OVIPOSITION BEHAVIOUR OF *NELTUMIUS ARIZONENSIS* SCHAEFFER (COLEOPTERA: BRUCHIDAE), A BIOLOGICAL CONTROL AGENT OF *PROSOPIS* SPP. IN SOUTH AFRICA

by

LORRAINE WALLACE STRATHIE

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Zoology Department
University of Cape Town

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FRONTISPIECE

A *Neltumius arizonensis* female depositing an egg onto the surface of a *Prosopis* pod. Most of the egg is already attached to the pod by means of an adhesive laid with the egg.

Scanning electron micrograph of a *Neltumius arizonensis* egg attached to the surface of a *Prosopis* pod. The surrounding adhesive attaching the egg to the pod surface is visible.

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ABSTRACT

Little has been documented on the biology of *Neltumius arizonensis* Schaeffer. This bruchid is native to the south-western United States of America and is a prospective biological control agent of *Prosopis* spp. in South Africa. The primary objective of this study was to examine and quantify the oviposition behaviour of *N. arizonensis* females under different conditions to determine whether eggs of conspecifics or of another bruchid species, *Algarobius prosopis* (LeConte), affected oviposition.

Diet, the number of mates, the duration of access to mates, and the variety of *Prosopis* pod provided for oviposition all affected the fecundity of *N. arizonensis*. Optimal conditions for *N. arizonensis* oviposition included: a diet of pollen pellets in solution, constant access to a limited number of mates, and mature, undamaged *Prosopis* pods of the 'mottled-purple' variety. The physical structure of the surface of *Prosopis* pods, observed by scanning electron micrography, did not reveal trends in characteristics among pod varieties that could be linked to the oviposition preferences of *N. arizonensis*.

The rate of oviposition in *N. arizonensis* peaked between the third and eighth day after emergence from pods and was highest during the first hour when females that had been deprived of pods for at least three days, were provided with pods. Each *N. arizonensis* female laid an average of about 80 eggs during her lifetime, which was about 35 days on average.

An event-recording computer programme was developed to quantify the oviposition behaviour of *N. arizonensis* when females were provided with one of four types of *Prosopis* pods for one hour: (a) pristine pods, (b) pods with conspecific eggs, (c) pods with *A. prosopis* eggs deposited within slits, and (d) pods with egg-free slits. Analyses of time budgets indicated that pod type had no significant effect on behaviour, although some activities differed significantly with the type of pod provided, but probably only as a result of the greater number of eggs laid on some pod types. Certain activities i.e. inspection of the pod surface, remaining stationary, and scraping of the ovipositor across the surface prior to egg deposition, occupied significantly more time on pods than other activities. Pod type did not affect the total time females spent on pods, nor the frequency of visits to pods. The availability of clean seeds did not affect the quantity of eggs deposited and the rate of oviposition did not differ significantly on pods of different types.

Neltumius arizonensis females displayed a high degree of discrimination among 'seeds' of different qualities as oviposition sites, although previous host deprivation resulted in less discrimination during the early stages of oviposition. Females on all pod types avoided laying eggs on aborted 'seeds', 'seeds' with emergence holes and 'seeds' that already carried one of their own eggs. An exceptionally high degree of preference for intact 'seeds' as oviposition sites was demonstrated on pods with conspecific eggs and, to a lesser and more variable extent, on pods with *A. prosopis* eggs within slits. Avoidance of 'seeds' with eggs would seem to be especially advantageous if there is competition for resources. No significant preference for intact 'seeds' was observed on pods with 'seeds' with egg-free slits.

Significantly more eggs were deposited, and probably accounted for the higher extent of utilisation of intact 'seeds', on pods with egg-free slits and on pods with *A. prosopis* eggs than on pods with conspecific eggs. Females inspected intact 'seeds' that were eventually accepted as oviposition sites for much longer than intact 'seeds' that were rejected, 'seeds' with conspecific eggs, 'seeds' from which conspecific eggs had been removed, and 'seeds' which carried a female's own eggs. The inspection time of an unsuitable 'seed' was only a few seconds. Eggs were usually deposited on a 'seed' that was not adjacent to the 'seed' on which a previous egg had been deposited.

The highly specialised, meticulous oviposition strategy of *N. arizonensis* enables avoidance of conspecifics as well as other bruchid species, and thus decreases the probability of intraspecific and interspecific competition.

CHAPTER 1.
INTRODUCTION

1.1 *Prosopis* spp.

Prosopis spp. (Fabaceae), commonly known as mesquites, are thorny, leguminous trees that are considered to be a major agricultural problem in many countries, e.g. south-western United States and northern Mexico (where they are native), because they have invaded large tracts of grazing land (DeLoach, 1985, 1988; Cordo and DeLoach, 1987). *Prosopis* spp. were originally introduced into South Africa from the United States of America (U.S.A.) towards the latter part of the nineteenth century. They were avidly propagated in southern Africa until about 1960, for their beneficial uses as shade trees and as a nutritious source of fodder for livestock, especially in the arid regions of the country (Harding, 1978; Zimmermann, 1991).

Subsequently, *Prosopis* spp. have formed dense, impenetrable stands on valuable grazing land, primarily in the dry north-western Cape regions of the country (Harding, 1978, 1987; Brown and Gubb, 1986). In the process, the desirable properties have been lost because smaller, thin-stemmed plants with fewer pods are produced in the dense thickets that have developed. The problem has been exacerbated because many of the seeds that are consumed by livestock are scarified by gut enzymes which increases the germination rate of seeds that are dispersed over large distances by ungulates (Swier, 1974; Coe and Coe, 1987 in Miller, 1994; Miller and Coe, 1993). Zimmermann (1991) postulated that the absence of seed-feeding insects had encouraged the rapid spread of *Prosopis* spp. in South Africa. In 1983, all *Prosopis* spp. were declared invasive weeds in South Africa according to the Agricultural Conservation of Natural Resources Act 43 (Peter and Zimmermann, 1987).

The taxonomy of *Prosopis* spp., all of which are exotic in southern Africa, is not clear (Zimmermann, 1991). Hybridisation of *Prosopis* species is a common phenomenon (Solbrig *et al.*, 1977) and has occurred extensively in South Africa (Peter and Zimmermann, 1987). Harding (1987) stated that six species were present in southern Africa: *P. pubescens*, *P. chilensis*, *P. juliflora*, *P. velutina*, *P. glandulosa* var. *glandulosa* and *P. glandulosa* var. *torreyana*. It appears that *P. glandulosa* var. *glandulosa*, *P. pubescens* and *P. chilensis* are not highly invasive, whereas *P. velutina* and *P. glandulosa* var *torreyana* are the most problematic in South Africa (Poynton, 1990; Harding and Bate, 1991) and they also have weed status in south-western U.S.A. (Cordo and DeLoach, 1987).

1.2 Control of *Prosopis* spp.

Many farmers in southern Africa are now in favour of control of *Prosopis* spp. because the problems of invasion outweigh the benefits of the plants. The primary control objectives are to arrest the spread of *Prosopis* spp. and so prevent the formation of impenetrable thickets, and to reduce existing thickets to levels where the beneficial attributes of the trees are restored (Zimmermann, 1991). Control by chemical or mechanical methods is unsatisfactory because of inadequate mortality, high costs, and the need for follow-up treatment (Smith and Ueckert, 1974).

1.3 Biological control agents of *Prosopis* spp.

Numerous phytophagous insects, associated with *Prosopis* spp. in the U.S.A. (Ward *et al*., 1977; Johnson 1983; Cordo and DeLoach, 1987), are potentially suitable for introduction into South Africa for the biological control of *Prosopis* spp. However, species that damage the vegetative parts of the plants, or the flowers or pods are not suitable because they would reduce the beneficial attributes of the plants. As a result, only seed-feeding insect species have been considered for the control of *Prosopis* spp. (Zimmermann, 1991). Moran *et al.* (1993) reported that, despite extensive seed destruction, the pods retain their nutritious value and can be utilised as livestock fodder.

Bruchidae, are obligate seed-feeding beetles, of which the majority feed on seeds of the family Fabaceae and are highly host-specific (Center and Johnson, 1974; Johnson and Slobodchikoff, 1979; Southgate, 1979; Johnson, 1981; van Tonder, 1985). The four or five larval instars (Parnell, 1966; Smith, 1992) of most bruchid species develop entirely within the seed of their legume host (Swier, 1974; Thiery, 1984). Each larva consumes either a portion or the whole of a single seed (Wasserman and Futuyma, 1981; Ernst *et al.* 1990a, 1990b; Mbata, 1993), or several seeds, depending on the size of the seeds and the bruchids (Bridwell, 1918 in Swier, 1974; Skaife, 1926; Howe and Currie, 1964; Johnson, 1968 in Swier, 1974, Center and Johnson, 1973 in Swier, 1974; Southgate, 1979). Pupation occurs either inside or outside the seed (Forister, 1971 in Swier, 1974). Before pupation, larvae chew a tunnel almost to the seed or pod surface, thus, a round emergence window of thin outer integument is visible prior to emergence (Swier, 1974; Southgate, 1979). Larvae enter a pre-pupal stage and pupate within the larval feeding cell, protected by the husk of the seed (Southgate, 1979). Adult bruchids chew or push away the thin circular window and pull themselves out of the exit hole onto the pod surface (Swier, 1974; Ernst, 1992, 1993).

Successive waves of several bruchid species develop and destroy the seeds of *Prosopis* spp. in the U.S.A. and each species has a specific behaviour and physiology that ensures exploitation of different portions of the vast seed resource (Swier, 1974; Conway, 1980). In Arizona, two bruchid species, *Algarobius prosopis* and *A. bottimeri* Kingsolver, account for about 90% of the seed damage of *Prosopis* spp. (Forister, 1970; Swier, 1974; Kingsolver *et al.*, 1977; Kistler, 1985). *Algarobius prosopis* was introduced into South Africa from Arizona in 1986 (Peter and Zimmermann, 1987) and *A. bottimeri* was introduced from Mission, Texas in 1985 and again in 1989 (Zimmermann, 1991). *Algarobius prosopis* is reported to be specific to *P. velutina* and *P. glandulosa* var. *torreyana*, whereas *A. bottimeri* is specific to *P. glandulosa* var. *glandulosa* (Kingsolver, 1964, 1986; Forister, 1970; Peter and Zimmerman, 1987). Both bruchid species oviposit on immature and mature pods. *Algarobius bottimeri* and *A. prosopis* are morphologically and behaviourally similar but because they vary in their host preferences and their geographical distribution in the U.S.A. (Peter and Zimmermann, 1987), it was suggested that in combination they would be better able to exploit the range of *Prosopis* spp. and hybrids in South Africa (Zimmermann, 1991). In South Africa, the bruchids presumably would be free from specific natural parasitoids and predators, and it was expected that the levels of damage observed in the U.S.A. would be even higher in South Africa (Peter and Zimmermann, 1987).

Since its release into South Africa, *A. prosopis* has proliferated and destroyed large quantities of *Prosopis* seeds (Moran *et al.*, 1993). In contrast, *A. bottimeri* has not become established, possibly because it is outcompeted by *A. prosopis* in the absence of specialist natural enemies (Hoffmann *et al.*, 1993a).

Biological control of *Prosopis* spp. in South Africa has been hampered by the consumption of bruchid-infested seeds because livestock devour the pods before the bruchids have destroyed the seeds (Zimmermann, 1991) and because digestive enzymes in the gut of ungulates destroy developing bruchids (Janzen, 1969; Halevy, 1974 in Miller, 1994; Lamprey *et al.*, 1974 in Miller, 1994; DeLoach, 1985; Coe and Coe, 1987 in Miller, 1994; Miller and Coe, 1993). High levels of seed destruction by *A. prosopis* can only be achieved when the pods are protected from ungulates (Zimmermann, 1991). This may be achieved by erecting fences around *Prosopis* infestations to exclude livestock until *A. prosopis* has destroyed almost all of the seeds in the pods on the ground. However, an alternative strategy is to introduce bruchid species that attack the seeds before the pods fall to the ground and become available to livestock. There are two bruchid species that could fill this role, *Mimosestes protractus* (Horn) and *Neltumius arizonensis* (Moran *et al.*, 1993). However, *M. protractus* is unsuitable because it is univoltine and synchrony with *Prosopis* spp. in South Africa would be problematic

(Moran, 1991). Although *N. arizonensis* is rare in its country of origin (Forister, 1970), it was considered suitable for biological control purposes (Moran, 1991).

There is a paucity of information on the biology of *N. arizonensis* and this species had never been successfully reared prior to its introduction into quarantine in South Africa. The taxonomy of the genus was dealt with by Kingsolver (1964), and Johnson (1978) reported on the ecology of a related species, *Neltumius texanus* Schaeffer. Kingsolver (1964) and Forister (1970) reported that *N. arizonensis* feeds primarily on *P. velutina* and occasionally on *P. chilensis*. Although previous studies had drawn conclusions from observations on relatively few individuals, the reports on the maturity of pods on which *N. arizonensis* oviposits stated that *N. arizonensis* attacks and completes its development on pods that are still attached to trees (Forister, 1970; Swier, 1974; Johnson, 1983; Kistler, 1985). As a result, *N. arizonensis* was introduced into South Africa during 1992, in an attempt to enhance the levels of seed damage caused by *Algarobius* spp.

A major difference between *A. prosopis* and *N. arizonensis* is the oviposition strategy each has evolved. Although the life histories of bruchids vary, most species, including *N. arizonensis* but excluding *A. prosopis*, lay flattened ovoid eggs which are deposited with an adhesive on the seed or pod surface (Swier, 1974; Southgate, 1979). The first instar larva, the locomotory stage (Zhu *et al.*, 1994), chews a hole in the ventral surface of the egg chorion, and tunnels through the pod wall or seed in search of food (Skaife, 1926; Parnell, 1966; Swier, 1974; Southgate, 1979, 1984). The egg chorion on the pod surface protects the entrance to the larval tunnel. As the larva chews, the chewed pod material is pushed by repetitive sinuous motions of the body into the egg case on the pod surface (Southgate, 1984), causing it to appear white. No material is ingested during this process (Southgate, 1984). Due to limited mobility as a result of poorly developed legs, the larva feeds only on the seed directly below the exocarp on which the egg is deposited (Swier, 1974).

Alternative oviposition strategies are utilised by other bruchid species e.g. *A. prosopis*, *A. bottimeri*, *Acanthoscelides obtectus* Say, *Bruchidius ater* Marsham, *B. sahlbergi* Schilsky and *B. uberatus* Fåhraeus (Prevett, 1971 in Swier, 1974; Swier, 1974; Ernst *et al.*, 1990b; Ernst, 1992). The oviposition behaviour of *Algarobius* spp. is unique among the bruchids that feed on *Prosopis* spp. (Conway, 1980). The eggs, each with two fibrous, adhesive strands, are deposited in clusters on the pod surface, in cracks and crevices in the exocarp or in the adult emergence holes of other bruchids (Bridwell, 1920a in Swier, 1974; Kunhikannan, 1923 in Forister, 1970; Kingsolver *et al.*, 1977; Zimmermann, 1991). By depositing eggs in clusters, levels of parasitism and predation, and the amount of exposure to ambient conditions may be lowered, hence a reduction in mortality may be achieved (Bridwell, 1920b in Forister, 1970; Johnson, 1978; Stamp, 1980). In *A. prosopis*, the neonate larvae possess well developed legs with setae and are able to travel among seeds, either over the pod surface or through the mesocarp, and select the seeds in which to continue their development (Swier, 1974; Pfaffenberger and Johnson, 1976). Swier (1974) suggested that if *Prosopis* spp. cotyledons were not sufficiently developed, *A. prosopis* larvae feed on the pod juices.

Successful oviposition contributes to the overall fitness of a population. The first larva that enters a seed usually excludes others by direct competitive encounters for space or indirect competition for food (Parnell, 1966; Janzen, 1969). Usually only one adult emerges per seed in *A. prosopis* and *N. arizonensis* (personal observation). The quantity of eggs laid on a seed determines the degree of larval competition (Dick and Credland, 1984; Smith and Lessells, 1985) and is especially important for species with relatively immobile larvae. Females of several bruchid species reduce the probability or intensity of competition between siblings or conspecifics by discriminating between seeds of different qualities (Mitchell, 1975; Messina and Renwick, 1985a, 1985b; Messina, 1989; Credland and Wright, 1990). Optimally, ovipositing females should also avoid seeds with eggs or larvae of other species.

The objective of this study was to observe the oviposition behaviour of *N. arizonensis* to determine whether the females responded in any way to eggs of conspecifics or of *A. prosopis*. Large populations of *A. prosopis* are already present in South Africa, thus if resource utilisation by this species and by *N. arizonensis* overlaps, it would be necessary for *N. arizonensis* to avoid competitive encounters and thus enhance its suitability as a supplementary biological control agent of *Prosopis* spp. in South Africa.

The following chapter deals with the factors that affect oviposition to determine the conditions that are optimal for oviposition by *N. arizonensis* in the laboratory. Chapter 3 examines the oviposition behaviour and the pattern of oviposition in *N. arizonensis*, under the optimal conditions that were determined in Chapter 2. The final chapter presents a synthesis of the results obtained from this study and discusses them with regards to the biological control of *Prosopis* spp. in South Africa.

GLOSSARY OF TERMS USED IN THE TEXT

The observations and experiments described in the following chapters, involve the responses of ovipositing bruchid females to *Prosopis* pods and seeds of different types. The terminology may be confusing, thus clarity is attempted below:

pod variety - the colour, texture (rough or smooth), pubescence, maturity of a pod.

pod type - refers to whether or not a pod bears eggs deposited by other bruchid females (either *N. arizonensis* or *A. prosopis*), or whether or not there are artificially-created slits across the pod surface (exocarp), in which some bruchid species oviposit (see definition below).

There are four pod types:
pristine pod - a pod which has not been oviposited upon by bruchid females, nor have any slits been made across the exocarp.

pod with conspecific eggs - a pod on which other *N. arizonensis* females have previously oviposited.

pod with *A. prosopis* eggs - a pod which bears slits created by scoring with a scalpel to simulate natural surface damage across the exocarp, and into which *A. prosopis* females have deposited eggs.

pod with egg-free slits - a pod which has slits across the surface but to which no bruchid females have been permitted access, so no bruchid eggs are present.

'seed' - is a term used to describe one of the successive swellings along the length of a pod, which represents a seed developing beneath the exocarp of the pod.

Each pod comprises a succession of 'seeds' as defined above. Where the bruchid females make oviposition choices on the basis of individual 'seeds' (as opposed to the pod as a whole), the terminology used is as follows:

'seed' type - refers to whether or not an individual 'seed' bears bruchid eggs and whether or not it has a slit across the exocarp.

The different 'seed' types are:

intact 'seed' - a 'seed' that bears neither eggs of other bruchid females, nor a slit across the exocarp.

'seed' with conspecific egg - a 'seed' that bears an egg that was previously deposited on the exocarp by a *N. arizonensis* female.

'seed' with removed conspecific egg - a 'seed' from which a previously-deposited egg of a *N. arizonensis* female has been scraped off from the exocarp.

'seed' with *A. prosopis* eggs - a 'seed' that has a slit across the exocarp, into which *A. prosopis* females have deposited eggs.

'seed' with egg-free slit - a 'seed' that bears a slit without *A. prosopis* eggs.

Some pod types contain only one type of 'seed', whereas other pod types contain a variety of 'seed' types:

- Pristine pods have intact 'seeds' only.
- In all cases, pods with conspecific eggs have intact 'seeds' in addition to 'seeds' with conspecific eggs.
- Pods with *A. prosopis* eggs have intact 'seeds', 'seeds' with eggs in slits and some have 'seeds' with egg-free slits.
- Pods with egg-free slits have intact 'seeds' and 'seeds' with egg-free slits.

The number of seeds of each type on pods of the same type, varies among individual pods.

Aborted 'seeds' and 'seeds' with emergence holes are also present on some pods.
aborted 'seed' - very little swelling on the exocarp of a mature pod, indicating poor development of the seed below.

'seed' with emergence hole - a 'seed' in which a bruchid has previously developed and emerged as an adult, indicated by a hole leading from the seed to the exocarp.

CHAPTER 2.
OPTIMAL CONDITIONS FOR OVIPOSITION BY *NELTUMIUS ARIZONENSIS*

ABSTRACT

In this chapter the factors that influence oviposition by *N. arizonensis* are examined. The objective was to determine which conditions are optimal for oviposition in the laboratory. A diet of pollen in solution, continual access to a single male and the provision of well-developed 'mottled-purple' pods resulted in optimal oviposition. Oviposition peaked from three to eight days after emergence from pods and continued for about 30 days, during which period about 80 eggs were laid. When females were deprived of hosts until peak-oviposition age, oviposition was highest during the first hour of exposure to the first new pod but declined during the following 10 hours.

INTRODUCTION

The process of oviposition in insects is highly complex and is influenced by many factors, both ecological and physical (e.g. humidity, temperature (El-Sawaf, 1956; Howe and Currie, 1964)), and the characteristics and availability of food, mates and hosts (Nwanze and Horber, 1976; Credland, 1986; Credland *et al.*, 1986)). Bruchid adults emerge with a high lipid content, the major source of metabolic energy, and do not require food and water to survive, mate or oviposit (Wightman, 1978b; Moller *et al.*, 1989a; Fox, 1993a; Fox and Dingle, 1994). However, the lifespan, fecundity, and egg-size may increase and the survivorship of progeny is substantially enhanced when females are provided with a diet of water, honey, sugar water, nectar or pollen (Larson and Fisher, 1924; Wightman, 1978b; Janzen, 1980; Dick and Credland, 1984; Johnson and Kistler, 1987; Moller *et al.*, 1989b; Thanthianga and Mitchell, 1990; Tucic *et al.*, 1990; Shimada and Ishihara, 1991; Fox and Dingle, 1994).

Females of most insect species mate frequently (Larson and Fisher, 1924; El-Sawaf, 1956; Ridley, 1990; Fox, 1993b). The presence of mates and mating may influence the physiology and fecundity of female bruchids by stimulating oogensis (Larson and Fisher, 1924; Menusan, 1935; El-Sawaf, 1956; Avidov *et al.*, 1965a; Pimbert and Pierre, 1983; Wilson and Hill, 1989). Many bruchid females, including *A. prosopis*, lay more eggs when they are provided with continual access to mates than if access to mates is restricted (Brauer, 1945; Hoffmann *et al.*, 1993b). However, oviposition may be reduced by interference during frequent mating attempts when there is an excess of males (Larson and Fisher, 1924; El-Sawaf, 1956; Credland and Wright, 1989; Ridley, 1990; Fox, 1993b).

The presence of seeds and pods of host plants have been shown to stimulate oogenesis and oviposition in many bruchids (Parnell, 1966; Labeyrie, 1978; Pouzat, 1978; Pimbert and Pierre, 1983; Credland and Wright, 1989). The physical structure of plants (e.g. the texture and maturity of host seeds or pods) influences the selection of oviposition sites in bruchids and in many, if not all, herbivorous insects (El-Sawaf, 1956; Prevett, 1966; Nwanze and Horber, 1976; Messina, 1984; Städler, 1986).

