and 30ºC

Soil		DT_{10}	DT_{50} (Days)	DT_{90}
CS 20ºC	*Sterile*	0.96	8.54e	77.70
	Non-sterile	0.33	2.98bc	27.10
CS 25ºC	*Sterile*	0.60	5.32cd	48.37
	Non-sterile	0.26	2.29b	20.82
CS 30ºC	*Sterile*	0.65	5.78d	52.56
	Non-sterile	0.16	1.44a	13.09

Means followed by different letters differ significantly (F -test, P=0.05)

Significant correlation was observed between temperature and DT_{50} and DT_{90} values for non-sterilized soils only (Figure 4.6).

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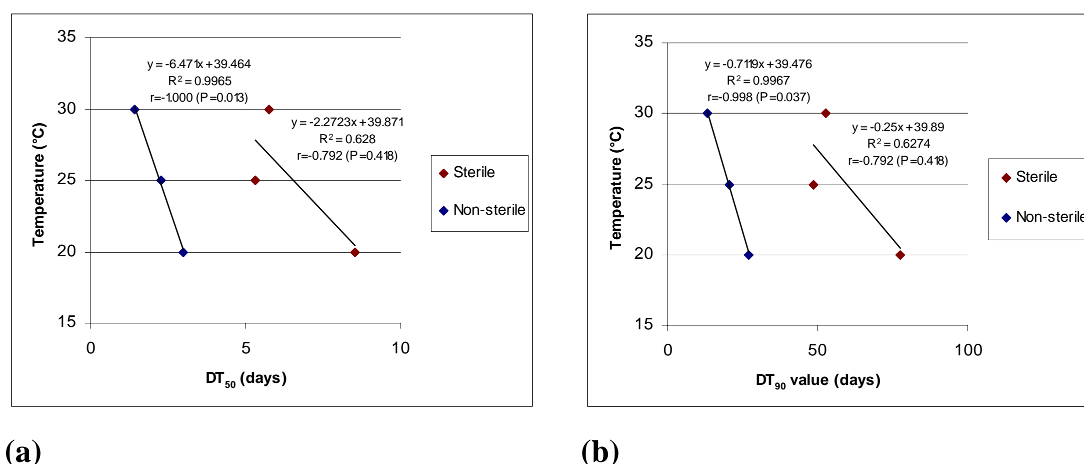

(a) **(b)**

Figure 4.6 Correlation between temperature and DT_{50} (a) and DT_{90} (b) values for sterile and non-sterile compost soil placed at 20, 25 and 30ºC

Schmidt & Ley (1999) postulated that allelochemicals may be prevented from building up to phytotoxic levels by microbial activity in natural soils. Limited work has been done on the microbial transformation of parthenin (Bhutani & Thakur, 1991) and further investigation into parthenin transformation and degradation products occurring in the soil will be necessary for increased appreciation of parthenin soil degradation mechanisms. Chemically transformed parthenin products may also display phytotoxic properties. Also, little is known of microbial sensitivity to parthenin and the influences of this on parthenin degradation in the soil. In the CS soil, parthenin DT_{50} was observed to be affected significantly by temperature and under natural conditions we can expect temperature, seasonal temperature fluctuation and amount of precipitation to affect the biochemical degradation of parthenin.

Different soil types also differed significantly with regard to DT_{50} values, reiterating the importance of soil characteristics in allelochemical degradation as has been reported (Dalton *et al*., 1989; Shibuya *et al*., 1994; Takahashi *et al*., 1994; Kobayashi *et al*., 2004). According to An *et al*. (2002), the potential phytotoxicity of plant residues ‘is dependent on numerous factors that together govern the rate of residue decomposition, the net rate of active allelochemical production and the subsequent degrees of phytotoxicity’. Although it is difficult to determine parthenin concentrations occurring under natural conditions, it is clear from the DT_{50} values that a continual replenishment of parthenin into the soil will be necessary in order for parthenin to have a phytotoxic effect on other plant species. Little is known about the

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necessary parthenin concentrations in the soil required to inhibit plant growth. Investigating the allelochemical dehydromatricaria ester (DME) from *Solidago altissima* L. (Asteraceae), Ito *et al.* (1998) observed that the DME concentration required for 50% growth inhibition was ten or twenty times greater in soil that in agar culture depending on soil type. Herbicide studies have shown that the phytotoxicity of herbicides in soils was highly correlated with soil water concentrations as opposed to amounts per whole soil mass (Kobayashi *et al*., 1994; Kobayashi *et al*., 1996). In studying the disappearance of isoalantolactone, a sesquiterpene lactone occurring mainly in species from the genera *Inula* and *Chrysanthenum*, Picman (1987) concluded that 'sesquiterpene lactones do not accumulate in the soil presumably because they are decomposed'.

