CHAPTER 5

EFFECTS OF INVASION AND CLEARING OF EXOTIC SPECIES ON SOIL SEEDBANKS AND REGENERATION

5.1 Introduction

The main mechanism of plant regeneration, other than vegetative regeneration through resprouting, is through the dispersal and establishment of new individuals via seed propagules. The regeneration of plant communities from seed depends on the seeds being in the right place at the right time while in the right physiological condition to germinate (Murdoch & Ellis 1992). In some species these requirements are satisfied by a regeneration strategy in which seeds germinate as they are shed. In others, seeds may remain dormant for periods of time and thus forming a seed bank.

When considering an individual seed in a seed bank one of three fates exist: emergence, persistence or disappearance by predation or death (Murdock & Ellis 1973). It is the proportion (or demography) of these fates that determine the reproductive success of a species in a particular environment or community. Reproductive success can be seen in two phases: pre-emergent (the number of seeds that enter the ambient environment) and post emergent (the percentage of progeny that survive to reproduce) (Wiens *et al.* 1987). The post emergent phase is typically used as a measurement of reproductivity, although it must still be taken into consideration that a number of survival limiting variables still occur.

5.1.1 Germination cues

Some seeds are able to stay in a quiescent state for a long period, and resume a normal metabolic rate under suitable conditions. It is these conditions that determine when a seed geminates. Generally, quiescent seeds need to be hydrated under conditions, which promote metabolism (oxygen and temperature) (Bewley & Black 1986). A number of other germination cues may determine seed germination. Examples of this are seed coat imposed dormancy (Baskin & Baskin 1989; Pierce & Moll 1994); animal digestion (Choinski & Tuohy 1991; Gardener *et al.* 1993); fire (Bewley & Black 1986; Bell *et al.* 1993; Bradstock & Auld 1995); smoke (Sutcliffe & Whitehead 1995) and density dependant germination (Smith 1983). Within the communities covered in this study the germination cues of light, temperature and density dependent germination would be expected to be prevalent.

5.1.2 Seed size and appearance

Seed size, shape and appearance is considered an important ecological attribute in seed persistence,

dispersal and survival. It has been observed that there is a connection between persistence in a seed bank, and seed size and shape (Thompson & Grime 1979; Garner & Witkowski 1997). Short-lived seeds tend to be larger, flattened and more elongate. Alternate studies have shown that other variables relating to persistence and seed morphology also occur (Stock *et al.* 1990; Witkowski & Garner 2000). One aspect is clear in all of the above-mentioned studies, burial is clearly an essential prelude to persistence (Thompson *et al.* 1993).

5.1.3 Seed attrition

Seed decay in the soil, especially in moist environments is a large source of seed bank attrition (Harper 1977). Age is another factor, as seeds become older there is more likelihood of death and hence attrition of the seed bank (Auld 1995). Depth of burial may be instrumental, as the successful germination of seeds is limited to the shallow depths of topsoil depth where germination cues still have effect and germination of seeds allows the seedling to reach the soil surface. It is for this last reason alone that disturbance, especially disturbance to the soil surface, plays such a large role in the community. Soil moisture, bacterial activity and temperature all play a role in the rate of seed decay in the soil. These variables are all altered by the biotic environment, which is subject to disturbances like invasion and clearing of vegetation. Thus, the seed bank and regeneration of seed reliant species is susceptible to this type of disturbance.

5.1.4 The role of seed banks in cleared and invaded communities

Any attempt to understand the population dynamics of a species must include factors that affect the fecundity of that species (Smith 1983). The spatial pattern of a species and its population size and density, depend on the dispensability of its seed and on the pattern and degree of seed germination, seed and seedling mortality (Smith 1983; Witkowski 2002). Thus, no investigation of the population of a plant species or community is complete without some understanding of its population dynamics during the seed stage of its life cycle.

Seeds are one of the principal means by which weed species spread and invade new and disturbed areas. It has been shown that annual seed production of invasive alien species usually exceeds that of indigenous vegetation (Rutherford *et al.* 1991), and a single weed plant commonly produces vast numbers of seeds, which can “infest” the soil by the hundreds (Anderson 1982; Kruger *et al.* 1991). Where natural vegetation is disturbed, it is apparent that the first plants to appear are pioneer species and typically of a weedy nature. Most of these plants have been found to arise from seeds lying dormant in the soil (Anderson 1982).

The species which dominate the seed bank within a community have an advantage when disturbance

creates regeneration opportunities for recruitment (Bond & Slingsby 1984). The world-wide problem of invasion of natural vegetation by alien plants may thus be seen as the large scale demonstration of the advantage that many weed species derive from seed or persistent propagules in the soil (Milton 1980). There is also evidence that compositional gradients in the seed bank and above-ground vegetation are highly correlated (Looney & Gibson 1995). Thus, in certain situations invasive species with the greatest number of viable seeds could become dominant and eventually replace the original vegetation (Dean *et al*. 1986), even after a disturbance to the standing vegetation.

5.1.5 Clearance and regeneration from soil-stored seed banks

The availability of resources (light, nutrients, water) plays not only a role in the seed persistence in the seed bank (Witkowski & Wilson 2000), but also reaches a peak soon after the removal of exotic species vegetation (Canham & Marks 1985). Consequently, the first plants established after a disturbance should encounter greater availability of resources, improving the probability of survival and establishment in the community. Species with seeds already in the disturbed environment have an advantage over species that are some distance away as they do not have the delay of dispersal (Leck 1989). Hence, seed banks have an important influence on plant succession (Leck 1989). The nature and severity of the disturbance will however determine the response of the seed bank through germination cues, resources and habitat modification to name but a few (McGee & Feller 1993).

Although the clearing of invasive species has, and is currently taking place, soil seed banks of exotic plants play an important role in re-invasion of cleared areas, both in terms of re-establishment of indigenous vegetation and follow up clearing operations (re-invasion). The objective of this chapter is to determine soil seed banks of indigenous and exotic species as a function of the degree of invasion prior to and after clearing, looking at seed bank composition and distribution with depth. In addition, further studies on seed bank persistence in *S. mauritianum* and *A. mearnsii* were done.

5.2 Materials and Methods

5.2.1 Field Sampling

Samples were situated within the sample design for vegetative sampling (refer to Chapter 2: Field experimental design). Sampling sites were identified within the 20 x 50 m quadrats using a random number table.

5.2.2 Soil Seed Bank Sampling

One hundred and sixty soil seed bank samples (four random samples per 20 m x 50 m plot) were collected in October & November 1996. Sampling used a fixed area steel quadrat of 30 by 30 cm area

and 10 cm in depth. The large quadrat size was justified by the low seed densities found in the soil (opposed to that typically seen in alien *Acacia* spp. (up to 900 per m^2 / cm depth)) observed by Holmes *et al.* 1987). Sample depth layers were marked at one cm intervals on the quadrat sides, thus enabling vertical sub-samples to be taken. Three vertical sub-samples were collected using a flat edged spade. The three vertical sub-samples consisted of a litter sample of all loose material on the surface, a 0-2 cm depth layer and a 2-4 cm depth layer.

Other studies have shown that more than 90 % of the soil seed banks are to be found in the surface and 0 - 4 cm depths (O' Connor, 1991; Garner & Witkowski, 1997; Custers, Witkowski & Shackleton, unpublished). This was in accordance with observations from Grice & Westby (1987) and Milton & Hall (1981) who stated that seeds are probably not buried deeper than 5-6 cm or 8 cm in sandy soils. The 4 cm depth level was considered a reasonable cut-off point for all species in this study, as it has been previously observed that seeds buried to greater depths, although viable, will probably not contribute to regeneration, as seedlings appear unable to emerge from depths greater than 4-5 cm. The 2 cm interval in sample depth (rather than 1 cm interval) was used in order to reduce time costs and to overcome non-normality typical of soil seed bank data.

5.2.3 Soil seed bank recovery

In the laboratory, a wet sieve technique was used to remove seeds from sub-samples. For a period of 10 minutes, each seed bank sub-sample (0-2 cm and 2-4 cm sub-samples only), was passed through a graded series of three sieves (namely: 2.0 cm, 1.0 cm and 0.1 cm) sequentially placed in decreasing coarseness on a sieve shaker and subjected to a constant flow of water and constant vibration. Litter samples were hand sorted without being sieved due to the large amounts of vegetation matter found in these samples, making sieving techniques unworkable. Samples were sorted under high light conditions for an average period of 15-20 minutes per sample. All seeds found were air dried and retained for identification. Seed species were identified using the Agricultural Research Committee resources and assistance from the Department of Agriculture Seed Unit. Those seeds that were not identifiable were isolated into groups and given reference names.

Seeds retrieved from the soil were air dried for two months and only intact propagules were counted as individuals constituting the soil seed bank.

5.2.4 Seed bank longevity of *S. mauritianum* and *A. mearnsii*

S. mauritianum seeds were collected from 20 individuals along the upper reaches of the Sabie river catchment during August 1996. These were collected from randomly selected plants and ripe berries still attached to the plant. *A. mearnsii* seeds were obtained from the Institute for Commercial Forestry

Research in December 1996 (ICFR - P.O. Box 100281, Scottsville 3209, Republic of South Africa).

For each species, 50 seeds were sealed in a 5 x 5 cm sachet of 75% Ultra violet (UV) cover shade net. The use of 75% UV shade net was deemed appropriate for two reasons: (a) the weave of the shade cloth was sufficiently fine to prevent seeds from escaping the sachet and; (b) the percentage UV protection offered by the sachet would be roughly the same as that offered by the herbaceous vegetation and litter debris found in the field. Twenty-one caches consisting of three sachets each, which were located at 0, 1 and 3 cm depths, were placed in the field. Twenty-one caches were also established in the greenhouse trial, but these caches consisted of two sachets each placed at the 0 and 1 cm depths.