Female age may influence egg quantity and host acceptance behaviour (Minkenberg *et al.*, 1992). Bruchids in stored products usually have a very short pre-oviposition period. Adult females emerge with mature eggs (Wilson and Hill, 1989) and peak egg-laying rate is achieved usually within the first two days after emergence from pods, with ninety percent of the eggs being laid within three to five days after emergence from pods (Larson and Simmons, 1923; El-Sawaf, 1956; Howe and Currie, 1964; Avidov *et al.*, 1965a, 1965b; Bellows 1982b; Giga and Smith, 1983; Dick and Credland, 1984; Credland and Wright, 1989; Ernst, 1993). As females age, the number and quality of eggs laid, decreases (Larson and Simmons, 1923; El-Sawaf, 1956; Howe and Currie, 1964; Giga and Smith, 1983; Wasserman and Asami, 1985; Fox and Dingle, 1994).

The factors that influence oviposition in *N. arizonensis* were investigated to determine the optimal conditions for oviposition in the laboratory, so that conditions could be standardised for subsequent observations on oviposition by *N. arizonensis*.

METHODS

A breeding colony of *N. arizonensis* was set up with 32 females and 31 males that were imported from Arizona, U.S.A. in September 1992. All adults utilised in experiments were selected from the third and subsequent generations of the founder colony. The beetles were maintained in a photoperiod of 12L:12D. Experiments were conducted at 25 ± 2 °C and 60 ± 10 % R.H.

All *Prosopis* pods used in this study were selected from two collections taken in February 1993 and January 1994 from Onderplaas farm (31° 59'S; 19° 15'E) in Bidou Valley, western Cape. Species and hybrids of *Prosopis* trees bear pods which differ visibly in terms of colour, surface texture, and size. Pods were specifically selected for these different characteristics and pods from each tree were kept separately. However, due to hybridisation, pod species could not be identified for this study.

2.1 Diet

In total, 67 *N. arizonensis* females were tested on seven different diets in no-choice situations, with eight to ten females per diet. The diets tested were:

-no food or water
-water only
-sugar and water solution
-honey and water solution
-Baker's yeast (*Saccharomyces cereviseae*) and water solution
-pollen (Pure Bio Ferment Pollen, i.e. non-*Prosopis* pollen, in homogenised pellet form) and water solution
-pollen, honey and water solution

All diets were supplied by soaking a small wad of cotton wool in the diet solution. A three-day old *N. arizonensis* adult male and female were confined in each 550ml plastic container with a gauze lid. Three *Prosopis* pods, each containing 10 healthy 'seeds', were provided to each pair of adults. The pods were removed and eggs were counted on the first, second, third, fourth and seventh days of the experiment. At the same time, the pods were replaced with pristine pods of the same variety and the food source was renewed. The total number of eggs laid throughout the week by each female was calculated.

2.2 Access to mates

To determine the effects of the number of mates and the duration of access to mates on the number of eggs laid by *N. arizonensis*, 60 three-day old virgin females were confined with either one, two or four males (i.e. 20 females per treatment). Within each of the three groups, 10 of the females had access to mates for the first two days (limited access), while the other 10 females in that group had access to mates for the duration of the experiment i.e. seven days (continual access).

Each female was provided with three pods, each containing 10 viable 'seeds', and a diet of pollen and water was provided. At the start of the third day, the males in 10 containers in each of the three groups (i.e. females with one, two or four males) were removed. The number of eggs laid by each female was examined on the first, second, third, fourth and seventh day of the experiment. At those times, the diet and the *Prosopis* pods were renewed. The total number of eggs laid by each female over the seven days was calculated.

2.3 Pod variety

From the large number of *Prosopis* pods collected in February 1993 on Onderplaas farm, four varieties, each from a different tree, were selected on the basis of their different appearance:

- 'mottled-purple' pods
- 'purple' pods
- 'rough-yellow' pods
- 'wide-yellow' pods with a smooth surface.

A choice of twenty pods (each containing 10 'seeds') of each variety were presented to 10 *N. arizonensis* males and 10 females, in a plastic box with dimensions of 35 x 15 x 12cm. This experiment was replicated four times. The boxes were shaken to provide a random distribution of pods. A diet of pollen pellets in solution was placed in the centre of each box. After six days, the total number of eggs laid on pods of each variety was counted.

2.4 Surface structure of pods

To determine whether the surface of different varieties of *Prosopis* pods varied and thus affected oviposition site selection by *N. arizonensis* females, scanning electron microscopy was used to examine the surface of the four pod varieties. Pod sections were mounted onto stubs, coated with 100µm gold palladium using a Pelaron sputter coater and viewed using a Cambridge S200 scanning electron microscope. The pods were examined at magnifications of about 28 x, 200 x, and 400 x, so that the attributes of the surfaces could be compared.

2.5 Pod maturity

To determine whether *N. arizonensis* females would oviposit on green, very immature pods (with 'seeds' at an early stage of development) in a no-choice situation, immature pods were collected in October 1993 from the eastern side of the Olifants River, near Clanwilliam (32° 9.5'S; 18° 53.5'E). Ten *N. arizonensis* females were provided with 20 immature pods, each with 10 'seeds'. The pods were removed after six days, the eggs were counted and the pods were replaced with 20 mature 'yellow' pods, with 10 'seeds' each. After three days, these pods were removed and the eggs laid were counted.

A second experiment was conducted to examine whether *N. arizonensis* females would lay eggs on unripe pods containing well-developed 'seeds' in a no-choice situation.

Twenty seven females, previously deprived of pods for at least three days, were placed in a container with 10 immature pods. After five hours, the eggs were counted.

2.6 Fecundity of *N. arizonensis*

To determine the number of eggs produced per female during her lifetime and to ascertain whether there was a peak in oviposition at any stage, 15 *N. arizonensis* females, newly-emerged from pods, were provided with a mate, a source of food and a daily supply of sections of 'mottled-purple' pods, amounting to 20 'seeds'. The number of eggs that were laid on pods was counted every day. Food and pods were replaced on a daily basis. After 35 days, or until a female died, the total number of eggs laid and the cumulative egg production was calculated.

2.7 Oviposition over a 10-hour period

To select a duration for detailed investigation of the oviposition behaviour of *N. arizonensis*, the pattern of oviposition was examined over a 10 hour period. Eighty-nine *N. arizonensis* females with prior access to mates and food, but deprived of *Prosopis* pods, were individually placed in large glass test tubes that were stoppered with porous foam bungs, so that oviposition could be monitored. Each female, at peak oviposition age, was provided with a single 'mottled-purple' pod with approximately 15 viable 'seeds'. The number of eggs laid was examined hourly for 10 hours.

In addition, to determine whether females may have been inhibited from oviposition by the presence of their own eggs on a pod, 48 *N. arizonensis* females were each provided with a pristine pod for five hours, after which the pod was removed and replaced with a second pristine pod for the following five hours. The number of eggs that were laid was monitored hourly.

The mean total number of eggs laid during 10 hours by females with access to one pod for 10 hours and by females with access to two pods for five hours each, was calculated. The mean number of eggs laid on non-viable 'seeds' and the mean number of 'seeds' with more than a single egg were calculated for females with one pod and those with two pods. The proportions of the total number of eggs which were laid in the first and the sixth hours were calculated for both treatments. In addition, the proportion of viable 'seeds' which were used during the first and sixth hour and after 10 hours, was calculated for each treatment.

Statgraphics version 6.0 was used for the statistical analysis of data throughout this study.

RESULTS

2.1 Diet

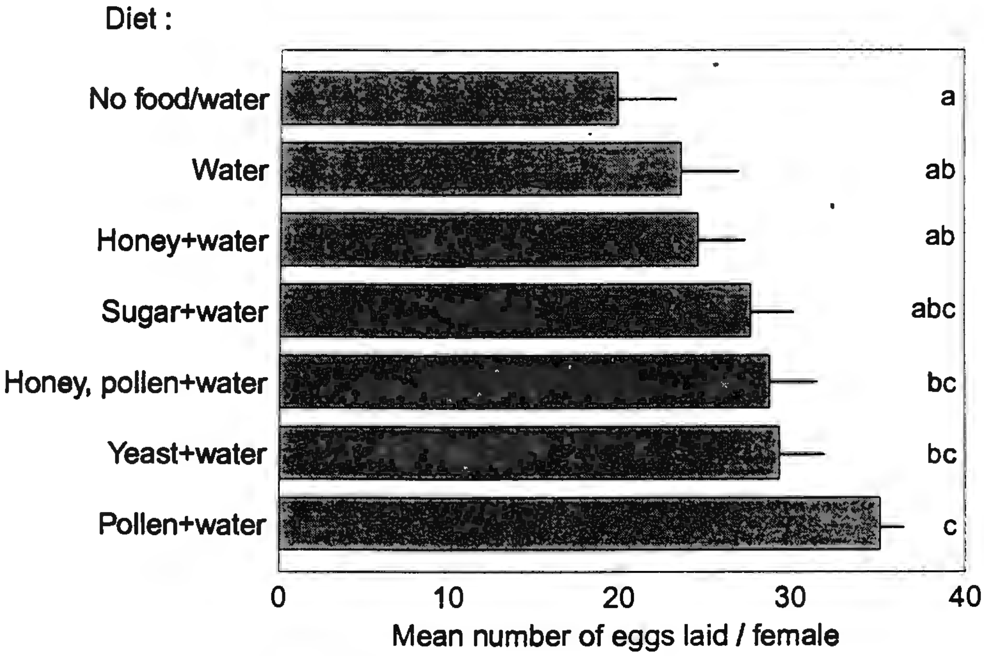


Fig. 1: Mean number of eggs (+ 1 S.E.) laid by *N. arizonensis* females maintained on various diets for one week. Different letters indicate significant differences between means.

Diet significantly affected egg production (one-way ANOVA, $F_{(6, 60)} = 2.839$, $p < 0.05$) (Fig. 1). Females that were deprived of both food and water produced the fewest eggs. Water, and the addition of honey or sugar to water, improved egg production while solutions of honey and pollen with water and yeast with water resulted in a further increase. Unexpectedly, a combination of honey and pollen did not result in the maximum number of *N. arizonensis* eggs. A diet of pollen pellets in solution resulted in maximal egg production.

2.2 Access to mates

Table 1: Mean number of eggs laid by *N. arizonensis* females during the first week after emergence from pods, given access to different numbers of mates for different durations. Ten females were used in each of the six groups. Means followed by different letters are significantly different.

Number of males	Mean number of eggs ± S.E. Access for 2 days	 Access for 7 days
1	12.80 ± 2.54 a	35.20 ± 1.80 c
2	11.90 ± 3.16 a	27.00 ± 1.16 d
4	21.20 ± 2.74 b	28.90 ± 1.82 d

The number of mates to which females had access, significantly affected egg production (two-way ANOVA, $F_{(2,\ 54)} = 3.190$, $p < 0.05$) (Table 1). In addition, the duration of access to mates significantly affected egg production (two-way ANOVA, $F_{(1,\ 54)} = 64.273$, $p < 0.01$); females with seven days of access to males laid double the number of eggs laid by females with access to mates for only two days. However, there is a significant interaction (two-way ANOVA, $F_{(2,\ 54)} = 5.056$, $p < 0.01$) between the duration of access and the number of mates. Therefore, the significant effect of the number of males is dependent on the duration of access, and the significant effect of the duration of access is dependent on the number of males with which females were provided. Thus, when females had access to males for only two days, females with four males produced the most eggs. In contrast, when females had access to males for seven days, females with four males laid fewer eggs than females with one male, possibly as a result of interruptions of oviposition by the surfeit of males. The greatest number of eggs were produced by females with access to one male for seven days.

2.3 Pod variety

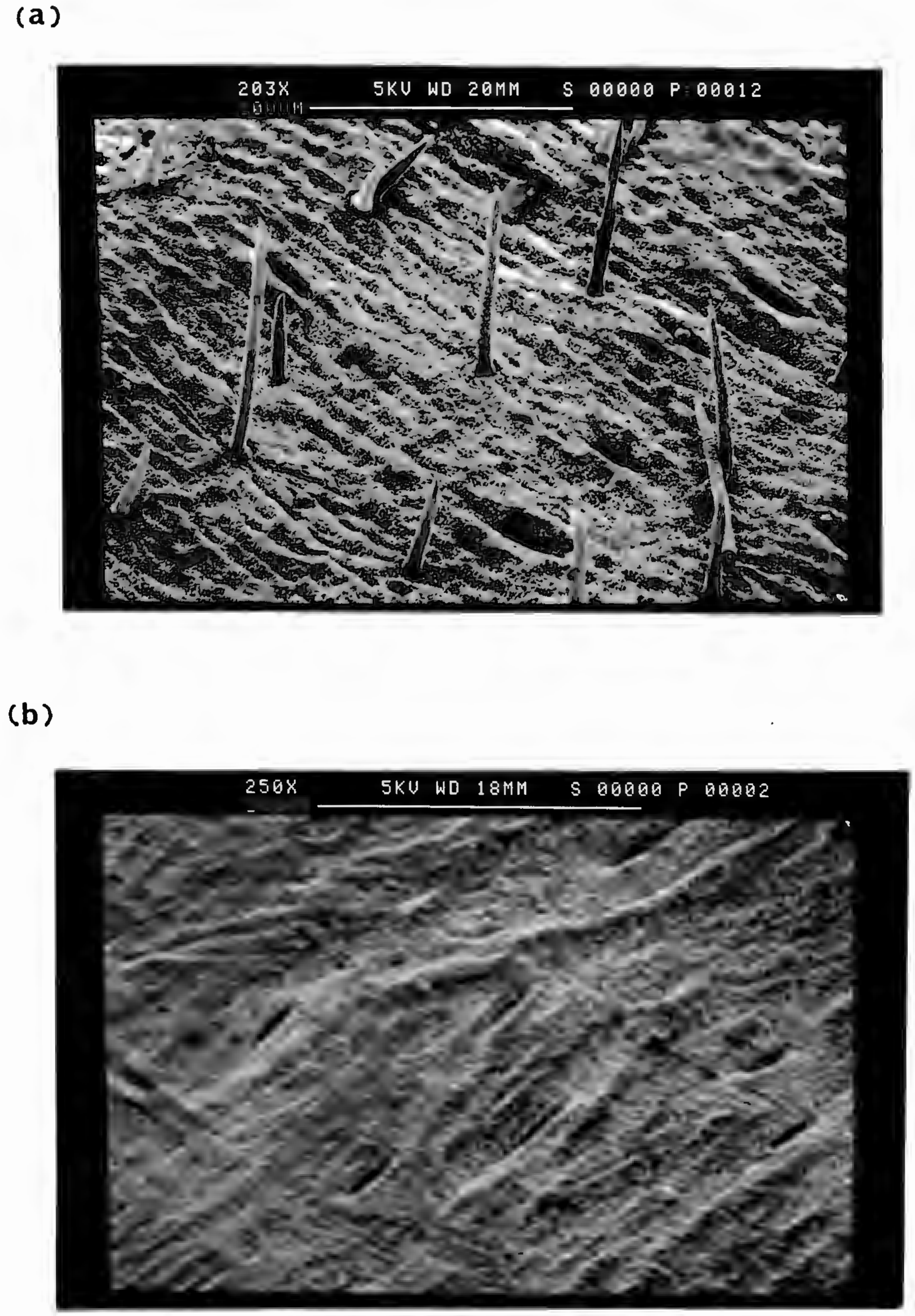
Table 2: Mean number of eggs laid on each of four varieties of *Prosopis* pods by 10 *N. arizonensis* females in six days. Each condition was replicated four times. Means followed by different letters are significantly different.

Pod variety	Total number of eggs ± S.E.
'Mottled-purple'	55.25 ± 4.21 a
'Purple'	45.25 ± 4.40 a
'Rough, yellow'	25.75 ± 5.66 b
'Wide, yellow'	25.25 ± 2.39 b

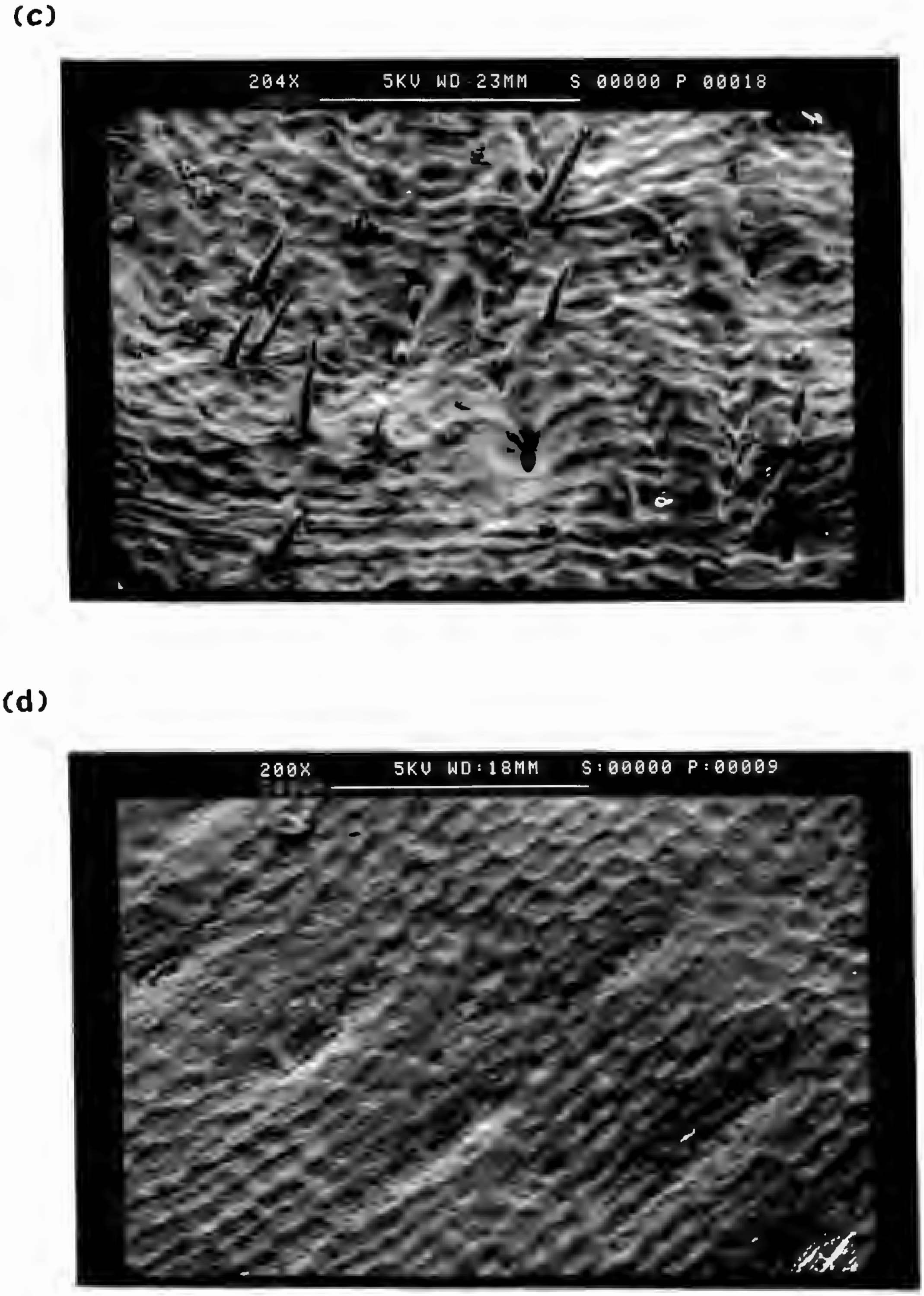
one-way ANOVA, $F_{(3, 12)} = 11.794$, $p < 0.01$

N. arizonensis females showed ovipositional preferences when provided with a choice of four pod varieties (Table 2). There was a significant effect of pod variety on the number of eggs laid. The females laid eggs on all of the pod varieties provided, but most eggs were laid on 'mottled-purple' pods.

2.4 Surface structure of pods



Figs. 2a-d: Scanning electron micrographs of the surface of four varieties of *Prosopis* pods taken at about 200x magnification. (a) 'mottled-purple' pod, (b) 'purple' pod. Stomata are visible as large, deep, dark indentations on the pod surface in Fig. 2a and as dark slits in Fig. 2b.



Figs. 2a-d: Scanning electron micrographs of the surface of four varieties of *Prosopis* pods taken at about 200x magnification. (c) 'rough-yellow' pod, (d) 'wide-yellow' pod. Stomata are visible as dark cavities in Fig. 2c and the guard cells of stomata are visible in Fig. 2d.

The micrographs (Figs. 2a-2d) are displayed in descending order of preference for *N. arizonensis* females. Of the four varieties of pods, the surface of the 'mottled-purple' pods had the roughest texture, and was highly ridged (Fig. 2a). Trichomes (hair-like outgrowths from the epidermis) were present on 'mottled-purple' pods and 'rough-yellow' pods. The surface of 'purple' pods was similar but less ridged than that of the 'mottled-purple' pods (Fig. 2b). The 'rough-yellow' pods had a smoother surface than the 'purple' or 'mottled-purple' pods (Fig. 2c). The identity of the bilobed structures on the 'rough-yellow' pods is not known; they are too big to be bacteria (J. Erasmus, personal communication) and too small to be pollen grains. The surface of the 'wide-yellow' pod had many indentations with surrounding ridges, in a honeycomb-like pattern (Fig. 2d), but it was generally smoother than that of the other pods, especially when viewed at 400x magnification.

2.5 Pod maturity

During the first six days when *N. arizonensis* females had access to immature pods with poorly developed 'seeds', only a single egg was laid on the pods but 37 eggs were laid on the container in which the bruchids were housed. During the following three days, 144 eggs were laid on mature pods by the same females. Thus, the transition from very immature pods to mature pods resulted in an eight fold increase in oviposition rate. Immature pods with poorly developed 'seeds' are not suitable for oviposition by *N. arizonensis*.

Fifty seven eggs were laid on unripe pods containing well-developed 'seeds', and adults were obtained from the eggs. Thus, *N. arizonensis* females did not avoid these pods.

2.6 Fecundity of *N. arizonensis*

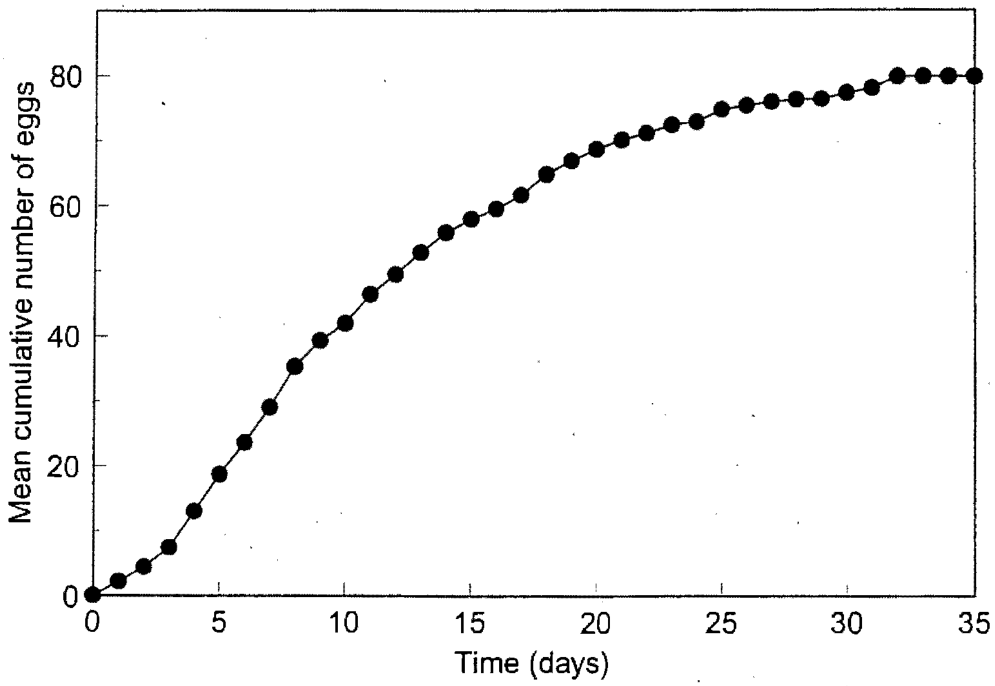


Fig. 3: The mean cumulative number of eggs laid by *N. arizonensis* females (n = 15) over a 35 day period following emergence from pods.