4.3.3.3 Conclusions

The disappearance of parthenin from soil can be a result of leaching from the soil, or chemical, biochemical or photochemical transformation. In this experiment, parthenin disappearance due to leaching or photochemical transformation can be ruled out. As parthenin disappeared faster in soils that had not been sterilized than in soils that were autoclaved, it is probable that microbial transformation of parthenin played a role.
Inderjit & Weiner (2001) suggested that in the field, effects of allelochemicals could be due to (i) direct effect of allelochemicals, (ii) effects of degraded or transformed products of the allelochemicals released, (iii) effect of allelochemicals on physical, chemical and biological soil factors, and (iv) chemical induction of release of active chemicals by a third species. Inderjit & Weiner (2001) further proposed 'that the behaviour of vegetation can be better understood in terms of allelochemical interactions with soil ecological processes rather than the classical concept of direct plant-plant allelopathic interference'. Although the phytotoxicity of parthenin on numerous test species has been well demonstrated, less is known about parthenin phytotoxicity in the soil and the effect of parthenin on soil ecology. This requires further research.

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CHAPTER V – EFFECT OF PURE PARTHENIN ON THE GERMINATION AND EARLY GROWTH OF THREE INDIGENOUS GRASS SPECIES

5.1 Introduction

The sesquiterpene lactone, parthenin, has been implicated as one of the major allelochemicals in *P. hysterophorus* allelopathy (see also CHAPTER III - 3.1); and is the main secondary metabolite of *P. hysterophorus*, possessing phytotoxic, cytotoxic, anti-tumour, allergenic, antimicrobial, anti-feedant and insecticidal properties (Datta & Saxena, 2001).

Parthenin has been observed to exhibit dose-dependent toxicity effects on a range of test species, including aquatic species (Patil & Hedge, 1988; Kohli *et al*., 1993; Pandey, 1996; Kraus, 2003). Batish *et al*. (1997) observed that parthenin caused a growth regulatory effect almost similar to indole-3-acetic acid (IAA) using *Phaseolus aureus* as test species. Batish *et al*. (2002b) found that parthenin significantly reduced germination and root and shoot length of *Avena fatua* and *Bidens pilosa*, with the latter species being more sensitive. The authors further observed that root and shoot growth as well as chlorophyll content was decreased when seedlings of *A. fatua* and *B. pilosa* were grown in soil to which parthenin had been added. Belz *et al*. (2006) observed a phytotoxic effect of parthenin on *Ageratum conyzoides*, *Echinochloa crus-galli*, *Eragrostis curvula*, *E. tef*, and *Lactuca sativa* as test species. The authors further calculated the contribution of parthenin to the overall phytotoxic effects of leaf extracts using model comparisons of dose-response relationships and observed that the contribution of parthenin varied from 16 to 100%.

The objective of this study was to determine the effect of pure parthenin on the germination and early growth of the three indigenous grass species (*E. curvula, Panicum maximum, Digitaria eriantha*) used in the field trial, and to observe whether differences in sensitivity to parthenin exist between them.

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5.2 Materials and Methods

Seeds for the three test grass species were obtained from Pannar (Pty) Ltd. in Pretoria, South Africa. The pure parthenin for the bioassay was supplied by the University of Hohenheim in Stuttgart, Germany, and was obtained from parthenium plants growing in the University glasshouses through the methods described by Belz *et al*. (2006) (see also CHAPTER III -3.2.2.2). A dose-response bioassay was conducted using a parthenin concentration series ranging from 0 – 500 µg g^{-1}. Each concentration in the series, including the control, contained 1% acetone. Due to differences in germinability between the grass species, 10, 25 and 30 seeds of *E.curvula*, *P. maximum* and *D. eriantha*, respectively, were placed into 9 cm diameter Petri dishes containing a single filter paper disc. A treatment volume of 5 ml was added to the Petri dishes and each concentration was tested in triplicate. Seeds were placed in a growth chamber and allowed to germinate in the dark at 20/30ºC alternating temperatures (12/12 h). Measurements were taken after 5 days for *E. curvula*, after 8 days for *D. eriantha* and after 10 days for *P. maximum*; germination percentage and radicle length were measured. Nonlinear regression analysis was done using SPSS® regression models and dose-response curves were compared using *F* test for lack-of-fit based on analysis of variances (P=0.05).

5.3 Results and Discussion

From the dose-response curves for radicle length and germination percentage (Figure 5.1), it can be observed that pure parthenin had a phytotoxic effect on all three grass test species. All three species displayed significant variation in response to pure parthenin, and none of the dose-response curves were parallel.

