Chapter 1

1. General introduction

1.1. Biological control of invasive alien plants

Biological control refers to the action of parasites, predators and herbivores in maintaining a pest (weed or insect) population at lower average densities than would occur in their absence (DeBach, 1964). It has been in practice for over a century worldwide (Keane and Crawley, 2002) and has been successfully employed against invasive alien plants, using insects and pathogens as control agents. Invasive alien plant species spread rapidly and become abundant in their non-native habitats partly because their co-evolved specialist herbivores and pathogens are absent (Hierro *et al.,* 2005; Keane and Crawley, 2002; Lake and Leishman, 2004; Strong *et al.,* 1984). In addition, a competitive advantage obtained from faster growth especially in disturbed areas, enables invasive (often ruderal) plant species to flourish in new habitats (Hierro *et al.,* 2005; Lake and Leishman, 2004), especially if a disturbance, for example fire, increases the availability of resources such as light and soil moisture (Lake and Leishman, 2004). Furthermore invasive plants may differ from native plants in resource acquisition, e.g. light and nutrients, hence they change ecosystems by altering the structure and composition of the plant community (Witkowski, 1991; Witkowski and Wilson, 2001).

Numerous weed biological control studies worldwide have evaluated the actual damage caused by biological control agents on the target weed (for example, the number of seeds or shoots destroyed) e.g. (Hoffmann and Moran, 1991; Moran *et al.,* 2003). It has been noted that the introduction of agents that damage weeds does not guarantee a change in a weed's population dynamics (Hoffmann 1990; Hoffmann and Moran 1991). Therefore one cannot deduce that successful biological control results simply from establishment of the agent and signs of damage on the plant. However one can assume successful biological control if the weed density and spread declines over time (Hoffmann 1990; Hoffmann and Moran 1991). For example the successful biological control of *Sida acuta* (Malvaceae) by *Calligrapha pantherina* Stal (Coleoptera: Chrysomelidae) in Australia was reported to be successful following a reduction in the density of the target weed by

up to 99% of the original densities within 10 years of release (Flanagan *et al.*, 2000). In Canada the biological control programme on diffuse knapweed (*Centaurea diffusa*) Lam (Asteraceae) was declared successful due to the reduced density of the weed following release of *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) from the beginning of 2000 onwards (Myers, *et al.*, 2007).

Biological control programs on weeds such as *Chromolaena odorata* (Asteraceae) and *Lantana camara* (Verbenaceae) in most tropical countries were considered failures by 1998 (McFadyen, 1998). These weeds are difficult to control biologically either because few or no agents established after release (Crawley, 1989), or the agent established but failed to succeed due to resistance of the target weed to the specific damage caused (Myers, 2000), such as in the case of *L. camara* in South Africa. Over 21 biocontrol agents had been released against *L. camara* since 1961 but only eight agents were reported to have established by 1999, and these failed to successfully control the weed (Baars and Neser, 1999). Inadequate insect establishment may be attributed to indigenous parasites or predators (Myers and Risley, 2000), which often attack poorly concealed endophagous insects more than those which are well hidden (Hawkins, 1990). Poorly concealed endophagous insects are in most cases unable to move away from chemical cues which are evidence of their feeding and are easily detected by attackers (Hill and Hulley, 1995). Although South African scientists have not broadly studied the effects of indigenous parasitoids on populations of introduced agents, it has been proposed that their influence is generally not strong enough to prevent establishment of introduced agents (Hill and Hulley, 1995).

Predators on the other hand may cause greater damage than parasitoids and are thus more implicated in influencing the outcome of biological control of weeds. Predation may not only deter establishment of introduced agents but may also decrease populations of already established biocontrol agents on a given weed (Goeden and Louda, 1976). For example in South Africa, insect predators such as the native coccinellid *Exochomus flavipes* Thunberg (Coleoptera: Coccinellidae) and an introduced coccinellid *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) reduced the

effectiveness of the cochineal insect *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) which was released against Prickly pear cacti (Pettey, 1948).

Recently, Hoffmann, (2007) urged that in order for scientists to avoid unnecessarily discrediting a given biological control agent when assessing success or failure, they should primarily consider what the specific weed problem would be like if the agent had not been introduced in the first place. In addition to considering what the weed problem would be like without the agent, it is important to be explicit about what measure is used to describe success in the first place.

1.2. Biological control in South Africa

South Africa, through the "Working for Water" program among others, has embarked upon a countrywide biological control campaign in an attempt to reduce the density of invasive alien plants in catchments, river courses and conservation areas (Moran *et al.*, 2005). The WfW program placed special emphasis on biocontrol following poor results from their initial aim (i.e. clearing alien trees through mechanical and chemical methods) which showed re-growth of weed populations from seeds in previously cleared areas (Moran *et al.,* 2000). By 2004, more than 117 biological control agents had been released against 49 weeds (Klein, 2004) in South Africa since 1913 (Olckers, 1999). Ideally, following two or more years of release of a biological control agent against its target weed, post release evaluations should be carried out to investigate the success or failure of the released agent against the target weed (Radford *et al.,* 2001). Post release evaluations therefore explore the performance of the agent, seeking to explain the factors that influence the impacts of the agent against a given weed (Radford *et al.*, 2001). The study presented here evaluates the impacts of *Sulcobruchus subsuturalis* (Pic) (Coleoptera: Bruchidae) on *Caesalpinia decapetala* (Roth) Alston within release areas in South Africa.

1.2.1. Taxonomy and description of *Caesalpinia decapetala*

There are approximately 100 tree species in the genus *Caesalpinia* growing in scrub rainforest in tropical and subtropical areas (China, Japan, Malaysia and India) and in

lowland rainforest in New South Wales in Australia (Polhill and Vidal 1981; Starr, *et al.*, 2003). *Caesalpinia decapetala* (native to China, Japan, Malaysia and India) is commonly known as “Mauritius Thorn” and “Mysore thorn” in South Africa and India respectively (Coetzer and Neser, 1999). It is a tremendously thorny, antagonistic perennial climbing shrub belonging to the family Fabaceae and the subfamily *Caesalpinioideae* (Fig 1.1). This evergreen woody species can climb trees over 10m high (Henderson, 1995). It forms large impenetrable thickets which can impale animals. For example, in Hawaii where *C. decapetala* was introduced as an ornamental fence/barrier plant, especially for ranches in 1888, a cow was found suspended dead on a thicket four feet high with its legs sticking straight up in the air (Starr, *et al.,* 2003). The shrub has become invasive in Australia, the United States of America, East Africa and South Africa (Coetzer, 2000).

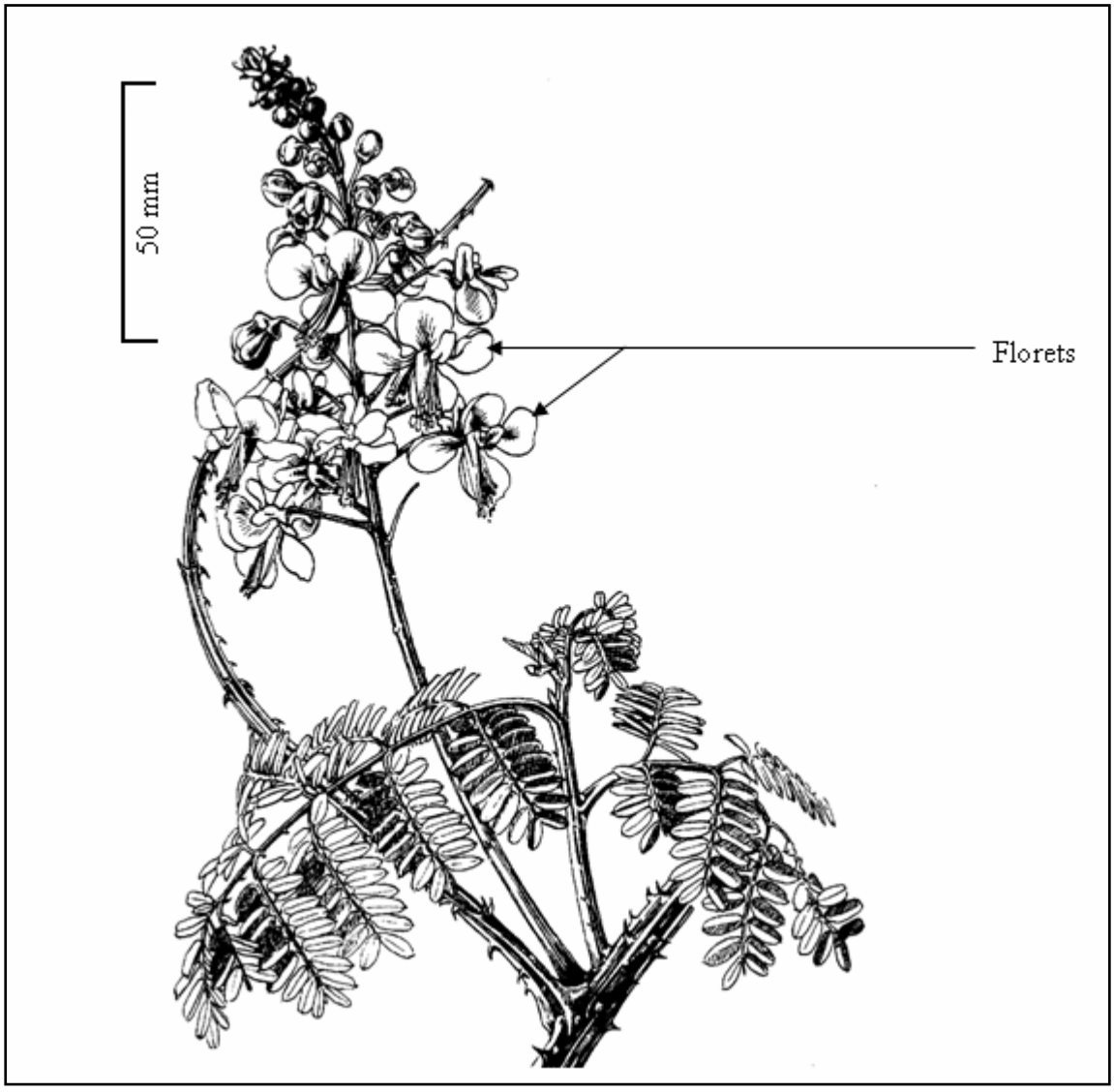


Figure 1.1: Diagram of *Caesalpinia decapetala* showing thorny branches and florets forming the inflorescence with a raceme pattern. (Drawing by: B. Connell, National Botanical Institute, Pretoria) (Henderson, 1995).

1.2.2. Biology and ecology of *C. decapetala*

Caesalpinia decapetala bears pale yellow flowers which are pollinated by various species of bees and other insects (Fig 1.2). The flowers are produced in long racemes from winter to spring (pers. obs; Turnbull, 2002). Because of the long period of flowering, this species is likely to have a very large total reproductive output and hence immense colonization potential (Lake and Leishman, 2004). The weed produces ellipsoid small

dark brown seeds, 8-12mm in length and 6-8mm in width. The woody leguminous pods containing seeds are produced between spring and summer (pers. obs; Turnbull, 2002). According to Turnbull (2002) *C. decapetala* seeds remain viable in the soil for up to 10 years. The cotyledon and embryo are enclosed in a hard (impermeable) seed coat which is typical of most legume species, which requires scarification prior to germination (Baskin & Baskin, 1998). Impermeable seed coats are scarified to facilitate more rapid germination under laboratory conditions (Baskin & Baskin, 1998). However past germination studies that have used scarified seeds have not elaborated how and when seed permeability and hence germination is controlled under natural conditions (Baskin & Baskin, 1998).

Figure 1.2: *Caesalpinia decapetala* flowers produced in long racemes between winter and spring as well as the compound leaves of this shrub. Note a potential pollinator on the flower (red circle). The photo was taken at a biocontrol agent release site in Ferncliffe Nature Reserve, KwaZulu-Natal province in June 2006.

1.2.3. *Caesalpinia decapetala* in South Africa

Although the year of the initial introduction of *C. decapetala* into South Africa is not known, the plant was grown for hedge purposes alongside other indigenous plants such as *Acacia ataxacantha* DC., *Dovyalis caffra* (Hook. F. & Harv.) and *Carissa macrocarpa* (Eckl.) DC. (Coetzer and Neser, 1999). *Caesalpinia decapetala* has been invasive in

South Africa since the 1960s, but was only officially declared a weed in 1983 (Coetzer and Neser, 1999). Today, the plant is a declared weed (Henderson, 2001), and threatens, amongst other things, the agricultural industry by occupying grazing land and injuring livestock. It invades commercial plantations of timber and natural areas such as riverine habitats, riparian vegetation, forest margins and savannas in the moist eastern parts of the country (Coetzer and Neser, 1999; Coetzer, 2000). The weed has invaded provinces of Limpopo Province, KwaZulu-Natal, Mpumalanga and the Eastern Cape (Coetzer and Neser, 1999; Henderson, 2001) (Fig 1.3 and 1.4). By 1998, *C. decapetala* was ranked number 20 out of a total of 25 invader species in South Africa and was estimated to utilize 33.82 million m^3 of water per annum (Versfeld *et al.*, 1999).

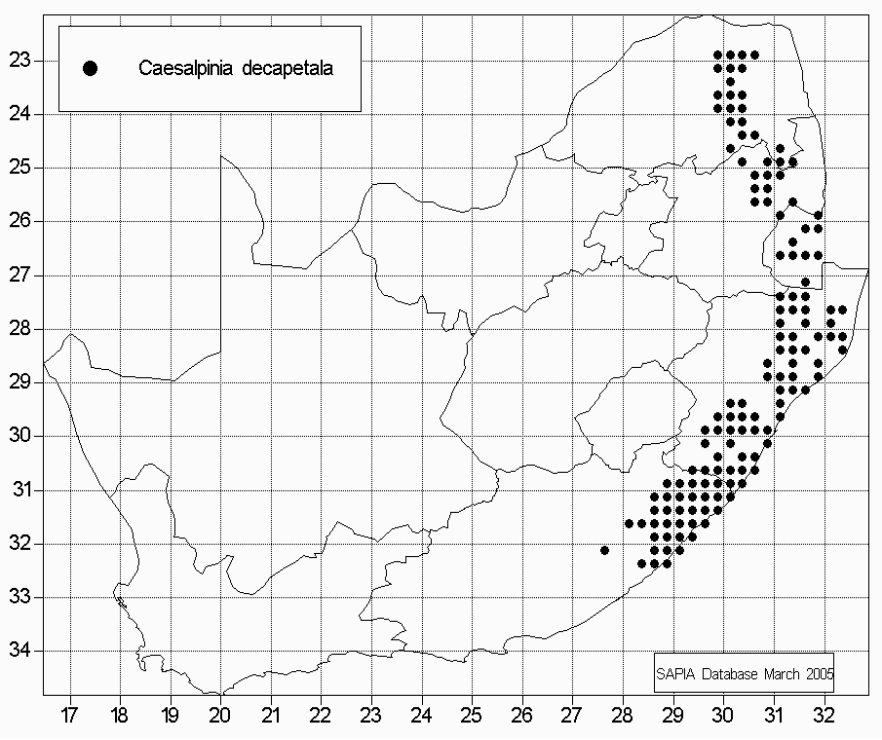


Figure 1.3: Distribution of *Caesalpinia decapetala* in South Africa. Each dot indicates presence within a ¼ degree grid. Source: SAPIA Database (Henderson, 2001).

Figure 1.4: A dense impenetrable *Caesalpinia decapetala* thicket at a biocontrol agent release site in Ferncliffe Nature Reserve, Kwazulu-Natal province, South Africa. Photo was taken in July 2006.

1.2.4. The seed feeder *Sulcobruchus subsuturalis*

Sulcobruchus subsuturalis (Fig 1.5), a seed feeding bruchid beetle was introduced into South Africa from India in 1996 to reduce the density of the invasive weed *C. decapetala* (Fig 1.4) (Coetzer, 2000). Although *S. subsuturalis* fed on *C. decapetala* seeds in India (Coetzer, 2000), according to Anton (1999), *S. subsuturalis* was originally first recorded developing in the seeds of *Dalbergia candenatensis* (Dennst.) Prain (Papilionaceae) and *Moullava spicata* (Dalz.) Nicolson (Caesalpiniaceae). This raises uncertainties as to whether *C. decapetala* is indeed the beetle's preferred host. Both *D. candenatensis* and *M. spicata* are perennial shrubs belonging to the family Fabaceae. Like *C. decapetala* both species are natives of Tropical Asia.

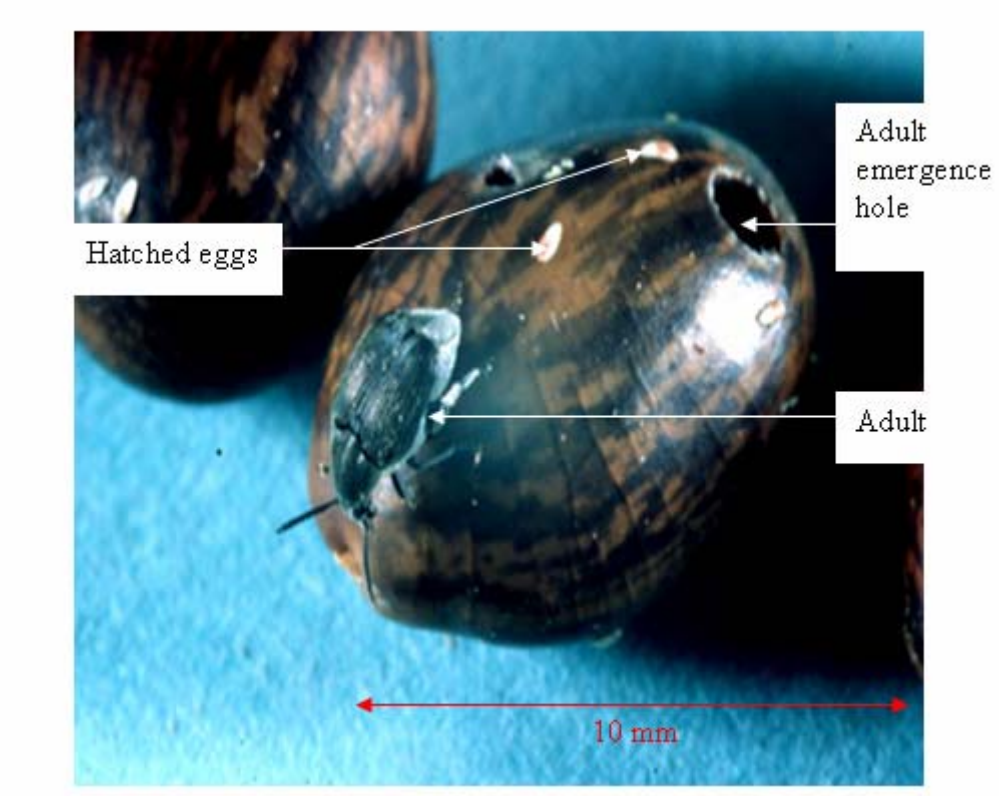


Figure 1.5: *Sulcobruchus subsuturalis*, a seed feeding bruchid beetle emerging from a seed of its host, *Caesalpinia decapetala*. Note the pale coloured hatched eggs attached to the seed.

Sulcobruchus subsuturalis adults are ± 4mm long, black with fine grey hair and are reported to live up to 65 days (Coetzer, 2003). Each female deposits a total of 73 to 111 eggs (Coetzer, 2003) on *C. decapetala* seeds. Incubation lasts about eight days and the immature stages take approximately 35 days to develop. The release of *S. subsuturalis* for biological control of *C. decapetala* in South Africa has been ongoing since 2000. Releases were undertaken each year from February 2000 to December 2006, but until this study, no post release evaluations had ever been conducted to examine the establishment or efficacy of the agent against the weed.

There is no literature concerning the biology of *S. subsuturalis* in relation to *C. decapetala*. Coetzer (2000) assumed that *S. subsuturalis* adults overwinter inside the walls of dehisced *C. decapetala* pods. Seeds which have been infested by the beetle exhibit white egg-case spots and adult emergence holes in the seed (between 1 and

1.5mm in diameter) signifying presence of larvae, pupae and adults. The beetle completes its lifecycle inside seeds and up to seven adults can emerge from a single seed (Kalibbala, 2005). According to Coetzer (2003), only mature seeds in dehisced pods are attacked by the beetle.

1.3. Seed feeding biological control agents

Seed feeders acting alone have a limited chance of reducing perennial weed density (Hoffmann and Moran, 1991; Myers and Risley, 2000), because the majority of plants produce a lot more seeds than are needed to replace an entire adult plant population and thus only extremely high levels of seed destruction can prevent regeneration (Kriticos *et al.,* 1999; Neser and Kluge, 1986). Hence plants are generally not seed limited. Furthermore, improved seedling survival due to reduced intra-specific competition may compensate for the reduction in seed densities, thus keeping the plant density high (Myers and Risley 2000). For example, the simulation model used by Myers and Risley (2000) on diffuse Knapweed, *Centaurea diffusa,* showed that the biocontrol agents (beetles and gall flies) were able to reduce seed production but due to increased seedling survival, the plant persisted. Nevertheless, releases of an additional biocontrol agent, *L. minutus* from 2000 onwards resulted into successful control of *C. diffusa* by 2007 (Myers, *et al.,* 2007).

Interference from herbivores (livestock and wild mammals), parasitoids and interspecific competition (Impson *et al.,* 1999) may also minimize chances of agent establishment. For example the two bruchid beetles *Algarobius prosopis* LeConte (Coleoptera: Bruchidae) and *Algarobius bottimeri* Kingsolver (Coleoptera: Bruchidae) which were introduced to South Africa in 1986 and 1989 respectively, against Mesquite, *Prosopis glandulosa var. torreyana* (Zimmermann, 1991), failed to establish (Hoffmann *et al.,* 1993). Herbivores feed on pods as soon as they fall onto the ground, denying seed attackers the opportunity to colonize the seeds inside the pods for biological control purposes (Moran *et al.,* 1993). Nevertheless, seed attackers can generally interfere with the potential of a specific plant species to reproduce even though other important elements of the plant remain intact and the entire population of the plant may not be affected (Andersen, 1989; Hoffmann and

Moran, 1991; Impson *et al.,* 1999; Witkowski and Garner, 2000; Botha *et al.,* 2004). Because only the weed's reproductive potential is compromised, the time period between agent introduction and weed density decline is usually long. Also, the level and durability (longevity) of the soil seed bank and the rate of death of surviving plants all determine the rate of weed density decline (Hoffmann and Moran, 1991; Witkowski and Wilson, 2001).

1.4. Vegetative and reproductive phenology

The term phenology refers to the seasonal timing of life cycle or biological events within a given year (Rathcke and Lacey, 1985). Time of occurrence, duration, amount of synchrony within a population and shape of the curve of events versus time are all factors that can describe phenological events (Rathcke and Lacey, 1985). Studies pertaining to plant phenology can either be at community, population, species or individual level and usually focus on reproductive (bud forming, flowering, fruiting and seed germination) and vegetative (leaf flushing and shedding) events (Sakai *et al.,* 1999). The seasonal timing of life cycle events e.g. flowering may vary among species and regions as a result of abiotic factors which directly (by hindering flower production) and indirectly (by affecting pollen vectors) limit flowering seasons. For example in the temperate regions flowering is limited during spring and autumn due to winter frost. In the seasonal neotropics many shrubs flower in the rainy season whereas trees flower in the dry season. Generally, the onset of flowering is initiated by three physical environmental factors namely, photoperiod, temperature and moisture (Rathcke and Lacey, 1985). In this study, examination of the phenology of *C. decapetala* and particularly when the plant produces seeds should indicate the appropriate time to release the seed feeding beetle.

1.5. Seed banks

Seeds stored in the canopy and under the canopy in the soil (on the soil surface and buried beneath it), all make-up the seed bank, which is a fundamental component of plant population dynamics (Witkowski *et al.,* 1991; Witkowski, 1994; Auld, 1995; Witkowski and Garner, 2000). When seeds are dispersed from the tree canopy, they may either remain on the surface of the soil or sink beneath it (Rotundo and Aguiar, 2004). Seeds

under a given tree canopy are therefore distributed vertically and horizontally in the environment. The deeper seeds are buried in the soil, the longer they tend to survive (Conn and Farris 1987; Witkowski and Wilson, 2001). This is because buried seeds rarely germinate due to poor germination conditions (lack of light and warm well-aerated moist soil). Depending on the burial depth, they would also survive various disturbances such as herbicide application as well as hot fire and diurnal soil surface temperature extremes (Thompson, 1992; Mbalo and Witkowski, 1997). Moreover the probability that seed attacking biological control agents may not reach buried seeds is high. In order to estimate seed production and the level of beetle establishment, this study focused on seeds from three sources: seeds within pods (pre-release canopy seeds) on the tree, seed rain (falling seeds) and seeds on and in the ground (vertical and horizontal soil seed bank). It is essential to understand the nature of soil seed banks if potential seedling recruitment events are to be identified (Auld, 1995). On the other hand, for some species some mature seeds remain in the tree canopy for extended periods of time (serotiny), building up the seed bank in the canopy (Lamont *et al.,* 1991), known as canopy seed storage. Once they are eventually released, they may germinate immediately, depending on the suitability of the conditions (Cowling and Lamont, 1987). However, if indeed *S. subsuturalis* attacks *C. decapetala* seeds within the pods in the canopy, it is highly probable that far fewer of these infested seeds will survive, and even fewer still will germinate, emerge and establish as seedlings after they drop on the soil surface, hence good seedling recruitment would not be expected under these circumstances.

1.6. Seedling recruitment

According to Andersen, (1989), seed densities in the soil may not necessarily determine actual levels of recruitment. This is because the densities of some species are not limited by seed numbers, but rather by availability of microsites or “safe sites” (Andersen, 1989; Lamont *et al.*, 1993). For such species, if suitable microsites are available, a seed feeder may fail to reduce target plant populations despite high proportions of damaged seeds (Szentesi and Jermy, 2003; Kean and Crawley, 2002). Seed predation, seed dispersal and disturbance determine seed and microsite availability (Eriksson and Ehrlein, 1992). Usually seedling establishment is attributed to successful dispersal of a seed to a suitable

site (microsite), followed by germination and survival of various disturbances (Eriksson and Ehrlein, 1992).

Seeds buried in shallow parts of the soil (1-2cm deep) germinate readily under appropriate conditions (warm well-aerated moist soil) (Schafer and Chilcote, 1970). However there is normally immense competition for resources at that soil depth thus seedlings may fail to establish in large numbers (Thompson, 1992), despite being part of a large seed bank.

1.7. Seed germination

Germination periods vary among plant species. For some species, germination is limited to either autumn, spring or the wet season whereas other species can germinate throughout the year (Baskin and Baskin 2001). Germination is influenced by factors such as temperature, moisture and light (intensity and quality) among others (Mayer and Poljakoff-Mayber, 1975). Soil temperature can be determined by insolation, ambient temperature, soil texture and structure, soil depth, water quantity, water evaporation conditions in the soil and plant cover (Mayer and Poljakoff-Mayber, 1975). The moisture content also varies between different soil types and at different times of the year. In desert ecosystems, soil moisture directly stimulates germination as well as the survival of seedlings (Mayer and Poljakoff-Mayber, 1975).

The abundance of light is normally limited to the soil surface unless the soil is covered by clear water (Mayer and Poljakoff-Mayber, 1975). The importance of light as a requirement for germination may differ depending on the status of the seed (dormant or nondormant) and the season (Baskin and Baskin 1988). For most plant species, if the seeds are not dormant, germination can occur either in light or darkness (Baskin and Baskin 1988). For some species seed germination may be greater in dark conditions (Baskin and Baskin 1988). Meanwhile seeds of some species such as the common milkweed, *Asclepias syriaca* L. strictly need light to germinate and therefore germination conditions are more suitable in spring following exposure to mild winter temperatures (Baskin and Baskin, 1977). Conversely, seeds of other species germinate more

favourably in autumn following exposure to high summer temperatures (Baskin and Baskin, 1982; Baskin and Baskin, 1988).

