Biological control of alien invasive mesquite species (*Prosopis*) in South Africa: the role of introduced seed-feeding bruchids.

by

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Thesis presented for the degree of DOCTOR OF PHILOSOPHY

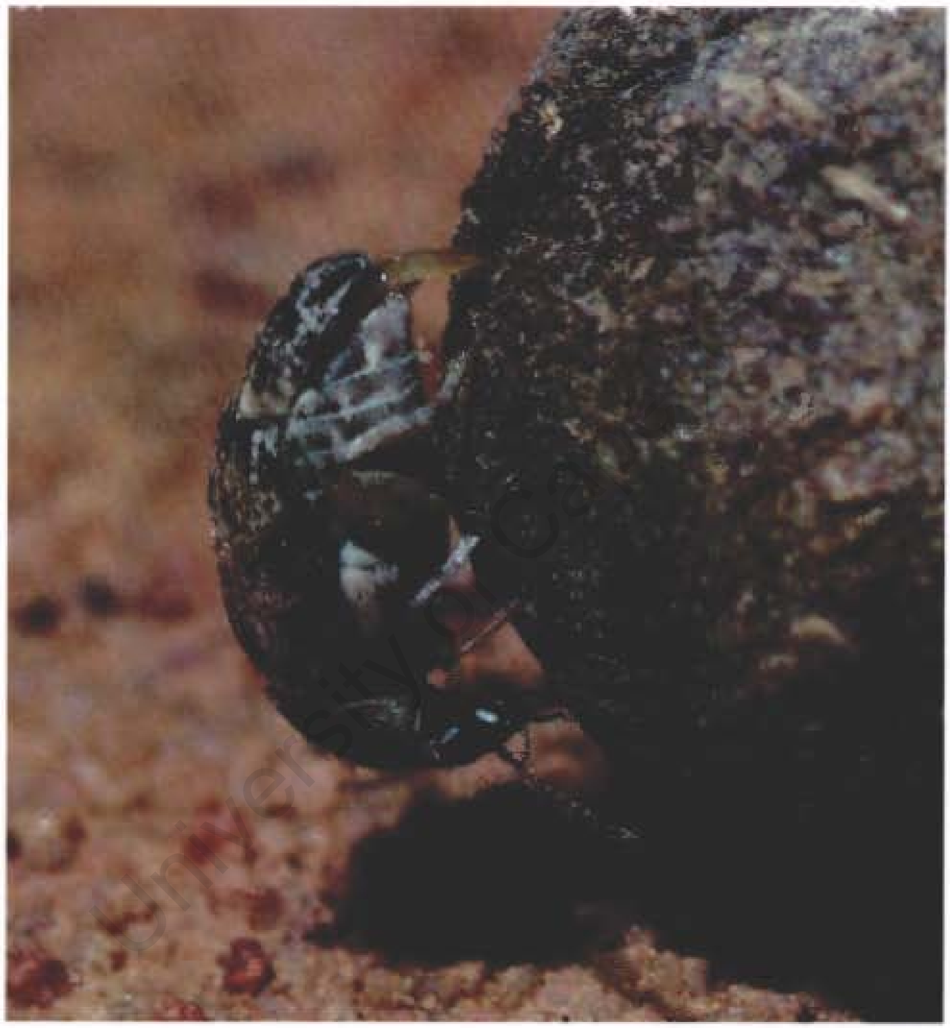
in the Department of Zoology,

University of Cape Town,

South Africa

August 2006

Frontispiece



Caught in the act! An *Algarobius prosopis* female ovipositing on a sheep dung pellet containing mesquite seeds - a novel or evolved behaviour enabling this bruchid species to persist on post-dispersed seed?

Contents

Declaration

Biological control of alien invasive mesquite species (*Prosopis*) in South Africa: the role of introduced seed-feeding bruchids.

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Acknowledgements

I would like to thank my supervisor John Hoffmann for his tireless support with this project and advice he ceaselessly made time to offer. His assistance with the editing of the first drafts was invaluable, as was his guidance when directionality of this project may possibly have swayed off-track.

I must thank my good friend Jonathan Colville for all the assistance in the field, someone who was always willing to offer constructive criticism and question the effectiveness of biological control. He, not being an advocate of biological control, gave me the drive to further my knowledge and efforts to elucidate to "non-believers" the benefits of biocontrol as a management tool.

Many thanks to Pete Bradshaw for his help in the field and with compilation of the distribution maps of mesquite in South Africa. Also thanks go to Lizelle Odendaal for her painstaking observational work in recording beetle behaviour.

Kobus and Berdine Vos kindly allowed myself and others to work on their farm Humansdam, in Vanwyksvlei, and so helpfully opened their house to me. Anton Bouwer and Louwtjie Steenkamp permitted me access to their properties in Modder River and allowed me to set up transects enabling long term monitoring of the infestations. Many thanks go to Kosie and Huffie Strauss, owners of Soetwater farm near Calvinia, on whose property the bulk of field work was conducted and without which much of the important information on the interactions of environment and beetle populations would have remained unanswered. Many thanks go to Beryl Wilson-Aitchison for all the help and for so kindly letting me stay with her during my many field trips.

I must also acknowledge Simon van Noort from the South African Museum and Ottilie Neser from the Agricultural Research Council for their assistance at short notice in identification of parasitoid species.

Thanks to Gillian Bowie and Norma Simmons from the radiometry unit at Groote Schuur Hospital for their time and patience, I hope the x-raying of pods provided a welcome distraction.

Many thanks also to Mike Picker, for his ideas and thought-provoking questions initiated over many a cup of tea. In addition, I would like to thank Carien Kleinjan for her willingness to hammer out ideas and also for the many valuable suggestions that she had to offer.

Coleen de Villiers from the South African Weather Services kindly and repeatedly dredged up historical rainfall and temperature data from their archives.

I must take this opportunity to thank Helmuth Zimmermann and Arne Witt from the Plant Protection Research Institute who supported this project from its inception, seeing it evolve from a two year to a five year programme. Also a big thank you to the Working for Water organisation who have funded this project and others like it, and for realising the inarguable necessity to invest resources into biological control.

Lastly I must thank my wife, Erin, for her willingness to help me in the field and the laboratory and for her support throughout this project from our early days of courtship, helping in the field, through to the compiling of my final thesis. Without her support and efforts this project would have undoubtedly been a more challenging task and one that would have seemed insurmountable.

Preface

The numbers of progeny left by plants depends on the amount of seed produced and on the number of 'safe sites' that allow establishment and survival of seedlings. If 'safe sites' are plentiful the plants may be seed limited but if 'safe sites' are scarce the plants will be site limited. The effectiveness of seed-feeding insects that are used for biological control will depend on whether or not the target weed is predominantly site or seed limited. The use of bruchid beetles for biological control of mesquite, *Prosopis* species, in South Africa is deemed to have failed because the beetles are supposed to be unable to compete with livestock which utilise the seed pods of mesquite extensively as forage. These assumptions have been made without any evidence as to how the beetles and livestock interact or as to whether mesquite is site or seed limited in South Africa. This study was initiated to test the hypotheses that the beetles are not coping with competition with livestock and that mesquite is site limited and not seed limited in South Africa. The results should elucidate the role that seed-feeding insects might play in the population dynamics of invasive perennial plant species outside of their native range.

Abstract

Two seed-feeding bruchids, *Algarobius prosopis* and *Neltumius arizonensis*, introduced in the late 1980s and early 1990s for the biological control of mesquite species (*Prosopis*) in South Africa have successfully established throughout the range of mesquite. This study serves as a post release evaluation of the bruchids in order to quantify the effects that these species may be having in reducing mesquite seed survival and in turn if there is any noticeable effect on annual recruitment of new plants. In most instances livestock, predominantly sheep, consume all the pods produced by mesquite before the bruchids can destroy the seed. Large numbers of seed are then disseminated within and away from mesquite infestations. This seed is still available to *A. prosopis*, which locates and oviposits on dung pellets that bear seeds, but not *N. arizonensis*, which only lays on pristine pods.

In order to quantify the proportions of pod- and dung-borne seeds that were destroyed annually by the bruchids, pods and dung were collected in the field over a three year period at various sites throughout the Western and Northern Cape provinces. Laboratory choice experiments were conducted to ascertain whether *A. prosopis* exploited one seed source more frequently than the other. Field monitoring of mesquite infestations were conducted over three rainfall seasons at sites across the country to determine if the bruchids were having an impact on the size of the soil seed banks and to measure levels of recruitment, if any, of new mesquite plants into the populations.

It appeared that the main factor limiting bruchid abundance was the continued consumption of pods by livestock. At sites in the arid region where livestock were

excluded, between 96.0 and 99.6% of the annual seed crop was destroyed by the bruchids, however, both species of bruchids appeared to be less damaging in the mesic regions and accounted for only 60% destruction of the annual seed crop, probably a consequence of unsuitable environmental conditions.

Algarobius prosopis was the dominant of the two species accounting for more than 90% of seeds destroyed. The acquisition of indigenous hymenopteran larval and pupal parasitoids did not appear to have a major influence on the populations of *A. prosopis* but may be negatively influencing the size of *N. arizonensis* populations. In addition, although egg parasitism of *N. arizonensis* appeared to have decreased since the initial introductions of the bruchids, the combined effects of egg and larval parasitism and the superior competitive ability of *A. prosopis* larvae were the likely causes for *N. arizonensis* populations remaining low.

Algarobius prosopis damaged substantially more than 50%, and in some instances this figure neared 100%, of dung-borne seed annually. Even in the presence of pods *A. prosopis* appeared to preferentially oviposit on dung, particularly if the ovipositing females had developed in dung. The utilisation of dung-borne seed was restricted to the vicinity of trees during summer when temperatures of exposed dung were lethal to developing larvae. The highest damage to dung-borne seed during winter occurred in the exposed areas, giving rise to two generations of *A. prosopis*. There appeared to be no relationship between distance from trees and levels of damage to dung-borne seed suggesting that *A. prosopis* were actively dispersing during the winter period. The use of dung-borne seed has not enabled *A. prosopis* to escape parasitism as 38-60% of the bruchid larvae in dung-borne seed were destroyed by two apparently specialist species of hymenopteran parasitoid (*Pteromalus* sp.), obtained from only dung collections.

In the arid region, the bruchids reduced the annual input of seed to the system at sites where livestock were excluded resulting in very low seed banks. Despite the ability to utilise dung-borne seeds, large seed banks have developed at sites where livestock have access to pods and were dung is concentrated in the shade below mature mesquite trees. In the mesic region, the failure of the bruchids to destroy >60% of the annual seed crop has resulted in the formation of large seed banks in both the presence and absence of livestock.

This study was conducted over a period during which below average rainfall was recorded in every year. Despite this, germination events occurred in two of three rainfall seasons but at only the mesic sites did any seedlings successfully establish. The evidence of distinct plant cohorts in the arid regions but not in the mesic regions supported the idea that recruitment in arid regions occurs only intermittently, in years of favourable rainfall, but that recruitment in the mesic regions occurs regularly. Despite the success of the bruchids in shifting mesquite infestations from being site to being seed limited in the arid region where livestock are excluded, the continued access of livestock to pods at most sites results in the mesquite populations remaining site limited and their ability to spread and densify has not been halted.

Introduction

The genus *Prosopis* consists of 44 species (Burkart, 1976) most of which are perennial trees and many of which are exploited commercially (see Pasiecznik, 2001 for a summary) as a source of excellent timber (Harding, 1987), shade in hot arid areas, fuel and large, nutritious seed pods (Pasiecznik, 2001) which are used extensively as forage by native and domestic animals (Zimmermann, 1991). Almost all of the species are of New World origin but three originate in the Old World, including one from Africa, *P. africana*, which occurs in the southern Saharan region. Many *Prosopis* species are considered phreatophytes (Mooney *et al.*, 1977) being able to utilise both surface water and ground water at great depths enabling them to thrive in harsh, arid environments.

The useful attributes, together with the ability of the plants to survive extreme conditions, has encouraged the use of *Prosopis* species in agroforestry projects around the world, particularly in Africa and Asia where many North American species have been cultivated. South Africa provides a classic example where six *Prosopis* species are currently recognized (see Harding 1987). Between 1897 and 1917, the Cape and Transvaal forestry commissions obtained a number of consignments of seed from various public and private sources in the U.S., Mexico and Hawaii (Poynton, 1987, cited in Zimmermann, 1991). Thousands of potted seedlings were produced from these seeds and widely distributed to farmers in the arid western parts of the country to be planted out on a large scale. The venture was an outstanding success and many landowners benefited from having fuel, shade and forage in abundance.

Two species, *P. velutina* Wootan and *P. glandulosa* var. *torreyana* L. Benson constituted many of the original seedlings distributed and are now thought to be predominant (Zimmermann, 1991). Subsequently there seems to have been extensive hybridisation between these two species and between them and the other introduced species within South Africa (Harding, 1987). As a result populations of plants are currently characterised by a range of overlapping morphotypes which are difficult to distinguish or assign to a particular taxonomic grouping. Most recent accounts make no attempt to ascribe a specific epithet when referring to the plants and only use the generic terms prosopis or mesquite, a convention that is used throughout this thesis.

In spite of the euphoria about the initial gains derived from mesquite, its use has come at a substantial environmental and economic cost. In many regions the introduced plants have spread from cultivation and become invasive and problematic due to the potential production of 9-20 tonnes ha^{-1} pods annually (Felker, 1979). At the height of invasions, mesquite form dense impenetrable thickets of spindly, multi-stemmed plants that render large areas useless for normal farming practices. These thickets are most common along water courses, dry river beds and depressions where ground water is located (Strain, 1970; Harding & Bate, 1991). When thickets form, pod production declines substantially and the crowded, stunted plants produce little usable shade. In addition, the plants use "copious quantities of scarce ground water" (Impson *et al.*, 1999). Their ability to access groundwater, even from a young age, has been to the detriment of the indigenous tree species, such as *Acacia erioloba*, a keystone species in the arid and semi-arid regions of South Africa, where even centuries-old trees have been shown to die as the water table lowered due to mesquite invasions (Woodborne *et al.*, 2000 *unpubl.*). The reduction of mean annual runoff as a result of mesquite invasions has been estimated at 192 million

m^3 (Le Maitre *et al.*, 2000). Vorster (1977) estimated the extent of invasion at 186 000ha and this subsequently increased to more than 1.8million ha by 2006 (Zimmermann *et al.*, 2006). The distribution of the plant is not limited to xeric (dry) areas and range expansion into the more mesic areas has been reported (Harding & Bate, 1991). However, despite the negative aspects many landowners still view mesquite as a beneficial plant and several industries that rely on resources from the plants have been established. Harding (1987) placed a value of R750ha^{-1} on charcoal production and projected a net worth of pods for livestock feed at a value of R120 million annually if the mesquite stands were to be managed correctly.

Attempts to control the mesquite problem in South Africa have met with difficulty. Mechanical and chemical control methods have had limited success because they are labour intensive and expensive, the costs often outweighing the value of the land that needs to be cleared (Vorster, 1987; Harding, 1987). Long-term, cost-effective control of the weed can probably only be achieved through biological control. However, constraints have been imposed on the types of agents that can be used because the plants are still exploited for their benefits. Ward *et al.* (1977) and Cordo & DeLoach (1987) produced checklists of more than 650 herbivorous insect species associated with *Prosopis* taxa in the New World. However, owing to the constraints imposed by parties in using the beneficial attributes of the plants it was decided that only seed feeding insects could be considered for introduction into South Africa. Seed reducing agents, by leaving the mesocarp undamaged, do not detract from the nutritional value of the plants and could possibly reduce the rate of spread of the weed (Zimmermann, 1991). Glendening and Paulsen (1955) found that bruchids destroy up to 75% of the mesquite seed crop in

Arizona: two of these considered suitable biocontrol agents for South Africa were *Algarobius prosopis* Le Conte and *A. bottimeri* Kingsolver. Although the two species are largely ecologically analogous (Kistler, 1995) they show differences in larval behaviour and morphology (Impson & Hoffmann, 1998) which could render them each more suitable for particular environmental conditions and therefore reduce competitive interactions.

Essentially, the adult females oviposit a number of eggs in crevices and blemishes on mature pods and after 8-10 days the eggs hatch and the larva burrows into the pod and through the mesocarp until a seed is located (refer to Swier (1974) for a more comprehensive account of larval behaviour). The larva chews through the seed coat entering the seed where it completes its development and pupation, emerging as an adult 22-25 days later providing temperatures are optimal (Zimmermann, 1991). Multiple generations occur through the year, however, the behaviour and physiology of adults and larvae are strongly affected by temperature (Kistler, 1985) and it is likely that both winter and summer temperatures affect the potential number of generations.

Following host specificity testing, *A. prosopis* and *A. bottimeri* were released in 1987 and 1990, respectively (Zimmermann, 1991). Mass releases of *A. prosopis* took place at numerous sites in the north-western Cape in 1987 and 1988 and of *A. bottimeri* in the Free State in areas where *A. prosopis* had not established by 1990. At present *A. prosopis* has successfully established throughout the range of mesquite in South Africa with up to 90 – 98% seed destruction in certain areas where livestock are excluded (Zimmermann, 1991; Roberts, 2003 unpubl. data, University of Cape Town) however, *A. bottimer*i has failed to establish (Impson & Hoffmann, 1998) possibly as a result of the superior competitive ability of *A. prosopis* (Hoffmann *et al.*, 1993).

Although trees are able to produce flowers and pods for most of the year, production of the vast majority of pods commences in October and pod-fall of the mature pods occurs from late January through till April across mesquite distribution (Fig. i). In years of ample rainfall the occurrence of a second flush of pods which mature in June/July is not unusual. One of the problems facing *A. prosopis* is that when mature mesquite pods fall to the ground they are often devoured within a short period by both livestock and wild animals.

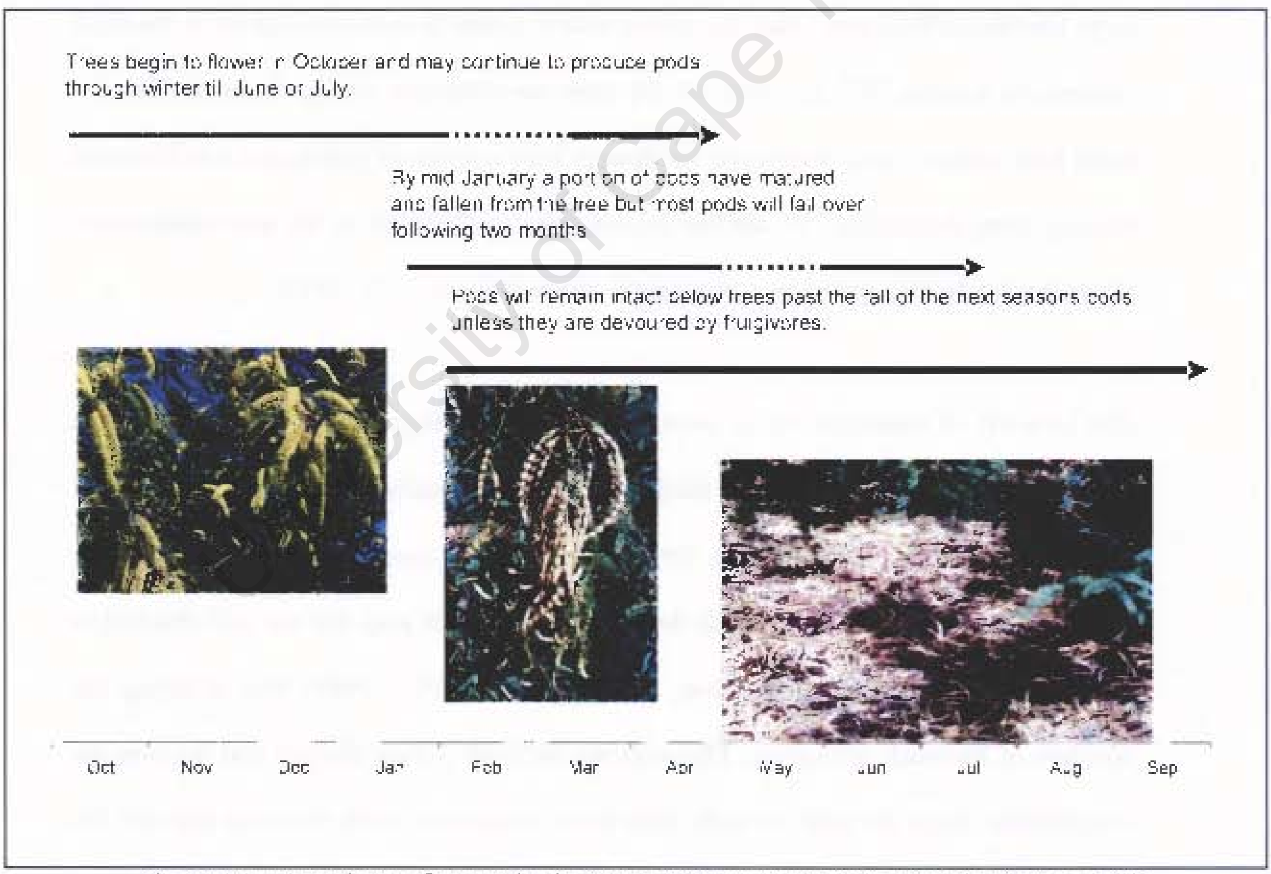


Fig. i: The phenology of mesquite in South Africa and the fate of pods after pod-fall. When livestock are excluded pods remain below the trees but, when allowed access, livestock readily remove the vast majority of pods.

Many seeds survive passage through the gut, the majority of these persist as "bare" seeds but a low proportion remain enveloped in the tough endocarp of the pod. A large number of viable seeds are deposited in dung away from the parent trees annually and are presumed to be unavailable to *A. prosopis* (Moran *et al.*, 1993). Ants, small rodents, porcupines, birds, antelope and domestic livestock are all responsible for removal of seed pods from below trees (*pers. obs.*) yet sheep are the primary consumers of pods and do so to a far greater degree than other frugivores and granivores. The high stocking rates of sheep on most farms where mesquite occurs results in removal of the entire annual seed crop. Harding (1991) concluded that sheep reduce potential seed populations in the field because on average 85% of seeds that are eaten are destroyed during digestion. Despite these high levels of seed destruction by sheep a large number of viable seed are dispersed through dung deposition. In addition to this, seed scarification in the gut passage may enhance germination probability of mesquite seeds (Peinetti *et al.*, 1993).

The removal of resources for *A. prosopis* through pod ingestion by foraging animals prompted the introduction of a third seed feeding bruchid, *Neltumius arizonensis* Schaeffer, in 1992. The rationale for this introduction was based on reports that *N. arizonensis* attacks and completes its development on green pods that are still attached to the plant (Forister, 1970 in Strathie, 1995 *unpubl.*; Kistler, 1985) thus avoiding the problem of livestock utilisation. Observations in South Africa showed that the original suppositions about the types of pods used by *N. arizonensis* were incorrect and that the beetles only utilise mature pods for oviposition and development (Strathie, 1995 *unpubl.*).

Neltumius arizonensis has become established in South Africa although their abundance is generally low relative to *A. prosopis*. These low numbers are thought to be due to a combination of factors, including: interspecific competition between the two introduced bruchid species (Impson & Hoffmann, 1998), and interference by native parasitoids (Hoffmann *et al.*, 1993; Coetzer & Hoffmann, 1997) with *N. arizonensis* subject to levels of egg parasitism as high as 80% by *Uscana* sp. (Trichogrammatidae) (Coetzer 1996; Roberts, 2000 *unpubl.*). Hoffmann *et al.* (1992) identified nine larval parasitoids (Chalcidoidea) emerging from bruchid larvae in mesquite pods collected in the Northern Cape but did not consider any to have a negative influence on the effectiveness of either bruchid species as biological control agents.

Algarobius prosopis has been found to utilise seeds located in sheep, antelope donkey, and porcupine faeces (Hoffmann & Roberts *pers. obs*, 1998). A report of a similar phenomenon was that of post-dispersal predation of *Acacia farnesiana* seeds by the bruchid *Stator vachelliae* in Central America (Traveset, 1990). The bruchid finds and oviposits on the seeds located in the faeces of horses and Ctenosaur lizards (Traveset, 1989) achieving on average 25% seed destruction. These observations prompted the need for further investigation into the extent to which *A. prosopis* locate and destroy post-dispersed seeds and to quantify if *A. prosopis* is having a greater effect on control of the spread of mesquite in southern Africa than was initially expected.

The use of dung-borne seeds by *A. prosopis* is advantageous for individuals in that they are able to utilise a portion of the larval food resource that would otherwise have been rendered unsuitable. From a biological control perspective the use of dung-borne seed is beneficial in that populations of the beetles may be sustained at higher levels and thus are able to destroy more seeds than would be possible if they were to rely solely on the low numbers of pod-borne seed that escape frugivores. In the absence of pods it would suit *A. prosopis* to utilise dung-borne seed for perpetuation of the populations because the period between pod-fall is a minimum of six months. Survivorship of *A. prosopis* on an artificial diet of sucrose and water under stable laboratory conditions has been shown to be 120 days at 20°C in the presence of pods (Kistler, 1985). Adult female survivorship may be extended under certain conditions such as at lower temperatures (Kistler 1985) or in the absence of pods (Kistler, 1985) or with the provision of pollen (Ernst *et al.*, 1990). However fecundity will likely decrease over extended periods of time (Ishahara & Shimada, 1995) and as a result their contribution to population maintenance would be reduced. Alternatively the beetles could persist in small isolated populations utilising the limited pods that have escaped livestock. However, the resulting concentration of resources could subject the bruchid larvae to higher levels of parasitism and competitive interactions, further compounding the effects of an already diminished population size. The use of dung-borne seed by *A. prosopis* may therefore enable an increase in fitness, escape from parasitism and secure alternative means of over-wintering, that is:

> <u>Increase in fitness</u>: a means by which *A. prosopis* individuals that utilise dung-borne seed increase their fitness by ensuring that a greater number of their offspring would survive than those individuals that relied solely on the use of pods for larval development. An increase in numbers may enable the perpetuation of *A. prosopis* populations in areas where pods are removed.

Escape from parasitism: Parasitoids that recognise pods as the source of hosts may not recognize dung as such and thus those larvae developing in dung may escape mortality arising through parasitism ("enemy-free space" see Lawton & McNeill, 1979 and Price *et al.*, 1980). In addition, the supposed restriction of parasitoids to the vicinity of mesquite trees that would normally support the highest concentration of host larvae, in pods within or below the trees, suggests that larvae in dung-borne seed away from trees may stand a higher chance of escaping parasitism.

Securing an alternative means of over-wintering: The use of dung-borne seed for larval development may enable the emergence of a post-winter cohort that will be more fecund and healthier than the over-wintering adults that have already oviposited to some degree and been subjected to environmental stresses (Ishahara & Shimada, 1995).

The aims of this study were to ascertain how much damage *A. prosopis* is causing on mesquite seeds in both dung and pods in South Africa and how this might be affecting soil seed banks and rates of recruitment of new plants into populations of the weed. In the process, the extent to which environmental conditions (including: arid or mesic habitats; seed exposed or shaded; seasonal effects; and presence or absence of livestock) influenced the abundance and activity of the beetles was investigated.

Chapter 1

The population dynamics of the introduced bruchids, indigenous parasitoids and the effects on seed survival of mesquite.

Abstract

High levels of bruchid damage to seed were consistently recorded at sites in the semi-arid regions with 95-99.6% of seed destruction over the period of one year. Seed damage at sites in the mesic region was lower, approximately 60%. Bruchid activity was strongly affected by temperature and the bruchids appeared to overwinter predominantly as inactive adults. In situations where pods were exposed to solar radiation during winter, bruchid adult activity and larval development rates increased as a result. *Algarobius prosopis* populations were consistently and markedly higher than those of *N. arizonensis*, accounting for 87-100% emergence from pods. *Neltumius arizonensis* numbers were lowest in the mesic region and none were reared from pods collections at the most north-easterly site. Despite the acquisition of additional indigenous hymenopteran parasitoid species, nine of 13 species reared from pod samples, larval/ pupal parasitoid numbers have remained low, generally below 1% of insect emergence at most sites. The levels of larval/pupal parasitism were observed to have peaked at the same time as the *N. arizonensis* populations suggesting that this species may be more susceptible to parasitism and if so these parasitoids may potentially be impacting negatively on *N. arizonensis* populations. Parasitism of *N. arizonensis* by the egg parasitoid *Uscana* sp. has continued to occur but at far lower levels than were previously reported and consequently the impacts during this study on *N. arizonensis* populations were not considered to be as important as in the past.

Introduction

There was continued debate in the 1970s and 80s as to the benefit of multiple species introductions of biological control agents over the introduction of single species for the control of invasive organisms. The argument being that interference from two or more enemies will reduce the total effectiveness to a level below that which the best one would achieve alone (Turnbull & Chant, 1961; Turnbull, 1967; Kakehashi *et al.*, 1984; Myers, 1985). Yet it has been shown in numerous studies that multiple introductions have had a greater impact on plant populations than would a single introduction alone (Huffaker *et al*, 1974; May & Hassell, 1981; Hoffmann & Moran, 1991; 1998; James *et al.*, 1992). Kakehashi *et al.* (1984) argued that in an heterogenous environment where there is no niche overlap or reduced competitive interactions then multiple introductions are suitable but where niche overlap exists for biocontrol agents that show aggressive responses then single species introductions are better.

Competitive interactions between herbivorous insects introduced for biocontrol of weeds can have a substantial effect on the level of success that each agent may achieve (Harris, 1989) yet a reduction in total effectiveness of the agents has not been shown to date. "Interspecific competition is most likely to occur between herbivorous insects that are closely related, introduced, immobile and dependent on discrete resources such as seeds" (Denno *et al.*, 1995). The larvae of the three bruchid species introduced for the control of mesquite fit all these criteria. Thus it may be postulated that the introduction of *N. arizonensis* resulted in either competitive displacement by the superior species (Turnbull, 1967), or a decrease in combined effectivity of the agents resulting from equal competitive outcomes (Kakehashi *et al.*, 1984) or there was an increase or little difference in the degree of over-all control of the weed (Huffaker *et al.*, 1974).

Competition between the two bruchid species:

Each mesquite seed is capable of supporting the development of only one bruchid larva which led Impson and Hoffmann (1998) to predict potentially high levels of competition between the three bruchid species that have been introduced into South Africa. Three times more *A. prosopis* than *N. arizonensis* emerged when equal numbers of neonate larvae of both species were placed simultaneously on seeds, showing that *A. prosopis* was able to out-compete and displace *N. arizonensis*, at least during the larval stages (Impson & Hoffmann, 1998). The larval morphology and behaviour of the two species differ. First-instar larvae of *A. prosopis* are capable of crawling between pods and tunnelling along the length of pods to locate suitable seeds while first-instar *N. arizonensis* larvae are immobile and only capable of utilising the seed immediately adjacent to where the egg is laid. (Pfaffenberger & Johnson, 1976; Kistler, 1995)

Interactions between larvae of the two species may be mediated by the oviposition behaviour of the adult beetles. *Neltumius arizonensis* is less fecund than *A. prosopis* (Kistler, 1995; Hoffmann *et al.*, 1993; Strathie, 1995 *unpubl.*) but the females are more meticulous in where they deposit eggs, seeking seeds around which the exocarp is pristine (Strathie, 1995 *unpubl.*). This fastidious behaviour enhances the chances of larval survival but restricts the numbers of seeds that are suitable for *N. arizonensis*. Females of *A. prosopis*, on the other hand, are less discerning and deposit eggs in many different situations, but mostly in seed pods that are blemished or weathered, allowing them to exploit almost any available seeds and hence a much larger pool of resources.

Under conditions of high pod availability it is expected that *N. arizonensis* populations will persist in the field, albeit at lower densities than *A. prosopis*. However, when pods are scarce, *A. prosopis* is expected to dominate the system and out-compete *N. arizonensis*.

Biotic and abiotic factors affecting seed quantity and quality and hence availability of seeds:

High levels of pod removal by herbivores during the pod fall period, before the bruchids are able to utilise the resource for population increase (Glendening & Paulsen, 1955), reduce seed availability for subsequent generations and enhance the chances of increased competition for an ever dwindling resource (Kistler, 1985; Keddy, 1989). Kistler (1985) found that in Arizona the bruchids are subject to population pressures induced by seed quality, quantity and lack of reproductive synchrony by the trees, creating heterogeneity in the availability of suitable seeds. In South Africa the limitations of seed quantity, induced by livestock removal of seeds, increase the constraints on bruchid populations which are likely to be more profound than those in Arizona yet any asynchrony in pod production may contribute to the perpetuation of bruchid populations.

Invertebrate herbivores may also impact negatively on seed availability and pod condition. In South Africa, Hemiptera (Alydidae) have been observed on mesquite pods (Hoffmann, *pers comm.*) from which they suck nutrients and destroy the developing seeds (Kistler, 1985). In Texas, Smith and Ueckert (1974) reported a 70% reduction in viable seeds on mesquite as a result of feeding by *Chlorochroa ligata* (Hemiptera: Pentatomidae). In contrast, wheat crickets, *Acanthoproctus* sp. (Orthoptera: Bradyporidae) in South Africa may enhance conditions for *A. prosopis* while diminishing suitable conditions for *N. arizonensis*. The crickets feed on and shred mesquite pods, exposing the meso- and endocarp, creating blemishes that are ideal oviposition sites for *A. prosopis* (Strathie, 1995 *unpubl.*) and creating conditions that are ideal for the beetles to conceal their eggs from predators and parasitoids (Swier, 1974). The effects of invertebrates on suitability of seed pods for oviposition and larval development are not investigated further in this study, other than generally as an inexorable component of the systems under consideration.

Pods lying on the ground often support prolific fungal growth with the onset of rains (Coetzer, 1996). Laboratory experiments showed that *N. arizonensis* seldom oviposits on fungus-covered pods and preferentially lays eggs on fungus-free pods (Coetzer, 1996) while *A. prosopis* readily utilises fungus-covered pods for oviposition (*pers. obs.*). In fact *A. prosopis* continues to emerge from pods for extended periods despite deterioration in pod condition. In South Africa, Zimmermann (1991) recorded adult emergences of *A. prosopis* from 10 month old pods in which 92% of the seeds had been destroyed and in Argentina, six years after pod fall, Baes *et al.* (2001) recorded 99% seed damage by the bruchid *Scutobruchus ferocis* on *Prosopis ferox*.

<u>Egg and larval parasitism of the bruchids:</u>

Although the genus *Prosopis* is not represented naturally in Southern Africa, a number of taxonomically and biologically similar *Acacia* species (Leguminosae) are present throughout the range of mesquite. As predicted and demonstrated by Hoffmann *et al.*, (1993), a number of indigenous parasitoids of bruchids associated with acacias were, at the time, utilising *A. prosopis* larvae as an additional host, albeit never at high levels (i.e. maximum parasitism levels never exceeded 5.4% and were on average 0.4%).

Although it is likely that *A. prosopis* and *N. arizonensis* share larval and pupal parasitoids, an essential difference between the two species is the fact that *N. arizonensis* eggs are exposed on the pod surface. The eggs in this situation have been exploited by a parasitoid species, *Uscana* sp. (Trichogrammatidae) which does not occur on *A. prosopis*. The impact of *Uscana* sp. could be having a substantial impact on *N. arizonensis* populations because parasitism rates of between 50-80% are frequently encountered (Coetzer & Hoffmann, 1997; Roberts, 1998 *unpubl.*).

In this part of the study I tested the assumption that *A. prosopis* will always be the numerically dominant species on mesquite in South Africa, because it is able to exploit seed pods in any condition and is less affected by parasitoids, as opposed to *N. arizonensis* which is confined to utilising recently-matured, pristine pods and which suffers high levels of parasitism. The possibility that *N. arizonensis* might become more abundant in some situations or seasons was also investigated but, in general, *A. prosopis* was expected to be by far the major contributor to biological control of mesquite seeds in South Africa with *N. arizonensis* playing a very minor or even insignificant role in the system.

Methods:

Seasonal and annual variation in seed damage and populations of bruchids and larval/pupal parasitoids

Because arid tolerant, mesquite grows successfully in climatically diverse regions and on many soil types (Jeffrey & March 1995), study sites were established across the major distribution of mesquite in South Africa along a 700km transect which ran broadly in a north east direction (Fig. 1.1). The distribution of mesquite can be separated into three main climatic zones namely, semi-arid winter rainfall (South-west region), semi-arid summer rainfall (Central region) and mesic summer rainfall (North-east region) (Fig. 1.2 & 1.3). A brief description of each study site follows:

South-west region: Winter rainfall. Natural vegetation in the region is classified as Semi-arid Upper Succulent Karoo (Low & Rebelo, 1996):

- **Citrusdal** (32° 33.800S, 19° 00.700E, elevation 182m, annual precipitation 460mm) – Situated to the north of the town Citrusdal. Although high rainfall occurs during winter, summer conditions are characterised by hot dry conditions. Sparsely dispersed mature mesquite trees occurring on clay soils with high incidence of grass cover between trees. No evidence of ongoing recruitment of young mesquite plants into the population. No livestock present.

- **Clanwilliam** (32° 10.000S, 18° 53.000E, elevation 75m, annual precipitation 210mm) – A small dense stand of mature mesquite trees occurring along an ephemeral stream, very low vegetation cover other than mesquite. Low numbers of juvenile plants present but estimated to all be older than 5-10years. Most of the surrounding area has been transformed through agriculture. No livestock present.
- **Driefontein** (32° 01.000S, 19° 12.000E, elevation 300m, annual precipitation 185mm) – Mesquite trees planted alongside a natural spring but have spread away from original plantation. Invasion situated on sands that lie above a shallow, hard calcrete layer. Surrounding vegetation on slopes dominated by succulent karoo but low lying sands dominated by indigenous grasses, predominantly *Stipagrostis namaquensis*. In general, a young invasion that has not as yet densified. Low density of Dorper sheep present.
- **Calvinia** (31° 29.155S, 19° 25.638E, elevation 987m, annual precipitation 200mm) – Mesquite planted along river banks and as borders to croplands on Soetwater farm. High levels of invasion by mesquite into the croplands have occurred in the past but ploughing from time to time destroys juvenile plants. Because of the high density of Dorper sheep, the bulk of field experiments were conducted at this site.

<u>Central region: Summer rainfall. Natural vegetation in the region is classified as Semi-arid Upper Nama Karoo (Low & Rebelo, 1996):</u>

- **Vanwyksvlei** (30° 28.544S, 22° 00.611E, elevation 962m, annual precipitation 177mm) – The farm (Humansdam) in which the site lies, contains a large and very dense mesquite invasion that infests the low-lying lands, that constitute much of the property, stretching for several kilometres either side of an ephemeral stream. There appears to be active invasion at this site as there are many size classes of juvenile and mature plants present. Although antelope are present the bulk of pods are removed by sheep except for those pods in a 1ha exclusion area of which a high proportion of seeds are eaten by rodents. The 1ha exclusion area was erected >20 years prior to this study. Within the boundaries of the mesquite invasion there is very little natural vegetation and bare ground and juvenile mesquite plants dominate the gaps between mature trees. However, within the livestock exclusion area there are mature and juvenile mesquite trees interspersed within a matrix dominated by natural vegetation.

- **Prieska** (29° 40S, 22° 44E, elevation 947m, annual precipitation 180mm) – Predominantly mature and late juvenile mesquite plants confined to a 100m wide strip along a drainage line. No livestock access to the site but high levels of seed removal presumably by rodents.

North-east region: Summer Rainfall. Natural vegetation in the region is classified as mesic Eastern Mixed Nama Karoo (Low & Rebelo, 1996):

- **Modder River** (29° 02.043S, 24° 45.647E, elevation 1360m, ann. precipitation 400mm) Mesquite infestation bordered on one side by the Modder river and on the other by croplands. Large and small size classes well represented. Invasion active at the sites with and without livestock present. Mesquite in monoculture with little to no natural vegetation represented within the area invaded by mesquite. Half the site on Rooidam farm, where no livestock were present and had been excluded for 15 years. Other half of the site on neighbouring farm, Gouskraal, where livestock, predominantly cattle, were present.

- **Kingswood** (29° 02.043S, 24° 45.647E, elevation 1260m, annual precipitation 508mm) – Mesquite in but not confined to a depression with little to no natural vegetation in association. Pods eaten by cattle at this site except for a small fenced area from which the cattle were excluded.

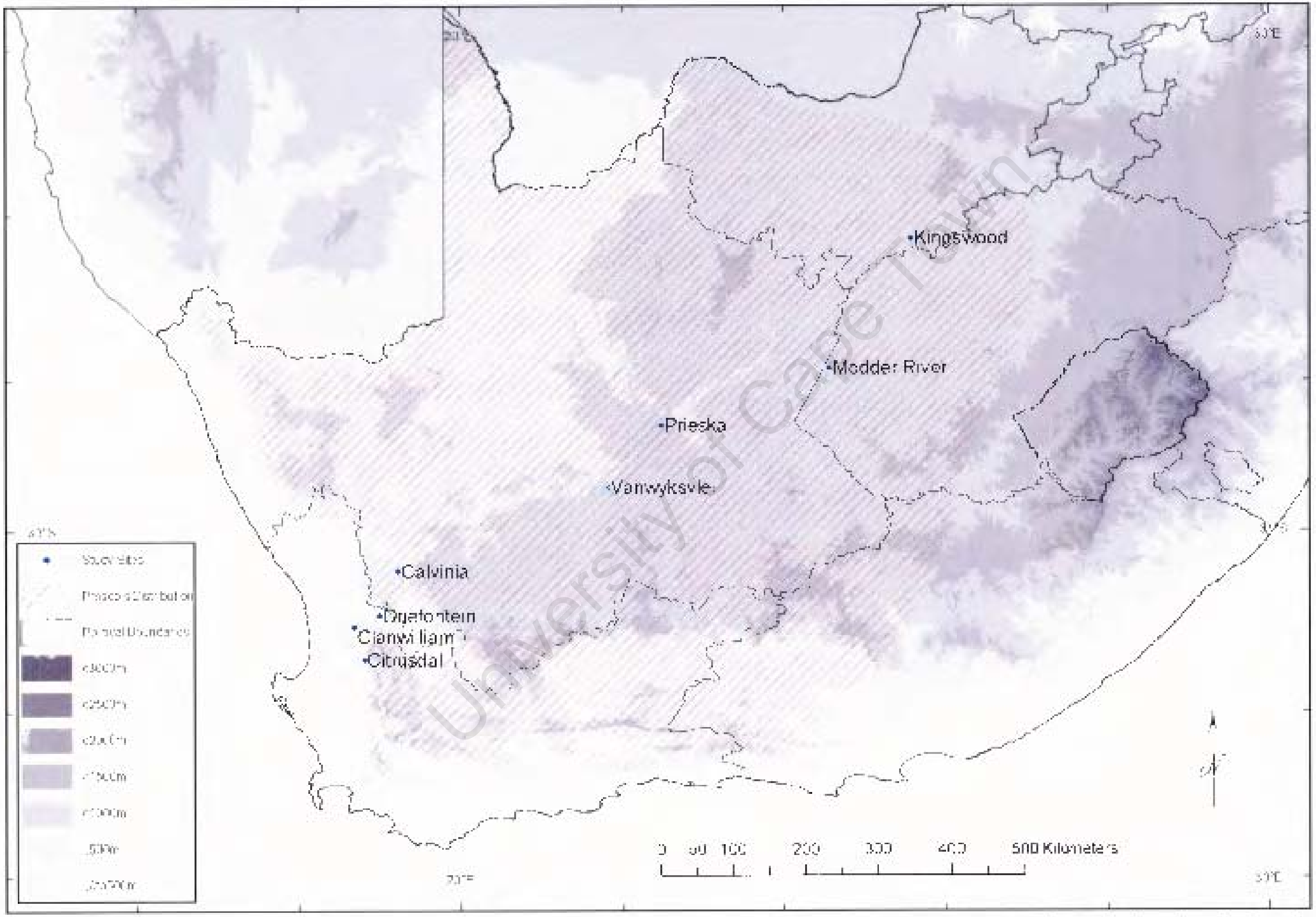


Fig. 1.1: The altitudinal map of South Africa illustrating the range of mesquite and the location of study sites in the South-west, Central and North-east regions.