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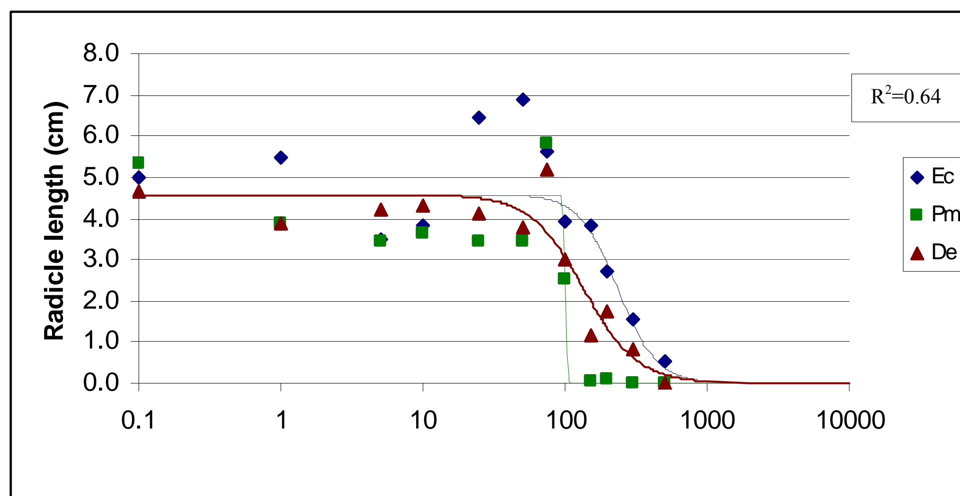
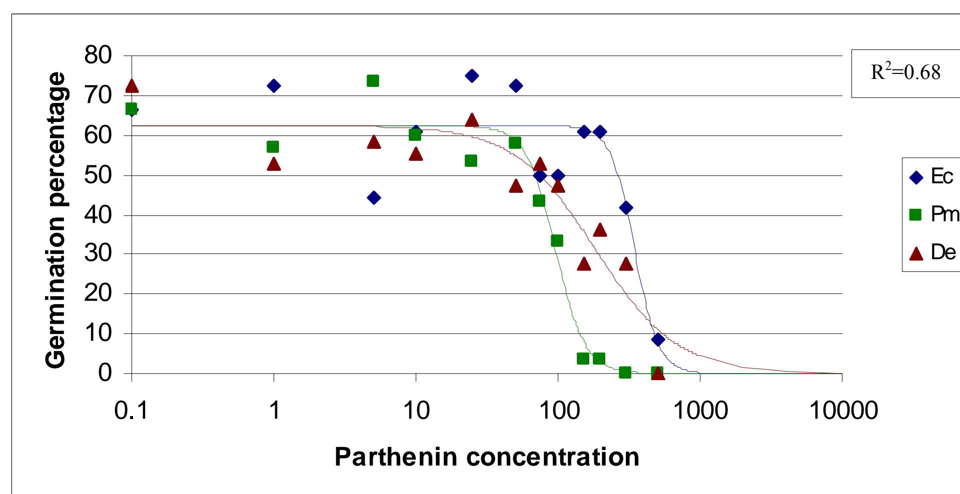


(a)



(b)

Figure 5.1 Effect of pure parthenin on radicle development (a) and germination percentage (b) of three indigenous grass species (Ec = *E. curvula*, Pm = *P. maximum*, De = *D. eriantha*)

Based on ED_{50} values calculated from dose-response curves for the parameters germination percentage and radicle length, *P. maximum* was observed to be the most sensitive species, followed by *D. eriantha*, with *E.curvula* being the least sensitive species (Table 5.1). Slope differences between curves may be due to variations in germination and seedling development between the grasses (Belz *et al.*, 2006). For radicle length, the *P. maximum* dose-response curve displayed a drastic reduction in length at the ± 100 µg ml^{-1} concentration. The reason for this is not clear. Complete germination inhibition and radicle development occurred at a concentration of 300 µg

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ml^{-1} for *P. maximum* and at a concentration of 500 µg ml^{-1} for *D. eriantha*. Complete inhibition of germination and radicle development for *E. curvula* did not occur at the highest concentration used. Greater inhibition on radicle growth than germination as observed in this experiment was also noted by Batish *et al*. (1997, 2002b) and Belz *et al*. (2006). Parthenin may therefore possibly only be regarded a 'rather weak germination inhibitor' (Belz *et al*., 2006), but may play a larger role in delaying germination (Kohli *et al*., 1996).

For *E. curvula*, Belz *et al.* (2006) observed ED_{50} values for germination percentage and radicle length at 491.3 and 167.8 µg ml^{-1}, respectively. Differences in ED_{50} values to those in this experiment may possibly be attributed to experimental conditions and/or purity of the parthenin used. Belz *et al.* (2006) reported a significant hormetic effect for *E. curvula* at low parthenin concentrations. *E. curvula* also displayed radicle growth stimulation in the current experiment, but this was not tested for significance. Belz *et al*. (2006) further observed that *E. curvula* was more sensitive to parthenin than the other monocot species tested, namely, *E. tef* and *Echinochloa crus-galli* (Appendix 5.1).

Table 5.1 Phytotoxicity of parthenin on three indigenous grass species

	ED_{50} (µg ml^{-1})	
Species	**Radicle length**	**Germination**
E. curvula	212.9a	345.9a
D. eriantha	144.7b	184.2b
P. maximum	100.6c	96.1c

Means followed by different letters differ significantly (F–test, α=0.05)

5.4 Phytotoxic potential of pure parthenin under natural conditions

Under field conditions, when *P. maximum* was established by transplanting seedlings raised in a greenhouse, together with transplanted *P. hysterophorus* seedlings, *P. maximum* was observed to be least sensitive to *P. hysterophorus* interference relative to the other two grass species (CHAPTER II). Yet *P. maximum* was observed to be the most sensitive species to pure parthenin. Ultimately the allelopathic potential of