The field trial consisted of one variable parameter, light, being used as shaded and unshaded treatments. Field experiments were located in the upper catchment of the Sabie river next to plot #1 (high altitude, cleared) (as the unshaded site), and below Horse-shoe Falls in uninvaded indigenous vegetation (as the shaded site) (for map location refer to Appendix 2). In the greenhouse trial, two variables were investigated, namely light and water. The light regime was varied as in the field trail with an unshaded (full sun) and a shaded (partial to full shade) treatment. For each of these treatments a high and a low watering regime was observed as the secondary variable.

Greenhouse high water regime caches received 494.8 ± 4.71 (S.E.) ml of water per week in daily application (± 70.69 ml/day), and low water regime caches received one 50 ml application once a week (the equivalent of ± 7.14 ml/day). Greenhouse temperatures reached a summer maximum and minimum of 44°C and 18°C respectively. In winter a maximum and minimum of 26°C and 5°C was recorded. This closely approximated the temperatures observed in the field sites. The greenhouse was located at the University of the Witwatersrand, Johannesburg.

The trials to determine the seed bank persistence of *S. mauritianum* and *A. mearnsii* were initiated in October 1996. An initial viability test for both species was done on the collected seed stock before initiation of the trials by randomly selecting seeds from the collected populations to be used in the experiment. This was done in order to determine the starting viability of the seed populations being used. Sampling intervals for seed persistence in the trial were once every three months with the first samples being retrieved in January 1997. Three caches per treatment per species were collected at each sample collection for both the field trial and the greenhouse trial over a 13-month period.

5.2.5 Seed persistence experiment viability testing

Once collected from the greenhouse / field, seeds were removed within 48 hours from each cached

sachet and counted. Seeds were cleaned under running water and allowed to air dry for a further 24 hours. Seeds were then scarified to allow seed imbibition to occur before viability testing. Different techniques of seed coat scarification were applied to each species. For *A. mearnsii,* seeds were placed on a hard board with the broadest (dorsal) side facing up. A hot soldering iron was touched to this surface for approximately 2 seconds. Seeds scarified using this technique required care in not damaging the embryo, and scarification was applied well away from the microphyle of the seed where the embryo is situated. *S. mauritianum* seed testas were scarified at the broadest diameter of the seed, away from the embryo's location, using a scalpel. Care was taken in both species to not damage any of the tissue surrounding the embryo (including the embryo itself). Seeds were tested for viability using tetrazolium staining (See section: 5.2.7 Tetrazolium staining).

5.2.6 *S. mauritianum* seed production

A random sample of 50 *S. mauritianum* plants of varied sizes were sampled along the upper altitude study area of the Sabie river. Each plant was noted for height using a clinometer. The number of inflorescences / infructescences on the plant were then counted and collected. The number of fruit/berries per inflorescence for that plant was also counted. A random sample of 30 ripe berries were then dissected and the number of seeds present per berry counted under high light conditions.

5.2.7 Tetrazolium staining

An aqueous solution of 1 % 2,3,5-triphenol tetrazolium chloride of pH 6.5-7.5 was used with no buffer required (ISTA 1996). Seeds were pre-moistened at 20°C for 22 hours (ISTA 1996). Staining of seeds was accomplished over a 24 hour period, in a dark cupboard, with seeds completely submerged in tetrazolium. No fungicides or antibiotics were used to control infection as very little infection was observed in preliminary trials.

After staining and imbibition a longitudinal cut was made through the middle of each embryonic axis using a scalpel in order to observe seed viability. Seeds were evaluated as either viable or non-viable based on the staining of the embryo tissue. Embryo's stained red in viable seeds while in non-viable seeds, no staining or staining of only the cotyledonous tissues was observed. The smaller seeds of *S. mauritianum* were observed for viability using a dissecting microscope (20 x magnification), while the larger seeded species were observed for viability with the naked eye.

5.2.8 Statistical analyses of data

Determination of seed persistence in A. mearnsii and S. mauritianum

Field samples were tested to determine the effects of depth and clearing on seed persistence using two way ANOVA's with repeated measures for each species. The repeated measures in this instance are

the time intervals 0, 3, 6, 9 and 18 months.

Greenhouse samples of *A. mearnsii* and *S. mauritianum* were tested between light intensity, water regime and depth using three way ANOVA's with repeated measures. Once again time intervals are the repeated measure. Ryans Q post hoc test was also used in all ANOVA interpretations for this section.

Soil seed banks

Numbers of viable seeds were obtained for three depth classes in each plot for all species with seeds larger than 0.1 cm in diameter. Soil seed densities were scaled up to 1 m^2 and 1000 m^2 (see section: Equations for determination of *in situ* seed bank characteristics). Data were transformed prior to statistical analyses using a $Log_{10}(X + 1)$ transformation. Three way ANOVA's with repeated measures were used to determine statistical differences for key species, i.e. *S. mauritianum*, *Eucalyptus grandis* and other species, at each depth interval in the soil between altitude, invasion intensity and clearing. Chi-square tests (χ^2) of number of seeds were also done for key species. Key species were identified as those species found present in more than 10 plots and those species deemed important to the clearing programme of the RDP, namely *S. mauritianum*, *Eucalyptus grandis* and *Pinus* spp. Species found in fewer than 10 plots were considered rare, and only the mean and standard errors per plot given. Statistical analyses were completed using SAS and Systat for windows packages. Regression analyses were also done on key species found in the soil seed bank. Propagule numbers were regressed against the invasion intensity for both cleared and uncleared sites.

Seed production of S. mauritianum

Numbers of berries per plant, inflorescences per plant, and seeds per plant were regressed against the standing height of plants in the field. Best-fit relationships from linear, power, logarithmic and exponential curves were determined and the best fit used to determine seed production against standing height.

5.3 Results

5.3.1 Soil seed bank species composition

Soil seed bank species richness

A total of 20 different seed species were distinguished from the soil seed bank sampling. Of these 20 different species, 5 were not identified and two were identified with some uncertainty (Table 5.1). It was found that high altitude sites typically had less species present in the soil seed bank than did low

altitude sites. Clearing of the exotic invasive vegetation appeared to lower the number of species found in the soil seed bank. The only exception to this was for high altitude low invasion sites where a higher number of species were found present in the soil seed bank with clearing (Table 5.2 & Appendix 12).

Soil seed bank species richness with depth

It was found that the number of species present in the soil seed bank increased with burial. However, more seeds were typically found in the 0 – 2 cm depth category than in either the litter or 2 – 4 cm depth categories (Table 5.2). This was also reflected in the number of species, with the 0 - 2 cm depth category having the highest number of seed species present.

5.3.1 Soil seed bank distribution

Total seed bank distribution

Comparing the total exotic soil seed bank (excluding *E. grandis* capsules) to the total indigenous seed bank and total soil seed bank density it can be seen that no clear pattern exists with clearing or any of the experimental categories observed (Figure 5.1). There appeared to be a higher exotic seed density for the low altitude high invasion cleared and uncleared treatment (Figure 5.1). This appeared to be due to the large numbers of *S. mauritianum* seeds found in the soil seed bank for these plots (Table 5.1).

Distribution of key seed species

Only four species of seed were found in the soil seed bank with a high enough frequency in all eight study categories and with depth for further statistical analysis. Other species of seed found in the soil seed bank tended to reflect the same distribution pattern as seen in the four key seed species identified. These species were: *Eucalyptus grandis* (capsules), *S. mauritianum*, *Clutia monticola* and an *Ipomoea* sp. (Table 5.1 & Table 5.3).

The *Ipomoea* sp. did not show any significant difference with invasion whereas the other three species did (Table 5.3). The differences in seed banks for the different species illustrated their life history traits and *E. grandis* and *S. mauritianum* both showed an increase in seed bank density with invasion (Table 5.1). The *C. monticola* showed the opposite effect with invasion, having more seed bank propagules in low invasion communities (Table 5.1). Both the *C. monticola* and *Ipomoea* sp. showed significant differences with clearing, the *C. monticola* seed bank being significantly lower in cleared sites and *Ipomoea* sp. significantly higher with clearing.

For each of the four seed species that occurred in high enough densities in the soil to be statistically compared (*E. grandis* (capsules), *S. mauritianum*, *C. monticola* and *Ipomoea* sp.), a regression curve

against invasion intensity was determined (Figures 5.2 - 5.5). *Ipomoea* sp. seed densities were low throughout with no relation to invasion intensity. With clearing treatment however, a trend of decreased seed density with increased invasion intensity occurred for the propagules of this species (Figure 5.3). *C. monticola* soil seed bank density was higher than *Ipomoea* sp. soil seed bank density but no relationship could be determined with invasion (Figure 5.2).

Solanum mauritianum seed bank density changed with increasing invasion intensity (Figure 5.4). A significant positive linear relationship of *S. mauritianum* seed density with increasing invasion intensity was found (Figure 5.4), with a projected maximum of approximately 300 seeds / m^2 (Figure 5.4) at 100 % invasion intensity. For uncleared plots, *S. mauritianum* seed bank density increased exponentially with invasion intensity, with over 1400 seeds/m^2 projected for 100 % invasion intensity (this was however not a significant relationship) (Figure 5.4). In all relationships, for both cleared and uncleared plots, *S. mauritianum* seed bank density did not start at the origin, but with an initial seed bank density at the 0% invasion category (Figure 5.4). This indicates a seed source outside the measured areas and the dispersal of seeds into these study areas.

In cleared plots, a positive polynomial relationship of *E. grandis* seed capsule density and invasion intensity was obtained, with a maximum projected capsule density in the soil seed bank at 100% invasion of 1000 capsules/m^2 (Figure 5.5). In uncleared plots, a linear relationship of *E. grandis* seed capsule density with invasion intensity was obtained, also with a projected maximum capsule density of around 1000 capsules/m^2 for 100% invasion intensity (Figure 5.5). Both relationships start near the origin with no capsules present at 0% invasion (Figure 5.5), indicating no dispersal of *E. grandis* capsules into uninvaded study plots. This however, does not necessarily mean seed dispersal did not occur.