The rate of oviposition by *N. arizonensis* was not constant (Fig. 3). The oviposition rate was relatively slow during the first three days after emergence. Thereafter the rate increased and an average of 5.5 eggs were laid per day for the following five days. More eggs were laid on a daily basis during this period, than at any other time during the 35 days of egg-laying. The rate of oviposition decreased from day nine onwards, and very few eggs were laid after 25 days. Each *N. arizonensis* female laid about 80 eggs in her lifetime, of which about 50% were laid during the first 10 days.

2.7 Oviposition over a 10-hour period

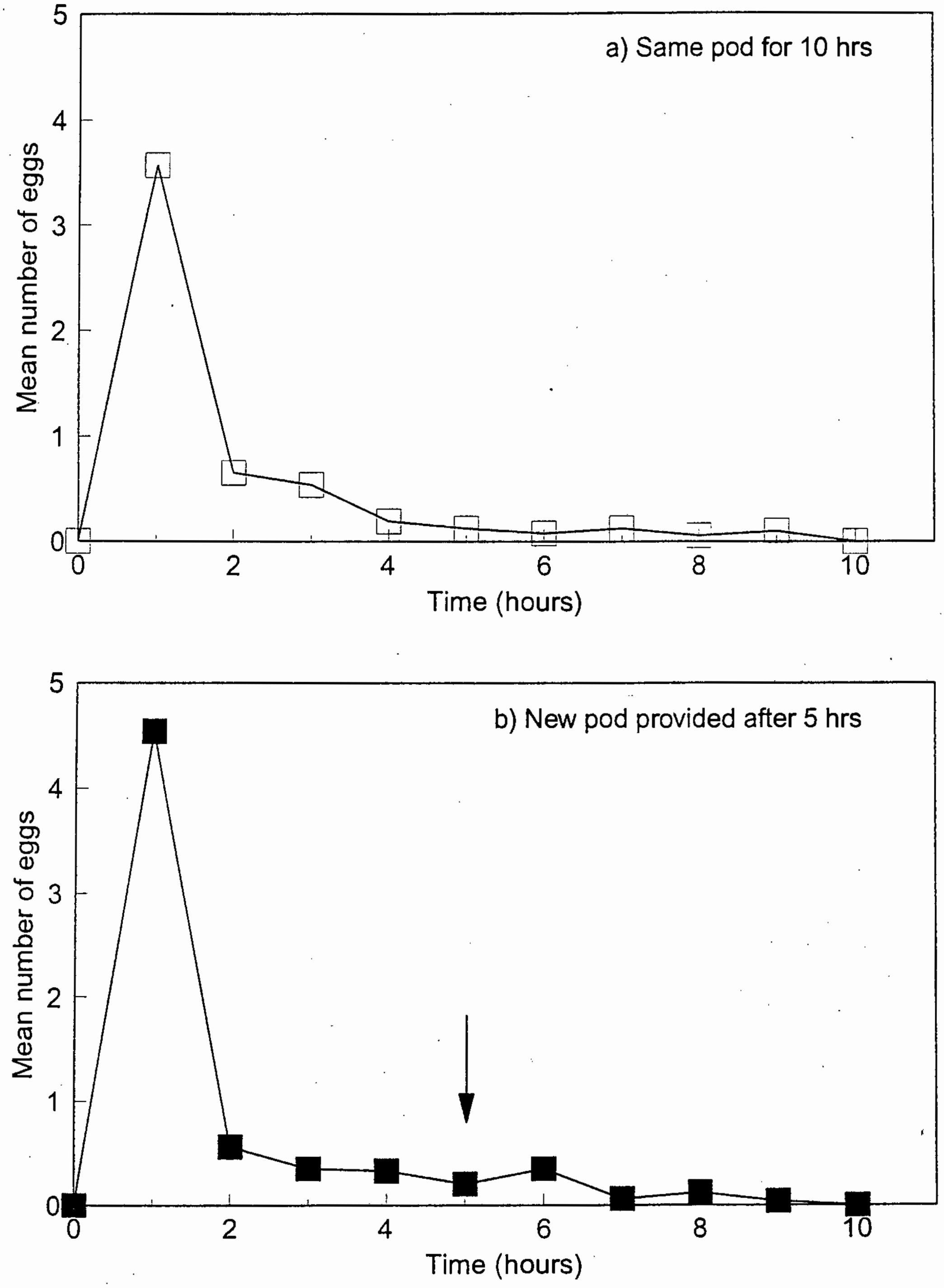


Fig. 4: Mean number of eggs laid by a *N. arizonensis* female during the initial 10 hours of exposure to a *Prosopis* pod. Standard errors range from 0.03 to 0.40. **(a)** Females (n = 89) were provided with a single pod for 10 hours. **(b)** Females (n = 48) were provided with one pod which was removed after five hours and replaced by a pristine pod (indicated by ↓) for the following five hours.

Table 3: Mean numbers ± S.E. and proportions ± S.E. of eggs laid and proportions ± S.E of viable *Prosopis* 'seeds' used by *N. arizonensis* females with access to either a single pod for 10 hours (n = 89) or one pod removed after five hours and replaced by another pristine pod for a further five hours (n = 48). The results of a one-way ANOVA or Kruskal Wallis test are shown as n.s. = no significance.

	1 pod for 10 hrs	2 pods for 5 hrs each	
Total no. eggs laid in 10 hrs	5.48 ± 0.31	6.58 ± 0.53	n.s.[1]
No. eggs on non-viable 'seeds'	0.09 ± 0.03	0.00 ± 0.00	-
No. 'seeds' with > 1 egg	0.17 ± 0.06	0.08 ± 0.07	n.s.[2]
% of total no. eggs laid in: 1st hr	63.16 ± 3.76%	76.43 ± 3.64%	-
6th hr	1.90 ± 0.84%	17.50 ± 5.45%	-
% of viable 'seeds' used in: 1st hr	31.29 ± 2.56%	35.88 ± 3.11%	-
6th hr	0.68 ± 0.28%	2.76 ± 1.12%	-
over 10 hrs	48.07 ± 2.87%	52.10 ± 5.12%	-

1: One-way ANOVA, $F_{(1, 135)} = 3.682$, $p > 0.05$

2: Kruskal Wallis test statistic = 1.445, $p > 0.05$

Neltumius arizonensis females laid most of their eggs during the first hour of encountering a pod (Figs. 4a and 4b; Table 3) and utilised about one third of the available viable 'seeds' during this period. Approximately four eggs, which is equivalent to 70-80% of the total amount laid per day during peak oviposition period, were laid during the first hour. Thereafter, the rate of egg-laying decreased substantially (Figs. 4a and 4b). When females were provided with a second pristine pod after five hours, slightly more eggs were laid, and thus more viable 'seeds' were utilised during the sixth hour (the first hour of exposure to the second pristine pod) than by females with a single pod. However, there was no significant difference in the total number of eggs laid and there was little difference in the proportion of viable 'seeds' utilised during 10 hours by females with access to only one pod and those with access to a second pristine pod

(Table 3). Thus, the provision of a second pristine pod after the first five hours did not substantially increase the number of eggs laid nor improve the oviposition rate. Therefore, the decline in egg-laying after the first hour was not due to a lack of acceptable oviposition sites, or to inhibition of oviposition by the presence of the females' own eggs on pods.

Very few eggs were laid on non-viable 'seeds' (i.e. aborted 'seeds' or 'seeds' with emergence holes) or on 'seeds' which had already been utilised as oviposition sites (Table 3).

DISCUSSION

The difference between the number of eggs laid as opposed to the number of eggs produced by a female bruchid has been emphasised by many authors. Wilson and Hill (1989) stated that measurements of daily or total egg output may be misleading as indicators of reproductive potential, because eggs may develop but be retained within the ovaries. The terms 'fecundity' and 'oviposition' refer to the number of eggs laid whereas the terms 'ovarian production' and 'maturation rates' refer to the sum of the number of eggs laid and the number of oocytes retained in lateral oviducts (Wasserman and Futuyma, 1981; Pimbert and Pierre, 1983; Wilson and Hill, 1989). This study focussed on the fecundity and oviposition of *N. arizonensis.*

Adults in breeding populations of *N. arizonensis* did not require water or food to survive and reproduce, but longevity was prolonged by providing water or food (personal observation). The number of eggs laid by *N. arizonensis* increased when females were provided with food and, or, water and the diet that optimised oviposition was pollen pellets dissolved in water. Therefore, female bruchids were provided with this diet for subsequent experiments.

Pollen derived from *Prosopis* flowers was not available but *N. arizonensis* oviposition may be further enhanced by *Prosopis* pollen. In the field, females surviving from the previous season may feed on the pollen in spring, as has been reported for other bruchids (Parnell, 1966; Johnson and Kistler, 1987; Bashar *et al.*, 1990). Pollen is known to increase the frequency of copulation, ovarian production, and increase and maintain the protein (vitellogenin) content of the haemolymph of many, but not all, bruchid females (Pajni and Sood, 1974; Huignard and Leroi, 1981; Pesho and van Houten, 1982; Ernst *et al.*, 1990b).

Neltumius arizonensis adults mated within hours of emergence from *Prosopis* pods (personal observation), although the frequency of mating thereafter was not observed. Unmated *N. arizonensis* females laid few eggs, all of which were non-viable. The duration of exposure of *N. arizonensis* females to mates had more .of an effect on oviposition than the number of mates with which they were provided. Oviposition was severely curtailed when females had limited access to mates and in females that were provided continual access to too many males. As a result, the largest number of eggs were laid by *N. arizonensis* females with continual access to one mate, and in subsequent experiments each *N. arizonensis* female was provided with continual access to one mate, with the exception of the brief periods in which detailed observations of behaviour were made.

This study examined whether certain pod varieties were selected as oviposition sites in preference to others. The number of eggs laid by *N. arizonensis* differed substantially on *Prosopis* pods of different varieties. However, the results were not meant to be exemplary of field situations where there is a much wider range of pod varieties. Field populations of *N. arizonensis* may prefer pod varieties other than those provided in the experiments. Survival of some bruchids is occasionally greater on hybrids of their host plants as opposed to the host plant species (Siemens and Johnson, 1992; Siemens *et al.*, 1994). It would be useful to investigate whether field populations of *N. arizonensis* distinguish between species and hybrids of *Prosopis* spp. in South Africa.

Speculation arose as to whether the apparent selection of 'mottled-purple' pods was based on the physical or chemical characteristics of pods. Physical properties of a pod such as the texture of the surface in terms of roughness or smoothness, may have been instrumental in directing oviposition. Contrary to previous studies that have shown that rough-textured seeds are avoided as oviposition sites by other bruchids (Nwanze and Horber, 1976; Giga *et al.*, 1993), *N. arizonensis* females preferred the rough-textured 'mottled-purple' pods. Smooth surfaces allow a firmer attachment of eggs than pitted, rough surfaces and larval entrance into pods is facilitated (Nwanze and Horber, 1976; Messina, 1984).

Neltumius arizonensis larvae are not particularly mobile, and they require firm adhesion of eggs to the pod surface so that they can use the egg case as a brace as they chew through the pod wall and enter the pod (personal observation). Similar to observations of *Acanthoscelides alboscutellatus* (Horn) made by Ott (1991), if *N. arizonensis* females do not have a secure tarsal hold on a pod during oviposition, eggs are only partially attached to the pod surface (Swier, 1974; personal observation). Although development occurred within the egg cases in those situations, the neonate larvae wandered about the pod surface and eventually died because they were unable to tunnel into the pods (Swier, 1974; personal observation). Therefore, it had been assumed that *N. arizonensis* females would select pods with the least surface irregularities. Resistance of plants to bruchid attack has been attributed to spines on seed coats (Raina, 1971 in Nwanze and Horber, 1976). However, trichomes present on some *Prosopis* pod varieties did not deter *N. arizonensis* oviposition. Although the physical attributes differed among the pod varieties, there were no obvious attributes that correlated with the trends in pod preference of *N. arizonensis* females. Chemical interactions between bruchids and legumes have been well documented (e.g. Birch *et al.*, 1989; Fox and Tatar, 1994) and the preferences of *N. arizonensis* for certain pod varieties may have been based, not on the physical attributes of pods but rather on their chemical attributes.

In concurrence with the information stated in Kingsolver *et al.* (1977), experiments in which *N. arizonensis* females laid most of their eggs on the container in which they were housed when supplied with immature *Prosopis* pods with poorly developed 'seeds', indicated a definite avoidance of those pods. The curvature of 'seeds' or the chemical composition of pods may have provided information on pod maturity. Perhaps volatile substances inhibited oviposition. Although eggs were laid on green pods with well-developed 'seeds' and development was successful, the adults that emerged from pods were much smaller than normal (personal observation). Fecundity increases with size in the females of many animal taxa (Berrigan, 1991), but it is not known whether fecundity was reduced in the 'miniature' *N. arizonensis* females. The results of the pod maturity experiments cannot be extrapolated to natural situations, and further investigation is required to determine the age at which pods in the field are selected for oviposition.

The fecundity of *N. arizonensis* was similar to that of many other bruchids (e.g. Howe and Currie, 1964; Giga and Smith, 1983; Dick and Credland, 1984; Moller *et al.*, 1989a; Tucic *et al.*, 1990). This study determined that the rate of egg-laying was not constant during the lifetime of *N. arizonensis*. Oviposition in *N. arizonensis* started later and was more prolonged than in most other bruchid species. Similar to other bruchids (Larson and Fisher, 1924), the number of eggs laid decreased gradually. Due to daily fluctuations in temperature, *N. arizonensis* females in the field may produce eggs over a longer period than one month. Constant temperatures and continual access to a mate, food and new oviposition sites in this study ensured that the observed decrease in *N. arizonensis* oviposition during one month was not experimentally-induced but probably a natural decrease due to age. *Neltumius arizonensis* females between three and eight days old, i.e. during peak oviposition period, were selected for subsequent experiments of oviposition behaviour.

Withholding or providing insufficient oviposition sites suppresses oviposition, for at least the first few days in bruchids (Ofuya, 1987; Credland and Wright, 1989; Mbata, 1993). The terminal eggs in the ovarioles, or even mature eggs in the oviduct, disintegrate and the contents are resorbed (Minkenberg *et al.*, 1992). Although *N. arizonensis* oviposition may have been suppressed by initial host deprivation until peak-oviposition age in subsequent experiments, the results were comparable because all females were deprived of *Prosopis* pods to ensure that sufficient oviposition events were observed.

It is not known whether the rapid egg-laying during the first hour of exposure to a pod, was a result of host deprivation prior to the experiments. Provision of a second pristine pod after five hours did not significantly improve oviposition, indicating that the decline in egg production during 10 hours was natural and not experimentally-induced. Therefore, to quantify the behaviour of ovipositing *N. arizonensis* females in subsequent experiments, it was necessary to observe them for only the first hour after provision of a pod. Based on the number of eggs laid during 10 hours, all pods that were provided to females in subsequent experiments, contained at least eight viable 'seeds' so that the number of 'seeds' available was in excess of the mean number of eggs laid during the first hour of access to a pod.

The oviposition behaviour of *N. arizonensis* and the selection of oviposition sites is investigated in greater detail in Chapter 3.

CHAPTER 3.
OVIPOSITION BEHAVIOUR OF *N. ARIZONENSIS*

ABSTRACT

The observations described in this chapter compare the behaviour of *N. arizonensis* females on pods of different types, to assess how the females respond to the presence of conspecific or of *A. prosopis* eggs. The type of pod did not alter the behavioural activities of *N. arizonensis* females, the total time they spent on pods, the frequency of visits to pods, or the rate of oviposition. The number of eggs laid was not affected by the availability of intact 'seeds' per pod, but it was affected by the type of pod. *Neltumius arizonensis* females avoided aborted 'seeds', 'seeds' with emergence holes, 'seeds' with their own, conspecific or *A. prosopis* eggs, and 'seeds' with egg-free slits and they selected intact 'seeds' for oviposition. Females that had been deprived of pods were initially less discerning about the surface on which they laid eggs, probably because host deprivation caused an accumulation of eggs in the oviducts and there was a need for them to be deposited by the females. The inspection time on intact 'seeds' that were accepted as oviposition sites was significantly longer than on 'seeds' that were rejected. Eggs were laid mostly on a 'seed' that was not adjacent to the 'seed' on which a previous egg had been laid.

INTRODUCTION

Oviposition preferences of insects are determined by a complex web of external and internal factors, and oviposition strategy is the result of a set of behavioural responses (Mitchell, 1975; Mangel and Roitberg, 1989). For phytophagous insects, environmental factors include the spatial distribution of potential host plants (Kareiva, 1984 in Messina *et al.*, 1992), the physical and chemical characteristics of hosts (Renwick, 1989 in Messina *et al.*, 1992), and the egg-load already present on the host (Roitberg and Prokopy, 1987). Endogenous factors that may influence oviposition include a female's oviposition experience and her physiological type (Papaj and Prokopy, 1989; Courtney and Kibota, 1990 in Messina *et al.*, 1992; Barton Browne, 1993).

The influence of the behavioural aspects of oviposition on the population dynamics of insect herbivores has been largely neglected (Minkenberg *et al.*, 1992; Marques *et al.*, 1994). Female preference for oviposition sites influences the development and survival of larvae and may thus affect population dynamics (Via, 1986; Marques *et al.*, 1994). High egg or larval densities result in the retardation of larval growth and a reduction in the number of emerging offspring (Crombie, 1942, 1943; Beaver, 1967 in Anderbrant *et al.*, 1985; Lekander, 1972 in Anderbrant *et al.*, 1985; Ogibin, 1973b in Anderbrant *et al.*, 1985; Berryman, 1974 in Anderbrant *et al.*, 1985; Peters and Barbosa, 1977; Wightman, 1978a; Thomson and Sahota, 1981 in Anderbrant *et al.*, 1985; Bellows, 1982a; Giga and Smith, 1991).

Natural selection could be expected to favour individuals that are able to assess food suitability for immatures, and the abundance of competitors and predators, and thus deposit their eggs on resources that are optimal for their progeny (Rausher, 1983 in Fox, 1993a; Thompson, 1988; Wilson, 1988; Blaustein and Kotler, 1993). This is particularly important in insects that do not feed as adults, where survival and fecundity is dependent on reserves accumulated during larval development, and in insects with relatively immobile immature stages (Mitchell, 1975; Tabashnik *et al.*, 1981; Parker and Courtney, 1984; Smith and Lessells, 1985; Wilson, 1988; Minkenberg *et al.*, 1992). It is therefore necessary for ovipositing females to minimise ovipositional mistakes that decrease the survival of progeny, by rejecting unfavourable hosts and by dispersing eggs so as to minimise intraspecific competition among larvae (Labeyrie, 1978; Wilson, 1988; Jones, 1991; Minkenberg *et al.*, 1992).

After locating a host, an insect must determine whether or not it is a suitable oviposition site and the number of eggs to be laid (Mangel, 1987). Females must balance a time/energy budget to locate potential oviposition sites, assess their suitability and deposit eggs. In the final stages of oviposition site selection, a considerable amount of time and energy may be spent on determining food availability and suitability (Matthews and Matthews, 1978).

Jermy and Szentesi (1978) stated that non-preference for oviposition substrates may be caused by a lack of oviposition stimulants, by the presence of visual or chemical oviposition deterrents, or by a combination of these. Pheromones that deter oviposition are termed oviposition markers, oviposition deterring pheromones or host marking pheromones, and they have been identified in many groups of insects (e.g. Diptera, Lepidoptera, Hymenoptera and Coleoptera (Price, 1974; Jermy and Szentesi, 1978; Szentesi, 1981; Tisdale and Wagner, 1991; Dempster, 1992; Pettersson, 1992; Bernays and Chapman, 1994)). Females, including bruchid females, discriminate against hosts that are or have previously been occupied by conspecifics, and between egg-loads that are already present (Avidov *et al.*, 1965a; Umeya, 1966; Oshima *et al.*, 1973; Mitchell, 1975; Rausher, 1979 in Singer and Mandracchia, 1982; Wasserman and Futuyma, 1981; Wright, 1983; Messina and Renwick, 1985a, 1985b; Wasserman, 1985; Averill and

Prokopy, 1987; Roitberg and Prokopy, 1987; Messina, 1989; Messina and Mitchell, 1989; Shimada and Ishihara, 1990; Jones, 1991; Minkenberg *et al.*, 1992; Pettersson, 1992; Mbata, 1993). This type of behaviour, described as egg-recognition, egg-load assessment or host discrimination (Messina *et al.*, 1991), is characteristic of species in which immobile larvae feed on small, discrete resources (Mitchell, 1975; Roitberg and Prokopy, 1987) and where a single host individual is unable to support more than one individual (Rausher, 1979 in Singer and Mandracchia, 1982).

Pheromones mediate uniform egg dispersal among available fruits and result in efficient resource partitioning for larval development (Oshima *et al.*, 1973; Mitchell, 1975; Prokopy *et al.*, 1976; Wasserman and Futuyma, 1981; Wasserman, 1981, 1985; Messina and Dickinson, 1993). Thus, host markers reduce intraspecific competition and they are expected in species where the probability of intraspecific encounter is high (Roitberg and Prokopy, 1987). The tendency to avoid occupied 'seeds' is genetically determined in some bruchids, although uniform egg-laying is the general trait (Messina 1989; Messina and Mitchell, 1989; Messina, 1991a, 1991b; Messina *et al.*, 1991; Mitchell, 1991). Different species (e.g. *Rhagoletis* spp.) and even individuals of the same species (e.g. *C. maculatus*) vary in their production or ability to recognise oviposition markers (Prokopy *et al.*, 1976; Messina and Dickinson, 1993).

Wilson (1988) proposed that the 'decision' to oviposit on a 'seed' is based on one of two rules. There has been much debate over which rule is correct. The first is a relative rule in which a female compares the number of eggs on a current 'seed' with the previous 'seed' or all 'seeds' previously inspected, and on the basis of this, decides whether or not to oviposit on the current 'seed' (Mitchell, 1975; Wilson, 1988). The second rule, an absolute rule, stipulates that a female only considers the egg load of the current 'seed' (Messina and Renwick, 1985a; Wilson, 1988). There are many possible variations on the two rules, each of which would result in a different distribution of eggs (Wilson, 1988). For example, a female may be able to discriminate between 'seeds' with different numbers of eggs (Messina and Renwick, 1985a). Alternatively, a female may only be able to discriminate between 'seeds' with and without eggs (Wilson, 1988). Some bruchids deposit eggs uniformly within patches of 'seeds' but not among patches (Credland and Wright, 1990; Mangel, 1990 in Messina *et al.*, 1992; Messina *et al.*, 1992). Females may compare egg loads within a patch but may not use this information when they move to other patches (Messina *et al.*, 1992).

Prior to this study, the only information on oviposition by *N. arizonensis* was that eggs are cemented to the surface of *Prosopis* pods and that larvae are relatively immobile and thus incapable of selecting their own food resources (Swier, 1974). Oviposition site selection is therefore presumably of prime importance in determining the fitness of *N. arizonensis* females and their progeny. If *N. arizonensis* is to be a successful biological control agent of *Prosopis* spp. in South Africa, in conjunction with *A. prosopis*, larval food resources must be partitioned, especially as *A. prosopis* larvae are known to be aggressive (Hoffmann *et al.*, 1993a).