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parthenin under completely natural conditions is of primary importance in understanding the role of this secondary metabolite in *P. hysterohorus* allelopathy. From the study described under CHAPTER III it was observed that a single mature parthenium plant can potentially introduce a total parthenin amount of greater than 236.15 mg into the environment in a single growing season. Parthenium plants have been observed to occur at different densities according to environmental factors. In India, Batish *et al*. (2002a) observed a parthenium density of 34.3±6.8 plants m^{-2}, while Pandey & Dubey (1989) observed densities of 14 plants m^{-2}, with other authors recording densities ranging between these values (Kanchan & Jayachandra, 1980b; Joshi, 1991b). In Skukuza, parthenium densities of 96 mature plants m^{-2} were observed. Total leaf dry mass of plants growing under natural conditions was observed to be 40% less than plants grown in the greenhouse. A parthenium stand of 96 mature plants m^{-2}, with each plant contributing 94.44 mg parthenin, could therefore potentially introduce a concentration of 2350 µg ml^{-1} in the top 2 cm layer of a soil (where most grass seed germination can be expected) such as the '2.1' soil tested in CHAPTER IV (see 4.2.2.1) if all the parthenin was in solution (Appendix 5.2). Parthenin released from the achene complex and other plant organs could increase this value. This concentration is above the ED_{50} pure parthenin concentration values for radicle length and germination percentage for all three test grass species. It therefore appears plausible that parthenin may have a phytotoxic effect and impede grass establishment under natural conditions.

A complex model would be required to investigate this matter further, however, incorporating a plethora of influential factors, including the parthenin release dynamics, adsorption capacity of soils for parthenin, and various other biotic and abiotic environmental factors. In CHAPTER IV (see 4.3.3.1) parthenin was observed to be easily degradable in soil, with DT_{50} values of 1.78 and 3.64 days in a loamy and sandy soil respectively (soils incubated at 20ºC, 40% WHC). A source of constant parthenin replenishment will therefore be required to keep parthenin concentrations at phytotoxic levels in the soil. The role of other allelochemicals, including phenolics, released by *P. hysterophorus* must also be considered in the overall allelopathic potential of the plant. In addition to direct effects on other plant species, the effect of the allelochemicals on soil ecology also needs further investigation.

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5.5 Conclusions

Pure parthenin was observed to have a phytotoxic effect on all three test species. *P. maximum* was the most sensitive species, and *E. curvula* the least sensitive. Radicle length was a more sensitive parameter then germination percentage for the three grass species. Based on the findings for parthenin production dynamics in *P. hsyterphorus* leaves and on the phytotoxic effect of parthenin, it is plausible that parthenin is phytotoxic under natural conditions. Further research is required to enable more accurate modelling of the phytotoxicty of parthenin under natural conditions. Knowledge gaps include the release mechanism of parthenin from the plant and the fate of parthenin in the soil.

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CHAPTER VI – GENERAL DISCUSSION AND CONCLUSIONS

Outside of its native range in tropical America, *P. hysterophorus* is a noxious weed and has become a menace to crop production, animal husbandry, human health and biodiversity in many countries throughout the world. Even over the two years of this study, the alarming rate at which *P. hysterophorus* can spread, and the extent of the threat it poses, is evident. Briefly defined, allelopathy is the chemical interaction between plants. Numerous bioassays have investigated the allelopathic effects of chemicals from one plant species on other test species. The challenge in these allelopathic studies is separating allelopathy and competition in plant-plant interference, and determining the phytotoxic effect of the allelochemicals, singularly and in conjunction with other allelochemicals, under completely natural conditions. In addition to direct plant-plant interactions, Inderjit & Weiner (2001) also stress the importance of allelochemicals on soil ecology processes to better understand vegetation behaviour. Parthenium plants growing on several different continents were classified into seven types by Picman & Towers (1982) according to lactone content, and parthenium plants growing in South Africa were classified into the 'parthenin group' which contain parthenin, coronopolin and tetraneurin A. Parthenin, a sesquiterpene lactone, is implicated as one of the primary allelochemicals in *P. hysterophorus* allelopathy (Patil & Hedge, 1988; Kohli *et al*., 1993; Pandey, 1996; Belz *et al*., 2006). Phenolics produced by the plant are also believed to play an important role in *P. hyserophorus* allelopathy.

A disturbed area (dumpsite) in Skukuza, Kruger National Park, which has naturally become infested with *P. hysterophorus* was used as a site for the field trial in which growth interference between *P. hysterophorus* and three indigenous grass species was studied. *P. maximum* showed best overall growth performance of the three grasses, with *E. curvula* and *D. eriantha* fairing less well. The poor performance of *E. curvula* and *D. eriantha* was attributed largely to the high soil pH which exceeded the preferences for the two grasses. Climatic factors were also implicated. *P. maximum* has a higher pH preference and is known to tolerate a wider range of climatic factors. For the first growth season (2003/2004), percentage of control data showed that *P. maximum* did not perform significantly different from *D. eriantha* at the 5 parthenium