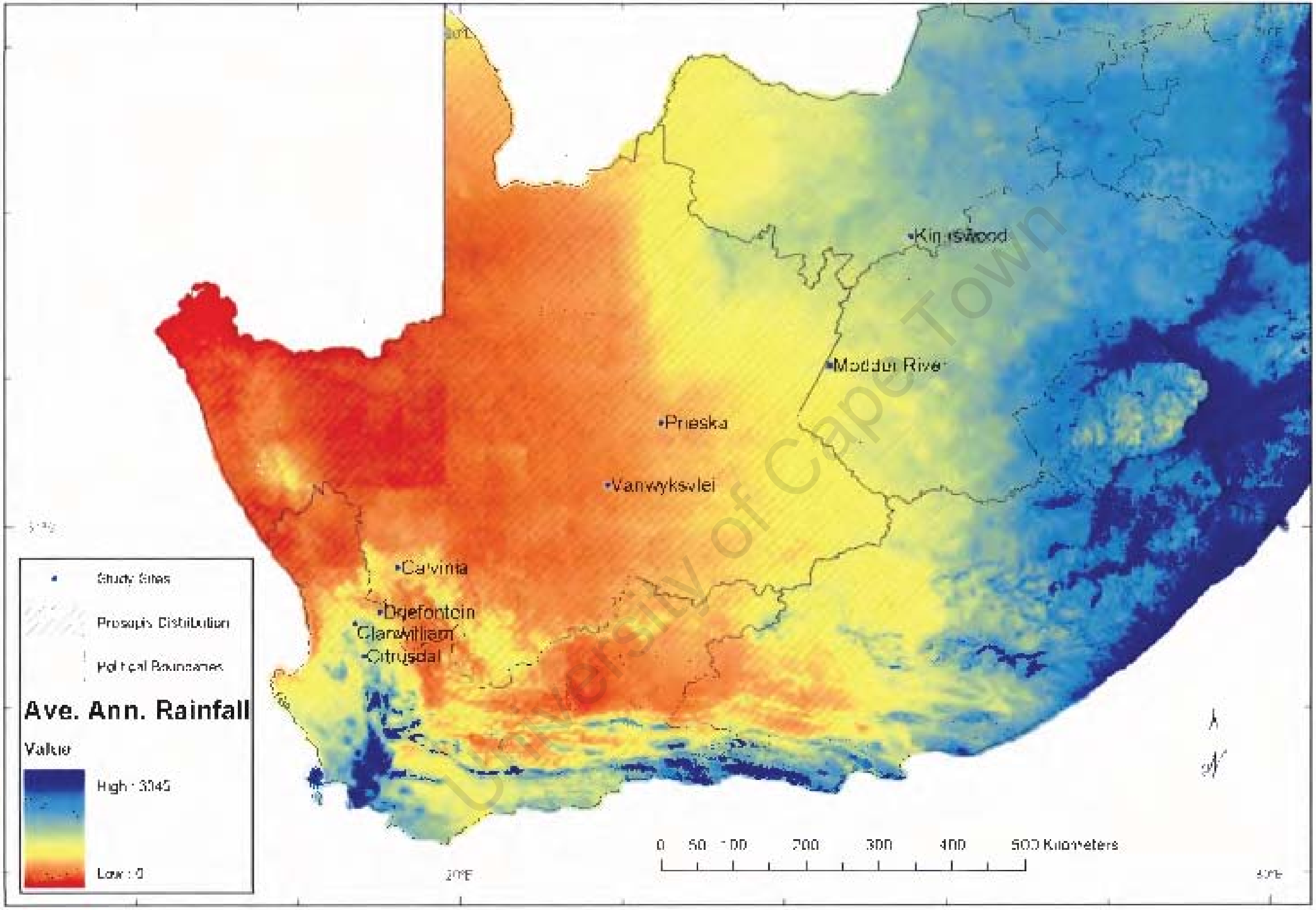


Fig 1.2: Location of study sites overlaid with average annual precipitation

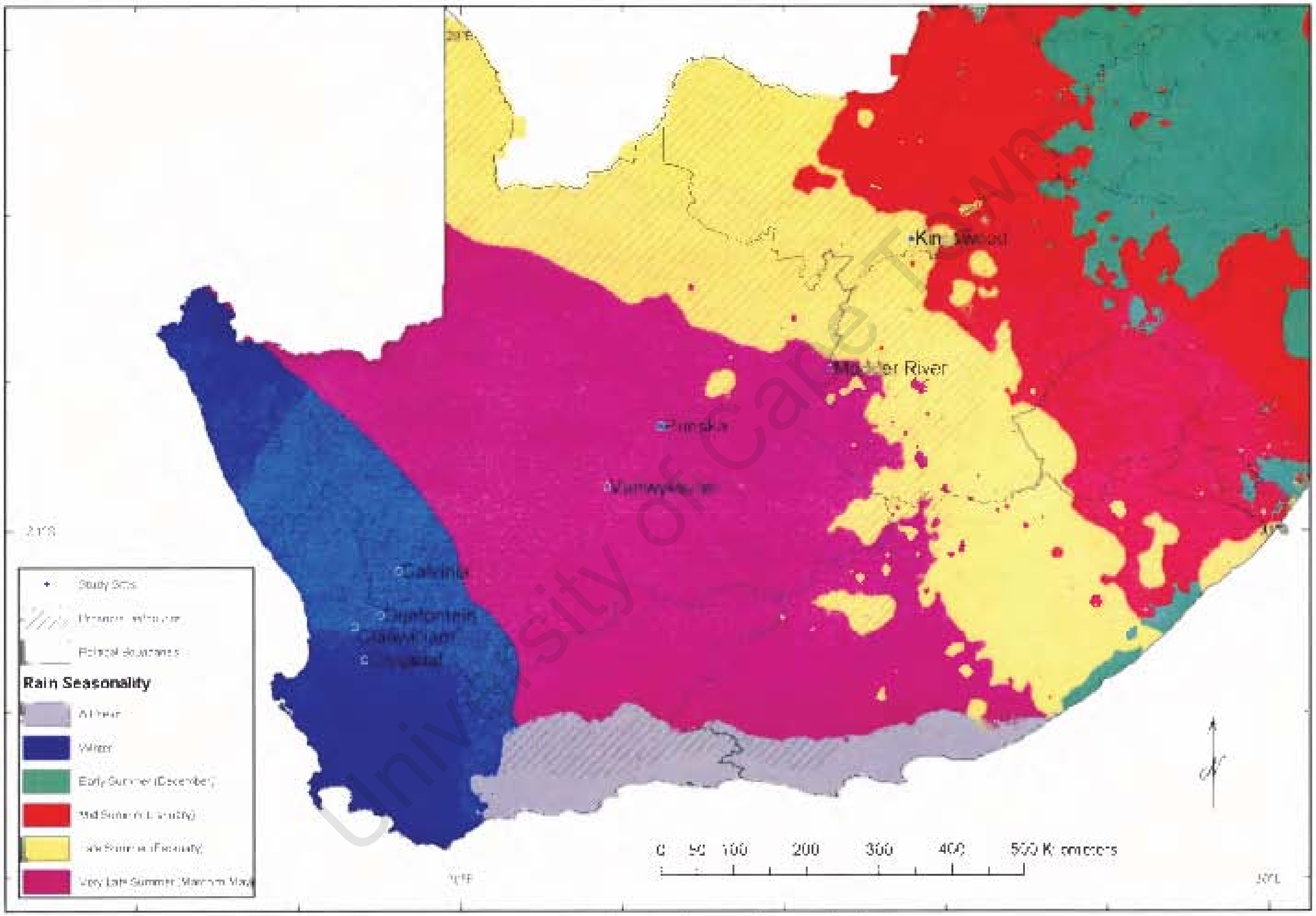


Fig. 1.3: Location of study sites and the seasonal rainfall experienced at each site.

Samples of pods were collected monthly for three seasons from the Clanwilliam and Driefontein sites commencing in January 2002 and ending in February 2004 and for two seasons from the Calvinia site commencing in January 2003 and ending in February 2004. At Vanwyksvlei, Prieska, Modder River and Kingswood, pods were collected every four months commencing February 2003 and ending in February 2005. Because of the indehiscent nature of the pods and their tough exocarp, mesquite pods which are produced in mid summer remain intact for many months or even years, unless removed by grazing animals or flood waters, (Baes *et al.*, 2001) making sequential sampling over a one year period possible. Pods from successive seasons could be easily differentiated because previous-season's pods were discoloured, dry and often fragmented, with advancing decay and surface weathering while the current-season's pods were naturally coloured, more fleshy, entire and with little surface weathering.

Pod samples, which included enough material to incorporate approximately 1200-1500 seeds, were brought back to the laboratory. A subsample of approximately 300 pods was removed and placed in a -20°C freezer in order to establish levels of seed damage in the field and levels of egg parasitism at the time the pods were collected. The remaining pods were placed in rearing containers (5 litre plastic boxes with gauze lids) and kept in a constant temperature room at 27°C ± 2°C and 40-60% relative humidity.

Emerging beetles and parasitoids were counted and removed from the rearing containers every 3-5days for a period of 35 days after which the pods were placed in a -20°C freezer. Terminating the experiments after 35 days ensured that all the individuals, including eggs, that were present in the pods at the time of collection had become adults, but that adults developing from eggs laid by adults that had emerged in the rearing containers would not be included in the counts.

In order to compare variations in bruchid/parasitoid activity and density between study sites and collection dates the numbers of bruchids (B) and larval parasitoids (P) emerging were standardised by converting the respective numbers to emergences per 1000 seeds (BStand and PStand). The total number of fully-developed seeds (STotal) and the total number of bruchids (BTotal) was the total count of bruchids that emerged during the 35 days after collection, so that:

$$\text{BStand} = 1000/\text{STotal} \times \text{BTotal} \qquad (1)$$

The conversions were made separately for each of the two bruchid species but all larval/pupal parasitoids emerging were considered collectively and irrespective of host species. It was assumed that the larval/pupal parasitoid species utilised both species of bruchid and because it was not possible to determine from which bruchid species they had emerged the counts were combined under one label 'parasitoids'.

In order to determine the seasonal population dynamics of the bruchids, levels of monthly seed damage were used to assess the availability of viable seeds on each collection date. Any decrease between months in the proportion of viable seeds in the samples indicated bruchids were utilising the pods during the previous month. Stasis of seed availability between months indicated either larval dormancy or absence of larvae in the seed pods. Seed availability (SAvail) at each collection date (t) was calculated as:

$$\text{SAvail}_{(t)} = \text{STotal}_{(t)} - \text{SHole}_{(t+1)} + \text{BTotal}_{(t+1)} \qquad (2)$$

where $STotal_{(t)}$ is the number of intact seeds in the sample (i.e. excluding those with emergence holes at the time of collection but including those containing immature bruchids on collection, as determined by the total number of bruchids, $BTotal_{(t+1)}$, that had emerged in the containers) and $SHole_{(t+1)}$ is the number of seeds with bruchid emergence holes after the laboratory emergence period..

All larval/pupal parasitoids that emerged were preserved in 80% alcohol to be identified later and compared to those collected by Hoffmann *et al.,* (1992) and Coetzer (1996), to establish if additional species of parasitoids had adopted the bruchids as hosts in the ensuing years.

Removal of pods by livestock at the Driefontein, Calvinia, Vanwyksvlei and Kingswood sites was prevented by either the erection of exclusion fences around individual trees, or by exclusion of livestock from certain paddocks. No livestock were present at the Clanwilliam and Prieska sites and at Rooidam farm at the Modder River site.

Variations in levels of seed damage between years:

As a result of the degradation of pods with time and the propensity for pods to accumulate fungal growth and become weathered, it became increasingly difficult to make accurate counts of the numbers of seeds in pods towards the end of the season. 'Old' pod samples, i.e. year-old pods, were therefore examined with X-rays. The pods were mounted on adhesive paper and placed in a Siemens – Elecma, Orbix X-ray machine. Seed exposure factors were 40KV, 32mAs and source image distance was 122cm. AGFA single emulsion film in a 24x30cm cassette with a 1x50 speed intensifying screen was used. Examination of the X-ray plates made it possible to accurately determine the numbers of undamaged, viable seeds in the sample of pods. However, this method was not suitable for identifying the cause of damage in seeds that were not intact, i.e. it was not clear whether the damage was as a result of bruchid usage, fungal decay or germination and subsequent decay. On average approximately 20% of ovules aborted early in their development on an annual basis at all sites. The resilience of the seeds with intact seed coats made it possible to assume that any damage to the remaining 80 % of seeds was initiated through bruchid larval penetration.

Chi-squared analyses were used to determine if there was any inter-annual variation in levels of seed destruction by the bruchids within and between sites in 2003 and 2004.

Results

Seed damage in one-year old pods

For most sites, high levels of seed damage were recorded in pods that had been lying on the soil for one year (Fig. 1.4). Bruchids continued to emerge from the year-old pods. Levels of seed destruction in 2002 and 2003 were similar for six of the eight sites, averaging approximately 97%, but two of the sites, Calvinia and Modder River, had significantly lower levels of seed damage (χ^2, $p<0.01$) than at other sites in both 2002 and 2003. Levels of seed damage were significantly higher at Driefontein and Citrusdal in 2002 than in 2003 ($\chi^2 = 9.29$, $p = 0.0023$ and $\chi^2 = 7.20$, $p = 0.0073$ respectively) which may have been due to the bruchid populations being affected by tree clearing operations which were conducted in 2003 at both of the sites. There was no significance in the levels of damage in 2002 and 2003 at any of the other sites.

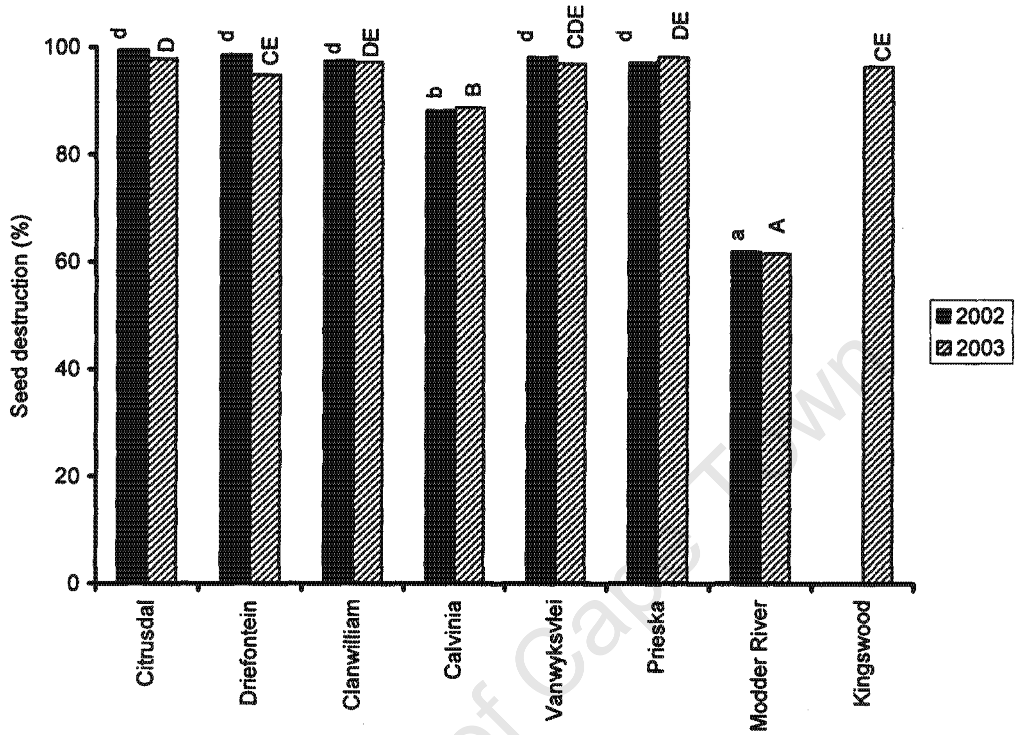


Fig. 1.4: Levels of seed damage to pod samples over the 2002 and 2003 seasons and collected in February of 2003 and 2004, one year after pod-fall at several sites across the distribution of mesquite. Bars with similar letters are not significantly different (χ^2, $p > 0.05$, lower case for comparisons in 2003 and upper case for comparisons in 2004).

Seasonal emergence patterns and subsequent decrease in seed availability

Winter rainfall region:

At all sites *A. prosopis* were numerically dominant (Table 1.1). The presence of livestock did not appear to affect the percentages of either bruchid species however, the percentages of larval/pupal parasitoids were marginally higher at sites where livestock were present.

Table 1.1: Emergence of each taxon as a percentage of the total insect emergence from pod collections at sites in the winter rainfall region in 2003.

		Percentage of total insect emergence		
Site	Livestock	*A. prosopis*	*N. arizonensis*	parasitoids
Clanwilliam	absent	99.1	0.9	<0.05
Citrusdal	absent	90.2	9.5	0.3
Driefontein	present	89.9	6.4	3.7
Calvinia	present	87.8	11.4	0.8

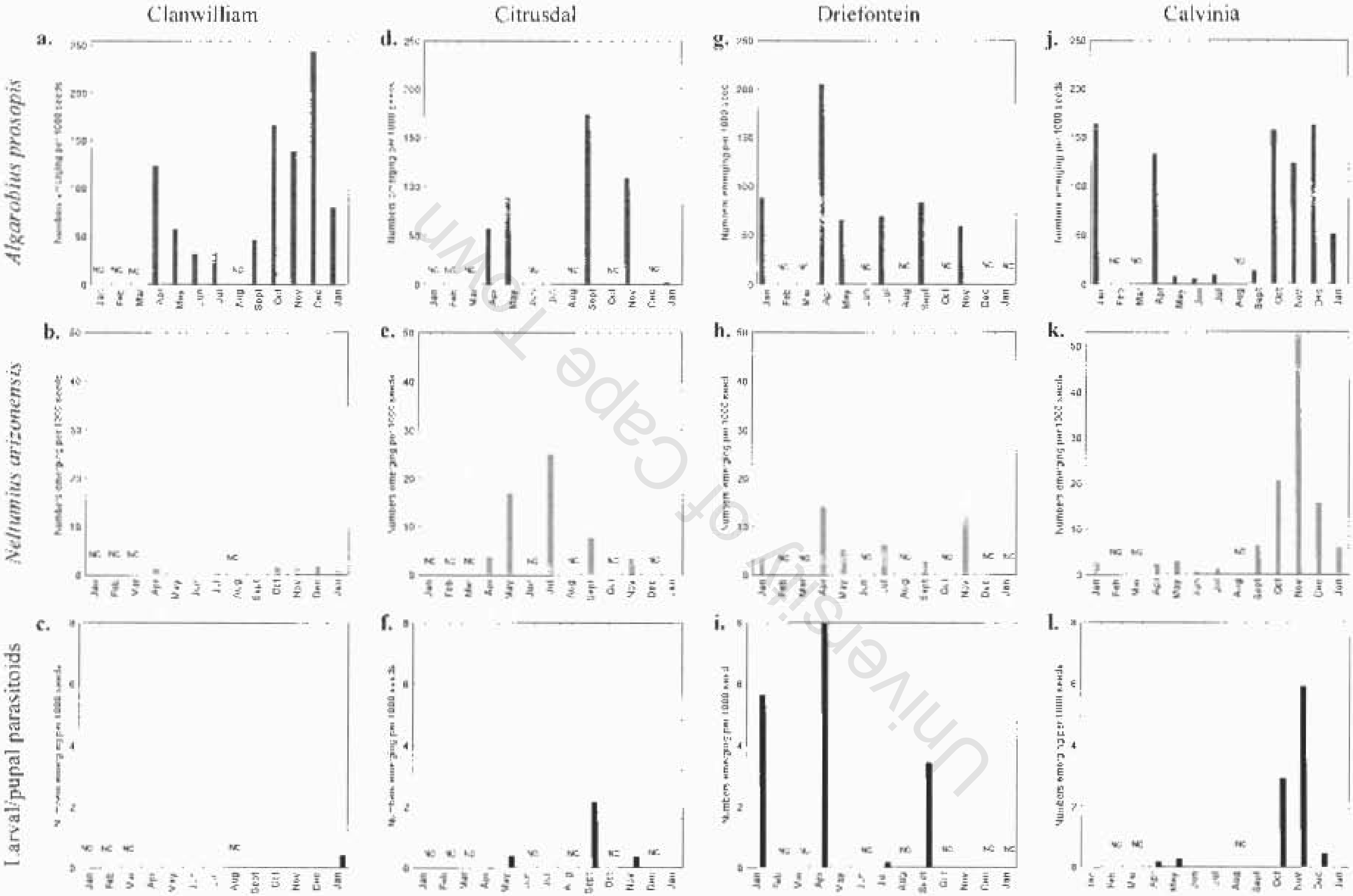
The numerical dominance of *A. prosopis* populations at Clanwilliam (99.1%) is possibly the norm in situations where livestock are absent. *Algarobius prosopis* numbers are consistently far higher than *N. arizonensis* averaging between 88-99% of total insect emergence and *A. prosopis* was responsible for approximately 30% of seed destruction by May, at the start of winter.

The patterns of emergence showed similarities and differences as follows (Fig. 1.5 a-l):

Algarobius prosopis:

Algarobius prosopis commenced ovipositing as soon as the pods matured and started emerging from pods during January. The numbers of *A. prosopis* that emerged from pods collected June-September, during winter, was similarly low between months suggesting there was little oviposition during this period. There was low early season activity at Citrusdal (Fig. 1.5 d) but high emergence during winter suggests higher *A. prosopis* activity during this period was a consequence of increased pod temperatures due to solar irradiation of pods after tree felling.

Emergence of *A. prosopis* during spring was higher than during autumn. The early pre-winter peak in *A. prosopis* activity at Driefontein (Fig. 1.5 g) was likely a result of limited pod availability due to active removal of pods by livestock and subsequent concentration of the insects on the remaining pods in areas where livestock were excluded. This high proportion of April emergence led to higher pre-winter oviposition by *A. prosopis* and subsequent high levels of emergence over winter in the laboratory. *A. prosopis* populations responded to increased seed availability, a result of the second flush of pods at Clanwilliam (Fig 1.5a) during winter, by maintaining high numbers, as evident from the second post-winter population peak seen in December.



<u>Fig.1.5 (a)-(l)</u>: *Algarobius prosopis* (row 1- a, d, g, j), *Neltumius arizonensis* (row 2 – b, e, h, k) and larval/pupal parasitoid (row 3 – c, f, i, l) emergence from monthly pod collections from Winter Rainfall Sites in 2003 at Clanwilliam (a-c), Citrusdal (d-f), Driefontein (g-i) and Calvinia (j-l). "NC" – No collections on these dates.

Neltumius arizonensis

The highest contributions of *N. arizonensis* to insect emergence in any month was 27% and 33% in May and September respectively at Calvinia (Fig. 1.5k), averaging 18.7% during the post-winter period at this site. *Neltumius arizonensis* populations overwinter as adults and not as larvae as there were very few adult *N. arizonensis* reared from winter pod collections except from those collected at Citrusdal (Fig. 1.5 e). There was evidence of pre- and post-winter oviposition by *N. arizonensis*, however post-winter populations were higher or at least oviposition on pods was highest, post-winter. This trend was most pronounced at Calvinia (Fig, 1.5 k). As with increased *A. prosopis* oviposition during winter at Citrusdal (Fig. 1.5 e), there was a noticeable increase in oviposition by *N. arizonensis* over this same period and adults contributed to 9.7% of the emerging insects.

Larval/pupal parasitoids

Parasitoid levels, as a proportion of bruchid emergence, were very low with between 0% and 3.7% of insect emergence. The highest levels of parasitism were recorded at Driefontein (Fig. 1.5i). Parasitism at Clanwilliam (Fig. 1.5c), Citrusdal (Fig. 1.5f) and Calvinia (Fig. 1.5l) was low and appeared to be highest early to mid-summer. Interestingly, despite relatively high mid-winter (July) populations of both *N. arizonensis* and *A. prosopis*, there was no parasitism at Citrusdal (Fig. 1.5f) over this period.

Seed availability

The most pronounced reduction in seed availability at all sites occurred post-winter, falling to below 20% by January of the following year (Fig 1.6). High bruchid activity at Calvinia reduced the available seed from 92% in November 2003 to 17% by January of 2004.

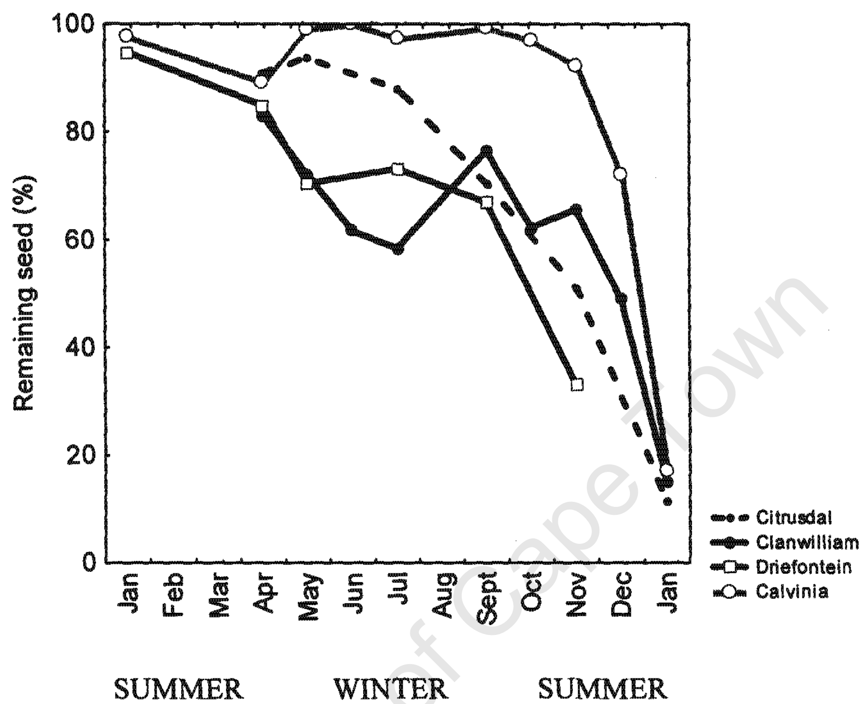


Fig. 1.6: The percentage of seed escaping bruchid damage during 2003 at four sites in the winter rainfall area.

The relative inactivity of the bruchids during winter resulted in stasis of seed availability over the winter period. This is demonstrated clearly by little change in available seed at Calvinia between May and September. The increase in available seed at the Clanwilliam and Calvinia sites during winter was a consequence of a second flush of pods that fell in July and May respectively.

The patterns of emergence in the winter rainfall region showed a general decline in bruchid activity during winter. This is reflected by the levels of seed availability in the field with numbers of available seed decreasing during periods when bruchids are active and remaining static when bruchids are inactive. Monthly emergence of bruchids in the laboratory reflected periods of oviposition in preceding months.

Summer rainfall region:

As with the winter rainfall region, *A. prosopis* accounted for high levels of seed damage (95-100%) in the summer rainfall region (Table 1.2), and *N. arizonensis* contributed far less to insect emergence in this region than in the winter rainfall region, accounting for only 0-5% of total number of insects that emerged.

Table 1.2: Emergence of each taxon as a percentage of the total insect emergence from pod collections at sites in the summer rainfall region in 2003.

		Percentage of total insect emergence		
Site	Livestock	*A. prosopis*	*N. arizonensis*	parasitoids
Vanwyksvlei	present	96.7	3.1	0.2
Prieska	absent	95.8	4.1	0.1
Modder River	absent	98.4	1.4	0.2
Kingswood	present	99.6	0	0.4

There was no record of *N. arizonensis* from the most easterly site, Kingswood. Larval/pupal parasitism was very low on all sampling dates accounting for 0.1-0.4% at the four sites occurring through the year but no parasitoids emerged from pods collected on final sampling dates.

The patterns of emergence showed similarities and differences as follows (Fig. 1.7 a-l):

Algarobius prosopis

Mid-winter (June) collections showed high *A. prosopis* emergence from all sites. The decrease in seed availability and increased emergence in the laboratory reflected *A. prosopis* activity during winter. However, high June emergence in the laboratory from Modder River (Fig. 1.7g) pods was not paralleled by a decrease in available seed in the field implying that larval development was slowed during winter at this site. Highest *A. prosopis* activity occurred post-winter as seen by the greatest decline in available seeds between October and February, most likely a consequence of high larval activity coupled with resulting seed degradation during periods of rain. Despite the high levels of available seed at Modder River one year after pod fall there was only low emergence from these pod collections. Six times as many bruchids emerged from recently matured 2004 pods than from the older 2003 pods collected in February of 2004.

Neltumius arizonensis

Emergence of *N. arizonensis* occurred from pods soon after pod fall, however, there was no emergence from pods at the end of the year when pod condition has deteriorated and there wais likely to be increased interspecific competition for remaining resources. The highest recorded emergence of *N. arizonensis* appeared to occur in spring

Larval/pupal parasitoids

Parasitism was low and appeared to occur in both winter and summer. The highest numbers of parasitoids emerged at the Kingswood site in spring (Fig. 1.7l) and likely

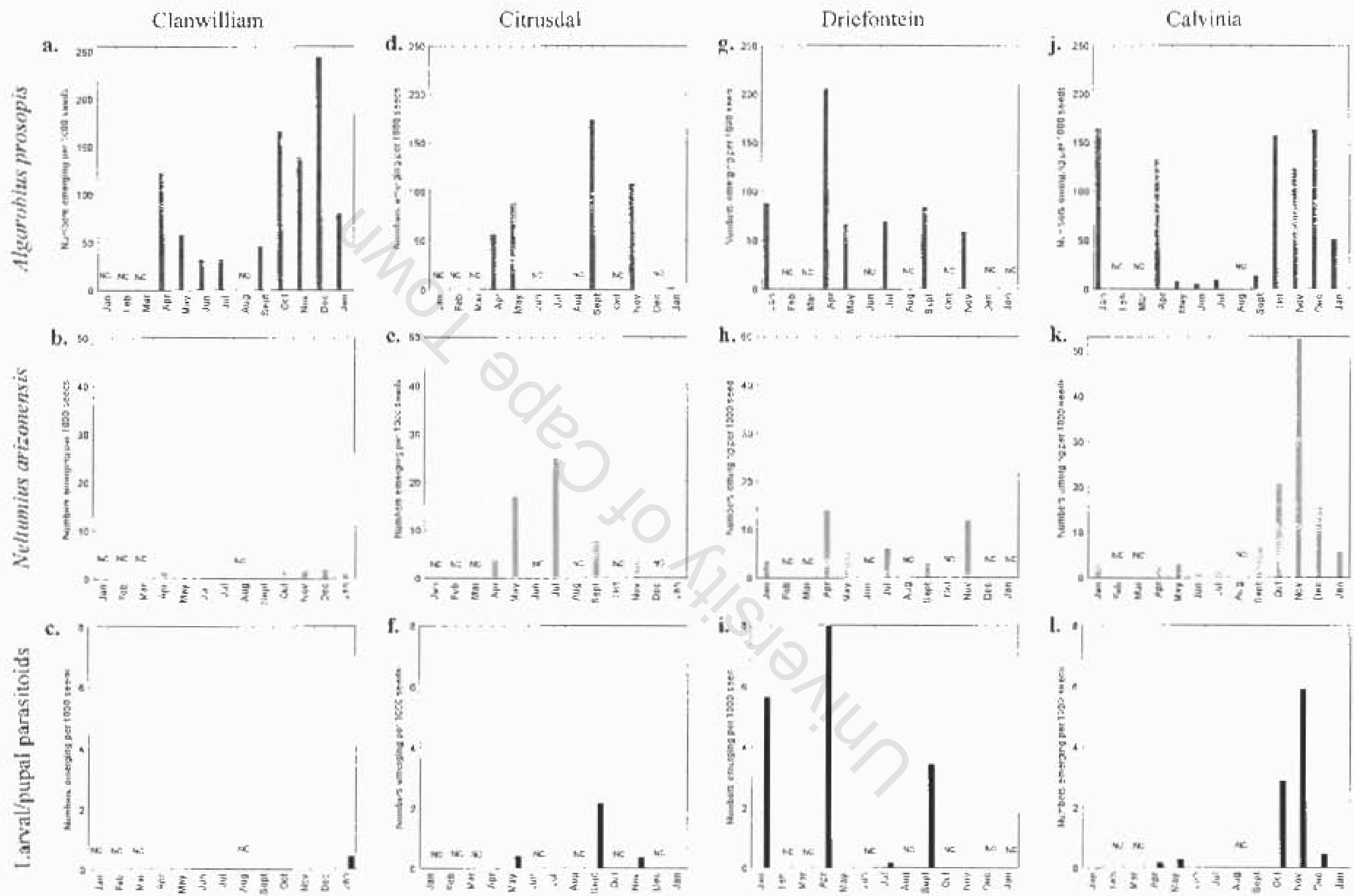


Fig.1.5 (a)-(l): *Algarobius prosopis* (row 1- a, d, g, j), *Neltumius arizonensis* (row 2 - b, e, h, k) and larval/pupal parasitoid (row 3 – c, f, i, l) emergence from monthly pod collections from Winter Rainfall Sites in 2003 at Clanwilliam (a-c), Citrusdal (d-f), Driefontein (g-i) and Calvinia (j-l). "NC" = No collections on these dates.

parasitised *A. prosopis* because no *N. arizonensis* were reared from pod collections from this site.

The pattern of emergence from the summer rainfall region differed from the winter rainfall region by having higher bruchid activity over winter with higher larval numbers within seeds and higher levels of seed destruction over this period.

Seed availability

At all sites there was a steady decline in available seeds as the season progressed (Fig.1.8), but because of having only data for one survey date in winter it was not possible to determine if there was a decrease in available seeds through winter. The high levels of emergence of *A. prosopis* from the June samples suggested that there was continuous ovipositing on pods over the winter period but the paucity of the information,

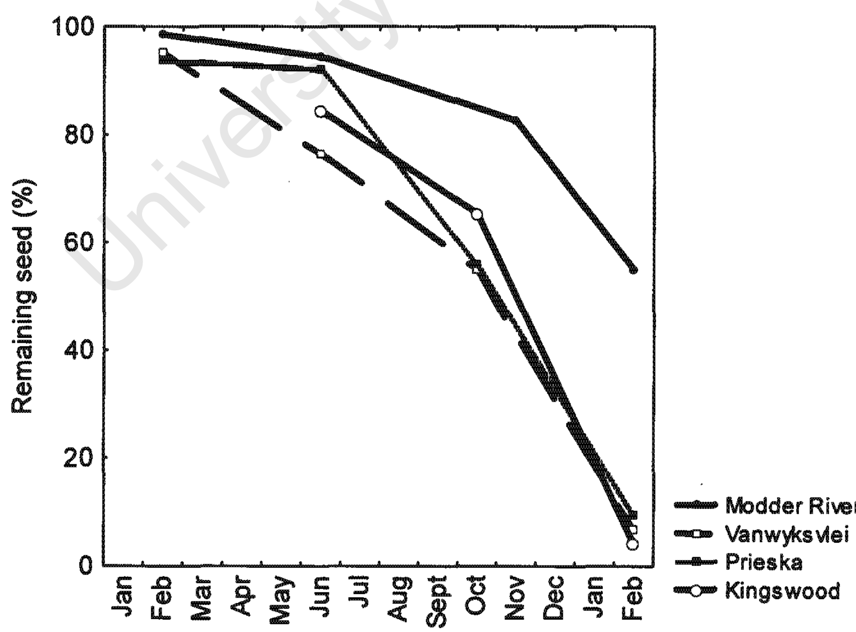


Fig. 1.8: The percentage of seed escaping bruchid damage during 2003 at four sites in the winter rainfall area.

due to staggered sampling, does not allow us to quantify the development of hatching larvae. However, the dramatic decline in numbers of available seeds between June and October at Prieska gives strong evidence for high bruchid activity during winter and a subsequent decrease in available seed. The highest period of bruchid activity was post-winter as seen by the greatest decrease in available seed over this period. Seed availability dropped to below 10% at Vanwyksvlei, Prieska and Kingswood after one year in the field but at Modder River it only decreased to 55% by this time. At the Kingswood site, livestock were slow to remove all fallen pods enabling bruchid populations to be maintained through the year, however, by late summer, pods were restricted to small areas from which livestock were excluded and competition for these remaining resources was likely intense.

A decrease in seed availability during summer in the summer rainfall region may not necessarily have reflected an increase in *A. prosopis* adult numbers but merely that there was an increase in the number of seeds containing bruchid larvae, a good proportion of which may have succumbed to mortality as a result of seed degradation.

New larval/pupal parasitoid associations:

Of the hymenopterans collected during the course of this study at least 9 out of a total of 13 were confirmed to be different to those collected by Hoffmann *et al.*, (1993) and Coetzer, (1996) and are considered new associations with the introduced bruchids (Table 1.3). The initiation of these "recent" associations cannot be determined conclusively due to variations in the intensity and duration of the sampling in the three studies, however, it does suggest that new parasitoid-host associations are continuing to be established. It is also strange that this study missed many parasitoids found previously suggesting that parasitism is not stable and that the parasitoids are opportunistic, except for possibly four of the species.

Table 1.3: Hymenopteran parasitoids of bruchid species reared from pod samples collected at sites across the distribution of mesquite tabulating those found in this study and those found in the studies by Hoffmann *et al.* (1993) and Coetzer (1996). Highlighted species may potentially be recent associations subsequent to those identified in the previous studies.

	This study	Hoffmann *et al.* (1993)	Coetzer (1996)	
Family	Genus	Genus	Genus	mesquite source
Torymidae	*Pseudotorymus sp.*			pods
		Antistrophoplex sp		pods
			Genus 1	pods
Eulophidae	*Aprostocetus sp.*			pods
	Entedon sp.1			pods
	Entedon sp.2	*Entedon sp.2*	*Entedon sp.2*	pods
		Entedon sp.3		pods
Encyrtidae	*Negenaspidus sp.*			pods
Eupelmidae	*Brasema sp.*			pods
	Eupelmus sp.1	*Eupelmus sp.1*		pods
	Eupelmus sp.2			pods
	Eupelmus sp.3			pods
	Eupelmus (Macroneura) sp.1			pods
	Eupelmus (Macroneura) sp.2			pods
		Eupelmus urozonus	*Eupelmus urozonus*	pods
			Eupelmus sp. 4	pods
		Genus 1	Genus 1	pods
			Genus 2	
Pteromalidae	*Dinarmus altifrons*	*Dinarmus altifrons*	*Dinarmus altifrons*	pods
		Dinarmus sp.1		pods
		Anisopteromalus sp.		pods
		Genus 1		pods
		Genus 2		pods
Eurytomidae	*Eurytoma sp.1*	*Eurytoma sp.1*		pods
		Bruchophagus		pods

The effect of egg parasitism on *N. arizonensis* populations:

The levels of egg parasitism at all sites was much lower than the values of 58-80% reported by Coetzer (1996) and Roberts (2000 *unpubl.*) respectively. Egg parasitism

Table 1.4: The percentage of *N. arizonensis* eggs parasitised and the numbers of eggs counted on pods (in parentheses) collected at four sites in the Western Cape a) Driefontein, b) Clanwilliam, c) Calvinia and d) Citrusdal. Blank = no sample.

	Citrusdal	Driefontein	Clanwilliam	Calvinia
Feb-02		33.3 (3)	0 (12)	
Mar-02		0 (9)	8 (16)	
Apr-02		9.1 (56)	0 (10)	
May-02		18.3 (128)	8.1 (14)	
Jul-02		5.9 (101)	0 (3)	
Aug-02		5 (49)		
Dec-02		5.3 (17)		
Jan-03		0 (2)	0 (1)	0 (0)
Apr-03	5.3 (6)	24.1 (39)	50 (2)	29.4 (6)
May-03	8.5 (25)	28.3 (46)	100 (1)	0 (2)
Jun-03			0 (4)	0 (1)
Jul-03	4.3 (41)	24.2 (67)	20 (4)	0 (4)
Sep-03	4.1 (58)	0 (30)	0 (1)	0 (2)
Oct-03				7.1 (71)
Nov-03	0 (162)	2 (124)	0 (1)	11.5 (113)
Dec-03			0 (9)	18.2 (29)
Jan-04			0 (0)	
Feb-04				0 (1)
Apr-04				0 (2)
May-04				0 (1)
Jun-04				35.7 (10)
Jul-04				0 (9)
Sep-04				0 (6)
Overall	4.5 (292)	11.9 (671)	13.2 (78)	6.0 (257)

appears to be highest in early winter, April and May (Table 1.4) and the highest recorded levels of parasitism, on months in which *N. arizonensis* eggs amounted to at least 10% of total egg count, was at Calvinia in June 2004 and April to July at Driefontein in 2003. The average levels of parasitism at these sites in all years sampled did not exceed 20%

and in most instances amounted to <10% of *N. arizonensis* eggs counted. There did not appear to be any correlation ($r = -0.166$, $p = 0.428$) between the percentage of eggs parasitised and the total number of *N. arizonensis* adults emerging (the one data point of 100% parasitism was excluded as there was only a single egg counted from that sample).

Discussion

Most New World Leguminosae have acquired at least one and sometimes many bruchid species that impact on seed output, often destroying the vast majority of the seed crop (Janzen, 1969). As a result of this, there are a number of adaptive features that enhance the chances of legume seeds 'escaping' from 'predators', including: 1) mast fruiting (Janzen, 1977); 2) asynchronous fruiting (Forget *et al.*, 1999); 3) synchronous fruiting (Swier, 1974); 4) seed dispersal mechanisms (Janzen, 1971a); and 5) seed characteristics (Janzen, 1975).

Synchronous production of high numbers of nutritious pods enables a portion of mesquite seeds to survive because 'predators' become satiated (Lloyd & Dybas, 1966), a process also known as resource concentration (Janzen, 1969). Predator satiation occurs when seeds are produced in abundance over a short time span and the natural enemies are unable to respond, either functionally or numerically (Janzen, 1971b), to utilise more than a portion of the available resource.

Selection pressures that enhance the attractiveness of seed pods to potential dispersers might in turn increase the survival probabilities of seeds through a process known as predator avoidance, i.e. whereby the seeds are removed from the fruit and dispersed at low densities and thus become less-easily detected by granivores (Janzen, 1971b). In the case of mesquite, the seeds benefit because most pods are devoured by frugivores and many of the seeds are dispersed in dung. The distance from fruiting conspecifics at which dispersers disseminate seed may affect the probability that a seed will 'escape' from seed predators (Janzen, 1970, 1972; Wilson & Janzen, 1972; Wright, 1983; Traveset, 1990; Fragoso *et al.*, 2003). In mast or synchronous fruiting periods, removal of seed pods from below fruiting trees by frugivores alters a system from one of predator satiation to one of resource limitation, resulting in increased predator pressure on remaining resources. The increased predator pressure results in extreme levels of damage among the seeds that remain on or around the parent tree (Janzen, 1971b).

Where pod removal by livestock limits the numbers of seeds available, seed destruction by bruchids should reach higher levels as a consequence of resource limitation. However, similar proportions of seeds were destroyed over a one year period at all of the sites regardless of presence or absence of livestock. At some of the sites, where pod removal by livestock was negligible, *A. prosopis* populations responded to the prolonged abundance of seed by maintaining elevated population levels throughout summer but despite this, the levels of seed destruction at Calvinia were repeatedly lower than at the other sites. Possible explanations for this anomaly will be dealt with in the following chapters.

The reason for the significantly higher numbers of seeds that escaped bruchid predation at Modder River may best be explained by the findings of Swier (1974) where weather conditions at sites in Arizona determined levels of bruchid damage to mesquite seeds. In

South Africa, the two most north-easterly sites, Modder River and Kingswood, receive higher rainfall and have colder winters than the other sites, a dissimilarity which may account for differences in bruchid population numbers. Because there was no removal of pods by livestock on the Rooidam farm at the Modder River site, pods were able to accumulate through the season. This resulted in predator satiation (Janzen, 1969) and the bruchid populations were unable to respond to the high availability of seeds after winter likely a result of poor over-wintering survivorship and consequently *A. prosopis* failed to respond numerically to seed availability prior to the production of the following seasons pods. By contrast, at Kingswood, where the climate is similar to Modder River, high levels of damage, similar to those at sites in the more xeric areas, were recorded. These differences are attributable to resource availability because most of the pods at Kingswood were removed by livestock. The bruchid populations were able to increase at the start of the podding season, before livestock gained access to the resource. As the season progressed, pod removal resulted in resource limitation, and subsequently higher levels of seed destruction on the few remaining pods, which were confined to a small area where livestock were excluded.

Another possible explanation for the low levels of seed destruction at Modder River was the non-target effect of insecticides through drift during spraying of adjacent agricultural lands. Hoffmann and Moran (1995) clearly demonstrated detrimental, non target effect of insecticidal sprays on a weed biological control agent. The close proximity of the Modder River study site to arable lands meant that bruchid populations may have been curtailed or repelled, or both, as a result of insecticidal spraying. However, emergence of *A. prosopis* from available seed in February 2004, from recently matured pods, was 13%

as opposed to 4% from old pods (i.e. from those produced during the 2003 season) that were collected at the same time. Numerically this represents 130 bruchids per 1000 seeds from new pods compared to 40 bruchids per 1000 seeds from old pods. Thus it seems *A. prosopis* in Modder River preferentially exploited seeds in new pods rather than old pods that are often degraded, moist and overgrown by vegetation. This does not rule out the possibility of decreased population size as a result of insecticides but does support the hypothesis that resource satiation was occurring at this site.

Temperature is likely to have major influence on bruchid behaviour and physiology in xeric environments, affecting life history parameters such as developmental rates, fecundity and survivorship. As a means of coping with harsh environmental conditions insects employ different strategies including behavioural (e.g. taking shelter in protected microhabitats or becoming inactive while conditions are harsh) and physiological (obligate or facultative diapause and aestivation) (Tauber *et al.*, 1984; Chapman, 1998). Diapause is known to affect life-history traits such as over-wintering survivorship and subsequent fitness where, for example, post-diapause females are less fecund and have prolonged oviposition periods compared to aestival non-diapause females (Ishahara & Shimada, 1995). However, diapause may potentially offer a means of escape for a vulnerable stage of an insect's lifecycle. For example, the relative inactivity of an over-wintering adult may result in it being more susceptible to predation or desiccation than a larva in diapause in a seed, where some degree of protection is afforded by the microenvironment within the seed coat. The strategies used by *A. prosopis* and *N. arizonensis* to survive through the harsh weather conditions in semi arid areas have not been ascertained but both species seem to be adaptable and thus are able to exploit the variable conditions that arise.