The method of *N. arizonensis* oviposition, which differs considerably from that of *A. prosopis*, may confer some degree of discriminatory behaviour for partitioning of resources. The primary objective of the work reported in this chapter was to observe, quantify and compare the oviposition behaviour of *N. arizonensis* on pods of different types to assess if the activity patterns observed on pristine pods altered when conspecific eggs or *A. prosopis* eggs were present. A second objective was to examine the pattern of egg deposition, to determine whether *N. arizonensis* females discriminate between 'seeds' of different qualities.

METHODS

It was necessary to quantify *Neltumius arizonensis* oviposition behaviour and egg deposition patterns in order to assess alterations in behaviour.

A computer programme was written in Turbo Pascal version 6.0 (refer to Appendix 1) to record events and quantify *N. arizonensis* oviposition behaviour. The behaviour of *N. arizonensis* females was examined under four no-choice conditions. Initially behaviour was quantified in what were deemed to be optimal pod conditions (i.e. on 'pristine' pods) and subsequently on different types of pods:

(a) Twenty five *N. arizonensis* females were observed individually on pods that did not bear eggs of any other bruchids, nor had the pod surfaces been tampered with. These were termed 'pristine' pods.

(b) The behaviour of 36 females was observed individually on pods on which other *N. arizonensis* females had oviposited. To manipulate the density of conspecific eggs on pods, the duration of exposure of the other females to the pods was varied. Comparisons were made between *N. arizonensis* behaviour on pristine pods and behaviour on pods with conspecific eggs.

(c) A third category of pods tested the effect of the presence of *A. prosopis* eggs on the oviposition behaviour of *N. arizonensis*. A single slit was scored in the exocarp of a 'seed' to simulate natural damage that is utilised as oviposition sites by *A. prosopis*. Between one and eight of the viable 'seeds' on each pod were scored on different pods.

The pods were then presented to *A. prosopis* females, who oviposited in some or all of the slits, depending on the duration of access to the pods. Investigation of the presence of *A. prosopis* eggs in slits required destructive methods and could only be checked after observations of *N. arizonensis* behaviour. Thorough examination of the slits revealed the number and location of *A. prosopis* eggs in slits and these data were recorded.

(d) A fourth type of pod was utilised as a control for the pods described in (c) above. The surface of between one and eight 'seeds' of pods were scored with a scalpel, but not subjected to oviposition by *A. prosopis*. The slits on these pods were termed 'egg-free'.

Observations were conducted at 25 ± 2 °C and 60 ± 10% R.H. The observation chamber was a plastic box of dimensions 21 x 15 x 8cm, with a glass lid. A single 'mottled-purple' pod with a minimum of eight viable 'seeds' was placed in the chamber, together with a *N. arizonensis* female that had been fed on pollen and had had access to mates but had not been permitted access to *Prosopis* pods. Each female was between three and eight days old (i.e. in peak egg-laying condition) and her behaviour was observed for an hour.

The following behavioural activities were distinguished: (i) walking about the observation chamber; (ii) walking across the pod without inspecting the surface; (iii) stationary (occasionally involved palpations or waving of the antennae in the air); (iv) grooming; (v) inspecting the pod (i.e. walking while thoroughly investigating the pod surface with the antennae as well as the maxillary and labial palps); (vi) scraping of the ovipositor on the pod surface; (vii) scraping of the ovipositor on the surface of the observation chamber; (viii) egg-laying (i.e. the release of an egg from the extruded ovipositor, onto the pod surface); and (ix) investigation of one of the female's own eggs. The process of egg-laying was separated into two stages because they were very distinct (i.e. scraping the ovipositor on the pod surface as opposed to laying an egg).

Amendments were made to the computer programme to examine the behaviour of females on pods other than pristine pods. An additional category of behaviour, namely inspection of an egg of another *N. arizonensis* female, was added when females were observed on pods with conspecific eggs (refer to Appendix 2). Modifications to the programme were also made to investigate *N. arizonensis* behaviour on pods with *A. prosopis* eggs in slits and on pods with egg-free slits (refer to Appendix 3). The additional categories recorded the time females spent inspecting individual slits.

During the observation hour, various keys on the computer were used to record the type and duration of each of successive behaviours on and off the *Prosopis* pod. In addition, the cumulative total duration was calculated for each behaviour. These data were converted into spreadsheet format for subsequent analyses.

3.1 Response to slits and other bruchid eggs

With the exception of pristine pods, the availability of intact 'seeds' differed among pods of the same type and it was necessary to determine whether this affected the number of eggs laid by *N. arizonensis*.

At the time of offering a pod with *A. prosopis* eggs to *N. arizonensis*, it was not known whether all slits contained *A. prosopis* eggs. It was necessary to determine whether there was a correlation between the proportion of slits that contained *A. prosopis* eggs and the proportion of the time on pods that *N. arizonensis* spent inspecting slits.

3.2 Total time spent on pod

The total time *N. arizonensis* females spent on pods of each type was calculated to determine whether this factor varied on the different pod types.

3.3 Number of visits to pods

The mean number of visits that *N. arizonensis* females made to pods was calculated for each pod type, to determine whether this factor varied on pods of different types.

3.4 Time budgets

Calculations were made of the time *N. arizonensis* spent in each behavioural activity on pods. These data were expressed as a proportion of the total time spent on pods. To examine whether the behaviour of females changed as a result of the type of pods, the time spent in individual activities on pristine pods was compared with that on pods with conspecific eggs. The time that *N. arizonensis* females spent in individual activities on pods with *A. prosopis* eggs was compared with that on pods with egg-free slits.

3.5 Number of eggs on pods

The mean number of *N. arizonensis* eggs laid on pods of each type was calculated to determine whether there was (a) a significant difference between the number of eggs laid on pristine pods and on pods with conspecific eggs, (b) a significant difference in the number of eggs laid on pods with *A. prosopis* eggs and on pods with egg-free slits and (c) a significant difference in the number of eggs laid among the four pod types.

3.6 Rate of egg-laying

To examine whether the type of pod affected the rate of oviposition by *N. arizonensis*, the time at which successive eggs were laid on pods of different types was compared. There was a large spread of data, so median values were used.

3.7 Number of eggs on different 'seed' types

Neltumius arizonensis females had a variety of 'seed' types to choose from under each set of conditions and it was necessary to determine whether there was a preference for particular 'seed' types. The availability of each type of 'seed' was expressed as a proportion of the total number of 'seeds' of all types on each type of pod (with the exception of pristine pods). Calculations and comparisons were made of the proportion of 'seeds' of each type that *N. arizonensis* utilised for egg-laying.

3.8 'Seed' type selected for deposition of successive eggs

Having examined the overall selection of 'seeds' by *N. arizonensis*, 'seed' selection for successive eggs on all except pristine pods was examined, to determine whether selectivity changed as time progressed. For each successive egg, calculations were made of the proportion that were laid on each 'seed' type.

3.9 Placement of eggs

Calculations were made of the proportion of *N. arizonensis* eggs that were laid on adjacent or non-adjacent 'seeds' to the previous egg, or on the same 'seed' as the previous egg. These data were calculated for all pod types, with the exception of pristine pods.

3.10 Inspection time on 'seeds' of different types

The amount of time that *N. arizonensis* spent inspecting 'seeds' of different types was examined. Twenty-two *N. arizonensis* females were each provided with a pod bearing intact 'seeds', 'seeds' with conspecific eggs and 'seeds' from which conspecific eggs had been removed by scraping. The time that individual females spent inspecting 'seeds' of each type, including 'seeds' on which they deposited eggs, was recorded using a timing device. The inspection time of intact 'seeds' that were accepted as oviposition sites and those that were rejected, was recorded separately.

3.11 Choice of pods

To determine whether *N. arizonensis* females would be selective of pods of different types in a choice situation, 19 females were each provided with a pristine pod, a pod with conspecific eggs and a pod with *A. prosopis* eggs, in plastic jars with gauze lids. Intact 'seeds' were available on all the pods that carried eggs of other females. The pods were removed after one hour and the newly-laid eggs were counted.

RESULTS

Oviposition behaviour

After being placed on the base of the observation chamber at the start of the one hour observation period, a *N. arizonensis* female invariably investigated the surface of the observation chamber. Occasionally the first (less than 12% of all first eggs), and sometimes the second egg (less than 4% of all second eggs), were laid on the surface of the observation chamber before a female had located the pod. After locating and palpating the pod, the female climbed onto it. She immediately began inspecting the pod surface, with her antennae and maxillary and labial palps in almost continual contact with the pod. Walking on a pod without inspecting the surface, usually preceded the departure of a *N. arizonensis* female from that pod. Inspection of a 'seed' involved palpating from side to side, while slowly walking to and fro across the surface of the pod. Females would often walk from one pod seam to the other and back numerous times on the same 'seed', palpating the surface with their antennae. Occasionally 'seed' inspection was rather cursory, and an egg was laid shortly after the female arrived on the 'seed'. The number of 'seeds' inspected before the first egg was laid varied from one 'seed' to several 'seeds'.

Fig. 5: A *N. arizonensis* female with her ovipositor extended while scraping it across the pod surface.

After a 'seed' had been selected as an oviposition site, the female extended her ovipositor onto the pod surface and scraped it in a backwards and forwards motion (Fig. 5), as also reported by Swier (1974). Sometimes the female remained at one site during this activity, while at other times, she walked about the 'seed' with the ovipositor extended, and scraped the pod surface at various sites. On a few occasions the ovipositor was withdrawn, another section of the 'seed' investigated, and the ovipositor was again extended. This sometimes occurred a number of times before an egg was laid. Immediately before an egg was laid, the ovipositor was extended to an even greater extent, the female was motionless for a variable period of up to 30 seconds, and the egg began to be extruded. As the pale yellow egg emerged slowly from the ovipositor, it was glued to the pod surface (i.e. unlike other bruchids (Parnell, 1966; Swier, 1974), the cement which attached the egg to the pod surface was deposited around the egg as it was being laid). Each *N. arizonensis* female shook her abdomen from side to side during the final stages of the egg deposition process, probably to aid the passage of the egg from the oviduct.

Following the adhesion of the egg to the pod, the female withdrew her ovipositor and rapidly left the area, without inspecting the newly-laid egg. The female immediately began investigating an adjacent 'seed' or other 'seeds', searching for another oviposition site.

Grooming occurred frequently but at irregular intervals. Females groomed themselves after being handled at the start of the observation period, between inspections of the surface of the pod, and occasionally for a short period after leaving the 'seed' on which an egg had been deposited. During grooming, either one or both of the third pair of legs were repeatedly brushed across the dorsal surface of the abdominal region, from the posterior end of the elytra to the tip of the pygidium. The soft second pair of wings were often extended and the legs were brushed across them. Much time was also spent grooming the head. One of the first pair of legs was used to wipe across the dorsal surface of the head region and one by one, the antennae were drawn towards the mouth. The antennae were meticulously and repeatedly groomed, particularly using the maxillary and labial palps.

3.1 Response to slits and other bruchid eggs

There was no significant correlation ($r^2 = 0.018$, d.f. = 34, $p > 0.05$) between the number of eggs laid by *N. arizonensis* and the number of 'seeds' with conspecific eggs. The positive correlation ($r^2 = 0.116$, d.f. = 34, $0.02 < p < 0.05$) between the number of *N. arizonensis* eggs laid and the number of 'seeds' with *A. prosopis* eggs was not highly significant and was disregarded. There was no significant correlation ($r^2 = 0.148$, d.f. = 10, $p > 0.05$) between the number of *N. arizonensis* eggs laid and the number of 'seeds' with egg-free slits. Consequently, it was not necessary to consider each female separately in the analyses of behaviour for each treatment.

There was no correlation ($r^2 = 0.003$, d.f. = 34, $p > 0.05$) between the proportion of the total time *N. arizonensis* spent inspecting slits on pods with *A. prosopis* eggs and the proportion of 'seeds' with slits that contained *A. prosopis* eggs. Thus, the fact that some pods with *A. prosopis* eggs had some slits without *A. prosopis* eggs, was disregarded.

3.2 Total time spent on pods

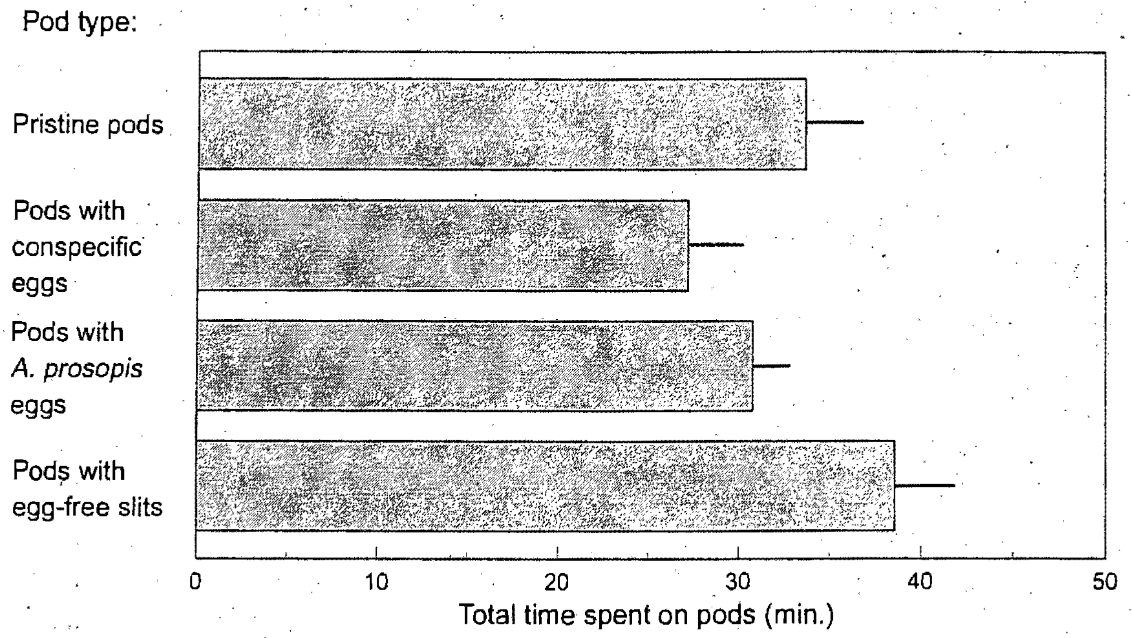


Fig. 6: Mean total number of minutes + 1 S.E. spent by *N. arizonensis* females during 60 minutes of exposure to *Prosopis* pods of different types. n = 25 for pristine pods; n = 36 for pods with conspecific eggs; n = 36 for pods with *A. prosopis* eggs in slits; and n = 12 for pods with egg-free slits.

Neltumius arizonensis females spent about half an hour on the pods (Fig. 6) and there was no significant difference (Kruskal Wallis test statistic = 3.972, n.s.) in the total time females spent on pods of different types.

3.3 Number of visits to pods

Table 4: Mean number of visits ± S.E. by *N. arizonensis* females to pods of different types during the one hour observation period. Values in parentheses indicate the numbers of females used in each treatment. The results of statistical analyses are shown, where n.s. = no significant difference.

Pod type	Mean number of visits
Pristine pods	2.40 ± 0.24 (25)
Pods with conspecific eggs	2.94 ± 0.31 (36)
Kruskal Wallis test statistic = 0.659, n.s.	
Pods with *A. prosopis* eggs in slits	3.58 ± 0.41 (36)
Pods with egg-free slits	3.17 ± 0.44 (12)
Kruskal Wallis test statistic = 0.012, n.s.	

There was no significant difference between the number of visits by *N. arizonensis* females to pristine pods and pods with conspecific eggs (Table 4). There was also no significant difference between the number of visits to pods with *A. prosopis* eggs and pods with egg-free slits.

3.4 Time budgets

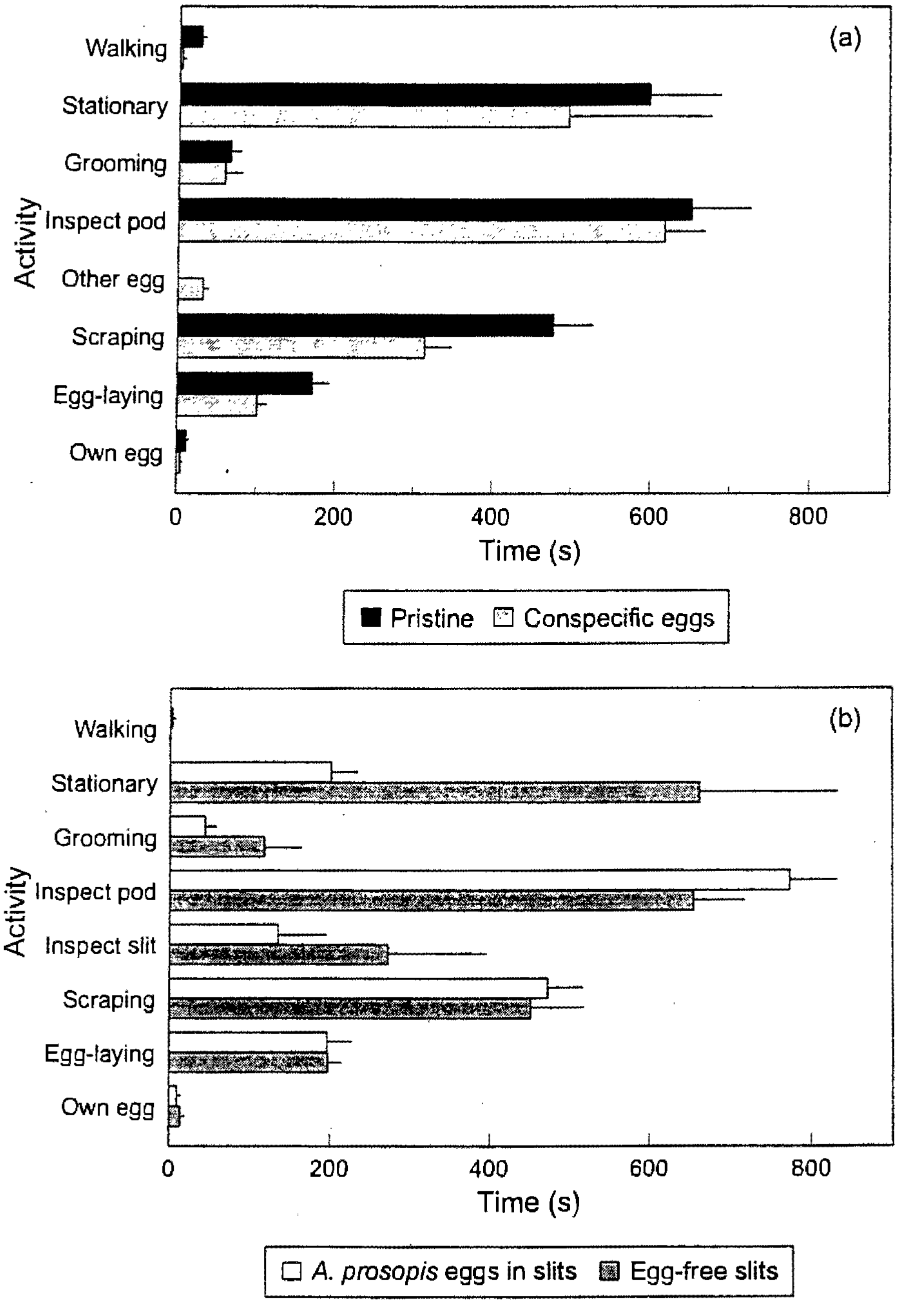


Fig. 7: Mean time (seconds) + 1 S.E. spent by *N. arizonensis* females in various activities while on pods of four different types: (a) pristine pods (n = 25) and pods with eggs of conspecifics (n = 36); **(b)** pods with slits in which *A. prosopis* females had oviposited (n = 36), and pods with egg-free slits (n = 12). The activities displayed are: walking = walking across the pod surface without inspecting it; stationary = inactive; grooming = grooming of body using legs; inspect pod = thorough investigation of the pod surface by palpations using the antennae and mouthparts; other egg = investigation of a previously-laid egg of a conspecific; inspect slit = investigation of slits with and without *A. prosopis* eggs; scraping = scraping of extruded ovipositor across pod surface, after thorough inspection of a 'seed' and prior to an egg being laid; egg-laying = process of egg being released from extruded ovipositor; own egg = inspection of one of female's own eggs laid during the observation period.

Table 5: Results of individual unpaired t-tests on the total time (seconds) spent in each activity by *N. arizonensis* females on pristine pods and females on pods with conspecific eggs, and by *N. arizonensis* females on pods with *A. prosopis* eggs and females on pods with egg-free slits. Results are displayed as n.s. = not significant, * = $p < 0.05$, *** = $p < 0.001$.

Activity	Pristine pods vs. pods with conspecific eggs (d.f. = 59)	Pods with *A. prosopis* eggs vs. pods with egg-free slits (d.f. = 46)
Walking	t = 4.203, ***	t = 1.311, n.s.
Stationary	t = 0.626, n.s.	t = 3.622, ***
Grooming	t = 0.257, n.s.	t = 2.146, *
Pod inspection	t = 0.359, n.s.	t = 1.048, n.s.
Slit inspection	-	t = 0.199, n.s.
Ovipositor-scraping	t = 2.606, *	t = 0.239, n.s.
Egg-laying	t = 2.603, *	t = 0.020, n.s.
Inspecting own eggs	t = 2.136, *	t = 0.801, n.s.

The time spent by *N. arizonensis* females on pods of all types was not equally apportioned among different behaviours (Figs. 7a and 7b). The majority of time on pods of all types was spent in three activities - stationary, inspecting the surface of pods and scraping the ovipositor across the pod surface. Inspection of the pod surface occupied about one third of the time spent on pods.

Females scraped their ovipositors on pristine pods for an average of 127.5 ± 8.0 (n = 91) seconds before laying an egg, whereas females on pods with conspecific eggs spent significantly less time on average in this activity (101.3 ± 5.5 seconds (n = 105)) (unpaired t-test, $t = 2.765$, $p < 0.001$). In comparison, ovipositor-scraping took about 85 seconds on pods with *A. prosopis* eggs and on pods with egg-free slits. On average, egg-laying took significantly longer (one-way ANOVA, $F_{(1, 202)} = 30.282$, $p < 0.001$) on pristine pods (47.7 ± 1.9 seconds, n = 89) than on pods with conspecific eggs (35.3 ± 1.4 seconds, n = 115). Females spent about 18% and 12% of the time scraping their ovipositor and then depositing eggs on pristine pods and on pods with conspecific eggs, respectively.

Neltumius arizonensis females examined the exterior and interior of slits on pods with *A. prosopis* eggs and on pods with egg-free slits. In total, *N. arizonensis* females examined slits for about 92-95% longer than they examined their own eggs. Egg-laying on pods with *A. prosopis* eggs and on pods with egg-free slits took 37.6 ± 0.6 seconds (n = 193) and 36.3 ± 1.5 seconds (n = 67), respectively.

The main differences between the behaviour of *N. arizonensis* females on pristine pods and that of females on pods with conspecific eggs were that females on pristine pods spent significantly longer (Table 5) walking across pods, scraping their ovipositor, laying eggs and inspecting their own eggs than females on pods with conspecific eggs. However, the difference in the time spent walking was probably due to an error in data collection at the outset of behaviour observations, where the time spent inspecting pristine pods was not differentiated from the time spent walking, and some data had to be extrapolated from later data. The only significant differences (Table 5) in the behaviour of *N. arizonensis* females on pods with *A. prosopis* eggs and on pods with egg-free slits, were in the time spent stationary and the time spent grooming. Females on pods with *A. prosopis* eggs spent less time stationary and grooming.