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m^{-2} density, but grew significantly better at the 7.5 parthenium m^{-2} density at the P<0.075 significance level. *E. curvula* displayed the poorest growth performance at both densities. Parthenium dry mass accumulation was observed to be highly significantly less (P<0.05) when growing on *P. maximum* plots as opposed to growing on *E. curvula* or *D. eriantha* plots. No significant differences were observed for parthenium dry mass accumulation for plants growing on plots containing the latter two grass species. In the following season, parthenium control plots at the 5 parthenim m^{-2} and 7.5 parthenium m^{-2} densities were included in the trial in order to allow for percentage of control data analysis. In the 2004/2005 growing season, *P. maximum* once again outperformed the other two grass species. *E. curvula* and *D. eriantha* performed far better than in the previous season, however, after having become better established, showing two- and four-fold increases in dry mass accumulation, respectively. For grass dry mass accumulation percentage of control, the main species effect was found to be significant, with *P. maximum* performing significantly better than *E. curvula*. For the second growing season, *P. maximum* once again most effectively interfered with parthenium growth. Parthenium plants growing together with *P. maximum* were observed to produce less seed relative to plants growing on adjacent plots, and in some instances parthenium plant mortalities occurred. *D. eriantha* and to a lesser extent, *E. curvula*, were only able to interfere with parthenium growth significantly at the 5 plans m^{-2} density. After the second season it was confirmed that *P. maximum* was the most suitable species to interfere with *P. hysterophorus* growth. The species can therefore potentially be used as an antagonistic species in an integrated control programme. It is unknown how well the species will establish from seed in a parthenium stand, however, and as the grass is highly palatable, it may have to be protected from grazers, initially at least, in order to allow it to become properly established.

Understanding the production of parthenin in the leaves of *P. hysterophorus* during the life-cycle of the plant is important for understanding the employment of this sesquiterpene lactone in the allelopathic interference strategy of the plant. Belz *et al.* (2006) observed differences in parthenin concentrations from leaves of the same parthenium plants harvested at different stages of growth. In this study it was observed that parthenin leaf concentration increased with plant age. At senescence, parthenium leaf dry mass was observed to contain a parthenin concentration of 34.7

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mg g^{-1}. Considering other plant parts, especially the flowers and achenes have also been observed to contain parthenin, it was calculated that under the experimental conditions, a single, mature parthenium plant has the potential of introducing an amount greater than 236.15 mg of parthenin into the environement in a single growth season. Belz *et al*. (unpublished) determined a parthenin concentration of 16.7 ± 1.8 mg g^{-1} in the dry leaves from flowering plants growing in the Kruger National Park. This corresponds with concentrations observed in this experiment for leaves from plants at the bud formation to beginning of flowering stages, indicating that parthenin levels in plants grown in greenhouses reflect those of plants growing in the wild. Attainment of highest parthenin concentration in the final three growth stages of the plant indicates a high resource allocation priority to this secondary metabolite. This accumulation of parthenin may indicate a strategy in which the plant employs residual allelopathy to inhibit or impede the recruitment of other species. Parthenin is also known for its anti-feedant and anti-microbial properties and accumulation of this compound in the plant until after the flowering process has been completed may play an important role in herbivore and pathogen defence.

For parthenin to have a direct phytotoxic effect on other plant species it must be available in the soil for plant uptake at sufficiently high concentrations. The fate and persistence of this compound in the soil will therefore be an important factor (Inderjit, 2001). Preliminary experiments showed that parthenin is easily degradable in soil, and parthenin added to the soil at concentration of 1 and 10 µg g^{-1} degraded faster than when added at a concentration of 100 µg g^{-1}. For the main experiment, the DT_{50} value for parthenin added at an initial concentration of 10 µg g^{-1} in the CS soil incubated at 20, 25 and 30ºC for sterilized soil was significantly higher in all circumstances than for non-sterilized soils. This may indicate that microbes play a predominant role in parthenin degradation. Furthermore, for non-sterilized soils, parthenin degradation occurred significantly faster in soil incubated at 30ºC than in soils incubated at 25 and 20ºC, with DT_{50} values of 1.44, 2.29 and 2.98 days, respectively. A significant correlation between temperature and DT_{50} and DT_{90} values for non-sterilized soils, but not for sterilized soils was observed. Microbial degradation may play an important role in preventing allelochemicals from reaching phytotoxic levels in natural soils (Schmidt & Ley, 1999). Analysis of parthenin degradation in different soil types showed that parthenin degradation occurred fastest in the loam soil and slowest in the

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sand. Significant difference for DT_{50} values were observed between the loam soil (3A) (1.78 days) and the very loamy sand (CS) (3.10 days), and the loam soil and sand (2.1) (3.64 days). No significant differences in DT_{50} values between the sandy loam (5M) (2.67 days) and any of the other soils was observed. Significant negative correlations for the 3A, 5M and 2.1 soils occurred between DT_{50} values and water holding capacity as well as soil cation exchange capacity, but not between DT_{50} values and soil pH and organic carbon percentage. Lack of correlation for the latter two parameters can possibly be attributed to similar pH values and low levels of carbon in the soils. Further research focuses should be aimed at determining parthenin concentrations in natural soils containing *P. hysterophorus* infestations, and investigating further concentration effects on parthenin degradation; as well as investigating the ability of varying microbial species populations found in different areas of the world on parthenin degradation.