Distribution of key seed species with depth

Of the four seed species focused upon, only *Ipomoea* sp. did not show a significant difference in propagule numbers with depth (Table 5.5), while all other species analyzed showed a significant increased seed bank with depth. Depth distributions of seeds were found to be significantly different for both *S. mauritianum* and *C. monticola* at the 0 - 2 and 2 - 4 cm depth classes with altitude (Table 5.4). *C. monticola* appears to have a higher number of seeds in the soil seed bank at high altitude while *S. mauritianum* appears to have a larger seed bank at low altitude (Table 5.4).

Table 5.1 Soil seed banks (mean seeds/m^2 ± S.E.) for species with seeds > 1 mm in diameter recovered from litter and 0 - 4 cm depth of burial in the soil. Values with the same superscript letter in each row were not significantly different (Ryan's Q multiple comparison test, $P < 0.05$).

Treatment	High Altitude				Low Altitude			
Species	High Invasion		Low Invasion		High Invasion		Low Invasion	
	Cleared	Uncleared	Cleared	Uncleared	Cleared	Uncleared	Cleared	Uncleared
Eucalyptus grandis (capsules)	467 ± 173^{ab}	1329 ± 279^{a}	372 ± 233^{ab}	113 ± 40^{b}	913 ± 269^{ab}	113 ± 47^{b}	723 ± 330^{ab}	201 ± 122^{b}
Solanum mauritianum	91 ± 30^{ab}	72 ± 30^{ab}	47 ± 14^{b}	66 ± 32^{b}	554 ± 228^{a}	376 ± 93^{a}	81 ± 20^{ab}	152 ± 49^{ab}
Clutia monticola	10 ± 9^{b}	14 ± 5^{ab}	23 ± 9^{ab}	71 ± 30^{a}	6 ± 2^{b}	2 ± 1^{b}	2 ± 1^{b}	20 ± 11^{ab}
Ipomoea sp.	16 ± 7^{a}	3 ± 1^{a}	4 ± 1^{a}	4 ± 1^{a}	17 ± 10^{a}	6 ± 3^{a}	20 ± 6^{a}	10 ± 5^{a}
Senna cf. *septemtrionalis*	0 ± 0	0 ± 0	9 ± 6	0 ± 0	0 ± 0	2 ± 2	1 ± 1	0 ± 0
Acacia ataxacantha	0 ± 0	39 ± 39	8 ± 8	2 ± 1	1 ± 1	1 ± 1	20 ± 15	7 ± 5
Bidens pilosa	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	4 ± 4
Passiflora edulis	2 ± 2	4 ± 2	14 ± 6	6 ± 4	3 ± 2	14 ± 13	3 ± 2	3 ± 2
Smilax anceps	0 ± 0	10 ± 8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2 ± 2
Eriosema sp.	18 ± 8	0 ± 0	14 ± 6	18 ± 6	0 ± 0	1 ± 1	6 ± 3	4 ± 2
Helinus sp.	2 ± 2	2 ± 1	4 ± 2	2 ± 1	0 ± 0	2 ± 2	5 ± 4	14 ± 11
Robinia sp.	17 ± 8	2 ± 2	8 ± 3	5 ± 3	1 ± 1	1 ± 1	0 ± 0	12 ± 12
Pavonia cf. *columella*	19 ± 9	17 ± 10	26 ± 16	362 ± 293	19 ± 18	0 ± 0	43 ± 29	7 ± 3
cf. *Entada* sp.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1
cf. *Turraea* sp.	0 ± 0	1 ± 1	7 ± 5	0 ± 0	3 ± 1	0 ± 0	14 ± 7	10 ± 6
Unknown Fabaceae sp.	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	16 ± 15	0 ± 0
Unknown Poaceae sp.	8 ± 8	2 ± 1	9 ± 8	1 ± 1	10 ± 9	14 ± 9	19 ± 13	4 ± 2
Big Brown seed sp.	0 ± 0	0 ± 0	5 ± 1	0 ± 0	3 ± 2	12 ± 7	3 ± 3	9 ± 8
Big round sp.	0 ± 0	3 ± 2	0 ± 0	1 ± 1	3 ± 2	0 ± 0	11 ± 5	21 ± 12
Round Black sp.	0 ± 0	8 ± 8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 1

Table 5.2 Species richness of the soil seed banks for both total and depth categories for each experimental category.

Treatment	High Altitude				Low Altitude			
Soil Seed bank depth class	High Invasion		Low Invasion		High Invasion		Low Invasion	
	Cleared	Uncleared	Cleared	Uncleared	Cleared	Uncleared	Cleared	Uncleared
Total Soil Sample	11	14	14	12	12	12	15	18
Litter	6	9	9	6	6	6	9	8
0 – 2 cm	8	11	13	10	10	9	13	13
2 – 4 cm	9	10	13	7	9	8	12	13

Table 5.3 Probability values for soil seed banks (litter plus 0 - 4 cm depth) of *E. grandis* (capsules), *S. mauritianum*, *C. monticola* and *Ipomoea* sp. Results from three way ANOVA analyses of invasion (INV), altitude (ALT) and management / clearing treatment (MAN). *d.f.* = 1,32 for all main effects and interaction terms.

Species	ALT	INV	MAN	ALT*INV	ALT*MAN	INV*MAN	ALT*INV*MAN
Eucalyptus grandis (capsules)	0.1078	**0.0104**	0.2325	0.8686	0.0731	0.7069	0.2599
Solanum mauritianum	**0.0001**	**0.0046**	0.9933	0.1108	0.6244	0.3021	0.9141
Clutia monticola	**0.0030**	**0.0201**	0.0514	0.1653	0.3262	0.1712	0.0591
Ipomoea sp.#	0.2016	0.6346	0.1044	0.3925	0.4043	0.6722	0.3705

Not an exotic species

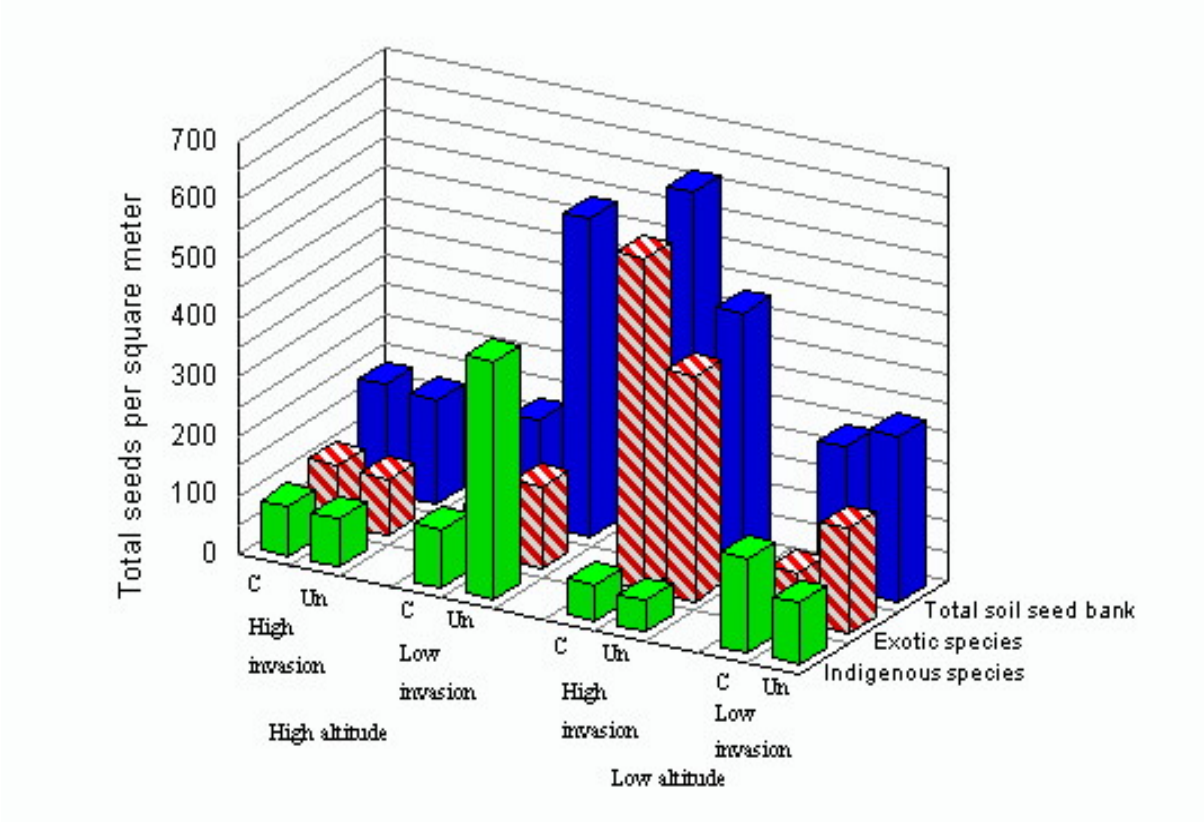


Figure 5.1 Soil seed banks of total indigenous and total exotic propagules (excluding *E. grandis* capsules) for each experimental category. C = Cleared; Un = Uncleared..

Both *S. mauritianum* and *C. monticola* also illustrated some significant differences with invasion intensity at the different soil depths in the seed bank (Table 5.4). *S. mauritianum* seed banks were significantly more abundant at high invasion intensity for both the litter and 2-4 cm depth classes. *C. monticola* soil seed banks at the 0 - 2 and 2 - 4 cm depth classes were significantly more abundant in the low invasion categories (Table 5.4).

Only *C. monticola* and *Ipomoea* sp. illustrated any significant differences with clearing of exotic species and this was only noted in the 2 - 4 cm depth class (Table 5.4). In both instances this reflected the results seen for the total seed bank of these species, with *C. monticola* having a higher abundance of propagules with clearing and *Ipomoea* sp. having the opposite distribution pattern (Table 5.5 & Appendix 13).