Although inter-year differences in levels of seed damage were not apparent between 2002 and 2003 at most sites, Driefontein and Citrusdal had significantly higher levels of seed damage in 2002 than in 2003, when the mesquite infestations in these areas were being cleared. The felling of trees left pods exposed to sunlight on the soil surface when they would normally have been shaded by the adult trees. The temperature of pods in full sun can be elevated by as much as 11°C above ambient (Kistler, 1995), affecting larval developmental rates and larval survival within. As a result, during winter, pods exposed to sun incur higher levels of seed damage than unexposed pods. Higher temperatures in pods exposed to sunlight during winter seemed to induce oviposition in a season when the beetles are normally inactive. This was apparent from a comparison of the Clanwilliam and Citrusdal sites, which have similar environmental conditions but showed very different bruchid emergence patterns in 2003. Clearing of trees at Citrusdal exposed the pods that had previously fallen in the shade below the canopies of the parent trees. As a result, there was a nine fold elevation in emergence of bruchids from pod collected in mid-winter (July), and a four fold elevation in late-winter (September), at Citrusdal when compared to Clanwilliam.

Both bruchid species increased their activity on pods exposed to the sun at Citrusdal during the winter period and *N. arizonensis* contributed more to total bruchid emergence (10%) over this period at Citrusdal compared to Clanwilliam (<1%). This observation supports the findings of Kistler (1995), where *N. arizonensis* populations were shown to peak under milder conditions and hence avoid temperature extremes that increase egg, larval and adult mortality. Kistler (1995) also found no effect of temperature on fecundity of either *A. prosopis* or *N. arizonensis* between 20-35°C. However, at 20°C adult metabolic rates of both species were very low, increasing significantly at higher temperatures. It is therefore likely that temperature effects are more pronounced during periods when there are marginal increases in temperatures around 20°C. Although the exposure of pods to sun resulted in increased adult oviposition rates, as reflected by increased bruchid emergence in the laboratory, the absence of a corresponding decrease in seed availability from field-collected pods implies that larval developmental rates in the field remained slow.

Kistler (1995) found larval developmental times of both *N. arizonensis* and *A. prosopis* to halve as temperatures rose from 20 to 25°C and larval survivorship increased from 20-80% over the same temperature range. Estimated developmental thresholds for both bruchid species were 14-15°C (Kistler, 1995). Thus, although adult activity increased in response to warmer daytime temperatures within pods, larval development rates did not increase accordingly because the advantages of high day time temperatures were offset by excessively cold evening temperatures. The maximum and minimum average temperatures for June at Clanwilliam were 21°C and 8°C respectively but daytime soil surface temperatures below trees may be as much as 11 degrees cooler than ambient (Kistler, 1995) and therefore below the larval developmental threshold. As a result, low temperatures in pods lying in the shade of trees limit adult bruchid activity and reduces larval development and survivorship in these situations during winter in the winter rainfall regions. Where pod temperatures are raised, i.e. through exposure to sun, bruchid oviposition takes place and larval developmental rates increase marginally.

Besides felling of trees, tree architecture and density determines the amount of irradiation received by pods lying on the ground. The mesquite infestations that were monitored in the summer rainfall region consisted mostly of scattered plants allowing more direct sunlight to penetrate through the canopy and reach the soil. In contrast, the infestations in the winter rainfall region were restricted to drainage channels where the plants were more densely clustered with denser shade and cooler below-canopy conditions. As a result, oviposition rates on pods in the winter rainfall region declined over the winter period while there were higher levels of oviposition from early to mid-winter in the summer rainfall region. These differences explain the higher occurrence of bruchids reared from pods collected over winter in the summer rainfall region compared to those collected over winter in the winter rainfall region.

Moisture availability may also explain the discrepancy between the winter rainfall region and the summer rainfall region in larval numbers in pods during winter. The summer rainfall regions are characterised by cool but extremely dry winter periods. In the winter rainfall region the adult bruchids are not subject to moisture deficits which may enable them to survive through winter in larger numbers and thereby rapidly exploit seed resources as they become available in summer. Over-wintering as larvae in seeds in winter rainfall regions may be less successful because of mortality induced by rotting-off of pods and damaged seeds, and increased susceptibility of larvae to disease, during the wet months (Janzen, 1975). Although adult longevity is enhanced with lower temperatures (Ishahara & Shimada, 1995; Kistler, 1995) there may be more desiccation-induced mortality of the adult bruchids due to lack of moisture. Under these conditions over-wintering as larvae in seeds would be more likely to succeed.

Larval and pupal parasitoid activity is an unlikely explanation for differences in bruchid larval numbers in the winter and summer rainfall regions because parasitism of bruchid larvae in seed is low during winter in both regions.

In the absence of livestock and using the Clanwilliam site as an example for winter rainfall regions and the Vanwyksvlei, Prieska sites as predictors for the summer rainfall regions, levels of seed damage over one year post pod-fall are shown below (Fig 1.9 and 1.10).

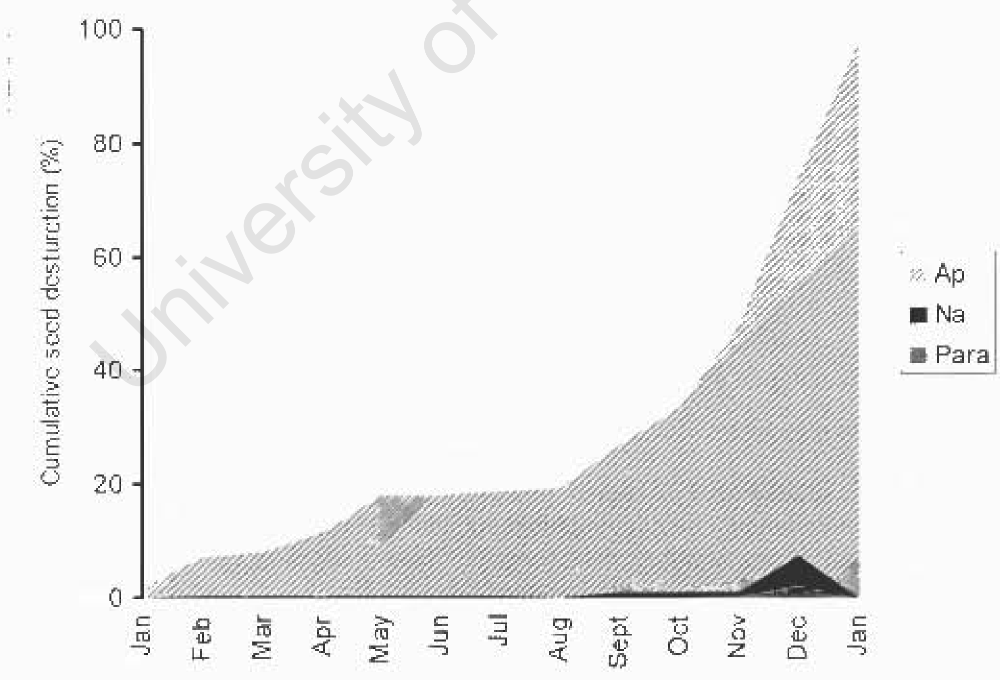


Fig 1.9: Estimations of annual variations in the proportions of *A. prosopis* (Ap) and *N. arizonensis* (Na) contributing to seed damage during the year following pod fall in the winter rainfall region. "Para" refers to the proportion of bruchids succumbing to parasitism over this period.

There is a west to east gradient of increasing rainfall and a change from winter to summer rains. As a result, in the winter rainfall region, bruchids exhibit a rapid functional and numerical response to available seeds and most of the seeds are exploited so that damage nears 100%. However, in the summer rainfall region, bruchids are deterred from exploiting on old degraded pods during summer rains, selecting to oviposit on new pods; thus as much as 40% of seed escapes bruchid damage.

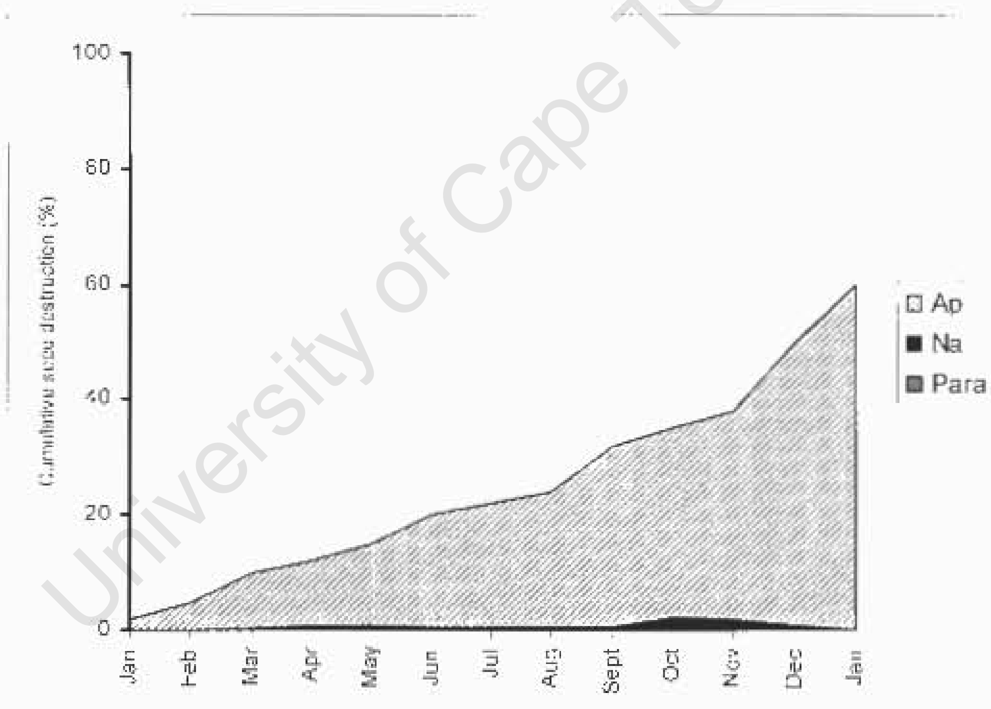


Fig 1.10: Estimations of annual variations in the proportions of *A. prosopis* (Ap), *N. arizonensis* (Na) contributing to seed damage during the year following pod fall in the summer rainfall region. "Para" refers to the proportion of bruchids succumbing to parasitism over this period.

Despite larval parasitoid numbers having remained low throughout this study, parasitoids have the potential to strongly impact populations of their herbivore host as evidenced by the extensive successful use of parasitoids for biological control of insect pests in agriculture (Hagler, 2000). The effectiveness of introduced herbivores, released for biological control of alien invasive plants, might be governed by the rate, and propensity, at which indigenous parasitoid species include the introduced species in their host range. Nine new parasitoid species were reared from pod collections and differed to the 10 species identified in two previous studies (Hoffmann *et al.*, 1993; Coetzer, 1996). Two possible reasons for this accumulation of additional parasitoids may be: 1) there was a period of adjustment needed, i.e. the biology or phenology of the parasitoid needed to become adapted for existence on the novel host; or 2) the geographical spread of the novel host having brought it into sympatry with an increasing number of parasitoid species some of which will be able to use it as a host (Cornell & Hawkins, 1993). The abundance of the individual parasitoid species will be an important factor when considering the importance of additional parasitoid-host associations and host population dynamics. Unfortunately the abundance of each species was not determined in this study as there was degradation of many of the specimens and these were not identified.

Although generalist predators have played a more demonstrable role in curbing agent efficacy (Goeden & Louda, 1976), the limited success of some weed biological control programmes has been attributed to high levels of parasitism by indigenous parasitoids on the biological control agents (e.g. Dodd, 1969; Goeden & Louda, 1976; Baars, 2003; Van Klinken, 2005). A good indicator as to the likelihood of introduced insects acquiring parasitoids in the new range is the number of closely related insect taxa in the country of introduction and susceptibility of insect herbivores to parasitism in their native range is a good predictor of their susceptibility in the area of release (Cornell & Hawkins, 1993).

Thus, weed biological control programmes are likely to be more successful when these criteria are not satisfied.

The cues by which these parasitoids locate the bruchid larvae is unknown but it is likely that these generalist parasitoids use one or more of several chemical cues such as the sex pheromones or oviposition markers of the adult bruchids (Mbata, 1992; Mbata *et al*, 2004) or from volatiles released by developing larvae and, or, the damaged seeds (Howard & Flinn, 1990; Mbata *et al.*, 2004) to locate bruchid immatures. The majority (70%) of the parasitoids reared out of mesquite pods in the study by Hoffmann *et al.* (1993) were generalist species, as was found with the study of the accumulation of native parasitoids on introduced species considered by Cornell and Hawkins (1993). This, together with the predisposition of introduced endophagous insects to acquire parasitoids (Cornell & Hawkins, 1993) explains the rapid acquisition of native parasitoids by the two introduced seed-feeding bruchids. The native parasitoid species on *A. prosopis* probably shifted onto *N. arizonensis* soon after its release in 1994 as the two bruchid species can be considered ecological analogues, especially during the larval stages in the seeds. It is possible that the early acquisition of these parasitoids by *N. arizonensis* prevented the beetles from ever becoming abundant and has kept the populations of the beetles at their current low levels, as happened with *Bactra truculenta* on nutsedge in Hawaii (Goeden & Louda, 1976) and *Procecidochares utilis* on crofton weed in Australia (Dodd, 1969).

Parasitism has the potential to alter the populations of two competing host species, in the case of dominance by either altering the competitive outcome (Yodzis, 1986) or by causing the exclusion of the inferior species at a local level (Price, 1980; Sikder, 1999).

Instances of changes in competitive outcome to multiple prey species when attacked by one or more shared enemy species, apparent competition (Holt, 1977), have been reported frequently in the literature (e.g. Holt, 1977; McClure, 1981; Howarth, 1991; Resteratis, 1991; Copeland & Craig, 1992; Holt & Lawton, 1994). Low levels of parasitism may have little effect on the dominant species locally but may compound unrelated deleterious effects that act on the inferior species. In light of the variations in larval competitive ability and resource availability, native parasitoids may be responsible for maintenance of the two bruchid species at densities found in this study. When the combined bruchid numbers are considered, i.e. those of *A. prosopis* and *N. arizonensis* together (Appendix 1.1), it is evident that overall larval parasitism is not sufficient to impact on the bruchid populations as parasitoids made up only 0.7% of total insect emergence in 2002 and 2003. However, the situation changes if one or other of the bruchid species is more susceptible to parasitism, especially if the competitively inferior *N. arizonensis* is more susceptible than *A. prosopis*.

If the parasitoids' host was confined to either one or other of the bruchid species, overall mortality through parasitism would have been 1.6% for *A. prosopis* or 20.4% for *N. arizonensis* during 2002 and 2003. At the highest levels of parasitism measured in a single month at one locality, parasitoids would have accounted for 75% of the *N. arizonensis* mortality compared to 8% for *A. prosopis*. Support for the assumption that parasitism may have been dissimilar on the two species comes from the Clanwilliam site where a 3-fold decrease in *N. arizonensis* populations from 2002 - 2003 corresponded to an 8-fold decrease in parasitoid populations despite constant levels of adult emergence of both bruchid species combined. There is a possibility that the relationship between high

N. arizonensis and parasitoid numbers resulted from optimum environmental conditions for parasitoids coinciding with those for *N. arizonensis* but not *A. prosopis*. However, this scenario is improbable as, despite Kistler's (1995) findings of temporal variation in population peaks in Arizona between the two bruchid species, we find corresponding peaks in populations of the two bruchid species in South Africa. Thus the susceptibility of both species of bruchids to parasitism is similar with regard to the time of year. It therefore seems likely that *N. arizonensis* is inherently more susceptible to larval/pupal parasitism than *A. prosopis* and even at low levels of parasitism (20%, see Janzen, 1975), the impact on population dynamics of *N. arizonensis* might be considerable.

Egg parasitism has not been detected on *A. prosopis* eggs but parasitism of *N. arizonensis* eggs have been shown to reach 50-80% in some months (Coetzer & Hoffmann, 1997; Roberts, 2000 *unpubl.*). The high levels of egg parasitism recorded by Coetzer (1996), at two of the sites also sampled in the current study, occurred at a time when *N. arizonensis* populations were double what they were in this study. Fluctuations in *N. arizonensis* populations occur within and between seasons and also between sites and it seems probable that levels of egg parasitism would do so too. Coetzer (1996) determined that in 1995, levels of egg parasitism varied from 0-58% between sites and that egg parasitism levels had dropped from 39% to 0% between 1994 and 1995 at one site. Over the course of this study parasitism at the four sites was on average approximately 6% in 2002 and 2003, much lower than the levels reported in 1995 and 2000 (approximately 35% and 45% respectively) (Coetzer, 1996; Roberts, 2000 *unpubl.*). *Neltumius arizonensis* populations remained low between 2002 and 2004. As with the findings of Coetzer (1996), levels of egg parasitism were density independent and in addition, there was no correlation between parasitism levels and emergence of *N. arizonensis* adults and thus it is unlikely that egg parasitism by *Uscana* sp. will currently be the major regulating factor of *N. arizonensis* populations.

Conclusions:

In xeric environments, high levels of seed destruction, approaching 100%, can be achieved over a one year period and it is probable that few seeds will escape bruchid damage and germinate. Low levels of seed damage in the higher rainfall areas are a consequence of sub-optimal environmental conditions for larval development and the chances of recruitment of new plants into the populations are increased.

Algarobius prosopis populations are consistently and markedly higher than those of *N. arizonensis*, accounting for 87-100% of bruchids emerging across the range of mesquite in South Africa. There does not seem to be any apparent affect of parasitism on *A. prosopis* populations. However, the combined effect of larval and egg parasitism incurred by *N. arizonensis*, its use of pods limited to those of pristine condition (Strathie, 1995) and the poor competitive ability of its larvae compared to that of *A. prosopis* (Impson & Hoffmann, 1998) are likely to be the main factors regulating *N. arizonensis* populations. In view of the afore mentioned constraints on *N. arizonensis* individuals throughout their lifecycle, it is surprising that their populations managed to establish and persist in South Africa. The contribution of *N. arizonensis* to overall seed damage is lower than was originally hoped and reflects the situation in its native range because neither release from competition nor release from parasitism has occurred in its country of introduction.

Environmental conditions, particularly temperature, are strong determinants of population dynamics of the two bruchid species. The abundance of both bruchid species may be altered by changing conditions. Temperature can strongly influence community structure and even leads, in some cases, to reversal in competitive outcomes (Park, 1954; Spence *et al.*, 1980). Yet when resource limitation occurs in mesic areas, despite unfavourable environmental conditions for both bruchid species, the competitive superiority of *A. prosopis* is exemplified and *N. arizonensis* is found to be excluded.

In conclusion, as Kistler (1995) stated: "When considering populations within a guild of mesquite bruchids, abundance is a function of resource availability and fecundity-mortality events, influenced to some extent by larval developmental rates." This is no better illustrated than by the population size and dynamics of the two bruchid species considered in the current study.

In summary, the removal of pods by livestock impacts on bruchids and mesquite populations both directly and indirectly:

1. Removal of seeds away from tree infestations enables a number of seeds to escape predation and potentially increases the chance of dispersal to safe sites. Ingestion of seed containing larvae results in seed and larval death. Increased competition for seeds that are not removed is intense resulting in total destruction of the seed in these pods by bruchids at most sites.
2. Resource limitation resulting from seed removal increases the likelihood of competitive interactions between the two bruchids and *N. arizonensis* populations suffer as a consequence.
3. The concentration of resources to livestock exclusion areas is likely to increase the pressures of parasitism on *N. arizonensis* populations because of reduced search times for the parasitoids.

Considering these points leads into my next chapter where I investigate the maintenance of *A. prosopis* populations despite the supposed absence of resources resulting from pod removal by livestock.

Chapter 2

Destruction of seed contained within dung by *Algarobius prosopis*

Abstract

Algarobius prosopis utilises dung-borne seed for larval development extensively through the year accounting for an estimated 50-70% reduction in dung-borne seed. Exploitation of dung-borne seed during winter was highest on exposed dung as the temperatures in these situations were closer to optimum. However exposed dung was not exploited during summer as temperatures were above the upper temperature limit of 45°C for one out of three days. During this period exploitation on shaded dung, below mesquite trees, was highest. There did not appear to be a distance effect on exploitation of dung-borne seed during winter as even dung placed 170m away from the closest mesquite stand were heavily exploited, suggesting that *A. prosopis* adults were active, dispersing during this period. Larval development during winter was continuous in exposed dung, giving rise to two generations and resulting in 81-99.6% damage to seeds. Larval development appeared to be greatly reduced in shaded dung during winter. High levels of parasitism of *A. prosopis* in dung-borne seeds in exposed areas were recorded over winter accounting for mortality of 38-60% of late-instar larvae and pupae. Two *Pteromalus* species were responsible for 88% of the parasitism of *A. prosopis* in dung-borne seed and neither of these wasp species was reared from pod collections.

Introduction

Only a small proportion of mesquite seeds are destroyed by *A. prosopis* whilst the pods are still hanging on the trees and most damage is caused while the pods are lying on the soil surface. However, wherever livestock have access, almost all of the pods are grazed within a short period of falling to the ground. The rapid removal of pods by livestock prompted Moran *et al.* (1993) to postulate that the vast majority of seeds produced each year never become available to the bruchids. Although approximately 85% of the seeds that are eaten by sheep are destroyed during chewing and passage through the digestive system (Harding, 1991), pod production is high enough to ensure that the surviving 15% that are passed in dung make a substantial annual contribution to the soil seed bank. Historical assumptions that seeds in dung are unsuitable for bruchid utilisation has proved to be incorrect and recent observations have shown that *A. prosopis*, but not *N. arizonensis*, emerges from seeds in sheep (*pers. obs.*) and donkey (Hoffmann, *pers. comm.*) dung collected in the field. Traveset (1990) noted a similar occurrence in Costa Rica where the bruchid *Stator vachelliae* emerges from seeds of the leguminous tree *Acacia farnesiana* contained within horse dung. The average levels of damage were 24.2% of seeds affected, but reached 57% in some dung piles (Traveset, 1990). *A. prosopis* females were observed oviposit one to many eggs in the cracks and crevices of dung pellets regardless of a seed being located with the ovipositor (*pers. obs.*). The eggs hatch and the mobile larvae tunnel through the dung pellet where upon finding a seed the larva tunnels in and remains for the remainder of its development.

The numbers of eggs oviposited on dung samples in the field and laboratory could not be accurately quantified as this would require destructive techniques and, in addition, predation of eggs in the field would affect actual counts and hence the term "exploitation" is employed and is a function of number of eggs laid and survival and development of immatures and is determined by the number of adults emerging.

Post-dispersal seed predation studies have demonstrated that frugivores may influence the probability that a seed is found by a granivore depending upon (1) the distance from a fruiting conspecific at which the seeds are disseminated with a decrease in seed damage with increasing distance (e.g. Janzen 1970, 1972; Connell 1971; O'Dowd & Hay 1980; Wright 1983, but see Forget *et al.*, 1999); (2) the habitat or microsite where the seeds land (Janzen 1971,1972, 1986; Schupp 1988); and (3) the local post-dispersal density of seeds where the levels of seed damage is positively correlated to seed density (Wilson & Janzen 1972; Janzen 1982). The extent of seed destruction by *A. prosopis* is therefore likely to be influenced by dispersal patterns of dung containing mesquite seeds.

Although seed damage by seed-feeding insects usually decreases with distance from the parent plant (Janzen, 1972; Janzen *et al.*, 1976; Wright, 1983; Howe *et al.*, 1985.), in the case of mesquite, livestock congregate and deposit high concentrations of dung with seeds (from here on referred to as "dung-borne seed") below trees (*pers. obs.*). The accumulation of seeds could provide an easily-accessible resource for the bruchids and result in higher levels of damage on seeds under trees. Indeed, Traveset (1990) found that levels of seed damage were not correlated with host plant position for bruchids that utilise *A. farnesiana* seed in horse dung. Seeds in dung that is deposited in low densities away from the parent plants may be more obscure and less likely to be located and utilised by the bruchids (Janzen, 1975, 1985). In addition, the degree to which utilisation of dung-borne seed occurs may vary if temperatures in the exposed pellets are extreme enough to affect survivorship of the larvae (Traveset 1990; Kistler, 1995).

Mesquite is mainly distributed in the arid to semi-arid areas of South Africa where daytime temperatures are extreme. The number of summer days where the maximum air temperature >31.5°C (=45°C in exposed dung pellets, *pers. obs.*) is 106 out of 182 and shade is generally sparse because trees are lacking in the landscape. Therefore, during summer months, high mortality of sessile larvae within seed in exposed dung was expected because of elevated temperatures through irradiation by sunlight. Traveset (1990) recorded levels of 97% mortality of larvae in seeds during the dry season, mainly due to heat and desiccation and more bruchids emerged from shaded or partly shaded dung piles than from exposed piles. Conversely in winter, warm temperatures in dung pellets exposed to sunlight was expected to lead to an increase in bruchid emergence because of extended periods of oviposition activity, and, or, increased larval survivorship in these warm situations compared to the colder environment in the shade of trees.

Temperature is likely to mediate bruchid activity in both winter and summer months through regulatory effects on both physiology and behaviour. Kistler (1995) studied the temperature relationships in *A. prosopis*. The optimal temperature for *A. prosopis* larval development, as determined by peak larval survivorship, was found to be between 30-

35°C but declined at higher temperatures. The developmental threshold for larvae was 14.6°C and only 20% of larvae survived at 20°C. Although cold periods strongly affected adult metabolic rates, females showed little variation in fecundity between 20-35°C. Temperature extremes are therefore likely to affect development and survivorship of the immobile, endophagous larvae to a greater degree than that of the adult bruchids. In light of the effects of temperature on larval physiology it is probable that the developmental rates and survival of larvae in dung-borne seed would vary as a result of seasonal and spatial differences in the internal temperatures of the dung pellets. Temperatures below 20°C may also affect larval survival indirectly by slowing development and increasing the chances of mortality due to an increase in exposure time to parasitoids (Clancy & Price, 1987) and infections (Anderson, 1979).

Bruchid emergence numbers should peak during periods when dung temperatures in the field are close to optimum. Conversely, larval mortality would be high when temperatures of dung in the field are extreme (i.e. dung exposed to full sun in summer or being fully shaded in winter) and not many adults would be expected to emerge from these samples of dung from these situations. The number of beetles that emerged from dung samples, which was the product of numbers of eggs laid and immature survival, was used to assess the extent to which bruchids were exploiting dung-borne seed. In addition, because temperature will determine developmental rates of larvae in the field, the timing of adult emergence in the laboratory from dung collected in the field will reflect the developmental stage of the larvae at the time of collection.

There are no reports in the literature of bruchids within seeds contained in dung being parasitised and it may be that bruchids in dung escape from parasitoids by occupying enemy-free space (Lawton & McNeill, 1979, Price *et al*., 1980). Because of the large structural difference between the pods and dung, parasitoids of larvae or pupae in dung-borne seed would need to rely on semiochemicals, rather than physical cues, to locate the bruchids (e.g. Law & Regnier, 1971; Mbata *et al*., 2004). It is possible that a certain percentage of the larvae might escape parasitism as only those in superficial seed in the dung pellet may be susceptible to parasitism and those in seeds away from the surface may be exempt from parasitism. Another possibility is that certain parasitoids have evolved an ability to utilise bruchids that develop in dung-borne seed of African acacia frugivores (see Barnes, 2001) and subsequently there may be an assemblage of parasitoids that exploit this niche.

It might be that the ability of the parasitoids that utilise bruchid immatures in dung-borne seed will be affected by factors such as the location of the dung, time of year and resource density and thus similar patterns of emergence of wasps and bruchids may appear.

Based on the prediction that *A. prosopis* populations will not decline in areas where livestock remove seed pods because the beetles are able to utilise dung-borne seed, the aim of this part of the study was to quantify the extent to which *A. prosopis* relies on dung-borne seeds as a food source when seeds in pods are scarce and how this use is mediated by temperature regimes in dung and by parasitoids.

Methods

Observations revealed that in no instances did *N. arizonensis* emerge from dung and so this species will not be considered at all when dealing with events in dung. In addition, owing to the distribution of mesquite in the arid and semi-arid areas of the country where livestock is predominantly sheep, dung of other livestock and indigenous mammals were not considered despite evidence that *A. prosopis* also utilises dung-borne seed from these animals (*pers. obs.*).

A. prosopis emergence from random field collections of dung

In order to establish the degree to which season and position of dung affected exploitation of dung-borne seed and larval development within this seed, samples of sheep dung were collected at monthly intervals from the Calvinia site between April 2003 and July 2004. This study site supported a large flock of sheep and although most dung was concentrated below the trees, dung was also deposited in reasonable abundance away from trees. The site had been ploughed at least five years prior to this study and supported no perennial vegetation apart from scattered mature mesquite trees with very few young plants. Dung samples were collected from below mature trees, referred to as "shade" from here on and away from trees, referred to as "exposed" areas. Exposed dung collections were at suitable distances, 10 metres or more, so as to be sufficiently distant from trees to avoid possible shading except from very early morning and late afternoon sun.

The soil below mature trees contained fragmented dung, accumulated and crushed by sheep trampling activity, interspersed with whole dung pellets of varying age. The broken-down dung lay above a compacted soil layer, similar to soil away from the trees. All dung and seeds situated below trees were contained within the uppermost 10cm layer. On deposition, sheep dung is concentrated initially in piles (approximately 15cm in diameter) containing approximately 50-120 pellets. These piles dry out and get trampled and scattered with time. To estimate the density of dung pellets, a 20 x 20cm quadrat was thrown below trees and into the exposed areas in September of 2003 and the numbers of pellets on the surface were counted. Averages for the number of pellets per m^2 were obtained from 5, 20 and 10 random throws of the quadrat below mature trees, 10-30m and 170m away from trees into the exposed areas respectively. Only surface dung was counted because dung buried below the surface was considered inaccessible to ovipositing bruchid adults.

Scoops of soil and dung from below mature trees containing on average 600g (1800 pellets) of dung were collected from Calvinia on each sampling occasion. These were sieved through a 5mm mesh sieve and any pod segments were removed to ensure that emerging bruchids did so solely from dung samples. The same process was repeated for dung in the exposed areas. These samples were placed in brown paper bags and sealed shut with staples for transport back to the laboratory. In the laboratory the samples were transferred to plastic rearing containers and kept in a constant temperature room at 27 ± 2°C and 40-60% relative humidity. Emerging beetles and parasitoids were counted and removed every 3-5 days for a 35 day period after which the containers were placed in a -20°C freezer to terminate further development of any larvae within the samples.

On removal of the dung from the freezer, sub-samples of between 40-70% of total mass of dung were crushed and sorted with a 2mm sieve to separate any seeds contained within. The samples were processed with care so as to avoid crushing the brittle hollow husks of seeds that had been utilised by the bruchids. Counts of damaged and undamaged seeds that were obtained provided a very close estimate of the total number of seeds in the dung sample and the proportion that had been destroyed by bruchids. Only seeds that were intact and assumed to be viable and seeds that were entire but with an adult bruchid exit hole were collected. Fragment of seeds that were destroyed during passage through the gut were not included in the counts. Adult bruchids that had not successfully emerged from the dung or from the seed within the dung were encountered at times and were included in the counts of the "total bruchids emerging". Their presence in the dung was attributable to the fact that they had developed at a slower rate than usual, or they had come from first-instar larvae that had taken a longer period to locate a suitable seed within the dung or they had ecloded but had become entrapped in the dung pellet or seed. The seeds that contained larvae or pupae were included in the "undamaged" category because these immatures would have originated from oviposition events in the laboratory.

To track the seasonal effects and availability of dung-borne seed on the population dynamics of *A. prosopis*, measurements were made of the levels of seed damage by bruchids that completed their development in the field (determined by the percentage of seeds with visible bruchid emergence holes), the percentage emergence of bruchid adults from available seeds (allowing estimates of exploitation of dung-borne seed in the field for the month prior to collection) and the average number of seeds per pellet.

To calculate the total number of seeds (STotal) in the original sample of dung from which adults had emerged, the mass of the whole sample of pellets (MTotal) was divided by the mass of the sub sample (MSs) and then multiplied by the numbers of damaged and undamaged seeds per sub-sample (SSs), so:

$$STotal = \frac{MTotal}{MSs} \times SSs \qquad (1)$$

The proportion of seeds available to the bruchids (SAvail) at each site on each collection date (t) was calculated with the same equation used in Chapter1:

$$SAvail_{(t)} = STotal_{(t+1)} - SHole_{(t+1)} + BTotal_{(t+1)} \qquad (2)$$

Where STotal is the total number of seeds in the count, ($SHole_{(t+1)}$) is the number of seeds containing emergence holes at the end of the experiment (t+1), and ($BTotal_{(t+1)}$) is the number of bruchids that emerged by the end of the experiment (t+1). Thus, on each collection date, the percentage of bruchids emerging from available seeds (BEm) was calculated by:

$$BEm = \frac{BTotal_{(t+1)}}{SAvail_{(t)}} \times 100 \qquad (3)$$

Seasonal and spatial variations in exploitation of dung-borne seed

In order to establish at what time of year and to what extent female bruchids exploit dung-borne seed in the field, batches of dung consisting of approximately 126 pellets (40g) were placed out at monthly intervals in the field at the Calvinia site. The batches were covered with rigid wire cages (dimensions 180 x 180 x 70 mm with 10x30 mm gauge mesh, (Plate 2.1) to prevent disturbance of the dung piles by sheep and any possible contamination through input of other dung.

Plate 2.1: Cages placed in field to house batches of dung and protect them from disturbance by livestock

The dung for this experiment had been collected from the Calvinia site in June 2003, the majority of which was from the 2003 podding season, and stored in a freezer at -20°C for a month to terminate any bruchid development in seeds. Twelve cages were placed below mature mesquite trees (designated as "shade" batches), 10 of which were shaded totally by the trees and two were placed at the edge of the canopy and were thus subjected to morning sun.

Seventeen cages were placed at varying distances away from the mature trees (designated as "exposed" batches), from a distance of 10 to 30m, into a previously ploughed area with no perennial vegetative cover. Another set of two cages was placed 170 m away from the mesquite stand in an area with no vegetation cover. Of the 17 cages placed between 10 and 30m from the trees, seven were artificially shaded by being covered with 80% shade cloth on 25 x 25 x 15 cm stands.

The dung samples were re-collected on a monthly basis and replaced with fresh samples. Any plants that had germinated within the cages since the previous collection date were removed but if they were mesquite seedlings that had germinated from the batches of dung, they were recorded and then removed. This experiment ran from July 2003 to September 2004 and a summary of collection dates and initiation of various treatments is given in Appendix 2.1.

On collection, the batches were sieved through a 5mm gauge sieve to remove soil and any adult bruchids that may have been on the pellets. The remains were then placed in self sealing plastic packets for transport back to the laboratory where the batches were transferred to 110 mm diameter clear plastic tubs with gauze lids and placed in a controlled environment room at 27°C ± 2°C and 40-60% relative humidity. Emerging insects were counted and removed every 3-5 days. Soon after being placed in the controlled environment room, and prior to the dung drying out, a number of seeds germinated from the moist dung collected during, or soon after, periods of rainfall. These seeds were removed and inspected for bruchid larval damage to the endosperm. After 35 days, the tubs were placed in a -20°C freezer to terminate any further development of larvae within the batches.

The batches of dung were sorted through a 2 mm mesh sieve and all retained seeds were inspected to determine the levels of bruchid damage and the condition of the seed. Seeds without emergence holes were dissected to count larvae, pupae and adults within. Larvae and pupae within the seeds at that stage must have originated from eggs laid by females that emerged in the laboratory and deposited eggs before being removed from the cages. Seeds with larvae and pupae were recorded as "undamaged" seeds because this was their status on collection. Seeds containing unemerged adults were included with "damaged" seeds because they would have had eggs or small larvae at the time of collection.

Seasonal variations in larval development in the field

The developmental rates of larvae in the field were determined from the emergence patterns of adult bruchids from randomly collected samples of dung (i.e. samples that were collected from the field at different times of the year). Development time from egg to adult is known to take about 38 days at 28°C (Kistler, 1985), the conditions in the insectaria where the dung samples were kept. Thus the timing of adult emergence from the dung samples will depend on the stage of development that the larvae were in on collection. Adults that emerged soon after collection were assumed to be late-instar larvae or pupae at the time of collection. Conversely adults that emerged almost 38 days after collection of the dung samples were presumed to be either eggs or early-instar larvae at the time of collection. The length of time taken for 50% of the adult bruchids to emerge from a dung sample was used to calculate an adult emergence index (AE_{50}), equivalent to the LD_{50} used to rate insecticides (Maddox, 1975). If the AE_{50} was close to 19 days (i.e. half the development time under insectaria conditions), the population of beetles in the dung sample was assumed to have an age distribution which included equal numbers of individuals in all stages of development. An AE_{50} below 19 days indicated that the sample had a high proportion of late-instar larvae and samples with AE_{50} values above 19 days had a high proportion of early-instar larvae. The development rates of the immature beetles in the field could be estimated by relating the AE_{50} values to exploitation patterns (see the previous section).

Survivorship of *A. prosopis* larvae in exposed sites during summer

To test whether the lack of *A. prosopis* emerging from dung was a consequence of larval death rather than avoidance of these dung piles by ovipositing females, the wire mesh cages used in the previous experiments were modified and a clear plastic covering was glued to the top and two of the sides of the cages. The remaining two sides were covered with gauze, small enough to prevent adult bruchids from escaping but large enough to enable airflow over the pellets. Four cages were placed out in the field for the month of

November, 2003. The dung placed in these cages originated from two different treatments: 1) dung from shaded areas and 2) dung from exposed areas. If the bruchid larvae in dung placed in the exposed areas survived, there would be either emergence in the cages or later in the laboratory. The samples were collected from the field after one month and, after being inspected for adult bruchids, transferred to rearing containers. The samples were left for a period of one month in the constant temperature room after which time they were crushed and all the seeds were removed and dissected to check for either bruchid larvae, pupae or adults.

Extent of seed damage in dung samples left in the exposed areas over winter.

Removal of dung samples on a monthly basis enabled estimations of the extent of exploitation by bruchids of dung-borne seed during those periods but failed to give an accurate account of the levels of seed destruction that may be achieved by *A. prosopis* over time. When ovipositing females are allowed continued access to a sample of dung in the laboratory, the larvae are capable of destroying 94.9% of the seed in the sample (*pers. obs.*) and it seems likely that the bruchids are potentially capable of destroying almost all of the seeds in the field over a season. In order to determine the levels of seed damage achieved by the bruchids during winter on seed in dung dispersed away from mature trees, eight rigid wire cages (dimensions 180 x 180 x 70 mm with 10x30 mm gauge mesh) housing dung were placed in the exposed areas, 30m from mesquite trees, during March 2005 and collected 4 months later during July 2005. Although this period did not cover the full winter season, it was deemed a suitable length of time to reflect the fate of dung-borne seed in the exposed areas during this season. On collection, the samples of pellets were removed by hand and placed into self sealing packets for transport back to the laboratory. Many seeds had fallen to the ground below the dung piles because a number of pellets had split open as a result of some seeds swelling through imbibing moisture or germinating. The fallen seeds were also collected and placed in the packets. On transferral of pellets from the packets to the rearing containers any loose seeds were removed and immediately dissected to assess the numbers of insects within and the condition of the seeds, any pupae of hymenopteran parasitoids were removed and placed in Petri dishes to rear to adulthood. The containers with dung samples were placed in a constant temperature room at 27°C ± 2°C and 40-60% relative humidity and checked on a daily basis for insect emergence. Any insects (including parasitoids) that emerged were recorded and removed daily. The experiment was run for a 35 day period after which time the containers were placed in the -20°C freezer to terminate any further larval development. Dung pellets were then carefully dissected by hand and the condition of the seed contained within was recorded.

Comparison between air temperatures and temperatures in the soil and dung: consequences for survival and development of larvae

On survey dates, prior to dung being collected from cages small holes were drilled into pellets using a 1.5mm drill bit. These pellets were placed back into the same environment from which they were taken and left for a minimum of 10 minutes to allow temperatures to equilibrate. Four replicates of six temperature readings were taken for: air (below trees); soil surface (below trees), 5mm below surface; soil surface (in exposed areas), 5mm below surface; dung pellet (below trees); dung pellet (in exposed areas);

dung pellet (below shade cloth). For dung temperatures in the exposed areas, two old/grey pellets and two fresh/dark brown pellets were included to establish a temperature range. This data allowed me to determine the relationship between air temperature and the temperature within the dung pellets and thus calculate a value for the slope of the relationship using least squares regression analysis. Maximum and minimum temperature data sourced from the South African Meteorological Department could then be used to estimate the maximum and minimum temperatures to which the larvae within dung would have been exposed in the different treatments while the samples were in the field. The monthly maximum and minimum temperature values were then correlated with bruchid emergence from those months in order to determine the effects of temperature on larval development in dung-borne seed.

<u>Upper temperature limits of larval development</u>: The upper temperature limits of *A. prosopis* are unknown, but a study by Lale and Vidal, (2000) on two other species of bruchid, *Callosobruchus maculatus* and *C. subinnotatus* found that there was 100% mortality of larvae exposed to a temperature of 50°C for 2hours. The upper temperature limit of *A. prosopis* larvae developing in dung-borne seed in the field was expected to be of a similar value.

Results

A. prosopis emergence from random field collections of dung

Dung pellets were more abundant below mature trees (2141 pellets.m^{-2}, σ = 857) where sheep congregated. By contrast, there were 358 pellets.m^{-2} (σ = 241) 10-30 m away from mature trees and no pellets were found 170m away from the mesquite stand. The fates of dung-borne seeds situated in shade and in exposed sites through the season are shown in Figs. 2.1 and 2.2. Dung samples from exposed and shaded areas are dealt with separately because of a marked difference in temperatures of these two situations through the year. Soil temperatures in the exposed areas can be double that in the shade of trees during summer months (*pers. obs.*), reaching 60°C compared to soil temperatures below trees that do not rise above 30°C.

The fate of dung-borne seed in shade (Fig. 2.1)

The emergence of *A. prosopis* in the laboratory varied through the year indicating variations in the degree to which the adults exploit dung-borne seed. *Algarobius prosopis* utilise dung-borne seed in the shade continuously through the year and although a higher percentage of dung-borne seeds in shaded areas are destroyed during summer than in winter (21.5%, σ=5.56 and 12.7%, σ=2.12 respectively), this is not significantly different (ANOVA, $F_{(1,13)} = 3.98$, $p>0.05$) and in only one summer month, November 2003, was emergence significantly higher than any recorded in winter (χ^2, $p < 0.05$). The highest

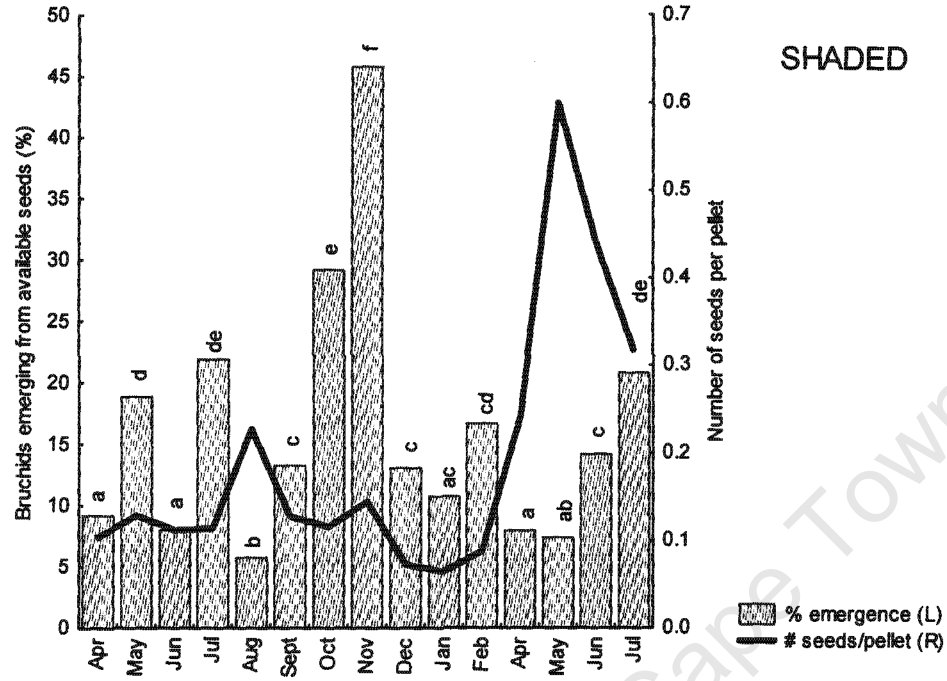


Fig. 2.1: Percentage emergence of *A. prosopis* from dung-borne seed collected from below mature mesquite trees, as a percentage of available seed, and number of seeds per pellet. Dung samples collected between April 2003 and July 2004. Bars with similar letters are not significantly different (χ^2, p>0.05)

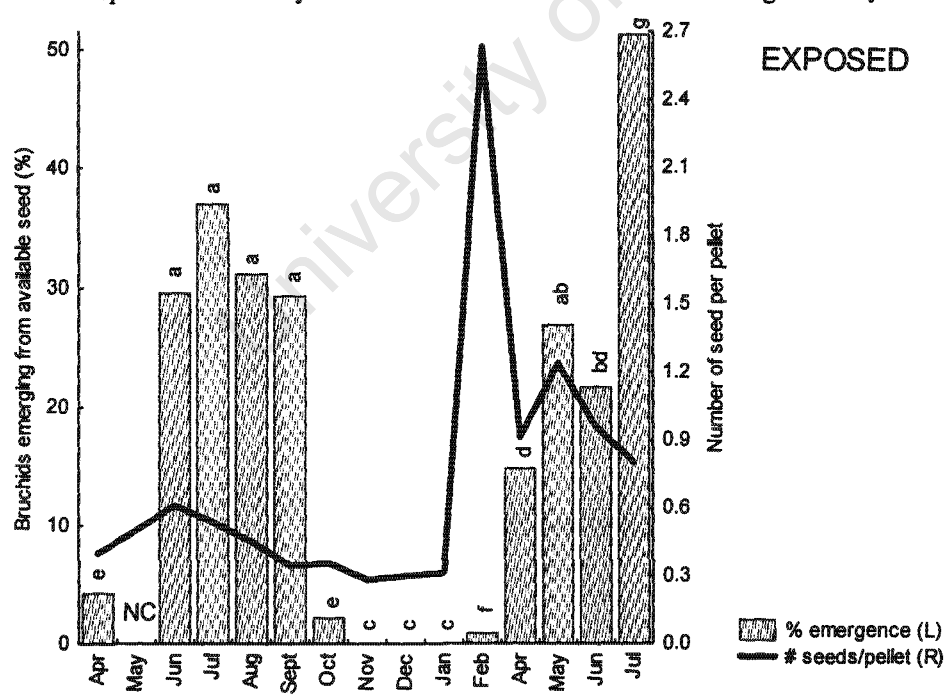


Fig. 2.2: Percentage emergence of *A. prosopis* from seed in dung collected from exposed areas, as a percentage of available seed and number of seeds per pellet. Dung samples collected between April 2003 and July 2004. Bars with similar letters are not significantly different (χ^2, p>0.05). "NC" = no collection on that date.

recorded emergence from dung-borne seed in shaded areas was in November 2003 where bruchids emerged from 45.8% of the available seeds and the lowest emergence recorded in shaded areas was 5.4% in August 2003.