Pure parthenin was observed to have a phytotoxic effect on *E. curvula*, *P. maximum* and *D. eriantha*. Only the sensitivity of *E. curvula* to pure parthenin had previously been assessed (Belz *et al*., 2006). Of the three grass species, *P. maximum* was observed to be the most sensitive species regarding germination percentage and radicle growth, followed by *D. eriantha* and then *E. curvula*. ED_{50} values for radicle length were 100.6, 144.7, and 212.9 µg ml^{-1}, respectively. Radicle length was observed to be the more sensitive parameter than germination percentage, as has been reported for other test species (Batish *et al*., 1997, 2002b; Belz *et al*., 2006). For *P. maximum* and *D. eriantha* complete inhibition of germination and radicle development occurred at parthenin concentrations of 300 and 500 µg ml^{-1}, while complete inhibition of germination did not occur for *E. curvula* across the concentration range tested. *P. maximum* displayed highest efficacy in interfering with *P. hysterophorus* growth in the field, but the relatively high sensitivity of *P. maximum* to pure parthenin may indicate that it will be challenging to establish *P. maximum* from seed in areas already infested with *P. hysterophorus.*

From work completed in this study it seems plausible that parthenin may have a phytotoxic effect on other plant species under natural conditions. Many further studies will be required to enable modelling that will more accurately determine the role of parthenin in *P. hysterophorus* allelopathy under natural conditions, however.

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Further objectives of this ongoing study are continuation of work to study the role of parthenin in *P. hysterophorus* allelopathy, and the long-term monitoring of *P. hysterophorus* spread in the Kruger National Park.

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Summary

The allelopathy of *Parthenium hysterophorus* may contribute significantly to the invasive potential of the plant. The allelochemical, parthenin, is hypothesized to play a leading role in the allelopathy of the weed and this study was conducted to further investigate the importance of parthenin in *P. hysterophorus* allelopathy; and to investigate the interference potential of the weed with indigenous grass species.

The first trial of the investigation involved a study on the interference of *P. hysterophorus* with three indigenous grass species, namely, *Eragrostis curvula*, *Panicum maximum* and *Digitaria eriantha*, under field conditions. The trial was established on an old dumpsite at Skukuza, in the Kruger National Park, where there is a naturally occurring *P. hysterophorus* infestation. Plots containing one of the grass species planted at a single density (16 tufts m^{-2}), and the weed planted at three different densities (0, 5, 7.5 plants m^{-2}), were first established in the 2003/2004 growth season and observed for two seasons. Grass and *P. hysterophorus* dry mass accumulation was monitored and subjected to statistical analysis. *P. maximum* clearly outperformed the other two grass species from the outset and was observed to be the species most adapted to the environmental conditions of the trial, especially soil pH. In the first growth season (2003/2004), despite considerably greater dry mass accumulation by *P. maximum* relative to the other grass species, significant differences ($P \leq 0.075$) for percentage of control data was only observed between *P. maximum* at both the 5 and 7.5 plants m^{-2} and *D. eriantha* at the 7.5 plants m^{-2} density. For *P. hysterophorus* dry mass data, the main species effect was observed to be significantly different with *P. maximum* significantly inhibiting the growth of *P. hysterophorus*. In the second growth season (2004/2005), *P. maximum* once again displayed the best performance, although the performance of the other two species greatly improved with increased adaptation to the environmental conditions. For percentage of control data, the main species effect was found to be significant ($P \leq 0.05$), with *P. maximum* performing significantly better than *E. curvula* across the two *P. hysterophorus* densities. For the second growth season (2004/2005), *P. hysterophorus* control plots were included in the trial and it was observed that all three grass species were able to interfere significantly with *P. hysterophorus* growth.

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As in the previous season, *P. maximum* was observed to interfere with *P. hysterophorus* growth most effectively and weed plants growing on the *P. maximum* plots were observed to produce less seed, and a large number of weed mortalities were observed. It was concluded that *P. maximum* therefore shows high potential for use as an antagonistic species in an integrated programme for control of *P. hysterophorus.*

In the second trial the production dynamics of parthenin over the life-cycle of *P. hysterophorus* was studied. Plants were grown in a greenhouse at the University of Hohenheim in Stuttgart, Germany, and the parthenin content in leaves harvested at different growth stages was monitored. Highly significant differences were observed for parthenin concentration in the leaves at different phenological stages. Highest parthenin concentrations occurred in the final three growth stages of the plant and it was calculated that a single plant can introduce >267.19 mg parthenin into the environment in a single growing season. This build-up of allelochemical (parthenin) content with age in the leaves may indicate that the plant utilizes residual allelopathy in its interference strategy, which may be aimed at limiting the recruitment of other or the same species.

In the third trial, the persistence of pure parthenin in soil was investigated. Four soils with different properties were utilized for the trial, and parthenin DT_{50} values were observed to range from 1.78 to 3.64 days when applied at an initial concentration of 10 μg g^{-1}. Degradation of parthenin was observed to be significantly faster in the loam soil than in the loamy sand or sand. Significant negative correlations were observed between DT_{50} values and the soil characteristics of soil water-holding capacity and soil cation exchange capacity, but not between DT_{50} values and pH and organic carbon percentage. Persistence of parthenin was also investigated in sterile and non-sterile loamy sand placed under different temperature regimes, and it was observed that parthenin degraded significantly faster in the non-sterilized soils, indicating that microbial degradation may play a predominant role in the disappearance of parthenin from soil. A significant correlation between DT_{50} values and temperature was only observed for non-sterilized soils.