3.5 Number of eggs laid on pods of different types

Table 6: Mean total number of eggs ± S.E. laid by *N. arizonensis* females during one hour on four types of pods. n indicates the number of female bruchids used in each experiment. Results are shown for one-way ANOVAs between (a) pristine pods and pods with conspecific eggs; (b) pods with *A. prosopis* eggs within slits and pods with egg-free slits. n.s. = no significant difference.

Pod type	Total number of eggs laid on pod	n
Pristine pods	3.64 ± 0.41	25
Pods with conspecific eggs	2.86 ± 0.34	36
	$F_{(1,59)} = 2.015$, n.s.	
Pods with *A. prosopis* eggs in slits	5.22 ± 2.93	36
Pods with egg-free slits	5.50 ± 0.86	12
	$F_{(1,46)} = 0.781$, n.s.	

Although slightly more *N. arizonensis* eggs were laid on pristine pods than on pods with conspecific eggs (Table 6), the difference was not significant. There was no significant difference in the number of *N. arizonensis* eggs laid on pods with *A. prosopis* eggs and on pods with egg-free slits. However, unexpectedly, significantly more eggs (one-way ANOVA, $F_{(3,105)} = 6.869$, $p < 0.001$) were laid on pods with *A. prosopis* eggs and on pods with slits than on pristine pods and pods with conspecific eggs.

3.6 Rate of egg-laying

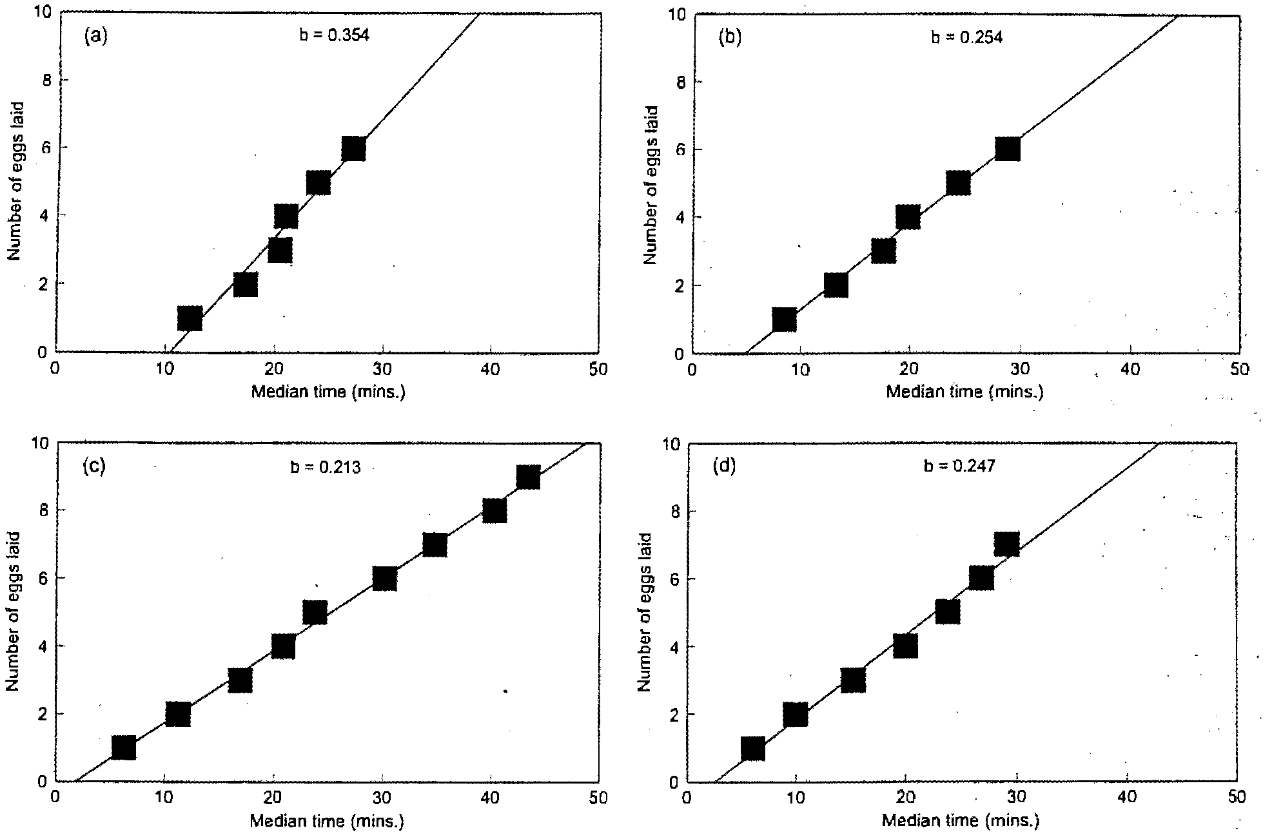


Fig. 8: Linear regressions of median time at which successive eggs were laid by *N. arizonensis* females, during one hour on pods of different types. (**a**) pristine pods (n = 25); (**b**) pods with conspecific eggs (n = 36); (**c**) pods with slits in which *A. prosopis* eggs had been laid (n = 36); and (**d**) pods with egg-free slits (n = 12). The values for the rates of oviposition are given as the slopes of the regressions, b.

Most *N. arizonensis* eggs were laid at regular intervals within the first half hour of the observation period on all types of pods (Figs. 8a-8d). The rate of oviposition on each type of pod was fairly constant. The first eggs on pristine pods (Fig. 8a) were laid between four and six minutes later than the first eggs that were laid on the other three pod types (Figs. 8b-8d). However, as indicated by the slopes of the regression lines, subsequent eggs were laid at a slightly faster rate on pristine pods than on the others. An analysis of covariance showed that there was no significant difference ($F_{(3, 20)} = 1.949$, n.s.) between the slopes of the four regression lines (i.e. the rate of egg-laying on the four pod types was not significantly different).

3.7 Number of eggs on different 'seed' types

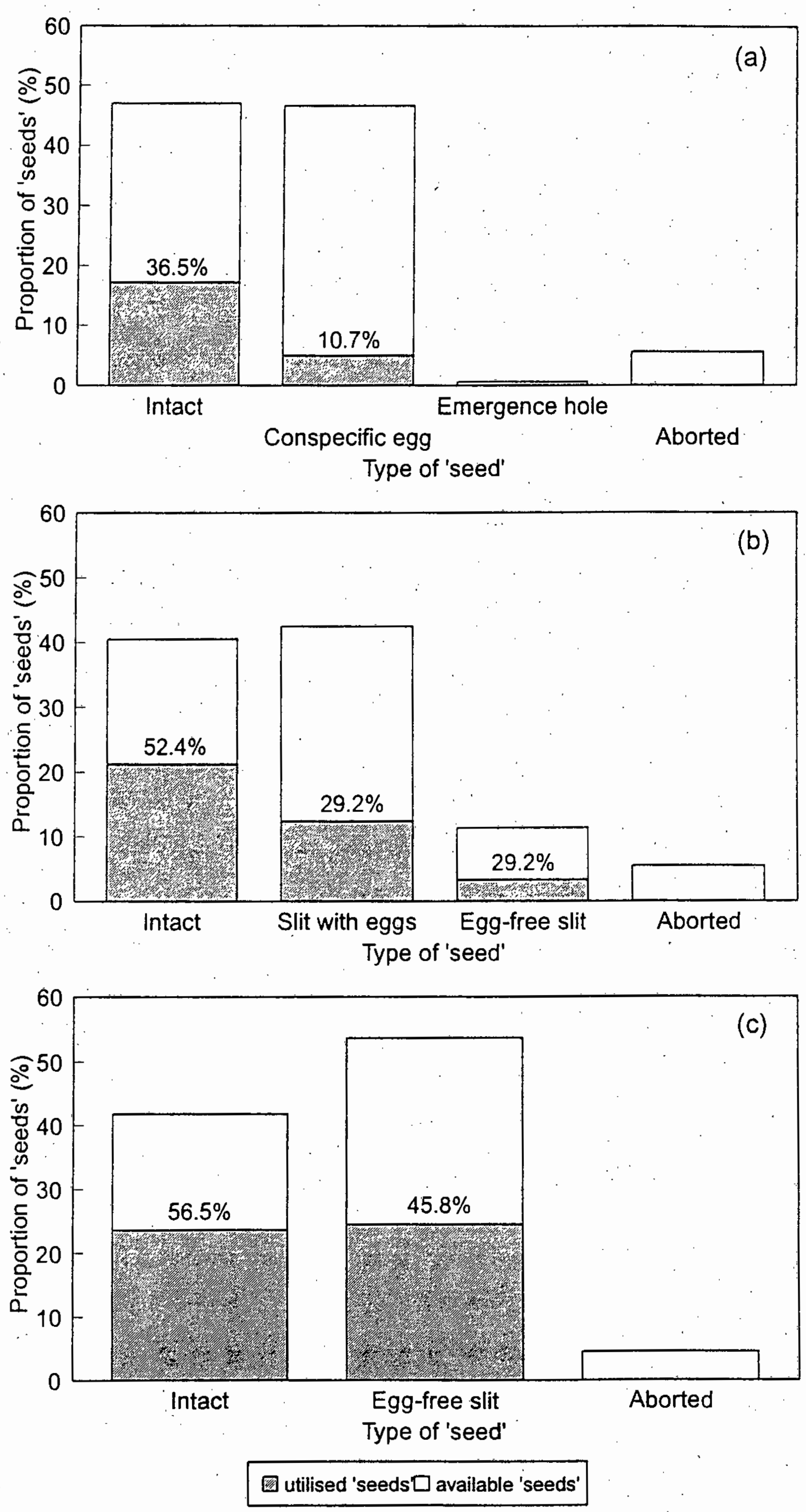


Fig. 9: The proportion of 'seeds' of each type available on **(a)** 36 pods with eggs of other *N. arizonensis* females; **(b)** 36 pods with *A. prosopis* eggs in slits; and **(c)** 12 pods with egg-free slits. The values and hatched areas indicate the proportion of 'seeds' of each type that were utilised as oviposition sites by *N. arizonensis* females.

Pods with conspecific eggs bore mainly intact 'seeds' and 'seeds' with conspecific eggs (Fig. 9a). The remainder of the 'seeds' were either aborted or had emergence holes of other bruchid adults; no eggs were laid on those 'seeds'. Although an equivalent number of intact 'seeds' and 'seeds' with conspecific eggs were available, there was a significant difference ($Chi^2 = 36.885$, $p < 0.001$) between the number of 'seeds' of each type that were utilised as oviposition sites. *Neltumius arizonensis* females preferred to oviposit on intact 'seeds'.

Four types of 'seeds' were available on pods with *A. prosopis* eggs in slits: intact 'seeds', 'seeds' with slits that contained *A. prosopis* eggs, 'seeds' with egg-free slits, and aborted 'seeds' (Fig. 9b). Very few 'seeds' on the pods were aborted, and *N. arizonensis* females did not utilise these as oviposition sites. Although about as many intact 'seeds' as 'seeds' with *A. prosopis* eggs were available, *N. arizonensis* utilised significantly more ($Chi^2 = 18.370$, $p < 0.001$) intact 'seeds' as oviposition sites than 'seeds' with *A. prosopis* eggs. *Neltumius arizonensis* also utilised significantly more ($Chi^2 = 7.170$, $0.025\ p < 0.01$) intact 'seeds' as oviposition sites than 'seeds' with egg-free slits. Therefore, *N. arizonensis* females preferred to oviposit mainly on intact 'seeds'. Although many more 'seeds' with *A. prosopis* eggs were available than 'seeds' with egg-free slits, there was no significant difference ($Chi^2 = 0.034$, n.s.) between the extent of utilisation of the two types of 'seeds'. It thus appeared that *N. arizonensis* avoided 'seeds' with *A. prosopis* eggs to the same degree as 'seeds' with egg-free slits.

There was a greater availability of 'seeds' with egg-free slits than intact 'seeds', on pods with egg-free slits (Fig. 9c). Although *N. arizonensis* females utilised more intact 'seeds' than 'seeds' with egg-free slits, there was no significant difference ($Chi^2 = 0.805$, n.s.) between the utilisation of the two types of 'seeds'. There were very few aborted 'seeds' and *N. arizonensis* females did not oviposit on them.

3.8 'Seed' type selected for deposition of successive eggs

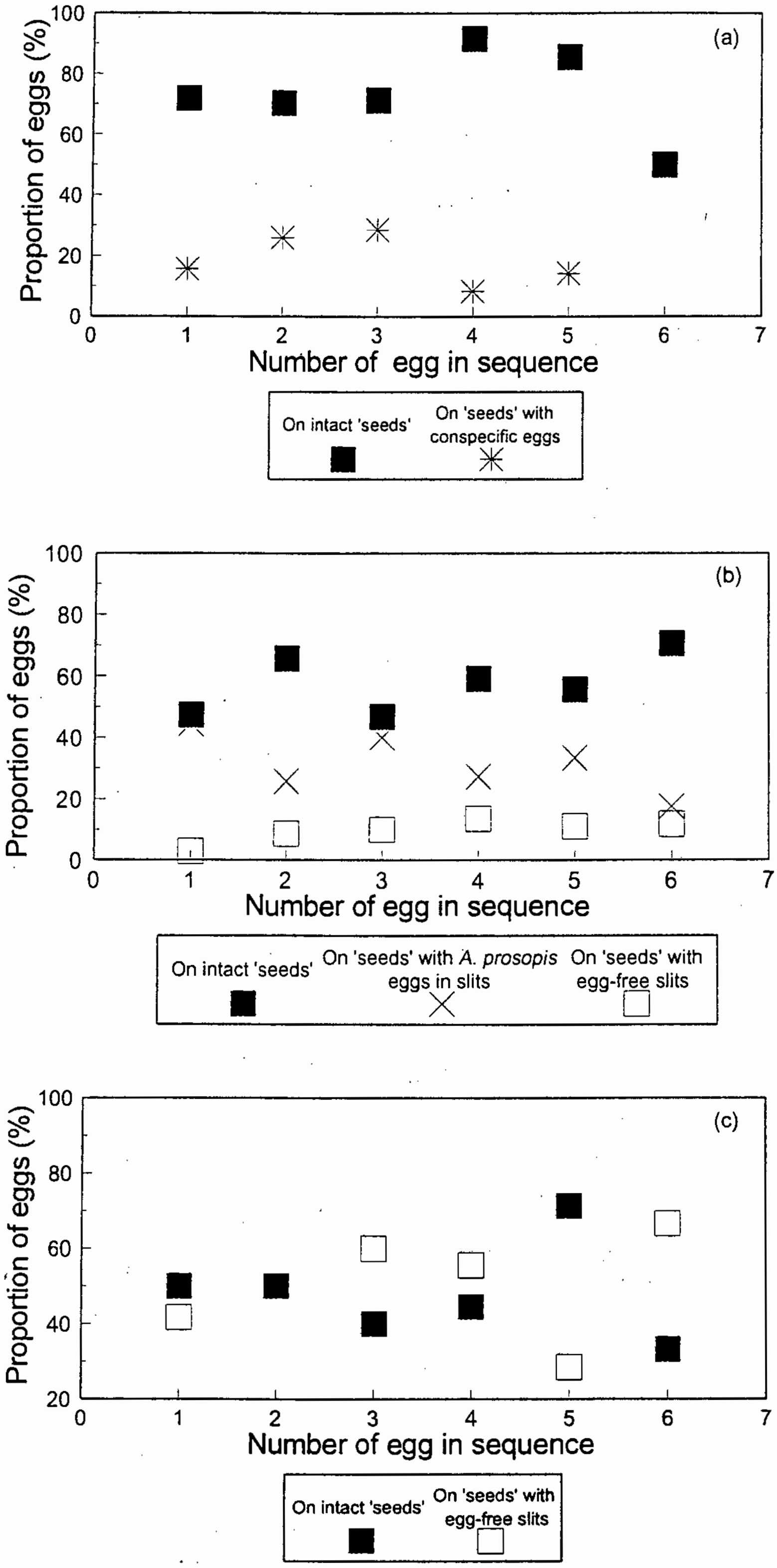


Fig. 10: Mean proportions of the total number of successive eggs laid by *N. arizonensis*, on **(a)** intact 'seeds' and on 'seeds' with conspecific eggs; **(b)** intact 'seeds', 'seeds' with *A. prosopis* eggs and on 'seeds' with egg-free slits; **(c)** intact 'seeds' and on 'seeds' with egg-free slits.

The proportions of the first few eggs in Figs. 10a and 10b do not sum to 100% because a small proportion of the eggs were laid on the observation chamber and were not included in the figures.

Most *N. arizonensis* females were highly selective on pods with conspecific eggs, preferring intact 'seeds' and usually avoiding 'seeds' bearing conspecific eggs (Fig. 10a). More of the fourth and fifth eggs were laid on intact 'seeds' than the first, second and third eggs, thus, *N. arizonensis* selection for intact 'seeds' on pods with conspecific eggs increased with time. The low selectivity for clean 'seeds' for the sixth eggs was an experimental artifact because only four sixth eggs were laid on pods with conspecific eggs.

Although *N. arizonensis* preferred to oviposit on intact 'seeds' on pods with *A. prosopis* eggs (Fig. 10b), a greater degree of selectivity for intact 'seeds' was shown at the early stages of oviposition on pods with conspecific eggs (Fig. 10a) than on pods with *A. prosopis* eggs. Selection for intact 'seeds' on pods with *A. prosopis* eggs increased with the number of eggs laid. Although it appeared that *N. arizonensis* laid eggs on 'seeds' with *A. prosopis* eggs in preference to those with egg-free slits, this was only because more 'seeds' with *A. prosopis* eggs were available; the same proportion of each type was utilised (refer to Fig. 9b).

There was no obvious selection for intact 'seeds' by *N. arizonensis* females on pods with egg-free slits (Fig. 10c). Occasionally more eggs were laid on 'seeds' with egg-free slits than on intact 'seeds', although mostly a similar number of eggs were laid on 'seeds' with egg-free slits as on intact 'seeds'.

3.9 Placement of eggs

Table 7: The proportion (%) of the total number of eggs laid at least one 'seed' away from the previous egg; on a 'seed' adjacent to the previous egg; or on the same 'seed' as the previous egg, on three types of pods. The values in parentheses indicate the proportion of previous eggs that had at least one adjacent intact 'seed' on which an egg could have been or was laid. Results of Chi^2 tests between the number of eggs laid on 'seeds' not adjacent to previous eggs and on 'seeds' adjacent to previous eggs, on each pod type, are shown below.

Placement of new egg	On pods with: conspecific eggs (n = 36)	*A. prosopis* eggs in slits (n = 36)	egg-free slits (n = 12)
not adjacent to previous egg	62.3% (41.9%)	77.4% (31.2%)	56.6% (36.6%)
adjacent to previous egg	30.4% [1] (52.4%)	21.0% [2] (38.4%)	43.4% [3] (56.5%)
on same 'seed' as previous egg	7.3% (0.0%)	1.6% (100.0%)	0.0% (0.0%)

1: $Chi^2 = 7.563, 0.01 < p < 0.005$
2: $Chi^2 = 40.164, p < 0.001$
3: $Chi^2 = 0.925$, n.s.

Despite the availability of at least one intact 'seed' adjacent to the previous egg in many cases, *N. arizonensis* females laid significantly more of their eggs on 'seeds' that were not adjacent to that of the previous egg than on 'seeds' that were adjacent to the previous egg, on pods with conspecific eggs and on pods with *A. prosopis* eggs, but not on pods with egg-free slits (Table 7). Eggs were very seldom laid on the same 'seed' as the 'seed' of the previous egg, on all types of pods.

3.10 Inspection time on 'seeds' of different types

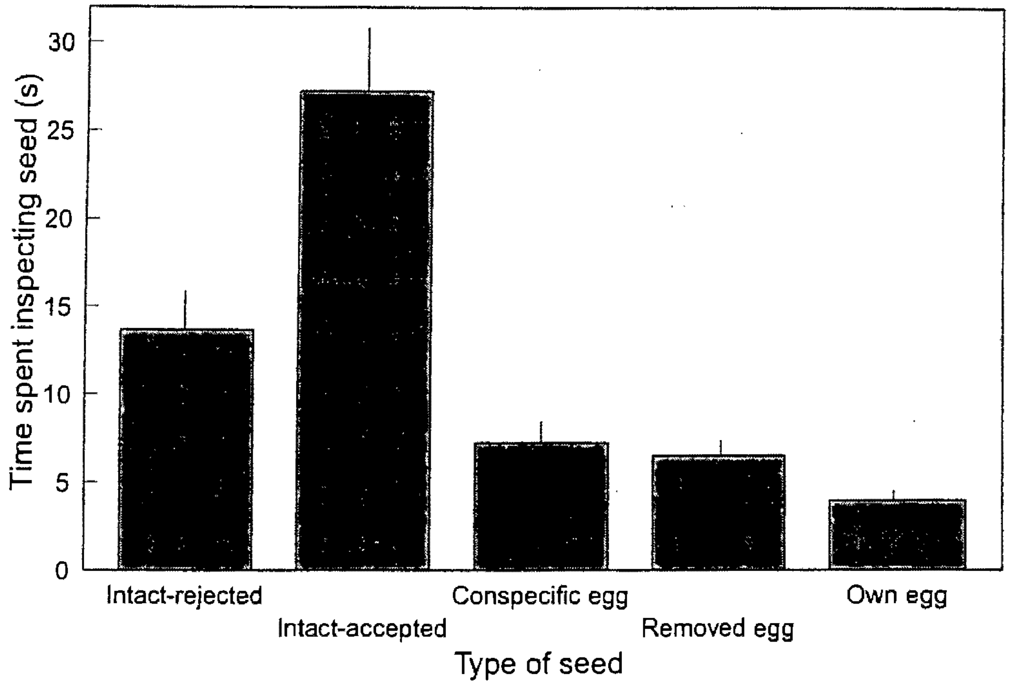


Fig. 11: Mean number of seconds + 1 S.E. spent by *N. arizonensis* inspecting intact 'seeds' that were rejected as oviposition sites (n = 64); intact 'seeds' that were accepted (n = 19); 'seeds' with a conspecific egg (n = 62); 'seeds' from which a conspecific egg had been removed (n = 38); and 'seeds' with the females' own eggs (n = 36).

There was a significant difference (Kruskal Wallis test statistic = 67.744, $p < 0.001$) in the time *N. arizonensis* females spent inspecting 'seeds' of different types (Fig. 11). The inspection time of intact 'seeds' that were accepted for oviposition was double the inspection time of intact 'seeds' that were rejected, and was much longer than the inspection time of any other 'seed' type. The inspection time of a 'seed' with a conspecific egg was similar to that of a 'seed' from which a conspecific egg had been removed. Females thoroughly investigated the exact areas from which eggs had been removed. The least amount of time was spent inspecting a 'seed' bearing one of the female's own eggs.

3.11 Choice of pods

Table 8: Mean number of eggs laid during one hour by *N. arizonensis* females (n = 19) with a choice between (a) pristine pods; (b) pods with conspecific eggs and (c) pods with *A. prosopis* eggs. n.s. = no significant difference, the result of a one-way ANOVA.

Pod type	Mean number of eggs ± S.E.
Pristine pods	1.63 ± 0.38
Pods with conspecific eggs	1.58 ± 0.45
Pods with *A. prosopis* eggs	2.26 ± 0.50
	$F_{(2,54)} = 0.726$, n.s.