The numbers of seeds per pellet followed a pattern, increasing during pod-fall and then generally decreasing with the lowest values recorded just prior to the next pod-fall. The increase in August 2003 coincided with the falling of a second flush of pods in June and July. The percentage of seed destroyed by *A. prosopis* in the field, estimated as the percentage of seeds containing adult emergence holes on collection varied through the year and there was no distinct cumulative increase in bruchid damage to dung-borne seeds between collection dates. Degeneration of seeds through weathering of dung pellets means that the use of proportions of intact seeds with emergence holes as an estimation of bruchid activity would lead to underestimations of the impact that *A. prosopis* would be having. Thus the decrease in number of seeds per pellet between pod-falls is a more accurate account of the impact of *A. prosopis* on seed numbers as, despite little evidence for similar proportions of adult emergence holes in the field, it is likely that larval penetration of a proportion of seeds resulted in their loss from the system without the need for larvae to develop to maturity.

<u>The fate of dung-borne seed in the exposed areas (Fig. 2.2)</u>

The usage of dung-borne seed by *A. prosopis* in the exposed areas was almost exclusively restricted to winter periods when temperatures of exposed dung pellets were not excessively high as during the summer months (November 2003 to February 2004) where significantly fewer bruchids emerged from dung-borne seed (ANOVA, $F_{(1,122)} = 19.44$, $p < 0.001$). The two highest peaks of emergence of *A. prosopis* occurred in July of 2003 and 2004.

The levels of seed destruction by *A. prosopis* varied through the year and was the inverse of that found for shaded areas. The numbers of seeds per pellet varied substantially with the availability of seed in the system and significantly higher numbers of seed occurred in pellets during the period of pod-fall (Mann-Whitney, $z = 2.89$, $p = 0.004$). High bruchid activity during winter would explain the 45% reduction in available dung-borne seeds between June and November of 2003. However, the most dramatic decrease in the number of seeds per pellet occurred between February and April 2004 with a 65% reduction. This decrease could not be attributed to bruchid activity as it occurred at a time when bruchids were largely inactive in the exposed areas. This large decrease in seed availability coincided with above normal and aseasonal summer rains at the site resulting in seed loss through germination and decay. Seed loss during winter was probably the result of a combination of both high bruchid activity and rainfall events.

Seasonal and spatial variations in exploitation of dung-borne seed

Through controlled manipulative experiments the patterns of bruchid emergence in the laboratory from seed in dung batches placed out and collected from the field reflected the seasonal and spatial patterns of exploitation of dung-borne seed (Fig 2.3) in the field. The three spatial situations were “exposed to full sunlight”, “exposed under shade cloth” and below trees = “shade”.

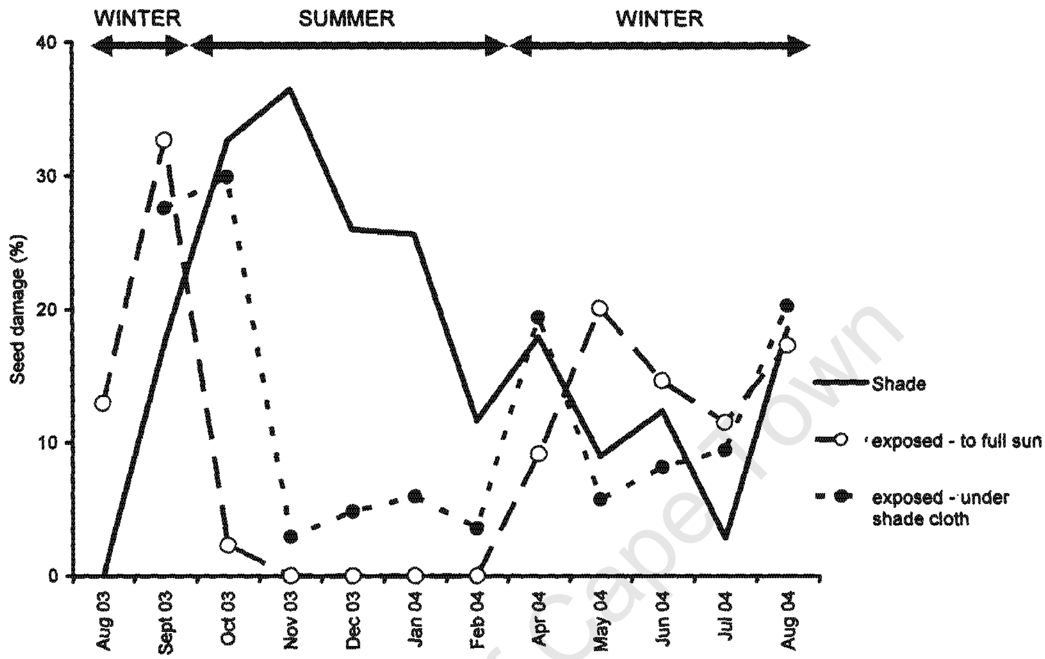


Fig 2.3: Seasonal variations in the exploitation of dung-borne seeds by *A. prosopis*, expressed as percentage seed damage in: shade (below mature trees); exposed – in full sunlight; and exposed – under shade cloth. Experiment conducted between August 2003 and August 2004 at the Calvinia site.

The effect of distance on monthly emergence of *A. prosopis*

Exploitation by *A. prosopis* of seed in dung exposed to full sunlight was restricted by high summer temperatures. During this period, there was no distance effect on exploitation by *A. prosopis* of seed in dung placed 10-30m away from mature mesquite trees in exposed areas during winter periods ($r^2 = 0.002$; $p = 0.65$) and therefore the data were combined for distance treatments (Fig. 2.3). However, there was a significant and positive correlation ($r^2 = 0.369$, $p = 0.036$) between distance and percentage emergence in one of three months in winter, June of 2004, when dung samples were also placed at 170m from the mesquite stand. The utilisation of dung-borne seed situated far from mesquite stands coincided with a period when usage of seed in dung in the proximity of mesquite trees was 4.5% lower than other stages during the winter period suggesting *A. prosopis* exploit seed contained in isolated piles of dung during this time.

The effect of treatment on monthly emergence of *A. prosopis*

The difference between levels of exploitation of dung-borne seed in exposed areas and shade was not found to be significantly different over winter (t-test for independent variables, $t = -0.572$, $p > 0.05$) but was highly significant during summer (t-test for independent variables, $t = -21.490$, $p < 0.0001$) (Figure 2.3). *Algarobius prosopis* avoided dung in exposed areas during summer. Dung placed below shade cloth in the exposed areas had significantly more eggs (t-test for independent variables, $t= -4.008$, $p=0.0001$) than exposed dung but significantly fewer eggs than dung below trees (t-test for independent variables, $t = 10.36$, $p < 0.0001$). A two-way ANOVA on levels of exploitation with different treatments and times of year showed that collection date ($F_{(8,276)} = 15.45$, $p < 0.001$), treatment ($F_{(1,276)} = 65.18$, $p < 0.001$) and the interaction of the two factors ($F_{(30,276)} = 14.33$, $p < 0.001$) are all highly significant for explaining differences in emergence levels.

Seasonal variations in larval development in the field

AE_{50} values for adult emergence from shaded dung remained well above the 19 day mark during most of winter in 2003 indicating slowed larval development (t-test of means for significance against 19 days, $t = 7.61$, $p<0.05$), and only toward the end of winter, when temperatures rose, was there any noticeable increase in larval developmental rates (Fig. 2.4). A similar trend was not found over the same period in 2004 despite the average maximum temperature for 14 days prior to collection being 23.0°C, 3.5°C higher in 2004 than in 2003 and with little difference in the average minimum between the two periods (data from South African Meteorological Department). These differences are likely a result of differences in rainfall where high winter rains occurred in 2004 but there was an almost absence of rain in 2003.

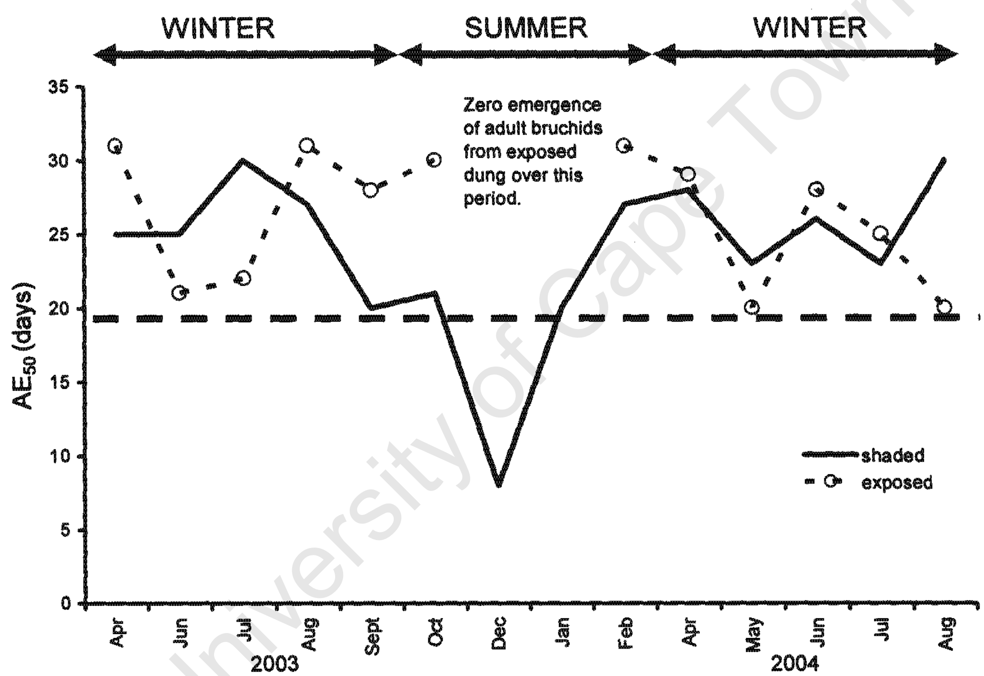


Fig. 2.4: The median of *A. prosopis* emergence (AE_{50}) from dung samples collected at the Calvinia site between April 2003 and September 2004, indicating winter and summer periods.

Between September and January, the AE_{50} was more frequently than not around the 19 day mark suggesting that there was an even distribution of instar stages during summer (t-test of means for significance against 19 days is not significant, $t = 0.251$, $p = 0.818$). However, December exhibited a higher proportion of late instar larvae possibly as a result of the shortening of developmental times and presumably more defined population peaks in summer compared to winter where larval development is prolonged and larvae may have arisen from multiple oviposition events, therefore, the timing of field collections in summer would strongly influence the AE_{50}.

Larval development in exposed dung during winter appeared to be continuous and hence the decline in the AE_{50} values from this dung between April and June 2003, April and May 2004 and July and August 2004 as these larvae matured and this may potentially have given rise to two generations of adults during the 2004 winter period. Active oviposition on seed in exposed dung and thus higher proportions of early instar larvae would explain the movement of the curve away from the 19 day mark during winter. There was no emergence from dung in the exposed areas during the hot summer months.

Survivorship of *A. prosopis* larvae in exposed areas during summer

On collection of dung samples left in cages in the field for the month of November 2003, there were neither adult *A. prosopis* in the cages nor was there any evidence of bruchid emergence from the dung. In addition, there was no emergence of adults in the laboratory over the 35 days that followed yet there was emergence from control samples in the laboratory. On dissection of the seeds in the samples left in the field, 3 - 13% of seeds contained bruchid larvae, originating from oviposition events prior to the samples being placed in the cages, but these were all dead, presumably as a result of extended exposure to sunlight.

The fate of seed in dung samples left in exposed areas over winter.

No unblemished seeds were found in a subsample of 686 seeds (≈ 50% of total seeds in dung pellets placed out in the field) that had fallen out of the pellets on or prior to collection. Dissection of the seeds showed that *A. prosopis* larval entry into seeds caused 81% of the damage (Table 2.1). The cause of damage for the remaining seeds (19%) was not established but bruchids or pathogens could have been responsible.

Table 2.1: The fate of the subsample of seeds removed from dung left in the field during four months of winter. The numbers of *A. prosopis* (=Ap) in various stages of larval development, number of parasitoids and the numbers of seed germinated as a result of larval entry are represented. No viable seeds were found in this subsample. Average percentage (± 1SD) that each category contributes to damage of seeds indicated.

	A.p. exit hole	Early A.p. instar	Late A.p. instar	A.p. Pupa	A.p. Adult	Dead A.p. larva	Parasit-oids	Degenera-ted seed, unknown cause	Seeds germi-nated with entry hole	Seeds germi-nated with larva	total
1	6	14	35	2	1	20	15	14	1	3	111
2	1	10	22	6	1	21	23	15	2	6	97
3	2	6	8	5	0	12	18	22	1	0	74
4	1	6	12	3	0	3	18	21	3	4	71
5	0	6	12	1	0	11	14	17	1	2	64
6	0	14	28	10	0	30	17	5	1	2	107
7	6	17	15	13	0	18	16	18	1	2	106
8	1	2	12	0	0	11	2	20	2	6	56
mean (%) ± 1SD	2.25 ± 2.21	10.19 ± 3.79	20.30 ± 6.65	5.27 ± 4.20	0.24 ± 0.45	17.74 ± 6.66	17.92 ± 7.40	21.41 ± 10.55	1.94 ± 1.28	4.01 ± 3.38	

Of the seeds that were classified as bruchid-damaged, 71% contained bruchids in various stages of development, 22% contained hymenopteran parasitoids (of which 99% were in the pupal stage and 1% were larvae), and the remaining 7% were seeds with partially developed radicles but that had failed to germinate and all of which contained larval entry holes and some still contained early instar larvae.

Over the 35 days in the laboratory the eight dung samples yielded 228 insects (Fig. 2.5). Of these 37.7% were hymenopteran parasitoids. The emergence patterns indicate that only late-instar larvae and pupae were suitable for parasitoid development because all the parasitoids emerged within 18 days. However, average parasitism levels during the first 14 days amounted to 61% of total insect emergence.

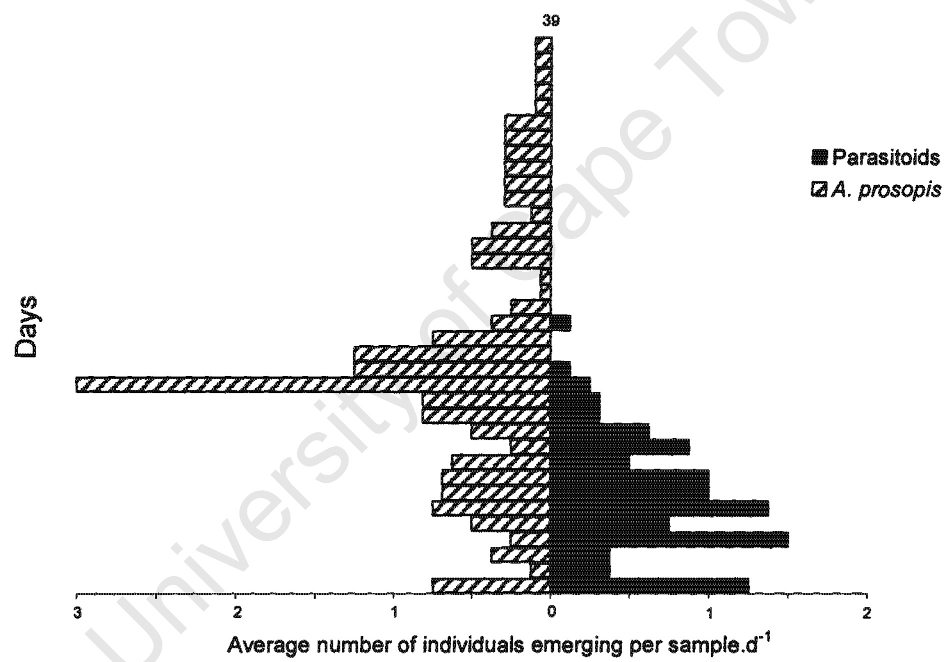


Fig 2.5: The numbers of *A. prosopis* and hymenopteran parasitoids, emerging daily in the laboratory from dung samples left out in the field during winter, March-July 2005.

Using the proportion of bruchids and parasitoids per seed obtained through seed dissection (see above), it was anticipated that the 714 seeds in the dung sample would yield 507 *A. prosopis* adults and 157 hymenopteran parasitoid adults. However, only 142 bruchids and 86 parasitoids emerged from the samples. Thus unaccounted pre-emergence mortality of the insects was high and amounted to 72% and 45% for *A.*

prosopis and the parasitoids respectively. The reason for the mortality of bruchids and parasitoids was not established but may be a result of infection by pathogens, drowning of larvae through water-logging of seeds or other unknown factors.

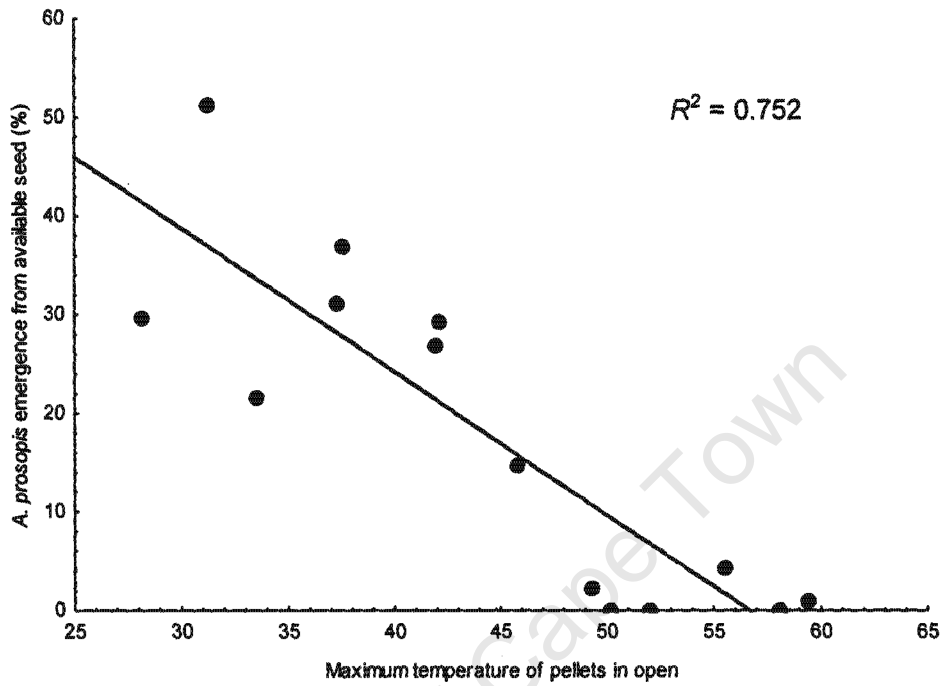
A lack of beetle larvae that were sufficiently mature for the ovipositing parasitoid females to use at the time that the samples were recovered would account for the lower than expected proportion of emerging parasitoid adults (37.7% rather than 61%). Of the 86 parasitoids that emerged 99% did so within 18 days (first record of Fig. 2.5 was on day three) and this was assumed to be the period of pupation as dissection of the seed revealed 99% to be in the pupal stage on collection. Identifications revealed that three pteromalid parasitoid species emerged from dung: two *Pteromalus* and one *Dinarmus* species but that the two *Pteromalus* species accounted for 87.7% of the total parasitoid emergence. The *Dinarmus* sp. was identified as the same species (*Dinarmus altifrons*) that was reared from pod collections. However, neither *Pteromalus* species had ever been reared from pod collections either in this study or others (Hoffmann *et al.*, 1993; Coetzer, 1996) and appeared to be confined to bruchids in dung-borne seeds. Only five viable seeds were found out of a total of 1400 seeds, a reduction of 99.6% of dung-borne seeds over the four month period in the field.

Effect of air temperature on soil and dung temperatures and the consequences for survival/development of larvae

Differences in the levels of exploitation of dung-borne seed situated in shaded and exposed areas may be determined by differences in the temperature regimes in these two situations. Kistler (1995) reported the optimum temperature for *A. prosopis* to be between 25 and 30°C with mortality increasing at lower and higher temperatures.

There were close correlations between ambient air temperatures and those of soil exposed to sunlight ($r = 0.927$), soil in the shade ($r = 0.956$), dung in sunlight ($r = 0.962$), dung in shade ($r = 0.974$) and dung under shade cloth ($r = 0.960$). It was assumed that by extrapolating meteorological air temperatures the temperatures of dung pellets could be gauged. Neither the extrapolated absolute minimum temperature nor the extrapolated average minimum temperature, obtained for a 50 day period prior to collection, correlated with the percentage emergence of *A. prosopis* from dung situated below trees ($r^2 = 0.158$, $p = 0.159$ and $r^2 = 0.007$, $p = 0.783$ respectively). By contrast, there was a significant and negative correlation of numbers of beetles emerging and the extrapolated maximum temperatures of dung pellets in exposed areas ($r^2 = 0.752$, $n = 14$, $p < 0.0001$) (Fig. 2.6a). However, there was no correlation between the numbers of bruchids emerging and the extrapolated maximum temperatures of dung in the shade ($r^2 = 0.023$, $n = 14$, $p = 0.61$) (Fig. 2.6b). The levels of damage caused by *A. prosopis* on dung-borne seeds were significantly lower in dung pellets that reached maximum temperatures in excess of 45°C one or more times than in dung pellets that never reached this temperature (Mann-Whitney, $U = 259.0$, $z = 8.36$, $p<0.001$).

a)



b)

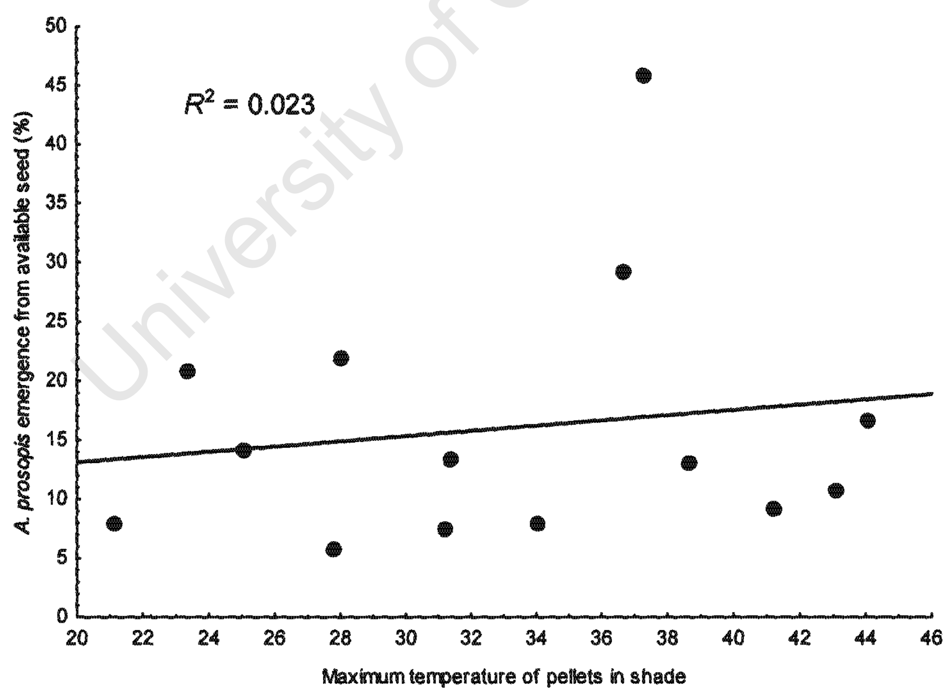


Fig. 2.6a & b: Percentage emergence of *A. prosopis* from dung-borne seed correlated with extrapolated monthly maximum temperatures of dung between April 2003 and July 2004 for dung in a) exposed areas and b) shade.

Discussion

The extent to which *A. prosopis* utilises dung-borne seed is governed by the time of year and the location of the dung i.e. below mesquite trees or in exposed areas. The highest recorded damage to dung-borne seed by *A. prosopis* in any one month was 35% and over the course of the year levels of damage would likely near 100% under ideal circumstances. However, trampling by sheep results in breakage of dung and burial of seed and thus the overall damage to seed by *A. prosopis* is reduced. In addition to bruchid damage, rainfall also contributes highly to removal of seed from the system such that: rain falling over summer periods when temperatures are high results in germination that has the potential to remove 65% of the available resources for the bruchids through enhanced seed germinability after passage through the gut of sheep (Peinetti *et al.*, 1993); alternatively, increased microbial action after rains (Janzen, 1975) in winter, when seeds do not readily germinate (Scifres & Brock, 1969), can lead to decay of those seeds where larval penetration of the seed coat has occurred.

Summer temperatures are too extreme for *A. prosopis* larvae to survive in exposed dung but seeds in dung located in shaded microsites away from mesquite trees are susceptible to bruchid damage during this period. Traveset (1990) reported that a greater proportion of *Acacia farnesiana* seeds contained in horse dung were damaged by the bruchid *Stator vachelliae* on shaded dung and that oviposition on seeds in exposed sites was much reduced. The effect of solar radiation on the survival of bruchids within stored leguminous crops has been investigated (Lale, 1998; Lale & Vidal, 2000; Chauhan &

Ghaffer, 2002). This study demonstrated *A. prosopis* larval survival to be substantially reduced at temperatures above 45°C, a temperature that exposed dung pellets reach in one out of three days during summer (October to March). However, at times when temperatures were mild it was beneficial for bruchids to utilise seeds in dung in exposed areas where solar radiation keeps dung temperatures close to optimal, circumventing the inhibitory effects of the colder temperatures of dung in shaded areas on larval development, and leading to damage of 99.6% of dung-borne seed in exposed areas.

Despite the majority of literature (Janzen, 1972; Janzen *et al.*, 1976; O'Dowd & Hay, 1980; Wright, 1983; Howe *et al.*, 1985, but see Forget *et al.*, 1999) on post-dispersal seed predation pointing towards reduced predation rates with increasing distance from fruiting conspecifics, it does not seem to be the case in this system and in fact to the contrary, in one month there was an increase in seed predation rate with increasing distance. Although utilisation of dung-borne seeds is restricted to shaded areas during summer, *A. prosopis* adults continue to disperse between stands of trees over this period, as shown by exploitation of seed in dung placed below shade cloth in exposed areas during summer.

The main questions to come out of this study are therefore "Why do *A. prosopis* utilise dung-borne seed during winter when these bruchid populations were previously thought to remain inactive over this period" and "What drives *A. prosopis* to search for dung away from mature mesquite trees"?

The first question essentially deals with the opportunistic behaviour of *A. prosopis*. As was demonstrated in the previous chapter, given suitable environmental conditions as when trees are felled, *A. prosopis* exploit pod-borne seeds in exposed situations during winter when they normally remain inactive. Thus, in absence of pods *A. prosopis* exploit dung-borne seed preferentially selecting dung below trees in summer when temperatures are too high and exploiting seed in exposed dung during winter when the temperature of the exposed dung is closest to optimum. But what drives the bruchids to search for dung-borne seed with increasing distances from mesquite trees? Is it to avoid intraspecific competition when oviposition intensity may be high around mesquite trees or is it to avoid parasitoids that are associated with these areas? Where dung is concentrated below mesquite trees parasitism may be expected to be higher because of a decreased host search time by parasitoids. Thus, the use of dung-borne seed dispersed away from mesquite trees may be a means of avoiding parasitoid related mortality, increasing the likelihood of escape of *A. prosopis* from parasitism by moving into enemy-free space (Oppenheim & Gould, 2002). However, the highest recorded levels of parasitism (38%, with estimates of 61%) were found to occur in dung-borne seed in the exposed sites during winter. Although a contributing factor to the high rate of parasitism observed in this study may have been that, by ensuring that the dung remained in piles, host search time by parasitoids was reduced yet the parasitoids were nevertheless able to overcome the hurdle of host dispersal and were still able to locate hosts that were at the suitable stage of development i.e. pupal stage. However, examination revealed that 87.7% of the parasitoids emerging from the dung were two species of the genus *Pteromalus* (Pteromalidae) found to have been collected only from dung samples and not pod samples. Therefore, the possibility exists that the parasitoids reared from *A. prosopis* in dung may have evolved to exploit bruchids in seed associated with dung and not in association with pods. This makes sense when considering that many of the African acacias have coevolved with seed dispersers and that in many instances the highest proportion of seed is contained within dung and not pods for much of the year.

The data arising from this study allow for predictions of the temporal escape of seed from *A. prosopis* and the consequences of variability of the timing of rainfall on the expansion of mesquite infestations.

Chapter 3

A Comparison of use of dung- and pod-borne seed by *A. prosopis*

Abstract

Algarobius prosopis emergence from pod and dung collections at sites where livestock were present, revealed that two thirds of the beetles emerging from the available seed did so from dung and this was the trend for most months. Laboratory studies also indicated that higher numbers of *A. prosopis* emerged from dung samples when females were given a choice of pods and dung, particularly if the females developed within dung-borne seed, suggesting that higher oviposition occurred on dung. The oviposition on sheep dung appears to be an inherent trait as even those beetles originating from populations that do not have to compete with sheep for the pod resource, showed higher offspring emergence from dung than from pods when parents were presented with a choice. Olfactory cues are an important means by which the *A. prosopis* females locate their host and the results of "Y"-tube experiments supported those of the oviposition trials showing that offspring emergence was indicative of female preference for oviposition on dung over pods.

Introduction

Many insect herbivores are generalists, utilising a variety of resources for larval development (Feeny, 1975). In some instances the larvae are able to utilise different plant species within and between instars but others may be restricted for their entire development to the plant on which the first-instar develops (Thompson, 1983). In most cases it is the maternal host choice that restricts the larva to a particular host for its development and which may be influenced by a number of proximate (behavioural and ecological) and ultimate (evolutionary) factors. Proximate factors that influence an individuals decision may include host plant on which the adults developed (e.g. Phillips, 1977; Jaenike, 1983; Barron, 2001), previous adult - host plant experience (Vet & van Opzeeland, 1984; Jaenike, 1988; Jaenike & Papaj, 1992; Vet *et al.*, 1995; Barron & Corbet, 2000), variability of host resource in time, space (e.g. Hunter & Price, 1992) and quality (e.g. Strong *et al*, 1984; Johnson & Kistler, 1987),and orientation of the resource (e.g. MacLellan, 1962; Atienza *et al.*, 1996; Kührt *et al.*, 2006) and probably the most important factor being innate host preference (Messina & Slade, 1997; Fox *et al.*, 2004). Factors that lead to the evolution of host choice include avoidance of inter- and intraspecific competitive interactions (e.g. Wilson, 1988; Minkenberg, 1992; Ohsaki & Sato, 1994; Strathie, 1995), and predator and parasitoid avoidance (e.g. Ohsaki & Sato, 1994; Tschanz *et al.*, 2005). All of these factors may be critical in insect population dynamics (Price *et al.*, 1990).

In turn, the choice of the adult as to where eggs are deposited may influence the fitness, survival, developmental rate and incidence of contact with competitors, predators and parasitoids, for immatures. Resource-use tactics of adult insects are critical in population dynamics of herbivorous insects (Price *et al.*, 1990) where variable resource-use can influence insect populations in two ways: performance and preference (Ohgushi, 1992). Performance is an immediate influence on survival and reproduction of the insects. In contrast, preference is defined as the evolutionary responses of insects to variable and heterogenous plant resources that improve individual fitness (Thompson, 1988). The relationship of adult and immature characteristics and the question of preference/performance has lent itself to many studies and debates on the evolution of host specificity (Wiklund, 1975; Bush & Diehl, 1982; Futuyama & Peterson, 1985), selection for enemy-free space (Lawton & McNeill, 1979; Price *et al.,* 1980; Ode, 2006) and host shifts in allopatric and sympatric insect populations (Bush, 1975; Futuyama & Mayer, 1980; Wood & Guttman, 1983; Thompson, 1988).

According to the oviposition-preference – offspring-performance hypothesis (Jaenike, 1978), oviposition-preference patterns are supposed to correspond to host suitability for offspring development (but see Scheirs *et al.*, 2000), which may have residual effects on performance for more than one generation (Andersson, 1978).

In the web of mesquite tree – sheep dung – *A. prosopis* interactions it is expected that female *A. prosopis* habitat and host selection are dictated by the suitability for offspring development. As *A. prosopis* are monophagous, "host" refers to the choice of pod- or dung-borne seed. It is assumed that larvae should develop equally well on dung-borne seed as on pod-borne seed because the seed will not have changed internally during passage through the gut of a sheep. Thus adults emerging from dung should be no different in terms of their fecundity to those emerging from pods.

Choice of oviposition sites by females would disclose which seed type (pod-borne or dung-borne) was most acceptable as a larval food source, and survival rates of the immature stages would disclose which seed type was most suitable, under different conditions. However, these could not be quantified separately because the samples would have had to have been destroyed to locate the concealed eggs and the endophagous larvae. As a result, the number of beetles that emerged from seed samples, which was the product of numbers of eggs laid and immature survival, was used to assess the extent to which bruchids were exploiting the different resources available to them.

The observations that *A. prosopis* utilise dung-borne seed for larval development raises the issue of whether or not this species is primarily associated with either pod-borne or dung-borne seeds and, if so, whether the females only resort to the alternate seed type when nothing else is available. If the beetles are not primarily associated with either seed type but use both equally, is parental choice influenced by the position of the seed in which larval development took place (i.e. do females that emerged from dung-borne seed show any preference for, or avoidance of, seeds in dung and do females that emerged from pod-borne seed prefer, or avoid, seeds in pods)? A series of choice experiments were conducted in the field and in the laboratory to provide answers to these questions. The results are presented in this chapter.

Methods

Seasonal and spatial variations in usage of seed in pods and dung

The following experiments were conducted at the Calvinia study site commencing in July 2003 and ending in September 2004 to establish the seasonal variation in exploitation of pod- and dung-borne seeds by *A. prosopis* females and the choices exhibited by these ovipositing females. Pods were placed in the field concurrently with dung samples as described in the previous chapter. Three pod allotments were placed below trees and four were placed at distances 10 - 30m away from adult trees. The pods had originally been collected at the beginning of the 2003 podding season and had been placed in a -20°C freezer to terminate any developing larvae originating from previous oviposition events in the field. Each pod allotment weighed 40g and contained on average 184 seeds and each dung allotment weighed 40g but contained on average only 35 seeds. The allotment sizes were determined on weight and not the amount of seeds they contained i.e. not 210g of dung that would likely yield a similar number of seeds, as this would create an unnaturally large dung pile and introduce another variable. The allotments were left in the field for a period of a month after which time they were collected and replaced with new allotments. On collection the pods and dung were placed in sealable packets for transport back to the laboratory. Once there, the allotments were transferred to 110mm diameter clear plastic tubs with gauze lids and placed in the constant temperature room at 27°C ± 2°C and 40-60% humidity. Emerging insects were counted and removed every 3-5 days.

Counts of emerging bruchids were used to establish seasonal trends in exploitation of pod- and dung-borne seed and if there were differences in the extent of exploitation in shaded or exposed sites, and if any differences existed between usage of pod or dung-borne seed for larval development.

Laboratory choice and performance experiments with pods and dung

The intention with these experiments was to investigate if the larval food source of an ovipositing female, pods or dung, influenced her host choice. In addition, the potential for choice reinforcement from one generation to the next was tested.

The pods used in these experiments were inspected and any aborted seeds or any having been damaged by bruchids were broken off and only segments containing 5-10 pristine seeds were used. Each pod segment had two sites for oviposition, one at each end (Swier, 1974), amounting to approximately 35-40 oviposition points in the pod samples. In order to establish the preferred oviposition sites of *A. prosopis* females, equal masses of pods and dung (20g) were placed in an arena and made available to females. The arena consisted of a 5 litre rectangular plastic box (34x15cm) with a gauze lid and when dung and pods had been placed inside there was a minimum distance of 20cm separating the allotments. Four one-day-old females were placed in the boxes together with two one- day-old males. The boxes were then placed in a constant temperature room at 27°C ± 2°C and 40-60% relative humidity and left for 5 days after which time the six adult bruchids were removed. The allotments, of dung and pods, were then placed separately into 110 mm diameter clear plastic tubs with gauze lids in which they were left for the remainder of the experiment. The tubs were checked on a daily basis and any emerging *A. prosopis* adults were counted and removed. This process continued until bruchid adults stopped emerging (35days) after which, the tubs were placed in a -20°C freezer to terminate any further development of bruchids within the samples. In conducting the experiments in the laboratory it was assumed larvae utilising seeds in pods and dung would have similar rates of survival and thus the numbers of adult bruchids emerging would be proportional to the number of eggs laid on the allotment.

To reduce the effects that may influence female oviposition choice and ensure that oviposition choices in the trials were the female's primary choice, several factors were adjusted for: i) the use of one-day-old females and allowing them only five days to oviposit was based on the work of Hoffmann *et al.* (1993) which showed female peak oviposition rates were only reached five days after mating (by limiting oviposition time, it was hoped that potential oviposition sites on one or other seed source would not have been limited by overcrowding of eggs and thereby limiting suitable "egg-free" oviposition sites on a particular choice); ii) only 2 males were placed in with the females so as to avoid oviposition and mating interference by unmated males - each male could mate more than once (*pers. obs.*) and mating frequency increases the numbers of eggs that *A. prosopis* lay (Hoffmann *et al.*, 1993).

Algarobius prosopis used in these experiments were sourced from two populations, the one population from Calvinia where sheep were present and removed the pods and the other from Modder River where there were no sheep.

The origin of the adult bruchids from Calvinia was from either dung or from pods collected in the field from the Calvinia site. Replicates of the choice experiments were run in 2003 and 2004 to determine if parent origin affected exploitation i.e. of dung- or pod-borne seed (Fig. 3.1). The process was then repeated using the bruchids emerging from the first experiments to determine if the larval food source of grandparents contributed to any variations in exploitation by parents. Limitations imposed by the numbers of bruchid females that emerged in the second generation did not allow the same number of replicates for the second set of choice tests (Fig. 3.1).

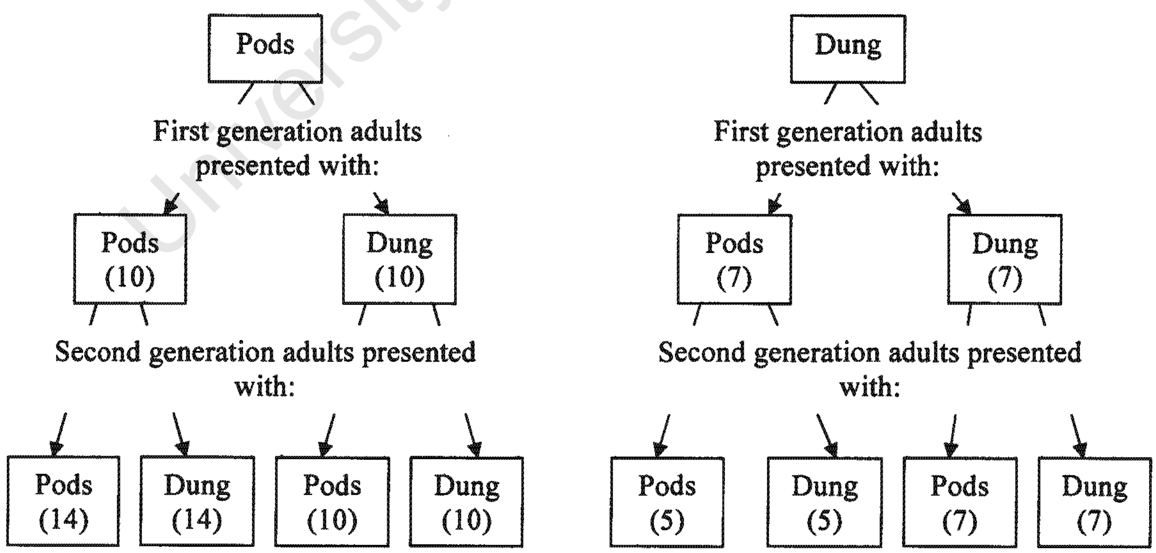


Fig. 3.1: Source of the first generation bruchids collected at Calvinia and the numbers of oviposition choice replicates (in parentheses) presented to them and to their offspring.

To confirm that bruchid emergence from dung reflected host preferences of the parents and not a result of forced oviposition arising from a lack of "egg-free" space on pods, the percentage of unused pod segments was determined. At the end of these experiments, the numbers of pod segments were counted. Each segment was considered as having two points of oviposition (i.e. the fractured ends), therefore, segments with emergence holes were counted as utilised and those with no emergence holes were considered unutilised. This was done to get an estimation of the percentage of oviposition sites on pods that were not exploited. It was assumed that any oviposition on a pod segment would give rise to successful development and emergence of bruchids from that pod segment under ideal controlled insectary conditions. The same was not done for dung as each pellet could be considered as having a multitude of potential oviposition points and many pellets may not have contained seeds.

The numbers of males and females emerging from dung and pods was measured for 12 of the replicates to confirm that host choice did not influence sex ratio.

The above experiments were repeated for *A. prosopis* originating from the Modder River site, where there was no removal of pods by sheep and thus no chance for *A. prosopis* to utilise dung-borne seed (Fig. 3.2).

Eleven replicates of the first generation choice tests were conducted but it was only possible to test bruchids emerging from pods. Choice tests (10 replicates) were then conducted on the next generation emerging from dung to see if the seed source in which the females developed influenced the degree to which they exploited each host choice (Fig. 3.2). The purpose of doing the choice test trials on two *A. prosopis* populations, from Calvinia and from Modder River, that have to contend with varying seed sources was to determine whether either population exhibited a stronger tendency to exploit dung-borne seed.

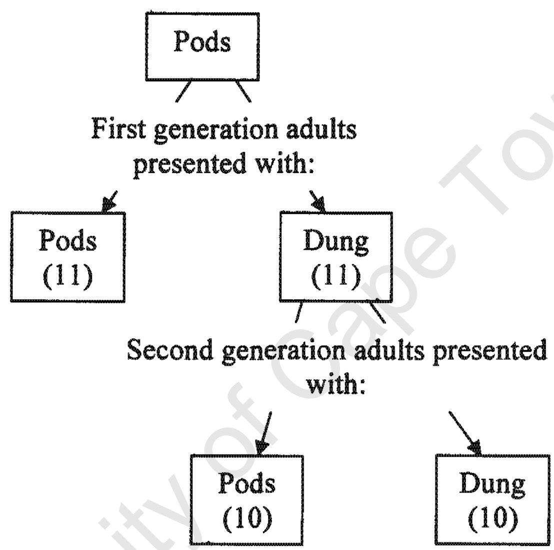


Fig. 3.2: Source of the first generation bruchids collected at Modder River and the numbers of choice replicates presented to them and to their offspring.

Olfactory preferences for dung or pods.

Mated *A. prosopis* females were used in an experiment in an attempt to determine if they use olfactory cues to distinguish pods from dung and if they exhibit preference for one or the other. A constant air flow of 16 mBar was passed through two chambers, each connected to an arm of a "Y" tube. The seed choices being presented to the bruchids were placed in the chambers. The three sets of choices (Left and Right) were:, nothing and pods, nothing and dung and finally dung and pods. A single bruchid female was introduced into the "Y" tube for each trial. Her first choice, the time it took to make this choice and her position after 5 minutes were noted. Twenty replicates were made for each set of choices. For each of the replicates, 10 were done with the seed choice in the left chamber and 10 with that seed choice in the right chamber to determine if there was any preference for one of the arms over the other. The same 20 bruchid females were used for each set of choices throughout the trial. The choices made were weighted such that a positive choice was allocated "+1", negative choice "-1" and no choice "0" and it was hypothesized that they would exhibit an even distribution of choices – non choices made i.e. a ratio of 1:1:1. The sums of choices were then used to demonstrate the proportions of choices made.

Oviposition on "non-mesquite" dung

A number of experiments were run to see if *A. prosopis*, having emerged from dung, would oviposit on dung that i) had no traces of mesquite in it and ii) came from sheep not exposed to mesquite but the pellets of which were hollowed, filled with 1-3 seeds and glued together using a water based wood glue. The number of seeds placed within these pellets was similar to the numbers of seeds typically found in the dung of mesquite-fed sheep. These experiments were done in order to establish if *A. prosopis* oviposition reflected their opportunistic behaviour and, in addition, if their larvae and emerging adults were able to tunnel through a possible barrier that "non-mesquite dung" might provide.