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In the fourth trial, the sensitivity of the three indigenous grass species used in the field trial to pure parthenin was assessed. Seeds were placed in Petri dishes and exposed to a parthenin concentration range (0-500 µg ml^{-1}). It was observed that *P. maximum* was the most sensitive species regarding germination and early radicle development. *D. eriantha* was the intermediate species, while *E. curvula* was the least sensitive species to pure parthenin. ED_{50} values for radicle length and germination, respectively, were 100.6 and 96.1 µg ml^{-1} for *P. maximum*, 144.7 and 184.2 µg ml^{-1} for *D. eriantha*, and 212.9 and 345.9 µg ml^{-1} *for E. curvula*.

Based on the findings from these trials it was calculated that a naturally occurring *P. hysterophorus* stand in Skukuza could potentially introduce a concentration of 2350 µg ml^{-1} in the top 2 cm layer of the soil. It therefore seems possible that parthenin alone can inhibit or impede the recruitment of indigenous grass species using allelopathy. It is acknowledged that allelochemicals other than parthenin may also be important in the allelopathy displayed by *P. hysterophorus*, and that competition by the weed is probably another important interference mechanism. Considering the sensitivity of *P. maximum* to parthenin, it may prove challenging to establish the grass from seed in *P. hysterophorus* stands when using the grass in an integrated control programme.

This ongoing study will continue to investigate the role of parthenin in *P. hysterophorus* allelopathy. The spread of this invader in the Kruger National Park will also be monitored.

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APPENDIX

Appendix 2.1 Abbreviated ANOVA table for the effect of species and parthenium density on grass dry mass accumulation over a period of 11 weeks expressed as percentage of control

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	8.07834260	1.61566852	4.65	0.0119
Error	13	4.52147834	0.34780603		
Corrected Total	18	12.59982094			
	R^2	C.V	Root MSE	Mean	
	0.641147	13.39973	0.589751	4.401215	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
spp	2	6.90293438	3.45146719	9.92	0.0024
par	1	0.62146505	0.62146505	1.79	0.2042
spp*par	2	0.15566820	0.07783410	0.22	0.8025

Appendix 2.2 Abbreviated ANOVA table for the effect of species and parthenium density on grass re-growth dry mass accumulation over a period of 4 weeks expressed as percentage of control

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2.07973276	0.41594655	0.43	0.8162
Error	11	10.55043148	0.95913013		
Corrected Total	16	12.63016423			
	R^2	C.V	Root MSE	Mean	
	0.164664	22.40880	0.979352	4.370391	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
spp	2	1.92697603	0.96348801	1.00	0.3975
par	1	0.00485808	0.00485808	0.01	0.9445
spp*par	2	0.22441916	0.11220958	0.12	0.8907

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Appendix 2.3 Abbreviated ANOVA table for the effect of species and parthenium density on grass dry mass accumulation over a period of 19 weeks expressed as percentage of control

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4.96181143	0.99236229	2.44	0.0908
Error	13	5.29132570	0.40702505		
Corrected Total	18	10.25313712			
	R^2	C.V	Root MSE	Mean	
	0.483931	18.09478	0.637985	3.525797	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
spp	2	2.07445954	1.03722977	2.55	0.1165
par	1	0.27947773	0.27947773	0.69	0.4223
spp*par	2	2.58295902	1.29147951	3.17	0.0755

Appendix 2.4 Abbreviated ANOVA table for the effect of grass species and parthenium density on parthenium dry mass accumulation over a period of 19 weeks

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	4840.794912	968.158982	8.27	0.0011
Error	13	1522.516667	117.116667		
Corrected Total	18	6363.311579			
	R^2	C.V	Root MSE	Mean	
	0.760735	25.87703	10.82205	41.82105	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
spp	2	4010.139394	2005.069697	17.12	0.0002
par	1	115.055217	115.055217	0.98	0.3397
spp*par	2	441.293333	220.646667	1.88	0.1912

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Appendix 2.5 Abbreviated ANOVA table for the effect of species and parthenium density on grass dry mass accumulation over a period of 14 weeks expressed as percentage of control

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1.31306050	0.26261210	3.55	0.0474
Error	9	0.66512700	0.07390300		
Corrected Total	14	1.97818751			
	R^2	C.V	Root MSE	Mean	
	0.663769	6.323438	0.271851	4.299102	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
spp	2	0.83822442	0.41911221	5.67	0.0255
par	1	0.37817593	0.37817593	5.12	0.0500
spp*par	2	0.09390834	0.04695417	0.64	0.5519

Appendix 2.6 Abbreviated ANOVA table for the effect of grass species and parthenium density on parthenium dry mass accumulation over a period of 14 weeks

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	1852.346970	264.620996	13.91	<.0001
Error	14	266.391667	19.027976		
Corrected Total	21	2118.738636			
	R^2	C.V	Root MSE	Mean	
	0.874269	36.28217	4.362107	12.02273	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
spp	3	1472.468500	490.822833	25.79	<.0001
par	1	245.707500	245.707500	12.91	0.0029
spp*par	3	323.049093	107.683031	5.66	0.0094

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Appendix 2.7 Skukuza Climatic Data

