



## Removal of post-dispersed seeds in *Acacia cyclops* thickets under biological control in South Africa

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### ABSTRACT

Biological control programmes have been mounted against all invasive Australian acacias with two agent species, *Melanterius servulus* (seed feeding weevil), and *Dasineura dielsi* (flower galling midge), being released on *Acacia cyclops* (rooikrans) in 1991 and 2002 respectively. Both of these agents are prolific and are causing high levels of damage, resulting in reduced seeding capacity of *A. cyclops* which in turn is expected to limit the invasiveness of the species. As part of an ongoing study to determine the long-term effectiveness of the biological control programme, we measured seed removal rates of *A. cyclops* by invertebrates and vertebrates, and the composition of granivorous species, to determine how these compare with earlier studies when there was no biological control. Results show that in *A. cyclops* thickets under biological control, 13% of seeds were removed by invertebrates 59% by rodents and 15% by ground-foraging birds and large mammals within 24 h. The removal rates of seeds with arils intact were double than those of seeds without arils. Camera traps captured 10 vertebrate species comprising six birds and four mammals including *Mellivora capensis* Storr (Cape ratel) and *Raphicerus melanotis* Thunberg (Cape grysbok) consuming seeds from stashes. The most frequent visitors were *Rhabdomys pumilio* (striped mouse), *Streptopelia capicola* (Cape turtledove) and *Cossypha caffra* (Cape robin-chat) (33%, 27% and 20% of visits respectively). We conclude that levels of granivory and the composition have not changed substantially with biological agents in the system.

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### 1. Introduction

Of the approximately 70 *Acacia* species that were introduced into South Africa, predominantly during the 19 century, at least 13 are currently recognised as invasive (Richardson et al., 2011; Van Wilgen et al., 2011). One of these, *Acacia cyclops* A.Cunn. ex G.Don (rooikrans), was introduced into southern Africa in 1835, primarily for stabilisation of sand dunes in the south-western Cape region (Shaughnessy, 1980). It has since successfully naturalized and invaded coastal regions across the southern Cape (Henderson, 2001). The proliferation of *A. cyclops* is ascribed to it having been widely propagated as a source of fuel wood for domestic and small-scale commercial purposes (Richardson et al., 2011; Van Wilgen et al., 2011) and to its prolific seed production (Milton and Hall, 1981; Impson et al., 2009). The nutritional, orange-reddish aril surrounding the seeds of *A. cyclops* is highly attractive to birds (Glyphis et al., 1981; Underhill and Hofmeyr, 2007) which affords long range dispersal of the seeds (Glyphis et al., 1981; Tucker and Richardson, 1995; Rejmanek, 2011). Ingestion of *A. cyclops* seeds by at least one bird species, *Onychognathus morio* Linnaeus (redwinged starling) has been shown to enhance germination (Glyphis et al., 1981; Impson, 2005).

Although seeds with fleshy arils in certain Australian *Acacia* species are adapted for vertebrate dispersal, especially by birds (Davidson and Morton, 1984; O'Dowd and Gill, 1986), most seeds fall under the canopy where they become a resource for animals which both disperse and destroy seeds (Milton, 1980; Holmes, 1990). Middlemiss (1963) identified an array of organisms foraging on *A. cyclops* seeds both in the tree canopy and on the ground.

In South Africa, rodents such as *Rhabdomys pumilio* Sparrman (striped mouse) consume substantial amounts of *A. cyclops* seeds (David, 1980; Holmes et al., 1987). Native invertebrates, including ant species (e.g. *Anoplolepis* species) that are important seed dispersers in fynbos vegetation (Bond and Slingsby, 1983; Pierce and Cowling, 1991), might also disperse *A. cyclops* seeds (Holmes, 1990). Hoarding by rodents, and possibly ants, burying *A. cyclops* seeds in underground stashes may also protect seeds from being scorched during fires and thereby enhance rates of invasion by the plant in burnt areas (Richardson and Kluge, 2008; Rusch, 2011).

Due to multiple threats associated with plant invasions in South Africa (Moran et al., 2011), different methods of control (e.g. mechanical, chemical and biological control) have been applied. Comparative assessments of weed management methods in South Africa contend that biological control is currently the most effective way of controlling invasive alien plants (Moran et al., 2011; Van Wilgen et al., 2012). Biological control entails the use of herbivorous insects and pathogens which suppress the vigour of weed species by destroying both the

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vegetative and reproductive parts (flowers and seeds) of their host plants (Neser and Kluge, 1986; Zimmermann et al., 2004; Moran et al., 2005). The importance of seed-damaging biological control agents for angiospermous alien plants has been emphasised by Neser and Kluge (1986), especially for massive seed banks for *Chrysanthemoides monilifera* and Australian *Acacia* species observed in their invasive ranges: Australia and South Africa respectively (Milton, 1980; Weiss and Milton, 1984). A standing example in South Africa is the substantial decrease in abundance of *Acacia longifolia* (Andr.) Willd., in the fynbos following release of *Trichilogaster acaciaelongifoliae* Froggatt (gall-forming wasp) in 1982 (Impson et al., 2011).

Ongoing efforts to determine the effect that two biological control agents, *Melanterius servulus* Pascoe (a seed-feeding weevil) and *Dasineura dielsi* Rübsaamen (a flower-galling fly) are having on *A. cyclops* include measurements of flower and seed mortality, seed bank dynamics and changes in host-plant population recruitment rates under conditions where seed production has declined substantially. An important component of these studies is being able to account for levels of activity in native organisms which destroy or disperse seeds of *A. cyclops* (Impson, 2005).