5.3.3 Regeneration potential of *S. mauritianum* and *A. mearnsii*

A high initial seed viability occurred for both *S. mauritianum* (78.89 ± 0.23) and *A. mearnsii* (91.89 ± 0.07). For both species (*S. mauritianum* and *A. mearnsii*), there was an initial slow decrease in the

percentage of viable seeds remaining through time for both the greenhouse and the field experiments, which increased, with time (Figures 5.6, 5.7 & 5.8). For *S. mauritianum*, there appears to be a significant decrease in the percentage of viable seeds after 6 – 9 months in all experimental categories, this was greatest in the litter or 0 cm depth class (Figure 5.6 & 5.8).

No differences in *S. mauritianum* seed viability occurred between the high and low light treatments for the 18 month period. High light conditions did however, reduce the number of viable *A. mearnsii* in the litter layer or 0 cm depth class of the seed bank (Figure 5.7). For the greenhouse experiment, no significant differences were apparent for the 0 cm depth class except for *S. mauritianum* under the high light and high water regime (Figure 5.8).

Viable seed half-life for *S. mauritianum* and *A. mearnsii* was calculated using regression relationships (Table 5.6, Appendix 14). Seed half life for *S. mauritianum* in both field and greenhouse trials illustrated that although differing water and light regimes do have different seed half lives in the soil, there is little variance, and half life ranges between 8 ½ months and 13 months (Table 5.6). Greenhouse high light conditions had a greater average seed half life for viable seeds of *S. mauritianum* than low light conditions, except for high water trials where water treatments exceeded that of annual rainfall in the field by almost twice (Trial: 2636 ml/year; Field: 1480 - 1105 ml/year). Under the same watering treatment but under low light conditions, a similar pattern was observed, where high water treatments decreased the average seed half-life of *S. mauritianum* (Table 5.6).

For *A. mearnsii* seeds buried in the soil, seed half-life ranged from 25 years to one year depending on depth of burial, water and light treatment (Table 5.6). Average seed bank half-life for *A. mearnsii* was highest under field high light conditions with a seed half-life of 8 years and 4 months (Table 5.6). Greenhouse high water conditions appeared to decrease the seed half-life for *A. mearnsii*, while low water high light conditions had the highest seed half-life for greenhouse trials (Table 5.6). Depth of burial of seeds tended to increase seed half-life for both *A. mearnsii* and *S. mauritianum* (Table 5.6).

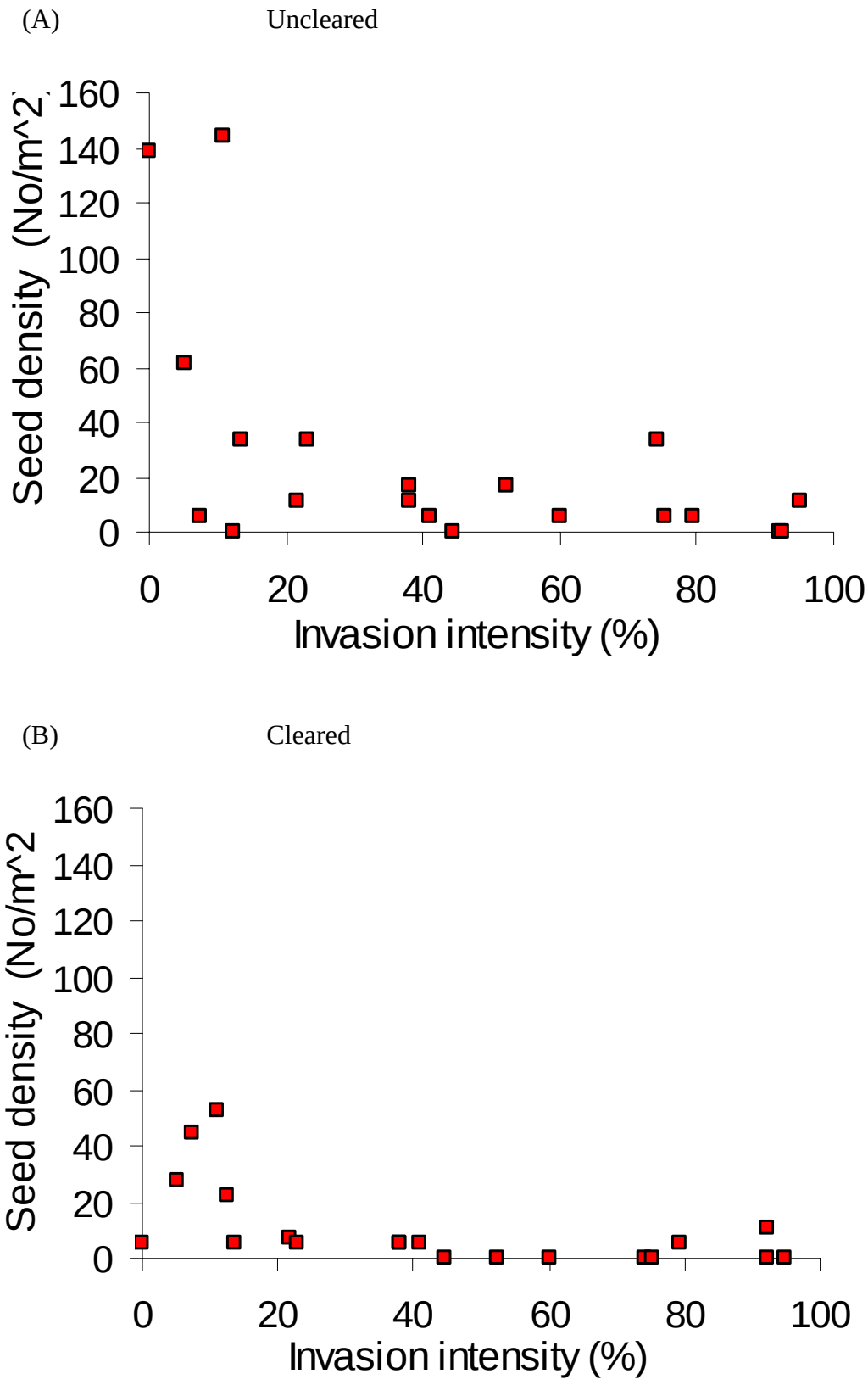


Figure 5.2 The relationship of *Clutia monticola* seed density in the soil with increasing invasion intensity (% aerial cover of exotic plants as calculated in section 4.4.2 of the Vegetation Chapter) on sites; (A) uncleared (r^2 = 0.25; P > 0.05) and (B) cleared of exotic plant species (r^2 = 0.17; P > 0.05) along the Sabie River, South Africa.

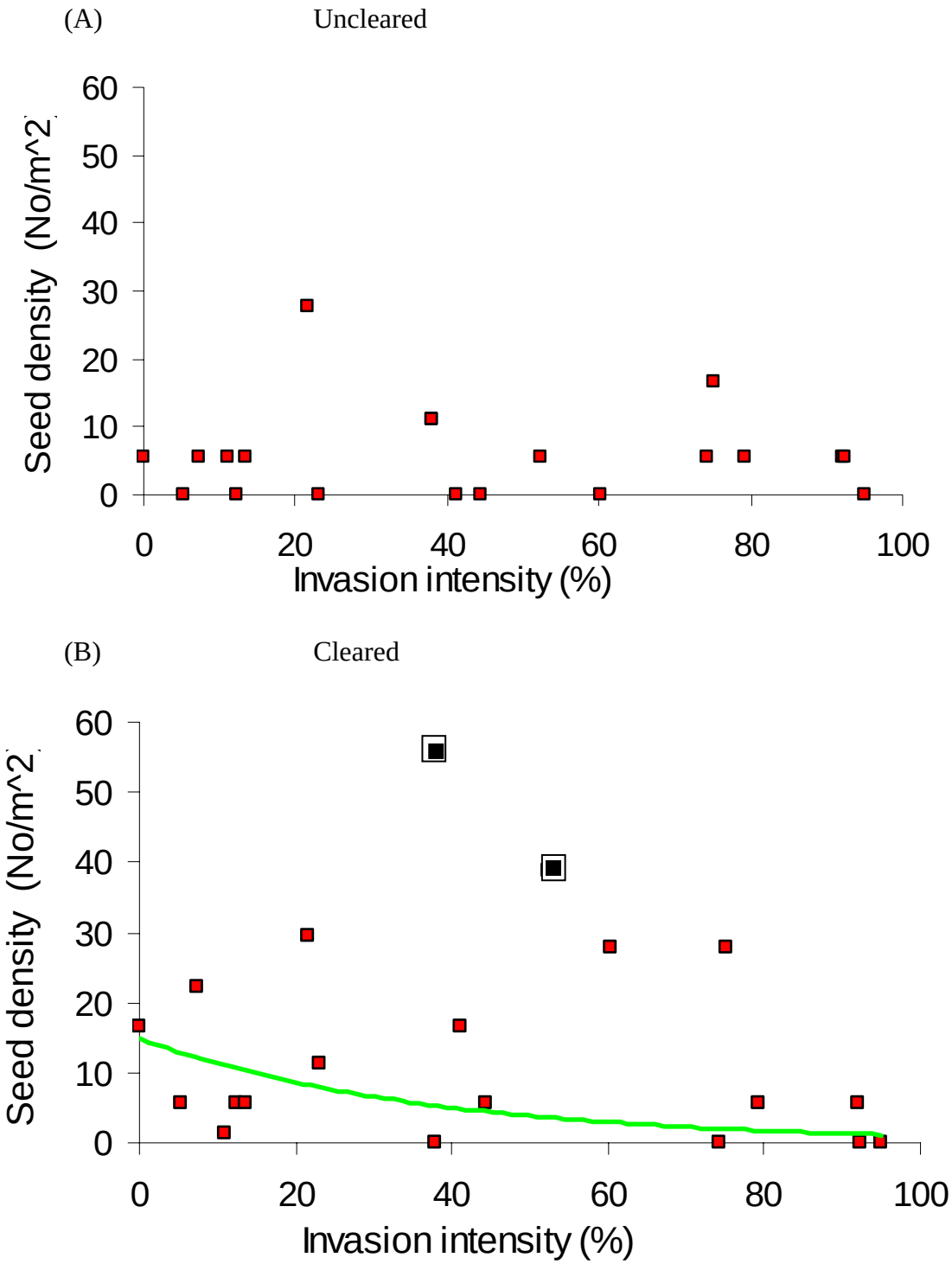


Figure 5.3 The relationship of *Ipomoea* sp. seed density in the soil with increasing invasion intensity (% aerial cover of exotic plants as calculated in section 4.4.2 of the Vegetation Chapter) on sites; (A) uncleared (r^2 = 0.03; P > 0.05) and (B) cleared of exotic plant species (r^2 = 0.21; P < 0.05) along the Sabie River, South Africa. ⊡ indicates outliers not included in regression analyses. Regression relationship (B) $Y = 14.130863^{(-0.006050041 * X)}$.

