

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.

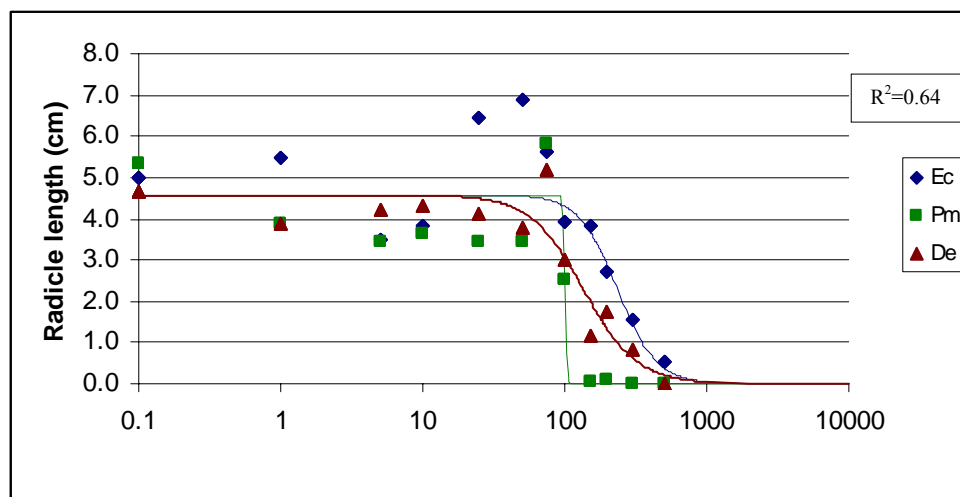
5.2 Materials and Methods

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 $\mu\text{g g}^{-1}$. 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 20/30°C 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 ($P=0.05$).

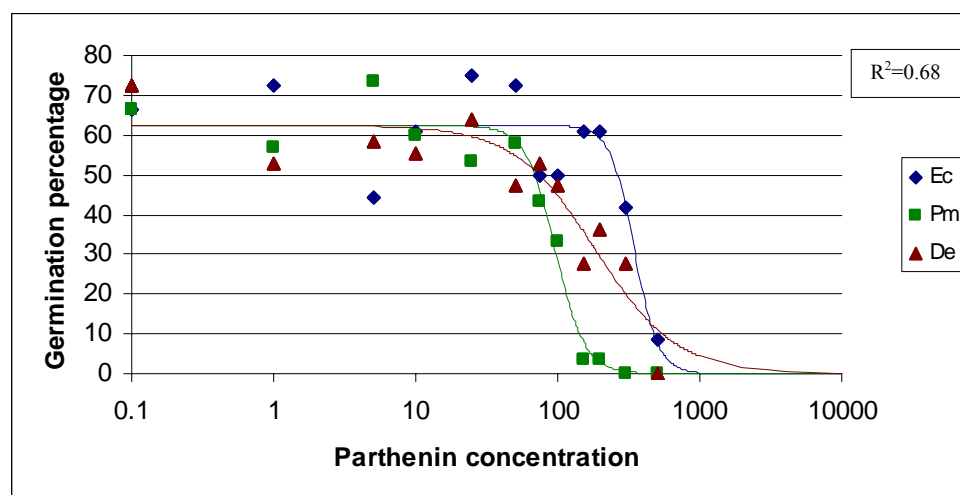
5.3 Results and Discussion

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 ED₅₀ 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 $\pm 100 \mu\text{g ml}^{-1}$ concentration. The reason for this is not clear. Complete germination inhibition and radicle development occurred at a concentration of $300 \mu\text{g}$

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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 ED₅₀ values for germination percentage and radicle length at 491.3 and 167.8 µg ml⁻¹, respectively. Differences in ED₅₀ 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

Species	ED ₅₀ (µg ml ⁻¹)	
	Radicle length	Germination
<i>E. curvula</i>	212.9a	345.9a
<i>D. eriantha</i>	144.7b	184.2b
<i>P. maximum</i>	100.6c	96.1c

Means followed by different letters differ significantly (E-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

PURE PARTHENIN BIOASSAY

parthenin under completely natural conditions is of primary importance in understanding the role of this secondary metabolite in *P. hysterophorus* 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 $2350 \mu g ml^{-1}$ 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 20°C, 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.

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 than germination percentage for the three grass species. Based on the findings for parthenin production dynamics in *P. hystrophorus* 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 phytotoxicity of parthenin under natural conditions. Knowledge gaps include the release mechanism of parthenin from the plant and the fate of parthenin in the soil.