The initial appearance of a seedling at the soil surface is referred to as seedling emergence (Forcella *et al.*, 2000). Soil water, soil temperature and light quality are among the factors affecting the emergence of seedlings (Forcella *et al.*, 2000). In this study, *S. subsuturalis* attack on seeds could also be held accountable for influencing seed germination and hence seedling emergence. The beetles feed on the entire cotyledon usually leaving behind an empty husk. The possibility of feeding on all internal organs of the seed increases as the number of beetles in the seed increases therefore limiting germination (Kalibbala, 2005).

1.8. Study sites and release strategies

There is a total of 233 *S. subsuturalis* release sites within the *C. decapetala* distribution area in South Africa. Fieldwork was conducted at 25 selected release sites (*C. decapetala* sites where *S. subsuturalis* larvae/or adults were released). The number of sites sampled varied among provinces. Between February 2006 and June 2007, three release sites were sampled in KwaZulu-Natal; 12 in Limpopo; three in Mpumalanga; and seven in the Eastern Cape. All sites sampled in Kwazulu-Natal and Mpumalanga are shown in table 1.1. However because Limpopo and Eastern Cape had so many sites (12 and seven respectively) in close proximity to one another, only those sites from which seeds were recovered are listed (Table 1.1 and Fig. 1.6).

Table 1.1: Geographical position of selected study sites including their respective mean monthly minimum and maximum temperatures and the number of agents released at specific times. Temperature data were provided by South African Weather Service. *** = data missing.

Site Location	Province	GPS coordinate	Mean monthly min. temp (ºC)	Mean monthly max. temp (ºC)	No. of agents released	Last release date
Bodupe	Limpopo	S23º 39' 19.7" E30º 15' 49.40"	15.8	25.8	17500	12/12/2006
Moshakga 1	Limpopo	S23º 39' 34.3" E30º 15' 55.60"	15.8	25.8	20500	21/12/2002
Moshakga 2	Limpopo	S23º 39' 42.0" E30º 15' 09.00"	15.8	25.8	19000	31/12/2002
Boughton	KwaZulu-Natal	S29° 36' 10.0" E30° 19’ 43.10"	13.3	26.1	2000	3/1/2003
Ferncliffe	KwaZulu-Natal	S29° 33' 55.2" E30° 19’ 51.7"	13.3	26.1	900	14/05/2001
Mtubeni valley	KwaZulu-Natal	S29° 53’ 59.9" E30° 06’ 44.0"	***	***	1917	30/12/2003
Nelsriver Bridge	Mpumalanga	S25º 25' 54.8" E30º 58' 03.80"	12.9	24.1	1000	14/03/2005
Riverwild	Mpumalanga	S25º 20' 19.0" E30º 38' 37.70"	10.7	21.3	1000	19/11/2002
Tropicado	Mpumalanga	S25º 19' 32.9" E030º 41' 51.9"	10.7	21.3	2000	19/11/2002
Nomvalo	Eastern Cape	S31º 31' 19.30" E29º 32' 23.00"	16.8	23.4	4800	28/09/2005
Tutor-Ngeleni-pass	Eastern Cape	S31º 34' 46.60" E29º 13' 11.60"	16.8	23.4	4000	28/09/2005
Tutor-Ndamase-pass	Eastern Cape	S31º 34' 54.00" E29º 13' 11.60"	16.8	23.4	4800	28/09/2005

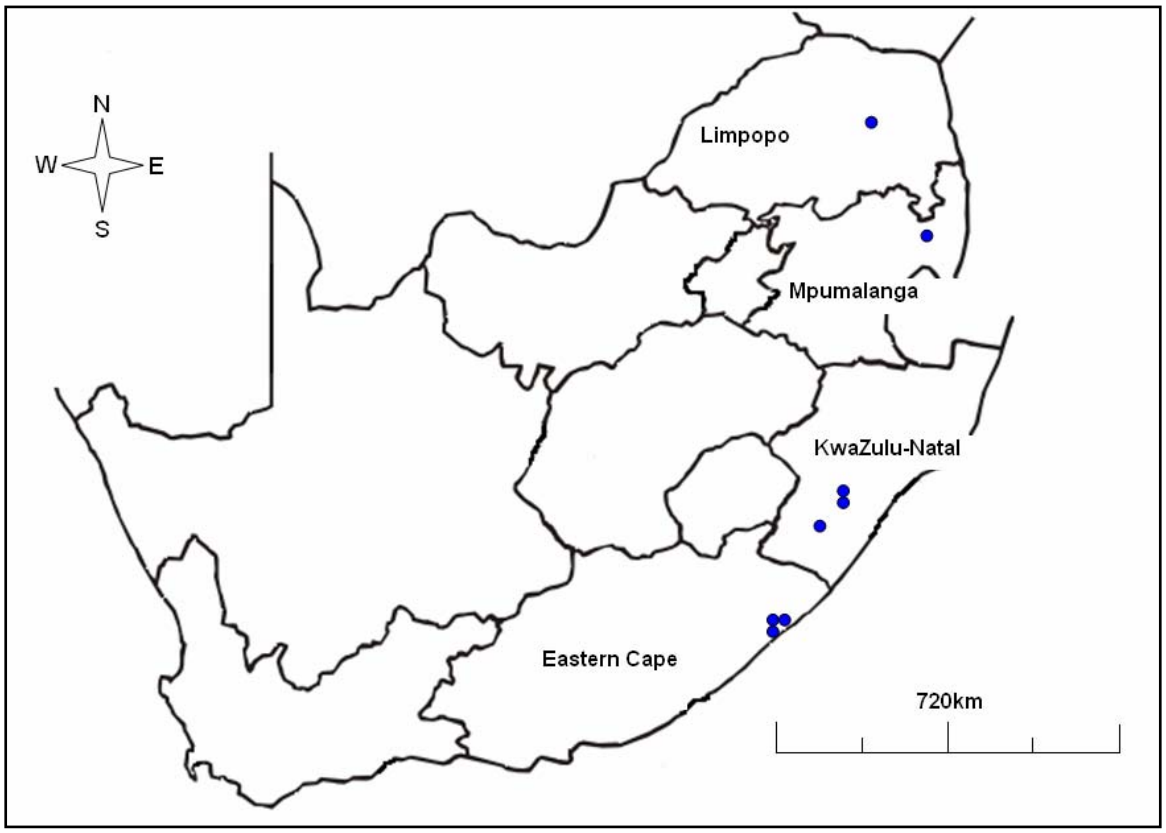


Figure 1.6: Sampled beetle release sites (●) in Limpopo, Mpumalanga, KwaZulu-Natal and the Eastern Cape province. Note that due to the scale (close proximity), some sampled sites in Limpopo, Mpumalanga and Eastern Cape could not be represented on the map.

Most field assessments were concentrated in KwaZulu-Natal province where monthly sampling focused on two release sites, Boughton and Ferncliffe which were the most accessible (Table 1.1) for a period of 15 months (February 2006 to April 2007). An additional release site (Mtubeni valley) was surveyed for beetle presence in May 2005 and February 2006. Other sites in Mpumalanga, Eastern Cape and Limpopo were also surveyed for beetle presence. Fieldwork in Mpumalanga took place in February 2006 and sampled sites included, Nelsriver Bridge situated in Nelspruit as well as Riverwild and Tropicado, both situated in Sudwala (Table 1.1). Fieldwork in the Eastern Cape took place in February 2006 and study sites were situated along the main highway between Umtata and Port St. John, specifically at Tutor-Ngeleni-pass and Tutor-Ndamase-pass (Table 1.1). The other study site was located in Nomvalo at Port St. Johns. In Limpopo,

fieldwork took place in June 2006 and all sampled sites were located in a small village near the Molototsi River in Tzaneen.

Inoculative releases were carried out at each of these field sites by Working for Water implementation officers in their respective provinces. Inoculative release refers to the release of relatively small numbers of natural enemies with the expectation that they will establish and multiply over several generations (Eilenberg *et al.*, 2001). The frequency and time of release varied among sites and provinces. In Ferncliffe and Boughton, KwaZulu-Natal, adults were released once only in May 2001 (winter) and January 2003 (summer) respectively. In the Mtubeni Valley, KwaZulu-Natal, two releases of adult beetles took place in February 2001 (summer) and December 2003 (summer). There was clear evidence of disturbance at the Boughton study site, which is located on a farm; i.e. in 2005 there was burning of vegetation and herbicide spot spraying has been ongoing since then (Symondson, G: pers. comm. 2005). The Ferncliffe study site, located within a Nature reserve, experienced no significant disturbance.

In Mpumalanga, containers with adults were emptied under *C. decapetala* canopies and releases took place once at each site, mostly in summer. Nelsriver Bridge was located along the Nelsriver which flooded during heavy rains in 2006 (Pers. obs.). The soil and pods on the surviving stands bore fresh ash from burnt vegetation which was evidence of fire in early 2006 following releases which had taken place in 2005. Most of the *C. decapetala* stands at the time of sampling were resprouting after the fire and were not mature enough to produce pods. Therefore all pods were collected from old stands. Riverwild was located on a gentle incline within the Sappi timber plantation. Due to the nature of the landscape at this site (gentle incline), most falling seeds dispersed down the slope and onto the road at the bottom of the incline, which is also inundated during heavy rainfall, washing many seeds even further away. This is an example of long-distance seed dispersal. Tropicado was located within a valley in an avocado plantation. In the Eastern Cape, at Tutor-Ngeleni-pass, both larvae and adults *S. subsuturalis* were released. Larvae were released by placing batches of seeds containing larvae at randomly selected positions under *C. decapetala* canopies while adults were released by emptying

containers of adults under randomly selected *C. decapetala* canopies. In Tutor-Ndamase-pass and Nomvalo, only larvae were released using a technique similar to that used at Tutor-Ngeleni-pass. In Limpopo, only adults were released by emptying containers under *C. decapetala* canopies at each site in summer. All study sites in Limpopo were situated within villagers' gardens. At least one-third (in area) of each site had previously been cleared for subsistence farming by the time of sampling.

1.9. Research aim and objectives

The aim of this study was to evaluate the impact of *S. subsuturalis* on seed germination and seedling recruitment of *C. decapetala* at the study sites. This includes an assessment of the beetle's lifecycle in relation to the plant's biology. This aim had the following objectives:

1. To describe and analyse the vegetative and reproductive phenology of *C. decapetala*.
2. To estimate the annual seed production of *C. decapetala*
3. To examine the level of *C. decapetala* seedling recruitment.
4. To determine the proportion of seeds attacked by *S. subsuturalis* at release sites
5. To examine the effect of *S. subsuturalis* on *C. decapetala* seed germination.
6. To examine the survival/mortality of each *S. subsuturalis* life stage in the field.

The above objectives were achieved by seeking answers to the following questions:

- When does *C. decapetala* produce pods?
- Has the beetle established within study sites?
- Where and when does *S. subsuturalis* attack *C. decapetala* seeds? On the ground and/or in the tree canopy?
- Does *S. subsuturalis* attack prevent *C. decapetala* seeds from germinating and hence establishing into seedlings?
- Is the beetle being attacked by indigenous predators and parasitoids?

Each of the following chapters attempted to address two or more of the study objectives. Chapter 2 covered the reproductive phenology of *C. decapetala* as well as estimating the number of seeds produced throughout the year and the density of *C. decapetala* seedlings. In addition to that, using data pertaining to the proportion of seeds attacked by the beetle at release sites, the level of beetle establishment was assessed. Therefore objectives 1, 2, 3 and 4 were addressed in chapter 2. Chapter 3 covered the biology of *S. subsuturalis* (longevity and oviposition) including its impact on seed germination. This addressed objective 5. Chapter 4 addressed predation and/or parasitism of the insect life-stages and therefore determined the survival/mortality within the life-stages in the field (objective 6). Chapter 5 integrated and synthesized the results of all the chapters by discussing the fundamental discoveries and providing conclusions and recommendations.

Chapter 2

Reproductive phenology of *Caesalpinia decapetala* and the establishment of *Sulcobruchus subsuturalis* at release sites

2.1. Introduction

2.1.1. Reproductive phenology and beetle releases

Synchrony in the phenology of a given biological control agent and its host plant is essential in order to correctly time beetle reproductive activities and hence avoid failure to establish (Coombs *et al.,* 2004). For instance *Acacia cyclops* (Fabaceae) phenology studies in South Africa showed that the green fully developed pods with soft seeds on which *Melanterius servulus* Pascoe (Coleoptera: Curculionidae) feeds and oviposits, peak in numbers in October when the weevil is fully active (Impson *et al.,* 2004). As such, *M. servulus* adults should be abundant on *A. cyclops* from October onwards. In North Carolina, the bruchid beetle *Bruchidius villosus* (F) (Coleoptera: Chrysomelidae) reduced 80% of *Cytisus scoparius* (Fabaceae) annual seed crop because there is synchrony between the plant's phenology and the beetle's biology (Redmon *et al.,* 2000). As *S. subsuturalis* adults only attacks mature seeds in dehisced pods in the tree canopy (Coetzer, 2003), beetle releases should take place when high seed densities are available and when conditions are appropriate for the beetle's reproductive activities.

In this chapter, studying the reproductive phenology of *C. decapetala* and hence determining when the plant produces flowers and pods (seeds), will indicate the appropriate time to release the seed feeder. More often than not, Working for Water implementation officers released *S. subsuturalis* during summer when beetles should presumably be active and seeds available for attack. No researchers had done a prior assessment of the availability of seeds in the field prior to beetle releases. For instance most releases at the study sites in most provinces took place mostly in March (late summer), September (spring), November (spring) and December (Early summer) (Table 1.1). There was also a winter (May) release in Ferncliffe, KwaZulu-Natal province. Moreover the number of agents released at *C. decapetala* infested sites ranged from 900

to 20500 adults per release (Table 1.1). Normally, the bigger the number of agents released in weed biocontrol, the better the chances of insect multiplication and hence establishment on the target plant (Grevstad, 1999).

2.1.2. Establishment of released biocontrol agents

Only when an agent develops self-sustaining populations on its target plant can it be considered established (Coombs *et al.*, 2004). Agents should be recovered from release sites three or more years following their release otherwise they may be considered failures (Harris 1991). Factors such as climate, predators, parasitoids, lack of synchrony between the agent and its host, poor release efforts (small numbers of agents) may all cause failure of released biological control agents to establish on their target plants. For instance in southern California, the stem and branch boring moth *Coleophora parthenica* Meyrick (Lepidoptera: Coleophoridae) failed to establish viable populations on Russian thistle *Salsola australis* R. Brown (Chenopodiaceae) due to a combination of poor host-plant synchronization, predator and parasitoids attacks (Muller *et al.*, 1990). In general, predation mostly by house mice, *Mus musculus* L. (Rodentia: Muridae) and parasitism by the hymenopterous parasitoids, *Norbanus perplexus* Ashmed (Hymenoptera: Pteromalidae) and *Eurytoma strigosa* Bugbee (Hymenoptera: Eurytomidae) led to a reduction in the *C. parthenica* (larvae) overwintering population by over 67.5%. Adult *C. parthenica* were mainly predated by two spider species namely; *Dictyna reticulate* Gertsch & Ivie (Araneae: Dictynidae) and *Diguetia mojavea* Gertsch (Araneae: Diguetidae) and their predation rate had reached 30.4% by the time the moth reached its third generation.

According to Day and Neser, (2000), in South Africa and Australia, 66.7% and 44% of biological control agents released against *L. camara* respectively had failed to establish by the year 2000 as a result of (a) Lack of naturally occurring *Lantana* species matching any phenotypes existing in South Africa and Australia as *L. camara* forms hybrids and thus has a variety of phenotypes. (b) *Lantana camara* also grows in various climatic areas therefore if introduced biocontrol agents are not collected from climatic areas similar to those in which releases are to take place, agents may fail to establish. (c) It was

speculated that inadequate agent release numbers were accountable for 80% (South Africa) and 45.5% (Australia) of failures in agent establishment on *L. camara*. The number of agents released in either country was low due to limited project funds and/or the dwindling number of laboratory cultures. Examples of agents that failed to establish on *L. camara* both in South Africa and Australia include: *Alagoasa parana* Samuelso (Coleoptera: Chrysomelidae); *Charidotis pygmaea* Klug (Coleoptera: Chrysomelidae); *Uroplata lantanae* Buzzi & Winder (Coleoptera: Chrysomelidae); *Teleonemia elata* (Hemiptera: Tingidae) all originally from Brazil and *Eutreta xanthochaeta* Aldrich (Diptera: Tephritidae) from Mexico (Day and Neser, 2000).

Biological control reports often highlight the number of agents released and the time of release. For reports on successful establishment of a released biocontrol agent, agent releases have typically taken place in summer or late winter and the release efforts vary among projects. For instance in South Africa, the seed feeder *Algarobius prosopis* was reported established on mesquite, *Prosopis* spp following releases of up to 20,000 adults at two sites and another 50,000 adults at two other sites in December (summer) 1987 and March (summer) 1988 respectively (Zimmermann, 1991). The seed feeding weevil *Erytenna consputa* Pascoe (Curculionidae: Erirhininae) successfully colonized *Hakea sericea* (Proteaceae) infested regions in South Africa after the release of 6208 agents at 102 sites in late winter (August/September) (Kluge and Neser, 1991). In the Australian Northern territory, *Calligrapha pantherina* Stal (Coleoptera: Chrysomelidae) established on *Sida acuta* (sida) (Malvaceae) Burman f. after the release of a total of 53,000 *C. pantherina* adults at 80 sites at the end of September (Spring) 1989 (Flanagan *et al.*, 2000).

In the case of *S. subsuturalis* releases, Working for Water implementation officers in their respective provinces maintain colonies of the beetle in insectaries from which they personally release mostly adults under *C. decapetala* canopies. In 2006 in Limpopo province, releases ranged between 17500, 19000 and 20500 adults per release per site. Other provinces released between 900 and 5000 biocontrol agents per release, per site between 2002 and 2006. Only data for one release per study site was provided by the

Working for Water implementation officers and therefore it is presumed that since the release campaign started on *C. decapetala*, only one release has taken place per site. In Limpopo, quarantine colonies of *S. subsuturalis* are destroyed annually following releases which are undertaken between September and December to avoid diseases caused by itch mites (pers. comm. Lemao Fickson 2007, working for water biocontrol officer, Limpopo Province). A new colony is then initiated from field cultures between January and March.

2.1.3. Seed banks and seedling recruitment

In this study, seed banks were analysed to determine the annual *C. decapetala* seed production and hence seedling recruitment. Usually soil seed banks and seedling recruitment are studied to facilitate the identification of potential seedling recruitment events (Auld, 1995). However the densities of some species are microsite limited, therefore seed densities in the soil may not necessarily influence actual levels of seedling recruitment (Andersen, 1989). For example limited microsites in *Colophospermum mopane* (Fabaceae) growing areas may hinder the plant's seedling establishment (Mlambo and Nyathi, 2004). More importantly, analyzing *C. decapetala* seed densities (especially seeds still in the canopy) in the present study plays an important role of indicating the season when seeds are available for *S. subsuturalis* to attack (assuming that they do not attack seeds on the ground). Although seed feeders acting alone have limited chances of reducing perennial weed density, it is hoped that with extremely high levels of seed destruction by the beetle, *C. decapetala* regeneration may ultimately diminish.

This chapter had the following objectives:

1. To describe and analyse the vegetative and reproductive phenology of *C. decapetala*.
2. To estimate the annual seed production of *C. decapetala*.
3. To examine the level of *C. decapetala* seedling recruitment.
4. To determine the proportion of seeds attacked by *S. subsuturalis* at release sites.

2.2. Materials and methods

2.2.1. Vegetative and reproductive phenology of *Caesalpinia decapetala*

In order to describe and analyse the vegetative and reproductive phenology of *C. decapetala*, eight $1m^2$ quadrats were sampled every month in both Boughton and Ferncliffe (KwaZulu-Natal Province) from April 2006 to April 2007. Four metres of contiguous $1m^2$ quadrats directed away from the tree trunk towards the edge of the canopy supporting *C. decapetala* were positioned under a randomly selected *C. decapetala* canopy at each site. This sampling technique was applied because it is difficult to distinguish between individual *C. decapetala* trees given that this species tends to root where its branches touch the ground. The other four metres of contiguous $1m^2$ quadrats were directed from the edge of the canopy outwards. The following monthly measurements were taken within each quadrat (for non-destructive sampling/ observations, except point 7 below) (Fig. 2.1).

1) Number of immature pods on the tree above each quadrat.
2) Number of mature pods on the tree above each quadrat.
3) Number of pods on the ground in the quadrat.
4) Presence or absence of flower heads in the canopy.
5) Colour of leaves.
6) Numbers of seeds on the ground (and from 0-6 cm in the upper soil layer) in adjacent 30 x 30cm seed-bank quadrats outside each $1m^2$ and from pods that have not yet dispersed seeds from the tree canopy. A different quadrat position was used at each sampling interval.
7) Number of seedlings of *C. decapetala* within the quadrat ($1m^2$).

Caesalpinia decapetala seed bank

Soil seed bank samples were collected off the ground in one, 30x30cm quadrat positioned outside each $1m^2$ quadrats each month from February 2006 to April 2007 (Figs. 2.1 and 2.2). By the end of the sampling period (February 2006 to April 2007) in KwaZulu-Natal,

a total of 120, 30 x 30cm seed bank quadrats had been sampled at each site (Boughton and Ferncliffe) over a 15 months period. Each month, a quadrat was placed at a new position outside each 1x1m quadrat to avoid resampling the same ground. Between February and August 2006, soil samples were collected from the depth of 0-6cm from each quadrat and sieved (using a 2mm sieve) to collect seeds. However in order to distinguish new seeds (seeds from the current season) from old seeds (seeds from previous seasons (one or more years)), the sampling technique was changed when flowering started, prior to seed fall and every month throughout the seed fall season. Hence, from September onwards, soil samples were collected systematically from different layers i.e. litter; 0-1cm; 1-2cm and 2-6cm and separately sieved through a 2mm sieve and the seeds counted. Seed densities were expressed as number per m^2. Wooden stakes were hammered into the ground to permanently demarcate the corner positions of the 1x1m quadrates which were maintained each month. Canopy seed samples were picked from 40 dry pods (split pods which have not yet released seeds) off branches 5m away from the non-destructive sampling area to avoid interference with phenology observations.

The sampling protocol elaborated above was also used to sample both ground and canopy seeds in other sites e.g. Mtubeni valley (KwaZulu-Natal); Riverwild, Tropicado and Nelsriver Bridge (Mpumalanga); Port St. Johns (Eastern Cape) and Tzaneen (Limpopo) between January (summer) and June 2007 (winter). Fieldwork in Limpopo, took place in June 2007 (mid winter), while the plant was still flowering and some pods were still immature and as a result only old pods (most still contained some of their seeds) from the previous season were picked. Seeds collected from all sites were transported in labeled paper bags to the laboratory to search for *S. subsuturalis* eggs, larvae, pupae and adults. The procedure used is elaborated in the following section (Establishment of *S. subsuturalis*).

Meanwhile seed rain experiments were set up under the *C. decapetala* canopy at Ferncliffe only. A total of six flower pots (25 cm in diameter and 20cm deep) with a wire mesh (2mm) base with additional holes (2mm in diameter) drilled in the base were

positioned singly adjacent to the 1 x 1m quadrats. The pots were suspended on PVC pipes 50cm above the ground to avoid vertebrate predator disturbance (Fig. 2.3). At the end of each month (November 2006 to March 2007) seeds were collected and counted. In addition to estimating seed rain (and hence production), this experiment was also used to determine whether seeds falling directly into the traps were infested with beetles which would be the case if the beetle attacked seeds prior to their dispersal from the pods in the canopy.

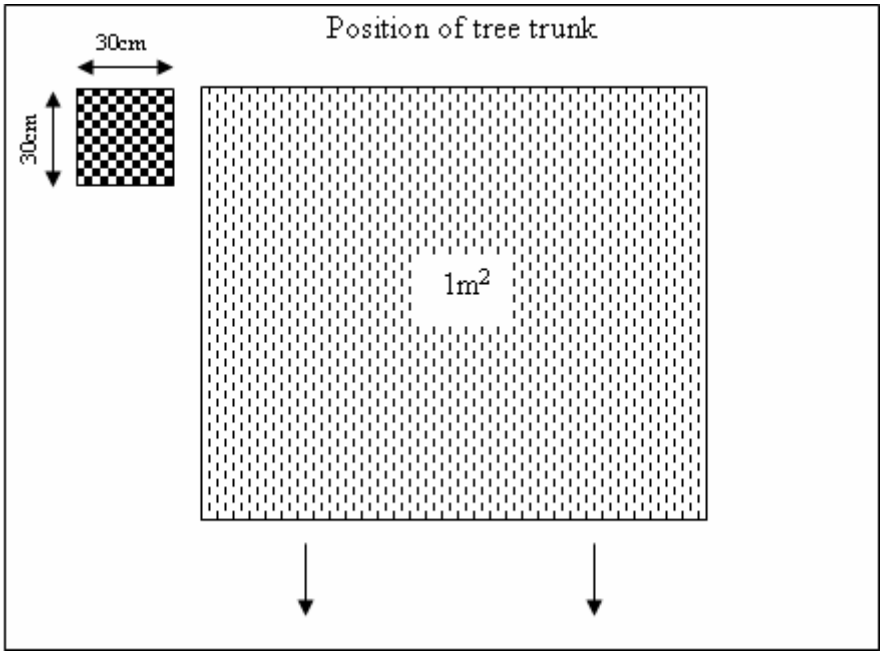


Figure 2.1: Field sampling layout used monthly, showing relative position of non-destructive sampling within 1 x 1 m quadrats and destructive sampling in adjacent 30 x 30 cm seed bank quadrats .

Figure 2.2: Monthly field sampling layout showing relative position of non-destructive sampling within 1 x 1m quadrats and destructive sampling in adjacent 30 x 30cm seed bank quadrats.

Figure 2.3: Flower pots suspended on PVC pipes 50cm above the ground positioned under *Caesalpinia decapetala* canopy adjacent to the 1x1m quadrats.

Caesalpinia decapetala seedling density and survival

The density of *C. decapetala* seedlings was measured by counting and recording the number of seedlings within each of the eight, 1 x 1m quadrats every month between February 2006 and April 2007. To determine survival of the seedlings in the field, 120 seedlings in batches of 30 seedlings were randomly selected from four 1x1m quadrats (two quadrats under canopy and two outside canopy) i.e. a total of 30 seedlings from each quadrat between January 2006 and April 2007. The selected seedlings were counted and marked using colour coded paper clips and monitored each month. The seedling survival determination was conducted at Ferncliffe only.

2.2.2. Establishment of *Sulcobruchus subsuturalis*

To investigate whether the beetle had established, seeds collected from all sites were analysed in the laboratory and the proportion of seeds infested with *S. subsuturalis* eggs, larvae, pupae or adults was determined. As demonstrated in the section (*Caesalpinia decapetala* seed bank) above, seeds were collected from two sources: seeds still within pods on the tree (not yet dispersed) and seeds on the ground within the eight metres of contiguous $1m^2$ quadrats under the canopy and outside the canopy.

In the laboratory, seeds with beetle eggs visible on the surface were separated from seeds without eggs. Seeds with eggs were placed singly in vials and maintained in the insectary at 20-25ºC under high humidity to observe any beetle emergence. Before placing seeds in the insectary, they were examined under the microscope to search for the larval entrance hole normally found on the seed. Entrance holes usually indicate that the larvae managed to penetrate the seed testa. Seeds without larval or adults exit holes were scarified using a bench grinder and then soaked for 24 hours to soften the seed coat for easy dissection. Seeds were dissected using a scalpel to search for larvae and adult beetles that had not yet emerged.