Unexpectedly, there was no significant difference between the mean number of eggs laid during one hour by *N. arizonensis* females when provided with a pristine pod, a pod with conspecific eggs and a pod with *A. prosopis* eggs in slits (Table 8). Therefore, *N. arizonensis* females were not selective of the type of pod their eggs were laid on when they were provided with a choice of pods.

DISCUSSION

Wilson (1988) stated that, almost without exception, studies of the oviposition strategies of phytophagous insects have been concerned with the end result of oviposition, and the inferences that have been made may have provided an inaccurate view about the behavioural mechanisms involved. With the exception of *Callosobruchus maculatus* (e.g. Ofuya and Agele, 1989a; Messina and Dickinson, 1993), detailed studies of bruchid behaviour have not been conducted. In this study I examined not only the outcome of oviposition in *N. arizonensis* under various types, but also the process and behavioural activities that were involved.

Behaviour

Neltumius arizonensis females spent a large proportion of their time in activities off *Prosopis* pods. They either walked about the observation chamber, groomed themselves, or occasionally deposited an egg on the chamber surface, but mostly, in common with *C. maculatus* (Wilson, 1988), they were stationary. *Neltumius arizonensis* females may have been waiting for the maturation and passage of eggs through the reproductive tract, during this time.

Contrary to expectations, the type of pods did not affect the total time *N. arizonensis* spent on pods, the frequency of their visits to pods or the time spent in various activities on pods. Although there were significant differences between some aspects of the behaviour of females on pristine pods and on pods with conspecific eggs, they were considered to be trivial because they were only a result of the marginally greater number of eggs laid on pristine pods. The significant difference in the time *N. arizonensis* spent grooming on pods with *A. prosopis* eggs as opposed to pods with egg-free slits, was also not considered to be important. Although there was a significant difference in the time *N. arizonensis* females were stationary on pods with egg-free slits and on pods with *A. prosopis* eggs, the total time spent stationary was equivalent on both pod types. Therefore, the type of pod provided had little effect on the behaviour of *N. arizonensis* females.

Neltumius arizonensis females spent a large proportion of time inspecting the surface of pods presumably because fitness is improved by the deposition of eggs on oviposition sites that allow successful larval development. During oviposition site selection, female bruchids use their antennae and maxillary and labial palps to explore the surfaces of pods to gain information on the texture, maturity and state of entirety of a pod, and to detect eggs (Jermy and Szentesi, 1978; Pimbert, 1980 in Pimbert and Pierre, 1983; Wasserman, 1985; Messina *et al.*, 1987; Ofuya, 1987). Chemoreceptors on ovipositors only play a minor role in the investigation process (Szentesi, 1976; Jermy and Szentesi, 1978). Olfactory receptors are present on antennae and taste receptors are present on maxillary and labial palps, thus, gustatory, olfactory and, or, tactile information regulate reproductive activity (Pimbert and Pierre, 1983). *Neltumius arizonensis* females regularly groomed themselves, especially their antennae and mouthparts, between the deposition of successive eggs. Perhaps grooming of bodyparts with sensory functions was necessary for their optimal performance.

Similar to *C. maculatus* (Wasserman, 1985; Messina and Dickinson, 1993), during inspection of a 'seed', *N. arizonensis* females walked about a 'seed' many times with their antennae and mouthparts in almost continuous contact with the pod surface.

Wassermann (1985) reported difficulty in quantifying inspection in *C. maculatus* because the number of 'seeds' inspected and the frequency of inspections before an egg is laid depends on the number of 'seeds' to which a female has access (Ofuya and Agele, 1989a; Messina and Dickinson, 1993). By examining many 'seeds' but spending less time on each 'seed', females may make better assessments of overall egg density and better disperse their eggs; otherwise less information may be gained about egg loads and eggs might be poorly dispersed (Ofuya and Agele, 1989a).

An efficient mechanism of egg recognition was observed in *N. arizonensis*. When a female encountered her own, conspecific or *A. prosopis* eggs, there was a very brief inspection period before the female left the 'seed' that already carried an egg. Inspection of the exact areas where eggs of conspecifics had been cemented but subsequently removed, indicated that *N. arizonensis* females were not only aware of the presence of intact eggs, but could also detect other features, possibly chemical, that remained after eggs had been removed. Perhaps *N. arizonensis* utilises an oviposition marker. In many bruchid species, a chemically-based oviposition marker is deposited with each egg (Oshima *et al.*, 1973; Mitchell, 1975; Wasserman and Futuyma, 1981; Messina and Renwick, 1985b) and in some species, the physical presence of eggs may also contribute to oviposition deterrence because 'seeds' with egg models or 'seeds' to which eggs have been glued are avoided (Messina and Renwick, 1985b). The long periods that *N. arizonensis* spent inspecting the damaged exocarp and exposed mesocarp on pods with *A. prosopis* eggs may indicate that either *N. arizonensis* females did not recognise, or were slow to detect, an oviposition marker of *A. prosopis*, or that *A. prosopis* does not produce an oviposition marker.

There may be several levels of oviposition site inspection, because intact 'seeds' that were rejected by *N. arizonensis* received less attention than intact 'seeds' that were accepted as oviposition sites. 'Seeds' seem to have been initially accepted or rejected on the basis of obvious characteristics. Once a 'seed' had been provisionally accepted as a potential oviposition site, more subtle attributes of the 'seeds' may have been examined.

Further exploration of the oviposition site may have occurred while the ovipositor was scraped across the pod surface. Hairs on the ovipositor of some bruchid species have a sensory function (Szentesi, 1976), but this needs to be demonstrated in *N. arizonensis*. There is circumstantial evidence that the ovipositor of *N. arizonensis* has a sensory function and females spent proportionately much more of their time scraping their ovipositors across the surface of the pod than other bruchid species (e.g. *C. maculatus* (Wasserman, 1985)). Some *N. arizonensis* females scraped their ovipositor across a 'seed' in preparation for egg-laying, but then walked away and began inspecting other 'seeds', indicating that they had been deterred by information gained during the scraping process.

As reported in other bruchids (Wasserman, 1985), *N. arizonensis* females were more likely to discontinue oviposition when disturbed by other bruchids during the 'seed' inspection or ovipositor-scraping phases than during the egg deposition phase, possibly because of the energy expended in patrolling 'seeds' and depositing the egg adhesive, or because of physiological reasons e.g. perhaps an egg must be expelled before the ovipositor can be withdrawn (Wasserman, 1985).

Unlike some bruchids (Wasserman, 1985; Messina and Dickinson, 1993), but in common with others (Parnell, 1966), once an *N. arizonensis* female had deposited an egg, she walked away from the 'seed' and there was a high probability that the following egg would be laid on a 'seed' not adjacent to the 'seed' on which the previous egg was laid, even when one or both adjacent 'seeds' were devoid of eggs. Perhaps there was an unavoidable delay between the deposition of consecutive eggs while the next egg was positioned in the oviduct, and the eggs were isolated from each other because of arbitrary movements by the females between depositions. Alternatively, *N. arizonensis* may deliberately disperse eggs, perhaps to minimise predation or parasitism.

Number of eggs laid & rate of oviposition

Bruchid females can probably only produce and retain a certain number of mature eggs before depositing them, after which there is an interval in which eggs mature. Bruchids lay between five and 11 eggs during one hour, depending on the bruchid species and the type of pods or seeds (Parnell, 1966; Wilson, 1988; Messina and Dickinson, 1993). *Neltumius arizonensis* inexplicably laid a significantly greater number of eggs, and thus utilised more intact 'seeds', on pods with *A. prosopis* eggs and on pods with egg-free slits than on pristine pods or pods with conspecific eggs.

The rate of oviposition in *N. arizonensis* was expected to be significantly higher on pristine pods because females on other pod types were expected to be occupied for longer, inspecting 'seeds' that carried slits or eggs of other females. However, the results did not concur with the expectations.

Host deprivation

Many eggs would have matured within *N. arizonensis* in the four days minimum between emergence and the observation period. The accumulation of eggs was probably responsible for the rapid initial egg deposition rate, the deposition of a small proportion of the first few eggs on the observation chamber surface, and the lack of effect of the availability of intact 'seeds' on the number of eggs that were laid on pods of the same type. The number of mature oocytes available for oviposition is a physiological parameter that has been hypothesised to affect host acceptance and many aspects of oviposition behaviour in herbivorous insects (Wasserman and Futuyma, 1981; Minkenberg *et al.*, 1992). Minkenberg *et al.* (1992) suggested that behaviour would maximise the rate of host encounter and utilisation when egg numbers were high, but when egg numbers were low, behaviour would instead maximise the quality of oviposition sites. A general decline in oviposition site discrimination has been reported for several insect species that have been deprived of hosts for a period (Avidov *et al.*, 1965a; Messina *et al.*, 1992).

Oviposition site selection

Neltumius arizonensis females avoided aborted 'seeds' and 'seeds' with emergence holes which would have resulted in decreased larval survivorship, as has been reported in several bruchids (Avidov *et al.*, 1965b; Mitchell, 1975; Jermy, 1972 in Szentesi, 1976; Mbata, 1993). Similar to some bruchids (Wasserman, 1985; Ofuya, 1987), *N. arizonensis* females frequently laid eggs on the cheek of the pod, but occasionally eggs were also laid on or near the seam or keel of pods. Swier (1974) noted that eggs of *N. arizonensis* were deposited randomly with no relation to the cotyledon.

Neltumius arizonensis females discriminated among 'seeds' of different types, although the degree of discrimination varied among pod types. Selection for intact 'seeds' may have been even greater on all pod types if *N. arizonensis* females had not been previously deprived of hosts. Low selection for intact 'seeds' on pods with egg-free slits may have occurred as a result of the greater availability of 'seeds' with egg-free slits, or because there was no indication of the presence of eggs of other females. *Neltumius arizonensis* avoided laying more than one egg per 'seed' because of the lower probability of a second egg's successful development. The high degree of discrimination for intact 'seeds' displayed when *N. arizonensis* females were restricted to a single pod did not extend to whole pods, and *N. arizonensis* showed no preference for pristine pods over those with conspecific eggs or *A. prosopis* eggs.

It seems that *N. arizonensis* oviposition behaviour is adapted to avoidance of competition among siblings and conspecifics to a greater extent than to the avoidance of larvae of other bruchid species. The selection pressure to avoid eggs of other species may be weaker than that to avoid conspecific eggs because of different temporal or spatial distributions of other species. In addition, the larvae of *A. prosopis* are highly mobile and often move among seeds, therefore, they may move from the seeds on which they are developing at the time of oviposition by *N. arizonensis*. However, there is presumably a trade-off because avoidance requires complex oviposition behaviour which is costly in terms of time and energy.

Moller *et al.* (1990) suggested that if only substrates that are covered with eggs or emergence holes are available, an insect should use assimilated resources to stay alive and should retain eggs until pristine oviposition sites are available. When bruchid females are offered only infested 'seeds', oviposition is inhibited in some species (Mitchell, 1991), but in others, the number of eggs laid does not differ from when only healthy 'seeds' are available; the females are only able to differentiate between 'seeds' that carry eggs and those that do not (Wright, 1983; Mbata, 1993). If bruchid females only encounter 'seeds' with many eggs, they may eventually accept those 'seeds' (Messina and Renwick, 1985a). Although some bruchids are reputed to recognise their own eggs and lay eggs on those 'seeds' rather than on equally loaded 'seeds' with eggs of another female (Ofuya and Agele, 1989b), this has been disputed (Messina and Tinney, 1991). Although very rare, two emergence holes were observed on a single 'seed' when there were exceptionally high densities of *N. arizonensis* adults in breeding cages.

In conclusion, *N. arizonensis* females have been shown to have meticulous oviposition behaviour which enables the females to discriminate between intact 'seeds' and 'seeds' with conspecific or other bruchid eggs, so that prime oviposition sites can be selected to minimise both intraspecific and interspecific larval competition. Having examined the oviposition behaviour of *N. arizonensis*, the effects of interactions between *N. arizonensis* and *A. prosopis* females with a limited number of oviposition sites, on the emergence of the first generation of offspring were briefly examined (refer to Appendix 4). This section of the study is relegated to an appendix due to its preliminary nature and because the experiments were hindered by the presence of mites. A thorough study of competitive interactions between *N. arizonensis* and *A. prosopis* would be essential to improve any prognoses of the eventual outcomes in the field.

The general discussion which follows, is an attempt to place the observations on *N. arizonensis* ovipositional behaviour into context and to provide a perspective on the potential role of *N. arizonensis* as a biological control agent of *Prosopis* spp. in South Africa.

CHAPTER 4.
GENERAL DISCUSSION

Other than taxonomic studies on non-economic species, research on bruchids has focussed primarily on species that are pests of crop legumes (Birch *et al.*, 1989). *Neltumius arizonensis* is one of a suite of phytophagous insects associated with *Prosopis* spp. in the countries of origin, North and South America (Ward *et al.*, 1977; Johnson 1983; Cordo and DeLoach, 1987). Prior to this study, other than a taxonomic description by Kingsolver (1964) and passing reference in theses by Forister (1970), Swier (1974), Conway (1980) and Kistler (1985), there was no information on the biology of *N. arizonensis*. Due to the scarcity of *N. arizonensis* in the U.S.A., conclusions on its biology were based on the few individuals that were available. In addition, attempts at rearing *N. arizonensis* had been unsuccessful. No study had concentrated on the biology and ecology of *N. arizonensis*, the knowledge of which is necessary for the determination of the suitability of *N. arizonensis* as a biological control agent of *Prosopis* spp. in South Africa.

Algarobius prosopis, the dominant bruchid on *Prosopis velutina* (C. Olson, personal communication) and other *Prosopis* spp. in the U.S.A., has successfully established large, widespread populations throughout the range of *Prosopis* spp. in South Africa. Despite the fact that *A. bottimeri* is potentially more fecund than *A. prosopis*, it failed to establish successfully after it was released in South Africa, possibly due to its competitive inferiority to *A. prosopis* (Hoffmann *et al.*, 1993a). Although levels of seed damage by *A. prosopis* are high (up to 90%) (Zimmermann, 1991), there is scope for achieving even greater levels of damage by introducing agents that will supplement the damage caused by *A. prosopis*.

Based on reports that *N. arizonensis* would attack young *Prosopis* pods (Forister, 1970; Swier, 1974; Kingsolver *et al.*, 1977; Johnson, 1983), it was hoped that, in introducing *N. arizonensis* into South Africa, this bruchid would attack the developing *Prosopis* pods, complementing the seed damage caused by *A. prosopis* and avoiding destruction by livestock feeding on the pods that fall to the ground. One of the dilemmas with multiple introductions of biological control agents is how species will interact, especially when they are ecologically similar.

In a natural community, one species may influence another, either directly or indirectly (Levine, 1976 in Tilman, 1987; Lawlor, 1979 in Tilman, 1987; Vandermeer, 1980 in Tilman, 1987). Competition can be defined as the negative effects which one organism has on another by consuming, or controlling access to, a resource that is limited in availability (Keddy, 1989). The response of the subordinate organism may be to tolerate the impact of the dominant organism, resulting in a low level of its presence, or the subordinate may avoid competition by spatial or temporal differentiation (Keddy, 1989).

The effects of competition are determined by the quality of the resource, its patchiness and the host-specialisation of the species involved (Keddy, 1989). If resources are of a high quality, much is gained from monopolising them and species have proportionately more nutrients to expend in defence of the nutrients (Keddy, 1989). If resources are patchy, there is more intense competition than when resources are evenly distributed, because individuals cluster around high-quality patches and encounters between competing individuals are more frequent (Keddy, 1989). Three levels of factors determine the outcome of interspecific competition: (a) characteristics of the individual e.g. fecundity, longevity and development duration, (b) characteristics of a single species, and (c) characteristics of each species when species are mixed (Park *et al.*, 1961 in Fujii, 1968).

Nicholson (1954 in Giga and Smith, 1991) defined two types of competition, scramble and contest, that occur when populations utilise the same resources. Scramble competition occurs within some bruchid populations, while contest competition occurs in others, and seed size is important in determining the type of competition that occurs (Bellows, 1982a; Giga and Smith, 1991; Messina, 1991b; Toquenaga and Fujii, 1991a, 1991b). When scramble competition occurs, there is no active aggression, and several individuals survive within a seed but produce adults that have reduced fecundity and weight, and there is increased mortality, due to over-exploitation of the resource (Smith and Lessells, 1985; Credland *et al.*, 1986; Credland and Dick, 1987; Toquenaga and Fujii, 1990; Giga and Smith, 1991). In contrast, after aggressive larval behaviour characteristic of contest competition, only one individual emerges from a seed (Toquenaga and Fujii, 1990; Giga and Smith, 1991). Smith and Lessells (1985) reported that avoidance of other larvae rather than attack, was more common among internal seed feeders. To assess whether populations undergo either contest or scramble competition, it is best to examine the number of survivors in conjunction with the initial density of competitors, direct larval interactions, as well as the patterns of resource utilisation and the mechanisms involved (Toquenaga and Fujii, 1990, 1991b).

Another well-known dichotomy in competition, proposed by Miller (1967 in Toquenaga and Fujii, 1990), is interference competition, where individuals prevent others from using a resource by aggressive behaviour or allelopathy, as opposed to exploitative competition, where each individual prevents the access of others by depleting the limited resources (Toquenaga and Fujii, 1990). Scramble and contest competition are the characteristics of populations, whereas interference and exploitative competition are the characteristics of individuals (Toquenaga and Fujii, 1990).

Competition has been extensively studied and its relative importance in the structuring of communities has been ardently debated for more than the past decade (e.g. Lawton and Strong, 1981; Roughgarden, 1983; Simberloff, 1983; Keddy, 1989; Denno *et al.*, 1995). There has been a de-emphasis on competition as an important factor in communities (Weins, 1977 in Kistler, 1985; Lawton and Strong, 1981; Simberloff and Connor, 1981 in Kistler, 1985; Strong *et al.*, 1984 in Keddy, 1989) and interspecific competition, intraspecific competition, predation, parasitism and abiotic factors are now considered to be important to community structure in different situations (Vadas, 1990).

Janzen (1975) stated that competition among bruchids may contribute to their host specificity because 90% of plants with bruchids only have one or two bruchid species associated with them, implying that interspecific competition becomes severe if numbers rise above two species. The intensity of competition is dependent on the density and distribution of individuals (Messina and Mitchell, 1989; Toquenaga and Fujii, 1991a; Woodson, 1994). Swier (1974) reported that bruchids utilised the top third of *Prosopis* trees to a greater degree than other areas of the trees, thus the probability of intraspecific and interspecific encounter is high. Bruchid emergence peaks bimodally in spring and in autumn on *Prosopis* spp. in the U.S.A., and competition is highest during spring when bruchid adults compete for the remainder of pods from the previous season (Swier, 1974). *Algarobius prosopis* and *N. arizonensis* both utilise *Prosopis velutina* (Kingsolver, 1964; Forister, 1970; Kingsolver, 1986), which is one of the most invasive *Prosopis* species in South Africa. There will be a temporal overlap in resource utilisation because *A. prosopis* and *N. arizonensis* use pods of the same age although *A. prosopis* also utilises pods that are younger than those used by *N. arizonensis* (Swier, 1974; Peter and Zimmermann, 1987; Chapter 2). Although the oviposition strategies of *A. prosopis* and *N. arizonensis* differ considerably, both are seed-feeders. Therefore, the potential for overlap in resource utilisation exists, although Kistler (1985) disputed this because mesquite seeds are abundant in the U.S.A. Due to the wide and successful distribution and extremely high population numbers of *A. prosopis* in South Africa, *N. arizonensis* may have to compete for seed resources.

Although competition between females for oviposition sites is often overlooked (Burk, 1988), this is the first level of interaction between species utilising the same resource. Fecundity is reduced in many insect species, as a result of substantial interference of egg-laying females by males, and by other females competing for oviposition sites (Crombie, 1942, 1943; Bellows, 1982a; Abrams, 1983). Females should choose to oviposit on seeds which offer their larvae the best prospects in terms of survival and fecundity (Parker and Courtney, 1984; Smith and Lessells, 1985). However, optimal oviposition behaviour may alter when other females utilise the same seeds and behaviour depends on whether females detect at the time of oviposition whether or not other eggs have already been laid (Parker and Courtney, 1984; Smith and Lessells, 1985).

One of the objectives of this study was to determine the conditions that would result in optimal oviposition by *N. arizonensis* in the laboratory, so that oviposition behaviour could be examined under those conditions. This study demonstrated that, similar to many other bruchid species, *N. arizonensis* is facultatively aphagous, but oviposition can be enhanced by the provision of water and, or, food (Chapter 2). Although a diet of pollen (from non-*Prosopis* spp.) pellets in solution produced optimal fecundity in the laboratory, it is possible that the pollen of *Prosopis* spp. may further enhance *N. arizonensis* oviposition in natural situations.

Under laboratory conditions, the duration of access to mates severely affected *N. arizonensis* oviposition and was more important than the number of mates to which a female had access (Chapter 2). It appeared as if multiple matings were necessary for the fertilisation of all eggs borne by *N. arizonensis* females. Similar results were obtained for *A. prosopis* and *A. bottimeri* by Hoffmann *et al.* (1993a). The implications are that if there is limited access to males in a natural situation, fecundity will be reduced.

Although *N. arizonensis* females preferred 'mottled-purple' pods, this may not be indicative of preferences under natural conditions where the variety of pods is much greater. Females probably selected pods mainly on the basis of their chemical characteristics, because the physical structure of the surface of pods, observed by scanning electron micrography, did not reveal features that could be related to the preferences of *N. arizonensis* (Chapter 2). The large proportion of time, and therefore energy, that *N. arizonensis* females spent investigating the surface of *Prosopis* pods (Chapter 3) indicated that the selection of oviposition sites was an important process. Palpation of the pod surface using antennae and palpi presumably provided information on the chemical and physical attributes of the pods and determined their suitability as oviposition sites.

The results of pod maturity experiments showed that, as mentioned by Forister (1970), Kingsolver *et al.* (1977) and Johnson (1983), *N. arizonensis* females oviposited on immature pods, but only once the seeds were well developed. *Neltumius arizonensis* also deposited eggs on mature pods. Although laboratory experiments are useful, field experiments are essential for the investigation of this aspect. Further investigation is vital to determine at what stage of the *Prosopis* fruiting season *N. arizonensis* utilises pods. This is crucial for the evaluation of *N. arizonensis* as a successful biological control agent in South Africa.

Oviposition in *N. arizonensis* is a time-consuming, meticulous process because 'seeds' are thoroughly inspected before a single egg is cemented to the pod exocarp. Observations of the oviposition behaviour of *N. arizonensis* females provided insight into the partitioning of time into various activities. *Neltumius arizonensis* females spent almost as much time off pods as on them, and while on the pods, there were significant differences between the time spent in various activities, indicating their relative importance. Contrary to expectations, the presence of slits or eggs of conspecifics or *A. prosopis* did little to alter the behaviour of *N. arizonensis* females, and the results up until this stage of the study had not been able to account for the time-consuming oviposition strategy.

This study indicated that it is beneficial to examine the resultant pattern as well as the process involved. By examination of the pattern of the deposition of successive eggs and the 'seeds' that were selected, it was obvious that the advantage of the oviposition strategy of *N. arizonensis* is that ovipositing females are able to discriminate between 'seeds' of different types. Host deprivation after emergence probably caused the initially lower discrimination by *N. arizonensis* females of the surface on which the first eggs were deposited, because discrimination improved after the first few eggs were laid. 'Seeds' that were unsuitable for oviposition (i.e. aborted 'seeds' and 'seeds' that were being utilised or had been utilised by conspecifics or *A. prosopis*) received only a cursory examination before being rejected. *Neltumius arizonensis* females select the 'seeds' that minimise larval competition so that development and survival of progeny is maximised and their own fitness is enhanced.