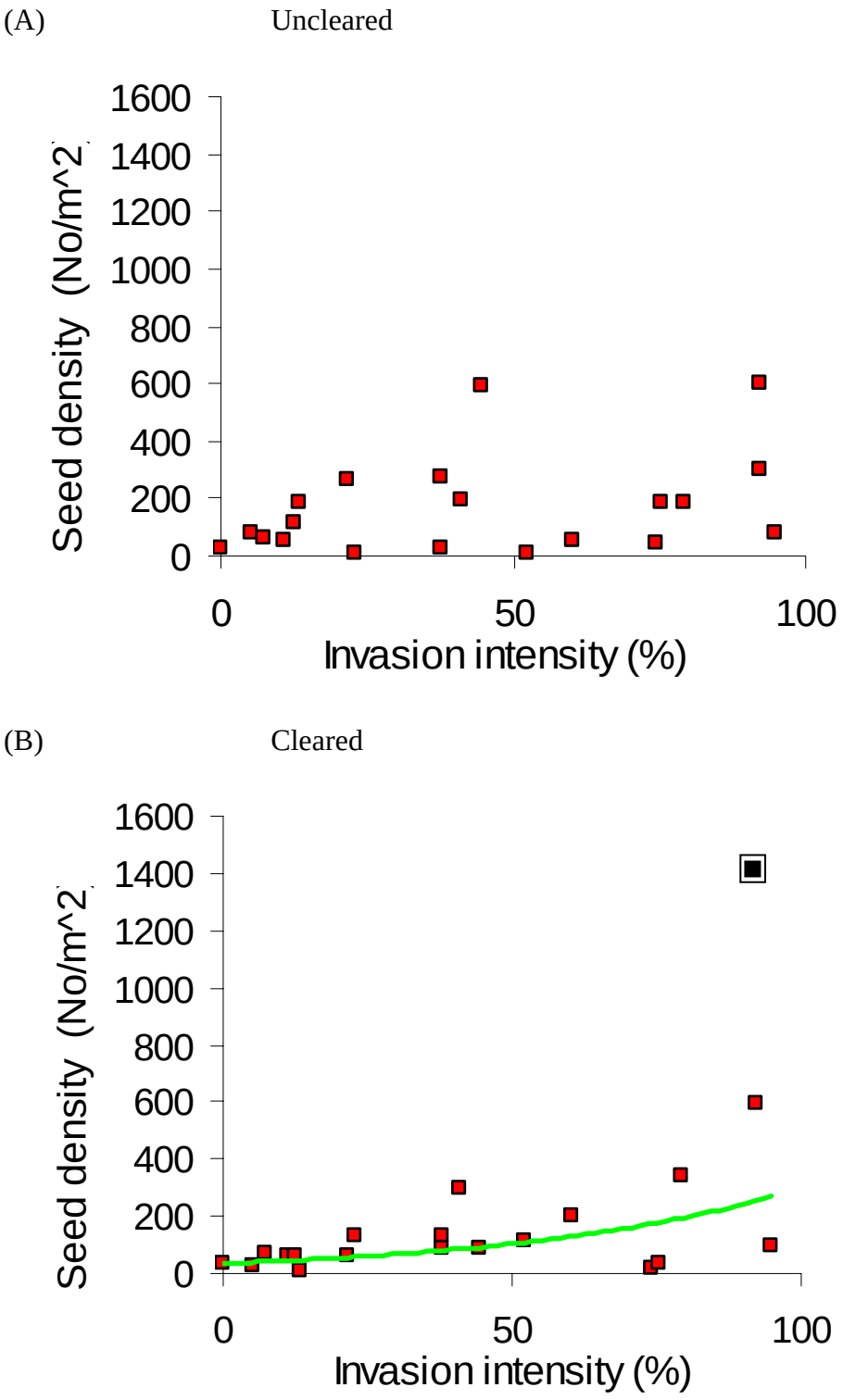


Figure 5.4 The relationship of *Solanum mauritianum* seed density in the soil with increasing invasion intensity (% aerial cover of exotic plants as calculated in section 4.4.2 of the Vegetation Chapter) on sites; (A) uncleared ($r^2 = 0.16$; $P > 0.05$) and (B) cleared of exotic plant species ($r^2 = 0.31$; $P < 0.05$) along the Sabie River, South Africa. ▣ indicates outliers not included in regression calculation. The regression relationship (B) is $Y = 49.94468^{(0.03681499 * X)}$.

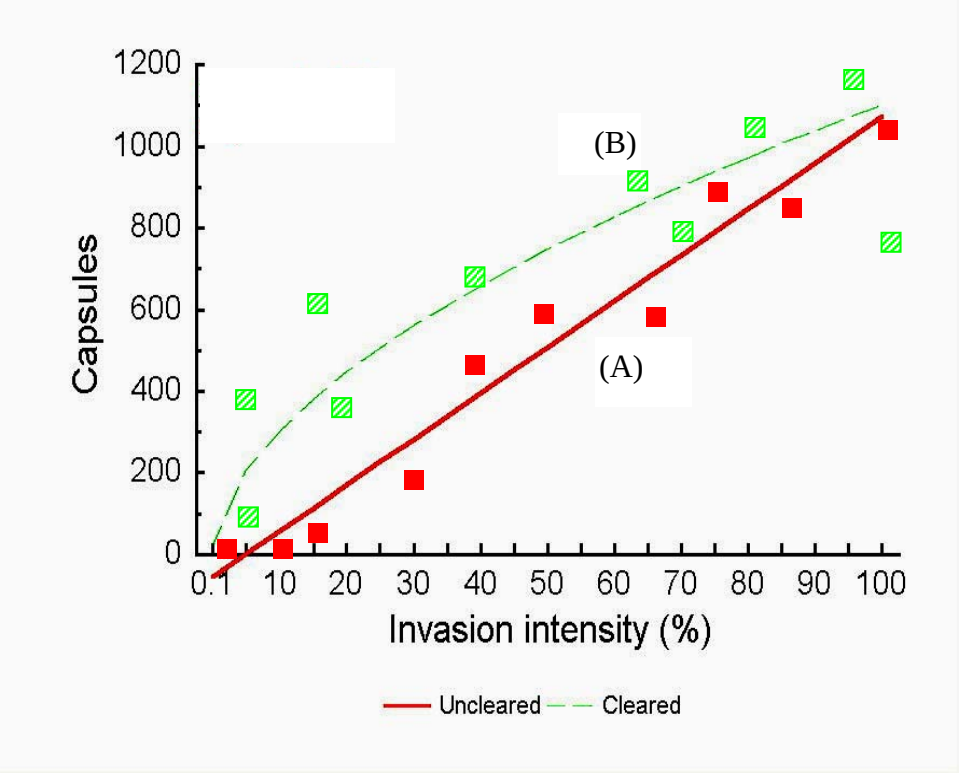


Figure 5.5 The relationship of *Eucalyptus grandis* capsule density in the soil with increasing invasion intensity (% aerial cover of exotic plants) on sites; (A) uncleared ($r^2 = 0.32$; $P < 0.05$) and (B) cleared of exotic plant species ($r^2 = 0.30$; $P < 0.05$) along the Sabie River, South Africa. The regression relationship (A) is $Y = (11.28 * X) - 55.3$ and (B) is $Y = 83.4 * X^{0.56}$.