To establish if *A. prosopis* oviposited on dung containing no traces of mesquite, two mated, day-old females were placed in each of five Petri dishes containing five sheep pellets collected from an area without mesquite. An additional six Petri dishes containing the same dung type had one mated, day-old female placed in each of them. The dishes were left for five days after which time the pellets were dissected under a dissecting microscope and the eggs counted.

To determine if the females could exploit mesquite seeds artificially inserted into non-mesquite dung containing no natural traces of mesquite, two day-old females and one male were placed in each of nine Petri dishes containing five pellets and left for five days after which time the adult bruchids were removed. These experiments were placed in the insectary to see if larvae developed successfully, and emerging adults were counted and removed daily. After 45 days, the dung pellets were crushed and the fate of the seeds recorded. A control of 5 replicates was run concurrently on dung, naturally containing mesquite seeds, which had been cut in half and glued back together to see if there was avoidance or an inhibitory effect of the dung containing glue.

Results

Seasonal and spatial variations in usage of seed in pods and dung

The numbers of beetles that emerged from both pod-borne seeds and dung-borne seeds varied from month to month (Fig. 3.3 a & b). It appeared that beetle exploited dung-borne seeds over pod-borne seeds as on average, 18.0 and 10.6 beetles emerged per 100 seeds in dung and pods respectively in shaded areas and 10.0 and 5.5 beetles emerged per 100 seeds in dung and pods respectively in exposed areas however, in neither shaded nor exposed areas were these differences significant (Mann-Whitney, $z = 1.41$, $p = 0.15$ and $z = 0.52$, $p = 0.60$ respectively). The numbers of beetles emerging per 100 seeds in pods exceeded emergence from dung-borne seed in only one month in both the shaded (February 2004) and exposed (October 2003) areas. The following section deals with normally distributed data and therefore ANOVA could be used for statistical tests: In the shaded areas, the highest emergence from pods and dung occurred during summer (October – February) where, although not highly significant ($P < 0.05$), it appeared that significantly higher numbers of bruchids emerged from dung (ANOVA, $F_{(1,61)} = 3.77$, $p = 0.056$). Although bruchids continued to utilise both pod- and dung borne seeds in the shade during winter, oviposition on pods was significantly less (ANOVA, $F_{(1,62)} = 7.14$, $p = 0.009$). In contrast emergence from pods and dung in exposed areas was restricted to winter, during which time significantly higher numbers of bruchids emerged from dung (ANOVA, $F_{(1,103)} = 21.32$, $p < 0.0001$). Low emergence from pods between May and August may be a consequence of the lower temperatures of the pods compared to dung in both the exposed and shaded situations and although there was no emergence from exposed pods for the majority of summer, the highest record of emergence on pods in exposed areas occurred during October 2003, at the start of summer. This happened at a time when temperatures of dung pellets would have been too extreme for larval survival while the pod temperatures, because of their light colour, were probably more suitable for the developing larvae. A factorial ANOVA revealed that: i) date; and ii) choice (dung- or pod-borne seeds) and location (in shade or exposed) both contributed significantly to the

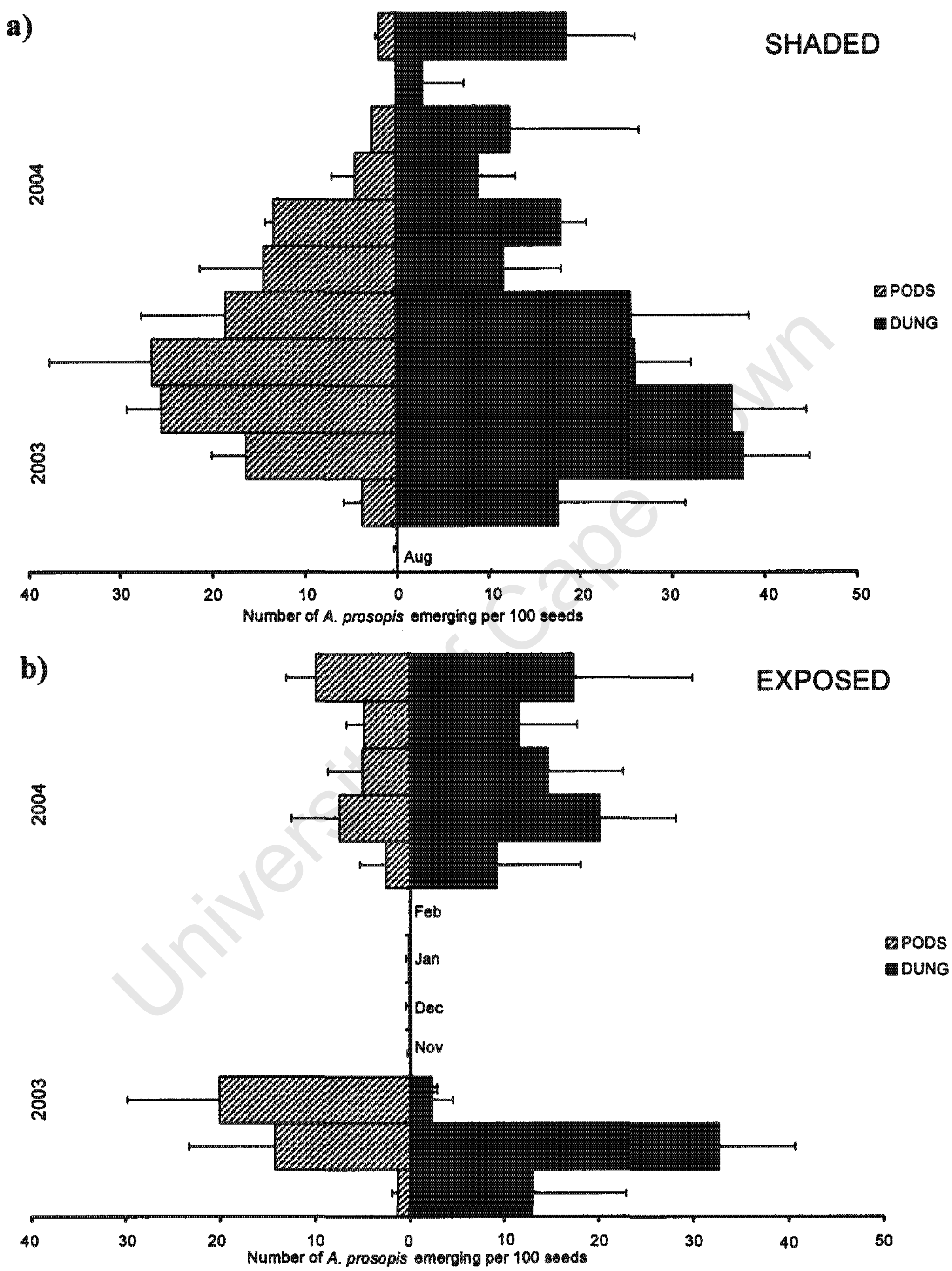


Fig. 3.3a & b: Seasonal trends in emergence of *A. prosopis* (mean + 1SD of numbers of beetles per 100 seeds) from dung and pods left in the field at Calvinia for one month in situations a)shaded from sunlight and b) exposed to sunlight. Trials conducted from August 2003 to August 2004.

variation in levels of seed damage ($p < 0.0001$ for all effects) (Table 3.1). There was also a significant ($p<0.0001$) interaction between the two factors. A post-hoc Tukey test of the combined factors, “date” and “choice and location”, revealed most variation was explained by the high levels of emergence from dung in September and from both dung and pods in October. This is probably due to the post-winter rise in adult emergence of *A. prosopis* in the area.

Table 3.1: Results of the factorial ANOVA of arcsine transformed percentage bruchid emergence from seeds and pods collected at the Calvinia site over the period of one year.

	SS	Degr. of freedom	MS	F	p
Intercept		0			
date	7330.70	10	733.07	14.82	0.000000
choice & location	6277.98	3	2092.65	42.31	0.000000
date*choice & location	30960.13	53	584.15	11.81	0.000000
Error	15724.67	318	49.44		

Using monthly beetle emergence values the cumulative effect of bruchid damage over the season could be estimated (Fig. 3.4). These values were determined from the emergence results between September 2003 and August 2004. Mature pods were first present in January and hence Fig. 3.4 spans the season from January to December. The rates of decline are probably overestimates because: i) in bringing the pod and dung samples back to the laboratory the impact of abiotic and biotic mortality factors on bruchid eggs and larvae are greatly reduced; ii) the placing out of fresh allotments of pods and dung every month would continually provide ideal seeds; iii) the dung piles were protected by the cages preventing the normal disruptive activity by sheep on dung and pods such as burial or trampling.

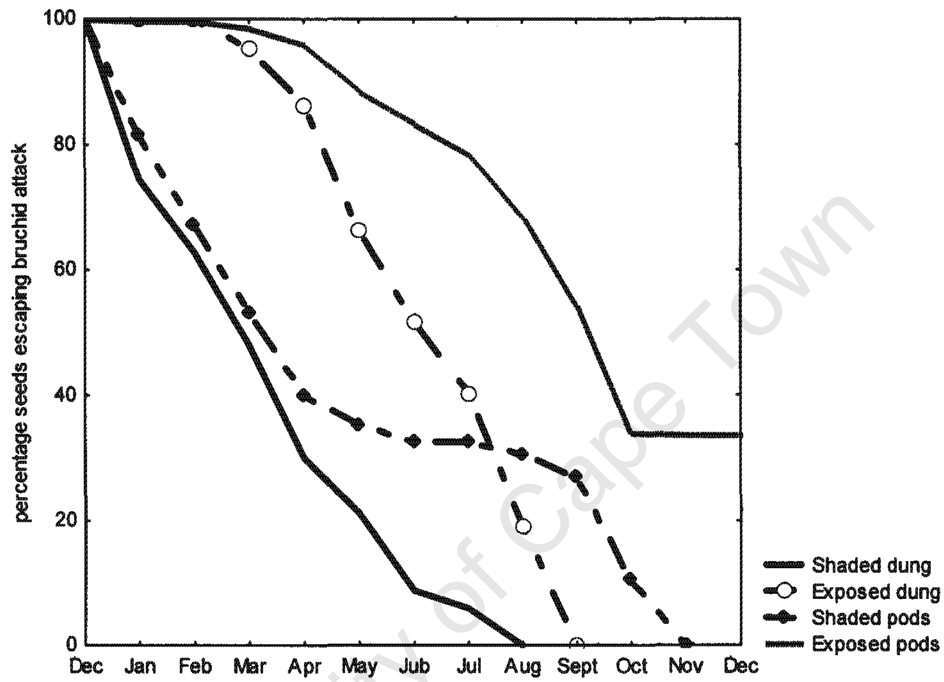


Fig. 3.4: The percentage of seeds in dung and pods escaping attack by *A. prosopis* over a one year period.

Despite emergence from pods and dung in shade being similar between January and April, there was very little emergence from pods in shade over the winter period and only after September were these pods properly utilised by the bruchids. According to these estimations although the use of seeds in unshaded dung is limited to winter, between April and September, during this six month period *A. prosopis* was capable of achieving 100% destruction of seeds despite high levels of pupal parasitism (see Chapter 2).

Laboratory choice and performance experiments with pods and dung.

There were no detectable effects of larval seed source of the grandparent on the host choice of parent as determined by the numbers of offspring emerging (t-tests for independent samples, $t=0.097$, $p=0.924$ and $t= -1.53$, $p=0.147$ for parents from pods and parents from dung respectively). This suggests that any variations in the exploitation of an host were a consequence of maternal origin and not that of previous generations and there is no detectable reinforcement from one generation to the next. The data for maternal choices of both generations from the same seed source were therefore combined (Table 3.2).

The emergence results from the Calvinia population showed significantly more bruchids emerged from dung over pods when the parents originated in dung but there was no significant difference in emergence from dung or pods if the parent originated in pods (paired t-tests; $t = 11.37$, $p < 0.0001$ and $t = 1.639$, $p = 0.114$ respectively). However, females from the Modder River population exploited dung-borne seed significantly more than pod-borne seed regardless of host for parent development (first generation from pods and second generation from dung) (t-tests for paired samples; $t = -2.32$, $p = 0.043$ and $t = -3.66$, $p = 0.005$ for adults from pods and dung respectively).

To confirm that variations in exploitation of pod- and dung-borne seeds (Fig. 3.1) was influenced by the host in which the parent developed, the numbers of offspring emerging from dung and pods were compared for parents from the two different hosts. Females

Table 3.2: Numbers of *A. prosopis* emerging from each of the paired choice tests for a) Calvinia and b) Modder River populations showing adult source and host choice and also indicating the average emergence (± 1 SE)

	a) Calvinia Adult source				b) Modder River Adult source			
	Dung		Pods		Dung		Pods	
Choice replicate	dung	pods	dung	pods	dung	pods	dung	pods
1	39	18	31	16	51	18	44	40
2	45	26	32	43	53	15	31	32
3	44	7	54	31	55	45	39	32
4	45	12	34	29	67	31	42	49
5	63	0	32	55	55	1	36	28
6	50	24	20	49	62	39	47	24
7	42	5	55	29	49	32	46	27
8	65	29	54	27	49	16	48	18
9	62	21	40	18	29	36	35	29
10	62	25	52	25	25	32	31	30
11	49	6	54	7			41	43
12	61	27	47	28				
13	43	11	26	49				
14	32	22	37	37				
15	56	18	41	35				
16	41	24	42	20				
17	56	26	36	34				
18	41	19	21	60				
19	42	23	44	45				
20			31	1				
21			42	30				
22			27	19				
23			42	25				
24			30	44				
25			30	48				
26			52	22				
Average	49.4 ± 2.25	18.1 ± 1.98	38.7 ± 2.08	31.8 ± 2.84	49.5 ± 4.16	26.5 ± 4.26	40.0 ± 1.84	32.0 ± 2.68

from both *A. prosopis* populations exploited dung-borne seed to a higher degree than pod-borne seed if the females developed in dung than if the females developed in pods (χ^2 = 107.8, p < 0.0001 and χ^2 = 14.9, p = 0.0001 for the Calvinia and Modder River populations respectively).

Offspring emergence from pods suggested that females from the Calvinia population that developed in dung and pods utilised only 62% (σ =26.0%) and 68% (σ =21.5%) of pod segments respectively, suggesting competition for "egg-free" space on pods was not intense between *A. prosopis* females. In addition, the proportion of pod segments that produced beetles was positively correlated with the total number of bruchids that emerged in each of the choice experiments (r^2 = 0.507, p=0.001 and r^2 = 0.522, p = 0.0005 for adults from dung and adults from pods respectively). In contrast, emergence of offspring of females from the Modder River population suggests these females may show a higher propensity to exploit pod seeds than females from the Calvinia population as 77.0% (σ =27.9%) and 79% (σ =12.5%) of pod segments provided to females that developed in dung and pods respectively, yielded adult beetles. Furthermore, the proportion of oviposition points on pods provided to females of the Modder River population did not correlate with the total number of bruchids emerging (r^2 = 0.38, p = 0.057 and r^2 = 0.33, p = 0.065 for parents from dung and pods respectively) as one might expect if increased exploitation of pods was a consequence of a decrease in availability of oviposition points on dung.

There were no significant differences in fecundity of parents that developed in either pods or dung, as was determined from the numbers of offspring emerging from each seed source (t-test for independent samples; t = 0.817, p = 0.418 and t = -0.674, p = 0.508 for bruchids from Calvinia and Modder River populations respectively). Neither did the sex ratios of progeny deviate from unity - male:female = 49:51 (t-test for dependant samples;

$t = -0.867$, $p = 0.40$) and 53:47 (t-test for dependant samples; $t = 1.156$, $p = 0.27$) for emergence from pods and dung respectively.

Olfactory preferences for dung or pods.

This experiment served to further confirm that females preferred dung to pods due to olfactory cues and that the strength of their response depended on the host in which they developed. The position of females after 5 minutes in the choice tests are shown and also the sums of choices were then used to show the proportions of choices made (Table 3.3).

Having developed in pods *A. prosopis* females showed a highly significant preference to orientate themselves in the arm of the tube in which pods were located ($\chi^2 = 15.861$, $p = 0.0004$) when provided with a choice of pods or nothing and although, when provided with the choice of dung or nothing they did show a tendency to move towards the dung, these values were not significant ($\chi^2 = 3.739$, $p=0.154$) (Table 3.3). In contrast, when the females had developed in dung-borne seed they exhibited a stronger tendency to orientate themselves in the tube that contained dung ($\chi^2 = 7.679$, $p = 0.022$) than those that contained nothing, and showed no preference to move towards pods ($\chi^2 = 0.709$, $p = 0.701$) when presented with pods or nothing (Table 3.3).

Table 3.3: Results of "Y"-tube choice tests of females originating in either pods or dung and their orientation after 5mins towards one or other seed source when presented with a choice of either pods or nothing, dung or nothing or pods and dung. Expected ratio of +ive: -ive: "0" is 1:1:1.

	Parent from pods		Parent from dung		Parent from pods	Parent from dung
Choice	Pods & nothing	Dung & nothing	Pods & nothing	Dung & nothing	Pods over dung	Dung over pods
+ive	15	10	7	12	4	11
-ive	2	3	5	2	5	7
"0"	3	7	8	6	11	2
Sum of Choices	13	7	2	10	-1	4

When parents originating in pods were presented with a choice of pods and dung (Table 3.3) marginally more positive choices were made towards dung (25%) than towards pods (20%) but these results did not differ significantly from the expected ratio (χ^2 = 4.345, p = 0.114). In comparison, the parents that developed in dung-borne seed showed a significantly stronger attraction to dung (55%) than was expected (33%) (χ^2 = 6.164, p = 0.046). The availability of both seed sources, pods and dung, on the response of females originating from different seed sources was varied: those that originated in pods appeared to be undecided whether to orientate to either dung or pods for much of the time and ended up failing to move towards either in 55% of the replicates, but females that developed in dung made a positive choice for either dung or pods in 90% of the replicates (χ^2 = 9.23, p = 0.002).

Oviposition on "non-mesquite" dung

Oviposition did occur on dung that contained no trace of mesquite, and over the 5 day period similar averages of 8.2 (σ =5.84) and 8.17 (σ =9.30) eggs were laid by each female on the available dung pellets in both trials i.e. those containing two females and those containing one female respectively. Despite the high standard deviation for the oviposition trials of the single female, a result of oviposition failure in 50% of the replicates, there was no significant difference in numbers of eggs laid per parent between the two trials (Mann-Whitney; $z = -0.091$, $p = 0.927$).

Oviposition on "non-mesquite dung" that contained artificially inserted mesquite seed and on "mesquite-dung" (i.e. naturally containing mesquite seeds) showed no significant difference in the percentage of bruchids emerging from available seed (Mann-Whitney; $z = 0.600$, $p = 0.549$) (Table 3.4).

Table 3.4: Emergence of *A. prosopis* from available mesquite seed in i) dung naturally absent of mesquite seeds and ii) dung naturally containing mesquite seeds and the percentage of seeds damaged. Number of replicates in parentheses.

	Seeds per pellet	Total seeds	Bruchids emerged	Damage
Non-mesquite dung	2.1	97 (9)	83	86%
Mesquite dung	2.0	48 (5)	44	92%

Discussion

In the field, dung-borne seeds were exploited to a higher degree by *A. prosopis* than were pod-borne seeds for most of the year and in only one month in both the open and exposed areas did the emergence from pods exceed that from dung. A possible explanation for the preference of dung over pods may be one of resource abundance (Price *et al.*, 1980; Thompson, 1988), whereby oviposition choice is affected by the availability of resources for larval development. *Algarobius prosopis* is able to exploit both resources and do so according to availability. As the abundance of dung-borne seeds exceeds that of pods for most of the year the bruchids might be expected to exploit dung-borne seeds when it is the dominant seed source. However, although pods account for the majority of new seed in the system between January and April, albeit at ever diminishing levels, the exploitation of pod-borne seeds over this period did not exceed that of dung-borne seed. In addition, the exploitation of pod-borne seeds decreased sharply at the beginning of winter. Possible explanations for this trend are that i) there was accumulation of new dung in the system with time, increasing its attractiveness as a resource (Price *et al.*, 1980; Thompson, 1988); ii) increasing numbers of females reared from dung and therefore conditioned to oviposit into it, either through pre-imaginal (Alloway, 1972; Tully, *et al.*, 1994; Ray, 1999) or imaginal conditioning (Vet & van Opzeeland, 1984; Jaenike, 1988; Jaenike & Papaj, 1992; Vet *et al.*, 1995; Barron & Corbet, 2000); iii) the drop in average temperatures as winter began made dung, particularly in the exposed areas, more useable (MacLellan, 1962; Kührt *et al.*, 2006); and iv) dung provided enemy-free space for developing larvae (Lawton & McNeill, 1979; Price *et al.*, 1980). Bruchid emergence from available pod-borne seeds during the summer months was not significantly lower than that from dung. Peak exploitation of pods occurred in October, at a time when pods were naturally absent from the system thus the resource abundance theory does not explain increased pod utilisation during the period between winter and mid-summer (pod-fall) and temperature is probably the main influence on host use. But are the bruchids driven to utilise resources that maximise enemy-free space, and what constitutes this enemy-free space – the use of dung as a resource or the oviposition on dung away from mesquite trees? Is the use of dung away from trees merely coincidental with seasonal temperature variations? The design of this experiment i.e. the regular monthly collection of pods and dung samples, was a means of establishing periods in which bruchids were most active on one or other resource and not to determine variations in parasitism levels. Results presented in the previous chapter show that parasitism of larvae in dung has the potential to be very high (≈ 40-60%). However, the bruchid larvae are only vulnerable for a brief period of their development, during the late instar larval or pupal phase and removing the samples from the field prior to this stage of development meant that levels of parasitism could not be recorded and there was no means of testing if this was a likely factor driving bruchids to use dung-borne seed. During winter, oviposition on exposed dung enhances the rates of larval development. Reduced developmental time decreases larval susceptibility to parasitism (e.g. Moran & Hamilton 1980; Price *et al.*, 1980; Grossmueller & Lederhouse, 1985, Clancy & Price, 1987; Benrey & Denno, 1997) formalised as the "slow growth – high mortality" hypothesis by Clancy and Price (1987). This theory was primarily established to interpret the effects of diet on larval development and subsequent parasitism but can be extended to include temperature effects on host-parasitoid interactions (Benrey & Denno, 1997). In the case of *A. prosopis* a factorial ANOVA, showed "date" and "location" to be the only combined factors that explained variability in oviposition and that a combination of "choice" with these two factors was inconsequential. This suggests that the bruchids are driven to exploit seed in different localities according to the time of year, a surrogate for temperature, and their choices are not governed by seed source. The results also suggest that if the choice to oviposit on dung is escape from parasitism for bruchid offspring then it is not the use of dung over pods that provides the enemy-free space but the location of the dung. However, the only seeds in exposed situations that would suitably increase the rates of larval development are those contained within dung.

Considering the reasons why bruchids may use dung-borne seed as a resource over pods (or in the absence of pods) i.e. enable perpetuation of the populations, escape from parasitism and secure alternative means of over-wintering, I question whether this is achieved. Is *A. prosopis* caught "between the devil and the deep blue sea" (Lawton & McNeill, 1979)? By relying solely on pods in areas where livestock remove the pod crop almost in its entirety it is unlikely that the *A. prosopis* populations would persist and if so, effect any suitable degree of damage on the seed crop for the brief period of availability, prior to consumption by livestock. The usage of dung-borne seed therefore enables the bruchids to circumvent the problem of diminished population size and contributes to the perpetuation of the bruchid populations. In addition, the use of dung-borne seed for over-wintering may enable the emergence of a post-winter generation with increased fitness, compared to over-wintering adults, which would be more likely to contribute to population increase. However, escape from parasitism of larvae developing in pods to enemy-free space which the novel host that dung was thought to provide, has not occurred. Despite this, the use of seed in dispersed dung for larval development during winter may help to reduce the overall levels of parasitism by altering patch dynamics and thus influencing search times/strategies of the parasitoids (Cook & Hubbard, 1977; Comins & Hassell, 1979; Godfray, 1994) and reducing developmental times of bruchid larvae as a result of temperatures closer to optimum (Kistler, 1995) thus further reducing their susceptibility to parasitism.

The field experiments showed that *A. prosopis* exploited dung-borne seed more frequently than pod-borne seed during the course of the year but that temperature was a major determining factor in which resource was used in different situations. As a consequence of this, choice experiments were conducted to determine if there was any evidence for host preference on dung over pods under controlled laboratory conditions.
Habitat selection is assumed to be determined by quality of the site for developing offspring (e.g. Jaenike, 1978; Ng, 1988; Thompson, 1988; Mayhew, 1997 but see Scheirs *et al.,* 2000 and Mayhew, 2001), with the majority of work investigating phenotypic differences in plant morphology and chemistry and its effect on larval development. But what would be the best strategy if resources were of similar nutritional value for the developing larva but in two different states? Under these conditions females could make choices on a spatial basis such as to conceal eggs to minimise predator or parasitoid encounters, or choose sites that would optimize larval developmental rates. Seeds of certain plant species are unavailable for attack by bruchids until the pod has undergone some change, usually in the form of removal of exocarp and the exposing of the endocarp

or seed e.g. *Scheelea* palm (Wilson & Janzen, 1972; Wright, 1983) and the palm *Maximiliana maripa* (Fragoso *et al.*, 2003). *Algarobius prosopis* appears to be adapted to use seeds in pods as its primary resource, the mobile larvae locate unoccupied seeds in pods out-competing other species if present and in addition the larvae are capable of living within the mesocarp for a period of time (Kistler, 1985) obtaining sustenance from this medium prior to seed entry. The high level of seed damage inflicted on pod-borne seeds by *A. prosopis* in its native and introduced ranges shows it is adapted to the use of pods. Seeds exposed on the dung surface might suffer a higher degree of larval entry (Traveset, 1990) to those deep within the dung which are in turn less susceptible than seeds contained in pods because the dung is thought to provide a non nutritional medium through which the larva must travel in order to locate a seed. The interaction of *A. prosopis* and seed contained within dung highlights a potential evolutionary strategy of a bruchid locating and preferentially ovipositing on post-dispersed seed.

The results of the choice experiments demonstrate that *A. prosopis* preferentially exploited dung-borne over pod-borne seeds, particularly when the parent developed within dung. The question therefore is where in the evolutionary history of *A. prosopis* was this behaviour initiated? Janzen and Martin (1982) considered the loss of New World megaherbivores and its impact on the distribution and dynamics of a number of tree species pre-adapted to seed dispersal by these megaherbivores in Central America. In their paper they discuss how exposed seed in megaherbivore dung is used by bruchids but that some seeds escape because they were scattered and discrete. Dispersal failure in absence of these megaherbivores has enabled bruchids to regularly destroy almost all of the annual seed crop. Reintroduction of large herbivores has enabled 'escape' of seed and the subsequent expansion and densification of mesquite stands (DeLoach, 1985). In light of this it seems plausible that *A. prosopis* evolved alongside megaherbivores and developed the ability to search for and utilise dung-borne seed.

The usage of dung-borne seed appears to be inherent to *A. prosopis* as the bruchids from the two populations, Calvinia and Modder River, were seemingly equally attracted to dung as a host despite the Modder River population not previously (since introduction) being exposed to dung-borne seed. Although females from the two bruchid populations both showed a preference to exploit dung over pods if they developed in dung, females from Calvinia population, having developed in dung, showed a significantly greater propensity to oviposit on dung than did those females that developed in dung from the Modder River population. Thus, it may be that subsequent to their introduction 18 years ago, *A. prosopis* populations that are continually subjected to pod removal by livestock and utilised dung-borne seed as an alternative seed source had been selected for and showed a more rapid and significant adjustment to the use of dung-borne seed if they developed in dung over those that developed in pods.

Female insects may use olfactory or visual cues or a combination of the two in order to locate suitable oviposition sites (Bernays & Chapman, 1994). Bruchids have been shown to locate host seeds at long distances from the parent plant (Wilson & Janzen, 1972; Wright, 1983; and the current study), however, the methods by which they locate these seeds is not fully understood. The bruchid *Callosobruchus chinensis* has been shown to respond positively to olfactory stimuli arising from undamaged seed and seed containing eggs of the cowpea and to show negative response to those seeds containing larvae

(Ignacimuthu *et al.*, 2000; Babu *et al.*, 2003). In the current study I set out to investigate whether *A. prosopis* females were able to orientate themselves towards a mesquite seed source using olfactory cues. The results did more than merely demonstrate that the females located seeds using olfactory cues but also that they exhibited preferences for one or other seed source showing trends which reflected preferences gleaned from the emergence results in the choice tests.

The results of these "Y"-tube experiments compliment those results obtained for the oviposition choices in the previous section and support the view that emergence patterns indicate oviposition preference.

Interestingly, *A. prosopis* oviposited on dung that contained no traces of mesquite pods or seed. Female insects are known to demonstrate time-dependant changes in host-acceptance threshold (Singer, 1982; Singer *et al.*, 1992; Browne & Withers, 2002), however, the female bruchids used in these experiments had not been oviposition site deprived and according to the findings of Hoffmann *et al.* (1993) were not at their peak oviposition age. Although it has been shown that *A. prosopis* females do exhibit preferences for host choice, oviposition on a medium that is not guaranteed to contain mesquite seed is not adaptive and implies *A. prosopis* is not very discerning in where it oviposits. Despite the high mobility of *A. prosopis* first instar larvae, it is unlikely that the larvae, having entered, searched and failed to locate a seed within a dung pellet would be able to continue the search in other pellets because this is probably an energetically expensive task and the dung is unlikely to compensate larva for this energy expenditure. In addition, pellets in the field may be scattered, further inhibiting the search ability of the larva. It is therefore likely that oviposition on a pellet containing no mesquite seed will result in the failure of the entire clutch oviposited on that pellet. This opportunistic oviposition strategy is far less conservative than that of *Neltumius arizonensis* where females carefully inspect seed pods for seed quality and eggs of conspecifics prior to oviposition, (Strathie, 1995). The oviposition on "non-mesquite" dung also suggests that there are other volatiles associated with sheep dung that attract and stimulate the females to oviposit.

The choice of dung as a host for larval development appears to be inherent to *A. prosopis* but what are the ecological consequences of these host choices and if host fidelity is occurring are we witnessing the formation of new host races? Host races are partially reproductively isolated, conspecific populations specialising on alternate hosts (Diehl & Bush, 1984). If adults show an increased preference for one or other host, whether an inherited or conditioned behaviour (Barron, 2001), it may be the first stage of a host shift. This study showed *A. prosopis* to exhibit a strong preference for oviposition on dung when adults themselves developed within dung-borne seed, but is this sufficient to induce host fidelity? Any temporal or spatial variation in the usage of hosts may lead to reproductive isolation and reinforcement of a host race. During summer *A. prosopis* females exploit both pods and dung to a similar extent but during winter the beetles are almost totally confined to utilising dung-borne seeds. Despite this, a number of factors are at work preventing the potential establishment of host races at the sites where livestock are active: i) the life history of *A. prosopis*, being multivoltine, excludes any chance of temporal reproductive isolation; ii) the effect of changing environmental conditions prevents maintenance of spatial separation; and iii) the temporal restriction of the bruchids' habitat selection to hosts associated with mesquite trees at a time when both hosts are available (mid-late summer).

The fact that *A. prosopis* originating from a population that is not exposed to sheep dung readily oviposit on dung, strengthens the view that these insects show an innate preference for dung over pods and that conditioning, such as pre-imaginal (Tully *et al.*, 1994; Ray, 1999; Barron, 2001) or imaginal conditioning (Vet & van Opzeeland, 1984; Jaenike, 1988; Jaenike & Papaj, 1992; Vet *et al.*, 1995; Barron & Corbet, 2000) is likely to be a secondary factor. The use of dung as an alternative source of mesquite seed, in the absence of pods, is possibly an evolved survival strategy allowing fuller exploitation of the resource while reducing competition and facilitating escape from parasitism. One generation reared on dung is sufficient to get a significant host shift onto dung in the next, however, bruchids reared on pods did not exhibit any preference to lay on pods. This indicates strong behavioural plasticity (Jaenike & Papaj, 1992) allowing *A. prosopis* individuals to readily utilise that resource which will maximise fitness. It is possible that *A. prosopis* evolved to utilise seed in the dung of extinct megaherbivores that consumed mesquite pods and that the host choice of mesquite pods may actually be a survival strategy in the absence of dung.

I feel that this study clearly demonstrated that *A. prosopis* readily oviposits on dung and, having developed in dung-borne seed, these females actually show a strong host preference for dung over pods. Despite females ovipositing on non-mesquite dung the data shows definite and replicable differences in host preference when presented with mesquite dung and mesquite pods in both the choice test arenas and the "Y"-tube tests.

Although oviposition on dung is likely an innate behaviour and selection for females that utilise dung in areas where sheep remove pods may have occurred, imaginal conditioning plays a strong if not dictating role in host choice.

The factors driving *A. prosopis* to utilise dung over pods in the field are not certain but it is likely a combination of i) a lack of pods and the resulting increase in attractiveness of dung through resource concentration, ii) the higher temperatures of dung during winter to enable an increase in larval developmental rates, iii) a means of over-wintering as larvae thus enhancing post-winter fitness, and iv) a partial escape from parasitism in time and space.

What is the impact of *A. prosopis* utilisation of dung-borne seed on mesquite infestations? The combined effects of pod removal by livestock and the destruction of pod- and dung-borne seed by *A. prosopis* on soil seed banks and recruitment rates are investigated in the following chapters.

Chapter 4

The impacts of seed usage by *A. prosopis* on soil seed banks.

Abstract

There was a minimal mesquite seed bank at sites in the semi-arid region where livestock were excluded which allowed for multiple generations of *A. prosopis* able to destroy large numbers of seeds and reduce the annual input of seed into the seed bank. In contrast, in the mesic region where bruchid activity was low the seed bank was markedly higher. In all instances, the presence of livestock prevented pre-dispersal destruction of mesquite seeds by the bruchids and consequently high seed banks were evident at these sites, despite *A. prosopis*' ability to locate and utilise dung-borne seed. Higher numbers of seed escaped bruchid damage, increasing the size of the soil seed bank which was concentrated in the vicinity of mature mesquite trees. Pod-borne seeds showed a higher degree of dormancy (95%) but passage through the gut of a sheep broke dormancy of 55% of this seed. Furthermore, larval penetration of the seed coat broke dormancy, aiding germination, but in most cases this window period was narrow and an extended period of larval development resulted in reduced seedling vigour and led directly or indirectly to seed death.

Introduction

Chapter 1 investigated the varying degrees to which the two bruchid species have reduced the seed output in mesquite stands on an annual basis when livestock were excluded. However, there are few situations where mesquite occurs without livestock and the next two chapters (2 & 3) then dealt with the ability of *A. prosopis* to locate and utilise seed contained in dung dispersed in the field and the potential effect this behaviour has on reducing mesquite seed banks. In this chapter the dynamics of soil seed banks are investigated.

Mesquite seeds are capable of remaining dormant for long periods of time as demonstrated in a long-term study where mesquite seeds were shown to survive 20 years burial in the soil in Arizona (Martin, 1970). In addition, seeds of herbarium specimens germinated after 44 years of storage (Martin, 1948). The ability of mesquite seed to remain dormant for long periods has substantial implications for land management and clearing operations. Any seed that is not destroyed by granivores, bruchids or microbial action and does not germinate has the potential to contribute to reinvasion of an area for long periods after seed input has ceased. In addition, the ability of hard-seed invasive weeds to remain dormant potentially allows for the establishment of large seed banks with time, thus off-setting the effectiveness of seed-reducing agents (Crawley, 1983, 1989; Myers & Bazely, 2003; Vivian-Smith *et al.*, 2006). In South Africa, in areas where livestock are excluded, the bruchids have continuous access to seeds in pods and are able to destroy almost all of the mesquite seeds that remain below the mature trees

(Zimmermann, 1991; and the current study), potentially resulting in a marked reduction in annual input of seed to the seed bank. However, seed damage in this situation probably has little effect on the population dynamics of the weed because seeds that remain below adult trees are expected to have little chance of ever becoming established plants for several reasons: i) temperatures in the shade are largely unsuitable for germination (Scifres & Brock, 1972), particularly in the winter rainfall region; ii) the photosynthetically active radiation levels in the shade of the parent trees are too low for seedling survival (Scifres *et al.*, 1973); iii) there is a higher incidence of natural enemies associated with these trees (Janzen, 1970; Harper, 1977); and iv) competitive exclusion is frequently highest below the canopy of parent trees (Harper, 1977). Therefore, the effects of seed removal from the vicinity of mesquite trees by dispersal agents may lead to seed deposition in microsites suitable for germination and seedling survival and enhance escape from seed-feeding species (Janzen, 1970, 1972; Connell, 1971; O'Dowd & Hay, 1980; Wright, 1983; Traveset, 1990).

Harding (1991) found that 85% of mesquite seeds ingested by sheep are destroyed during passage through the gut. Despite this, the 15% that remain intact are sufficient to allow high numbers of seeds to be dispersed in dung. Although many of these seeds are deposited in conditions that are ideal for germination and establishment of seedlings (Brown and Archer, 1989), their contribution to the next generation is not guaranteed because they remain a resource for *Algarobius prosopis* to utilise for larval development.

Seeds may form part of either a transient seed bank, not lasting more than one year, or be incorporated into a persistent seed bank exhibiting varying degrees of dormancy (Thompson & Grime, 1979). The seed that does escape bruchids and granivores but fails to germinate with the onset of rains will probably be incorporated into this persistent seed bank. The question arises, are the levels of damage caused by *A. prosopis* on seeds dispersed in dung sufficient to offset the advantages that the seeds have gained by being dispersed in dung as opposed to lying in the shade of the parent trees where they are destined to fail anyway? In addition, does seed dispersal enable the establishment of large seed banks away from parent trees?

Seeds that constitute the soil seed bank may be viable but may not necessarily be germinable due to the effects of dormancy from: physical barriers preventing water uptake (Baskin & Baskin, 1998); negative responses to light (Pons, 1992); temperature (Probert, 1992); and the chemical environment (Karssen & Hilhorst, 1992). However, germination in mesquite seed can be induced simply by compromising the intact seed coat (Shiferaw *et al.*, 2004). The effect of a bruchid larva on a seed may therefore not always be negative. A number of seed-feeding insects are known to promote recruitment by compromising the seed coat and interfering with the mechanism by which the seeds remain dormant (e.g. Karban & Lowenberg, 1992; Ollerton & Lack, 1996; Takakura, 2002; Vivian-Smith *et al.*, 2006). Some species of "hard-seeded" plants may even require bruchid entry of the seed to break dormancy (Takakura, 2002). Therefore the entry of a bruchid larva into a mesquite seed may result in a number of outcomes: destruction of the seed by the developing larva; decay of the seed through microbial activity; swelling of the seed and failed germination if environmental conditions are unsuitable; and lastly successful germination of the seed. All these factors will result in removal of the seed from the seed bank.

In this chapter the following hypotheses were investigated:

1. In spite of damage caused by the bruchids, their introduction has been too recent to have had an effect on the persistent, long lived banks of seeds which have accumulated over many years and are unavailable to the introduced bruchids.
2. Because of variability in the extent of seed damage that *A. prosopis* achieves throughout the distribution of mesquite in South Africa we would expect that seed banks would vary accordingly such that seed banks in the South-western and Central Regions will be smaller than in the North-east region;
3. Seed banks will be larger in areas where livestock have access than where they are excluded because the bruchids are more efficient at locating and destroying pod-borne seeds than dung-borne seeds;
4. Seed numbers will always be greater beneath than away from mature trees because in areas were livestock are excluded the seeds have no dispersal agency and where livestock are present animals tend to shelter and deposit most of their dung in the shade of the trees;
5. Seed in areas with live stock will be more germinable compared to seed in areas without livestock because passage through the gut of a sheep will break dormancy;

6. Although extensive bruchid feeding diminishes the overall viability of seeds, initial colonisation of seed will not be immediately detrimental and the germinability of recently colonised seeds will be enhanced because the perforation of the seed coat will break dormancy.

Methods

Soil seed banks

Soil cores were taken at a number of sites in order to quantify the size of soil seed banks in different regions and in the presence and absence of livestock. Two permanent 50 m transects were set up through mesquite infestations in each of three regions: "South west" – semi-arid Upper Succulent Karoo (winter rainfall); "Central" semi-arid Upper Nama Karoo (summer rainfall); and "North-east" - mesic Eastern Mixed Nama Karoo (summer rainfall) (vegetation types by Low & Rebelo, 1996). Sheep were present in some of the sites but absent from others. Soil cores were taken at intervals using a handheld circular corer of 8.3cm diameter to a depth of 10cm (a surface area of 54.1cm^2) on a number of sampling occasions (Table 4.1). Cores were taken every 2m along the 50m transects through the infestation thus incorporating areas in the shade of mesquite trees, open ground and in the shelter of indigenous shrubs. If the transects did not incorporate enough samples of the shade or open category additional cores were taken randomly alongside the transects so as to increase the sample numbers. The cores were taken from different points along transects on the different survey dates so as to never sample the same point on the transect more than once. The counts from each of the sites were

Table 4.1: Date of survey trips on which soil cores were taken in the three regions SW – south west, Cent – Central, and NE – north east under differing land-use patterns, L – livestock and NL – no livestock. The number of soil cores taken on each sampling occasion shown in parentheses.

	2002		2003		2004		2005	
	summer	winter	summer	winter	summer	winter	summer	winter
SW – L			31 Jan (40)		27 Jan (25)	16 May (26), 6 Sept (27)	21 Mar (26)	5 July (26)
SW – NL	12 Dec (27)			3 Apr (25)				
Cent - L			11 Feb (78)		25 Feb (52)		4 Feb (25)	
Cent - NL			11 Feb (78)		25 Feb (52)		4 Feb (25)	
NE – L			14 Feb (85)				2 Feb (71)	
NE-NL			14 Feb (71)		27 Feb (123)			

averaged so as to accommodate seasonal fluctuations in the seed bank size resulting from seed loss or gain.

Each soil core was placed in a labelled sealable packet for transport back to the laboratory where the packets were opened immediately and allowed to dry. Occasionally mesquite seedlings would germinate within the packets if the samples were exceptionally moist on collection. These seedlings were noted and considered to have arisen from "free" seed i.e. seed not within either dung or pods. Once dry, the samples were sieved through a 2-mm sieve so as to extract any "free" seed. Some samples required crushing if soils had high clay content. If any pods, endocarps or pellets were present these were counted and removed prior to sieving and the numbers of viable seed within them was recorded and included in the total seed count. This seed was kept for later checks of viability and germinability. Seeds found in pods segments and endocarps, were included in the calculation of seeds per m^2. On all sampling occasions the bulk of the pod segments were fragments from recently fallen pods and many of the seeds contained within would probably have been destroyed by bruchids as the season progressed or be eaten by livestock. Because of the inclusion of the pod-borne seeds the calculations of the soil seed bank size would probably be overestimates. The exclusion of pod-borne seeds when calculating seed bank size would be justifiable in regions where bruchid activity was high, but not in the North-east where bruchid activity was low; however, there were too many uncertainties to warrant their exclusion.

Most soils at the sites were firm and compacted and seed was contained within a few centimetres of the surface so that cores to a depth of 10cm would ensure most of the seed within that core area was collected.

Viability and germinability of seed

Viability of seed in the soil seed bank.

Shiferaw *et al.* (2004) found mechanical scarification of viable *P. juliflora* seed resulted in 100% germination. Seeds collected in soil cores throughout the mesquite distribution in South Africa were tested for viability. To do this, the seeds were "clipped" i.e. an incision was made with a scalpel into the micropyle to remove a small piece of the seed coat and expose the underlying cotyledon. The seeds were then placed 5mm deep in fine river sand in polystyrene seedling trays housed in a controlled temperature room at 27°C ± 2°C, the optimum germination temperature for *Prosopis* species in South Africa, (Scifres & Brock, 1969; 1972), and watered daily. Seeds that imbibed moisture caused a bulge on the surface of the river-sand. When the bulge was prominent, the underlying seed was excavated and inspected. Those that were viable had produced a radicle. Seeds that had imbibed moisture but had not produced a radical were replanted and rechecked daily for the remainder of the experiment. Sub-samples of approximately 50 seeds from each site, representing free seed and pod- and dung-borne seed, were tested for viability in this manner.

Currently no accurate method exists for establishing the age of seeds but mesquite seeds turn from a light yellow-brown colour to a dark brown with age and exposure to environmental elements (*pers. obs.*). A note of the seed colour was made to determine whether the darker (= older) seeds germinated at different rates to light (= younger) seeds. The experiments were run for three days because earlier trials had shown that viable seeds germinated within this time.

<u>Germinability of soil-, pod- and dung-borne seed.</u>

Seeds collected in soil cores were in three states: contained in dung (dung-borne), contained in pods (pod-borne) and "free" seed (soil-borne). To ascertain the germinability of the seeds in the three states, trials were run using clipped and unclipped seeds. The use of clipped seeds was to confirm that the seeds used were viable and that germination failure was as a result of dormancy and not of the seeds being unviable. The same procedure as that outlined above was used except that the experiments were run for 14 days (after Baskin & Baskin, 1998) and not for 3 days. Seeds were assumed to have germinated if there was growth of the radicle, once this was noted the seeds were removed from the trial. At the end of 14 days unclipped seeds that failed to germinate were considered dormant.

Effect of larval penetration of the seed coat on germinability.