It is possible that *N. arizonensis* utilises an oviposition marker to enable rapid identification of 'seeds' that are already occupied by conspecifics. Oviposition markers are more advantageous to species whose females select the seeds on which their progeny develop, to deter subsequent oviposition by other females. Thus, it is less likely that *A. prosopis* utilises an oviposition marker because larvae migrate away from the oviposition sites.

It appeared as if *N. arizonensis* is better adapted to avoidance of conspecifics than of *A. prosopis*, because the degree of discrimination among 'seed' types was lower on pods with *A. prosopis* eggs than on pods with conspecific eggs. Due to their concealment beneath the pod surface, *A. prosopis* eggs may occasionally not have been detected by *N. arizonensis* females. However, it is encouraging, in the context of biological control, that *N. arizonensis* females frequently avoided 'seeds' bearing *A. prosopis* eggs because if *N. arizonensis* and *A. prosopis* utilise the same pods in the field, it appears that *N. arizonensis* is likely to avoid interspecific larval encounters. *Neltumius arizonensis* females may have displayed the lowest degree of 'seed' discrimination on pods with egg-free slits as a result of the absence of eggs of other bruchid females. Contrary to expectations, when provided with a choice between pristine pods, pods with conspecific eggs and pods with *A. prosopis* eggs, the selectivity for intact 'seeds' did not extend to selectivity among pods, because *N. arizonensis* females displayed no discriminatory ability in the selection of pristine pods as oviposition sites.

Neltumius arizonensis laid their eggs on 'seeds' that were not adjacent to the 'seed' on which the previous egg had been deposited. The implications of this are that fitness may be increased by broader dispersal of eggs and, if one egg is subject to predation or parasitism, other eggs may escape mortality. In addition, if an egg is laid in an area where, unknown to the *N. arizonensis* female, numerous *A. prosopis* eggs have previously been deposited, the probability of interspecific competition will be reduced if subsequent eggs are further dispersed.

Algarobius prosopis larvae frequently have to move a long way through the pod before locating a healthy seed in which they can develop, but they only need to penetrate the seed coat and not the tough exocarp, at an age when the larvae are most vulnerable due to their small size. *Neltumius arizonensis* larvae do not need to search for healthy seeds but they have to penetrate the pod wall, the mesocarp and the seed coat of the 'seed' on which the egg was laid. The results of interspecific larval competition experiments demonstrated that *N. arizonensis* was outcompeted by *A. prosopis* when first instar larvae of the two species were placed on the same seed (F. Impson, personal communication). *A. prosopis* larvae were aggressive and killed *N. arizonensis* larvae and *N. arizonensis* was deemed to be competitively equivalent to *A. bottimeri* (F. Impson, personal communication). However, it is hoped that the avoidance of 'seeds' with *A. prosopis* eggs by ovipositing *N. arizonensis* females will help to reduce the likelihood of this situation in the field.

On average, a *N. arizonensis* female laid 80 eggs during 35 days (Chapter 2), whereas an *A. prosopis* female lays about 200 eggs during a similar period (Hoffmann *et al.*, 1993a). Thus, in contrast to the findings of Kistler (1985), *A. prosopis* is more fecund than *N. arizonensis*. Higher fecundity confers a competitive advantage on *A. prosopis* because more seeds will be utilised, leaving fewer available for *N. arizonensis*. Uniform egg dispersal may be costly by investing foraging time and energy in marking hosts, rejecting marked hosts and spending time searching for 'seeds' with below-average egg-loads (Messina, 1989; Messina and Mitchell, 1989), thus *N. arizonensis* females may have less time or fewer resources to lay as many eggs as *A. prosopis*. Species that use oviposition markers usually lay fewer eggs than nonmarkers (Roitberg and Prokopy, 1987).

The timing and sequence of oviposition are often important determinants of the outcome of competition between two species, therefore, developmental duration directly influences competitive ability (Toquenaga and Fujii, 1990; Wai and Fujii, 1990). If two species have similar patterns of resource utilisation, and if one has a more rapid development rate, that species is able to consume more of the resources and a competitive advantage is gained each generation over the other species (Crombie, 1944; Fisher, 1961 in Keddy, 1989; Fujii, 1965, 1968; Bellows and Hassell, 1984; Abrams, 1990; Toquenaga and Fujii, 1991a). Species that are scramble competitors can evade species that are contest competitors by developing more rapidly so that critical periods of interference in which contest competitors outcompete scramble competitors, are avoided (Toquenaga and Fujii, 1990, 1991a). Large populations of *A. prosopis* are already widely dispersed throughout South Africa, therefore *A. prosopis* already has a numerical advantage over *N. arizonensis*. This will increase each generation as a result of the more rapid development of *A. prosopis* (Appendix 4).

Laboratory experiments can indicate what might occur under natural conditions, and whether postulated relationships could occur under specific sets of conditions (Keddy, 1989). Although there were some interesting preliminary results from the interspecific interactions experiments, which may reflect the field situation, firm conclusions could not be drawn because of predation by mites. However, it appeared that in many situations, under conditions that were optimal for oviposition by *N. arizonensis*, as many, but never more, *N. arizonensis* than *A. prosopis* progeny per parent female emerged as adults. Results may differ under natural conditions and further investigations into the interactions between *N. arizonensis* and *A. prosopis* should involve multiple-generation experiments in the field. Investigations into the survival rates of eggs and larvae would provide information on whether the the number of eggs that are laid are related to the emergence of adult offspring in competitive situations.

Parasitoids may reduce the effectiveness of biological control agents by reducing the population levels (Hoffmann *et al.*, 1993b). It was expected that native egg and larval parasitoids would attack *Algarobius* spp. and *N. arizonensis*, because several parasitoids in South Africa are known to attack a diversity of bruchid species (Rasplus, 1989 in Zimmermann, 1991). In addition, there are numerous bruchid parasitoids in the areas of release of the biological control agents of *Prosopis* spp. (Hoffmann *et al.*, 1993b). Zimmermann (1991) postulated that a shift of indigenous egg parasitoids from native species to introduced bruchids feeding on *Prosopis* spp. was more detrimental than a shift of indigenous larval parasitoids because seeds would be damaged to a lesser degree. Although mites were problematic in laboratory breeding populations of *A. prosopis* and *N. arizonensis*, their impact may have been greater because of the close confinement of bruchids in the laboratory, than it will be on the field populations. *Neltumius arizonensis* adults appeared to be more susceptible than *A. prosopis* to attack by mites, perhaps because of the frequently observed aggregation of *N. arizonensis* adults in inactive groups. Hoffmann *et al.* (1993b) stated that in 1990, parasitoids of bruchids were scarce on mesquite and levels of parasitism of *A. prosopis* were low. The parasite load on *N. arizonensis* populations should be carefully monitored in the field.

Algarobius prosopis does not waste time or energy in producing and depositing cement with eggs. The cracks and crevices on pods of any stage are utilised, whereas other bruchids of the *Prosopis* guild require pods of certain ages (Swier, 1974). Although the oviposition behaviour of *A. prosopis* is considered to be more primitive than that of other bruchid species associated with *Prosopis* spp. (Swier, 1974), it may result in reduced egg mortality (Kistler, 1985). Eggs that are deposited in cracks and crevices are less vulnerable and less susceptible to climatic effects (Parnell, 1966), and to attack by predators and parasites (e.g. ants, mites and trichogrammatid wasps) (Bridwell, 1920b in Swier, 1974; Hinckley, 1961 in Swier, 1974). If parasites locate eggs, possibly only the uppermost eggs will be attacked and the remainder will be protected. In contrast, *N. arizonensis* eggs are conspicuous and very exposed because they are cemented to the surface of pods, thus, as in other bruchids with similar oviposition strategies (Hinckley, 1960, 1961 in Swier, 1974; Parnell, 1966; Southgate, 1979; Giga and Smith, 1983; Traveset, 1990; Siemens and Johnson, 1992), the probability of temperature and desiccation-related mortality and attack by predators and parasites is high.

Kistler (1985) reported that *A. prosopis* is better adapted in terms of longevity, reproduction, development rate, and larval metabolic rate, than *N. arizonensis* to extreme temperatures that coincide with peak pod production, and to a range of temperatures from 20-35 °C. *Neltumius arizonensis* is more limited by abiotic factors (Kistler, 1985). *Neltumius arizonensis* avoids temperature extremes, but there are fewer resources at the end of the pod season, and lower temperatures result in a lower population growth rate and parasitoid populations have accumulated by then. As this did not appear to be an optimal strategy, Kistler (1985) argued against a competitive explanation for the structure of the mesquite bruchid guild.

Other than problems caused by mite predation, very large numbers of *N. arizonensis* were easily reared within the laboratory. Between August 1993 and

January 1994, mass releases of *N. arizonensis* adults were made near Piketberg (32° 50'S; 18° 49'E), Clanwilliam (32° 9'S; 18° 53'E) and exceptionally large releases were made on Onderplaas farm in Bidou Valley (31° 59'S; 19° 15'E) in the north-western Cape. A smaller release of *N. arizonensis* adults was made in March 1994 near Molopo (20° 30'E; 27° 3'E) in the northern Cape, South Africa. As with all biological control programmes, evaluation studies of the post-release success of a biological control agent are essential. It is recommended that further releases of *N. arizonensis* should be made early in the *Prosopis* fruiting season, when immature pods have well-developed seeds. Thus, harsh temperatures and high levels of parasitism may be avoided. In addition, *N. arizonensis* should be released in areas where *A. prosopis* populations are known to be relatively small. To prevent grazing of pods by livestock and improve the demise of *Prosopis* spp., farmers should be encouraged to fence off areas in which populations of the biological control agents are known to be high.

The information obtained from this study of the biology and oviposition behaviour of *N. arizonensis* indicates that this species is highly specialised. Despite the discriminatory behaviour of *N. arizonensis* females in selecting intact 'seeds' for oviposition, *A. prosopis* may be competitively superior due to its generalist-type strategy. The shorter development and greater fecundity of *A. prosopis* will result in the utilisation of many resources before *N. arizonensis* has emerged. *Neltumius arizonensis* may initially establish small populations that could decline as *A. prosopis* increases its numerical advantage with time. The main dilemma associated with the control of *Prosopis* spp. in South Africa is that farmers wish to reduce the invasiveness of the plant but retain its beneficial attributes i.e. shade and a nutritional source of fodder for livestock. If *N. arizonensis* fails to enhance the current levels of seed damage caused by *A. prosopis*, the situation will require re-evaluation. It may be necessary to introduce host-specific insects that feed on plant structures other than the seeds to effectively control the rate of spread and the invasiveness of *Prosopis* spp. in South Africa.

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APPENDIX 1

The programme used to quantify and record the behaviour of *Neltumius arizonensis* females on pristine *Prosopis* pods:

```
program Behaviour;

Uses Crt, Dos;

Var
   Key              : CHAR;
   OutFile          : TEXT;

   Starthour,
   Startminute,
   Startsecond,
   Startsec100,
   Stophour,
   Stopminute,
   Stopsecond,
   Stopsec100,

   WalkInChamSecond,
   TotalWalkInChamSecond,
   WalkInChamMinute,
   TotalWalkInChamMinute,

   WalkOnPodSecond,
   TotalWalkOnPodSecond,
   WalkOnPodMinute,
   TotalWalkOnPodMinute,

   StatSecond,
   TotalStatSecond,
   StatMinute,
   TotalStatMinute,

   GroomSecond,
   TotalGroomSecond,
   GroomMinute,
   TotalGroomMinute,

   InspectSecond,
   TotalInspectSecond,
   InspectMinute,
   TotalInspectMinute,

   ScrapeSecond,
   TotalScrapeSecond,
   ScrapeMinute,
   TotalScrapeMinute,

   ScrChamSecond,
   TotalScrChamSecond,
   ScrChamMinute,
   TotalScrChamMinute,
```

```
   EggLaySecond,
   TotalEggLaySecond,
   EggLayMinute,
   TotalEggLayMinute,

   OwnEggSecond,
   TotalOwnEggSecond,
   OwnEggMinute,
   TotalOwnEggMinute: WORD;

PROCEDURE Initialise;

BEGIN
   WalkInChamSecond := 0;
   TotalWalkInChamSecond := 0;
   WalkInChamMinute := 0;
   TotalWalkInChamMinute := 0;

   WalkOnPodSecond := 0;
   TotalWalkOnPodSecond := 0;
   WalkOnPodMinute := 0;
   TotalWalkOnPodMinute := 0;

   StatSecond := 0;
   TotalStatSecond := 0;
   StatMinute := 0;
   TotalStatMinute := 0;

   GroomSecond := 0;
   TotalGroomSecond := 0;
   GroomMinute := 0;
   TotalGroomMinute := 0;

   InspectSecond := 0;
   TotalInspectSecond := 0;
   InspectMinute := 0;
   TotalInspectMinute := 0;

   ScrapeSecond := 0;
   TotalScrapeSecond := 0;
   ScrapeMinute := 0;
   TotalScrapeMinute := 0;

   ScrChamSecond := 0;
   TotalScrChamSecond := 0;
   ScrChamMinute := 0;
   TotalScrChamMinute := 0;

   EggLaySecond := 0;
   TotalEggLaySecond := 0;
   EggLayMinute := 0;
   TotalEggLayMinute := 0;

   OwnEggSecond := 0;
   TotalOwnEggSecond := 0;
   OwnEggMinute := 0;
   TotalOwnEggMinute := 0;

   ASSIGN( Outfile, 'A:\DATA.OUT');
   REWRITE( Outfile );
```

```
END; { of PROCEDURE Initialise }


PROCEDURE ElapsedTime; FORWARD;


Procedure Intro;

Begin
     CLRSCR;
     WRITELN('Record of bruchid behaviour:');
     WRITELN('----------------------------');
     WRITELN;
     WRITELN('Use the following keys for timing
     behaviour:');
     WRITELN(' (1) - Walking in chamber ');
     WRITELN(' (2) - Walking on pod ');
     WRITELN(' (3) - Stationary ');
     WRITELN(' (4) - Grooming ');
     WRITELN(' (5) - Inspecting pod ');
     WRITELN(' (S) - Scraping ovipositor on pod ');
     WRITELN(' (H) - Scraping ovipositor on chamber ');
     WRITELN(' (E) - Laying an egg ');
     WRITELN(' (I) - Investigating own egg ');
     WRITELN(' (Q) - QUIT     ');
     WRITELN;
     WRITELN('Use the key as a toggle - Press once for ON');
     WRITELN('                        - Press again for
     OFF');
     WRITELN;
     WRITELN('N.B. Make sure Caps Lock is ON !!');
     WRITELN;
     WRITELN('Press a key.........:');
     WRITELN;
     REPEAT
       Key := UPCASE(READKEY);
       IF (Key IN ['1', '2', '3', '4', '5', 'S', 'H', 'E',
       'I',])
         THEN
            BEGIN
               GETTIME(StartHour, StartMinute, StartSecond,
                Stopsec100);
               IF Key In ['1'] then WRITELN('Walking in
               chamber (1):');
               IF Key In ['2'] then WRITELN('Walking on pod
               (2):');
               IF Key In ['3'] then WRITELN('Stationary
               (3):');
               IF Key In ['4'] then WRITELN('Grooming
               (4):');
               IF Key In ['5'] then WRITELN('Inspecting pod
               (5):');
               IF Key In ['S'] then WRITELN('Scraping
               ovipositor on pod (S):');
               IF Key In ['H'] then WRITELN('Scraping
               ovipositor on chamber (H):');
               IF Key In ['E'] then WRITELN('Laying an egg
               (E):');
               IF Key In ['I'] then WRITELN('Inspecting own
               egg (I):');
```

```
                Key := READKEY;
                GETTIME(StopHour, StopMinute, StopSecond,
                Stopsec100);
                ElapsedTime;
             END;
    UNTIL Key = 'Q';
End;


Procedure ElapsedTime;

   Procedure TimeDif(VAR Sec, Min:WORD);
   {This procedure calculates the time difference}
   {between Start and Stop and returns the answer as}
   {elapsed seconds to the calling line.}
   begin
   If StopSecond >= StartSecond then Sec := StopSecond -
   StartSecond
      Else Sec := 60-(StartSecond-StopSecond);
   If StopMinute >= StartMinute then Min := StopMinute -
   StartMinute
      Else Min := 60-(StartMinute-StopMinute);
   If StopSecond < StartSecond then Min := Min-1;
   end;  {Of TimeDif Procedure}

Begin    {of Procedure ElapsedTime}

 CASE Key OF

   '1' : BEGIN
           TimeDif(WalkInChamSecond, WalkInChamMinute);
{On Screen}WRITELN('Seconds ', WalkInChamSecond);
           TotalWalkInChamSecond := TotalWalkInChamSecond +
           WalkInChamSecond;
           If (TotalWalkInChamSecond > 60) then
             begin
               TotalWalkInChamSecond:=
               TotalWalkInChamSecond - 60;
               TotalWalkInChamMinute :=
                TotalWalkInChamMinute + 1
             end;
           WRITELN('Minutes ', WalkInChamMinute);
           TotalWalkInChamMinute := TotalWalkInChamMinute +
           WalkInChamMinute;
           WRITELN(Outfile, '1, ', WalkInChamMinute, ', ',
           WalkInChamSecond, ', ',
           TotalWalkInChamMinute, ', ',
           TotalWalkInChamSecond);
           WRITELN('TotalMinutes = ',
           TotalWalkInChamMinute);
           WRITELN('TotalSeconds = ',
           TotalWalkInChamSecond);
           WRITELN('--------------------------------------')
         END;

    '2': BEGIN
           TimeDif(WalkOnPodSecond, WalkOnPodMinute);
           WRITELN('Seconds ', WalkOnPodSecond);
           TotalWalkOnPodSecond := TotalWalkOnPodSecond +
           WalkOnPodSecond;
```

```
        If (TotalWalkOnPodSecond > 60) then
          begin
            TotalWalkOnPodSecond := TotalWalkOnPodSecond
            - 60;
            TotalWalkOnPodMinute := TotalWalkOnPodMinute
            + 1
          end;
        WRITELN('Minutes ', WalkOnPodMinute);
        TotalWalkOnPodMinute := TotalWalkOnPodMinute +
        WalkOnPodMinute;
        WRITELN(Outfile, '2, ', WalkOnPodMinute, ', ',
        WalkOnPodSecond, ', ',
        TotalWalkOnPodMinute, ', ',
        TotalWalkOnPodSecond);
        WRITELN('TotalMinutes = ',
        TotalWalkOnPodMinute);
        WRITELN('TotalSeconds = ',
        TotalWalkOnPodSecond);
        WRITELN('-------------------------------------');
      END;

'3' : BEGIN
        TimeDif(StatSecond, StatMinute);
        WRITELN('Seconds ', StatSecond);
        TotalStatSecond := TotalStatSecond + StatSecond;
        If (TotalStatSecond > 60) then
          begin
            TotalStatSecond := TotalStatSecond - 60;
            TotalStatMinute := TotalStatMinute + 1
          end;
        WRITELN('Minutes ', StatMinute);
        TotalStatMinute := TotalStatMinute + StatMinute;
        WRITELN(Outfile, '3, ', StatMinute, ', ',
        StatSecond, ', ',
        TotalStatMinute, ', ', TotalStatSecond);
        WRITELN('TotalMinutes = ', TotalStatMinute);
        WRITELN('TotalSeconds = ', TotalStatSecond);
        WRITELN('-------------------------------------')
      END;

'4' : BEGIN
        TimeDif(GroomSecond, GroomMinute);
        WRITELN('Seconds ', GroomSecond);
        TotalGroomSecond := TotalGroomSecond +
        GroomSecond;
        If (TotalGroomSecond > 60) then
          begin
            TotalGroomSecond := TotalGroomSecond - 60;
            TotalGroomMinute := TotalGroomMinute + 1
          end;
        WRITELN('Minutes ', Groomminute);
        TotalGroomMinute := TotalGroomMinute +
        GroomMinute;
        WRITELN(Outfile, '4, ', GroomMinute, ', ',
        GroomSecond, ', ',
        TotalGroomMinute, ', ', TotalGroomSecond);
        WRITELN('TotalMinutes = ', TotalGroomMinute);
        WRITELN('TotalSeconds = ', TotalGroomSecond);
        WRITELN('-------------------------------------');
      END;
```

```
'5':  BEGIN
        TimeDif(InspectSecond, InspectMinute);
        WRITELN('Seconds ', InspectSecond);
        TotalInspectSecond := TotalInspectSecond +
       InspectSecond;
       If (TotalInspectSecond > 60) then
          begin
            TotalInspectSecond := TotalInspectSecond -
            60;
            TotalInspectMinute := TotalInspectMinute + 1
          end;
        WRITELN('Minutes', InspectMinute);
        TotalInspectMinute := TotalInspectMinute +
        InspectMinute;
        WRITELN(Outfile, '5, ', InspectMinute, ', ',
        InspectSecond, ', ',
        TotalInspectMinute, ', ', TotalInspectSecond);
        WRITELN('TotalMinutes = ', TotalInspectMinute);
        WRITELN('TotalSeconds = ', TotalInspectSecond);
        WRITELN('------------------------------------');
      END;

'S':  BEGIN
        TimeDif(ScrapeSecond, ScrapeMinute);
        WRITELN('Seconds ', ScrapeSecond);
        TotalScrapeSecond := TotalScrapeSecond +
        ScrapeSecond;
        If (TotalScrapeSecond > 60) then
          begin
            TotalScrapeSecond := TotalScrapeSecond - 60;
            TotalScrapeMinute := TotalScrapeMinute + 1
          end;
        WRITELN('Minutes ', ScrapeMinute);
        TotalScrapeMinute := TotalScrapeMinute +
        ScrapeMinute;
        WRITELN(Outfile, 'S, ', ScrapeMinute, ', ',
        ScrapeSecond, ', ',
        TotalScrapeMinute, ', ', TotalScrapeSecond);
        WRITELN('TotalMinutes = ', TotalScrapeMinute);
        WRITELN('TotalSeconds = ', TotalScrapeSecond);
        WRITELN('--------------------------------------');
      END;

'H':  BEGIN
        TimeDif(ScrChamSecond, ScrChamMinute);
        WRITELN('Seconds ', ScrChamSecond);
        TotalScrChamSecond := TotalScrChamSecond +
        ScrChamSecond;
        If (TotalScrChamSecond > 60) then
          begin
            TotalScrChamSecond := TotalScrChamSecond -
            60;
            TotalScrChamMinute := TotalScrChamMinute + 1
          end;
        WRITELN('Minutes ', ScrChamMinute);
        TotalScrChamMinute := TotalScrChamMinute +
        ScrChamMinute;
        WRITELN(Outfile, 'H, ', ScrChamMinute, ', ',
        ScrChamSecond, ', ',
        TotalScrChamMinute, ', ', TotalScrChamSecond);
```

```
           WRITELN('TotalMinutes = ', TotalScrChamMinute);
           WRITELN('TotalSeconds = ', TotalScrChamSecond);
           WRITELN('-------------------------------------');
         END;

   'E':  BEGIN
           TimeDif(EggLaySecond, EggLayMinute);
           WRITELN('Seconds ', EggLaySecond);
           TotalEggLaySecond := TotalEggLaySecond +
           EggLaySecond;
           If (TotalEggLaySecond > 60) then
             begin
               TotalEggLaySecond := TotalEggLaySecond - 60;
               TotalEggLayMinute := TotalEggLayMinute + 1
             end;
           WRITELN('Minutes ', EggLayMinute);
           TotalEggLayMinute := TotalEggLayMinute +
           EggLayMinute;
           WRITELN(Outfile, 'E, ', EggLayMinute, ', ',
           EggLaySecond, ', ',
           TotalEggLayMinute, ', ', TotalEggLaySecond);
           WRITELN('TotalMinutes = ', TotalEggLayMinute);
           WRITELN('TotalSeconds = ', TotalEggLaySecond);
           WRITELN('-------------------------------------')
         END;

   'I':  BEGIN
           TimeDif(OwnEggSecond, OwnEggMinute);
           WRITELN('Seconds ', OwnEggSecond);
           TotalOwnEggSecond := TotalOwnEggSecond +
           OwnEggSecond;
           If (TotalOwnEggSecond > 60) then
             begin
               TotalOwnEggSecond := TotalOwnEggSecond - 60;
               TotalOwnEggMinute := TotalOwnEggMinute + 1
             end;
           WRITELN('Minutes ', OwnEggMinute);
           TotalOwnEggMinute := TotalOwnEggMinute +
           OwnEggMinute;
           WRITELN(Outfile, 'I, ', OwnEggMinute, ', ',
           OwnEggSecond, ', ',
           TotalOwnEggMinute, ', ', TotalOwnEggSecond);
           WRITELN('TotalMinutes = ', TotalOwnEggMinute);
           WRITELN('TotalSeconds = ', TotalOwnEggSecond);
           WRITELN('------------------------------------');
         END;
    END; { of CASE }

END; { of PROCEDURE ElapsedTime }

BEGIN { actual program }

   Initialise;
   Intro;
   CLOSE(OutFile);

END. { of PROGRAM }
```