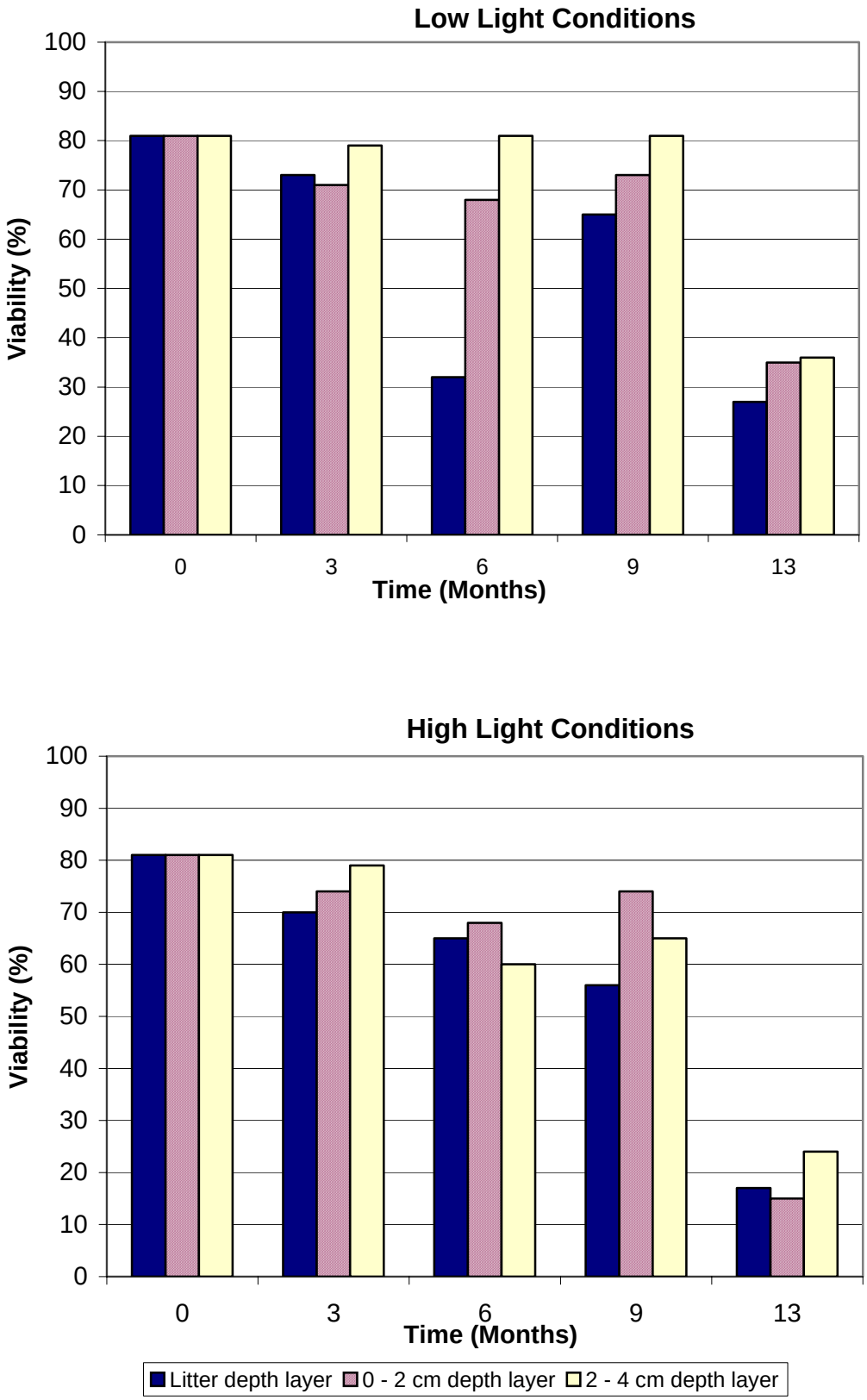


Figure 5.6 Percentage viable *Solanum mauritianum* seeds remaining through time for different depth layers under field conditions of high (unshaded) and low (shaded) light conditions.

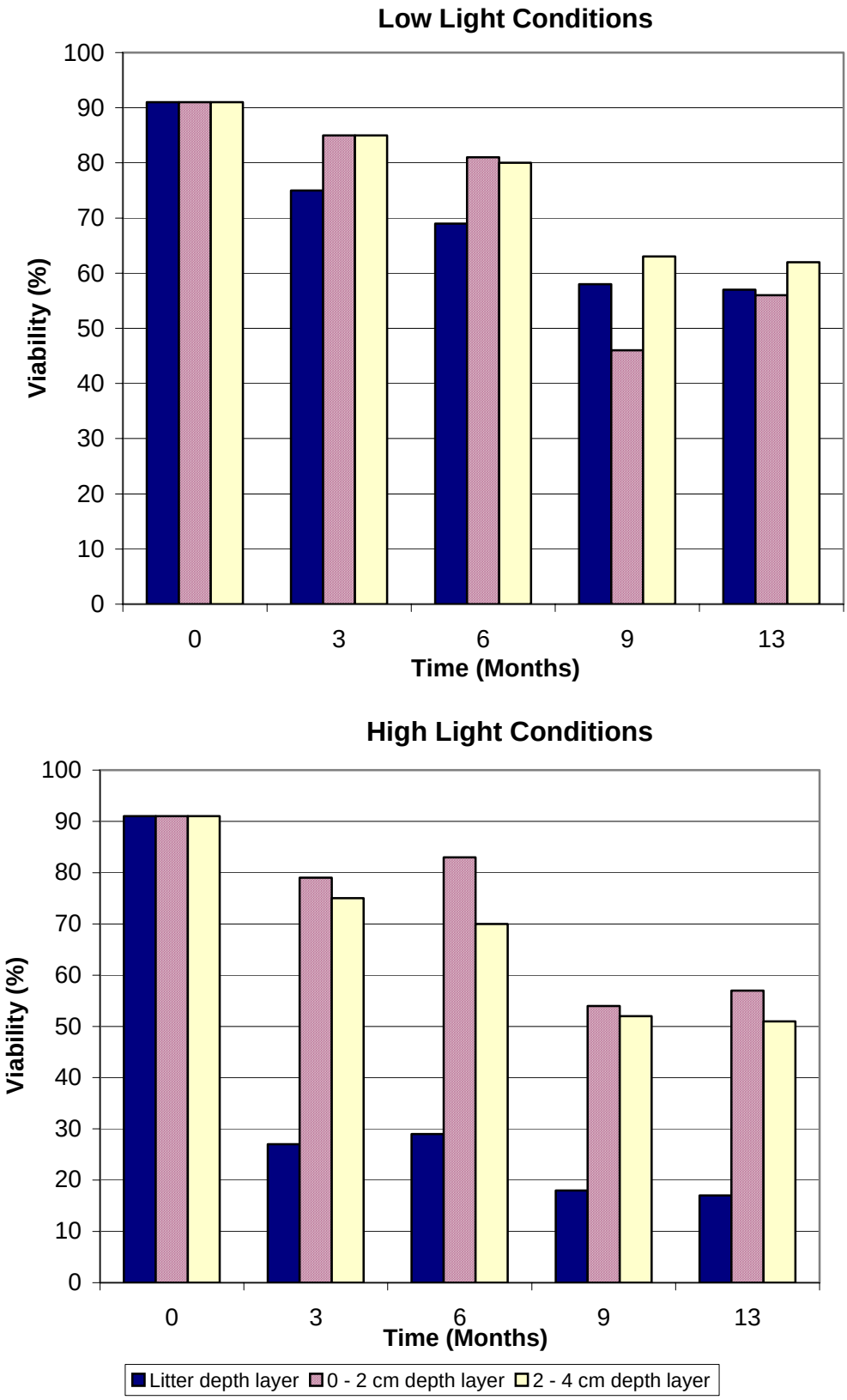
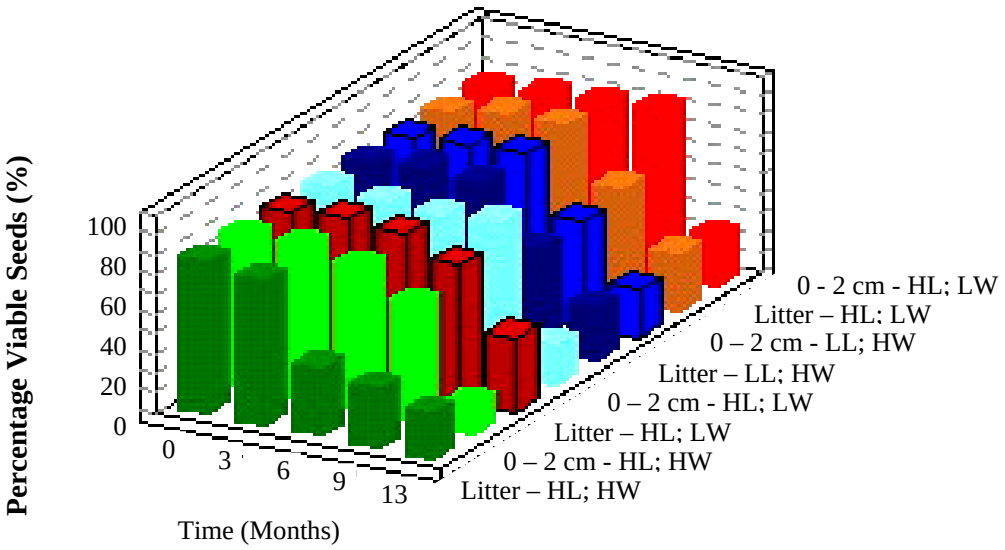


Figure 5.7 Percentage viable *Acacia mearnsii* seeds remaining through time for different depth layers under field conditions of high (unshaded) and low (shaded) light conditions.

S. mauritianum



A. mearnsii

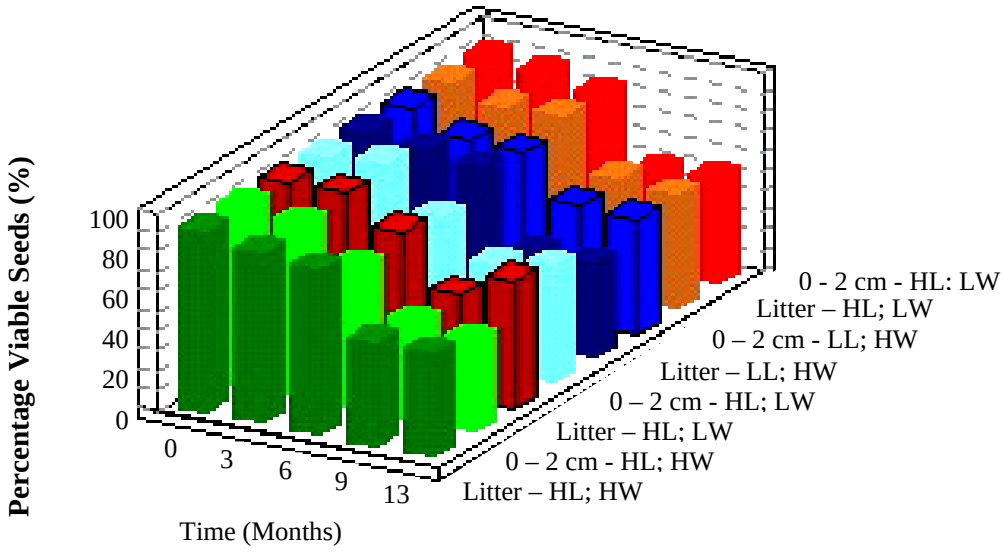


Figure 5.8 Viable seed persistence through time and with burial (Litter & 0 – 2 cm) under greenhouse water and light trial conditions for *S. mauritianum* and *A. mearnsii*. HL - high light; LL - low light; HW - high water; LW - low water.