In these experiments I set out to determine if seeds from dung into which a bruchid larva had penetrated, germinated at a different rate to clipped and unclipped dung-borne seeds. Mated *A. prosopis* females and cleaned seeds from dung (placed into pods with split endocarps to encourage oviposition) were placed into Petri dishes. After two days the adult bruchids were removed and the seeds, housed at a constant temperature of 27°C ± 2°C, checked for larvae every day thereafter. The first larvae were noted eight days after introduction of the females to the seeds. Seeds containing a larva were removed and the larva was allowed to develop within the seed for differing lengths of time: 1 day or 7, 14 or 21 days after which time the seeds were placed in the freezer to terminate further larval development. Seeds in which larvae were allowed to develop for only one day (n=57) were then tested for germinability using the same procedure as outlined above. After a radicle had developed the seeds were removed. The experiment continued for 14 days.

Seeds in which larvae were allowed either 7, 14 or 21 days to develop were clipped and tested for viability, however, the germinated seeds were not removed from the experiment but allowed to develop for the full 14 days to determine whether they were able to develop into seedlings with fully expanded cotyledons. The numbers of seed with larvae of 7-, 14- or 21-days old were 11, 21 and 17 respectively.

Results

Soil seed banks

In both the South-west and Central regions soil seed banks at sites where livestock were present were substantially higher than the seed bank at sites where livestock were absent (30.1 times and 114.3 times higher, respectively) shade and open combined (Fig. 4.1). In contrast, in the North-east region the soil seed bank was 10.8 times larger in areas where livestock were excluded than where livestock were present. At all the sites the highest density of seeds occurred below mature mesquite trees except for the "no-livestock" site in the Central region where the highest densities of seed were located away from the mature trees, below indigenous shrubs, often in middens at the entrances to rodent burrows. The soil cores from shrubs and open areas were considered together as, although significantly higher numbers were found below shrubs than in both areas below trees (shaded) and in the open (Kruskal-Wallis, $H = 5.14$, $p = 0.02$ and $H = 12.31$, $p < 0.001$ respectively), seeds in open and below shrub areas constituted dispersal away from the parent plants. A Kruskal-Wallis test showed that of the open areas only the livestock site in the South-west region contained significantly higher numbers of seeds per m^2 ($p < 0.01$) and that all the others were not significantly different. Similar numbers of seed per m^2 were found

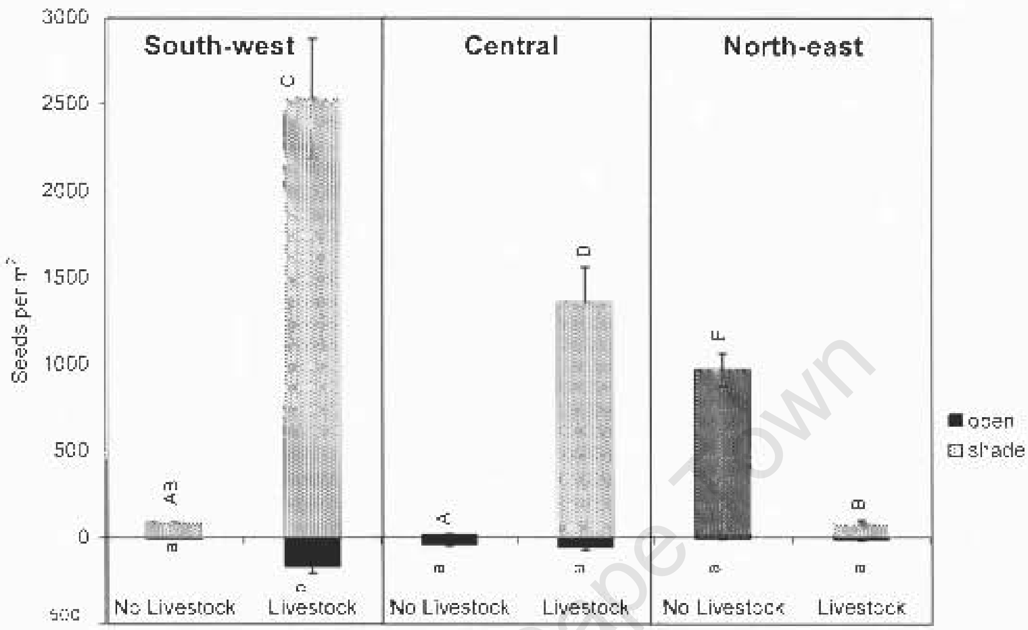


Fig. 4.1: Mesquite seeds per m^2 derived from the soil cores taken intermittently in the three regions (South-east, Central and North-east), between 2002 and 2005, from sites where livestock were present and absent. Sites with differing letters (upper case for shade, lower case for open) are significantly different (Kruskal-Wallis, $p<0.05$). Whiskers indicate the S.E.

below trees in the South-west and Central regions where livestock were absent and in the North-east region where livestock were present (Kruskal-Wallis, $p>0.05$). Where livestock were present, soil cores in the open areas showed there to be 170.1, 57.2 and 15.8 seeds per m^2 contributing to 6.3, 4.1 and 17.4% of the total soil seed bank in the South-west, Central and North-east regions respectively. In contrast, where livestock were absent, the numbers of seeds in the open amounted to 7.4, 45.0 and 8.9 seeds per m^2 contributing to 8.3, 78.4 and 0.9% of the soil seed bank in the South-west, Central and North-east regions respectively. The percentages of seed existing in dung as opposed to pods and "free" standing seed in shade and in the open are presented in Table 4.2 below.

Table 4.2: The percentages of seed in various states i.e. free, dung-borne and pod-borne seed that constitute the seed bank in shade and open areas in the South-west, Central and North-east regions. Seeds found below indigenous shrubs included with open samples as they had been dispersed away from mesquite trees.

	Livestock						No livestock					
	Shade			Open			Shade			Open		
	free	dung	pods	free	dung	pods	free	dung	pods	free	dung	pods
South-west	81.0	14.4	4.6	62.5	31.9	5.6	0	0	100	100	0	0
Central	83.5	13.0	3.5	57.1	38.1	4.8	33.3	0	66.7	75	0	25
North-east	23.4	0	76.6	16.7	16.7	66.7	68.5	0.2	31.3	75	0	25

Because of the inclusion of the pod-borne seeds the calculations of the soil seed bank size would be overestimates as for example, if seeds in pods were excluded from combined shade and open areas, the soil seed bank would decrease by as much as 80% at the "no-livestock" site in the South-west region, a decrease from 30 to 5 seeds per m^2.

To demonstrate the fluidity of the soil seed banks Fig. 4.2 demonstrates how sampling on any one date may yield an estimate of the soil seed bank that may potentially vary by several factors on different sampling occasions.

The numbers of seeds found in the open in January 2004 were almost 9X greater than the numbers that were found in January 2003. A difference of a factor of 33 existed between the lowest and highest levels of soil seed bank size in the open. Contrasting to this, the largest difference between estimations of seed bank size in shaded areas was only a factor of 4. The higher turnover of seeds in the open areas was probably a consequence of longer exposure to bruchids (because the pellets were not buried below the surface by sheep activity) and higher germinability induced by warmer temperatures resulting from increased solar insolation.

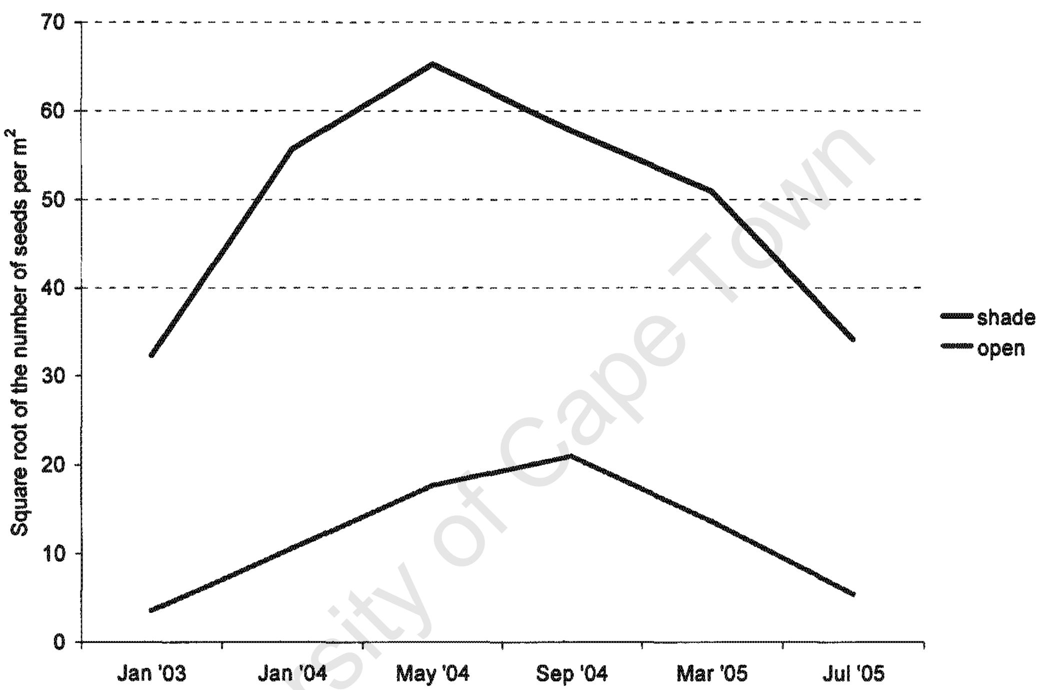


Fig. 4.2: The number of seeds per m^2 at Calvinia, in the South-west region, calculated from soil cores taken intermittently between January 2003 and July 2005.

Viability and Germinability of seed

Viability of seed in the soil seed bank.

Mesquite seeds are reputed to be long-lived in the soil within their natural range (Martin, 1970) but the viability of soil-borne seed in South Africa has not been investigated. In most regions, because seed banks in the presence of sheep were far higher than in areas where sheep were absent and almost all infestations have sheep present, most of the soil-borne seed within the system had passed through the gut of a sheep before entering the seed bank. Viability of seeds from soil was >98.7% across all of the sites so there was no need to consider viability when measuring seed banks by counting seeds.

Germinability of soil-, pod- and dung-borne seed

The age of seed obtained through soil coring was not known and there are no techniques for determination of seed age. There was no significant difference in germination rates between old (dark) seed and young (light) seed (Mann-Whitney, $z=-0.754$, $p=0.451$). The sensitivity of soil-borne seeds to germination is likely to vary to those seeds that have remained within the protected environment of a pod.

Of the unclipped seeds from soil 40.6% germinated within 4 days of being planted (Fig. 4.3a). Another 5% of the seeds germinated on day eight of the trial but the remaining 54.4% of the seeds from soil were dormant and showed no signs of germination. Significantly fewer unclipped seeds germinated than did clipped seeds where 100% germinated by day three ($\chi^2 = 383.5$, $p < 0.001$).

The average percentage of unclipped seeds from dung that failed to germinate was 44.4 (Fig. 4.3b). This value is not significantly different to that obtained for germinability of unclipped seeds from soil ($\chi^2 = 2.86$, $p = 0.091$). This supports the idea that the majority of soil-borne seed within infestations originates from sheep dung. Passage through the gut of sheep enhances germination for 55.6% of the surviving seeds, a far higher value than has been demonstrated in other studies (13% for the study by Cox *et al.*, 1993). All borne seed within the system had passed through the gut of a sheep before entering the seed bank. Viability of seeds from soil was >98.7% across all of the sites so there was no need to consider viability when measuring seed banks by counting seeds.

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Germination of seeds removed from pods and clipped was markedly slower than the germination rates of clipped seed from dung and soil, although after 13 days 100% of seed had germinated (Fig 4.3c). More than 60% of the clipped seeds from pods germinated within 48hr but the remaining seeds required an additional 11 days to germinate. Significantly fewer of the unclipped seeds from pods germinated than did clipped seeds from pods or unclipped seeds from dung and soil ($\chi^2 = 181.0$, $p < 0.001$, $\chi^2 = 36.7$, $p < 0.001$ and $\chi^2 = 34.6$, $p < 0.001$ respectively) where only 5 out of a 100 unclipped seeds germinated within the 14 day trial.

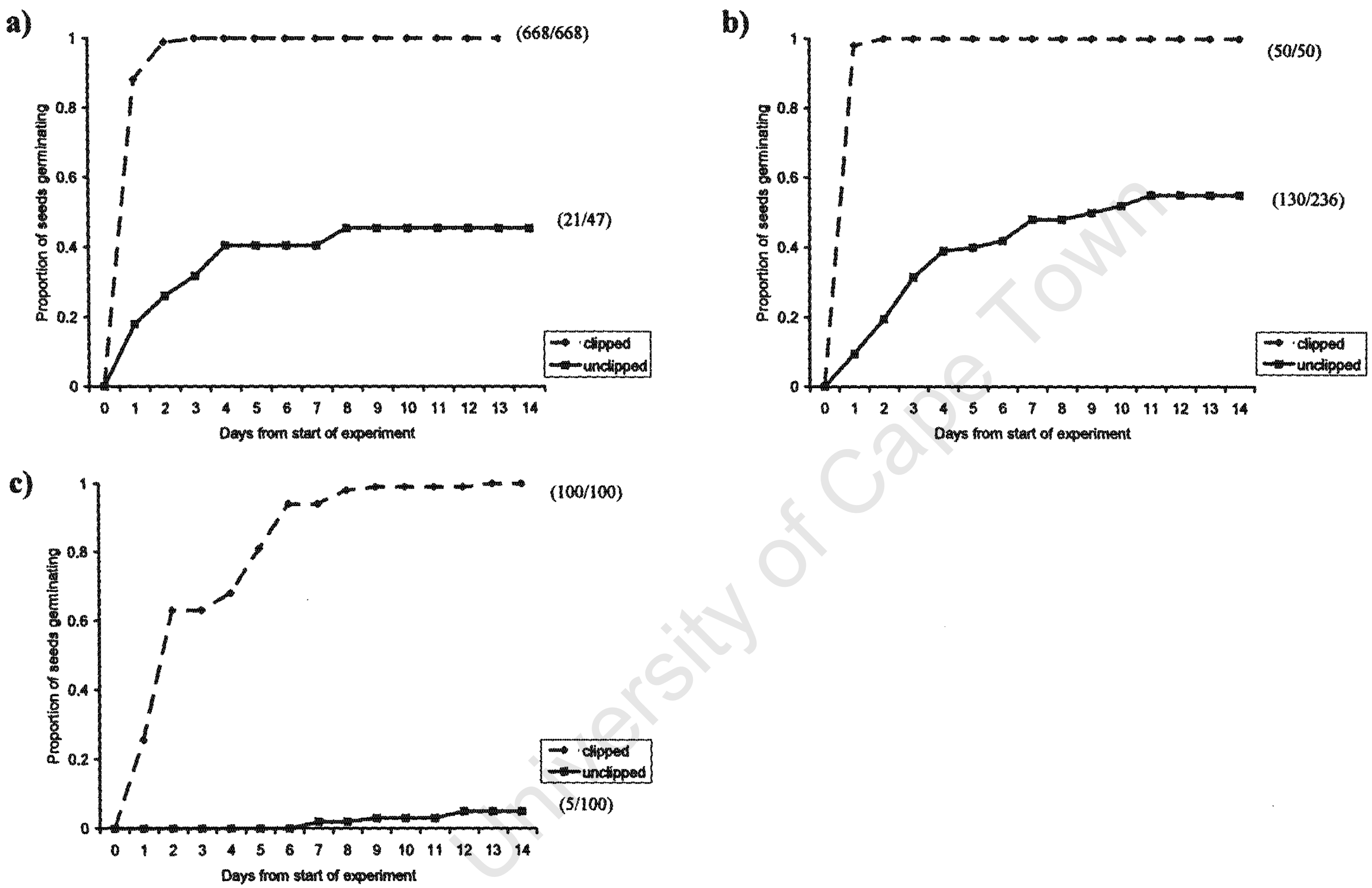


Fig. 4.3: Two week germination trial investigating the germination (clipped) and dormancy (unclipped) rates of a) seed from soil, b) seed from dung and c) seed from pods. Values in parentheses are the numbers that had germinated at the end off 14 days (first values) out of the total number at the start (second value).

Effect of larval penetration of the seed coat on germinability

Larval penetration of the seed coat had a dramatic effect on germination rates (Fig. 4.4). All of the seed from dung that contained 1-day old larva germinated within 72 hours, a similar germination rate to that of clipped seeds from dung and significantly higher than unclipped seed from dung (Fig. 4.3b) ($\chi^2 = 64.86$, $p < 0.001$).

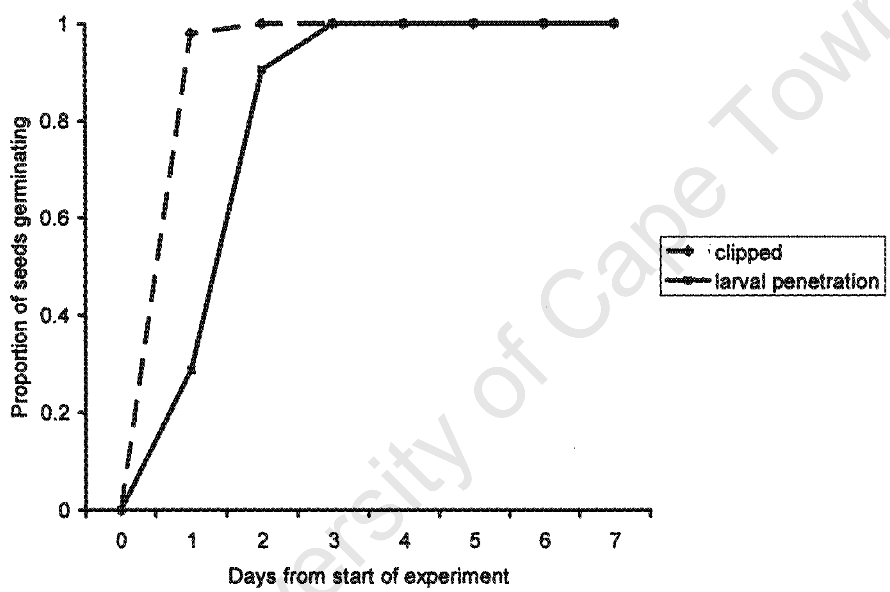


Fig 4.4: The effect of larval penetration of the seed coat on germination rates of dung-borne seed in comparison to the germination rates of clipped dung-borne seed.

All of the seeds in which larvae had been allowed to develop for seven or 14 days germinated within three days but none of the seeds containing 21-day old larvae germinated. Survival was significantly higher among seedlings that developed from seeds that contained seven-day old larvae (90.1%) than among seedlings that contained 14-day old larva (47.6%) ($\chi^2 = 5.77$, $p = 0.016$). Obvious damage from larval feeding was noticeable in the cotyledons, particularly in the seeds containing 14-day old larva.

Discussion

As predicted, at sites where livestock had access to the pods in the South-west and Central regions the soil seed banks were substantially higher than when livestock were excluded. Thus despite *A. prosopis'* ability to utilise dung-borne seed, a substantial amount of seed escaped both bruchids and granivores and a large seed bank had accumulated in the areas with livestock. In contrast there were small seed banks in areas where livestock were excluded because the bruchids had continued access to pods throughout the year which enabled the population to develop through multiple generations and thereby destroy the vast majority of seed before they became incorporated in the seed banks.

In all of the regions, most of the seeds were concentrated below mesquite trees even though seed predation would be expected to be highest in these areas (Janzen, 1970, 1972; Connell, 1971; O'Dowd & Hay, 1980; Wright, 1983; Traveset, 1990). Where sheep were present their activity around the trees had several consequences for seed distribution and survivorship: i) dung was concentrated below trees to where sheep congregated to feed on pods and use the shade during the heat of the day; ii) trampling by the sheep disturbed the soil and buried dung containing seed below the soil surface where it was inaccessible to the bruchids; and iii) trampling of dung by the sheep broke up the pellet releasing the seeds which were then less suitable for the ovipositing *A. prosopis* females. In the South-west region, where sheep were absent the indehiscent fruits were not dispersed and seeds were largely confined to the immediate vicinity of the parent tree. However, at sites in the Central region where livestock were excluded rodent activity resulted in the concentration of seeds below indigenous shrubs where rodent middens were located. Presumably the rodents foraged for pods which were taken from below the trees and carried to the more concealed environment of shrubs to be eaten or hoarded. In addition, seeds from dung were observed being taken into the nests of harvester ants (*Mesor* sp.) and harvester termites (*Microhodotermes viator*) in the Central region (*pers. obs.*). The movement of seeds by dispersal agents other than livestock may have implications for mesquite demographics and the perpetuation of this invasive weed in some areas but this was not considered further in this study.

In determining the size of the soil seed banks, seeds contained in pod segments and endocarps were included in the counts. Although seeds in pods have the ability to remain viable for extended periods of time (Baes *et al.*, 2001) it is unlikely that these seeds would escape bruchid predation and their presence was likely to be transient and consequent on sampling dates. Inclusion of pod-borne seed in seed bank counts overestimated the extent of the persistent seed bank size but this conservative bias was considered a more valid option than exclusion of the seeds which would have introduced an underestimate bias.

The soil seed banks in the North-east region were lower in areas where livestock were present than in areas where livestock were absent, a trend that differed from the South-west and Central regions. Despite the livestock in this region being predominantly cattle, through which most seed consumed passes intact and which displays only 3% enhanced germinability (Cox *et al.*, 1993), the seed banks were unexpectedly low at the livestock site. The limitations on seed accumulation in these livestock areas could have resulted from: i) increased annual rainfall inducing germination over extended periods; ii) increased seed decay in animal droppings due to higher rainfall moisture levels; and iii) dung removal by dung beetles (Scarabidae) and seed destruction by termites (Termitidae).

A number of processes act on the seed in the soil seed bank giving rise to a dynamic system with fluctuations in the size of the soil seed bank on different sampling dates. Soil samples taken intermittently at Calvinia in the South-west region between January 2003 and July 2005 showed the size of the soil seed bank varying by a factor of 33 on different sampling occasions from open areas and a factor of 4 from shaded areas (Fig. 4.2). This showed that careful consideration must be given to the timing of soil surveys and how the results are interpreted when gauging the extent of mesquite seed banks. In the mesquite-livestock-bruchid system in South Africa the timing of livestock access to pods, timing of rainfall events and seasonal variability in bruchid activity all played a part in affecting the size of the soil seed bank. In addition, the broad distribution of mesquite, encompassing various ecological and climatic conditions, affected the dynamics of the seed banks between the regions. The proportion of seed collected that was transient as opposed to persistent depended on the timing of the sampling relative to seasonal rainfall in the region. Only frequent and regular (Zaman & Khan, 1992, cited in Baskin & Baskin, 1998) or seasonal sampling (Gilfedder & Kirkpatrick, 1993) would allow for accurate measures of the persistent seed bank and because of irregular sampling in this study, the sizes of the temporary and persistent seed banks could not be established. The

January samples taken in the winter rainfall region may most closely reflect the true size of the persistent seed bank in these regions but even these numbers fluctuated annually. Germination of seed was responsible for removal of a high proportion of the seed in the transient but it was likely to remove only a small proportion of the seed from the persistent seed bank annually. The effects of granivory and bruchid damage would be more negatively impacting on seed in the persistent seed bank. Soil cores taken prior to pod fall may give the most reliable measures of the size of the persistent seed bank but this is not guaranteed, particularly in arid and semi-arid areas where rainfall events may be sporadic and not suitable for germination. During extended periods of zero germination the seed bank will accumulate until such time as a suitable rainfall event does take place. Thus there is a need for regular and frequent sampling to establish more sound estimates of seed bank dynamics and the size of the persistent seed bank.

The long-lived nature of mesquite seeds (Martin, 1970) potentially enables the formation of persistent seed banks and it is argued that the capacity of invasive species to do this decreases the effectivity of seed feeding insects introduced for biological control (Crawley, 1983, 1989; Vivian-Smith *et al.*, 2006). The dormancy of mesquite seeds, in extreme cases, may diminish the effects of the persistent seed bank in short term population dynamics thereby decreasing the invasive potential of mesquite. The results showed that most seed in the soil seed bank was viable. Unfortunately no techniques are currently known to enable suitable estimates of seed age and it was therefore not possible to establish if the seed tested had originated pre-or post-introduction of the bruchids. The indications were that a mix of seed of varying age was present yet all seeds responded positively to clipping and readily germinated. However, this does not tell us anything about the potential influence of the soil seed bank on recruitment events. Germinability trials showed that germination of seeds was enhanced by approximately 50% once they had travelled through the gut of a sheep. Previous studies showed that passage through the gut of livestock only enhanced germination marginally, by 13% in the case of sheep (Cox *et al.*, 1993) and by 8% in the case of goats (Baes *et al.*, 2002). There appears to be a degree of endogenous dormancy (Nikolaeva, 1977, 2001), likely to be physiological dormancy, of seeds contained within pods as seen by the gradual germination of these seeds over a 14-day period compared to the rapid germination of most of the dung-borne seeds within 72 hours. Passage through the gut of sheep altered this state of physiological dormancy and germination of mesquite species in South Africa is governed primarily by exogenous dormancy (i.e. related to external properties of the seed coat). Mesquite seeds also exhibit polymorphism (Baskin & Baskin, 1998), in terms of germination rates. Because a range of seeds from different sources were monitored it cannot be said if this polymorphism is within or between trees, or within and between populations or is due to hybrid formation (Baskin & Baskin, 1998).

Despite enhanced germinability of dung-borne seed, a population of dormant seed would remain after germination events and those seeds that escape bruchids and granivores would be incorporated into the persistent seed bank. Unclipped seeds from soil and dung showed similar rates, and success, of germination, a result that was expected because in most areas the soil-borne seeds had at one time been consumed by sheep and passed intact. It is possible that running the experiment for 14 days in the laboratory did not truly show the proportion of seed that would germinate in the field where abiotic factors such as continued wetting and drying or temperature fluctuations (Baskin & Baskin, 1998) may affect germination. However, the trials did show that a proportion of the seeds are dormant. The resultant damage to the seed coat of dormant dung-borne seed by *A. prosopis* larvae and its potential to enhance seed germinability may be critical in reducing the proportion of seed that would enter the seed bank. Larval entry into a seed increased the germination success of dung-borne seed dramatically over seeds from dung that had not been clipped and the germination rate was similar to that of clipped dung-borne seeds. Thus larval entry into a seed may influence mesquite population dynamics as, for example, a rapid germination response to a rainfall event may not coincide with an ideal time for seedling establishment and the resultant seedlings may succumb to premature death. Alternatively, perforation of the seed coat may enable microbial entry and lead to seed decay. Therefore, the proportion of dung-borne seeds that bruchid larvae are able to penetrate prior to the onset of rains or to the breakdown of dung may have a telling effect on the effectiveness of *A. prosopis* as a biological control agent. The situation will be compounded by the timing of rainfall events. Late rains after larval development has progressed will reduce seed survivorship while early rains when larval development is recent will enhance seed germination and seedling establishment and *A. prosopis* would serve as a recruitment enhancer.

In conclusion, throughout the semi-arid areas in South Africa the introduced bruchids were able to dramatically reduce the annual input of seed into the seed bank where livestock were excluded and multiple bruchid generations occurred in a year. Where bruchid activity was low as a result of unsuitable environmental conditions the seed banks were markedly higher. The exclusion of livestock from mesquite infestations is the exception and consequently the vast majority of the annual pod production is consumed and the surviving 15% of seed is confined in dung. Despite *A. prosopis*' ability to utilise this dung-borne seed, high proportions of seeds escaped bruchid damage and became incorporated in the soil seed bank particularly in the immediate vicinity of mature mesquite trees.

A large proportion of the seeds in the seed banks exhibited some degree of dormancy which was broken when the seed coat had been compromised by a bruchid larva.

The next chapter investigates the next stage in the dynamics of mesquite, namely annual recruitment of seedlings and their survivorship. The survival of young plants, more than a year old, was also considered and size classes, or cohorts, were also determined in order to show patterns of recruitment in the plant populations.

Chapter 5

The demographics and dynamics of mesquite populations

Abstract

Despite below average rainfall over the course of this study, germination events occurred at most sites in all years. Significantly less seedlings occurred where livestock were absent as the seed banks associated with these areas were small. At most sites seedlings were concentrated in the immediate vicinity of mature mesquite trees but the presence of livestock increased the proportion of seedlings occurring in open areas. No seedlings survived at the xeric sites during this study period but some recruitment of new plants was recorded at the mesic sites despite below average rainfall. Most mesquite plants that survived for more than one year established into the population. Stem diameters of plants in arid areas revealed definite size classes which represented cohorts, implying recruitment occurred intermittently in years of favourable rainfall and that little above ground growth occurred in years of poor rainfall. Recruitment at sites in the mesic region appears to have been regular and regardless of livestock presence or absence. Mesquite populations seem to be seed limited at sites in the arid and semi arid areas where livestock are absent, otherwise the populations are site limited.

Introduction

Recruitment of new plants into populations is dependent on seedlings developing in sites that are suitable for their survival and maturation, termed safe sites. Recruitment in some plant populations is constrained by the number of sites that are suitable for seedling development (site limited) while others are constrained by the number of seeds available to fill the suitable sites (seed limited) (Harper, 1977). The distinction between the two limitations is not species specific but is determined by a complex of interacting biotic and abiotic factors.

Insects that reduce the seed output have been shown to play an important role in the population dynamics of perennial plants (e.g. Louda, 1982, 1983; Kelly & Dyer, 2002). Biological control of introduced weeds provides some extreme examples where insects that reduce seed output may have substantial effects on plant population dynamics (Rees, 1977; Neser & Kluge 1986; Kluge & Neser, 1991; Hoffmann & Moran, 1998; Maron *et al*., 2002). Although the effects of seed-reducing insects on long-lived perennial population dynamics, particularly in the field of biological control, have been questioned in the literature (Wilson, 1964; Duggan, 1985; Andersen, 1989; Myers *et al*., 1990; Paynter *et al*., 1996) the compensatory responses by the parent plant to reductions in seed output, frequently used to dispel arguments on the effects of seed reduction, are not valid when dealing with post-dispersed seed (Janzen, 1971c).

Despite doubt about the wisdom of using seed-feeding insects as biological control agents, investigations into and releases of seed-feeding agents on exotic weed species continue, yielding mixed degrees of success. Often the definition of "success" is not clarified and it may not be the proportions of seed destroyed by an agent but the ratios of germinating seeds to seedlings establishing that is the determining factor in the agent being considered successful (Fowler *et al.*, 1996; Paynter *et al.*, 1996). The seedling is the most vulnerable stage of a plant's life history and successful seedling establishment is governed by a number of independent and interacting factors (Scifres & Brock, 1969, 1972; Ueckert *et al.*, 1979). Brown and Archer (1999) argued that mesquite recruitment into mesic grasslands is not contingent upon unusual or episodic rainfall. However, recruitment events of species in arid and semi-arid regions are usually highly dependent on rainfall (Harrington, 1991; Meyer & Pendleton, 2005) which may be sporadic, low and may even fail in certain years. In circumstances where rainfall favourable for recruitment is episodic, seed feeders might be important if they suppress the establishment of a seed bank capable of responding to these episodic events (Andersen, 1989). In addition, germination events do not necessarily translate into recruitment events as unsustained rainfall may result in germination but not seedling survival and establishment of young plants.

Besides mortality due to a lack of rainfall, herbivory from mammals, insects, molluscs, and millipedes and decay by pathogens have been shown to make a major contribution to seedling mortality (Crawley, 1983, 1989; Mills, 1983). Although Weltzin *et al.*, (1998) demonstrated that mesquite seedlings can withstand repeated defoliation providing it occurs above the cotyledonary node, damage below this node is fatal to seedlings (Scifres & Hahn, 1971). The effect of herbivory on seedling losses and plant population dynamics may be greater in years of low seedling emergence (Blundell & Peart, 2004) either directly, through seedling removal, or indirectly, through energy, nutrient and regeneration limitations imposed by defoliation (Weltzin *et al.*, 1998).

Besides the size of the soil seed bank, the numbers of seeds germinating may also be relative to the dispersion of seeds in suitable microsites. Seeds require that several environmental conditions are met prior to initiation of germination but where and when germination conditions are optimum, "prompt and complete seedling stands" may form (Crocker & Barton, 1957). Scifres & Brock (1969, 1972) determined the rate and extent of germination of *Prosopis glandulosa* seeds to be dependent on soil surface temperatures. At a soil temperature of 24°C seeds germinated readily but at 18°C germination was minimal, additionally, newly germinated seedlings were less tolerant of water stress at higher temperatures (Scifres & Brock, 1969). The timing of rainfall events will therefore determine the extent of seedling establishment. Rain coinciding with low temperatures (below 18°C) will result in little seedling establishment because of continued dormancy of the seeds through an inability to imbibe water and germinate. In addition high post-germination temperatures will subject seedlings to lethal water stresses. In the winter rainfall areas where periods of moisture availability might not coincide with suitable temperatures for germination, seeds in areas exposed to higher solar radiation, should germinate in greater proportions than those in shaded areas. Conversely, in summer-rainfall regions, seedlings that grow in exposed situations will suffer greater water stress than those growing in shaded situations.

This interconnectedness in rainfall, germination and seedling establishment coupled with the episodic and unreliable rainfall patterns of arid and semi-arid areas will only allow seedlings to become established intermittently and in most years new recruits will fail to survive beyond the seedling stage. Therefore it is likely that the demographics of mesquite infestations in arid regions will be characterised by cohorts of individuals of the same age. In addition, the dispersion of plants may suggest that successful seedling establishment may require placement of a seed into suitable microsites. Monitoring of plant populations may reveal where recruitment events of mesquite occur within the matrix of indigenous shrubs, mesquite thickets and exposed ground and whether recruitment is random or contagious (Kershaw 1973). Svedberg (1922) and Kershaw (1973) considered the dispersion of plants within a population to be random if they followed a Poisson distribution and if not, plants were contagiously dispersed (clumped) implying that recruitment occurs within microsites and suggesting that the population may be site limited.

The aim of this part of the study was to determine whether, in spite of damage caused by *A. prosopis*, there are still sufficient seeds in the system for mesquite populations to be sustained or to increase in density. Of particular interest was whether the use of pods by livestock has affected recruitment rates of mesquite and whether the location of post-dispersed seed has affected probabilities of survivorship and dispersion of established plants. The role of rainfall on recruitment events and the effects of its variability across mesquite distribution were also investigated.

Methods

Permanent transects were set up at sites where livestock were either present or absent in each of the three regions – South-east, Central and North-east. These transects were monitored annually. Transects were set up at Driefontein and Citrusdal in April 2002 and resurveyed in 2003 and at the remaining sites the transects were established in July 2002 and the subsequent survey trips were conducted in February of 2003, 2004 and 2005, toward the end of the rainy season.

The transects were monitored to ascertain survivorship and growth of plants from year to year and annual recruitment into the populations. At each site two transects of 50m in length and 2m wide were established and all plants falling within the 2m-wide ribbon transect were marked and recorded and mature plants with stems outside of the transect but with a canopy over the transect were also noted. Four categories of plants, determined by stem diameter at the soil surface using Vernier callipers, were marked with different coloured plastic coated wire, including: **seedlings**, with stem diameters of approximately 1mm, generally with cotyledons or cotyledonary scars visible (plate 5.1); **juveniles** with rigid stems and a stem diameter of 1-2.9mm, usually with no secondary branching ; **sub-adults** with stems of 3-5.9mm, often with secondary branching; a second group of **sub-adults** that were larger and more branched and with a stem diameter of 6-9.9mm. The remaining plants > 10mm were not marked but were recorded and monitored annually. On each survey date, plants were re-measured and any newly established plants were tagged with a different coloured wire. Any plants that had germinated before the previous survey were promoted from the seedling to the juvenile

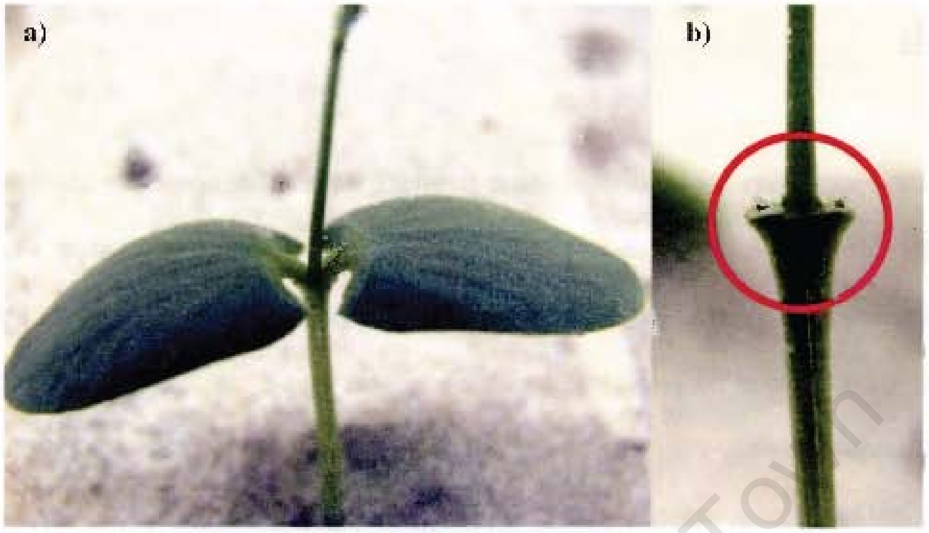


Plate 5.1: Young mesquite seedling showing a) cotyledons and b) cotyledonary scars

category and any juveniles from the previous survey were promoted to the sub-adult category. The maximum percentage of seedlings surviving was determined from the total seedling count on the previous survey trip, likely overestimates in all instances as many seedlings that emerged were probably missed. Annual surveys meant that survivorship and advancement into higher size classes of tagged plants could be done to allow for estimations of population growth of these invaded stands from year to year and also over the entire study period. Only the sub-adults and adult categories were used for estimations of population growth as these classes were stable. No deaths of adults were recorded and neither were there any advancements from sub-adult to adult categories over the study period.

The transects in the South-west region were within an affordable transport distance and could be monitored more frequently. Provision was made to survey these sites after rainfall events in order to monitor events in the region where climatic conditions suitable for recruitment were most unpredictable.

During the course of this study there were some disruptions to two of the study sites where landowners authorised mesquite clearing operations to commence. As a result, only one transect at the Driefontein site in the South-west region remained intact and could be monitored continuously between 2002 and 2004. Removal of most of the adult trees in the area must have resulted in a marked reduction in annual seed input to this system but the extent of the reduction was not quantified. At the Modder River "livestock" site, tree felling operations in the vicinity of the transects were in progress when the February 2004 surveys were due. Although the transects had not been affected, no surveys were made at the time because the plants in the transects were apparently about to be cleared and poisoned. However, the clearing operations were discontinued shortly after and before any of the transects were affected and surveys were made in February 2005. The stand of mesquite trees in which the transects lay was not disrupted so the annual input of seed would not have been affected to the same extent as was the case with the clearing operations at the Driefontein site.

Transects at a second site in the South-west region (Calvinia) were established to monitor the survival of a large cohort of seedlings that germinated after heavy rains in April 2004. This site was monitored in June and September of 2004 and again after February rains in 2005. A final survey was conducted in March 2005 to observe survivorship of the 2004 and 2005 seedlings.

No seedlings, juveniles or sub-adult plants were recorded from the non-livestock site (Citrusdal) in the South-west region in 2002 or 2003 and because seed cores revealed that the soil-seed bank was low (<7 seeds per m^2), observation of these transects was discontinued after two seasons.

Annual surveys of the transects revealed where recruitment events occurred relative to mature trees. Considering recruitment events over a long time span makes it possible to determine whether the dispersion of plants fits a Poisson distribution, (i.e. randomly distributed) or not (i.e. contagious) (Kershaw 1973). A comparison of observed versus expected frequencies will reveal if the populations follow a Poisson distribution (Kershaw 1973 after Svedberg, 1922). Thus from the series e^{-m}, me^{-m}, $m^2/2!\ e^{-m}$, $m^3/3!\ e^{-m}$..., the expected number of quadrats containing 0, 1, 2... individuals can be calculated (Kershaw, 1973) where:

$$\text{Mean density of the population, } m = \frac{\text{total \# of individuals}}{\text{\# of quadrats}}$$

The assumption follows that particular qualities of a microsite, natural or induced, enhance establishment of new individuals leading to a clumped distribution. In order to extract the frequency data from the 50x2m transects, the transects were divided into 100 quadrats, each 1x2m in size, and the numbers of young plants (juvenile and sub-adults) within each quadrat were determined.

Results

The number of seedlings recorded on each survey date is shown in Fig. 5.1. The missing data points (represented by MD) indicate either that the transects were not surveyed on those particular dates or that the presence of unaccounted juvenile plants in one year suggested post-survey germination giving rise to the juvenile plants detected on the next survey. The inclusion of data from the two "livestock" sites in the South-west Region (Figs 5.1a & b) demonstrated the importance of livestock for the continued replenishment

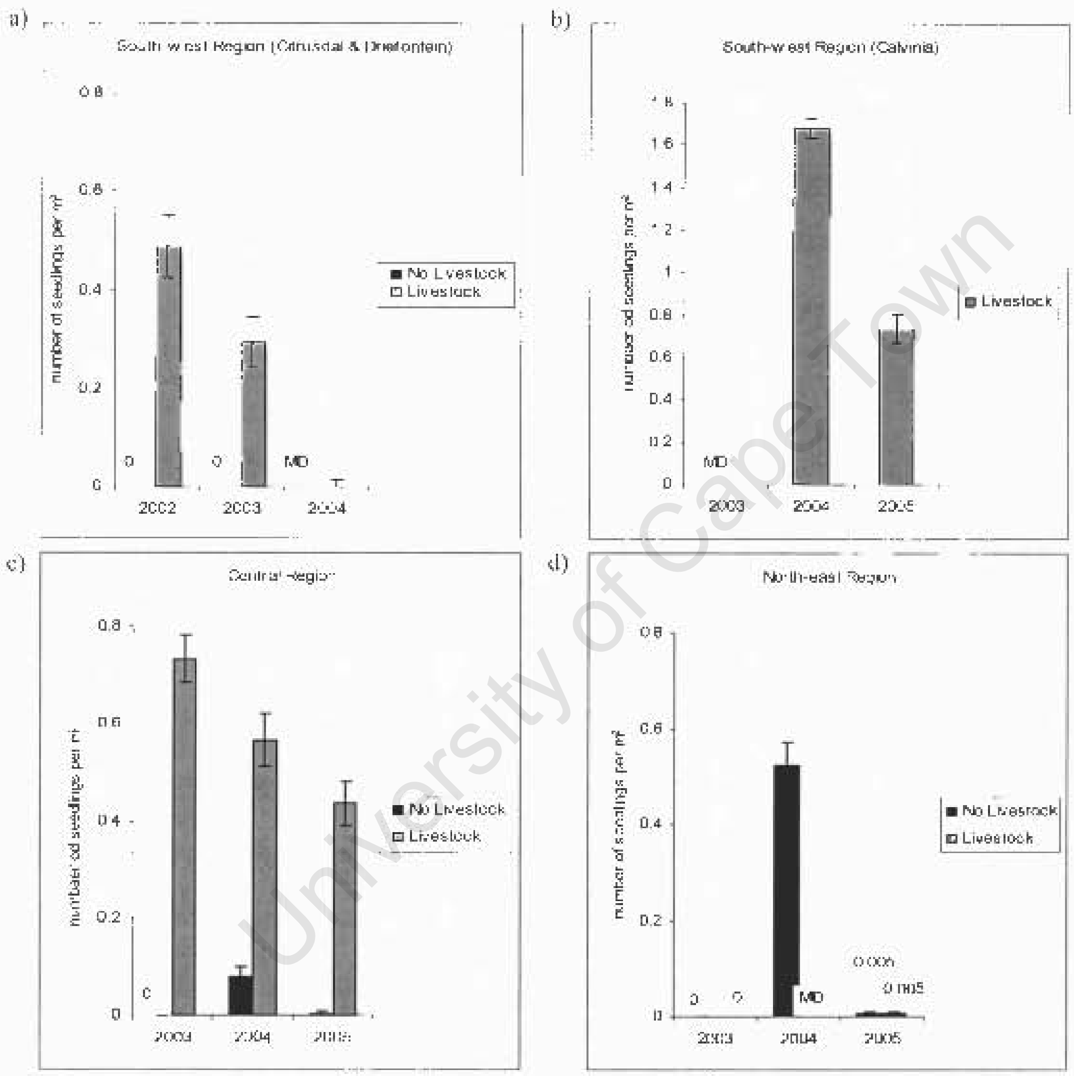


Fig 5.1: Number of seedlings (mean log n+1 m^{-2}) in presence and absence of livestock within three regions: a) South-west (Driefontein livestock; Citrusdal no livestock); b) South-west (Calvinia); c) Central; and d) North-east, between 2002 and 2005. Missing data points are indicated as "MD", Whiskers indicate standard errors.

of seed to the soil seed bank annually. Usage of pods by livestock at the Calvinia site replenished seeds lost to mortality and germination. At the Driefontein site, the felling of trees disrupted seed and was probably responsible for the absence of seedlings in 2004. The 45% decrease in the number of seedlings at Driefontein between 2002 and 2003 was not significant (Mann-Whitney, $z=1.413$, $p=0.158$). However, the 95% decline between 2003 and 2004 was highly significant (Mann-Whitney, $z=2.358$, $p<0.001$) and happened despite the region receiving pre-winter rains that were suitable for germination as seen by high levels of germination at another site in the region (Fig 5.1b). The high level of seed germination at the Calvinia site in 2004 was probably a consequence of increased seed availability owing to poor rains in 2003 enabling "carry-over" of seeds to the next year.