There are no reports on measurements of levels of granivory on *A. cyclops* seeds in areas where biological control agents have suppressed seed production for a prolonged period. To address this gap, we measured removal rates of *A. cyclops* seeds on the ground to determine whether, after 20 years of biological control, there have been changes in: (i) levels of granivory compared to the findings by Holmes (1990), and (ii) the composition of granivorous species using *A. cyclops* seeds (*sensu* Middlemiss, 1963).

## 2. Material and methods

### 2.1. Study site

The study was conducted in an *A. cyclops* thicket located in the Koeberg Nature Reserve (33°40'32.97"S 18°26'29.45"E), Western Cape Province, South Africa. The plants were all growing in deep sand. The climate is Mediterranean with 75% of rain falling between April and September. Winters are mild but summer days are hot and dry with morning fog a common occurrence in the coastal location of Koeberg. The thicket consisted of large trees interspersed with open bare space and occasional indigenous shrub species. The height of most trees was at least 2.5 m and the canopy cover was approximately 75%.

### 2.2. Removal of *A. cyclops* seeds

Seeds that were used for feeding trials were harvested from Koeberg Nature Reserve and from Hout Bay (34°01'43.51"S 18°20'35.94"E), Zeekoevlei (34°05'00.55"S 18°31'40.80"E) and Stellenbosch (33°59'37.27"S 18°45'19.38"E) between January and July 2012.

Exclosure cages were used to measure removal rates of seeds by different combinations of invertebrates, rodents and birds. Each cage covered a 0.25 m<sup>2</sup> area of soil surface and was 5 cm high. The cages consisted of metal frames which were covered with different configurations of mesh to allow different levels of access to seeds. To expose seeds to invertebrates only, the sides and top of the cages were entirely enclosed in mesh (5 mm diameter). To expose seeds to mice and invertebrates but exclude birds, the top and two opposite sides of the cage were enclosed with mesh leaving two sides open. To determine combined seed removal rates by birds, rodents and invertebrates together, the patch was left uncaged and therefore 'open to all'.

In preparation for placement of seeds, both within the caged exposure and in the 'open to all' treatment, a sheet of polyester gauze 'shade cloth' was laid out and covered with a 2 cm layer of sand. This formed an isolating layer which ensured that extraneous seeds from the seed bank already in the sand did not become incorporated in the trials, and that placed-out seeds that became covered with sand could

be recovered before counts were made at the end of the exposure periods. The cages were pegged down within the patch of sand covering the shade cloth.

Eight sets of traps were placed at 10 m intervals along a 100 m transect which extended inwards from the periphery of the *A. cyclops* thicket. Each set comprised an 'exposed to all', an 'exposed to invertebrates only' and an 'exposed to rodents and invertebrates' treatment, placed 3 m apart. At the start of each trial, 30 arillate seeds were uniformly spread within the confines of each cage and in the 'open to all' treatment. Seed removal was monitored at 24-hour intervals over 4 days. Seed removal was defined by the difference between the original number of seeds and the number of seeds remaining after 24 h. After the counts were made, fresh seeds were added to the patch to replenish those that had been removed and to start each 24-hour monitoring period with a full complement of 30 seeds. To test the influence of arils on seed removal rates, the procedure was repeated over three days using seeds from which the arils had been removed. Since vegetation cover influences small mammals' foraging activity (Van Hensbergen et al., 1992; Pons and Pausas, 2007), seed removal rates between treatments at different locations in a transect were compared.

To determine seed removal by rodents only, the numbers of seeds removed from the 'exposed to invertebrates only' treatment was deducted from that of the 'exposed to rodents and invertebrates' treatment. To determine seed removal by birds and large mammals, the numbers of seeds removed from the 'exposed to rodents and invertebrates' treatment was deducted from that of the 'exposed to all' treatment.

### 2.3. Identifying granivores and visitation frequency

Surveillance digital camera traps are the best method for studying wildlife in a nonintrusive manner (Kays et al., 2011). Digital camera traps (Scoutguard SG550V-31) with infrared motion sensors were set up at different locations during the feeding trial period to identify the birds, large mammals and rodents that were removing the seeds from the 'open to all' treatment, and to determine their visitation frequency. Camera traps provided both digital images and video sequences of the organisms that removed seeds from the feeding station, both night and day.

### 2.4. Statistics

Seed count data were square root transformed and a one-way generalized linear model analysis of variance (GLM-ANOVA) tested for differences between and among seed removal rates for *A. cyclops* per day between treatments during feeding trials with arillate and non-arillate seeds. Significantly different means at  $P \leq 0.05$  were distinguished with a Duncan's Multiple Range test in UNISTAT version 6.0.

## 3. Results

During each 24 hour exposure period, 87% of seeds were removed from the 'exposed to all' treatment. 72% of the seeds were removed from the 'exposed to rodents and invertebrates' treatment and 13% were removed from the 'exposed to invertebrates only' treatment. Difference between seed removal rates from the 'exposed to invertebrates only' and the 'exposed rodents and invertebrates' showed that rodents alone removed 59% of the available seeds while the difference between the 'exposed to all' treatment and the 'exposed rodents and invertebrates' showed that 15% of the seeds were removed by birds and large mammals (Table 1).

Seed removal was significantly higher for arillate seeds than for non-arillate seeds regardless of level of exposure to granivores ( $F_{(1, 46)} = 26.94$ ,  $p < 0.0001$ ) (Fig. 1). For arillate seeds, there was a significant decline in the proportion of seeds removed as the level of exposure decreased ( $F_{(2, 309)} = 178.14$ ,  $p < 0.0001$ ; Fig. 1 A). For non-arillate

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