2.2.3. Statistical analyses

Statistical analyses were performed using SAS enterprise guide 3.0 and STATISTICA 6.0. Most data were continuous and hence descriptive statistics (means and standard

deviations) were determined and a normal distribution was fitted to test for normality or skewness of the data. Repeated measures ANOVA and Tukey post hoc tests were applied to test for significant differences in seed and seedling densities between the two release sites Boughton and Ferncliffe over the sampling period. A t-test for independent variables was applied to test for significant differences in seed densities under canopy and outside the canopy per site. One way ANOVA and Tukey post hoc tests were applied to test for significant differences in the number of mature pods in the tree canopy and the number of pods on the ground between months. A regression was applied to test for the relationship between (a) rainfall amount and seedling densities (b) number of agents released and number of agents recovered.

2.3. Results

2.3.1. Vegetative and reproductive phenology of *Caesalpinia decapetala*

Although its leaves fall throughout the year, *C. decapetala* never sheds all its leaves even during winter. Nevertheless, in Ferncliffe, leaves maintained a yellowish-green colour between April and August (towards the end of summer and throughout winter). From September 2006 onwards leaves were green. In 2006, the plant flowered between July and September at Ferncliffe (Fig 2.4). There was an average of 11.8 inflorescences/m^2 throughout the flowering period in Ferncliffe. The plant produced the greatest density of inflorescences in September with a mean of 21.5 inflorescences/m^2 per. The number of florets per inflorescence ranged between 19 and 44. New pods were present from the end of September 2006 until the end of March 2007 (Fig. 2.4).

The number of pods produced from a single inflorescence were normally far less than the number of florets previously available. For example in Ferncliffe, in September, only 16.6% of the 120 florets survived to develop into pods. The number of dispersed pods (pods that have already dispersed their seeds) collected on the ground was not significantly different between months ($F_{(12, 39)} = 2.8772$; $P = 0.006$). Meanwhile the number of mature pods collected in the tree canopy was significantly different between months ($F_{(12, 39)} = 10.487$; $P < 0.0001$). Tukey post hoc test results revealed that May, June and July had significantly more mature pods in the tree canopy than any other

sampling month (Appendix I). However all the pods in the tree canopy before September 2006 were old from the previous season and had dispersed most of their seeds. Seeds (seed rain) were recovered from the seed rain experiments in February, March and April 2007 (Fig. 2.4).

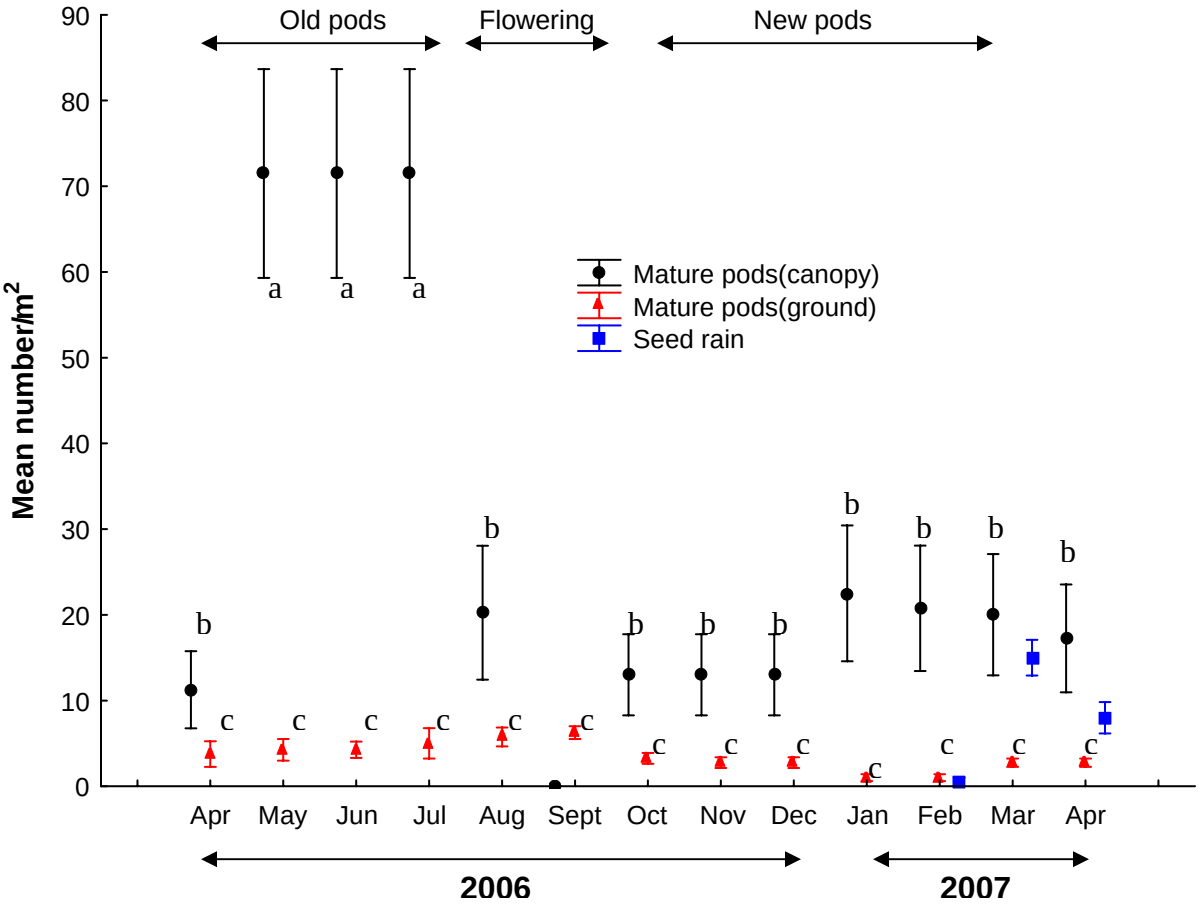


Figure 2.4: Seasonal distribution of the number of *Caesalpinia decapetala* pods and seeds at Ferncliffe represented as mean (± SE) per square metre. Immature and mature pods in the canopy were from both the current and previous season. Mature pods on the ground were mainly dispersed from the previous season. For each time period, bars with different letters (a,b) are significantly different at $P = 0.05$. Note that the y-axis in this figure is 10 times more than the y-axis in figure 2.5.

In Boughton, leaves maintained a yellowish-green colour between April and September (towards the end of summer throughout winter to spring). From October onwards leaves were green again. No flowering was observed in Boughton possibly due to disturbance in form of burning of vegetation and herbicide spot spraying. However, small patches of *C.*

decapetala roughly 1 km away from the main site were flowering. Since *C. decapetala* did not flower at the sampled site, no pods were produced. However, mature pods observed in the tree canopy over the sampling period had been produced from the previous season (2005) (Fig. 2.5). There was no significant difference in the number of mature pods collected from the tree canopy between months ($F_{(12, 39)} = 1.797945$; $P = 0.083$). However there was a significant difference in the number of pods collected from the ground between months ($F_{(12, 39)} = 5.48333$; $P < 0.0001$). Tukey post hoc test results revealed that the months of April to September 2006 were significantly different from October to December 2006 and January to April 2007 in terms of mature pod density on the ground (Fig. 2.5). That is, there were no mature pods on the ground in 2007.

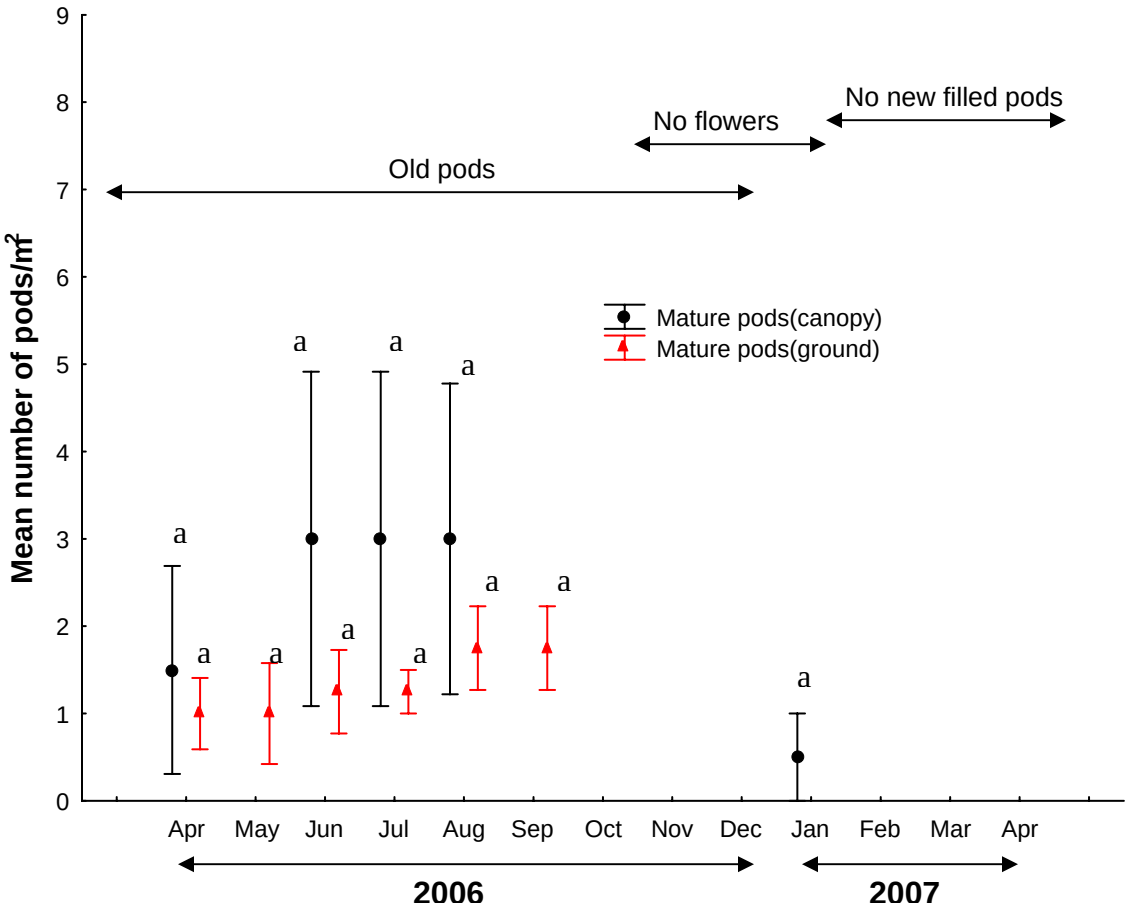


Figure 2.5: Seasonal distribution of the number of *Caesalpinia decapetala* pods at Boughton represented as mean (± SE) per square metre. Mature pods in the canopy are from the previous season. Note that Boughton produced no pods in 2006 because the plant at the site did not flower. Mature pods on the ground were also dispersed from the previous season. a = means followed by the same letter are not significantly different at $P = 0.05$. Note that the y-axis in this figure is 10 times less than the y-axis in figure 2.4.

Canopy seeds

In Ferncliffe, during and after the flowering period, there was production of new seed-filled pods and the number of seeds per pod was highest in September 2006 with 5.9 seeds/pod but gradually decreased and by March 2007 there was an average of 1.9 seeds/pod (Fig. 2.6). Most pods in the tree, before the flowering period, were from the previous season and are here referred to as old pods, most of which contained less than three seeds on average (Fig. 2.6). In Boughton, most pods in the tree canopy were from the previous season and contained less than three seeds on average (Fig. 2.6). Canopy seeds were therefore available throughout the year at both sites.

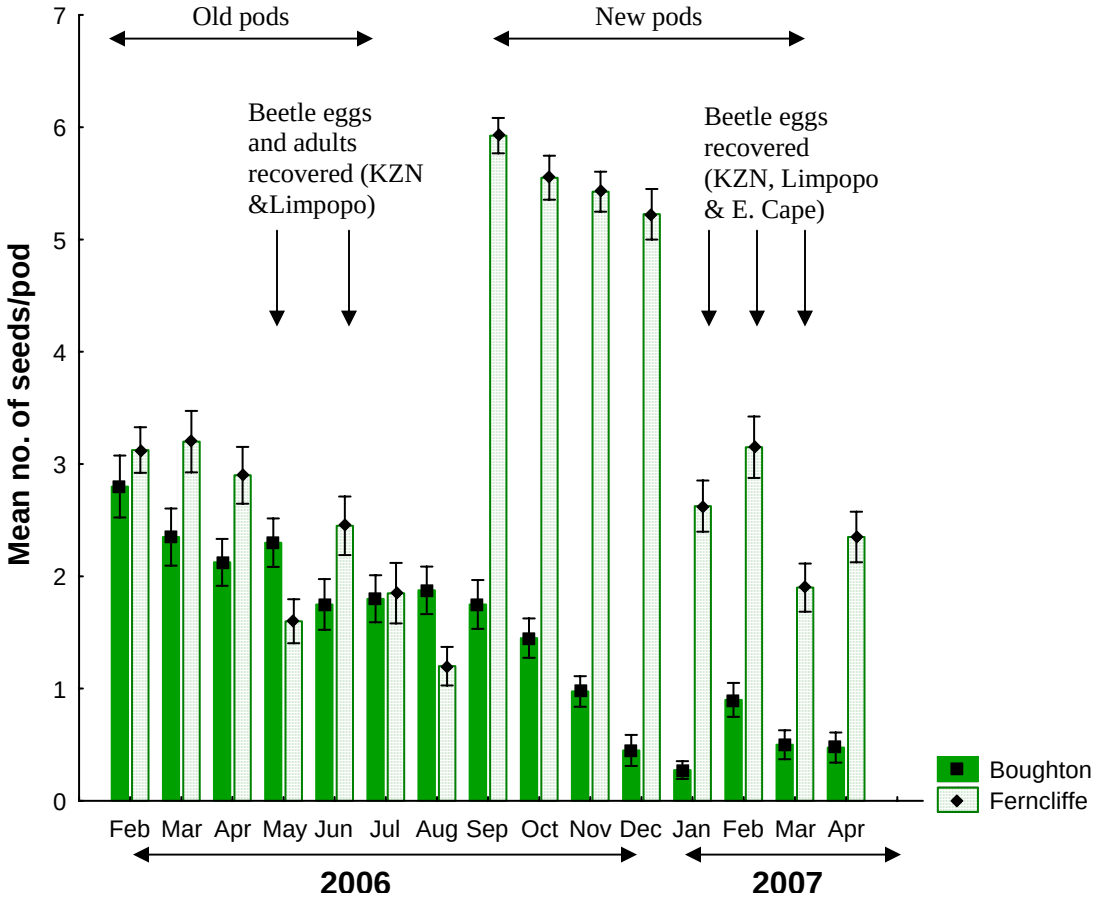


Figure 2.6: Mean (± SE) seeds per mature pod in the canopy observed over a period of 15 months at Ferncliffe and Boughton. Note that Boughton did not produce new pods in 2006 because the plants did not flower, hence the number of seeds per pod continued to decrease throughout the year. Data collected from n = 40 pods sampled per month per site.

***Caesalpinia decapetala* seed bank**

Soil seed bank densities under canopy

Ferncliffe showed a greater variation in seed densities under the canopy, on the ground and out of pods over the 15 month sampling period (Fig. 2.7). Seed densities started increasing from March to September (2006) but decreased between October and December 2006. In February 2007, seed densities again began increasing but declined again in March 2007. At Boughton, the mean number of seeds in the soil seed bank was always less than 25 seeds/m^2 (Fig. 2.7). Generally, there were nearly always more seeds in Ferncliffe than Boughton. Statistical results revealed that seed densities were higher at Ferncliffe than Boughton ($F_{(1, 6)} = 110.02$; $P<0.0001$) and differed between the various months ($F_{(14, 84)} = 4.1418$; $P<0.0001$). Seed densities were significantly different between Ferncliffe and Boughton from March 2006 to April 2007 but not February 2006.

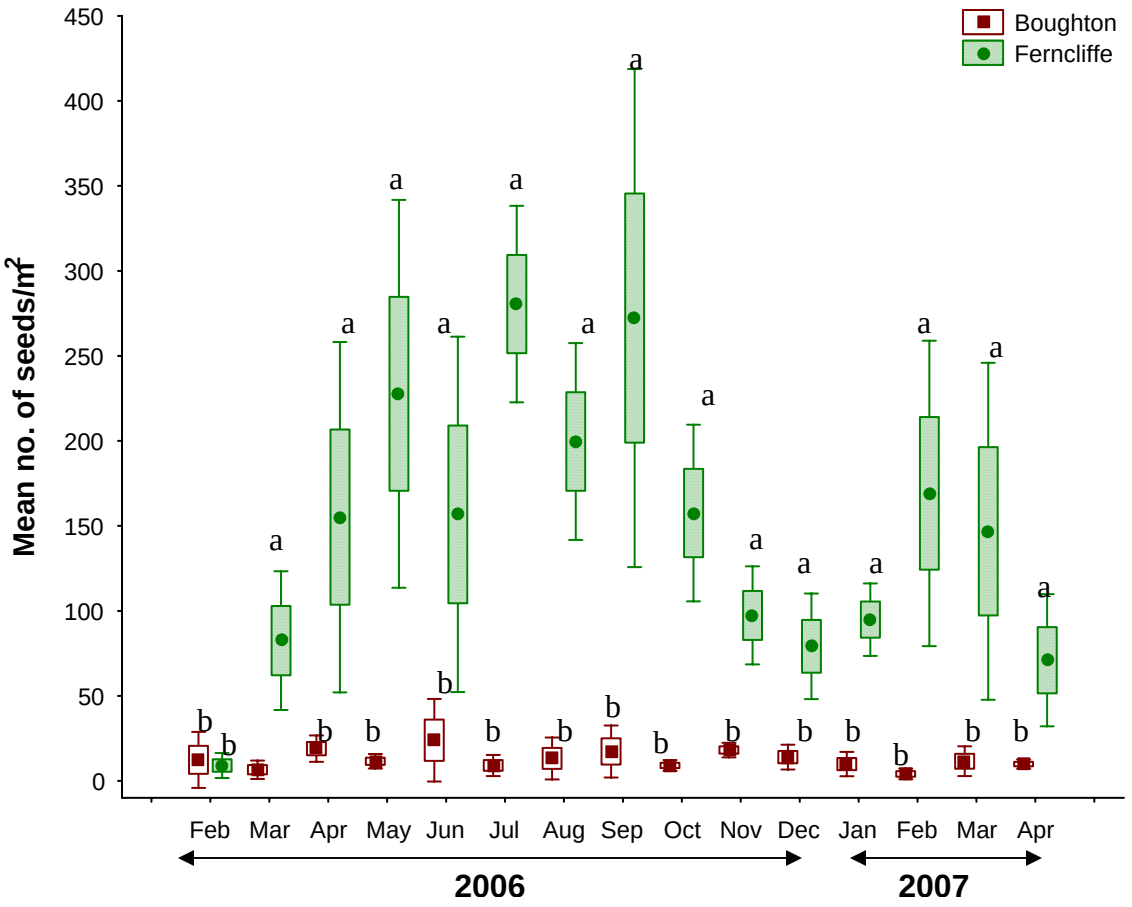


Figure 2.7: Under canopy mean (± SD) seed bank densities of *Caesalpinia decapetala* at Boughton and Ferncliffe over a period of 15 months. Boxes = SE of the mean; whiskers = SD of the mean. Seeds were collected from the litter layer plus the 0-6cm soil depth. For each time period, bars with different letters (a,b) are significantly different at $P = 0.05$

Soil seed bank densities outside the canopy

Seeds were present throughout the year outside the canopy in both Ferncliffe and Boughton but were always less than under the canopy. The mean number of seeds collected outside the *C. decapetala* canopy did not exceed 50 seeds/m^2 throughout the 15 month sampling period at either Ferncliffe or Boughton (Fig. 2.8). At Ferncliffe, between February and April, seed densities were low (less than one seed/m^2 on average) but began increasing and reached a peak in July and August 2006, following which seed densities steadily declined from September to December 2006. There was a slight rise in numbers in January 2007, corresponding to the seed rain and under-canopy increase in seed

density. By April 2007 there was less than three seeds/m^2 outside the canopy. Mean seed densities outside the *C. decapetala* canopy differed significantly between months ($F_{(14, 84)}$ = 4.5128; P<0.0001) but there was no significant difference between Ferncliffe and Boughton ($F_{(1, 6)}$ = 0.0232; P = 0.8839).

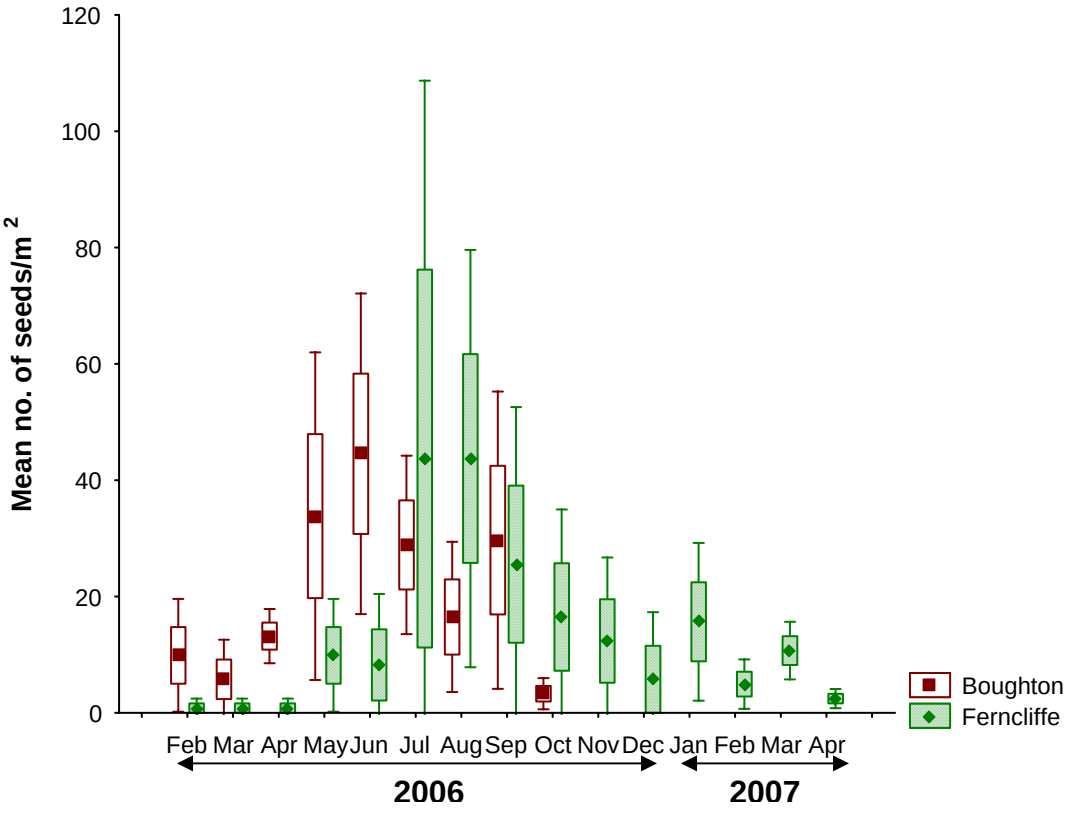


Figure 2.8: Mean (± SD) seed densities of *Caesalpinia decapetala* outside the canopy, at Boughton and Ferncliffe over a period of 15 months. Boxes = SE of the mean; whiskers = SD of the mean.

All seeds sampled from the soil seed bank before mid October 2006 had dispersed from the previous season's pods whereas seeds sampled from October 2006 onwards were dispersed from both old and new pods (pods from the current season).

Under canopy versus outside canopy seed densities at Ferncliffe and Bougthon

Overall, at Ferncliffe, there was a significant difference between seed densities under the canopy and outside canopy (P < 0.0001) (Figs. 2.7 and 2.8) implying that seeds only

dispersed short distances. At Boughton there was no significant difference between seed densities under canopy and outside canopy ($P = 0.9212$) (Figs. 2.7 and 2.8).

Seed bank at different soil depths

At Ferncliffe, September 2006 showed the greatest number of seeds in the litter layer. By the end of October 2006, more seeds were found 2-6cm deep than in the litter layer (Fig. 2.9). September was the month where flowering was at its peak and for some individual plants, pods started developing. Seeds collected from the litter layer down to 6cm depths between September and October are presumed to have fallen within the previous season (old seeds). By December 2006 there were again more seeds in the litter layer compared to other soil depths (Fig. 2.9) (for that month and January 2007). There was a significant difference between soil depths in terms of seed densities ($F_{(3, 12)} = 6.30675$; $P = 0.008$). The seeds in the litter had fallen off the tree in that season. In general, seed densities declined between September 2006 and April 2007. There was a significant difference in the mean number of seeds in the seed bank between months in Ferncliffe ($F_{(7, 84)} = 6.6423$; $P < 0.0001$). Tukey post hoc test results revealed that there was a significant difference in the mean number of seeds collected at several soil depths (Litter layer, 0-1cm, 1-2cm and 2-6cm) at Ferncliffe (Fig. 2.9).

In Boughton there were more seeds collected within the 2-6cm depths (at least >5seeds/m^2) compared with the litter layer between October and November (Fig. 2.10). These were regarded as seeds from the previous season (old seeds). Between October and December 2006, more seeds were found at deeper depths but these disappeared in the following months. However there was no significant difference in the mean number of seeds in the seed bank between months in Boughton ($F_{(7, 84)} = 1.38195$; $P = 0.2236$). Overall, no new seeds were produced at this site, simply because the plant did not flower in 2006. Nevertheless seeds were available throughout the eight month sampling period.

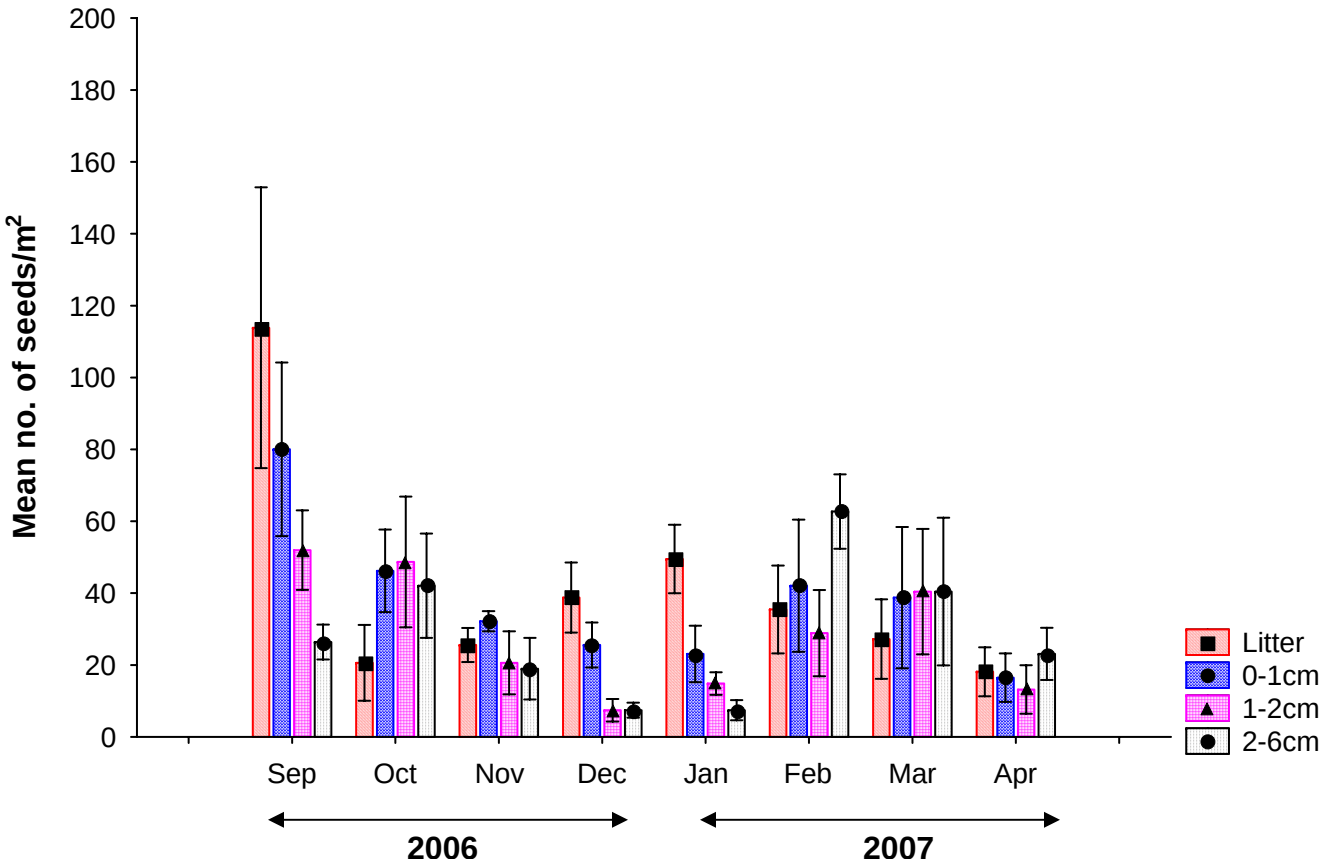


Figure 2.9: Soil seed-bank densities of *Caesalpinia decapetala* sampled between the litter layer and 0-6cm soil depths under the plant canopy at Ferncliffe. Seeds were sampled every month for a period of 8 months (September 2006 to April 2007). Error bars = SE of the mean. Note that the y-axis in this figure is 10 times more than the y-axis in figure 2.10.