Levels of seedling recruitment in the South-west and Central regions were substantially higher at sites where seed was dispersed by livestock compared to sites where livestock were absent (Mann-Whitney, $z = 2.48$, $p = 0.013$ and $z = -2.63$, $p = 0.009$ respectively). Although the relationship between seedling recruitment and the size of the soil seed bank was not significant ($r^2 = 0.277$, $p = 0.065$) there was a positive correlation across mesquite distribution despite variations in suitability of environmental conditions that initiated germination. There was no correlation between the number of seedlings that survived to become one-year-old plants (Table 5.1) and the initial number of seeds that germinated per m^2 ($r^2 = 0.0396$, $p = 0.637$).

Table 5.1: The number of seedlings per m^2 that survived for one year to become juvenile plants between 2002 and 2005. The maximum possible percentage seedling survival, where known, is shown in parentheses.

	2002 Seedling survival	2003 Seedling survival	2004 Seedling survival	2005 Seedling survival
South-west No Livestock	0 (?)	0 (?)		
South-west Livestock (Driefontein)	0.13 (6.17)	0 (0)	0 (0)	
South-west Livestock (Calvinia)			0.19 (0.40)	0.01 (0.22)
Central No Livestock	0 (?)	0 (0)	0 (0)	
Central Livestock	0 (?)	0.01 (0.23)	0.02 (0.93)	
North-east No Livestock		0.1 (?)	0.11 (4.7)	
North-east Livestock		0.03 (?)	0.05 (?)	

Counts of seeds in the soil (Chapter 2) showed that seed densities at sites where sheep had access to pods were highest around mature mesquite trees where the sheep congregated and deposited most of their seed-bearing dung. This would account for the general pattern where significantly higher numbers of seedlings emerged in the vicinity of mature trees compared to open areas (Table 5.2). The one exception was at Driefontein where the transect traversed an infestation of young mesquite trees which were too small to provide shelter for the sheep and there was no difference in the size of the soil seed bank below these trees and the open areas.

Table 5.2: Mean (± S.E.) number of seedlings per m^2 across a climatic gradient - South-west (Cit = Citrusdal, Drie = Driefontein and Calv = Calvinia sites); Central (Van = Vanwyksvlei); North-east (Mod = Modder river), between 2002 and 2005. Kruskal-Wallis ANOVA for comparison of seedling numbers in shade and open areas on each survey date: ns = not significant; * $p < 0.05$; ** $p< 0.01$.

Region	Site	Livestock	Position	2002 Mean ± SE	2003 Mean ± SE	2004 Mean ± SE	2005 Mean ± SE
South-west	Cit	absent	Shade	0	0		
			Open	0	0		
			P	ns	ns		
	Drie	present	Shade	13.84 ± 6.14	8.5 ± 4.9	0	
			Open	3.95 ± 1.16	1.9 ± 0.8	0	
			P	ns	ns	ns	
	Calv	present	Shade			138.1 ± 12.8	22.8 ± 12.8
			Open			78.1 ± 8.8	4.1 ± 0.7
			P			**	**
Central	Van	absent	Shade		0	0.43 ± 0.23	0.03 ± 0.03
			Open		0	0.38 ± 0.22	0.02 ± 0.02
			P		ns	ns	ns
	Van	present	Shade		19.00 ± 11.1	11.07 ± 2.95	7.24 ± 2.73
			Open		1.48 ± 1.29	1.22 ± 0.62	0.72 ± 0.50
			P		*	**	**
North-east	Mod	absent	Shade		0	8.25 ± 1.44	0
			Open		0	0.75 ± 0.26	0.02 ± 0.02
			P		ns	**	ns
	Mod	present	Shade		0.021 ± 0		0.02 ± 0.02
			Open		0.019 ± 0		0
			P		ns		ns

Comparisons showed that seedling emergence was strongly influenced by the year in which surveys were conducted (Kruskal-Wallis, $p < 0.001$ for five of the six sites). Despite the observed differences in patterns of seedling emergence, neither the year of germination nor the position of the seedling relative to the shade of mature trees significantly affected the probability of seedling survival (main effects ANOVA, $F_{(1,12)} = 2.626$, $p = 0.156$ and $F_{(1,6)} = 1.00$, $p = 0.363$ respectively) over the course of this study.

The presence of cattle in the North-east region did not affect the densities of young plants (juveniles and adults) at the two sites (Kruskal-Wallis, N=200, H=3.13, p=0.08). In contrast, there were significantly more plants in areas where sheep were present in the more arid South-west and Central regions (Kruskal-Wallis, $N=100$, $H=44.4$, $p<0.0001$ and $N=200$, $H=20.17$, $p<0.0001$ respectively).

Figures 5.2 (a & b) show the densities of young plants occurring below and away from mature trees. In the North-east region the absence of cattle resulted in a higher number of plants situated below mesquite trees compared to the site where livestock were present.

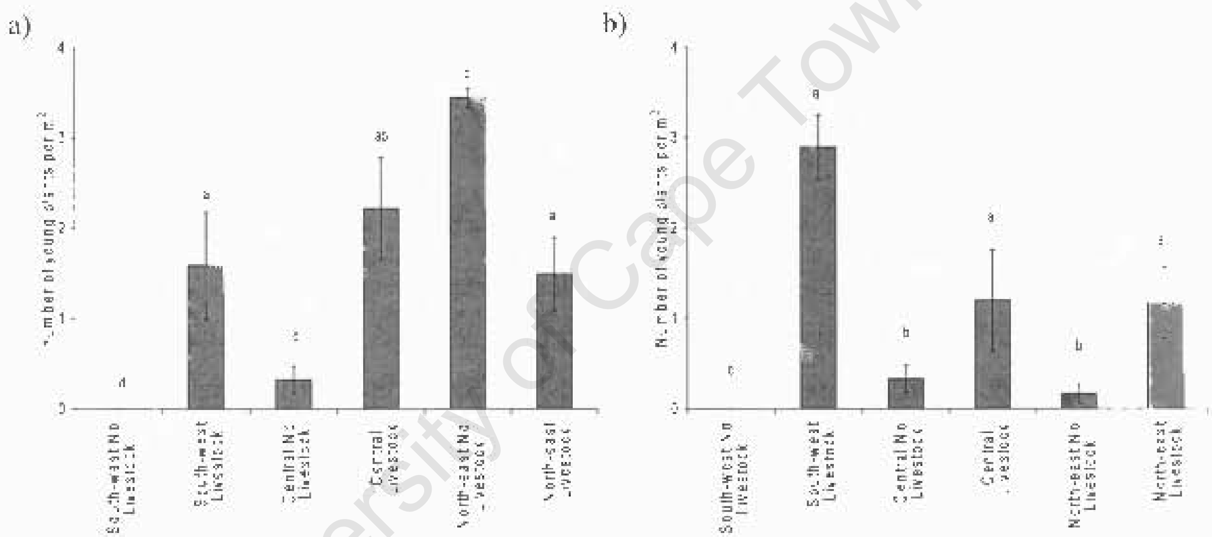


Fig 5.2 a & b: The densities (mean ± SE) of young plants (juveniles and sub-adults combined), in a) shade and b) open areas, at sites across mesquite distribution and under different land-uses in 2004. Sites with the same letters above bars are not significantly different (Kruskal-Wallis, $p > 0.05$).

However at most sites there was no significant difference between recruitment in open areas and below trees and only in the North-east region did the absence of an active seed disperser, cattle, result in there being significantly more plants below trees (ANOVA; $F_{(1,98)} = 20.65$, $p<0.0001$).

To determine if the dispersion of plants was random or contagious (clumped) observed versus expected frequencies of young plants occurring within the transects were compared. The χ^2 goodness of fit showed that at no sites did the dispersion of young plants fit the Poisson series (Table 5.3) and therefore all populations were considered to be contagious.

The population dynamics of the mesquite infestations over three consecutive rainfall seasons showing recruitment and survivorship of established plants for all sites (except South-west, "no-livestock") are represented in Figs. 5.4 – 5.9. The "no-livestock" site (Citrusdal) in the South-west region was excluded because in no year were any seedlings or juvenile plants observed. Each site is dealt with individually.

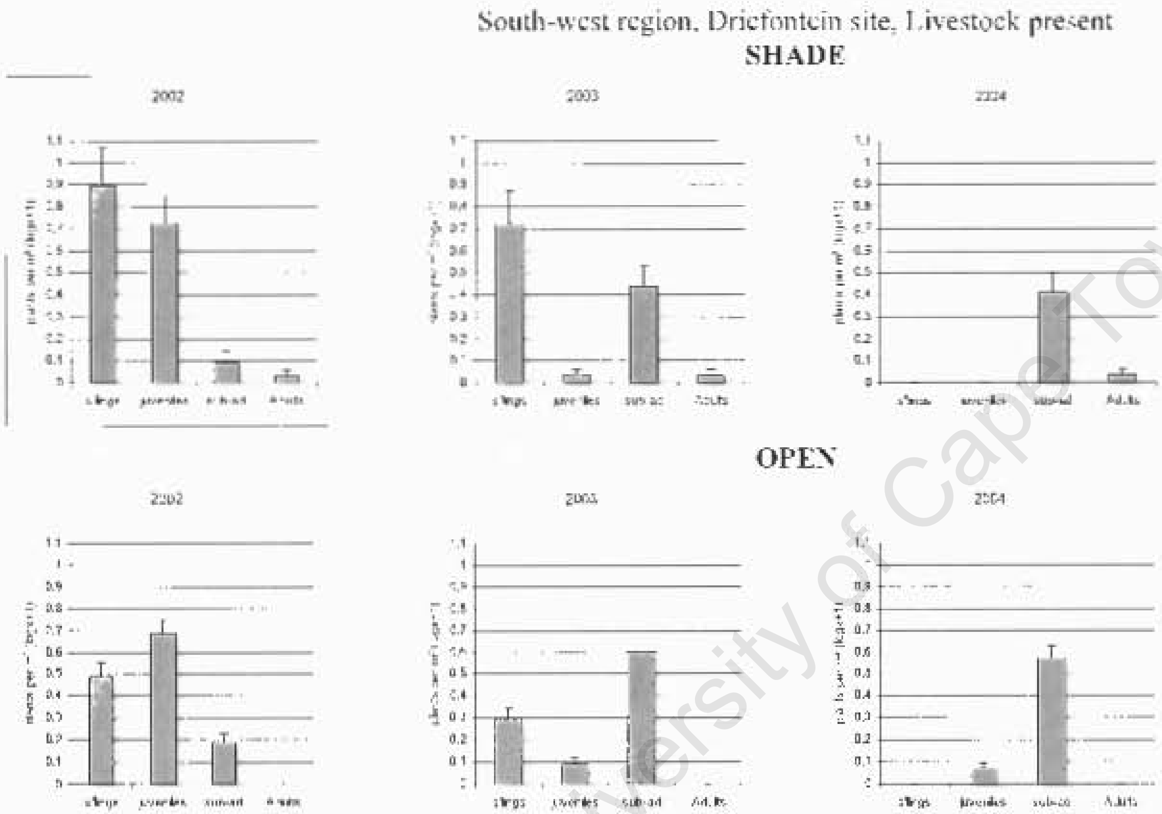


Fig 5.4: Population demographics of mesquite infestations at Driefontein (livestock present) in the South-west region on April 2002, September 2003 and September 2004 showing numbers of plants per m^2 associated with (shade) and away from (open) mature trees and annual changes in classes. The y-axis gridlines are included in the graphs to highlight subtle inter-annual fluctuations in plant densities.

<u>South-west region, Driefontein, livestock present (Fig. 5.4):</u>

Although germination was high in 2002, few seedlings survived and only a low percentage of seedlings became juveniles in 2003 (maximum 2.4% in the shade, and 7.6% in the open). Tree felling at this site commenced late in 2002 so there was no addition of seed to the system in 2003 or subsequently. Seedlings still appeared from seeds in the seed bank in 2003 but inadequate rainfall meant that none of the seedlings that germinated in 2003 survived to become juveniles in 2004 and there was no recruitment of new individuals in that year.

Although this site was not surveyed after summer rains in April 2004, surveys in September of that year indicated that no recruitment had occurred. The soil seed-bank declined from 25 seeds per m^2 in October 2002 to 0 seeds per m^2 in September 2004 (Chapter 4) and it is probable that the cessation of seed production after tree felling accounted for the lack of seedlings in 2004. Despite a number of juvenile plants making the transition into the sub-adult category (34.6% below mature trees and 65% in the open areas) between 2002 and 2003 mortality of young plants in the juvenile and sub-adult categories in 2003 caused a 59.6% reduction in individuals below mature trees and a 25.6% reduction in the open. 2004 yielded a further reduction of 10% of juveniles and sub-adults in both open and shaded areas.

Although seed and seedling counts per m^2 were higher below mature trees compared to the open, proportionally more young plants (>1yr) survived in open areas (0.63) than in the shade (0.53) although this difference was not significant (ANOVA, $F_{(1,23)} = 1.50$, $p = 0.23$).

There was a large recruitment event in 2001 as a large number of juveniles were recorded in 2002 resulting in rapid population growth between 2002 and 2003 ($\lambda = 6.18$) but there was negative population growth from 2003-2004 ($\lambda = 0.90$). There was a net population increase over the study period ($\lambda = 5.56$).

<u>South-west region. Calvinia, livestock present (Fig. 5.5):</u>

Seedling establishment was high in both 2004 and 2005 but very few of these seedlings survived (0.40% and 0.22% respectively). Failure of continued rains, post-germination, caused almost total seedling mortality in one month between February and March 2005. Germination levels were lower in 2005 than 2004, which may have been a consequence of lower livestock activity (*pers. obs.*) and therefore less available seed when the rains fell. Establishment of seedlings was marginally higher in the open than away from mesquite thickets but was very low in both situations (0.4 and 0.2% respectively). Herbivory is likely to have contributed to seedling mortality as many seedlings were noted to have been grazed to below the cotyledonary node. The lack of sub-adult plants in the population was a consequence of the site having been ploughed in the late 1990s. Despite low survival of seedlings there was a large population increase between 2004 and 2005 ($\lambda = 4.5$).

South-west region, Calvinia site. Livestock present

SHADE

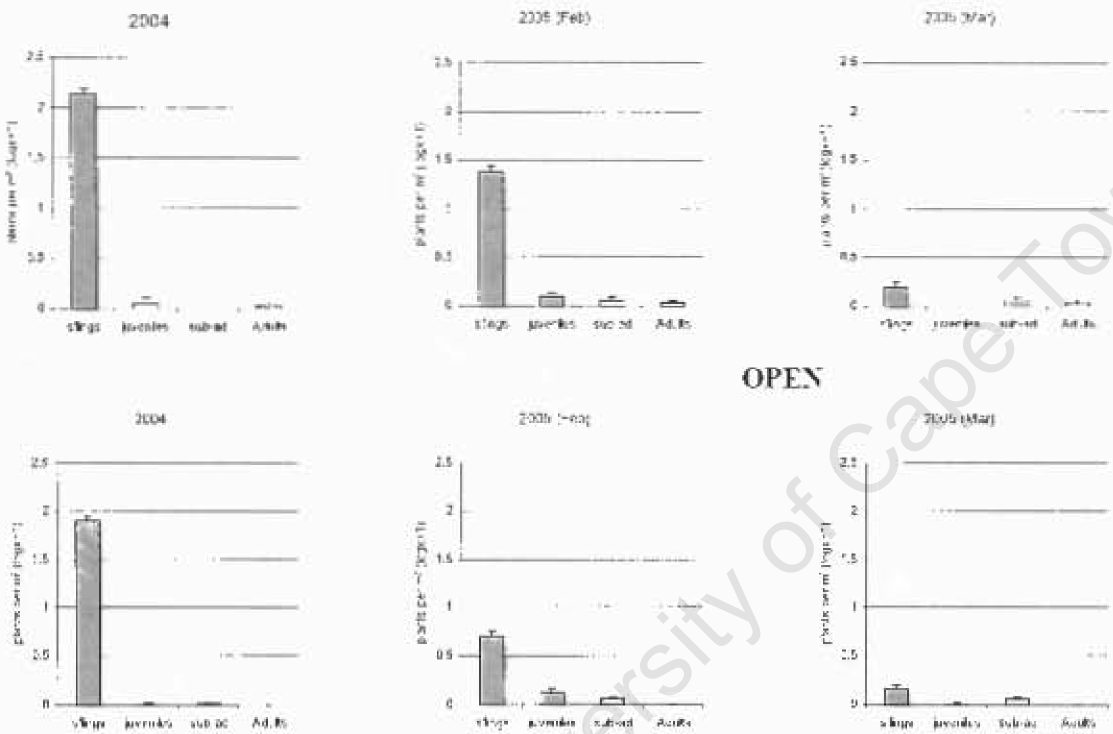


Fig 5.5: Population demographics of mesquite infestations at Calvinia (livestock present) in the South-west region on April 2004, February 2005 and March 2005 showing numbers of plants per m^2 associated with (shade) and away from (open) mature trees and annual changes in classes. The y-axis gridlines are included in the graphs to highlight subtle inter-annual fluctuations in plant densities.

Central region, Livestock absent

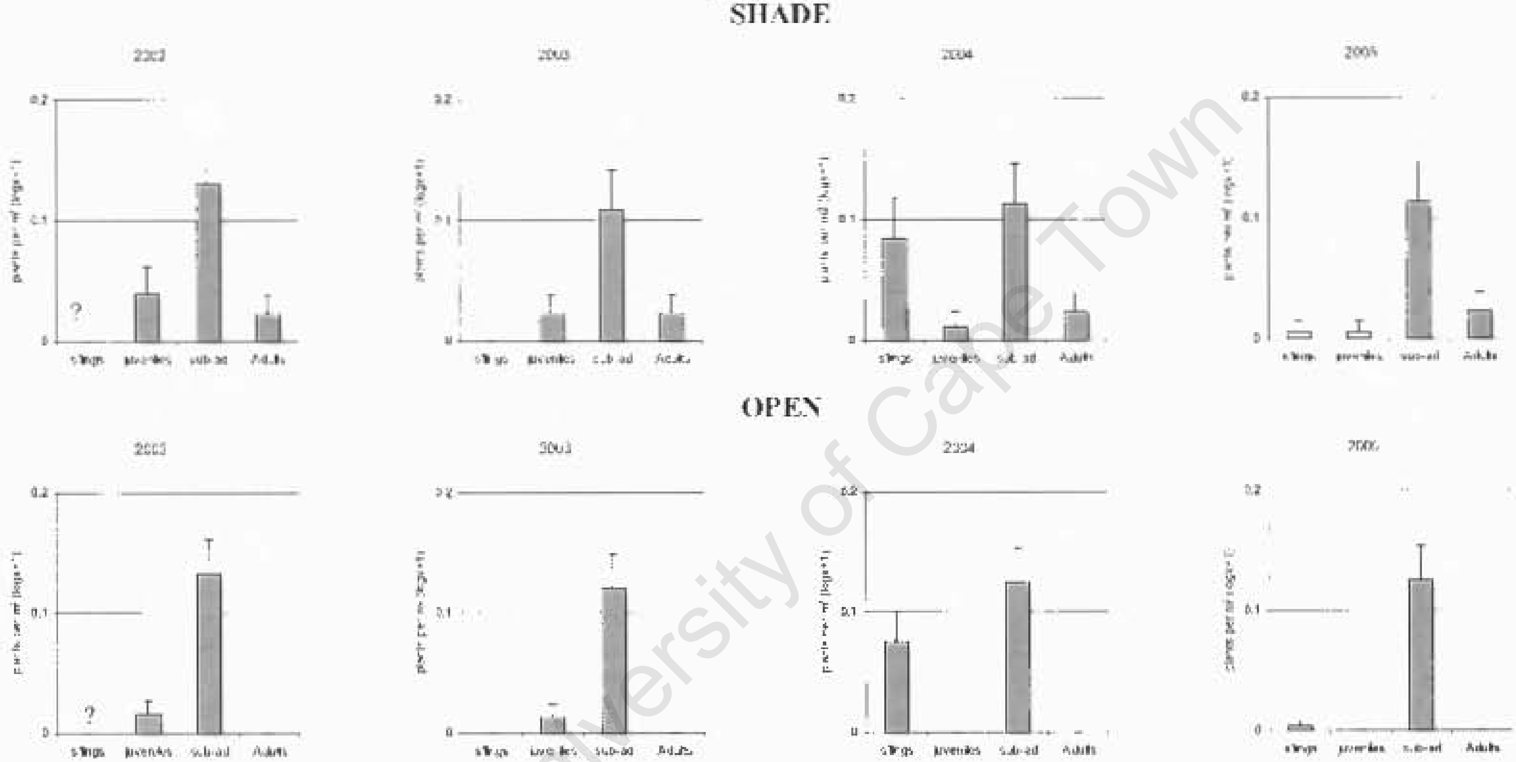


Fig 5.6: Population demographics of mesquite infestations at Vanwyksvlei (livestock absent) in the Central region on July 2002, February 2003, February 2004 and February 2005 showing numbers of plants per m^2 associated with (shade) and away from (open) mature trees and annual changes in classes. The y-axis gridlines are included in the graphs to highlight subtle inter-annual fluctuations in plant densities.

Central region, Vanwyksvlei, Livestock absent (Fig. 5.6):

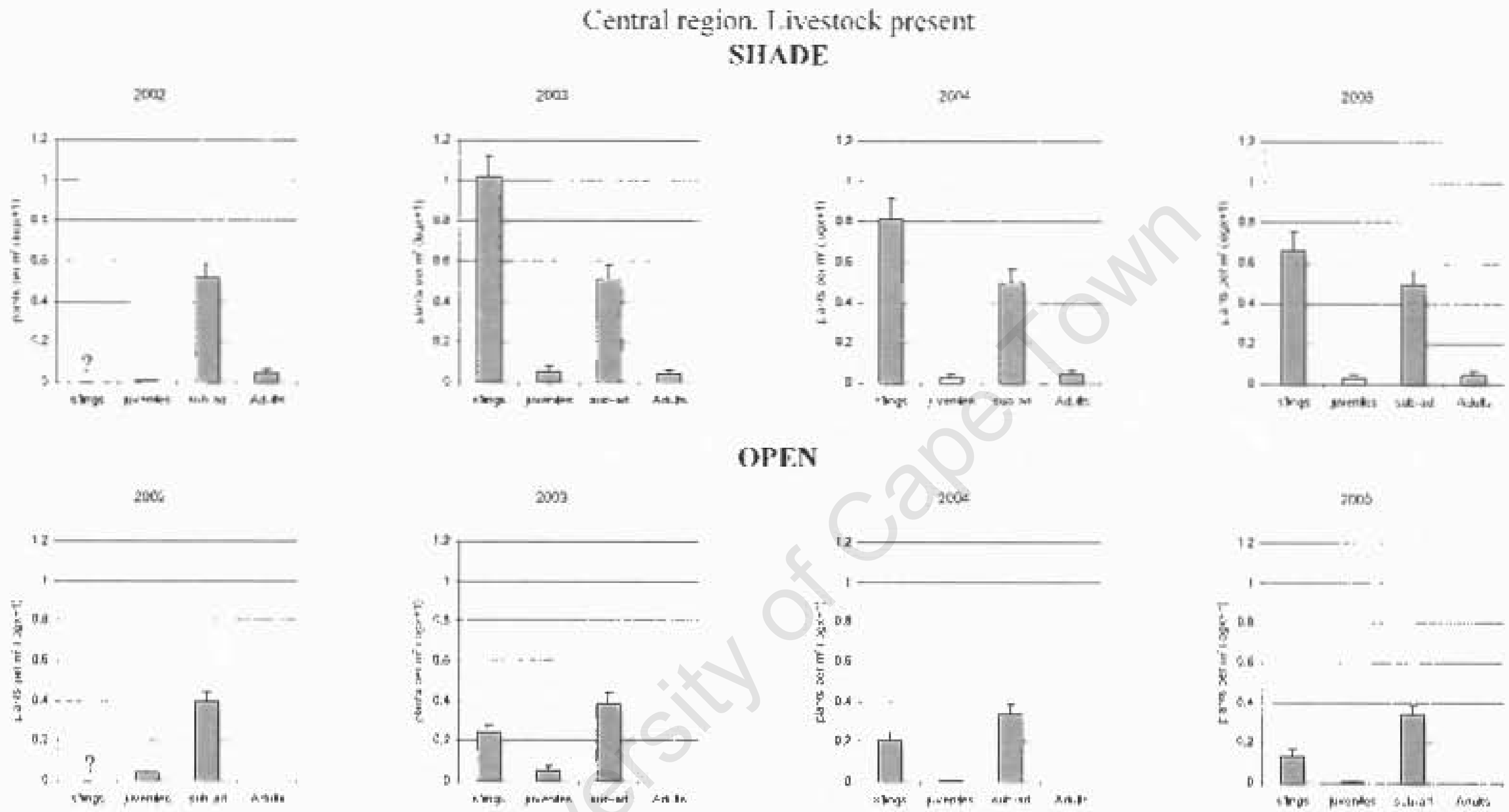
No seeds were observed to have germinated in 2003. Seed dispersal away from trees (thought to be predominantly by rodents) resulted in similar levels of seedling germination in the open and below mature trees in 2004 and 2005 but this was very low in both years. No seedlings survived between July 2002 and February 2005.

Densities of sub-adult plants were similar in open areas and below mature trees (0.33 and 0.32 plants per m^2 in 2004). The overall size of the population decreased from 2002-2005 (with an overall $\lambda = 0.88$ and an annual average of $\lambda = 0.96$) with the greatest population decline occurring in 2003 ($\lambda = 0.91$) and proportionally more young plants being lost below mature trees.

Central region, Vanwyksvlei, Livestock present (Fig. 5.7):

Germination occurred at this site in 2003, 2004 and 2005 with decreasing numbers of seed germinating in each year. Substantially more seed germinated below mature trees in all three years. Between February 2003 and February 2005, no seedlings became established in the open and only 0.1 and 0.9% became established below mature trees in 2003 and 2004.

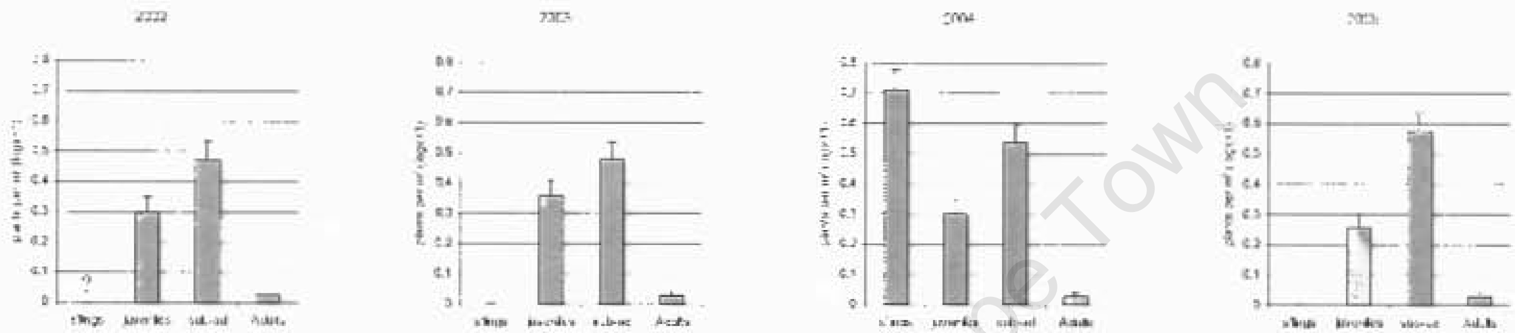
There were approximately twice as many juvenile and sub-adult plants below mature trees than in the open. The population increased from 2002 to 2003 but between 2002 and 2005, despite recruitment of new individuals into the population, there was an overall population decline as a result of mortality of juvenile and sub-adult plants. (the overall $\lambda = 0.86$ and average annual $\lambda = 0.95$). Mortality of young plants was higher in the open than below mature trees. The density of plants was more than four times higher at this site compared to that where livestock were absent (0.35 and 1.65 plants per m^2 respectively).



<u>Fig 5.7</u>: Population demographics of mesquite infestations at Vanwyksvlei (livestock present) in the Central region on July 2002, February 2003, February 2004 and February 2005 showing numbers of plants per m^2 associated with (shade) and away from (open) mature trees and annual changes in classes. The y-axis gridlines are included in the graphs to highlight subtle inter-annual fluctuations in plant densities.

North-east region. Livestock absent

SHADE



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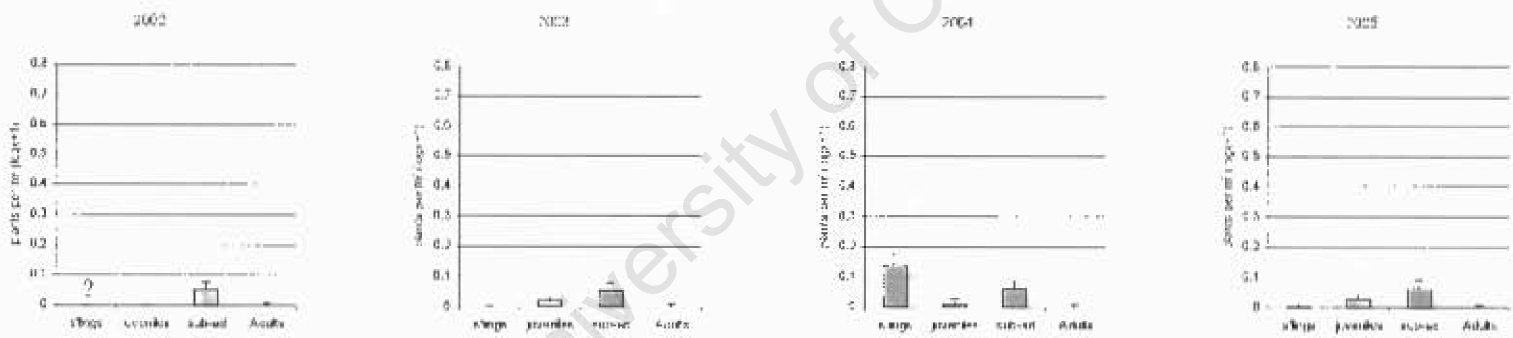


Fig 5.8: Population demographics of mesquite infestations at Medder River (livestock absent) in the North-east region on July 2002, February 2003, February 2004 and February 2005 showing numbers of plants per m^2 associated with (shade) and away from (open) mature trees and annual changes in classes. The y-axis gridlines are included in the graphs to highlight subtle inter-annual fluctuations in plant densities.

North-east region, Modder River, Livestock absent (Fig. 5.8):

No seedlings were observed in 2003 but a number of seeds were found to have germinated below mature trees in 2004. Despite an absence of livestock from this site indigenous animals contributed to dispersal of a small proportion of the seed and may have been responsible for the low numbers of seed that were found to have germinated in the open. Despite no seedlings having being recorded in 2003 a recruitment event did occur between February 2003 and February 2004 as a number of juvenile plants were recorded on the latter date.

Transition of plants from seedlings to juveniles and juveniles to sub-adults occurred annually and there was little to no mortality each year. A far higher number of plants occurred in association with mature trees than in the open (3.51 and 0.18 plants per m^2 respectively). The population at this site grew annually between July 2002 and February 2005 (with an overall $\lambda = 1.38$ and average annual increase $\lambda = 1.12$).

North-east region, Modder River, Livestock present (Fig. 5.9):

This site was not checked in 2004. On the survey dates in February of 2003 and 2005, low numbers of seedlings were recorded from below mature trees and in the open. All of the seedlings recorded in February 2003 failed to establish. However, the presence of new juveniles and sub-adults observed in 2005 indicated that there had been successful recruitment in 2003 and 2004.

Although higher densities of plants were found to occur below mature trees than in the open (1.66 and 1.23 plants per m^2 respectively) these proportions were lower than at the Modder River site where livestock were excluded. However, the overall averages of young plants per m^2 were similar at 1.92 and 1.44 for the livestock-absent and livestock-present sites respectively (this data is not normally distributed and hence non parametric tests are used: Kruskal-Wallis, $H = 3.13$, $p = 0.077$). The population at this site grew between 2003 and 2005 ($\lambda = 1.35$)

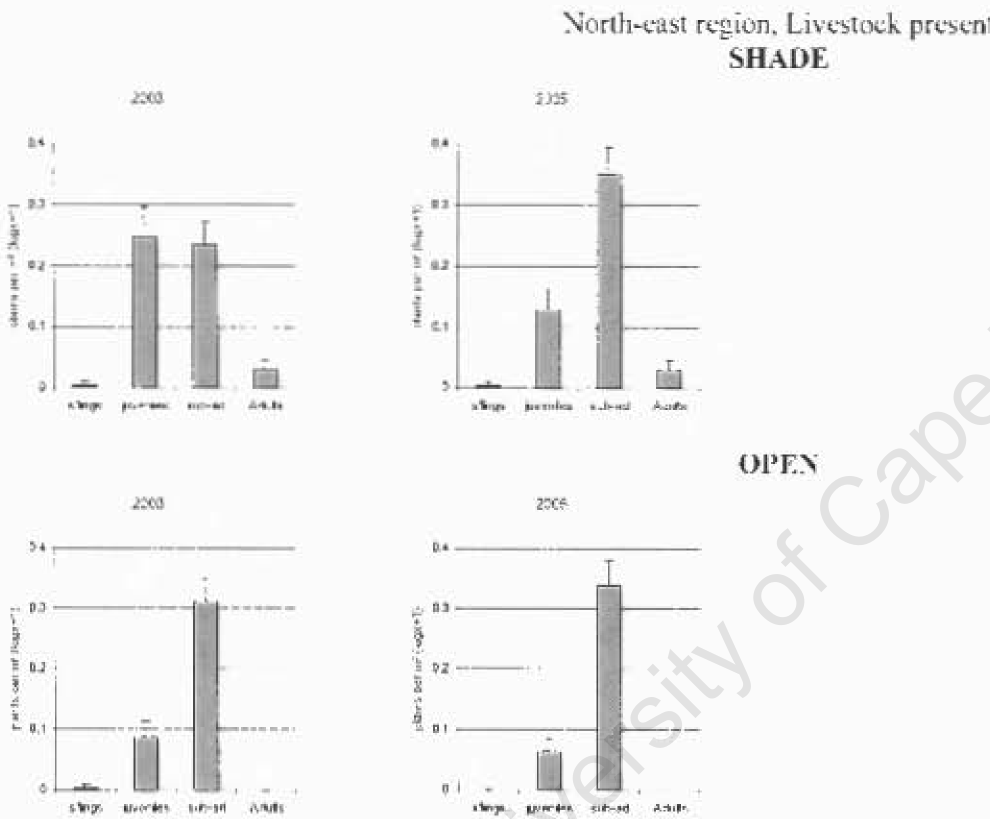


Fig 5.9: Population demographics of mesquite infestations at Modder River (livestock present) in the North-east region on February 2003 and February 2005 showing numbers of plants per m^2 associated with (shade) and away from (open) mature trees and annual changes in classes. The y-axis gridlines are included in the graphs to highlight subtle inter-annual fluctuations in plant densities.

To confirm whether pulses of recruitment were apparent in the size classes of populations of plants, the frequency distributions of individual stem diameters of the plants that were measured are represented below (Figs. 5.10-5.12). Distinct size classes would be evident if recruitment occurred in some years and not in others.

South-west region, Driefontein, livestock present (Fig. 5.10):

This appeared to be a young invasion with the main representation in size classes <1cm and very few individuals in the larger size classes. The high frequency of small plants showed that there was an active invasion taking place but poor representation in size classes 0.1 and 0.2cm indicated a period of poor recruitment in the recent past. Fifty percent of individuals in the population accounted for the <0.4cm size classes suggesting a recruitment event estimated to 2-4yrs previously. It was not easy to discern distinct cohorts but there was little growth of the plants between 2002 and 2004 implying that the plants established on separate occasions.

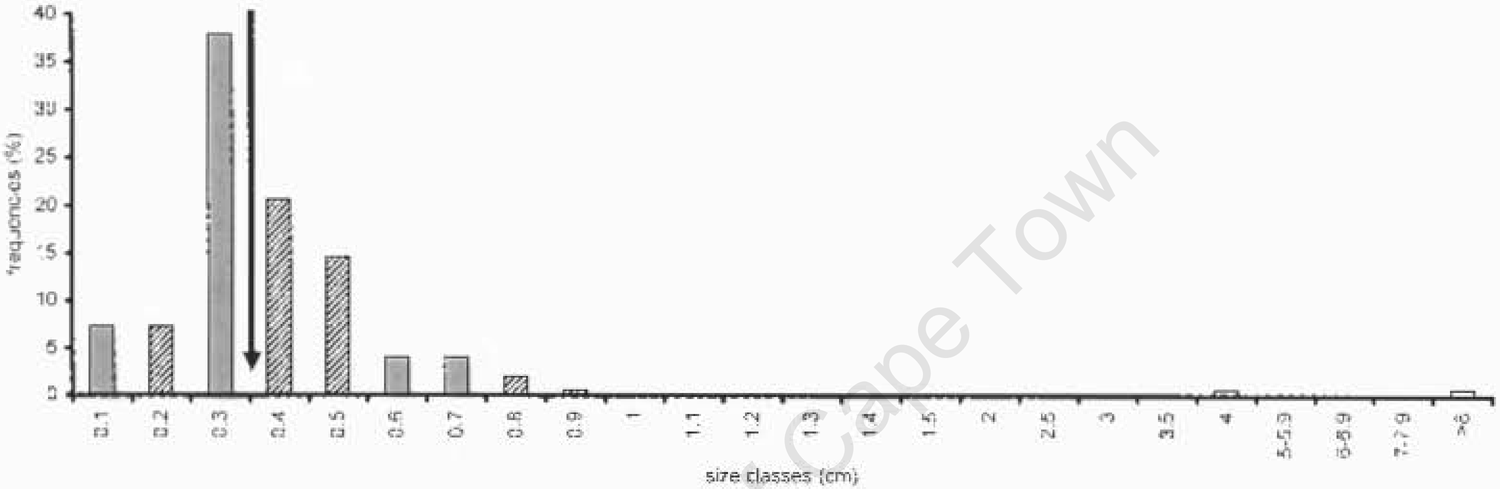


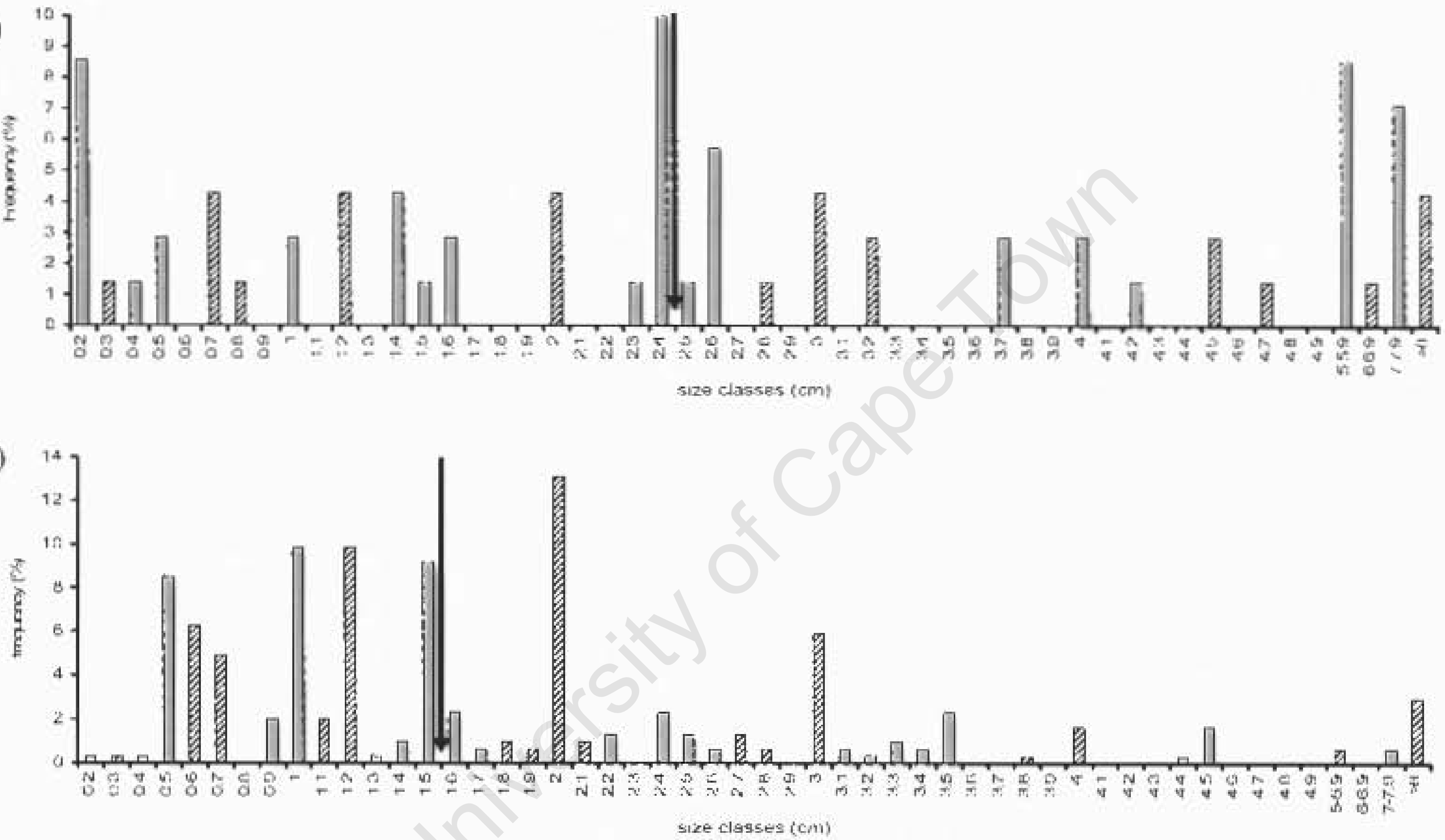
Fig. 5.10: Frequencies size classes (stem diameter in cm) of mesquite plants in a population, in the South-west region (Driefontein) where livestock were present. Alternating changes in shading with increasing values along the x axis indicate probable distinct cohorts. The arrow represents the median of the frequency distribution.

Central region, Vanwyksvlei (Figs. 5.11 a & b):

The mesquite invasion at this site has occurred since the 1940s with the introduction of the first trees (Harding & Bate, 1991) and evidence for a long period of invasion stems from the fact that older age classes are well represented. The peaks of certain size class frequencies and absence of individuals in others support the supposition that recruitment is intermittent and not continuous. Invasion in areas where livestock were present compared to areas where livestock were absent indicated that size class frequencies were proportionally similar. However, recent recruitment where livestock were present appeared to be higher as the median was lower at this site.

a)

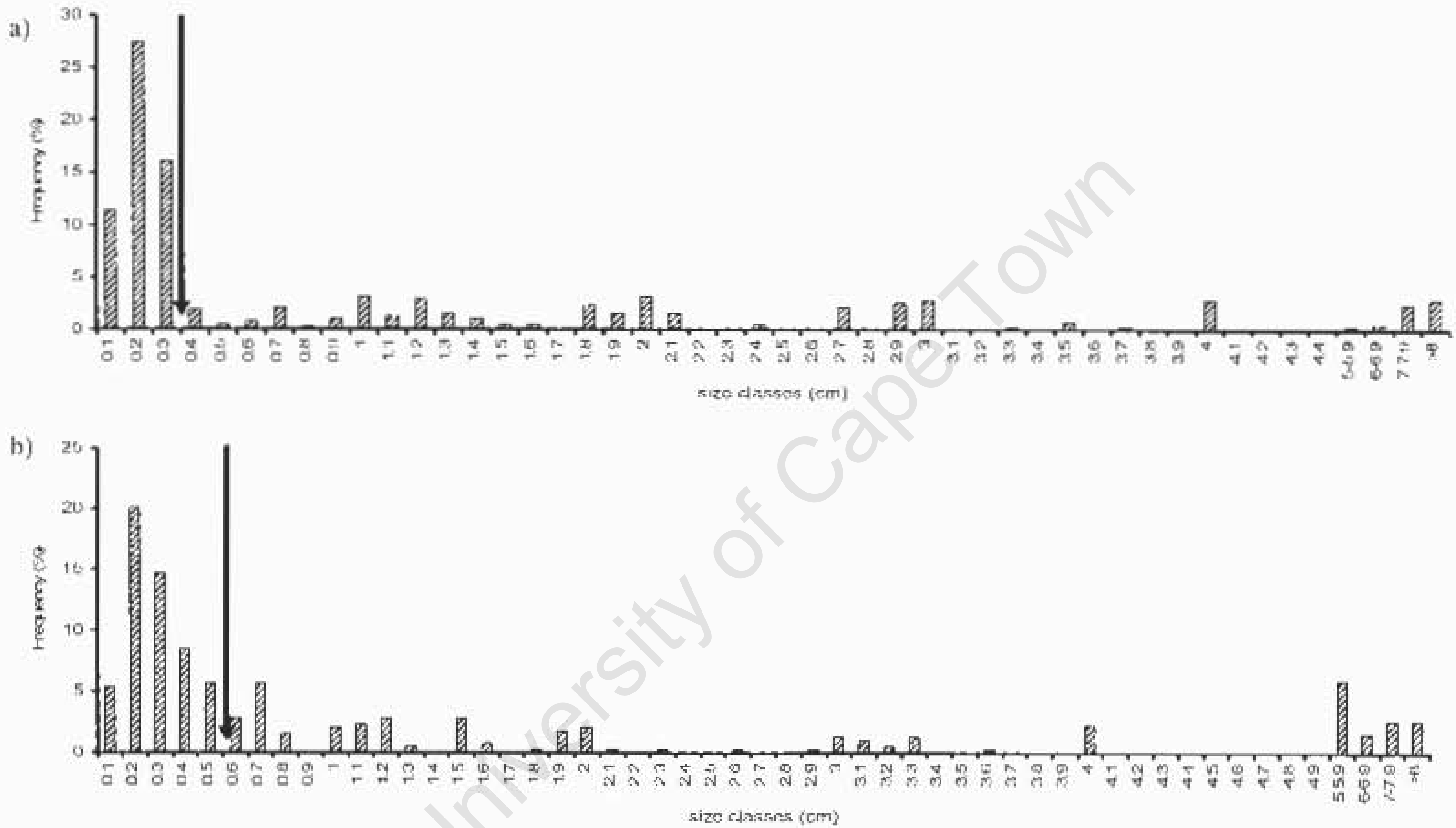
b)



Figs. 5.11 a & b: Size class distribution (stem diameter in cm), represented as frequencies of total plant count, in the Central region (Vanwyksvlei) where livestock were a) absent and b) present. Alternating changes in shading with increasing values along the x axis indicate probable distinct cohorts. The arrow represents the median of the frequency distribution.