2004

Month	Ave max temp (ºC)	Ave min temp (ºC)	Rainfall (mm)
January	32.7	21.3	208.2
February	31.6	20.9	153.7
March	29.2	19.5	84.7
April	28.6	16.4	59.6
May	27.5	9.6	0.6
June	25.6	5.2	13.9
July	25.0	5.2	24.3
August	28.5	10.0	6.3
September	29.3	11.5	33.4
October	31.2	16.3	36.2
November	33.1	19.5	252.4
December	32.8	20.2	132.4

2005

Month	Ave max temp (ºC)	Ave min temp (ºC)	Rainfall (mm)
January	33.3	21.9	130.9
February	34.1	20.7	53.6
March	31.9	18.6	52.9
April	30.8	16.2	31.1
May	***	***	6.5

*** data not available

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Appendix 2.8 Field trial soil sample analysis results

	pH (water)	P Bray mg kg^{-1}	Ammonium acetate extractable Ca	K	Mg	Na
			mg kg^{-1}			
Sample 1 (plot 7)	7.5	58.2	4683	422	479	22
Sample 2 (plot 17)	7.9	37.9	4913	1071	455	57

Appendix 2.9 Field trial layout for 2003/2004 and 2004/2005 growth seasons (Factorial experiment: 3 grasses × 3 parthenium densities × 4 replicates)

36	**35**	**34**	**33**	**32**	**31**	**30**	**29**	**28**
19	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**10**
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

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2004 Growth season

Species	Parthenium m^{-2}	Plot no.	Species	Parthenium m^{-2}	Plot no.	Species	Parthenium m^{-2}	Plot no.
E.curvula	0	32	***P. maximum***	0	36	***D. eriantha***	0	19
	0	29		0	24		0	16
	0	7		0	3		0	1
	0	5		0	11		0	31
	0	15		0	8		0	25
	0	23		0	28		0	26
	5	7		5	9		5	18
	5	17		5	20		5	33
	5	27		5	4		5	6
	7.5	13		7.5	12		7.5	35
	7.5	14		7.5	34		7.5	21
	7.5	10		7.5	30		7.5	22

2005 Growth season

Species	Parthenim m^{-2}	Plot no.	Species	Parthenium m^{-2}	Plot no.	Species	Parthenium m^{-2}	Plot no.
E.curvula	0	32	***P. maximum***	0	36	***D. eriantha***	0	19
	0	5		0	24		0	16
	0	7		0	3		0	1
	0	23		0	11		5	18
	5	17		0	8		5	33
	5	15		0	28		5	6
	5	2		5	9		7.5	26
	7.5	13		5	20		7.5	21
	7.5	14		5	4		7.5	31
	7.5	10		7.5	12			
				7.5	34			
				7.5	30			

Species	Parthenim m^{-2}	Plot no.
Parthenium	5	22
	5	27
	7.5	25
	7.5	29
	7.5	35

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Appendix 3.1 Abbreviated ANOVA table for the effect of growth stage on leaf parthenin concentration

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	59.01385049	5.36489550	22.33	<.0001
Error	64	15.37696962	0.24026515		
Corrected Total	75	74.39082010			
	R^2	C.V	Root MSE	Mean	
	0.793295	22.69907	0.490168	2.159421	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
growth stage	11	59.01385049	5.36489550	22.33	<.0001

Appendix 5.1 Phytotoxicity of parthenin on five different plant species (Taken from Belz *et al*., 2005)

Species	ED_{50} [a] [µg ml^{-1}]	
	root length	germination
Ageratum conyzoides	51.8 (38.7-64.8) [b]	289.9 (253.7-326.2)
Echinochloa crus-galli	220.6 (200.8-240.4)	645.8 (514.3-777.2)
Eragrostis curvula	167.8 (146.0-189.7)	491.3 (396.2-586.3)
Eragrostis tef	226.7 (200.6-252.8)	687.5 (211.5-1163.5)
Lactuca sativa	328.4 (296.4-360.3)	450.4 (399.4-501.5)

[a] concentration causing 50% response; [b] asymptotic 95% confidence interval.

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Appendix 5.2 Calculation of potential parthenin concentration in soil

Amount of soil in top 10 cm of 1 m^2: 100 cm x 100 cm x 2 cm
= 20 000 cm^3

For the 2.1 soil (sand):

Weight per volume (g 1000 ml^{-1}) → 1390 ± 37
Water holding capacity (g 100 g^{-1}) → 34.7 ± 5.0

Mass of 2.1 soil: 20 000 cm^3 x 1.39 g
= 27 800 g

Water holding capacity (g 100 g^{-1}) → 34.7 ± 5.0

H_2O in 2.1 @ 100% WHC = 9646.6 ml
H_2O in 2.1 @ 40% WHC = 3858.6 ml

1 parthenium plant (maturity) → 236.1 mg parthenin * 0.4 = 94.44 mg parthenin

Assuming a stand of 96 parthenium plants m^{-2} → 9.07 g parthenin

Therefore potential [parthenin] in top 2 cm of soil @ 40% WHC →
9.07 g/3859 ml
= 9070 mg/3859ml
= 2.35 mg ml^{-1} = 2350 µg ml^{-1}