Table 5.4 Probability values for soil seed banks for *E. grandis* (capsules), *S. mauritianum*, *C. monticola* and *Ipomoea* sp. for three way ANOVA's of seeds recovered for each depth class in the soil in relation to invasion (INV), altitude (ALT) and management / clearing treatment (MAN). *d.f.* = 1,32 for all main effects and interaction terms.

Species	*Eucalyptus grandis*			*Solanum mauritianum*			*Clutia monticola*			*Ipomoea* sp.		
Treatment	Litter	0 - 2 cm depth	2 - 4 cm depth	Litter	0 - 2 cm depth	2 - 4 cm depth	Litter	0 - 2 cm depth	2 - 4 cm depth	Litter	0 - 2 cm depth	2 - 4 cm depth
ALT	01809	0.7687	0.8475	**0.0110**	**0.0020**	**0.0001**	0.1157	**0.0250**	**0.0069**	0.7060	0.9699	0.0628
INV	0.074	0.1803	0.1564	**0.0006**	0.1057	**0.0332**	0.5915	**0.0402**	**0.0156**	0.7060	0.8361	0.1884
MAN	0.4287	0.2470	0.1336	0.7344	0.3920	0.9602	0.7583	0.0694	**0.0175**	0.1632	0.9699	**0.0393**
ALT*INV	0.8524	0.5189	0.0594	0.4352	0.4653	0.1614	0.5915	0.1749	0.4707	0.9973	0.2876	**0.0004**
ALT*MAN	**0.0398**	0.0544	0.0677	0.1491	0.8400	0.7716	0.7583	0.2676	0.4999	0.7060	0.1935	0.6399
INV*MAN	0.3790	0.5885	0.9543	0.3313	0.3853	0.1502	0.6061	0.3874	0.1360	0.3015	0.2876	0.9122
ALT*INV*MAN	0.3163	0.0603	0.1402	0.3656	0.8334	0.9107	0.4501	0.8463	0.1896	0.9973	0.4536	0.1273

Table 5.5 Probability values of the three way ANOVA's with repeated measures (depth) for soil seed banks for four species in relation to invasion (INV), altitude (ALT) and management / clearing treatment (MAN). *d.f.* = 1,32 for all main effects and interaction terms.

Species	*Eucalyptus grandis*	*Solanum mauritianum*	*Clutia monticola*	*Ipomoea* sp.
Treatment				
ALT	0.4718	**0.0001**	**0.0020**	0.1506
INV	**0.0348**	**0.0010**	**0.0128**	0.4194
MAN	0.1861	0.7705	**0.0234**	**0.0378**
ALT*INV	0.7010	0.1761	0.1945	0.0844
ALT*MAN	**0.0260**	0.6428	0.2932	0.3991
INV*MAN	0.5880	0.4984	0.1583	0.8768
ALT*INV*MAN	0.0930	0.6270	0.2854	0.1285
Depth	**0.0001**	**0.0090**	**0.0001**	0.0709
Depth*ALT	0.3227	0.3829	0.4039	0.3799
Depth*INV	0.1445	0.2164	0.1736	0.5147
Depth*MAN	0.0758	0.5811	0.1564	0.3561
Depth*ALT*INV	**0.0125**	0.8698	0.6790	**0.0019**
Depth*ALT*MAN	0.9075	0.2775	0.7363	0.4047
Depth*INV*MAN	0.5421	0.1407	0.6597	0.3613
Depth*ALT*INV*MAN	0.5495	0.6985	0.6406	0.5639

5.3.4 Seed production of *S. mauritianum*

Regression analysis of numbers of viable *S. mauritianum* seeds per plant and inflorescences per plant in relation to height were used to determine the relationship between plant size and reproductive output (Figure 5.9). Number of berries per plant was not plotted as this was directly related to the number of seeds per plant. The number of seeds per berry was high (193 ± 0.68 (SE)) for *S. mauritianum* plants with a CV of 98%. An initial viability of *S. mauritianum* seeds was found to be 78.65%.

Both the viable seeds per plant and inflorescences per plant relationships with height illustrated a critical height at which seed production begins. This critical height was 1.65 m above ground (Figure 5.9). Seed production is large, with plants of 15 m (maximum recorded height) producing in the region of 68 inflorescences and approximately 25 000 viable seeds per annum (Figure 5.9).

Resprouting and clearing effectiveness

The proportion of resprouting individuals to all individuals present for cleared plots was determined (Table 5.7). *Pinus patula* individuals that were clear felled did not resprout, but *E. grandis* individuals did resprout, and with a greater vigour at low altitude (Table 5.7). *S. mauritianum* had a higher proportion of resprouting individuals than did any of the other two species measured. The greatest proportion of resprouting *S. mauritianum* individuals was found in the cleared low altitude sites (Table 5.7).

Death of *S. mauritianum* individuals clear felled was not related to diameter at cut height as no significant difference was found between resprouting individuals and dead individuals for both the high (*d.f.* = 98; t = 0.30; P = 0.38) and low altitudes (*d.f.* = 98; t = 0.32; P = 0.37). The mortality of individuals was related however, to the height at which each individual *S. mauritianum* stem was cut . This mortality versus height cut was found to be significant at both high (*d.f.* = 98; t = 4.95; P = 0.001) and low altitudes (*d.f.* = 98; t = 5.65; P = 0.001). *S. mauritianum* individuals at high altitudes appear to survive when cut at a lower height (40.05 ± 2.63 cm (Mean ± S.E.)) above the ground than those individuals at low altitude (47.85 ± 2.36 cm). Similarly, for those individuals that died at high altitude sites, the cutting height was slightly lower (28.19 ± 2.13 cm) than observed for low altitude individuals (29.05 ± 4.08 cm).

Table 5.6 Seed half-life for seeds of *A. mearnsii* and *S. mauritianum* for different light, water and depths of burial from greenhouse and field trials. Regression equations used in the calculation of the seed half-life are to be found in Appendix 15.

	Field conditions			
Depth	**low light (shaded)**		**High light (unshaded)**	
	S. mauritianum	*A. mearnsii*	*S. mauritianum*	*A. mearnsii*
0 cm	9 ½ months	25 years	8 ½ months	5 years
2 cm	11 ½ months	1 year 1 month	9 ½ months	1 year 2 months
4 cm	13 months	6 years 4 months	9 ½ months	25 years
Average	11 months	11 years 3 months	9 months	8 years 4 months
	Greenhouse high light (unshaded) conditions			
Depth	**High water (50.69 ml/day)**		**Low water (5.14 ml/day)**	
	S. mauritianum	*A. mearnsii*	*S. mauritianum*	*A. mearnsii*
0 cm	9 months	11 ½ months	10 months	1 year 1 ½ months
2 cm	5 months	1 year 1 month	11 ½ months	1 year 3 months
Average	8 ½ months	1 year	11 months	1 year 2 months
	Greenhouse low light (shaded) conditions			
Depth	**High water (50.69 ml/day)**		**Low water (5.14 ml/day)**	
	S. mauritianum	*A. mearnsii*	*S. mauritianum*	*A. mearnsii*
0 cm	9 ½ months	1 year 4 months	10 ½ months	1 year ½ month
2 cm	9 ½ months	1 year	9 ½ months	1 year 3 months
Average	9 ½ months	1 year 1 month	10 months	1 year 1 ½ months

Table 5.7 Proportion coppicing exotic individuals (mean ± S.E.), and proportion exotic vegetation "missed" or germinated after clearing operations for *E. grandis, P. patula* and *S. mauritianum*.

	Proportion coppicing individuals after clearing		Proportion standing vegetation missed by clearing or newly germinated individuals	
Species	High altitude	Low altitude	High altitude	Low altitude
E. grandis	0.19 ± 0.09	0.33 ± 0.21	0.24± 0.20	0.48 ± 0.25
P. patula	0	0	1.00 ± 0.00	1.00 ± 0.00
S. mauritianum	0.31± 0.19	0.41± 0.28	0.85 ± 0.51	0.07 ± 0.04

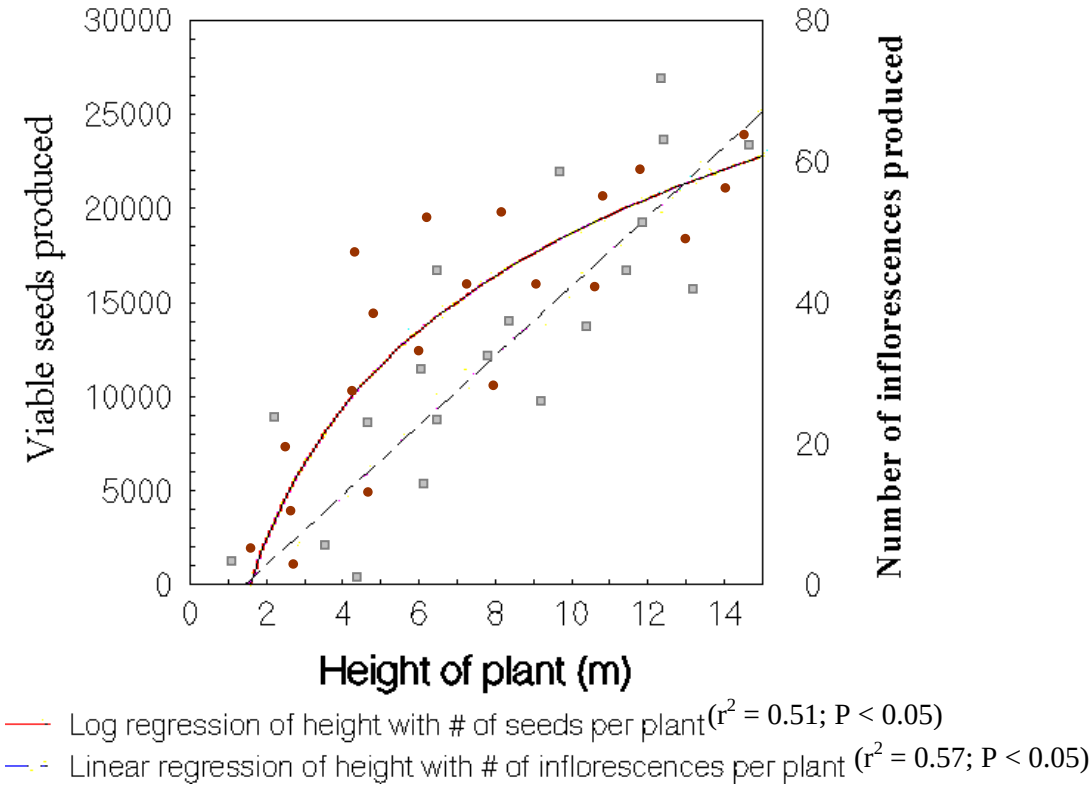


Figure 5.9 Regression relationships of viable seeds and inflorescences of *S. mauritianum* with measured height of the adult plant.