North-east region, Modder River (Figs. 5.12 a & b):

A spread of individuals across several size class indicates that invasion at this site has occurred over an extended period of time. However, the high frequencies of individuals in the small size-class and the median being very low suggest that invasion has intensified in recent years.



Figs. 5.12 a & b: Size class distribution (stem diameter in cm), represented as frequencies of total plant count, in the North-east region (Modder River) where livestock are a) absent and b) present. No changes in shading as cohort divisions not easily designated. The arrow represents the median of the frequency distribution.

Discussion

For woody plants with potentially long life-spans and low post-establishment mortality rates, seedling recruitment is a critical life-history stage (Scholes & Archer, 1997). However, successful recruitment in arid ecosystems is a rare event (Kemp, 1989) and high losses of seedlings occur in most years. Considering the high numbers of seedlings emerging annually at most sites it appears that the mesquite populations are not currently seed limited. However, of the seedlings recorded 99.1% suffered mortality over the study period. Infrequent surveys at most sites would have resulted in the failure to record a large number of seedlings that may have germinated and subsequently died between survey dates and therefore annual mortality during this study period was likely to be closer to 100%.

The high proportions of seedlings lost annually may be inconsequential for perennial plants with long-lived seed banks, where dormancy ensures that some seeds persist through unfavourable years and only germinate when conditions are suitable for seedling survival. In this study on average only 1.68% of seed in the soil seed bank was found to germinate annually yet the remaining ungerminated seed was not accounted for in later survey dates and there was no evidence of an accumulation of seed. Granivory, although not investigated in this study is likely to be one of the major causes of annual seed loss.

Brown *et al.* (1979) argue that mesquite seed loss through granivory by rodents, ants and birds in North American arid and semi-arid regions greatly exceeds that by bruchids and other insects and strongly influences the size of soil seed banks. I found evidence for removal of high proportions of seed by ants and rodents. In the central karoo system, it appears that in the absence of livestock rodents acted as the primary seed disperser, removing seed away from mesquite trees to below indigenous shrubs. In the presence of livestock, harvester ants (*Mesor* sp.) were seen to be major secondary dispersers of mesquite seed deposited in sheep droppings in the South-east and Central regions where as many as 10 seeds per minute were observed being taken into a single colony entrance (*pers. obs.*).

Plants in several families have evolved alongside ants which are often the major dispersers of seeds e.g. *Proteaceae* in South Africa (Midgley & Bond, 1995), *Acacia* in Australia (Whitney, 2002; Edwards *et al.*, 2006). However, in desert ecosystems ants may have significant impacts on seed banks affecting both seed and plant densities (Whitford, 1978). Owing to the inability of mesquite seed to germinate from >5cm below the soil surface (Scifres & Brock, 1972) it is unlikely that seed burial by ants or rodents may be beneficial to the plants as has been documented for other plant species (Midgley *et al.*, 2002).

Dissemination of seed away from fruiting conspecifics to below indigenous shrubs may reduce the rates of damage by natural enemies and enhance chances of seedling establishment by being situated below a "nurse" plant (e.g. Nathan & Muller-Landau, 2000; Baraza *et al.*, 2006). It was evident that where livestock were excluded in the Central region, germination of seed below indigenous shrubs accounted for the highest proportion of seedling emergence.

In arid to semi-arid regions where livestock are absent high proportions of seeds were damaged by bruchids (Chapter 1) and the soil seed banks were sparse (Chapter 4). The introduction of the bruchids appears to have shifted the mesquite populations in the arid South-west region from being site limited to now being seed limited and recruitment has been halted as no seedlings were recorded over two years of observations. However in the Central region, despite very high levels of seed damage by bruchids the removal of seed pods by rodents away from the parent tree may enable the escape of a small proportion of seeds from bruchid attack. This results in 78% of the soil seed bank being associated with rodent middens below indigenous shrubs and although only a fraction of the annual seed production, this small seed bank gave rise to low numbers of seedlings in two of three years. Although no seedlings became established during the study period, in some years when conditions were suitable the mesquite stands in these areas may still expand and densify.

In arid and semi-arid regions where livestock are present and 85% of seed is destroyed by sheep, bruchids caused further damage to a high proportion of the seeds within dung annually (Chapter 2). In spite of the cumulative damage, the soil seed banks were higher than in areas where livestock were excluded and under these circumstances mesquite does not appear to be seed limited. Despite the enhancement of germination with passage through the gut of sheep resulting in high numbers of seedlings emerging annually at sites with livestock, less than 1% of these seedlings survived to become recruited into the mesquite populations. Only a small proportion of the seeds that did not germinate became incorporated in the seed bank because of the combined effect of bruchid damage, decay and removal by granivores. Evidence for this came from the Driefontein site where seedling recruitment stopped and the soil seed bank was depleted two years after the mature trees had been felled. With a better understanding of the soil seed bank processes, such as the proportions of seeds germinating and levels of damage caused by bruchids and granivores, Fig. 5.13 explains how soil seed banks are affected in areas where livestock are present, particularly at the winter rainfall sites such as Calvinia.

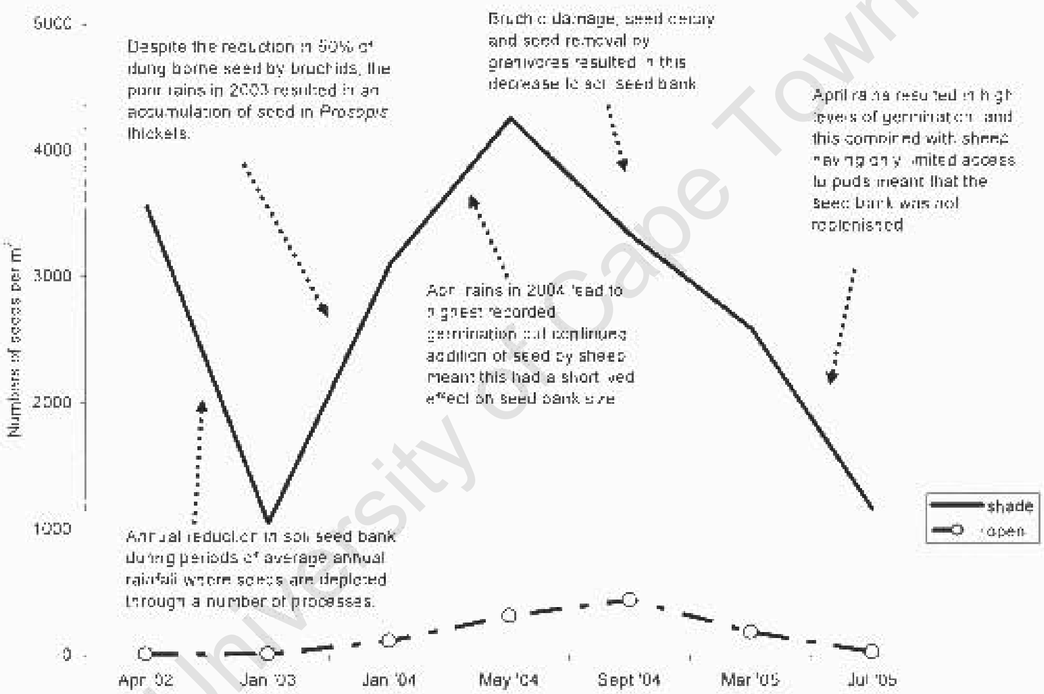


Fig. 5.13: Mesquite soil seed bank dynamics in shaded (below mature trees) and open areas at a site with livestock in the winter rainfall region (Calvinia).

At sites in the winter rainfall region with livestock, in years with average rainfall, there was an approximate decrease of 75% in the soil seed bank situated below mature trees and a 94% decrease in open areas. Only 2.5 and 10.0% of the seeds were found to have germinated in each situation respectively. Thus it seems that germination of seed is not the driving factor responsible for annual decreases in soil seed banks. It was shown in Chapter 2 that 99.6% of seeds in dung pellets placed in open areas in the field over winter were destroyed and of which 81% were confirmed as having bruchid larvae within. It is probable that the remaining damaged seeds decayed as a result of unconfirmed bruchid entry. Unlike the open areas, an estimated 50% of dung-borne seed situated below mesquite trees escaped bruchid damage (Chapter 4), and thus larger seed banks developed in these areas.

In the more mesic North-east region, where bruchids failed to inflict high levels of seed damage, a large seed bank was found to have formed in both the presence and absence of livestock. Seedling recruitment occurred annually and survival was estimated at a maximum of 4.7% despite below average rainfall during this study period. These results are similar to those of Brown and Archer (1999) where recruitment in Texas, a subtropical area with average annual rainfall comparable to this study site, proved to be continuous even in years of below average rainfall. Two processes appeared to be at work in this region that may enhance seed germination: although passage through the gut of cattle, the main livestock in this region, has been reported to enhance germination only marginally (Cox *et al.*, 1993) the excision of seed from the tough, protective endocarp could increase germination over seed that remains enclosed in the indehiscent pods. In the absence of livestock, termites (probably *Odontotermes* sp.) may perform a similar action of seed exposure by constructing covered runways over pods (*pers. obs.*) and removing the pod exocarp and pericarp thus accelerating pod degeneration. In mesic areas, unlike xeric sites, soil seed banks were lower in the presence of livestock than in their absence. Seedling recruitment showed the same trend but whether this was a consequence of seedling herbivory or disturbance by livestock or merely reduced recruitment due to a smaller seed source will need to be investigated further. Across mesquite distribution, despite the general trend where higher densities of seeds and seedlings occurred below mature trees, recruitment was similar in both the open and shaded areas.

To determine whether the patterns of seedling survivorship observed in this study were typical or the result of consecutive below-average rainfall years, the dispersion of immature plants i.e. those more than 2 years old was examined. If the dispersion of seeds and seedlings is skewed a similar dispersion of immature plants is expected to show within a longer time frame. The data showed that for most sites no significant differences existed between the numbers of immature plants below mature trees or in the open, both in the presence and in the absence of livestock. There was also no correlation between distance from mature trees and the numbers of immature plants suggesting there is a regular dispersion of plants in the field. However, the frequency data showed dispersion to be contiguous (clumped) at all sites. This suggests that successful seedling establishment is enhanced within scattered microsites and that the plant is site limited.

In the xeric region, distinct cohorts of plants, and failure of recruitment of new individuals at most sites during the course of this study, suggest that in addition to requiring suitable microsites, extended periods of above average rainfall are necessary for seedling establishment. Below average rains in 2003 resulted in the mortality of 10-15%

of established sub-adult plants in the xeric regions. This did not occur in mesic areas suggesting that under wetter conditions, providing seedlings establish and survive till the next rainy season, it is unlikely that they will be lost from the system. In all regions the seed and seedling stages are the most vulnerable but have the potential to strongly influence population growth, λ, in certain years. However, mortality of immature plants in the xeric regions will likely have a greater influence on λ during the periods between recruitment events.

There was little growth of immature plants in all regions over the period of study, so much so that over three years of surveying most individual plants showed no detectable increase in stem diameters or noticeable increase in height and it seems probable that most resources were directed to below-ground growth (Glendening & Paulsen, 1955). This does not negate the idea of cohorts as, although above-ground growth is retarded, the development of roots ensures that in periods of good rain the plant will respond accordingly to its ability to take up soil moisture. Immature mesquite plants seem to be able to remain in an immature state for long periods of time (Brown & Archer, 1989), as with some savannah tree species (Menaut *et al.*, 1990), and may only move from this state given favourable conditions, this being termed the "Gulliver effect" by Bond and van Wilgen (1996) for tree-grass interaction and the suppression of above-ground tree growth.

Slow growth of plants has ramifications for projections of invasion rates, particularly those surmised from aerial or satellite images where the bulk of the plants in the population may be too small to be detected. This suggests that work involving slow growing, long-lived invasive perennials and projections of their invasive rates needs to incorporate both multi-year field monitoring and aerial or satellite imagery to accurately predict rates of spread.

In conclusion recruitment in arid and semi-arid areas is episodic and requires extended periods of above average rainfall or alternatively periods of well timed rainfall to carry the seedlings through the season. However, it appears that these are rare occurrences. In contrast, recruitment in mesic areas is continuous even during periods of below average rainfall. Bruchid damage to seed, seed germination and granivory prevent the establishment of large soil seed banks in the xeric regions and although these processes occur in the mesic region these seed-reducing factors are not sufficient to extensively decrease the size of these soil seed banks. As a consequence of the reduction in the size of the soil seed bank recruitment in the arid and semi-arid areas has been reduced dramatically when livestock are excluded and this may be primarily attributed to seed destruction by the biocontrol agent and in these regions the system can be considered as having been shifted from site to seed limited. However, where livestock have access to pods recruitment is more continuous and although it may be episodic it has not prevented the infestations from densifying and these mesquite populations remain site limited. The interference primarily by livestock has reduced the ability of the bruchids to destroy pre-dispersed seed to sufficient levels to prevent further invasion. Under current management practices where livestock have access to pods, despite the bruchid's ability to destroy high portions of post-dispersed seed, they are ineffectual in stopping the spread and densification of current infestations although the rate of spread may have been slowed.

Recruitment in the mesic region is continuous regardless of livestock presence or absence and the invasion of these areas appears to be accelerating as recently recruited plants constitute the highest proportion of the population. The continuous recruitment that occurs in these areas corresponds to that of Kenya where mesquite became a problem in one third of the time it originally took in South Africa (Zimmermann *et al.*, 2006). The current agents appear to be ineffectual in reducing the seed crop sufficiently to halt the spread of this weed and careful consideration must be given when considering new agents to ensure that they are able to be suitably damaging in both the mesic and xeric regions.

SYNOPSIS

At sites where livestock were excluded the combined effect of bruchid damage to mesquite seeds was high in most regions, approaching 100% in the xeric environments but only low levels (60% damage) were realised in the higher rainfall areas, probably a consequence of sub-optimal environmental conditions for bruchid larval development. *Algarobius prosopis* populations were consistently and markedly higher than those of *N. arizonensis*, accounting for 87-100% of bruchid adults emerging across the range of mesquite in South Africa.

Despite the apparent "new host associations" between the bruchids and indigenous parasitoid species, where nine out of 13 parasitoids reared from pod collections were considered recent associations, there remained little effect of pupal parasitoids on the success of bruchid populations in reducing seed output. The levels of parasitism on *N. arizonensis* eggs were markedly lower than the levels reported in earlier studies and it seems unlikely that they are having a large impact on the beetle populations. However, the combined mortality due to larval and egg parasitoids on *N. arizonensis*, its reliance on pods in pristine condition, and the poor competitive ability of its larvae compared to those of *A. prosopis* all militate against *N. arizonensis* ever becoming a very effective biological control agent of mesquite in South Africa.

There are few instances where pods are not exposed to livestock so most pods produced by mesquite in South Africa are consumed soon after pod fall and prior to bruchids being able to damage the seed. Foraging renders most of the resources unavailable to *N. arizonensis* but the ability of *A. prosopis* to locate and utilise dispersed dung-borne seed as a resource for larval development may enable large populations of this species to persist in the absence of pods. Although the utilisation of dung-borne seed by *A. prosopis* was governed by the time of year and the location of the dung relative to mesquite trees, there did not appear to be any effect of distance on exploitation of dung-borne seed. The annual reduction in input of dung-borne seed to the system as a result of bruchid usage was estimated to be between 50 and 70%.

Temperature played a major role in determining bruchid activity. The utilisation of dung-borne seed dispersed away from mesquite trees enabled the *A. prosopis* populations to persist during winter as the temperature of dung in exposed areas was close to optimum thus reducing the risk of parasitism by allowing maximal larval developmental rates. Two generations of beetles emerged from dung-borne seeds during winter.

The use of dung-borne seed by *A. prosopis* does not appear to be a recent behavioural adaptation and is likely an evolved survival strategy to cope with pod removal by an associated assemblage of extinct mega-herbivore seed dispersers in the region of origin. The preference for dung-borne seed over pod-borne seed by ovipositing females, particularly if the females had developed in seed in dung, suggests that *A. prosopis* might have evolved to utilise post-dispersed seed, further explaining the opportunistic oviposition behaviour that characterises this species.

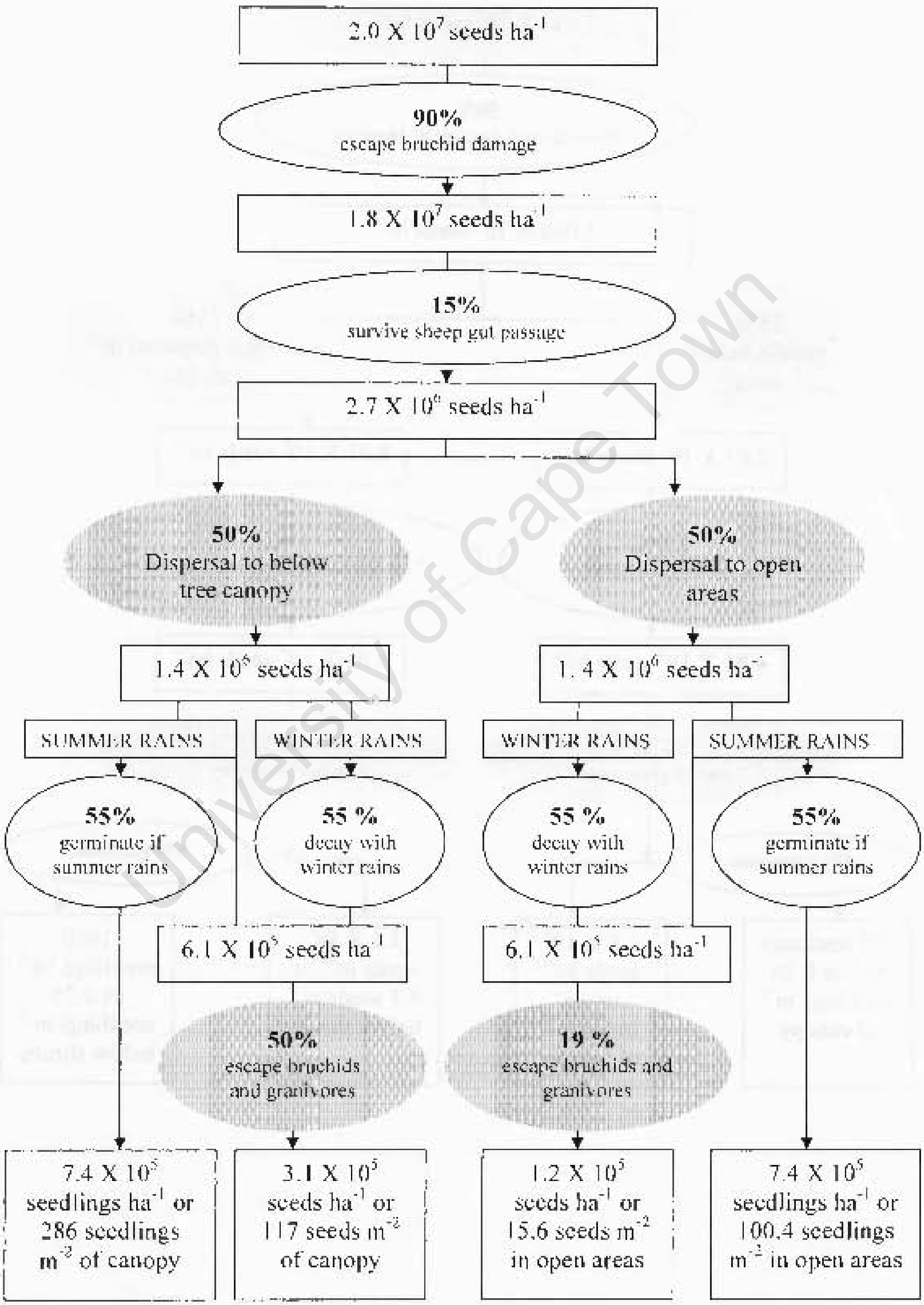
The primary aim of this project was to determine if the utilisation of dung-borne seed has had any effect on the ability of the beetles to curb populations of mesquite, where recruitment failure occurred in most years in xeric regions. Passage through the gut of sheep destroys 85% of mesquite seed and germination rates of the remaining seeds are enhanced by more than 50% through gut passage. For the bruchids to reduce the numbers of seed potentially available for germination, sufficient numbers must be damaged prior to rainfall. In addition, seeds partially damaged by *A. prosopis* larvae germinate readily and this process may enhance recruitment depending on the time of rainfall. Thus the timing of rainfall would be crucial in determining whether *A. prosopis* is able to reduce seed numbers sufficiently to prevent recruitment events.

Another factor that was not investigated here is the role that granivores play in preventing the development of large seed banks. The absence of large seed banks in areas where the bruchid damage was limited and the numbers of seeds germinating annually did not amount to the realised decrease in seed bank size, provided evidence that granivores may contribute substantially to seed removal.

The following figures summarize the factors acting on seed banks. Although there was need for speculation on the levels of seed removal (highlighted) or factors responsible for the disappearance of the seed that were not measured in this study, the estimates of seedling emergence and potential for annual recruitment in most instances reflect those observed in the field. However, the estimates are only for a period of one year and are probably overly conservative regarding the amounts of seed-loss due to bruchids and granivores because longer-term projections based on the estimates would produce much larger seed banks than those that were observed. Although it has been demonstrated that the hard seeds of mesquite are capable of remaining dormant for several decades resulting in the accumulation of large seed banks, this appears not to be the case in South Africa as the cessation of seed input has been shown to result in the depletion of the seed bank within a few years.

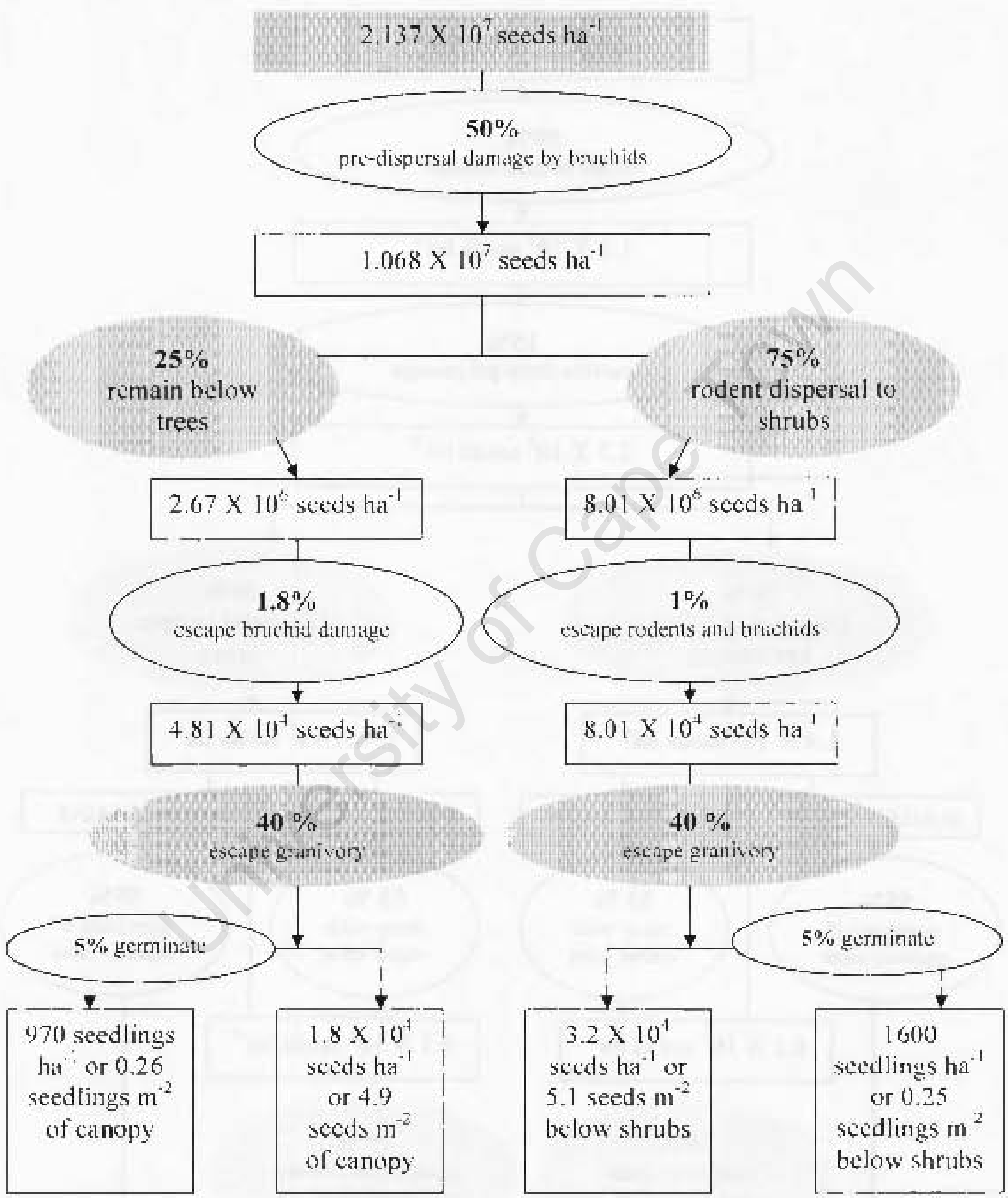
SOUTH-WEST REGION: LIVESTOCK

$2600 m^2$ of canopy in 1ha @ 7700 seeds m^{-2}



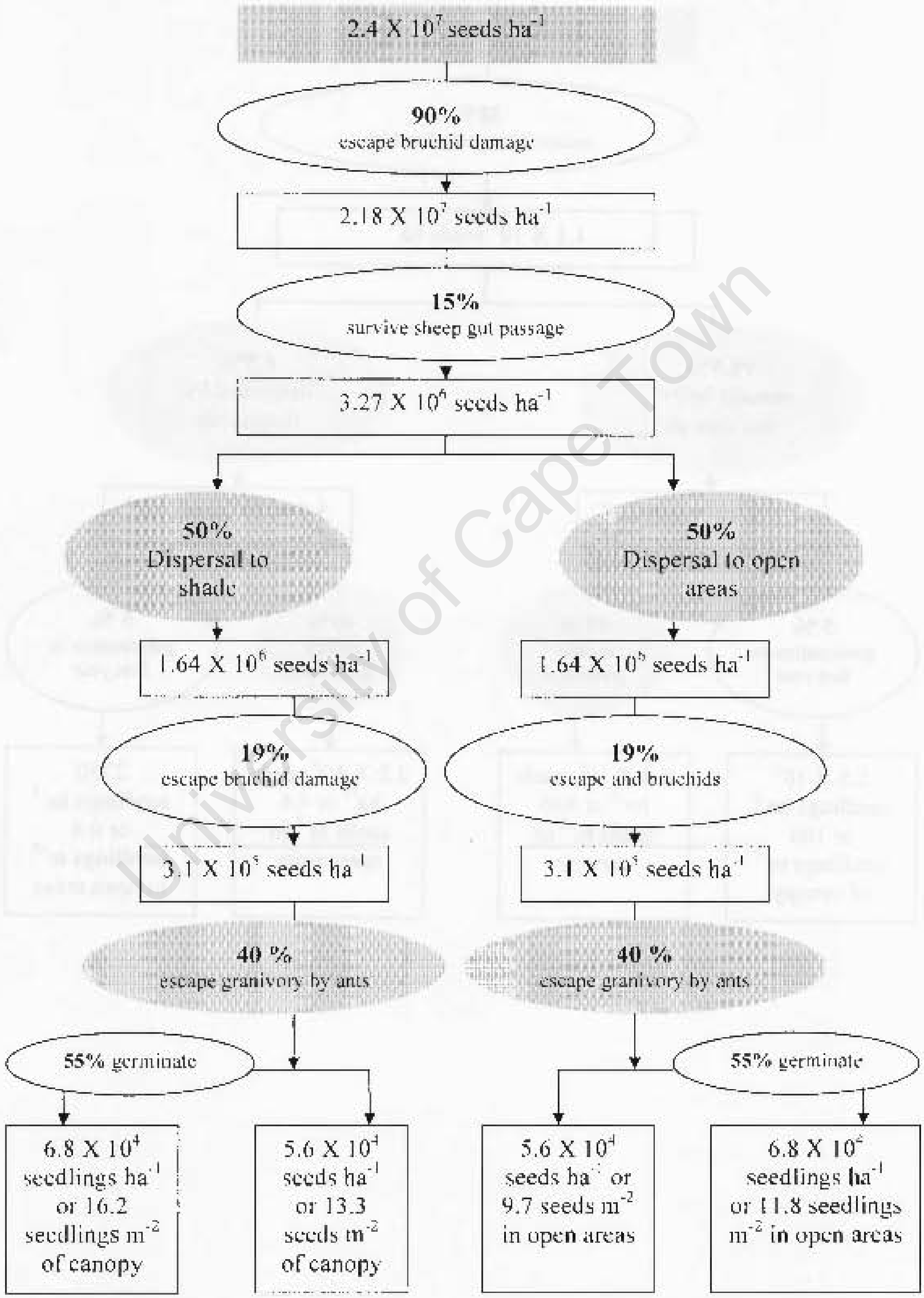
CENTRAL REGION: NO LIVESTOCK

$3700 m^2$ of canopy in 1ha @ 5775 seeds m^{-2}



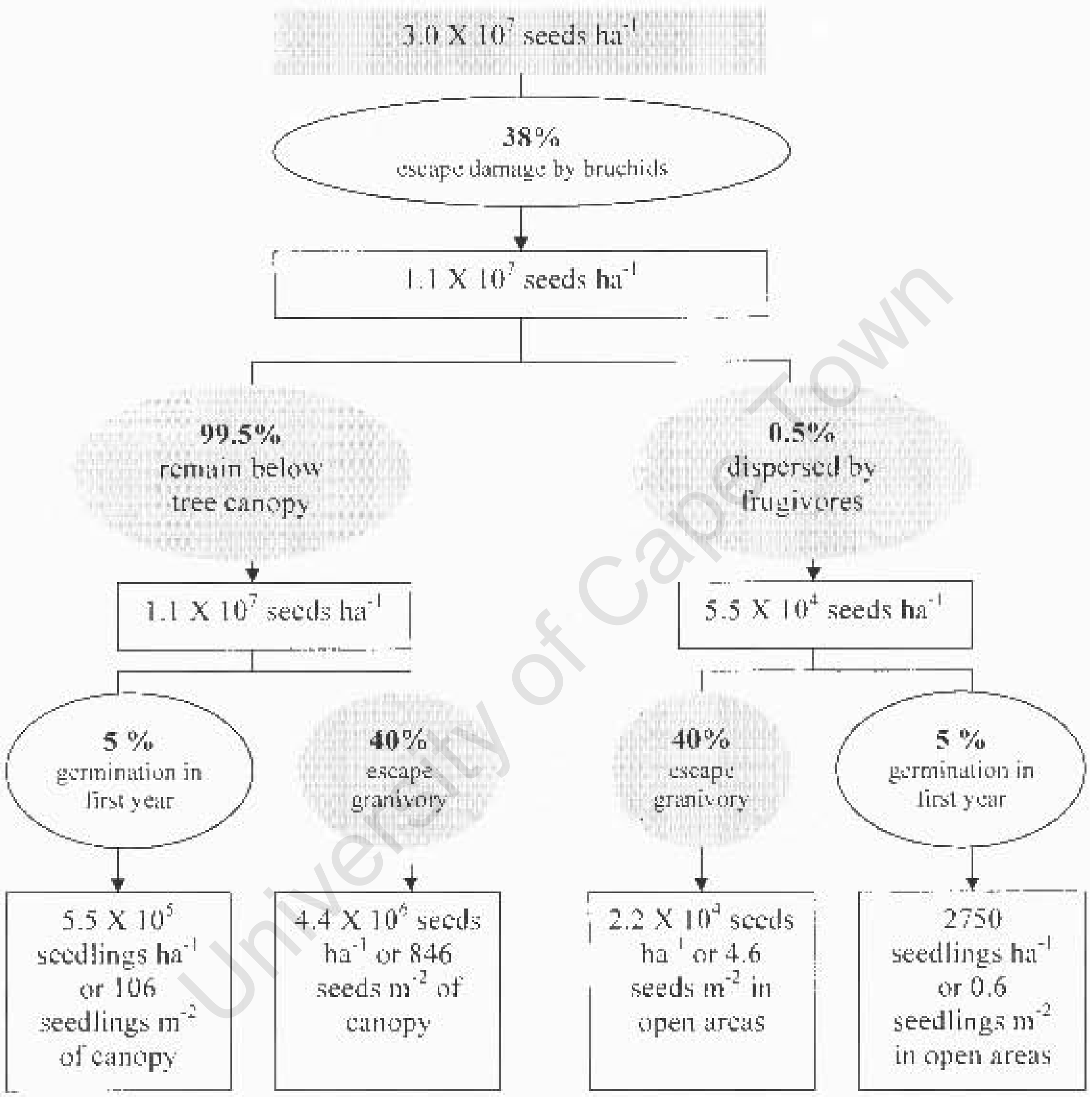
CENTRAL REGION: LIVESTOCK

4200m^2 of canopy in 1ha @ 5775 seeds m^{-2}



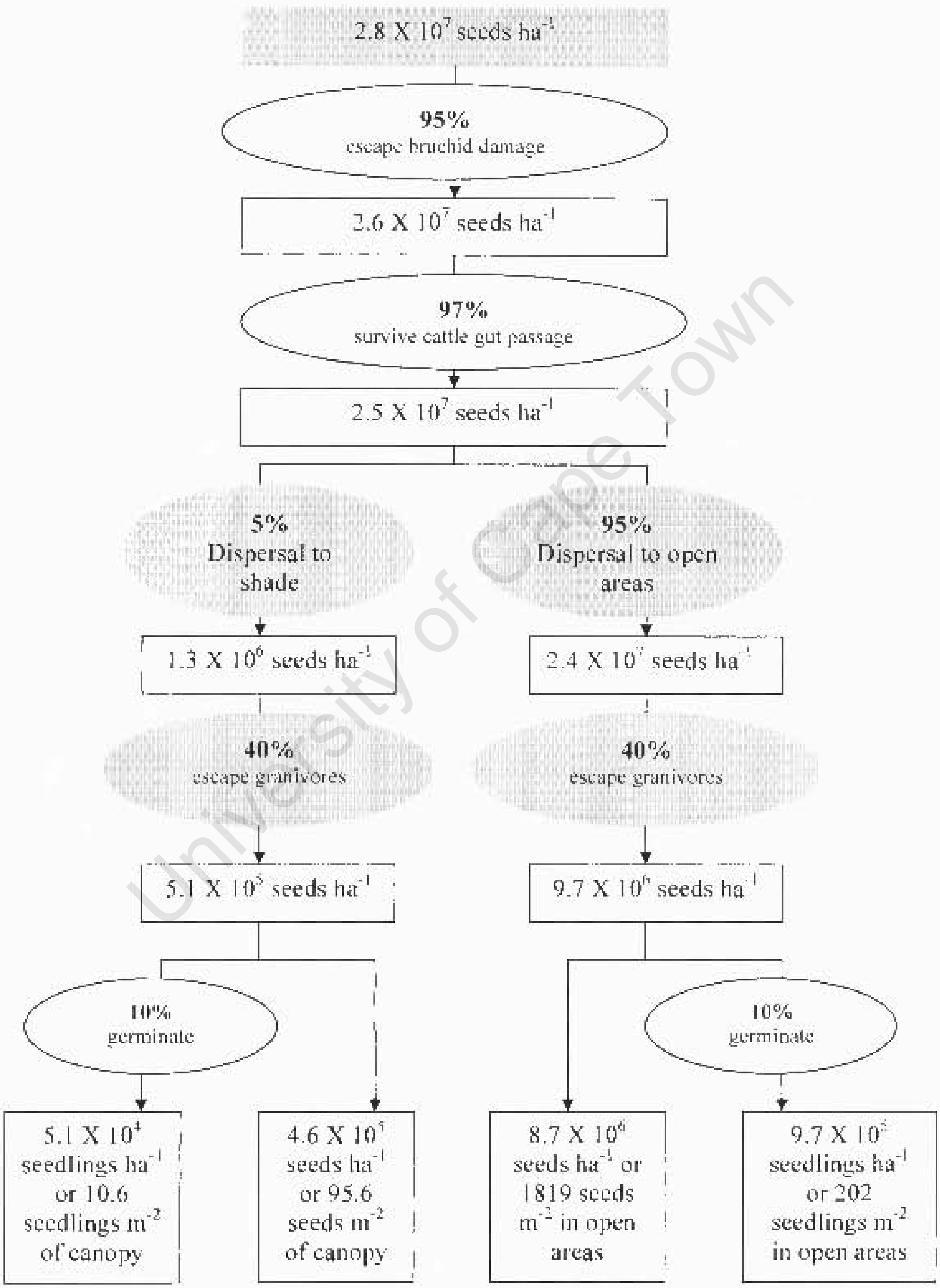
NORTH-EAST REGION: NO LIVESTOCK

5200m^2 of canopy in 1ha @ 5775 seeds m^{-2}



NORTH-EAST REGION: LIVESTOCK

$4800m^2$ of canopy in 1ha @ 5775 seeds m^{-2}



In the absence of livestock, I feel the introduction of *A. prosopis* for the control of mesquite in xeric areas would have added to the few success stories of a seed-feeding insect having a noticeable effect on the populations of a long-lived perennial weed. Despite total recruitment failure in most years in arid regions, *A. prosopis* has reduced the size of the soil seed banks sufficiently to prevent high levels of recruitment in years of favourable rainfall. Although mesquite is long-lived and once established an individual plant will persist for several decades, recruitment levels and rates of spread of the weed have been reduced dramatically as a result of the introduction of *A. prosopis* in the arid areas. However, both of the agents have failed to have any impact in the mesic region as high numbers of seed escape bruchid damage annually and recruitment in this region appears to be continuous.

Despite the ability of *A. prosopis* to utilise dung-borne seed dispersed in the field, within and away from mesquite infestations, a large number of seeds "escape" bruchids and enter the seed bank. Although individual seeds do not appear to persist for many years, seed banks are maintained through annual replenishment, resulting in sufficient seedling recruitment in favourable years to allow densification and spread of the weed. Where livestock are present mesquite continues to be site limited, throughout its distribution in South Africa.

Despite *A. prosopis* achieving higher levels of damage to annual seed production in the presence of livestock than was originally presumed, these are not sufficient to curb the spread of mesquite and there is a need to introduce additional agents. The current host specificity research on the Apionid, *Coelocephalapion gandolfoi*, that damages seeds in the green pods, looks promising as it can develop on pods of several *Prosopis* species in the Algorobia section (Witt. *pers. comm.*) and the two main invasive species in South Africa are *P. velutina* and *P. glandulosa* var. *torreyana*, both in this section. The benefit of this agent will be to reduce the seed available to livestock. Another potential agent is an *Asphondylia* species (Diptera: Cecidomyidae), a mesquite gall midge that develops in the flower buds preventing the formation of pods. Although flower gallers are not considered to be ideal agents because of high levels of parasitoids they acquire (Harris & Shorthouse, 1996; Macfadyen & Spafford Jacob, 2003) and also the fact that most flowers produced by mesquite trees abort anyway, there have been instances of seed production of perennial weeds being markedly reduced such as with *Dasineura dielsi* on *Acacia cyclops* in South Africa (Hoffmann, *pers comm.*).

It is hoped that if these agents are introduced and became established the pod production of mesquite will be reduced sufficiently and there will be less desire by landowners who view mesquite as a valuable resource for livestock fodder to retain mesquite on their properties, giving support for the introduction of additional agents that will damage the vegetative parts of plants (Moran *et al.*, 1993). A leaf tying moth, *Evippe* sp., in Australia causes high levels of defoliation and reduced plant vigour of mesquite (van Klinken *et al.*, 2003) and this species should be one of the primary agents considered as and when stakeholders concur.

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Appendix 1.1

Numbers of *A. prosopis (Ap)*, *N. arizonensis (Na)* and larval parasitoids (para) per 1000 seeds emerging from pod collections in 2002 and 2003. Also the percentage of combined *A. prosopis* and parasitoids, of *N. arizonensis* and parasitoids and of total insects emerging that were parasitoids. Samples from survey dates where no parasitoids emerged are not included.

Site	Date	# of *A.prosopis* per 1000 seeds	# of *N.arizonensis* per 1000 seeds	# of parasitoids per 1000 seeds	Percentage of combined Ap+para that were parasitoids i.e. # para/(#Ap + # para)*100	Percentage of combined Na+para that were parasitoids i.e. # para/(#Ap + # para)*100	Percentage of total insects that were parasitoids
Driefontein	Feb 02	39.52	3.33	0.16	0.41	4.70	0.38
	Mar 02	106.49	5.29	0.35	0.33	6.22	0.31
	Apr 02	168.82	21.34	0.34	0.20	1.57	0.18
	May 02	145.15	36.14	4.43	2.97	10.93	2.39
	Jul 02	144.20	32.56	12.04	7.70	26.99	6.37
	Aug 02	112.62	10.79	7.45	6.20	40.84	5.69
	Oct 02	149.16	1.42	2.16	1.43	60.28	1.41
	Jan 03	88.31	3.40	5.63	6.00	62.36	5.79
	Apr 03	205.14	13.99	7.99	3.75	36.35	3.52
	Jul 03	69.38	6.20	0.17	0.24	2.61	0.22
	Sept 03	83.61	2.94	3.43	3.94	53.85	3.81
Clanwilliam	Feb 02	154.59	2.66	1.07	0.69	28.63	0.67
	Mar 02	117.91	3.23	0.05	0.05	1.64	0.04
	Apr 02	132.38	5.53	0.12	0.09	2.18	0.09
	Jul 02	87.75	1.21	0.19	0.21	13.31	0.21
	Dec 02	63.92	5.10	2.14	3.24	29.54	3.00
	Dec 03	80.02	0.98	0.43	0.53	30.22	0.52
Calvinia	Apr 03	132.62	2.37	0.18	0.13	6.91	0.13
	May 03	7.81	2.95	0.28	3.46	8.66	2.54
	Oct 03	156.88	20.55	2.89	1.81	12.33	1.60
	Nov 03	123.85	52.53	5.89	4.54	10.09	3.23
	Dec 03	163.15	15.73	0.45	0.28	2.78	0.25
	Feb 04	125.30	1.21	2.09	1.64	63.41	1.63
	Jul 04	35.64	4.51	0.11	0.31	2.37	0.27
Citrusdal	May 03	88.68	16.89	0.41	0.46	2.38	0.39
	Sept 03	173.96	7.63	2.14	1.22	21.92	1.17
	Nov 03	108.29	3.36	0.36	0.33	9.62	0.32
Vanwyk svlei	Feb 03	141.19	0.74	0.26	0.18	25.75	0.18
	Jun 03	156.15	3.21	0.37	0.24	10.32	0.23
	Oct 03	51.63	8.54	0.21	0.41	2.44	0.35
Prieska	Jun 03	130.50	5.51	0.21	0.16	3.66	0.15
Modder River	Feb 03	56.67	0.11	0.32	0.56	74.89	0.56
	Nov 03	101.72	4.49	0.21	0.21	4.47	0.20

Appendix 2.1:

Collection/placement dates of dung samples in the field for shaded and exposed treatments. Four ranges of distance placements exist for the exposed treatments: 10-14m, 15-19m, 25-30m and 170m.

Collection dates	Treatment: Below tree – Shade	Treatment: Below tree – Morning sun	Treatment: Exposed – Sun	Treatment: Exposed – Shade cloth
22-Aug 2003	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m	
22-Sep 2003	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m	2 @ 10-14m, 3 @ 15-19m, 2 @ 25-30m
30-Oct 2003	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m	2 @ 10-14m, 3 @ 15-19m, 2 @ 25-30m
21-Nov 2003	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m	2 @ 10-14m, 3 @ 15-19m, 2 @ 25-30m
30-Dec 2003	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m	2 @ 10-14m, 3 @ 15-19m, 2 @ 25-30m
27-Jan 2004	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m	2 @ 10-14m, 3 @ 15-19m, 2 @ 25-30m
29-Feb 2004	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m	2 @ 10-14m, 3 @ 15-19m, 2 @ 25-30m
13-Apr 2004	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m	2 @ 10-14m, 3 @ 15-19m, 2 @ 25-30m
16-May 2004	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m	2 @ 10-14m, 3 @ 15-19m, 2 @ 25-30m
21-Jun 2004	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m, 2 @ 170m	2 @ 10-14m, 3 @ 15-19m, 2 @ 25-30m
26-Jul 2004	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m, 2 @ 170m	2 @ 10-14m, 3 @ 15-19m, 2 @ 25-30m
6-Sep 2004	10	2	5 @ 10-14m, 2 @ 15-19m, 3 @ 25-30m, 2 @ 170m	2 @ 10-14m, 3 @ 15-19m, 2 @ 25-30m