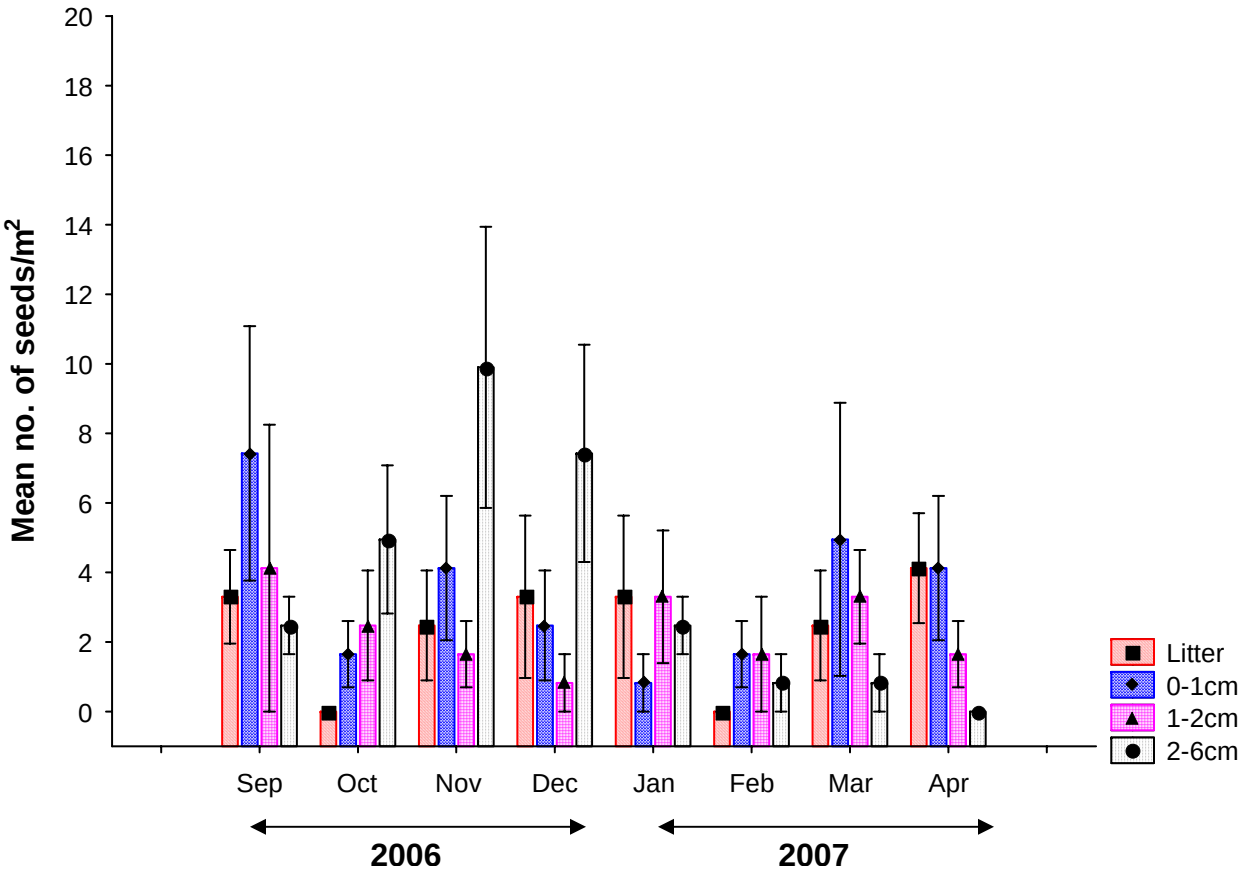


Figure 2.10: Soil seed-bank densities of *Caesalpinia decapetala* sampled between the litter layer and 0-6cm soil depths under the plant canopy at Boughton. Seeds were sampled every month for a period of 8 months (September 2006 to April 2007). Error bars = SE of the mean. Note that the y-axis in this figure is 10 times less than the y-axis in figure 2.9.

Seedling densities of *C. decapetala* at study sites

Under canopy

In Ferncliffe, there was an average of 40 seedlings per square meter in February 2006 followed by a decline over the following months and by July there was less than two seedlings per square meter (Fig. 2.11). Between August and December 2006, no seedlings were recorded but from January to April 2007 the number of seedlings was at its highest i.e. more than 50 seeds/m^2. Seedling survival at Ferncliffe was relatively high between January and April 2007 i.e. approximately 70% of the 120 sampled seedlings within the $4m^2$ sampling area survived. In Boughton the only months in which seedlings were available was February to April 2006 (Fig. 2.11). In 2006, the rainy season was from January to May and from August through to December onwards. The monthly rainfall from January 2007 to April 2007 when seedling recruitment was highest ranged from 25 to 193 mm (Fig 2.11). Overall, seedling density and rainfall at Ferncliffe tend to increase and decrease together (r = 0.04).

The mean number of seedlings under canopy was significantly different between months (March to November 2006 and January to April 2007 at Ferncliffe) and ($F_{(11, 66)}$ = 42.4540; $P < 0.0001$) and between Ferncliffe and Boughton ($F_{(1, 6)}$ = 53.3463; P = 0.0003).

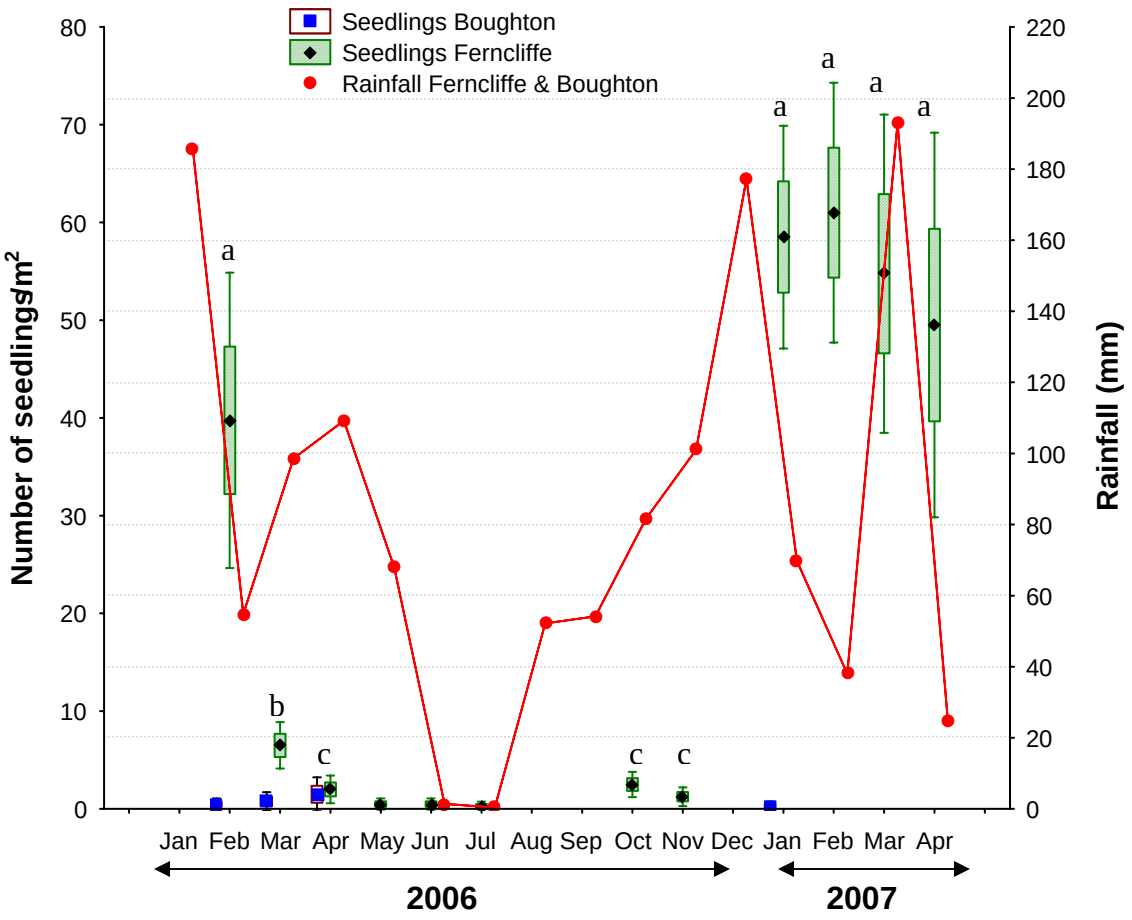


Figure 2.11: Mean number of seedlings of *Caesalpinia decapetala* under the plant canopy, and monthly rainfall for Ferncliffe and Boughton. Boxes = SE of the mean; whiskers = SD of the mean. The data were collected between 2006 and 2007. For each time period, bars with different letters (a,b) are significantly different at $P = 0.05$. Rainfall data provided by South African Weather Services.

Outside canopy

Ferncliffe had an average of at least 13 seedlings/m^2 in February 2006 but by the end of March 2006 less than three seedlings/m^2 on average were recorded. Seedlings were observed again ten months later (January 2007) during the rainy season and seed rain period (Fig. 2.12). Generally, seedling density and rainfall at Ferncliffe increase and decrease at the same time (r = 0.07). Boughton on the other hand showed very low seedling densities (less than 6 seedlings/m^2) outside the canopy although in general the number of seedlings outside the canopy was greater than the number of seedlings under canopy (Fig. 2.11 and 2.12). However, as the plant did not flower and hence produce

seeds at this site during the study period, the few seedlings were recorded in 2007 and were assumed to have originated from seeds from the previous season. The mean number of seedlings outside the canopy was not significantly different between the analysed months ($F_{(9, 54)} = 1.109187$; $P = 0.3723$) or between Ferncliffe and Boughton ($F_{(1, 6)} = 1.136603$; $P = 0.3274$).

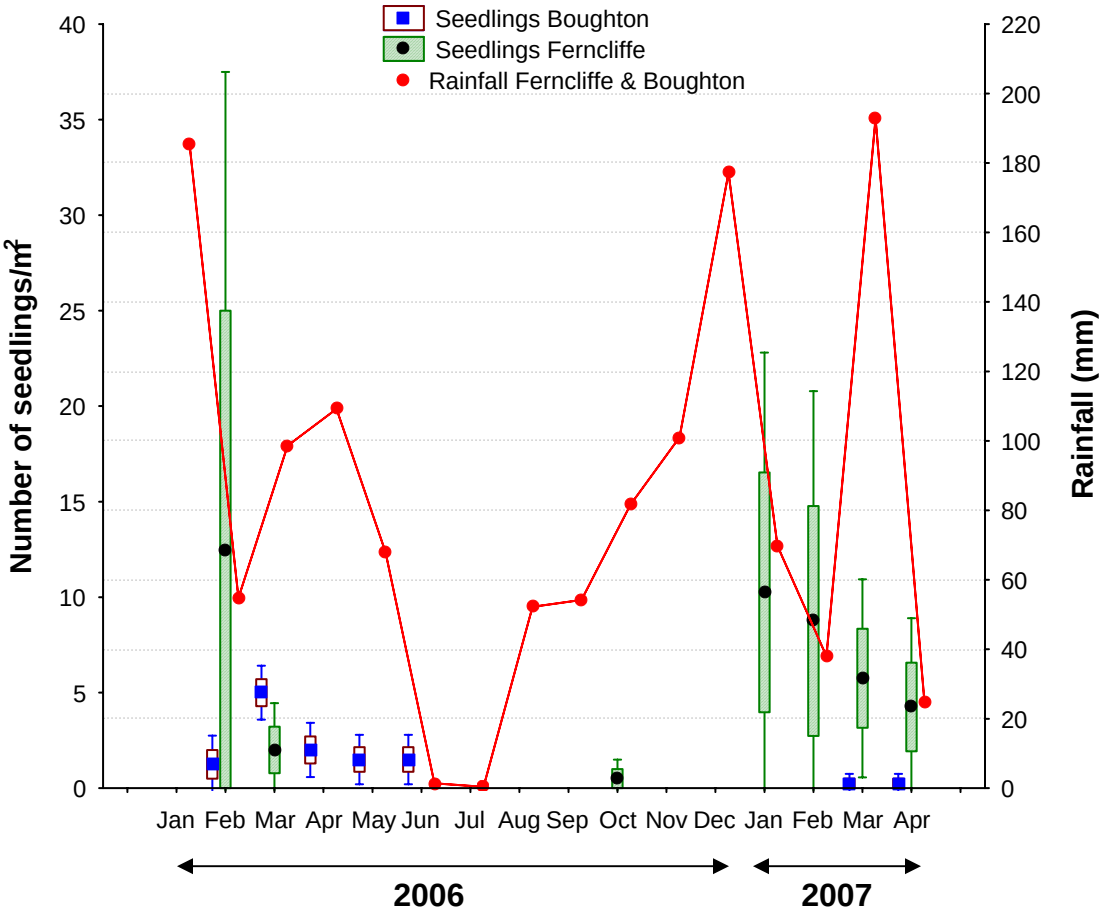


Figure 2.12: Mean number of seedlings of *Caesalpinia decapetala* outside the plant canopy and monthly rainfall for Ferncliffe and Boughton. Boxes = SE of the mean; whiskers = SD of the mean. The data were collected between 2006 and 2007. Rainfall data provided by South African Weather Services.

2.3.2. Establishment of *Sulcobruchus subsuturalis*

All infested seeds came from pods picked from trees and no beetle eggs were found on seeds collected from the ground (Table 2.1).

Table 2.1: The presence of the biocontrol agent (eggs and adults) in the main study sites (Ferncliffe and Boughton) in KwaZulu-Natal and other sites around South Africa

Site Location	No. of agents released	Release Date	Sample date	No. of canopy seeds sampled	No. of Seeds bearing eggs (%)	No. of adults recovered (%)
Boughton(KZN)	2000	Jan, 2003	May, 2005	179	0.6	0.56
Ferncliffe(KZN)	900	May, 2001	May, 2005	90	0	0
Mtubeni(KZN)	1917	Dec, 2003	May, 2005	22	13.6	9
Mtubeni(KZN)	1917	Dec, 2003	Jan, 2007	210	0.5	0
Nomvalo(E. Cape)	4800	Sep, 2005	Feb, 2007	224	4.5	0
Nelsriver Bridge (Mpumalanga)	1000	Mar, 2005	Feb, 2007	33	0	0
Riverwild (Mpumalanga)	2000	Nov, 2002	Feb, 2007	144	0	0
Tropicado (Mpumalanga)	2000	Nov, 2002	Feb, 2007	333	0	0
Boughton(KZN)	2000	Jan, 2003	Mar, 2007	20	5	0
Moshakga1 (Limpopo)	20500	Dec, 2002	Jun, 2007	214	13.6	1.4
Moshakga2 (Limpopo)	19000	Dec, 2002	Jun, 2007	103	15.5	0.97
Bodupe (Limpopo)	17500	Dec, 2006	Jun, 2007	374	6.4	0.53

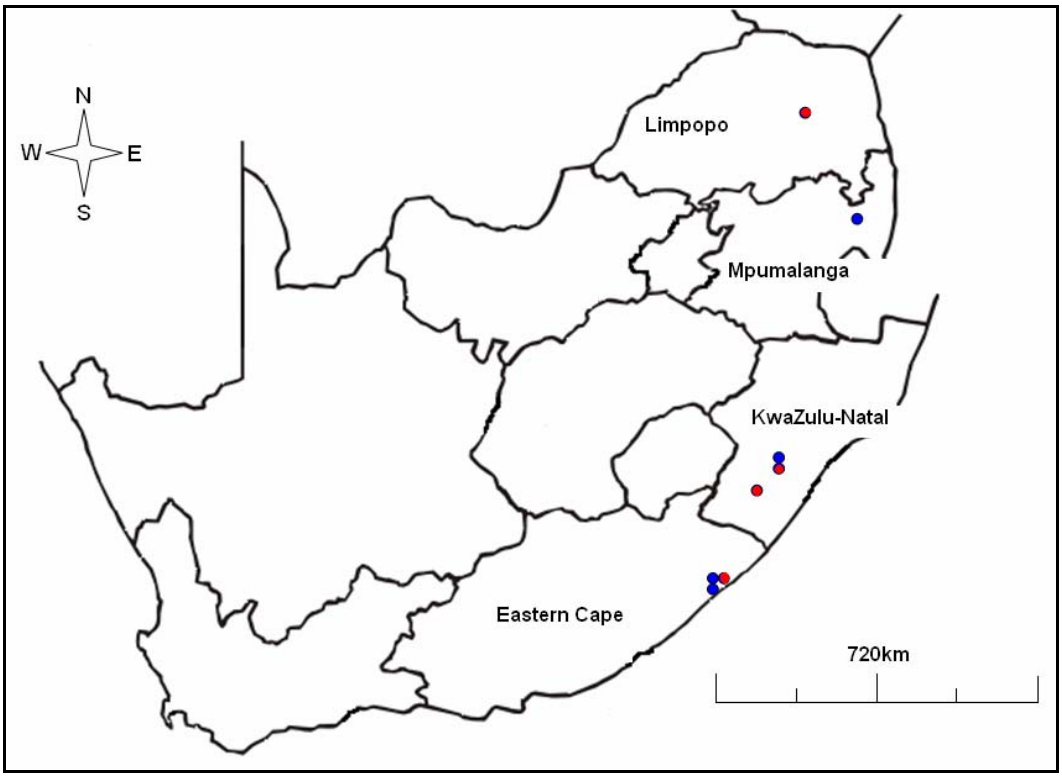


Figure 2.13: Map showing sampled release sites where *Sulcobruchus subsuturalis* eggs and/or adults were recovered (●). The other sites from which no beetles were recovered are also indicated (●).

Seeds infested with *S. subsuturalis* beetles were recovered from Limpopo, KwaZulu-Natal and the Eastern Cape but not Mpumalanga (Fig. 2.13). The number of infested seeds collected varied among provinces. The highest proportion of seeds bearing beetle's eggs was collected from Limpopo, followed by KwaZulu-Natal while Mpumalanga had had no seeds carrying eggs (Table 2.1). All eggs had hatched by the time of collection from the field sites. No infested seeds were collected in KwaZulu-Natal in 2006 but in Feb 2007, 0.5% of 210 sampled seeds in Mtubeni valley carried eggs. In addition, 5% of the 20 sampled seeds in March 2007 from Boughton carried eggs (Table 2.1). No adults were recovered from the February and June samples. Except for Limpopo, all the adults recovered from the other three provinces were dead. Generally, the number of agents released tend to influence the number of adults recovered ($r = 0.72$). All adults were found inside seeds in winter and none were obtained in summer (Table 2.1).

2.4. Discussion:

2.4.1. Vegetative and reproductive phenology of *Caesalpinia decapetala*

At Ferncliffe, pods containing seeds were available throughout the year (Fig. 2.4). Plants at Boughton did not flower in 2006 and as a result no new pods were produced but old pods were still present (Fig. 2.5). In general, mature seeds in pods were available throughout the 15 months sampling period at both Ferncliffe and Boughton because of the carry-over from the previous season's seed production (Fig. 2.6). At Ferncliffe, September 2006 revealed the highest number of seeds per pod while at Boughton, February 2006 showed the highest number of seeds in the canopy (Fig. 2.6). If indeed *S. subsuturalis* attacks seeds mainly in the pods then September onwards would be appropriate to release the biocontrol agent at Ferncliffe. However, at Boughton, there were limited seed numbers throughout 2006 therefore the site would never have been particularly suitable to release biocontrol agents.

The availability of both old pods and newly filled pods which dispersed seeds throughout the 15 months of sampling (Fig. 2.6) explains the greater seed bank densities under the canopy at Ferncliffe than Boughton (Fig. 2.7). Seeds collected under the canopy at Boughton were being dispersed from old pods only. Moreover the site lay on a gentle

incline and as such a lot of seeds released from the canopy rolled down the slope towards the outside canopy area (Fig. 2.8). In Ferncliffe, where the landscape was relatively flat, the number of seeds stored under the canopy was much greater than outside the canopy, which is typical for most weeds, as seed distribution is usually clumped around the parent plant. This could imply that if undisturbed by e.g. predators, parasitoids, fire, the biological control agent could colonise many seeds, hence potentially leading to high levels of agent infestation without having to disperse.

There was a large number of seeds stored in the litter layer especially at Ferncliffe (Fig. 2.9). If *S. substuralis* is able to attack ground seeds in the field, this may allow beetles to attack large numbers of seeds at the soil surface. According to Witkowski and Garner, (2000), seeds stored at 2-6cm or deeper are older seeds relative to those stored in the shallow parts of soil (0-2cm soil depths). In this case, *S. subsuturalis* would be expected to mainly attack new seeds in the litter layer from the current season's production. The large number of seeds recorded at Ferncliffe revealed no seed damage caused by *S. subsuturalis* in the field and consequently seedling recruitment was plentiful.

Caesalpina decapetala seedling densities

Ferncliffe showed the greatest seedling densities, firstly because the number of seeds in the seed bank was high and available throughout the year, and many germinated during the rainy season when conditions were favourable (Fig. 2.11). The seed rain period was from mid October 2006 to April 2007 and therefore it was assumed that seedlings that emerged between January and April 2007 emerged from both the current season's seeds (new seeds) and the previous season's seeds (old seeds) (Fig. 2.11). Given that Boughton did not produce seeds in 2006, seedling densities were generally low throughout the 15 months sampling period both under canopy and outside canopy (Fig. 2.11). In Boughton seedlings were assumed to have emerged from seeds from the previous season (old seeds) Low seed production may limit seedling recruitment as observed at Boughton. Seedlings that emerged outside the canopy in Ferncliffe between January and April 2007 are presumed to have emerged from the current season's seeds (dispersed during the seed rain period) (Fig. 2.12). This indicates that *C. decapetala* seeds may have germinated

immediately after being dispersed from the tree and ultimately established into seedlings. Low levels of seedling recruitment normally result from poor annual seed production (Lamont *et al.,* 2001).

However according to Andersen, (1989), seed densities in the soil may not necessarily determine actual levels of seedling recruitment as some species densities are determined by availability of suitable microsites rather than seed availability. Setterfield, (2002) further reports that abundance of both seeds and microsites can increase seedling densities in several communities such as deciduous woodlands, coniferous woodlands and calcareous grasslands. However the importance of rainfall in determining seedling recruitment, especially in savanna ecosystems should not be ignored (Wilson and Witkowski, 1998; Higgins *et al.,* 2000). Results obtained in Ferncliffe show that *C. decapetala* seedling density is highest during the rainy season (Fig. 2.11). The same was reported for *C. mopane* whose seedling establishment depends on the pattern of rainfall during the rainy season (Mlambo and Nyathi, 2004). All in all, the large number of seedlings at Ferncliffe leads to two hypotheses: (a) The beetle had no effect on seedling recruitment. A preliminary study presented results showing normal levels of seedling recruitment (Kalibbala, 2005) (b) The beetle failed to successfully establish on the weed possibly due to poor release efforts and failed to overcome other factors such as ant predation of beetle life stages as well as parasitism by native parasitoids. These two hypotheses will be examined in chapters 3 and 4 respectively.

Sulcobruchus subsuturalis infestation of Caesalpinia decapetala seeds in the field

All *S. subsuturalis* infested (eggs and adults) seeds were collected from pods in the tree canopy and none on the ground. This implies that the beetle attacks mature seeds in dehisced pods. Furthermore, Coetzer, (2003) reported that *S. subsuturalis* only attacks mature seeds in dehisced pods on the tree. Although Coetzer, (2003) did not demonstrate how he arrived at this conclusion, no beetle infested seeds were collected anywhere else other than the tree canopy in this study. The stage at which pods (young or mature) are attacked varies among various seed feeding agents. For instance, larvae and adults of the seed feeding bruchid *M. servulus* feed on green developing *A. cyclops* seeds which are

available between June and January with a peak in October when female weevils are most active (Impson *et al.*, 2004). Consequently in the case of *A. cyclops* it was important for the biocontrol agent to be in abundance in the field when green pods were mostly available and the weevil active. In 1998, post release monitoring showed that *M. servulus* adults and larvae together destroyed 95% of *A. cyclops* seeds (Impson *et al.*, 2000). This level of seed destruction increases the chances of success (decreasing weed density) of the seed feeding agent against the host plant as the biocontrol agent is able to destroy a large proportion of the annual seed crop.

If the proportion of seeds destroyed in a year is small, the seed feeding biological control agent may fail to significantly reduce the weed density regardless of the level of damage caused to seeds. In New Zealand, the seed feeding weevil *Exapion ulicis* (Forster) (Coleoptera: Brentidae) (formally known as *Apion ulicis*) which only oviposits in spring, infested up to 90% of *Ulex europaeus* L., Fabaceae pods produced in spring but ultimately failed to significantly decrease the weed density as pods produced before or after spring were not attacked (Hill *et al.*, 1991). In the present study *C. decapetala* pods filled with mature seeds are available throughout summer (highest densities in September) when *S. subsuturalis* is presumably reproductively active, therefore high levels of infestation would be expected during this period. As such, it is imperative to ensure that *S. subsuturalis* adults are available in the field during summer (September to March). Nevertheless live *S. subsuturalis* adults in this study were only recovered inside seeds in the field in May and June (winter) and none in summer. However *C. decapetala* seed filled pods are barely present during winter (Fig. 2.6) and as a result adults may fail to survive in large numbers to speed up the re-establishment process when new pods are produced in summer (Zimmermann, 1991). In North Carolina, *B. villosus* adults (which complete their lifecycles inside *C. scoparius* seeds) occur on the plant in the field from early April to late August when pods filled with seeds are present. Because the presence of the weevil adults correlates with the availability of pod seeds, *C. scoparius* seed production was reduced by 80% (Redmon *et al.*, 2000).

2.4.2. Establishment of *Sulcobruchus subsuturalis*

In terms of release efforts, Limpopo province was at the top of the list with total agent releases of between 17500, 19000 and 20500 agents in a single release at a site. In the Eastern Cape, 4800 agents were released per site in a single release. Mpumalanga sites received between 1000 and 2000 agents (total) per release whereas in the KwaZulu-Natal sites, the total number of agents released ranged between 900 and 2000 per release (Table 2.1). The data provided by the respective implementation officers shows only the last date of release although KwaZulu-Natal and Limpopo implementation officers state that more than one release had taken place since efforts began in 2000 at some sites. Quarantine colonies of *S. subsuturalis* in Limpopo are destroyed every year after releases between September and December in order to prevent attacks by an unnamed pest (itch mite), of the beetle as well as labourers. A new beetle colony from infested seeds collected from the field is initiated between January and March the following year (pers. comm. Lemao, F., 2007). Data collected at release sites (June 2007) in Tzaneen (Limpopo) are presented (Table 2.1) to demonstrate the establishment of *S. subsuturalis*. Normally, beetle establishment is determined by the increased presence of released agents in two or more successive years (Syrett *et al.,* 2000).