5.4 Discussion

5.4.1 Soil seed bank species composition

It has been observed that soil seed banks are sometimes highly correlated (Looney & Gibson 1995) and sometimes poorly correlated (Leck 1989) to the species composition of the vegetative community from which they are extracted. During this study however this was not found to be the case. The lower species richness in the soil seed bank observed for high altitude sites did not correspond with the higher total plant species richness observed (see Chapter 6). Tree species richness did however correlate with the seed bank species richness. There could be many reasons for this, as many propagules may be more rare in the environment and have "escaped" the sampling technique. A large proportion of species in the community are either grasses, sedges or herbaceous species which either propagate by vegetative growth or have exceptionally small seeds.

5.4.2 Soil seed bank distribution

Seed persistence in the environment corresponded with the literature in the sense that propagules were more abundant below the soil surface than in the litter. Many of the seeds found in the seed bank were also of a small size and not excessively flat or elongated, hence satisfying the desired seed shape for persistence in a seed bank. Seed persistence of *A. mearnsii* and *S. mauritianum* in the seed bank and in the experimental trials also showed the need for a hard seed coat to ensure seed persistence. The softer *S. mauritianum* seeds, although more abundant, did not persist for as long in the soil seed bank.

No clear pattern of total seed bank density was determined with clearing or invasion. This was found to be due to the exotic soil seed bank "making up" for the indigenous soil seed bank density "lost" when invasion occurs. In addition, the short time interval between clearing of the exotic vegetation and the sampling done in this study does not allow for the lag effect of seed attrition in the soil seed bank. This could also be complicated by the fact that the composition of the soil seed bank may be independent of the standing vegetation and related more to a regional species composition and the functioning of dispersal vectors. Further sampling through time, along with a more in depth investigation into the dispersal vectors for the key seed species, would be needed to clarify this.

Distribution of key seed species

Of the four species of seed found with a high enough frequency in the soil seed bank, a clearer pattern of distribution emerges than for total seed bank composition. This mainly illustrates the life history of the species being observed, but also gives insight into the effects of invasion and clearing on these life histories. For example, the *Ipomoea* sp. was not affected by exotic species invasion whereas the other three key species, *E. grandis*, *S. mauritianum* and *C. monticola* were. This species (*Ipomoea* sp.) is an indigenous pioneer species and would occur in any community where disturbance offers the opportunity for establishment. Both clearing and invasion are a form of disturbance to the community and would afford this species the opportunity to establish and generate a soil seed bank.

Eucalyptus grandis (capsules) and *S. mauritianum* both showed an increase in seed bank content with invasion and can be related directly to the abundance of these species in the community as would be expected from an aggressive invasive species. Both of these species illustrated very large projected seed banks at high invasion intensity and an initial seed bank density of up to 5% of the total seed bank at low invasion categories. This indicates a seed source outside the measured areas and the dispersal of seeds from outside the study sites into these areas. *C. monticola*, an indigenous shrub species showed the

opposite effect of invasion, having more seed bank propagules in low invasion communities where invasion would not have resulted in the exclusion of the species due to overtopping. This would be a typical example of many of the indigenous species found under conditions of invasion and clearing.

5.4.3 Regeneration potential of *S. mauritianum* and *A. mearnsii*

Both *S. mauritianum* and *A. mearnsii* illustrated high initial seed viabilities typical of invasive species. Experimental seed banks illustrated that this viability dropped off steadily but had a significant drop at 6 – 9 months for both species after being in the seed bank. There was only one external combination of variables, which highlighted a particular reason for this decline in *S. mauritianum*. The high light and high water regime set in the green house trials showed increased attrition. This was not reflected in *A. mearnsii* but this could be attributed to the morphology of the seed as *A. mearnsii* seeds have an exceptionally hard seed coat which would afford propagules of this species better protection against these elements compared to the softer *S. mauritianum* propagules.

Once again the attrition in the litter layer was found to be higher and this affected seed half-life. *S. mauritianum* seed half life ranged from 9 to 13 months with very little variance observed for different light and watering regimes. This lack of variation between treatment would be explained by the short half-life of the seed, which would mask the effects of the experimental variables. A more aggressive application of the variables applied in the trial may however more effectively show the negative effects on propagule half-life. The problem with this is relating it back to actual environmental variables seen in the community.

Two years may seem a short period for seed viability in the soil of such a prolific invasive species such as *S. mauritianum*. If however, the reproductive strategy of the species is looked at in a more holistic manner then the short seed viability makes sense. Firstly, consider the seed size, this is small and contains very little in terms of endosperm and cotyledonous material (germination reserves). The longer the propagule stays in the soil the deeper the propagule is buried, and the germination of a propagule buried too deeply in the soil, would not result in seedling emergence from the soil. In addition to this, the large numbers of seeds generated per plant by *S. mauritianum* (up to 25 000 seeds per plant per annum) more than makes up for any seed attrition in the soil especially in the first 2 years of invasion. In addition the short-term seed half life probably implies that a small proportion of the seeds may be viable for well over 2 years.

A. mearnsii had a significantly longer seed half life of up to 25 years, which corresponds with the seed life span observed in the literature. Seed viability in *A. mearnsii* seed banks was negatively affected by high light and high water regimes, especially on the surface or litter layer. This once again corresponds with the literature where the burial of seeds is an essential for persistence.

Resprouting of key weed species and clearing effectiveness

Clearing operations utilized the method of clear felling exotic plant individuals and applying herbicide to the stump. Clear felled individuals either died or resprouted after this treatment. By determining the number of individuals of exotic species remaining in the community and classifying whether they originated from seed, were missed or resprouted, the problem species, or those species that will be persistent in the environment after clearing can be identified. This information on regeneration shows an important aspect of their reproductive strategy, which needs to be focused upon in these species for better control.

Resprouting or coppicing is used extensively in the timber industry for the regeneration of plantations after harvesting. The approach is however different for the Working for Water Programme as stumps are sprayed with herbicide with the intension of eradication of the individuals. Generally, *P. patula* individuals did not resprout once treated, but a few individuals were found, having been missed or established since the clearing of the study sites. For *E. grandis,* up to a third of the cut stems coppiced and almost half of the individuals found had been either missed or were present as a consequence of propagation through seed. This generally occurred with greater vigour at low altitude, as this is the highest production area for this species (A. Poulter *Pers. Comm.*1996).

Similarly, *S. mauritianum* illustrated the same effect with even higher resprouting, as well as “missed” or seeded percentages. This occurred to such an extent that in the high altitude sites up to 85% of the individuals encountered were either missed or newly seeded. This poor eradication percentage means that a follow up programme will have to be employed to continue the removal process to make the programme successful.

From a clearing effectiveness perspective, the height at which *S. mauritianum* is cut plays a role. When cut below 28 cm from the ground, the attrition rate of individuals was found to be significantly higher. This has important repercussions for the application of mechanical clearing techniques for this species and could significantly reduce the re-invasion of communities after clearing. By removing the coppicing

proportion of the *S. mauritianum* population, the total invasion intensity after clearing can be reduced, and as *S. mauritianum* had a higher proportion of resprouting individuals than did any of the other species measured, it should have a significant effect on the final post-clearing community.

5.5 Conclusion

Soil seed banks in the study community were found to be limited in their species richness. The soil seed banks were dominated by propagules of four species, two exotic, one indigenous shrub and one indigenous herbaceous species. The total soil seed bank did not show any relationship to total species richness, but did relate to woody species richness. The soil seed bank did not relate to the invasion intensity or clearing thereof, but rather the individual species propagules within the soil seed banks related to invasion intensity and clearing according to their life history traits. This means that invasive species propagules increased with invasion intensity. Weedy species, which typically take advantage of a highly disturbed disturbance regime, were more abundant in the seed bank in the high invasion cleared sites and indigenous species propagules generally declined in high invasion categories.

The soil seed banks of the key weed species, *A. mearnsii* and *S. mauritianum,* once again illustrated that burial is a prerequisite for persistence in the soil seed bank. Seeds of these species were found to have different half-life's, with *A. mearnsii* having up to a 25 year half-life and *S. mauritianum* up to 13 months half-life in the soil. This has significant repercussions for the Working for Water clearing programme, as, coupled with the very large seed bank of *S. mauritianum* and reinvasion figures collected from cleared sites, it shows that the programme is only in the region of 15% effective in eradication in some cases, due to reestablishment from seed.

Coppicing is also a large regenerative property of the key invasive species found within the study sites. This was generally not an issue for *P. patula*, but definitely plays a role for *E. grandis* and *S. mauritianum.* By modifying the mechanical clearing techniques for a key species such as *S. mauritianum*, a significant improvement in the success of clearing can be achieved.