Generally the greatest number of beetle infested seeds where collected in Limpopo where the greatest release efforts occurred. Anecdotal results reveal that there is a correspondence between the number of agents released and the number of beetles recovered. Although large numbers of the biocontrol agent were released when seeds inside pods, and on the ground were available in Tzaneen (Limpopo), the proportion of infested seeds recovered from the field was low, that is only 6.4%, 13.6% and 15.5% seeds bearing eggs in Bodupe, Moshakga1 and Moshakga2 respectively (Table 2.1). Low agent establishment could be due to ant predation of beetle life stages as well as parasitism by native parasitoids. These predictions will be tested in chapter four. In KwaZulu-Natal and Mpumalanga, small numbers of released agents could have reduced chances of establishment in large numbers on their host. This might be as a result of the following:

- In cases of disturbances such as fire and floods (e.g. at Nelsriver Bridge and Riverwild in Mpumalanga), the nascent agent population might be destroyed.
- Reproduction rates may decrease as the ratio of male to female is likely to skew toward males. At all release sites, adults were released at random.
- Inadequate genetic diversity may prevent agents from adapting to their new environments (Grevstad, 1999).

On the other hand, where the above factors do not exist, small numbers of agents can establish, for example in New Zealand, 500 and 1000 *B. villosus* adults were released against *C. scoparius* at two neighboring sites in five different regions and agents established at both sites of four out of the five regions (Syrett *et al.,* 2000). According to Memmott *et al.,* (1996), releasing biological control agents in small numbers and more frequently is likely to be more efficient than releasing biocontrol agents in large numbers but less frequently. Neither of the above techniques was used in any of the sites in South Africa. Instead all releases either of small or large numbers of agents have only been made once since the campaign started.

Seeds bearing eggs and adults were collected in May 2005 (winter) in KwaZulu-Natal. In January 2007 (summer) another batch of seeds bearing only eggs were obtained in KwaZulu-Natal. In February 2007 (summer) seeds bearing eggs were collected in the Eastern Cape but no adults were recovered. In March 2007 (summer), 5% of seeds collected from Boughton bore eggs but no adults. Finally in June 2007 (winter) in Limpopo, seeds carrying eggs and adults were recovered. As all adults were recovered inside seeds in winter and none were obtained in summer, it can be concluded that *S. subsuturalis* overwinters as an adult inside seeds mostly in the pods in the tree canopy. However filled pods are barely present during winter and therefore adults may fail to survive in large numbers to speed up the re-establishment process when new pods are produced in summer. This could also affect populations of *S. subsuturalis* on *C. decapetala*. Nonetheless the above conclusions may not be definite due to the differences in the sampling times at different sites.

2.5. Conclusions

Although the density of canopy seeds and seeds on the ground varied among sites at Ferncliffe and Boughton, seeds were available throughout the year. The highest number of canopy seeds was sampled in summer (September to December) in Ferncliffe when the beetle is expected to be reproductively active. *Sulcobruchus subsuturalis* adults were mostly released in summer and in high numbers. As such one would expect the beetle to maintain viable populations in the field but this does not seem to be the case as few beetle-infested seeds were collected. In addition, the high level of seedling densities, especially at Ferncliffe, is attributed to the availability of large numbers of seeds and the possibility that the beetle had no effect on seedling recruitment because it did not successfully establish or that the beetle infested seeds can still germinate. Various factors such as parasitism and predation by native parasitods and ant predation of beetle eggs, larvae, pupae and adults could be responsible for the poor establishment of *S. subsuturalis* on *C. decapetala* in the field. Furthermore, even though large numbers of agents were released especially in Limpopo, very few infested seeds were recovered in the field. Therefore *C. decapetala* may not be the preferred host for *S. subsuturalis*. The following chapter assesses *S. subsuturalis* oviposition preference and efficacy.

Chapter 3

Oviposition and Longevity of *Sulcobruchus subsuturalis* and its effect on seed germination and seedling recruitment

3.1. Introduction

Bruchid beetles

Sulcobruchus subsuturalis is a bruchid beetle. The Bruchidae consist of about 1300 species, most of which are known to feed and breed on only seeds of leguminous plants (Southgate, 1979). For example in North America, most *Algarobius* species attack only Mesquite (*Prosopis* species) (Johnson 1983; Kingsolver 1986). According to Janzen, (1980), bruchids are mostly host specific and can attack seeds in either dehiscent (e.g. *S. subsuturalis* on *C. decapetala* (Coetzer, (2003)) or indehiscent (e.g. *Bruchidius sahlbergi* Schilsky (Coleoptera: Bruchidae) on *Acacia erioloba* E. Meyer, Fabaceae) pods. Those attacking dehiscent pods deposit eggs directly onto the surface of the seed and when the egg hatches, larvae begin feeding on the seed immediately. Meanwhile those attacking indehiscent pods lay eggs on the surface of the pod, therefore larvae have to first penetrate the pod and then begin feeding on the seed (Southgate, 1979). In Chile, 68% of indehiscent *Acacia caven* (Mol.) Leguminosae pods were attacked by *Pseudopachymeria spinipes* (Erickson) (Coleoptera: Bruchidae) adults. This was measured by counting the number of adult emergence holes on the pods (Rojas-Rousse, 2006). However counting adult emergence holes to indicate the intensity of attack by the bruchid on its host is unreliable as some adults may leave the pod by the first pierced hole whereas others may not (Rojas-Rousse, 2006).

The majority of bruchids typically complete their lifecycles inside seeds and when the larvae are ready to pupate, they create a window by feeding close to the seed testa so as to make an emergence route for adults to emerge (Southgate, 1979). Bruchids' adult longevity has been reported and ranges between 4, 20 and 40 days (Ernst *et al.*, 1990; Rojas-Rousse, 2006). *Sulcobruchus subsuturalis* adults have been reported to live up to 65 days (Coetzer, 2003). Bruchids can damage a large proportion of the seed crop of an

attacked plant if undisturbed by fire, floods, predation as well as parasitism. For example in Botswana, the seed feeding beetle *Bruchidius uberatu*s (Fahraeus) (Coleoptera: Bruchidae) was reported to damage 60% of *Acacia nilotica* L. Willd seeds (Ernst *et al.*, 1990).

Generally most bruchids (either univoltine (one generation a year) or multivoltine (two or more generations a year)) are capable of showing high levels of establishment on their target plants and cause severe damage to seeds if uninterrupted by disturbances such as fire, floods and predation as well as parasitism in their natural field environments (Southgate, 1979). Under controlled laboratory conditions, bruchids can also cause high levels of host infestation, especially when a good number of seeds are kept for long periods of time (Coe and Coe, 1987).

In certain regions (e.g. the Mediterranean), nearly all bruchid beetles are dormant during winter when temperatures are low but become reproductively active from spring onwards (Southgate, 1979). For the multivoltine bruchid beetle *Kytorhinus sharpianus* Bridwell (Coleoptera: Bruchidae) in the United States of America, females emerging immediately after winter (from diapausing larvae) do not lay eggs unless they feed, whereas those from the non-diapause generation can oviposit eggs immediately after emerging without having to feed (Ishihara and Shimada, 1994). In North Carolina *B. villosus* spends the winter period in the soil and becomes active from spring and throughout summer (Redmon *et al.*, 2000). The period when beetles are active coincides with the period when seeds are available for beetles to breed. There have been limited reports concerning the biology of *S. subsuturalis* in relation to *C. decapetala.* However chapter two of this study indicates that seeds are available inside pods; on the ground and at various soil depths throughout the year. This chapter investigates whether *S. subsuturalis* can deposit eggs on *C. decapetala* seeds placed under various conditions (inside pods, soil surface and buried).

The effect of bruchids on seed germination and seedling recruitment

Because most bruchid beetles complete their lifecycle inside seeds, they have the capability to consume the entire contents of the cotyledon, and hence prevent or reduce seed viability and the potential to germinate (Coe and Coe, 1987; Southgate 1979). For example the development of the bruchid beetle *B. sahlbergi* in *A. erioloba* seeds led to the destruction of 9 to 100% of the cotyledon of a single seed (Ernst, 1992). In Australia, larvae of the seed feeding bruchid beetle *Penthobruchus germaini* (Pic) (Coleoptera: Bruchidae) destroyed 90-100% of the cotyledon of *Parkinsonia aculeate* L. (Caesalpiniaceae) seeds therefore preventing germination (Briano *et al.,* 2002).

The ability of infested seeds to germinate and establish into seedling declines with the level of damage caused. In some cases if the embryo of the seed is not destroyed a seed may germinate but may not necessarily establish into a seedling because of reduced reserves caused by bruchid attack (Coe and Coe, 1987; Okello and Young, 1999). Even though the entrance and exit holes created by bruchid larvae and adults respectively allow water imbibition, hence speeding up the germination process by breaking the seed coat-imposed dormancy especially in seeds of some *Acacia* species, seed germination and seedling establishment may still remain low (Mucunguzi, 1995; Okello and Young, 1999). A seedling is a young plant still dependant on food reserves stored in the cotyledons (Kitajima and Fenner, 2000). As such, for seedlings to emerge there should be sufficient food reserves in the seed. However infestation of seeds by seed feeders may bring about scarcity of food reserves. For instance 100% germination was recorded in seeds of *Acacia albida* (Del.) A. Chev. (Leguminosae) infested by seed feeding bruchids (not specified), however the majority of the seedlings did not survive due to limited reserves remaining in the seed (Hauser, 1994). On the other hand some researchers working on *Acacia* seeds reported no significant differences in the rate of germination between infested and intact seeds (Miller, 1994; Mucunguzi, 1995).

This chapter had the following objectives:-

- To examine the biology (oviposition and adult longevity) of *S. subsuturalis*

- To examine the effect of *S. subsuturalis* on *C. decapetala* seed germination as well as seedling recruitment.

3.2. Materials and methods

3.2.1. Oviposition period and adult longevity

To determine the length of time during which eggs were laid in the lifecycle of *S. subsuturalis* (oviposition period) and adult longevity of *S. subsuturalis*, 20 pairs of adult beetles were added to a plastic container (12.6 x 6.6 x 7.9cm). Each container contained 100 *C. decapetala* seeds and cotton wool soaked in 10% sugar water on which adults feed. The containers were kept in the insectary at 20-25ºC under high humidity. The 100 seeds were replaced on the second or fourth day depending on whether the beetles had laid eggs on the seeds. The number of eggs laid per seed were counted and recorded. Furthermore the day on which the first beetle eggs were observed on seeds was recorded. Seeds carrying *S. subsuturalis* eggs were used to determine adult longevity. Seeds carrying *S. subsuturalis* eggs were placed in separate plastic containers (12.6 x 6.6 x 7.9cm) in the insectary at 20-25ºC under high humidity and monitored for beetle emergence. The time of emergence and death of beetles was recorded. The experiment ran for approximately 35 days and there were three replicates.

To determine if *S. subsuturalis* is univoltine or multivoltine under laboratory conditions, a total of 300 field collected seeds carrying *S. subsuturalis* eggs (provided by the Working for Water implementation officer, Abbie Heunis) were monitored in the insectary and emerging adults were provided with fresh/new (intact) seeds for oviposition and hence development of the following generations. Successive beetle generations were recorded as follows: -

- Parental generation: Emerged from the first batch of 300 seeds
- F1 generation: Emerged from the second batch of 100 seeds carrying eggs deposited by beetles from the parental generation
- F2 generation: Emerged from the second batch of 100 seeds carrying eggs deposited by beetles from the F2 generation.

3.2.2. Oviposition preference

To examine the oviposition preference of *S. subsuturalis*, a total of 30 seeds in batches of 10 were respectively placed on (a) the soil surface, (b) 2cm deep and (c) 4cm deep in a plastic container (12.6 x 6.6 x 7.9cm) (treatment) (Fig. 3.1). Seeds were placed in 1 container at all three depths. Soil used in this experiment was collected from Boughton and Ferncliffe in Pietermaritzburg, KwaZulu-Natal. As a control, a batch of 10 other seeds was placed in a plastic container without soil (Fig. 3.1). Five pairs of adult beetles were then placed in each plastic container (treatment and control) with cotton wool soaked in 10% sugar water. Each container was covered with a lid containing small (0.5mm) holes for aeration. The containers were kept in quarantine at 20-25ºC under high humidity. There were three replicate containers and trials ran for 30 days at the end of which the number of eggs laid on seeds was counted.

Seeds inside pods

In order to determine whether *S. subsuturalis* can attack seeds inside dehisced pods, 14 pods containing 146 seeds were hung in a cage into which five female and five male beetles were added. A piece of cotton wool soaked in 10% sugar water was also placed within the cage. There were two replicate cages (Height 42cm x Length 25.8cm x Width 25.8cm) and ran for 14 days at the end of which the number of eggs laid on seeds was counted.

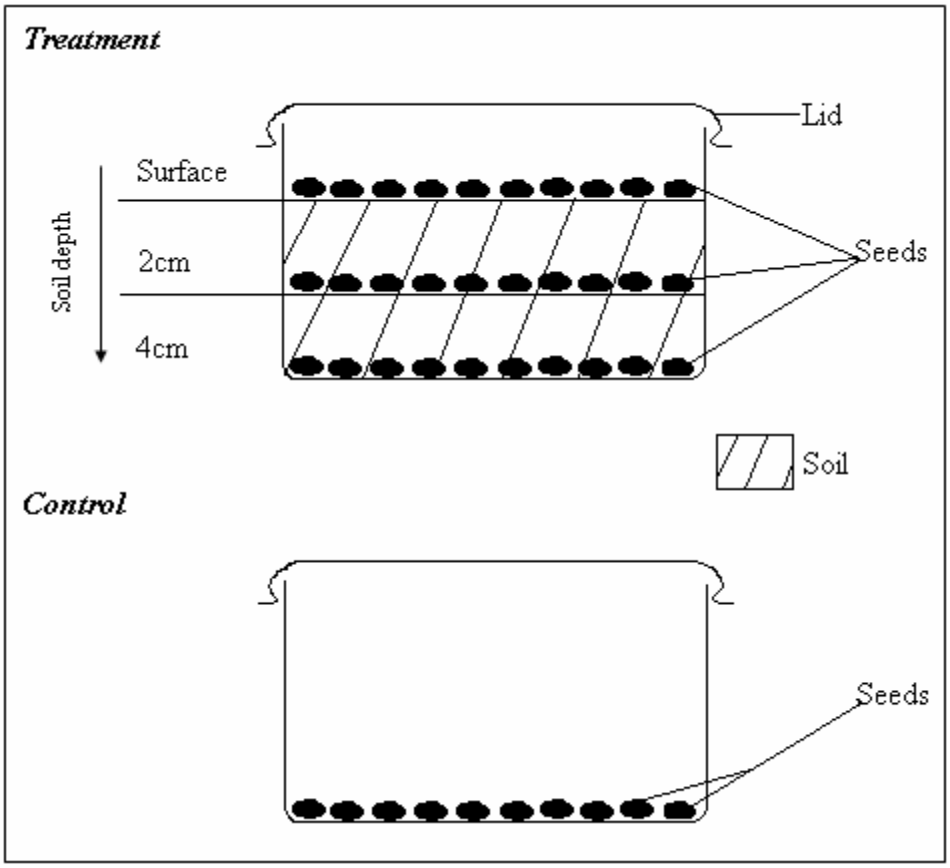


Figure 3.1: Experiment to test whether *Sulcobruchus subsuturalis* adults can attack *Caesalpinia decapetala* seeds at different soil depths. In the treatment seeds are placed in a plastic container at different soil depths (surface, 2cm and 4cm). In the control seeds are placed in a plastic container without soil.

3.2.3. Effects of *Sulcobruchus subsuturalis* on seed germination and seedling recruitment

In order to determine the effect of *S. subsuturalis* on seed germination, infested seeds and uninfested seeds were germinated in growth chambers and the glasshouse. All seeds were collected from the field (from pods on the trees) from either Boughton or Ferncliffe throughout 2006.

Seed germination experiment

Prior to germination, all seeds were scarified using a bench grinder to hasten the germination process as *C. decapetala* seeds possess a tough impermeable testa which impedes water uptake. Seeds were also surface sterilized using a solution of 10% sodium hypochlorite for 10 minutes to minimize fungal growth.

Germination and seedling emergence

This experiment used a total of 192 seeds which were divided into four groups of 48 seeds each. Seeds were placed singly in 24-well ELISA plates containing distilled water. The seeds were germinated in a growth chamber for 14 days then transferred to a glasshouse. Monitoring of seed germination in the glasshouse continued for one month in order to determine the effect of the beetle on seedling emergence. Day time temperatures in both the glasshouse and the growth chamber were set between 25-27ºC and night time temperatures were set at 18ºC. Each group of seeds was categorized as; Treatment (a) seeds containing larvae, (b) seeds containing adults and/or emergence holes and (control) (c) uninfested seeds. Beetle eggs take about eight days to hatch and *S. subsuturalis* larvae penetrate the seed once eggs hatch thus seeds carrying eggs more than eight days old were assumed to contain larvae. While inside the seed, mature larvae create a window by feeding close to the seed surface for adults to emerge (Southgate, 1979). Seeds bearing that window were assumed to contain adults. Seed germination was recorded after 14 days. In order to examine seedling emergence, all 192 seeds were then transferred to the glasshouse in labeled polystyrene cups (10.8cm deep, 8cm diameter) containing soil collected from Boughton and Ferncliffe. Two seeds were planted at 1-2cm depth in each polystyrene cup. Seeds were watered everyday at 12h00.

3.2.4. Statistical analyses

A Chi-square test using a 2 x 2 contingency table was performed to test the difference between infested (treatment) and unifested (control) seeds in terms of germination. The significant difference between infested and unifested seeds in terms of seedling emergence was also tested by performing a Chi-square test using a 2 x 3 contingency table. The oviposition and adult longevity sample sizes were too small to analyse statistically.

3.3. Results

3.3.1. Oviposition period and adult longevity

The oviposition period lasted over 26 days and a single female deposited approximately 33.2 eggs on average (Table 3.1). Adult longevity varied among beetles. While some beetles (10%) lived for three days, others (58%) lived for 15 days, moreover by day 30 all (100% of 120 beetles) had died (Figure 3.2).

Table 3.1: Mean number of eggs deposited by a female on 100 exposed seeds per replicate within the oviposition period. n = 20 females per replicate.

	Day on which first beetle eggs were observed	Total no. of days of oviposition	Total no. of eggs	No. of eggs/female	No. of eggs/seed
Replicate 1	3	30	895	44.75	8.95
Replicate 2	3	27	302	15.1	3.02
Replicate 3	2	22	794	39.7	7.94
Mean±SE	2.7±0.3	26.3±2.3	664±183.2	33.2±9.2	6.6±1.8

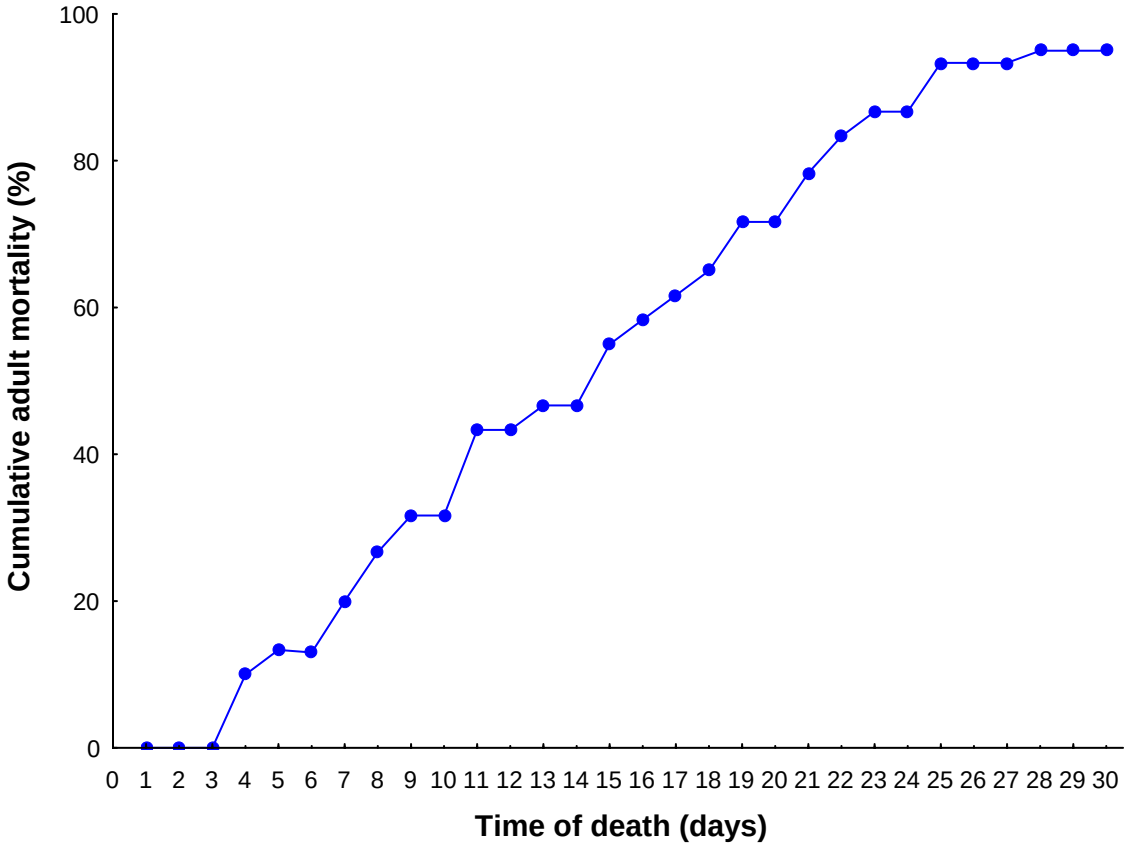


Figure 3.2: Cumulative percentage of dead *Sulcobruchus subsuturalis* adults over time (days). n= 120 adults.

Beetle longevity over three generations

There were three successive generations within a period of 10 months in the laboratory which implies that under laboratory conditions *S. subsuturalis* is multivoltine (Table 3.2).

Table 3.2: The total number of adult beetles emerging per successive generation over time under laboratory conditions. Monitoring took place from June to December 2006.

	No. of seeds carrying eggs	**Total no. of adult beetles**	**Beetle emergence period (days)**
Parental generation	300	320	182
F1 generation	100	141	70
F2 generation	100	84	48

3.3.2. Oviposition preference

There were only 1.7 eggs laid per seed on the batch of seeds placed on the soil surface, and no eggs were laid on seeds buried 2 and 4cm below the soil surface (Table 3.3). On the other hand there were 7.5 eggs laid per seed in the containers without soil (control) (Table 3.3). For seeds inside hanging pods, there was a total of 19 eggs from 10 females. Therefore each female laid 1.9 eggs and there were only 0.1 eggs laid per seed.

Table 3.3: Egg frequencies recorded on *Caesalpnia decapetala* seeds (n = 30 seeds/treatment) placed in containers with soil on the soil surface, 2 and 4cm depths and in containers without soil. n = 15 females per experiment.

Treatments	Total no. of eggs	No. of eggs/female	No. of eggs/seed
Soil surface	52	3.5	1.7
2cm deep	0	0	0
4cm deep	0	0	0
Control (no soil)	225	15	7.5

3.3.3. Seed germination

There was 75% germination of uninfested seeds while only 52.1% of seeds bearing beetle eggs germinated (Fig 3.3). There was a significant difference between uninfested and infested seeds (bearing eggs only) in terms of germination (df = 1; χ^2 = 5.4407; P = 0.0197) (Fig 3.3). All eggs had hatched by the time germination trials were carried out which implies the larvae had already tunneled through the seed testa. The assumption here is that larval entrance holes decreased the probability of seed germination. Furthermore there was a significant difference between uninfested seeds and seeds containing adults in terms of germination (df = 1; χ^2 = 43.885; $P < 0.0001$). Seeds containing larvae and adults showed low levels of germination (Fig. 3.3). There was no significant difference between seeds containing larvae and seeds containing adults in terms of germination (df = 1; χ^2 = 0.9241; P = 0.3364).

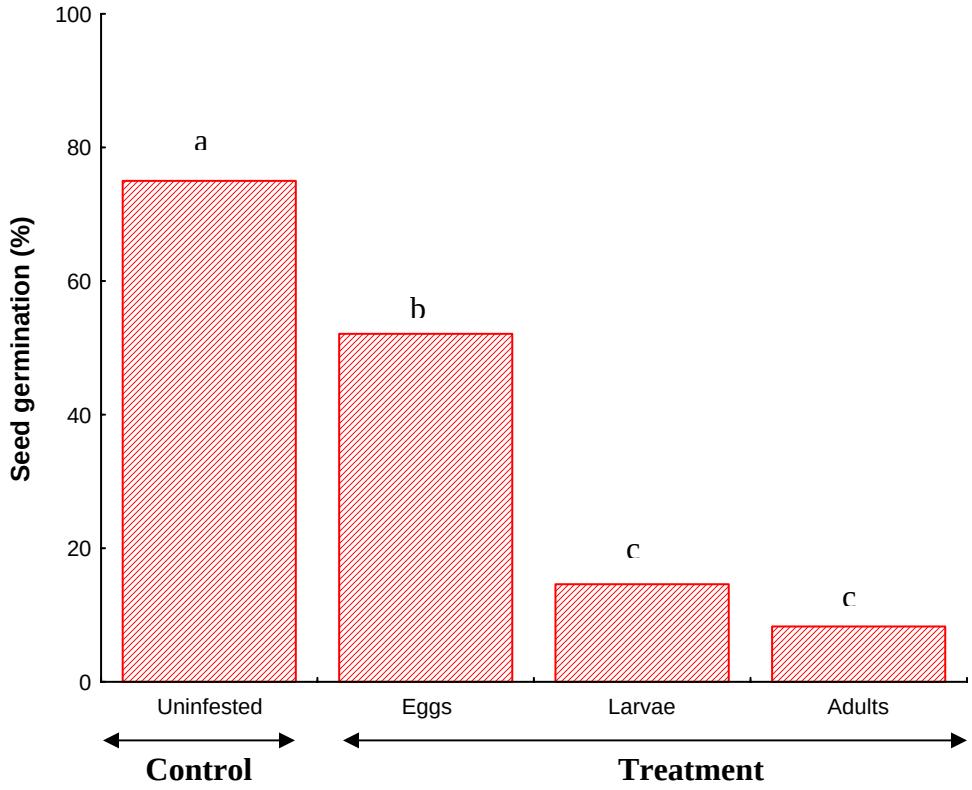


Figure 3.3: Percentage seeds germination after predation by different stages of *Sulcobruchus subsuturalis.* n = 48. Bars with different letters (a,b) are significantly different at 0.05 level of significance based on the chi-square test (contingency tables).

Seedling emergence

Only 35.4% of uninfested seeds (of the starting total) established as seedlings (Fig. 3.4). Percentage seedling emergence from seeds with hatched eggs was 6.3%. Only 2.1% and 6.3% of seeds with larvae and adults respectively emerged as seedlings (Fig. 3.4). As expected, there was a significant difference between unifested seeds and seeds containing larvae or adults (df = 2; χ^2 = 25.4216; P < 0.0001). However there was no significant difference between seeds containing eggs, larvae and adults in terms of seedling emergence (df = 2; χ^2 = 1.2013; P = 0.5485).

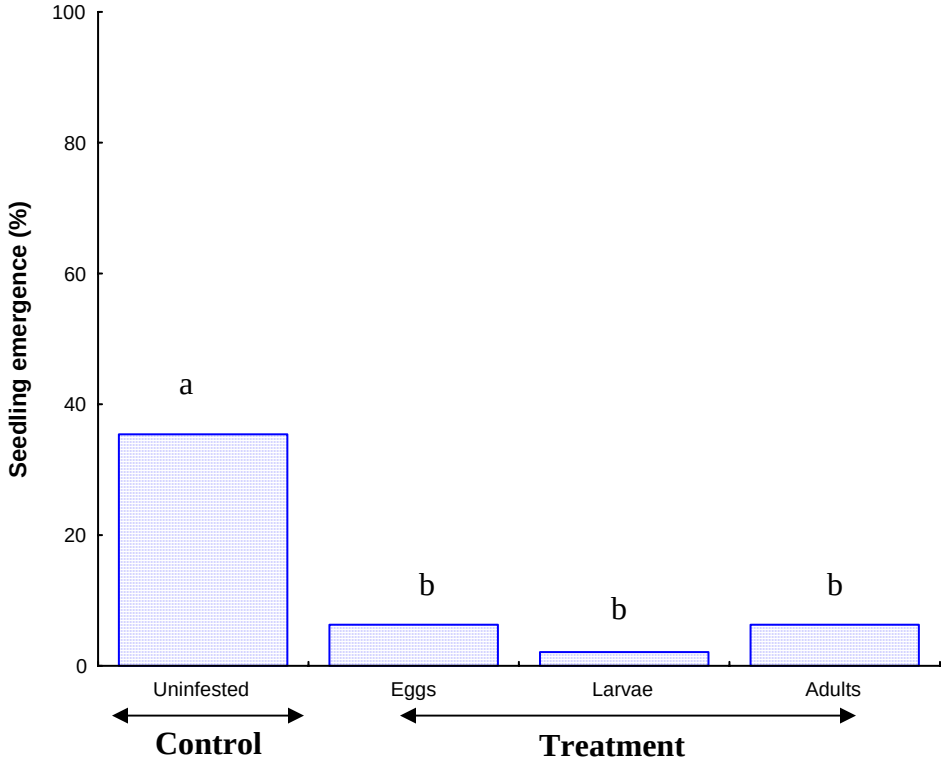


Figure 3.4: Percentage seedling emergence after attack by different stages of *Sulcobruchus subsuturalis.* n =48. Bars with different letters (a,b) are significantly different at 0.05 level of significance based on the chi-square test (contingency tables).

3.4. Discussion

3.4.1. Oviposition period and adult longevity

Following confinement with males, female beetles normally started laying eggs on the third day (i.e. three days after emerging) and the oviposition period lasted 26.3 ± 2.3 (mean ± SE) days (Table 3.1). Each *S. subsuturalis* female laid 33 ± 9.2 eggs (Table 3.1). Coetzer, (2003) reported that each *S. subsuturalis* female deposits a total of 73 to 111 eggs. Nevertheless Coetzer, (2003), did not demonstrate how he determined this. According to Janzen, (1980), a female bruchid beetles typically deposits between 50 and 100 eggs in her life time, frequently depositing one or a few eggs on a single seed. In the present study, females laid 6.6 ± 1.8 (mean ± SE) eggs per seed on average (Table 3.1). For biological control purposes, if all the eggs hatch and the larvae penetrate the seed to continue their development, damage to the *C. decapetala* seed crop would be severe.

However very few *C. decapetala* seeds were attacked by *S. subsuturalis* in the field (Table 2.1) and as a result annual seed production was high (Figs. 2.6 and 2.7).

Up to seven *S. subsuturalis* adult beetles can emerge from a single *C. decapetala* seed which is typical of bruchids and their hosts especially if the beetle is small relative to the size of the seed (Kalibbala, 2005 unpublished data). The emergence holes of *S. subsuturalis* (± 4mm long) are normally 1 to 1.5mm in diameter and a *C. decapetala* seed is 8-12mm long and 6-8mm wide. A seed is likely to survive if a single beetle develops inside the seed and the embryo remains intact.

Adult longevity varied between 3 to 30 days for *S. subsuturalis* in this study (Fig. 3.2). The life span of adults of the bruchid beetle *B. uberatus* on *A. nilotica* in Botswana ranges between 4 to 40 days (Ernst *et al.,* 1990). In Chile, *P. spinipes* adult longevity on *A. caven* is approximately 20 days (Rojas-Rousse, 2006). However Coetzer, (2003) reported that *S. subsuturalis* adults live up to 65 days but again he did not show how he arrived at that figure. It has been demonstrated that under favourable conditions, *S. subsuturalis* is multivoltine (Table 3.2). According to Coe and Coe, (1987), beetles can go through several generations under controlled conditions and ultimately cause high levels of infestation of their host. However, at this point it is not yet clear how many generations *S. subsuturalis* undergoes under natural conditions in the field. It was reported in Chapter two that seeds were available throughout the year with a peak of pod-seeds between September and December 2006, and hence it appears that there should be enough *C. decapetala* seeds within the pods to support two *S. subsuturalis* generations in the field as there was no seed production in the first year (2006) at Boughton. In Australia, mature *P. aculeate* seeds within pods were available for the multivoltine seed-feeding bruchid beetle *P. germaini* whose adults lay eggs on mature pods between October and January (Van Klinken, 2005). Due to factors such as pollination, resource availability (for fruit and seed ripening) and seed predators, the flowering peak and hence the seed peak period of individual plants of a given species may vary year after year (Brody, 1997; Pico and Retana, 2001).

3.4.2. Oviposition preference

Sulcobruchus subsuturalis did not lay eggs on seeds buried below the soil surface and had difficulty laying eggs on seeds lying directly on the surface of the soil (Table 3.3). However beetles deposit large numbers of eggs on seeds placed in containers without soil (Table 3.3). Few eggs were laid on seeds inside hanging pods in the laboratory but the few adults available did manage to find seeds and lay eggs on them. In the field, all seeds carrying eggs were collected inside pods from the tree canopy but not on the ground (chapter two). Studies of bruchid beetle attacks on several *Acacia* species have reported seed attacks ranging from fresh green pods to fully mature dry pods on the tree (Ernst *et al.,* 1990; Miller, 1994; Impson *et al.,* 2004). *Melanterius servulus* specifically feeds and breeds on *Acacia cyclops* seeds in fully developed but soft green pods (Impson *et al.,* 2004). In Australia, *P. germaini* oviposits eggs on *P. aculeata* mature pods (tree canopy and ground) and seeds within pods in the tree canopy as well as seeds on the ground (rarely though). For instance up to 94% of sampled seeds within pods contained eggs as opposed to only 1.3% of 1727 seeds sampled on the ground (Van Klinken, 2005). Beetle eggs could be rubbed off seeds collected from the ground. But in the present study, (a) no *S. subsuturalis* eggs were found on seeds collected from the ground in the field (b) the beetle can not find buried seeds for oviposition and (c) there were very few eggs laid in seeds on the soil surface in the laboratory. These findings strongly suggest that *S. subsuturalis* usually lays eggs on seeds in pods in the tree canopy and that high numbers in the laboratory cultures are an artifact of the rearing process.

3.4.3. Effects of *Sulcobruchus subsuturalis* on seed germination and seedling recruitment

For most bruchid beetles, when eggs hatch, larvae begin to burrow through the seed/pod, forming entrance holes in the pods and/or seeds and ultimately the adults create emergence holes to exit the seed/pod. Although larval entrance holes and adult emergence holes allow rapid penetration of water into the seed, thus stimulating germination, seed germination still remains low as a result of non-viable seeds (Miller, 1994). Only 52.1% and 6.3% of *C. decapetala* seeds carrying hatched *S. subsuturalis*

eggs germinated and then emerged as seedlings respectively (Figs. 3.3 and 3.4). Seed germination may be relatively high but possibly due to limited reserves in the beetle damaged seed, seedling emergence was minimal. *Sulcobruchus subsuturalis* completes its lifecycle inside *C. decapetala* seeds and when the adult emerges from the seed is likely not to germinate due to seed damage during beetle development. Therefore only 8.3% of seeds containing adults germinated and 6.3% emerged into seedling (Figs. 3.3 and 3.4). These seeds germinated regardless of beetle damage probably because beetles had not destroyed major generative parts such as the embryo.

However, in general, percentage seedling recruitment was much lower than percentage seed germination, possibly due to lack of reserves to sustain seedling growth (Coe and Coe, 1987). Conversely 75% of undamaged seeds germinated (9 times higher than uninfested seeds) and 35.4% (5.6 times higher than uninfested seeds) established into seedlings (Figs. 3.3 and 3.4). This is understandable as seeds were intact and there are several explanations for the lack of germination or seedling recruitment in the remaining proportion e.g. some seeds could still have been in a dormant state or maybe resources (light, water) in the soil at the time could not support seedling recruitment.

The lower germination percentage in beetle-damaged seeds can be compared to other studies involving bruchid beetles and seeds of A*cacia* species. For example Halevy (1974) demonstrated that the germination percentage of *Acacia pachyceras* seeds damaged by bruchids was only 6% as opposed to 68% in undamaged seeds. In contrast some studies have shown the opposite, for instance Mucunguzu, (1995) reported that 17% of beetle damaged *Acacia sieberana* seeds germinated and 0% germination in intact seeds. However other studies (e.g. Miller, 1994) showed no difference between bruchid beetle damaged and undamaged seeds of some *Acacia* species in terms of germination percentange. Overall the germination of most leguminous plant seeds can be influenced by damage caused by bruchid beetles among other factors.

3.5. Conclusions

Sulcobruchus subsuturalis is multivoltine under controlled laboratory conditions and therefore can potentially build up a large population to infest a large number of seeds. Because infestation levels were high, the impacts of the beetle on seed germination as well as seedling recruitment were severe in the laboratory. Although there was some degree of germination and seedling emergence, even among infested seeds, it is unlikely that seedlings emerging from beetle damaged seeds would survive competition from those emerging from undamaged seeds in their natural environments. This is due to limited food reserves in beetle damaged seeds.

Beetle egg abundance on seeds placed in containers without soil was higher than that on seeds buried (zero eggs laid) in the soil in the laboratory therefore the beetle may not be able to successfully attack seeds on the ground both under controlled laboratory conditions and uncontrolled field conditions. However in the field all seeds carrying eggs were collected inside pods on the tree. Generally infestation levels in the field were very low despite the large number of agents released, especially in Limpopo province. Infestation levels in the field were low because the beetle did not successfully establish. It would appear from the data presented here that the beetle is restricted to laying eggs on seeds in the tree canopy. These seeds will continue to be dispersed from the pods as the season continues and the fate of the eggs on these seeds remains to be examined. Parasitism and predation of beetle eggs and larvae may account for the poor establishment of the biocontrol agent on *C. decapetala* in the field. The following chapter will assess predation and parasitism of *S. subsuturalis* life stages in the field.

Chapter 4

Ant predation and parasitism by native parasitoids on *Sulcobruchus subsuturalis* life stages.

4.1. Introduction

4.1.1. Predation and parasitism

Several early studies implicated indigenous predator and parasitoid activities in influencing the effectiveness of biological control agents on their target weeds (Dodd, 1940; Pettey, 1948; Dodd, 1961 and Bornemissza, 1966). Nevertheless Goeden and Louda, (1976), later argued that indigenous parasitoids cannot exclusively prevent biocontrol agent establishment. On the other hand predation may prevent biocontrol agent establishment on the target and negatively affect the efficacy of an already established biocontrol agent (Goeden and Louda, 1976). Most predators for biocontrol agents are generalists and range from small mammals to birds and insects. In Australia between 1930 and 1982, there were many introductions of *Tyria jacobaeae* L. (Lepidoptera: Arctiidae) on *Senecio jacobaea* L. (Asteraceae) yet all of them resulted in unsuccessful establishment because of larval and adult mortality of the cinnabar moth (Bornemissza, 1966; Field, 1989). Larval and adult mortality was caused by predation from the indigenous mecopteran *Harpobittacus nigriceps* (Selys) (Mecoptera: Bittacidae) among other factors (Currie and Fyfe, 1938). In South Africa, the native coccinellid *Exochomus flavipes* caused 13% and 53% mortality in colonies of *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) on Prickly pear cacti in the Karoo and outside the Karoo respectively (Pettey, 1948).

Various species of ants have also been implicated as important factors hindering the activities of different biological control agents on their hosts in South Africa. This is because these generalist predators can attack all life stages of a given biological control agent, including eggs, larvae, pupae and adults. In so doing they may prevent sustainable establishment of a biological control agent on the target weed. For example ants such as *Anoplolepis custodiens* (Smith) (Hymenoptera: Formicidae) and *Anoplolepis*

steingroeveri (Forel) limited the effectiveness of the moth *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) which was introduced for the biological control of *Opuntia megacantha* Salm-Dyck (Cactaceae) (synonym of *Opuntia ficus-indica*) in South Africa (Pettey, 1948). Hoffmann (1981) reported that due to the mortality of 80% of eggs of the moth *Tucumania tapiacola* Dyar (Lepidoptera: Pyralidae) on *Opuntia aurantiaca* Lindley (Cactaceae) by ants, the moth failed to successfully establish in South Africa. Nonetheless ants may not be a threat to the successful establishment of moths on cactus weeds in other parts of the world. For example in Australia, the same moth *T. tapiacola* successfully established on the same weed *O. aurantiaca* (Dodd, 1940). Other studies, such as that of Robertson (1985), Robertson (1988) and Robertson and Hoffmann (1989) have also extensively examined the damage caused by ants on the eggs, larvae and pupae of *C. cactorum* on cactus weeds in South Africa. Predation caused egg mortality of 56.6% (summer generations) and 53.5% (winter generations) on *Opuntia ficus-indica* (L.) Miller (Cactaceae). In *O. aurantiaca* egg mortality due to predation was 74% and 72.4% in summer and winter generations respectively. Adult mortality was 45.3% (summer generations) and 84% (winter generations) (Robertson and Hoffmann, 1989). However, in Australia, *C. cactorum* successfully controlled *O. inermis* (DC) (Cactaceae) and *O. stricta* Haworth, most probably because ants did not interfere with the establishment of the moth. Overall, in South Africa there were more ant species attacking *C. cactorum* than in Australia (Robertson, 1985). In a study by Traveset, (1990), in Costa Rica, ants e.g. *Crematogaster brevispinosa* Mayr (Hymenoptera: Formicida) continued removing egg shells (hatched eggs) even after larvae had penetrated the *Acacia farnesiana* (L.) Willd (Fabaceae) pods. The same scenario was expected for hatched *S. subsuturalis* eggs.

Aside from native predators, native parasitoids could also hinder the success of an introduced biological control agent on a given target weed. Parasitoid attacks on biocontrol agents might decrease agent development on the target weed (Hoffmann, *et al.,* 1993). By 1995, parasitism mainly by indigenous parasitoids was found in approximately 40% of released weed biocontrol agents in South Africa (Hill and Hulley, 1995). Numerous bruchids on *Acacia* species were parasitized by mostly hymenopterous parasites and percentage parasitism was between 1.1 and 78.7%. Moreover there were

only 0.4% to 5.3% parasitoid attacks on bruchid infested mesquite pods (Hoffmann *et al.*, 1993). The parasitoid *Dinarmus actifirons* (Walker) parasitized *Algarobius prosopis* infested mesquite pods in 1989 and by 1990 nine more native parasitoids were recovered, parasitizing *A. prosopis* inside mesquite pods (Hoffmann *et al.,* 1993). But parasitoids recovered from mesquite weeds infested with bruchids did not affect the survival and development of *A. prosopis*, therefore they were not a threat to biocontrol (Hoffmann *et al.*, 1993). Hill and Hulley (1995) recommended that biological control agents susceptible to native parasitoid attacks need not be rejected as the levels of parasitism are normally low. On the other hand some scientists have reported failures of biocontrol agents to establish due to parasitism: e.g. the attack by native parasitoids on a gall forming moth, *Frumenta* (undescribed) on *Solanum elaeagnifolium* (Solanaceae), resulted in the failure of the moth to establish on its target weed (Olkers, 1995). In Australia, eight native parasitoids had a negative impact on the performance of *Procecidochares utilis* stone (Diptera: Tephritidae), which had been released against Crofton weed (Dodd 1961). The present study examines the level of predation and parasitism of *S. subsuturalis* on *C. decapetala* in South Africa.

In this study, chapter two reported that all seeds bearing eggs were collected from pods in the tree canopy and none on the ground. Moreover chapter three indicates that *S. subsuturalis* lays few eggs on seeds on the ground in the laboratory. However, even if eggs are laid on ground seeds or seeds carrying eggs fall on the ground, it is predicted that they will be predated and/or removed by ants. Hence, the objective of this chapter is to examine the removal/predation and parasitism of *S. subsuturalis* life stage (eggs, larvae and adults) in the field.

4.2. Materials and Methods

4.2.1. Removal/predation of *S. subsuturalis* life stages by native ants

To determine the removal/predation of *S. subsuturalis* eggs, larvae and adults in the field, infested *C. decapetala* seeds from the laboratory were divided into three batches of 20 seeds each containing either eggs, larvae or adults. Each batch of seeds either carrying/containing eggs, larvae or adults was placed in the field under the *C. decapetala*

canopy and monitored every second day for a period of 34 days between 10th March and 11th April 2007 at Boughton and Ferncliffe. Prior to exposure in the field, the number of eggs on the seeds was counted. Because larvae and adults were inside seeds, they could not be counted. Instead, seeds containing larvae or adults were counted. Therefore every second day of monitoring, the following were counted: (a) the number of eggs removed; (b) the number of seeds containing larvae predated (c) the number of seeds containing adults predated. Experiments at Boughton and Ferncliffe were conducted concurrently. Each batch of 20 seeds was positioned approximately 1m apart. It takes about eight days for beetle eggs to hatch (chapter 3) and by the start of the trial most of the beetle eggs had hatched (pale coloured). *Sulcobruchus subsuturalis* larvae penetrate the seed as soon as eggs hatch, and therefore seeds carrying eggs more than eight days old were assumed to contain larvae. When inside the seed, mature larvae create a window by feeding close to the seed surface for adults to emerge (Southgate, 1979). In this experiment, seeds bearing that window (which would ultimately become the adult emergence hole) were assumed to contain adults. Every second day, any seeds that had been predated were discarded and only intact seeds were left exposed for further monitoring. There were three replicates of 20 seeds at each site. Hatched eggs were used for most trials because of the lack of unhatched eggs at the time of the study. However, two batches of seeds carrying 28 and 34 unhatched eggs were added as a separate treatment in Boughton and Ferncliffe respectively. It was predicted that unhatched eggs would be predated faster than hatched eggs as they are more attractive as a food item than hatched eggs (Hamish, R., pers. comm. 2008).

To prevent attacks on infested seeds by ants, a control was also established. Three batches of 10 seeds infested with either hatched beetle eggs, larvae or adults were placed in petri-dishes and surrounded by “ant-stop”, a sticky barrier against ants and crawling insects. This control treatment was also replicated three times at each site.

4.2.2. Native parasitoids

Field collected seeds carrying eggs from Limpopo province only were examined under the microscope and the number of eggs parasitized was recorded at each site. Seeds carrying eggs were also dissected to search for more evidence of parasitism.

4.3. Statistical analyses

A Chi-square test using a 2 x 3 contingency table was performed to test the difference in the number of eggs attacked by parasitoids at Bodupe, Moshakga1 and Moshakga2 in Limpopo province. Predation data did not require statistical analyses.

4.4. Results

4.4.1. Removal/predation of *S. subsuturalis* life stages by native ants

Egg removal/predation

At Boughton, the ant *Pheidole megacephala* Fabricius (Hymenoptera: Formicidae) and *Messor natalensis* Mayr (Hymenoptera) and three species of *Crematogaster* were recovered inside beetle infested seeds on two occasions. Beetle egg disappearance from seeds started by day two (with approximately 56% egg removal) (Fig. 4.1). Cumulative percentage egg removal/predation gradually increased with time and by day 10 egg removal was 100% (Fig. 4.1). When seeds containing unhatched eggs were positioned under the *C. decapetala* canopy all eggs were removed within 4 and 6 days at Ferncliffe and Boughton respectively (Table 4.1). The ant species *Crematogaster* and *Tetramorium avium* Bolton (Hymenoptera: Formicidae) were captured near exposed seeds bearing beetle eggs at Ferncliffe and some were feeding inside the seeds. Results of ant removal/predation obtained from Ferncliffe showed a similar trend to those obtained from Boughton (Fig. 4.1).

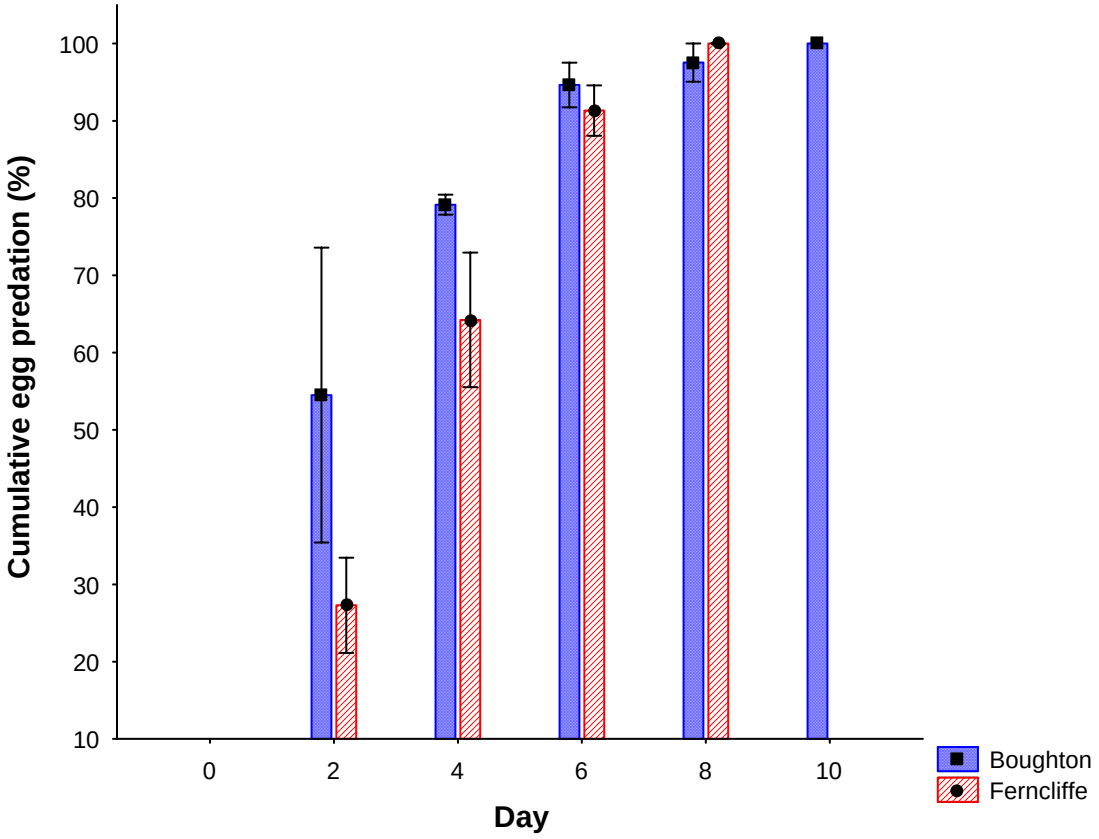


Figure 4.1: Cumulative percentage removal/predation of *Sulcobruchus subsuturalis* eggs between March and April 2007 at Boughton and Ferncliffe. Error bars = SE of the mean. Data from; n = 4 trials, mean = 68.8 eggs/trial (Boughton): n = 3 trials, mean = 66.3 eggs/trial (Ferncliffe).

Table 4.1: Removal/Predation of unhatched *Sulcobruchus subsuturalis* eggs at Boughton and Ferncliffe. n = 1 trial = 28 unhatched eggs (Boughton): n = 1 trial = 34 unhatched eggs (Ferncliffe).

Day	**Total no. of unhatched egg**		**Cumulative egg predation**		**Cumulative percentage egg predation (%)**	
	Boughton	Ferncliffe	Boughton	Ferncliffe	Boughton	Ferncliffe
0	28	34	0	0	0	0
2	28	34	1	30	3.6	88.2
4	27	4	27	34	96.4	100
6	1		28		100	

***Sulcobruchus subsuturalis* eggs protected from ants**

Egg removal/predation at Boughton and Ferncliffe

Even though seeds bearing eggs were protected from ants using "ant-stop" in the control, eggs were still removed/predated to some extent at Boughton. However the percentage of egg removal/predation observed was much lower (less than 45%) compared to the exposed treatment (100%). Egg removal/predation did not continue beyond day 12 in the control (Fig. 4.2). A few ants and flies were found trapped in the sticky substance. Egg removal/predation at Ferncliffe was much lower (less than 25%) than that observed at Boughton (Fig. 4.2). By day two no removal/predation had occurred, but day four showed removal/ predation at a low percentage with a gradual increase in percentage egg removal/predation (Fig. 4.2).

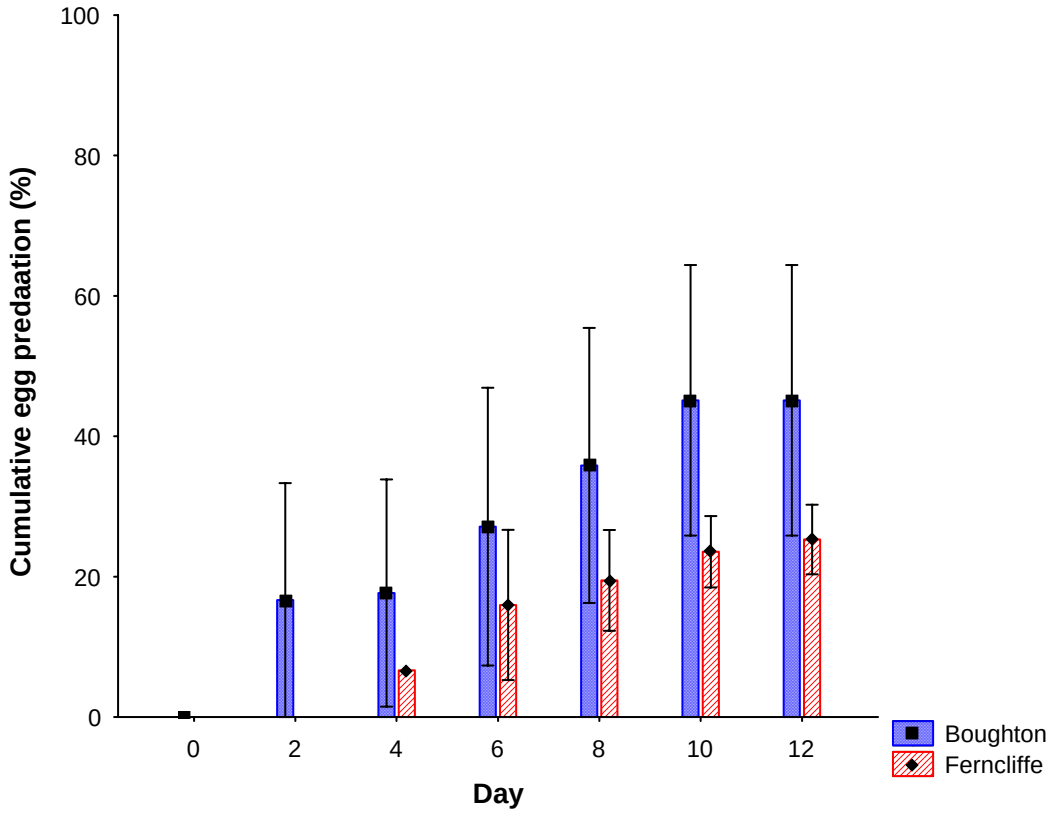


Figure 4.2: Cumulative percentage removal/predation of *Sulcobruchus subsuturalis* eggs from 'ant-protected' seeds in April 2007 at Boughton and Ferncliffe (control). Error bars = SE of the mean. n = 3 trials, mean = 22.7 eggs/trial (Boughton): n = 3 trials, mean = 25.6 eggs/trial (Ferncliffe).

Larval removal/predation at Boughton and Ferncliffe

It was observed at Boughton that removal/predation of seeds containing larvae began at day two and by day 12 all seeds (100%) containing larvae had been removed/predated (Fig. 4.3). At Ferncliffe, larval removal/predation increased rapidly such that by day eight all seeds (100%) containing larvae had been predated (Fig. 4.3).

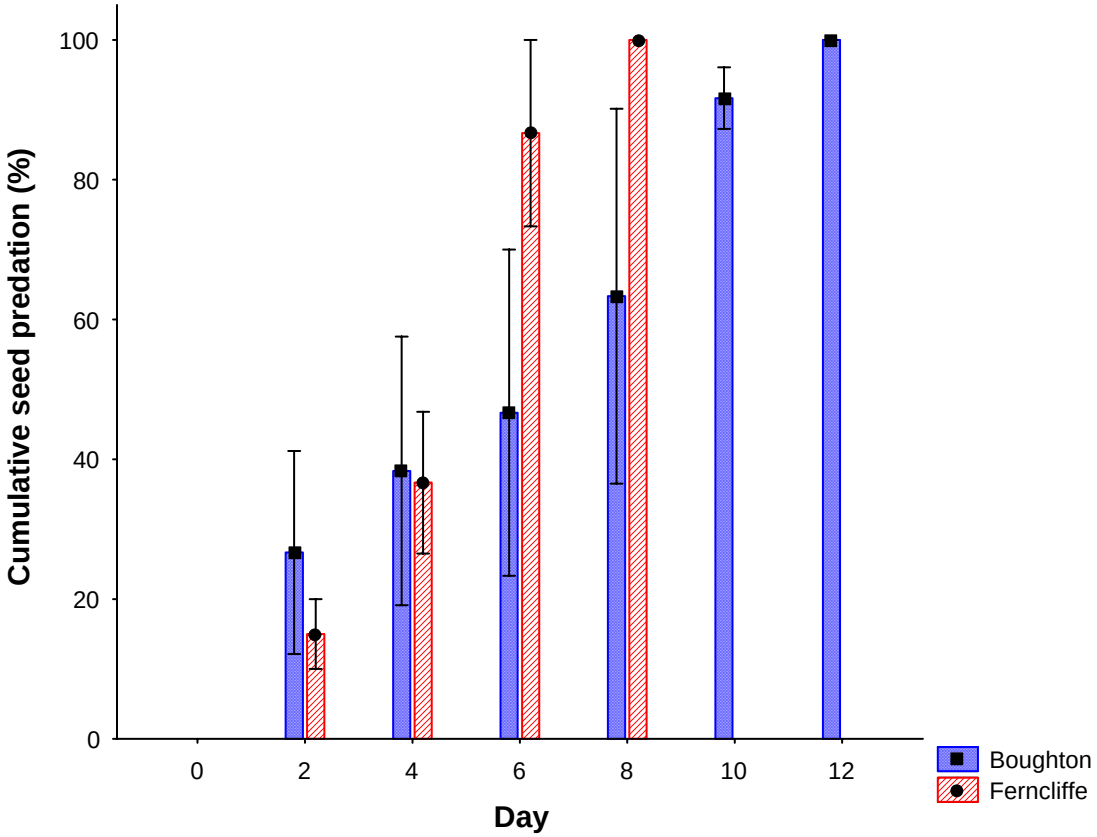


Figure 4.3: Cumulative percentage predation of seeds in April 2007 at Boughton and Ferncliffe. Seeds contained larvae of *Sulcobruchus subsuturalis*. n = 3 trials (Boughton). Error bars = SE of the mean. n = 4 trials (Ferncliffe). n = 20 seeds/trial.

Adult removal/predation at Boughton and Ferncliffe

Seeds used in this experiment contained small circular windows created by larvae for adult emergence. All seeds containing one or more of such windows were assumed to contain/ have contained adults. These windows were easily penetrated by ants which readily burrowed through seeds and attacked the adult beetles. The predation of seeds containing adults increased with time and ranged between 39 and 100% i.e. between day two and eight when all seeds had been predated (Fig. 4.4). At Ferncliffe, the trend

observed was similar to that observed at Boughton except it took 12 days for all seeds containing adults to be predated, and predation was between 17 (day 2) and 100% (day 12) (Fig. 4.4).

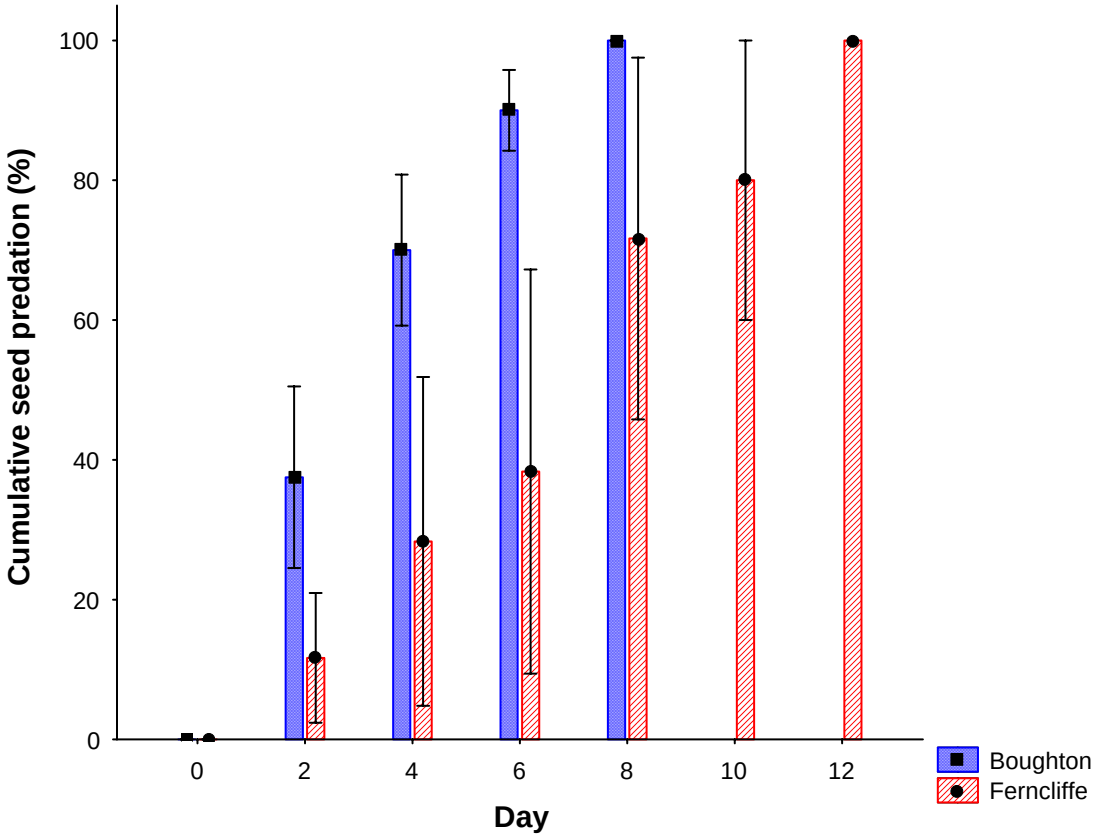


Figure 4.4: Cumulative percentage predation of seeds in April 2007 at Boughton and Ferncliffe. Seeds contained *Sulcobruchus subsuturalis* adults. n = 4 trials (Boughton). Error bars = SE of the mean. n = 4 trials (Ferncliffe). n = 20 seeds/trial.

4.4.2. Parasitoids of beetle eggs and adults

A few eggs had a small exit hole on the dorsal surface which was possibly evidence of egg/larval parasitism. The proportion of eggs parasitized was high at the three sites in Tzaneen (Limpopo) i.e. 81.3%, 82.4% and 93.1% at Bodupe, Moshakga1 and Moshakga2 respectively (Fig. 4.5). There was no significant difference in the number of eggs parasitized between these sites (df = 2; χ^2 = 2.1034; *P* = 0.3493). In Bodupe, 4.2% of the 24 beetle egg carrying seeds contained a parasitic wasp which was identified as *Dinarmus altifrons* (Walker) (Hymenoptera: Pteromalidae). It was found developing

inside one seed and was presumed to be feeding on the immature stage of the beetle. There was no evidence of parasitism from seeds carrying beetle eggs collected at other release sites.

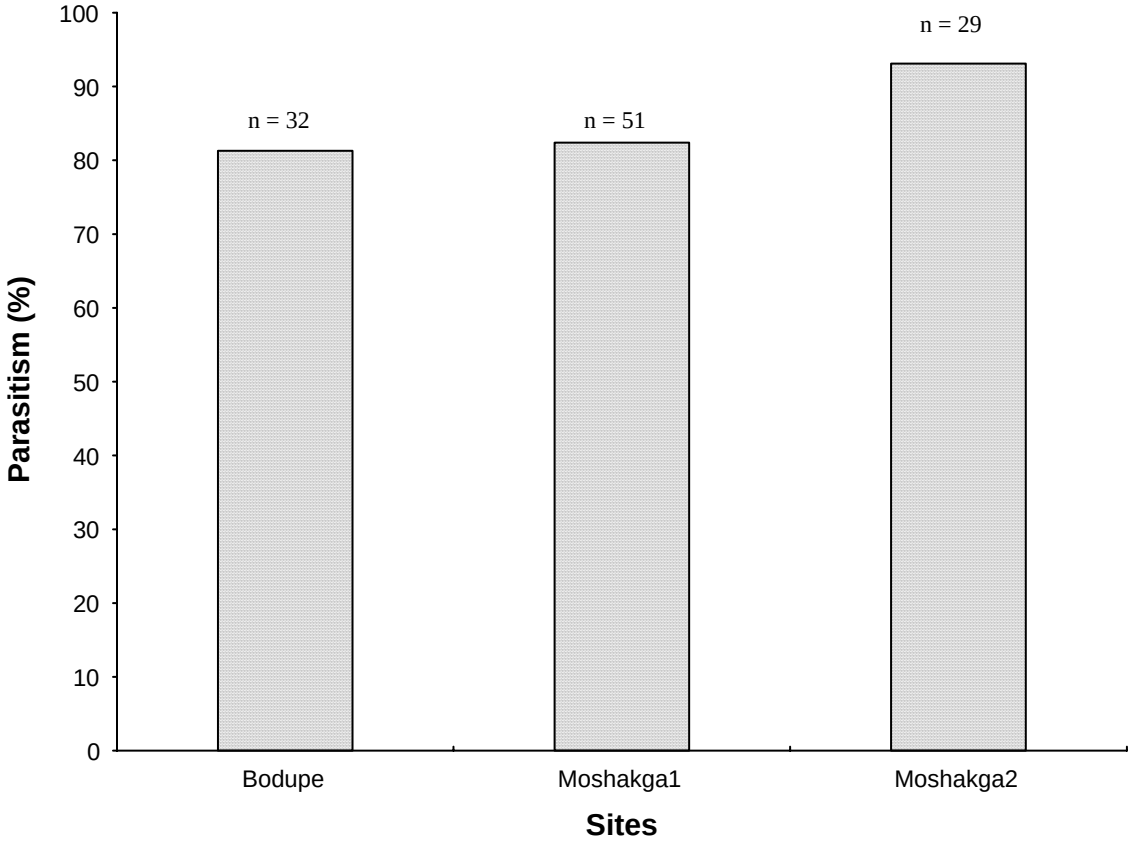


Figure 4.5: Percentage egg parasitism at Bodupe, Moshakga1 and Moshakga2 in June 2007. n = number of eggs sampled/site.

4.5. Discussion

By 1990, very few studies had presented actual data on beetle egg predation by ants (Hinckley, 1961; Nickerson *et al.*, 1977; Hoffmann, 1981; Robertson, 1988) and the topic of egg predation by ants had generally not been well studied (Traveset, 1990). In the present study, no specific experiment was conducted to observe ant activities in the field but on more than one occasion ant species such as *P. megacephala*, *M. natalensis*, *T. avium* and *Crematogaster* species (identified by Hamish G. Robertson director of the

Natural History collections, Iziko Museum, Cape Town) were observed feeding through beetle entrance/emergence holes of seeds. It was therefore assumed that these generalist ants were responsible for the removal of *S. subsuturalis* eggs, larvae and adults. Similar species of ants were the main cause of egg mortality in *C. cactorum* on *Opuntia ficus-indica* (L.) Miller (Cactaceae) and O. *aurantiaca* (Robertson and Hoffmann, 1989). It is important to note that season and prevailing temperatures have a strong influence on egg predation, especially by ants (Robertson, 1985). In the present study trials were only conducted in summer (March to April 2007).

Coetzer, (2000) reported that it takes about eight days for *S. subsuturalis* eggs to hatch and in this study exposed eggs had been completely removed/predated within 10 days at Boughton and eight days at Ferncliffe (Fig. 4.1). Hatched egg removal/predation ranged between 28 and 100% from day two to day 10 (Fig. 4.1). The longer eggs were exposed in the field, the greater their chances of removal/predation. Overall over 90% of hatched eggs at both sites had disappeared by day eight (before hatching) which implies that even if an adult manages to deposit eggs on a seed, eggs may not last on the seed long enough to hatch. Moreover, table 4.1 showed 100% removal/predation of unhatched eggs by day six and four at Boughton and Ferncliffe respectively. Unhatched eggs were predated in a shorter time period than hatched eggs presumably because they are a preferred food item (Hamish, R., pers. comm. 2008). This time period of unhatched egg predation is comparable to that reported from Costa Rica where ant species such as *C. brevispinosa*, *Camponotus rectangularis* Emery (Hymenoptera: Formicidae) and *Pseudomyrmex sericeous* Mayr (Hymenoptera: Formicidae) caused up to 45% mortality of *Mimosestes nubigens* (Mots) (Coleoptera: Bruchidae) and *Mimosestes mimosae* (Fab) (Coleoptera: Bruchidae) unhatched eggs on *A. farnesiana* pods within five days (Traveset, 1990). In Hawaii, ant species of the genus *Monomorium* predated 40% of *Mimosestes sallaei* Sharp (Coleoptera: Bruchidae) eggs on *A. farnesiana* pods (Hinckley, 1961).

Even with the application of the sticky barrier against ants, egg removal/predation was observed at both experimental sites although it was at a low percentage (less than 50% overall) (Fig. 4.2). There are two possible explanations for this: (a) Ants may not be the

only generalist predators responsible for the removal of eggs but flying insects may also have played a role. Various species of crickets, flies and of course the identified ants were found trapped in the sticky barrier at both experimental sites in KwaZulu-Natal (b) Abiotic factors such as rainfall could have contributed to the removal of eggs as experiments were conducted during the rainy season.

Even if the eggs survive to hatch and larvae manage to penetrate the seed, it is probable that ants feed through larval entrance holes and in the process attack larvae and adults developing inside seeds. This seemed to be the case at Boughton and Ferncliffe where exposed seeds containing larvae had all been predated within 12 days at Boughton and eight days at Ferncliffe (Fig. 4.3). It took eight and 12 days for exposed seeds containing adults to be predated at Boughton and Ferncliffe (Fig. 4.4). Ants presumably eat all of the seed contents, including adult beetles. Therefore no adults could emerge from those seeds in the field. Anecdotal evidence showed that ants fed inside seeds containing adults. The high level of removal/predation of *S. subsuturalis* life stages in the present study can be compared to that reported in *C. cactorum* (eggs, larvae, pupae and adults) on cactus weeds in South Africa (Robertson, 1985; Robertson, 1988; Robertson and Hoffmann, 1989). This high level of removal of beetle eggs on the ground could also explain why no seeds carrying eggs were collected on the ground (as reported in chapter two of this study) even if some egg carrying seeds might have fallen from the pods.

Nevertheless, all these predators make up just a small fraction of the problems hindering the survival of *S. subsuturalis* on *C. decapetala* in South Africa. For example in Limpopo province (Bodupe release site), parasitoid entrance/exit holes observed on the surface of eggs, including wasp cocoons in and around parasitized eggs, were signs that eggs were being parasitized. Results showed high level of egg parasitism in Limpopo i.e. between 81.3 and 93.1% (Fig. 4.5). Furthermore the parasitic wasp *D. altifrons* was recovered developing inside a *C. decapetala* seed infested with *S. subsuturalis*. Hoffmann, *et al.*, (1993) reported that in 1989, *D. altifrons* was recovered inside mesquite seed pods infested with *A. prosopis* and apparently reduced the population density of the already established bruchid beetle. It is probable that this parasitoid could have the same effects

on *S. subsuturalis* hence preventing the beetle's development and survival on *C. decapetala*. This could explain the low level of beetle establishment throughout the country i.e. between 0 and 15.5% of seeds bearing eggs across the site (Table 2.1, Chapter 2).

Parasitoids such as *Uscana chiliensis*, (Pintureau and Gering) (Hymenoptera: Trichogrammatidae), *Uscana espinae* (Pintureau and Gering) (Hymenoptera: Trichogrammatidae) and *Dinarmus simus* (Girault) (Hymenoptera: Pteromalidae) are generally host specific and normally search for seeds within pods of their hosts rather than the specific biological control agent inside seeds (Hetz and Johnson, 1988). When parasitoid biology is synchronized to the phenology of their hosts, parasitoid attacks on the plant and hence the plant biological control agent(s) may be high. For instance in Australia parasitism of the seed feeding bruchid beetle *Penthobruchus germiaini* on *Parkinsonia aculeate* reached 70.5% of all eggs. Parasitoids included several *Uscana* species (undescribed) and the larval parasitoid *D. simus* (Van Klinken 2005). In Chile, parasitism of eggs and larvae of the bruchid beetle *Pseudopachymeria spinipes* on *Acacia caven* reached 100%. While *U. espinae* parasitized beetle eggs, *Monoska dorsiplana* (Boucek) (Hymenoptera: Pteromalidae) parasitized beetle larvae (Rojas-Rousse, 2006). Conversely lack of synchrony between parasitoid biology and the phenology of the plant and the biocontrol agent may result in low levels of parasitism. For example in North Carolina, parasitoids such as *Dinarmus* species (undescribed) only attacked later larval stages of *Bruchidius villosus* a biological control agent for *Cytisus scoparius* with parasitism ranging between 6 and 14% at two sites (Redmon *et al.*, 2000). The level of parasitism was low because by the time the parasitoids attacked the bruchid beetle, they (beetles) had already consumed most of the occupied seeds. In the present study, parasitism levels by generalists which ranged between 81.3 and 93.1% at sites in Limpopo could indicate that there was synchrony between parasitoids biology and the phenology of *C. decapetala*.

4.6. Conclusion

The high levels of *S. subsuturalis* (eggs, larvae and adults) predation, mainly by native ants, and evidence of egg and larval parasitism reported in this study could explain the low proportions of *S. subsuturalis* infested seeds recovered from release sites in Limpopo, Eastern Cape, KwaZulu-Natal and Mpumalanga (Chapter 2). Given that chapter two also reports that no seeds carrying eggs were collected on the ground and chapter three suggests that the beetle does not oviposit eggs on seeds on the ground, it can be concluded that once seeds bearing eggs fall on the ground from pods, they are removed/predated by ants. Therefore no eggs were collected on the ground firstly because, adults do not lay eggs on seeds stored on the ground and secondly, ants remove/predate eggs on the ground.

The largest number of beetle infested seeds was recovered in Limpopo province which also showed the greatest release efforts. This implies that release efforts play an important role in the establishment of *S. subsuturalis*, notwithstanding the problems caused by predators and parasitoids.

The level of damage caused by indigenous parasitoids on *S. subsuturalis* is not yet known but can be estimated as large from these data. A combination of parasitism and predation may cause enormous damage which may limit survival and development of a weed biological control agent, hence preventing its establishment. Although the problem of parasitism and predation still stands, it is recommended that beetles (*S. subsuturalis*) should be released in much greater numbers and more frequently. In addition, parasitism of *S. subsuturalis* by native parasitoids needs to be studied in greater depths by thoroughly analyzing large samples of *S. subsuturalis* infested seeds collected from beetle release sites in Limpopo, KwaZulu-Natal, Mpumalanga and the Eastern Cape. This will help measure the extent of the problem pertaining to parasitism. Furthermore, large numbers of unhatched eggs on seeds could be placed in the tree canopies to assess the ability of wasps to parasitise the biocontrol agent. Meanwhile, *S. subsuturalis* has failed to establish in large numbers on *C. decapetala*, therefore the beetle is not yet or may not be an effective biological control agent

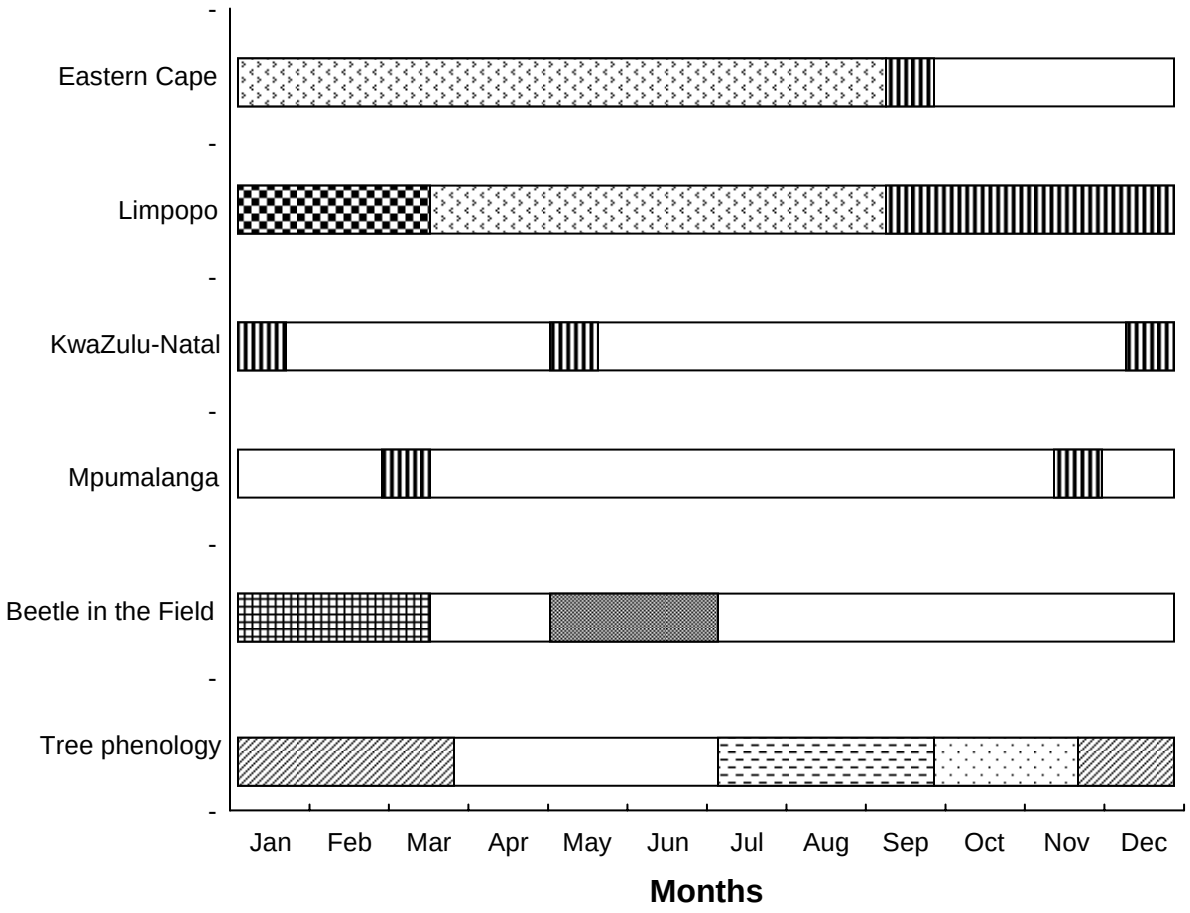
Chapter 5

5.1. General discussion

In South Africa, *C. decapetala* was officially declared a weed in 1983 and by 1998 it was ranked number 20 out of a total of 25 invader species (Coetzer and Neser, 1999; Versfeld *et al.*, 1999). Most of these invasive plants reduce water supply in areas where they invade (riparian vegetation, forest margins and riverine habitats), for instance *C. decapetala* was estimated to use up to 33.82 million m^3 of water a year (Versfeld *et al.*, 1999). Along the Sabie river (riverine zone in Mpumalanga province) alone, the density of *C. decapetala* was estimated to be 400 plants/ha in 2005 (Beater, 2006). In 1996, the seed feeder *S. subsuturalis* was introduced into South Africa from India in an attempt to reduce the density of this invasive weed *C. decapetala* (Coetzer, 2000). Records obtained from the Working for Water (WfW) implementation officers indicate that release activities in *C. decapetala* infested regions along the Eastern coast of South Africa in the provinces Limpopo, Mpumalanga, KwaZulu-Natal and Eastern Cape began in February 2000. The latest releases took place in December 2006 in Limpopo province. Normally, inoculative releases were carried out at each site by WfW. Beetles were reared in local quarantine facilities and released under tree canopies in thousands, mostly during summer except in KwaZulu-Natal where one release took place in winter (Fig. 5.1).

Generally, in the Eastern Cape, beetles are reared in quarantine between January and August and releases take place in September (early spring/summer) (Fig. 5.1). In Limpopo, Infested seeds are collected from the field between January and March and reared in quarantine between April and November. Releases in the field take place between September (early spring/summer) and December (summer). Soon after beetle releases in the field, the quarantine colonies are destroyed. It would have been helpful if the implementation officers in KwaZulu-Natal and Mpumalanga had recorded more detailed information regarding their release activities. Only the dates on which agents were released in the field were provided. In KwaZulu-Natal releases took place in May (winter) 2001; January (summer) 2003 and December (summer) 2003. In Mpumalanga releases took place in November (summer) 2002 and March (summer) 2005. More often

than not releases were undertaken in summer when the beetle is expected to be reproductively active and when mature seeds are available on the tree (spring throughout summer) (Fig. 5.1).



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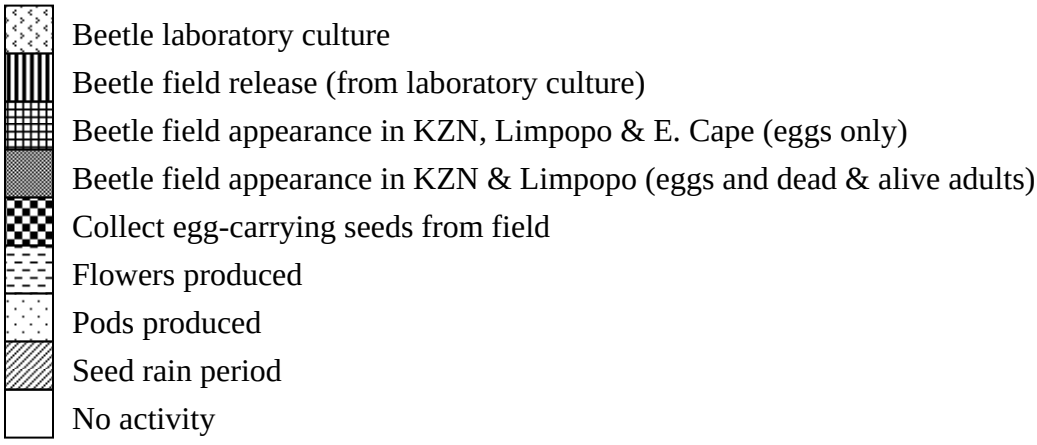

Figure 5.1: Provincial *Sulcobruchus subsuturalis* release activities, appearance of the beetle in the field and the phenology of *Caesalpinia decapetala*.

Initially seeds were sampled each month from two main study sites (Boughton and Ferncliffe) in KwaZulu-Natal from February 2006 to January 2007. The main objective was to determine the presence of *S. subsuturalis* and therefore examine its effect on *C. decapetala* seed density as well as seed germination and seedling recruitment. However no beetles were recovered throughout that sampling period. Consequently, with the same objective, additional sites i.e. one in KwaZulu-Natal and three in each of the remaining provinces (Mpumalanga, Eastern Cape and Limpopo) were sampled. The additional study site (Mtubeni Valley) in KwaZulu-Natal was sampled in January 2007. Study sites in Mpumalanga (Nelsriver Bridge, Riverwild and Tropicado) and the Eastern Cape (Nomvalo, Tutor-Ngeleni-pass and Tutor-Ndamase-pass) were sampled in February 2007. Study sites in Limpopo (Bodupe, Moshakga1 and Moshakga2) were sampled in June 2007. Meanwhile sampling continued at the two main study sites (Boughton and Ferncliffe) in KwaZulu-Natal between February and April 2007. Therefore in total there were 15 months (February 2006 to April 2007) of sampling at Boughton and Ferncliffe.

Caesalpinia decapetala flowered between July and September. Pods are produced from the end of September to mid November and seeds started falling from mid November onwards (Fig. 5.1). Because *C. decapetala* can retain pods containing seeds for extended periods of time, canopy seeds were generally available throughout the year. However the highest number of canopy seeds was sampled in summer (September to December) in Ferncliffe. Moreover summer is also the period when the beetle is expected to be reproductively active. Field results showed that the beetle occurs in the field in the winter months of May and June (recovered eggs and adults (overwintering inside seeds)) and summer months of January, February and March (recovered eggs only) (Fig. 5.1). Approximately 1% of all overwintering *S. subsuturalis* adults were alive at the time of recovery and the rest were dead. No live adults were captured at release sites in summer. This chapter summarises the overall findings with regard to the establishment of *S. subsuturalis* and its effect on *C. decapetala* as the first post release evaluation of the agent.

Sulcobruchus subsuturalis has failed to establish in large numbers on *C. decapetala*

Sulcobruchus subsuturalis adults were mostly released in summer and in high numbers (900 agents in some sites in KwaZulu-Natal and up to 20500 agents in others in Limpopo). As such one would expect the beetle to maintain viable populations in the field, especially in Limpopo, but this was not the case. The proportion of infested seeds collected from release sites in Limpopo, KwaZulu-Natal and the Eastern Cape was low (Table 2.1). No infested seeds were found from Mpumalanga province. These low proportions of infested seeds obtained from release sites, even after large numbers of biocontrol agent releases especially in Limpopo province, clearly indicate that *S. subsuturalis* populations are not thriving on *C. decapetala*. It appears that both indigenous parasitoids and predatory ants may be accountable for the low beetle infestation levels. Additionally, poor release efforts may also be the cause. For instance the data provided by WfW implementation officers show that only one release took place per site. Other scientists e.g. Evans and Tomley, (1996) and Julien and Griffiths, (1998) reported that the mis-match between agent native range plant biotype and introduced range plant biotype was accountable for the failure of the agents to establish.

In this study, because *S. subsuturalis* did not establish in large numbers at release sites, it may not have any effects on seed and seedling densities which were high, especially at Ferncliffe (KwaZulu-Natal) where measurements were taken (Figs 2.7 and 2.11). Furthermore *C. decapetala* may not be the preferred host of *S. subsuturalis*. Additional agents attacking different vegetative parts (buds, roots and stems) may be needed to control the weed. In East Africa (on Lake Victoria), it was not after the combined release of the two most important biological control agents, *Neochetina bruchi* Hustache (Coleoptera: Curculionidae) and *Neochetina eichhorniae* Warmer (Coleoptera: Curculionidae) that the reduction in water hyacinth, *Eichhornia crassipe* (Martius) Solms-Laubach became evident in Uganda in 1998 and Kenya 1999 (Center *et al.,* 2002). The release of *N. eichhorniae* alone in 1974 had been ineffective (Cilliers, 1991). In South Africa, a combined of release of all three different weevils i.e. *Trichapion lativentre* Beguin-Billecoeq (Coleoptera: Curculionidae) feeding on buds; *Rhyssomatus marginatus* Fahraeus (Coleoptera: Curculionidae) attacking seeds and *Neodiplogrammus*

quadrivittatus Olivier (Coleoptera: Curculionidae) a stem borer, was the most effective way of controlling *Sesbania punicea* (Cav.) Benth (Fabaceae) as weed density tremendously reduced (Hoffmann, 1990). *Trichapion lativentre* and *R. marginatus* reduced the seed bank of the weed whereas *N. quadrivittatus* destroyed mature plants eventually leading to limited seedling recruitment (Hoffmann, 1990).

Sulcobruchus subsuturalis mostly attacks seeds in open pods in the tree canopy

Since the beetle did not establish in large numbers in the field, an experiment to determine where *S. subsuturalis* attacked *C. decapetala* seeds were conducted in quarantine. Results revealed that very few eggs were laid per seed on the soil surface and no eggs were laid on seeds buried 2 and 4cm below the soil (Table 3.3). The number of eggs laid per seed in the control, where seeds were placed in containers without soil, was high. Seeds inside hanging pods were exposed to beetles and very few eggs were laid per seed. However, in the field all infested seeds were collected from pods in the tree canopies only. Although a few eggs were laid on seeds placed on the surface of the soil in the laboratory, the beetle may not be able to attack ground seeds under uncontrolled field conditions, given that no infested seeds were picked from the ground in the field throughout the entire sampling period. Eggs on seeds on the ground are exposed to predation. Overall *S. subsuturalis* attacks seeds in the tree canopy. Other studies of bruchid beetle attacks on several *Acacia* species have also reported seed attacks in pod seeds on the tree (Ernst *et al.,* 1990; Miller, 1994; Impson *et al.,* 2004). Considering that *S. subsuturalis* attacks mature seeds on the tree, seeds that are attacked dispersing from the tree are unlikely to germinate and hence establish into seedlings. However, *C. decapetala* may not be the preferred host of *S. subsuturalis* as the proportion of infested seeds collected from open pods in the tree canopies at release sites was low. Moreover the beetle originally fed on seeds of *Dalbergia candenatensis* and *Moullava spicata* (Dalz.) Nicolson in India (Anton, 1999).

Sulcobruchus subsuturalis attack prevents *C. decapetala* seeds from germinating and hence establishing into seedlings

Given that the abundance of a given biocontrol agent does not necessarily result into control of the target weed, quantifying its impact on the target weed is vital (Dhileepan, 2003). Nevertheless, *S. subsuturalis* has failed to establish in large numbers in the field therefore its impact on seed germination and seedling recruitment in the field was not observed. This is because seed density and seedling recruitment were maintained at relatively high levels throughout the 15 months sampling period, mostly at Ferncliffe, KwaZulu-Natal where sampling took place. It is also not clear whether the beetle is multivoltine or univoltine in its natural environment. However *S. subsuturalis* is multivoltine in the laboratory, and therefore infestation levels were high and its impact on *C. decapetala* seed germination as well as seedling emergence was observed (Figs. 3.3 and 3.4). There was generally much lower seed germination and seedling recruitment among beetle damaged seeds compared to undamaged seeds, possibly due to limited reserves in beetle damaged seeds. Nevertheless other factors such as dormancy, light quality, soil temperature, soil water and soil air quality may also influence seed germination and seedling emergence (Forcella, *et al.,* 2000). Studies on several *Acacia* species have also reported more or less similar bruchid beetle impacts on seed germination (Halevy, 1974; Mucunguzu, 1995). In high numbers *S. subsuturalis* can inflict high levels of damage on seedling output. However, these high numbers were not seen in the field and consequently the impact on the population structure of the weed is low.

The above situation can be related to instances where agents are collected from a specific plant biotype in their native range but released against a different plant biotype in the introduced range. In this case biocontrol agents may cause great damage to the target weed in the laboratory (because the system is simple) but may totally fail to establish in the field possibly because in the field, the target weed can not stimulate female beetles to oviposit eggs (McFadyen, 1985; McFadyen, 1987). For instance *Lantata camara* with over twenty nine biotypes in Australia alone has proven difficult to control biologically (Smith and Smith, 1982). On the other hand some agents are difficult to rear in the

laboratory yet they thrive in the field. For example the univoltine flower feeding weevil *Apion brunneonigrum* Beguin-Billecocq (Coleoptera: Curculionidae) which was introduced to Nigeria (1970-75), Ghana (1975) and Guam (1984) from Trinidad for biocontrol agent of *Chromolaena odorata* is difficult to rear in the field and as such only field collected adults can be released in *C. odorata* infested regions (Muniappan and Bamba 2000).

Sulcobruchus subsuturalis is being attacked by indigenous predatory ants and parasitoids

Attack of the *S. subsuturalis* life stages by ants and native parasitoids prevented the beetle's survival and development on *C. decapetala*. *Sulcobruchus subsuturalis* eggs larvae and adults were attacked by various ant species, mostly belonging to the genus *Monomorium* in KwaZulu-Natal province, where predation experiments were carried out. In most cases all eggs were removed from seeds within 10 days (Fig 4.1 and table 4.1). Even when eggs were protected from ants using an ant stop substance, egg removal was still observed (Fig. 4.2) suggesting that other organisms are also involved. All exposed beetle infested seeds were predated within 12 days at both Boughton and Ferncliffe (Fig. 4.3 and 4.4). In Guam, predation of *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) by ants such as *Solenopsis geminate* Fabricius (Hymenoptera: Formicidae) among other organisms (various species of spiders, a toad species and skinks) in the 1970s and 1980s caused failure of the agent to establish on *C. odorata* (Seibert, 1989). *Sulcobruchus subsuturalis* is also attacked by native parasitoids, e.g. *D. altifrons* which is presumed to parasitize beetle larvae, pupae and adults.

5.2. General conclusion

Biological control practitioners have always concentrated on agent host-specificity testing and therefore ensuring safety in biocontrol. However they have ignored assessing the efficacy of biocontrol agents before releases take place in the field (McEvoy and Coombs, 2000). It is no wonder the majority of introduced weed biocontrol agents establish on their target plants but only a fraction succeed as control agents. (Julien and Griffiths, 1998). For instance McFadyen, (2003) showed that only 55% of the 98 established agents were successful in controlling a total of 38 weeds worldwide. In

Canada, by 2002, the stem miner *Microplontus edentulous* (Schultze) (Coleoptera: Curculionidae) had established on *Matricaria perforate* Merat (Asteraceae) but had no significant impact on the target weed population (McClay *et al.,* 2002). McFadyen, (2003) argued that agent pre-release efficacy assessment is an additional cost and can lead to wrong predictions thus increasing the risk of rejecting weed damaging biocontrol agents. However a simulation model showed that pre-release efficacy assessment can be done on condition that it is less expensive than the host specificity testing procedure (McClay and Balciunas 2005).

It is not ethical releasing biocontrol agents that might not be sufficiently damaging to the target weed yet this can be avoided by assessing their efficacy prior to release. The efficacy of *S subsuturalis* on *C. decapetala* was never assessed prior to release in South Africa moreover according to Anton, (1999), in India, the beetle also targeted other weeds (*D. candenatensis* and *M. spicata*). This raises concerns that *C. decapetala* might not be the preferred *S. subsuturalis* host. The high level of seedling recruitment, especially at Ferncliffe, is attributed to the availability of large numbers of seeds in the soil. This is because the beetle is not an efficient biocontrol agent (causes no damage to seeds) as it has failed to establish on *C. decapetala* in large numbers in the field. Therefore its efficacy should have been assessed prior to releases in the field.

Nonetheless, this study shows that attacks by native predators and parasitoids as well as poor release efforts prevented *S. subsuturalis* from establishing on *C. decapetala*. Poor release efforts could involve releasing agents in low numbers at the wrong time of the yeah (when beetles are inactive and when seeds are unavailable in the tree canopy) and too infrequently. This may lead to low levels of beetle establishment. The largest number of infested seeds was recovered in Limpopo province, which also showed the largest number of agents released. However because there was only one release a year at each site in all provinces, release efforts were considered poor. The following are recommendations to improve release efforts and possibly increase levels of beetle establishment.

- Since *S. subsuturalis* is multivoltine in the laboratory, large numbers of agents should be reared in the laboratory and released in the field (inundative releases).

- Releases need to take place every year between September and March (summer) when pods filled with mature *C. decapetala* seeds are available in relatively high densities on the tree and when the beetle is expected to be reproductively active. This is expected to increase *S. subsuturalis* infestation levels.

- WfW implementation officers should endeavor to involve land owners, farmers and nature reserve authorities (in *C. decapetala* infested regions) in their release activities so as to release in as many areas as possible.

- Further studies on parasitism of *S. subsuturalis* by native parasitoids are needed at a wider spatial scale than in this study.

- *Caesalpinia decapetala* may not be the preferred host for *S. subsuturalis* and therefore an additional/another agent should be sought for release against this invasive weed.

- It is also highly recommended that the efficacy of the next biocontrol agent to be released against *C. decapetala* should be examined before releases take place in the field. The examination could be based on (a) impact experiments conducted in India, the native range (b) databases and (c) Mathematical and experimental models (McEvoy and Coombs, 2000).

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Appendix I

Release sites in Limpopo and KwaZulu-Natal provinces. A few sites were selected for this research project in each province. Sampled sites were selected based on accessibility.

Latitude	Longitude	Release date	Location	Site Name	Province	No. of agents released
23.38.752	30.21.575	5-Feb-03	Tzaneen	Seopeng1	Limpopo	20000
23.38.926	30.21.524	5-Feb-03	Tzaneen	Seopeng2	Limpopo	23500
23.40.611	30.19.273	29-Nov-06	Tzaneen	Relela assembly	Limpopo	19850
23.40.004	30.18.639	29-Nov-06	Tzaneen	Masebotja	Limpopo	23000
23.29.898	30.15.233	22-Nov-06	Tzaneen	Mamphakathi assembly	Limpopo	23000
23.39.343	30.55.600	21-Dec-02	Tzaneen	Moshakga1	Limpopo	20500
23.39.197	30.15.494	12-Dec-06	Tzaneen	Bodupe	Limpopo	17500
23.39.420	30.15.090	31-Dec-02	Tzaneen	Moshakga2	Limpopo	19000
23.39.234	30.16.369	22-Nov-06	Tzaneen	Seoka Panel Beaters	Limpopo	22000
23.38.544	30.17.066	21-Dec-06	Tzaneen	Mokwasele	Limpopo	19600
29 45.169	29 51.422	2/26/2001	Bulwer	Nkumba farm	KwaZulu-Natal	1100
29 53.830	30 06.440	2/26/2001	Richmond	Mtubeni valley	KwaZulu-Natal	1917
29 50.897	30 42.915	3/28/2001	Shongweni	Shongweni RR	KwaZulu-Natal	5165
29 35.725	30 37.770	4/17/2001	Wartburg	Nagle dam	KwaZulu-Natal	3500
29 33.552	30 19.517	5/14/2001	Pietermarizburg	Ferncliffe	KwaZulu-Natal	900
29 45.274	30 16.044	6/4/2001	Baynesfield	Baynesfield	KwaZulu-Natal	1000
29 18.904	30 32.023	3/22/2002	New Hanover	Elandspruit	KwaZulu-Natal	2622
30 38.407	29 51.273	9/25/2002	Harding	Sheepwalk farm	KwaZulu-Natal	470
28 10.329	31 57.047	10/24/2002	Hluhluwe	Hluhluwe River	KwaZulu-Natal	2000
28 03.637	32 07.510	10/31/2002	Hluhluwe	Magengeni	KwaZulu-Natal	700
28 02.254	32 05.087	11/18/2002	Hluhluwe	Manzibomb\vu	KwaZulu-Natal	500
28 55.236	31 11.441	12/6/2002	Eshowe	Mbongolwane	KwaZulu-Natal	500
29 36.100	30 19.431	1/3/2003	Pietermarizburg	Woodlyns	KwaZulu-Natal	2000
27 30.341	31 21.08	3/19/2003	Louwsberg	Ithala	KwaZulu-Natal	1500

Appendix II

Release sites in Mpumalanga and the Eastern Cape provinces. A few sites were selected for this research project in each province. Sampled sites were selected based on accessibility. The GPS coordinates of sites marked *** in Mpumalanga are unknown.

Latitude	Longitude	Release date	Location	Site Name	Province	No. of agents released
25.20.190	30.38.370	19-Nov-02	Sudwala	Riverwild	Mpumalanga	1000
25.19.329	30.41.510	19-Nov-02	Sudwala	Tropicado	Mpumalanga	2000
***	***	26-Nov-02	Bushbuckridge	Save the Sand	Mpumalanga	2000
***	***	3-Feb-03	Sabie	Tweefontein	Mpumalanga	2000
***	***	3-Feb-03	Sabie	Sabie Plantation Falls	Mpumalanga	2000
***	***	3-Feb-03	Hazyview	Kiepersol	Mpumalanga	2000
***	***	17-Mar-03	Nelspruit	Penryn College	Mpumalanga	500
***	***	16-Sep-04	Burgersfort	Burgersfort	Mpumalanga	1000
***	***	11-Feb-05	Barberton	Eureka City - Dynamite	Mpumalanga	1000
***	***	2-Mar-05	White River	Quartzberg	Mpumalanga	1000
25.25.548	30.58.030	14-Mar-05	Nelspruit	Nelsriver Bridge	Mpumalanga	1000
***	***	8-Dec-04	Burgersfort	Burgersfort	Mpumalanga	1500
***	***	8-Dec-04	Burgersfort	Burgersfort - Lepelle	Mpumalanga	1500
31.31.223	29.32.309	28-Sep-05	Port St. Johns	PSJ bridge (345.6), Lusikisiki (386.0), R61 = 359.4	Eastern Cape	4800
31.31.595	29.32.460	28-Sep-05	Port St. Johns	PSJ bridge (345.6), Lusikisiki (386.0), R61 = 358.5	Eastern Cape	4800
31.34.288	29.14.381	28-Sep-05	Port St. Johns	Umtata Total (251.2) , PSJ bridge (345.6), R61 = 309.4	Eastern Cape	4800
31.34.511	29.13.292	28-Sep-05	Port St. Johns	Umtata Total (251.2) , PSJ bridge (345.6), R61 = 306.7	Eastern Cape	4800
31.34.543	29.15.045	28-Sep-05	Port St. Johns	Umtata Total (251.2) , PSJ bridge (345.6), R61 = 310.7	Eastern Cape	4800
31.34.786	29.15.297	28-Sep-05	Port St. Johns	Umtata Total (251.2) , PSJ bridge (345.6), R61 = 311.4	Eastern Cape	4800
31.34.926	29.13.182	28-Sep-05	Port St. Johns	Umtata Total (251.2) , PSJ bridge (345.6), R61 = 305.7	Eastern Cape	4800
31.35.515	29.17.041	28-Sep-05	Port St. Johns	Umtata Total (251.2) , PSJ bridge (345.6), R61 = 315.4	Eastern Cape	4800
31.37.024	29.21.184	28-Sep-05	Port St. Johns	Umtata Total (251.2) , PSJ bridge (345.6), R61 = 323.9	Eastern Cape	4800
31.37.313	29.21.906	28-Sep-05	Port St. Johns	Umtata Total (251.2) , PSJ bridge (345.6), R61 = 325.3	Eastern Cape	4800
31.34.466	29.13.116	28-Sep-05	Port St. Johns	Tutor Ngeleni pass (road between Port St. Johns and Umtata)	Eastern Cape	4000
31.31.19	29.32.230	28-Sep-05	Port St. Johns	Nomvalo	Eastern Cape	4800

Appendix III

Average minimum and maximum temperatures (ºC) for station 0239698 5 – Pietermaritzburg measured at 8h00 between 1999 and 2007. Temperature data was provided by the South African weather services.

Min

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1999	18.9	18.6	18.2	14.8	10.5	6.7	8.2	9.4	11.8	13.8	17.3	19.1
2000	18.1	20	18.8	13.8	9.7	7.2	6.1	10.2	11.8	15.1	15.9	17.4
2001	17.3	17.5	17.3	14.9	10.3	7.7	7.1	9.6	11.7	15.2	17.4	18
2002	19.1	18.5	18.5	15.6	9.9	7.5	6.5	11.3	12.5	14.4	14.5	18.4
2003	19.3	20	16.9	15.5	9.7	6.2	5.7	7.5	12.4	13.9	15.7	16.5
2004	18.3	18.4	16.9	14	10	5.7	5.6	10.1	10.3	13.8	17.6	18
2005	18.5	18.9	16.6	13.8	9.4	6.9	6.3	11.1	12.2	14.4	16.1	15.4
2006	18.8	19.4	14.9	13.5	7.3	4.8	6.3	7.9	11.4	14.8	15.1	16.5
2007	17.6	18.5	16.7	14.5	8.1	6.4						

Max

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1999	29.2	28.6	30.5	28.3	25.3	23.9	24.8	26	26.3	26.5	28.4	28.3
2000	26.6	28.7	28.8	24.9	21.9	23.7	23.6	26	25.3	24.3	24.6	26.2
2001	26.6	26.8	29.2	24.4	24.5	23.8	22.5	25.3	24.9	26	27.5	27
2002	29.2	27.3	29.8	29	25	22	22	23.4	24	26.6	27.2	28.3
2003	29.9	31.2	30	26.8	23.2	20.8	22.7	23.5	23.7	27	27.5	28.7
2004	28.4	28.3	27.2	27.5	26.7	23.2	21	25.1	23.7	26.7	29.3	28.7
2005	27.7	29.3	26.7	26.2	26.1	24.1	24.2	25.8	26.9	26.9	26.6	27
2006	28.4	28.3	26	25.2	21.3	21.6	24.4	22.7	24.8	25.6	25.6	27.1
2007	29.1	30.7	27.5	26.1	26.6	22.6						

Appendix IV

Monthly daily rainfall (mm) for station 0239698 5 –Pietermaritzburg measured at 8h00 between 1999 and 2006. Rainfall data was provided by the South African weather services.

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1999	146.6	128.2	49.4	12	29.6	1.4	0.2	2	21.6	111.1	98.8	358.8
2000	157.2	69.2	97.6	0	83.8	7.2	0	0	81	72.8	94.8	147.4
2001	74.4	101	47.6	139.6	12	1	0.2	9.8	132.4	152.4	156.6	142.3
2002	136.8	35.2	34.6	75.8	16.4	20.4	82	93.2	56.8	39	64.2	145
2003	76.4	53.8	130.2	83.6	45	8.2	0	23.6	35.8	17.6	83	49.4
2004	54.2	191	59.6	11.8	0.2	22.6	38.2	15.2	70	70	183.4	189.8
2005	180.4	84	121.2	8.2	0.8	3.2	1	10.7	24.4	67	71.4	102.2
2006	185.6	54.8	98.6	109.2	68	1.4	0.4	52.2	54.2	81.6	101	177.2

Appendix V

Repeated Measures Analysis of Variance (ANOVA) for seeds under canopy and seeds outside canopy at Ferncliffe and Boughton. Sigma-restricted parameterization. Effective hypothesis decomposition.

Under canopy

Effect	SS	Degr. of freedom	MS	F	p
Intercept	761103.6	1	761103.6	155.4957	0.000016
Sites	538519.2	1	538519.2	110.0211	0.000044
Error	29368.2	6	4894.7		
M	166939.5	14	11924.3	4.1418	0.000019
M*sites	163757.8	14	11697.0	4.0629	0.000024
E	241836.4	84	2879.0		

Outside canopy

Effect	SS	Degr. of freedom	MS	F	p
Intercept	20046.68	1	20046.68	12.82562	0.011625
Sites	36.30	1	36.30	0.02322	0.883871
Error	9378.11	6	1563.02		
M	15909.38	14	1136.38	4.51287	0.000006
M*sites	7725.55	14	551.82	2.19144	0.014475
E	21152.01	84	251.81		

Appendix VI

Univariate Tests of Significance for seeds at different soil depths in December 2006 at Ferncliffe. Sigma-restricted parameterization. Effective hypothesis decomposition.

Soil depths Dec-06 Ferncliffe

Effect	SS	Degr. of freedom	MS	F	p
Intercept	6272.640	1	6272.640	42.40491	0.000029
Soil depths	2798.730	3	932.910	6.30675	0.008181
Error	1775.070	12	147.923		

Appendix VII

Repeated Measures Analysis of Variance (ANOVA) for seeds in the seed bank under canopy at Ferncliffe and Boughton respectively. Sigma-restricted parameterization. Effective hypothesis decomposition.

Ferncliffe under canopy

Effect	SS	Degr. of freedom	MS	F	p
Intercept	148015.6	1	148015.6	140.5262	0.000000
Sites	4160.9	3	1387.0	1.3168	0.314450
Error	12639.5	12	1053.3		
M	30887.4	7	4412.5	6.6423	0.000003
M*sites	25264.5	21	1203.1	1.8110	0.030178
E	55801.4	84	664.3		

Boughton under canopy

Effect	SS	Degr. of freedom	MS	F	p
Intercept	1105.675	1	1105.675	61.30189	0.000005
Sites	41.858	3	13.953	0.77358	0.530807
Error	216.439	12	18.037		
M	149.397	7	21.342	1.38195	0.223651
M*sites	434.579	21	20.694	1.33998	0.174448
E	1297.271	84	15.444		

Appendix VIII

Repeated Measures Analysis of Variance (ANOVA) for seedlings under and outside canopy at Ferncliffe and Boughton. Sigma-restricted parameterization. Effective hypothesis decomposition.

under canopy

Effect	SS	Degr. of freedom	MS	F	p
Intercept	13066.67	1	13066.67	55.70820	0.000298
Sites	12512.67	1	12512.67	53.34628	0.000336
Error	1407.33	6	234.56		
M	15560.58	11	1414.60	42.45404	0.000000
M*sites	15775.58	11	1434.14	43.04062	0.000000
E	2199.17	66	33.32		

Outside canopy

Effect	SS	Degr. of freedom	MS	F	p
Intercept	621.613	1	621.6125	3.396558	0.114903
Sites	208.013	1	208.0125	1.136603	0.327380
Error	1098.075	6	183.0125		
M	347.763	9	38.6403	1.109187	0.372297
M*sites	536.363	9	59.5958	1.710726	0.109058
E	1881.175	54	34.8366		

Appendix IX

Table analysis results of seed germination

Unifested seeds and seeds bearing eggs

The FREQ Procedure

Frequency Col Pct	Table of Seeds by Germination		
Seeds	**Germination**		**Total**
	No	**Yes**	
Eggs	23 65.71	25 40.98	48
No beetles	12 34.29	36 59.02	48
Total	35	61	96

Statistics for Table of Seeds by Germination

Statistic	DF	Value	Prob
Chi-Square	1	5.4407	0.0197
Likelihood Ratio Chi-Square	1	5.5109	0.0189
Continuity Adj. Chi-Square	1	4.4965	0.0340
Mantel-Haenszel Chi-Square	1	5.3841	0.0203
Phi Coefficient		0.2381	
Contingency Coefficient		0.2316	
Cramer's V		0.2381	

Unifested seeds and seeds containing adults

The FREQ Procedure

Frequency Col Pct	Table of Seeds by Germination		
Seeds	Germination		Total
	No	Yes	
Adults	44 78.57	4 10.00	48
No_beetles	12 21.43	36 90.00	48
Total	56	40	96

Statistics for Table of Seeds by Germination

Statistic	DF	Value	Prob
Chi-Square	1	43.8857	<.0001
Likelihood Ratio Chi-Square	1	48.8847	<.0001
Continuity Adj. Chi-Square	1	41.1857	<.0001
Mantel-Haenszel Chi-Square	1	43.4286	<.0001
Phi Coefficient		0.6761	
Contingency Coefficient		0.5601	
Cramer's V		0.6761	

Unifested seeds and seeds containing adults

The FREQ Procedure

Frequency Col Pct	Table of Seeds by Germination		
Seeds	Germination		Total
	No	Yes	
Adults	44 51.76	4 36.36	48
Larvae	41 48.24	7 63.64	48
Total	85	11	96

Statistics for Table of Seeds by Germination

Statistic	DF	Value	Prob
Chi-Square	1	0.9241	0.3364
Likelihood Ratio Chi-Square	1	0.9345	0.3337
Continuity Adj. Chi-Square	1	0.4107	0.5216
Mantel-Haenszel Chi-Square	1	0.9144	0.3389
Phi Coefficient		0.0981	
Contingency Coefficient		0.0976	
Cramer's V		0.0981	