APPENDIX 2

The programme used to quantify and record the behaviour of *Neltumius arizonensis* females on *Prosopis* pods with conspecific eggs:

```
program Behaviour;

Uses Crt, Dos;

Var
   Key             : CHAR;
   OutFile         : TEXT;

   Starthour,
   Startminute,
   Startsecond,
   Startsec100,
   Stophour,
   Stopminute,
   Stopsecond,
   Stopsec100,

   WalkInChamSecond,
   TotalWalkInChamSecond,
   WalkInChamMinute,
   TotalWalkInChamMinute,

   WalkOnPodSecond,
   TotalWalkOnPodSecond,
   WalkOnPodMinute,
   TotalWalkOnPodMinute,

   StatSecond,
   TotalStatSecond,
   StatMinute,
   TotalStatMinute,

   GroomSecond,
   TotalGroomSecond,
   GroomMinute,
   TotalGroomMinute,

   InspectSecond,
   TotalInspectSecond,
   InspectMinute,
   TotalInspectMinute,

   OtherEggSecond,
   TotalOtherEggSecond,
   OtherEggMinute,
   TotalOtherEggMinute,

   ScrapeSecond,
   TotalScrapeSecond,
   ScrapeMinute,
   TotalScrapeMinute,
```

```
   ScrChamSecond,
   TotalScrChamSecond,
   ScrChamMinute,
   TotalScrChamMinute,

   EggLaySecond,
   TotalEggLaySecond,
   EggLayMinute,
   TotalEggLayMinute,

   OwnEggSecond,
   TotalOwnEggSecond,
   OwnEggMinute,
   TotalOwnEggMinute: WORD;

PROCEDURE Initialise;

BEGIN
   WalkInChamSecond := 0;
   TotalWalkInChamSecond := 0;
   WalkInChamMinute := 0;
   TotalWalkInChamMinute := 0;

   WalkOnPodSecond := 0;
   TotalWalkOnPodSecond := 0;
   WalkOnPodMinute := 0;
   TotalWalkOnPodMinute := 0;

   StatSecond := 0;
   TotalStatSecond := 0;
   StatMinute := 0;
   TotalStatMinute := 0;

   GroomSecond := 0;
   TotalGroomSecond := 0;
   GroomMinute := 0;
   TotalGroomMinute := 0;

   InspectSecond := 0;
   TotalInspectSecond := 0;
   InspectMinute := 0;
   TotalInspectMinute := 0;

   OtherEggSecond := 0;
   TotalOtherEggSecond := 0;
   OtherEggMinute := 0;
   TotalOtherEggMinute := 0;

   ScrapeSecond := 0;
   TotalScrapeSecond := 0;
   ScrapeMinute := 0;
   TotalScrapeMinute := 0;

   ScrChamSecond := 0;
   TotalScrChamSecond := 0;
   ScrChamMinute := 0;
   TotalScrChamMinute := 0;
```

```
    EggLaySecond := 0;
    TotalEggLaySecond := 0;
    EggLayMinute := 0;
    TotalEggLayMinute := 0;

    OwnEggSecond := 0;
    TotalOwnEggSecond := 0;
    OwnEggMinute := 0;
    TotalOwnEggMinute := 0;

    ASSIGN( Outfile, 'A:\DATA.OUT');
    REWRITE( Outfile );

END; { of PROCEDURE Initialise }


PROCEDURE ElapsedTime; FORWARD;


Procedure Intro;

Begin
     CLRSCR;
     WRITELN('Record of bruchid behaviour:');
     WRITELN('----------------------------');
     WRITELN;
     WRITELN('Use the following keys for timing
     behaviour:');
     WRITELN(' (1) - Walking in chamber ');
     WRITELN(' (2) - Walking on pod ');
     WRITELN(' (3) - Stationary ');
     WRITELN(' (4) - Grooming ');
     WRITELN(' (5) - Inspecting pod ');
     WRITELN(' (O) - Investigating egg of another female ');
     WRITELN(' (S) - Scraping ovipositor on pod ');
     WRITELN(' (H) - Scraping ovipositor on chamber ');
     WRITELN(' (E) - Laying an egg ');
     WRITELN(' (I) - Investigating own egg ');
     WRITELN(' (Q) - QUIT     ');
     WRITELN;
     WRITELN('Use the key as a toggle - Press once for ON');
     WRITELN('                        - Press again for
     OFF');
     WRITELN;
     WRITELN('N.B. Make sure Caps Lock is ON !!');
     WRITELN;
     WRITELN('Press a key.........:');
     WRITELN;
     REPEAT
       Key := UPCASE(READKEY);
       IF (Key IN ['1', '2', '3', '4', '5', 'O', 'S', 'H',
       'E', 'I',])
         THEN
            BEGIN
               GETTIME(StartHour, StartMinute, StartSecond,
                Stopsec100);
               IF Key In ['1'] then WRITELN('Walking in
               chamber (1):');
               IF Key In ['2'] then WRITELN('Walking on pod
               (2):');
```

```
                IF Key In ['3'] then WRITELN('Stationary
                 (3):');
                IF Key In ['4'] then WRITELN('Grooming
                (4):');
                IF Key In ['5'] then WRITELN('Inspecting pod
                (5):');
                IF Key In ['O'] then WRITELN('Investigating
                egg of another female (O):');
                IF Key In ['S'] then WRITELN('Scraping
                ovipositor on pod (S):');
                IF Key In ['H'] then WRITELN('Scraping
                ovipositor on chamber (H):');
                IF Key In ['E'] then WRITELN('Laying an egg
                (E):');
                IF Key In ['I'] then WRITELN('Inspecting own
                 egg (I):');
                Key := READKEY;
                GETTIME(StopHour, StopMinute, StopSecond,
                Stopsec100);
                ElapsedTime;
             END;
    UNTIL Key = 'Q';
End;


Procedure ElapsedTime;

   Procedure TimeDif(VAR Sec, Min:WORD);
   {This procedure calculates the time difference}
   {between Start and Stop and returns the answer as}
   {elapsed seconds to the calling line.}
   begin
   If StopSecond >= StartSecond then Sec := StopSecond -
   StartSecond
      Else Sec := 60-(StartSecond-StopSecond);
   If StopMinute >= StartMinute then Min := StopMinute -
   StartMinute
      Else Min := 60-(StartMinute-StopMinute);
   If StopSecond < StartSecond then Min := Min-1;
   end;  {Of TimeDif Procedure}

Begin    {of Procedure ElapsedTime}

 CASE Key OF

   '1' : BEGIN
           TimeDif(WalkInChamSecond, WalkInChamMinute);
           WRITELN('Seconds ', WalkInChamSecond);
           TotalWalkInChamSecond := TotalWalkInChamSecond +
           WalkInChamSecond;
           If (TotalWalkInChamSecond > 60) then
             begin
               TotalWalkInChamSecond:=
               TotalWalkInChamSecond - 60;
               TotalWalkInChamMinute :=
                TotalWalkInChamMinute + 1
             end;
           WRITELN('Minutes ', WalkInChamMinute);
           TotalWalkInChamMinute := TotalWalkInChamMinute +
           WalkInChamMinute;
```

```
       WRITELN(Outfile, '1, ', WalkInChamMinute, ', ',
       WalkInChamSecond, ', ',
       TotalWalkInChamMinute, ', ',
       TotalWalkInChamSecond);
       WRITELN('TotalMinutes = ',
       TotalWalkInChamMinute);
       WRITELN('TotalSeconds = ',
       TotalWalkInChamSecond);
       WRITELN('-------------------------------------')
     END;

'2': BEGIN
       TimeDif(WalkOnPodSecond, WalkOnPodMinute);
       WRITELN('Seconds ', WalkOnPodSecond);
       TotalWalkOnPodSecond := TotalWalkOnPodSecond +
       WalkOnPodSecond;
       If (TotalWalkOnPodSecond > 60) then
         begin
           TotalWalkOnPodSecond := TotalWalkOnPodSecond
           - 60;
           TotalWalkOnPodMinute := TotalWalkOnPodMinute
           + 1
         end;
       WRITELN('Minutes ', WalkOnPodMinute);
       TotalWalkOnPodMinute := TotalWalkOnPodMinute +
       WalkOnPodMinute;
       WRITELN(Outfile, '2, ', WalkOnPodMinute, ', ',
       WalkOnPodSecond, ', ',
       TotalWalkOnPodMinute, ', ',
       TotalWalkOnPodSecond);
       WRITELN('TotalMinutes = ',
       TotalWalkOnPodMinute);
       WRITELN('TotalSeconds = ',
       TotalWalkOnPodSecond);
       WRITELN('-------------------------------------');
     END;

'3': BEGIN
       TimeDif(StatSecond, StatMinute);
       WRITELN('Seconds ', StatSecond);
       TotalStatSecond := TotalStatSecond + StatSecond;
       If (TotalStatSecond > 60) then
         begin
           TotalStatSecond := TotalStatSecond - 60;
           TotalStatMinute := TotalStatMinute + 1
         end;
       WRITELN('Minutes ', StatMinute);
       TotalStatMinute := TotalStatMinute + StatMinute;
       WRITELN(Outfile, '3, ', StatMinute, ', ',
       StatSecond, ', ',
       TotalStatMinute, ', ', TotalStatSecond);
       WRITELN('TotalMinutes = ', TotalStatMinute);
       WRITELN('TotalSeconds = ', TotalStatSecond);
       WRITELN('-------------------------------------')
     END;

'4': BEGIN
       TimeDif(GroomSecond, GroomMinute);
       WRITELN('Seconds ', GroomSecond);
```

```
          TotalGroomSecond := TotalGroomSecond +
          GroomSecond;
          If (TotalGroomSecond > 60) then
            begin
              TotalGroomSecond := TotalGroomSecond - 60;
              TotalGroomMinute := TotalGroomMinute + 1
            end;
          WRITELN('Minutes ', Groomminute);
          TotalGroomMinute := TotalGroomMinute +
          GroomMinute;
          WRITELN(Outfile, '4, ', GroomMinute, ', ',
          GroomSecond, ', ',
          TotalGroomMinute, ', ', TotalGroomSecond);
          WRITELN('TotalMinutes = ', TotalGroomMinute);
          WRITELN('TotalSeconds = ', TotalGroomSecond);
          WRITELN('--------------------------------------');
        END;

  '5' : BEGIN
          TimeDif(InspectSecond, InspectMinute);
          WRITELN('Seconds ', InspectSecond);
          TotalInspectSecond := TotalInspectSecond +
          InspectSecond;
          If (TotalInspectSecond > 60) then
            begin
              TotalInspectSecond := TotalInspectSecond -
              60;
              TotalInspectMinute := TotalInspectMinute + 1
            end;
          WRITELN('Minutes', InspectMinute);
          TotalInspectMinute := TotalInspectMinute +
          InspectMinute;
          WRITELN(Outfile, '5, ', InspectMinute, ', ',
          InspectSecond, ', ',
          TotalInspectMinute, ', ', TotalInspectSecond);
          WRITELN('TotalMinutes = ', TotalInspectMinute);
          WRITELN('TotalSeconds = ', TotalInspectSecond);
          WRITELN('--------------------------------------');
        END;


   'O': BEGIN
          TimeDif(OtherEggSecond, OtherEggMinute);
          WRITELN('Seconds ', OtherEggSecond);
          TotalOtherEggSecond := TotalOtherEggSecond +
          OtherEggSecond;
          If (TotalOtherEggSecond > 60) then
            begin
            TotalOtherEggSecond := TotalOtherEggSecond -
            60;
            TotalOtherEggMinute := TotalOtherEggMinute + 1
          end;
          WRITELN('Minutes ', OtherEggMinute);
          TotalOtherEggMinute := TotalOtherEggMinute +
          OtherEggMinute;
          WRITELN(Outfile, 'O, ', OtherEggMinute, ', ',
          OtherEggSecond, ', ',
          TotalOtherEggMinute, ', ', TotalOtherEggSecond);
          WRITELN('TotalMinutes = ', TotalOtherEggMinute);
          WRITELN('TotalSeconds = ', TotalOtherEggSecond);
```

```
         WRITELN('-------------------------------------');
       END;

  'S': BEGIN
         TimeDif(ScrapeSecond, ScrapeMinute);
         WRITELN('Seconds ', ScrapeSecond);
         TotalScrapeSecond := TotalScrapeSecond +
         ScrapeSecond;
         If (TotalScrapeSecond > 60) then
           begin
             TotalScrapeSecond := TotalScrapeSecond - 60;
             TotalScrapeMinute := TotalScrapeMinute + 1
           end;
         WRITELN('Minutes ', ScrapeMinute);
         TotalScrapeMinute := TotalScrapeMinute +
         ScrapeMinute;
         WRITELN(Outfile, 'S, ', ScrapeMinute, ', ',
         ScrapeSecond, ', ',
         TotalScrapeMinute, ', ', TotalScrapeSecond);
         WRITELN('TotalMinutes = ', TotalScrapeMinute);
         WRITELN('TotalSeconds = ', TotalScrapeSecond);
         WRITELN('-------------------------------------');
       END;

  'H': BEGIN
         TimeDif(ScrChamSecond, ScrChamMinute);
         WRITELN('Seconds ', ScrChamSecond);
         TotalScrChamSecond := TotalScrChamSecond +
         ScrChamSecond;
         If (TotalScrChamSecond > 60) then
           begin
             TotalScrChamSecond := TotalScrChamSecond -
             60;
             TotalScrChamMinute := TotalScrChamMinute + 1
           end;
         WRITELN('Minutes ', ScrChamMinute);
         TotalScrChamMinute := TotalScrChamMinute +
         ScrChamMinute;
         WRITELN(Outfile, 'H, ', ScrChamMinute, ', ',
         ScrChamSecond, ', ',
         TotalScrChamMinute, ', ', TotalScrChamSecond);
         WRITELN('TotalMinutes = ', TotalScrChamMinute);
         WRITELN('TotalSeconds = ', TotalScrChamSecond);
        'WRITELN('-------------------------------------');
       END;

  'E': BEGIN
         TimeDif(EggLaySecond, EggLayMinute);
         WRITELN('Seconds ', EggLaySecond);
         TotalEggLaySecond := TotalEggLaySecond +
         EggLaySecond;
         If (TotalEggLaySecond > 60) then
           begin
             TotalEggLaySecond := TotalEggLaySecond - 60;
             TotalEggLayMinute := TotalEggLayMinute + 1
           end;
         WRITELN('Minutes ', EggLayMinute);
         TotalEggLayMinute := TotalEggLayMinute +
         EggLayMinute;
```

```
                WRITELN(Outfile, 'E, ', EggLayMinute, ', ',
                EggLaySecond, ', ',
                TotalEggLayMinute, ', ', TotalEggLaySecond);
                WRITELN('TotalMinutes = ', TotalEggLayMinute);
                WRITELN('TotalSeconds = ', TotalEggLaySecond);
                WRITELN('--------------------------------------')
              END;

        'I': BEGIN
                TimeDif(OwnEggSecond, OwnEggMinute);
                WRITELN('Seconds ', OwnEggSecond);
                TotalOwnEggSecond := TotalOwnEggSecond +
                OwnEggSecond;
                If (TotalOwnEggSecond > 60) then
                  begin
                    TotalOwnEggSecond := TotalOwnEggSecond - 60;
                    TotalOwnEggMinute := TotalOwnEggMinute + 1
                  end;
                WRITELN('Minutes ', OwnEggMinute);
                TotalOwnEggMinute := TotalOwnEggMinute +
                OwnEggMinute;
                WRITELN(Outfile, 'I, ', OwnEggMinute, ', ',
                OwnEggSecond, ', ',
                TotalOwnEggMinute, ', ', TotalOwnEggSecond);
                WRITELN('TotalMinutes = ', TotalOwnEggMinute);
                WRITELN('TotalSeconds = ', TotalOwnEggSecond);
                WRITELN('------------------------------------');
              END;
        END; { of CASE }

END; { of PROCEDURE ElapsedTime }


BEGIN { actual program }

   Initialise;
   Intro;
   CLOSE(OutFile);

END. { of PROGRAM }
```

APPENDIX 3

The programme used to quantify and record the behaviour of *Neltumius arizonensis* females on *Prosopis* pods with *Algarobius prosopis* eggs within slits and on pods with egg-free slits:

```
program Behaviour;

Uses Crt, Dos;

Var
   Key             : CHAR;
   OutFile         : TEXT;

   Starthour,
   Startminute,
   Startsecond,
   Startsec100,
   Stophour,
   Stopminute,
   Stopsecond,
   Stopsec100,

   WalkInChamSecond,
   TotalWalkInChamSecond,
   WalkInChamMinute,
   TotalWalkInChamMinute,

   WalkOnPodSecond,
   TotalWalkOnPodSecond,
   WalkOnPodMinute,
   TotalWalkOnPodMinute,

   StatSecond,
   TotalStatSecond,
   StatMinute,
   TotalStatMinute,

   GroomSecond,
   TotalGroomSecond,
   GroomMinute,
   TotalGroomMinute,

   InspectSecond,
   TotalInspectSecond,
   InspectMinute,
   TotalInspectMinute,

   Slit1Second,
   TotalSlit1Second,
   Slit1Minute,
   TotalSlit1Minute,

   Slit2Second,
   TotalSlit2Second,
   Slit2Minute,
   TotalSlit2Minute,
```

```
      Slit3Second,
      TotalSlit3Second,
      Slit3Minute,
      TotalSlit3Minute,

      Slit4Second,
      TotalSlit4Second,
      Slit4Minute,
      TotalSlit4Minute,

      Slit5Second,
      TotalSlit5Second,
      Slit5Minute,
      TotalSlit5Minute,

      Slit6Second,
      TotalSlit6Second,
      Slit6Minute,
      TotalSlit6Minute,

      Slit7Second,
      TotalSlit7Second,
      Slit7Minute,
      TotalSlit7Minute,

      Slit8Second,
      TotalSlit8Second,
      Slit8Minute,
      TotalSlit8Minute,

      ScrapeSecond,
      TotalScrapeSecond,
      ScrapeMinute,
      TotalScrapeMinute,

      ScrChamSecond,
      TotalScrChamSecond,
      ScrChamMinute,
      TotalScrChamMinute,

      EggLaySecond,
      TotalEggLaySecond,
      EggLayMinute,
      TotalEggLayMinute,

      OwnEggSecond,
      TotalOwnEggSecond,
      OwnEggMinute,
      TotalOwnEggMinute: WORD;

PROCEDURE Initialise;

BEGIN
   WalkInChamSecond := 0;
   TotalWalkInChamSecond := 0;
   WalkInChamMinute := 0;
   TotalWalkInChamMinute := 0;
```

```
WalkOnPodSecond := 0;
TotalWalkOnPodSecond := 0;
WalkOnPodMinute := 0;
TotalWalkOnPodMinute := 0;

StatSecond := 0;
TotalStatSecond := 0;
StatMinute := 0;
TotalStatMinute := 0;

GroomSecond := 0;
TotalGroomSecond := 0;
GroomMinute := 0;
TotalGroomMinute := 0;

InspectSecond := 0;
TotalInspectSecond := 0;
InspectMinute := 0;
TotalInspectMinute := 0;

Slit1Second :=0;
TotalSlit1Second := 0;
Slit1Minute := 0;
TotalSlit1Minute := 0;

Slit2Second := 0;
TotalSlit2Second := 0;
Slit2Minute := 0;
TotalSlit2Minute := 0;

Slit3Second := 0;
TotalSlit3Second := 0;
Slit3Minute := 0;
TotalSlit3Minute := 0;

Slit4Second := 0;
TotalSlit4Second := 0;
Slit4Minute := 0;
TotalSlit4Minute := 0;

Slit5Second := 0;
TotalSlit5Second := 0;
Slit5Minute := 0;
TotalSlit5Minute := 0;

Slit6Second := 0;
TotalSlit6Second := 0;
Slit6Minute := 0;
TotalSlit6Minute := 0;

Slit7Second := 0;
TotalSlit7Second := 0;
Slit7Minute := 0;
TotalSlit7Minute := 0;

Slit8Second := 0;
TotalSlit8Second := 0;
Slit8Minute := 0;
TotalSlit8Minute := 0;
```

```
   ScrapeSecond := 0;
   TotalScrapeSecond := 0;
   ScrapeMinute := 0;
   TotalScrapeMinute := 0;

   ScrChamSecond := 0;
   TotalScrChamSecond := 0;
   ScrChamMinute := 0;
   TotalScrChamMinute := 0;

   EggLaySecond := 0;
   TotalEggLaySecond := 0;
   EggLayMinute := 0;
   TotalEggLayMinute := 0;

   OwnEggSecond := 0;
   TotalOwnEggSecond := 0;
   OwnEggMinute := 0;
   TotalOwnEggMinute := 0;

   ASSIGN( Outfile, 'A:\DATA.OUT');
   REWRITE( Outfile );

END; { of PROCEDURE Initialise }


PROCEDURE ElapsedTime; FORWARD;


Procedure Intro;

Begin
     CLRSCR;
     WRITELN('Record of bruchid behaviour:');
     WRITELN('---------------------------');
     WRITELN;
     WRITELN('Use the following keys for timing
     behaviour:');
     WRITELN(' (1) - Walking in chamber ');
     WRITELN(' (2) - Walking on pod ');
     WRITELN(' (3) - Stationary ');
     WRITELN(' (4) - Grooming ');
     WRITELN(' (5) - Inspecting pod ');
     WRITELN(' (Z) - Inspecting slit1 ');
     WRITELN(' (X) - Inspecting slit2 ');
     WRITELN(' (C) - Inspecting slit3 ');
     WRITELN(' (V) - Inspecting slit4 ');
     WRITELN(' (B) - Inspecting slit5 ');
     WRITELN(' (N) - Inspecting slit6 ');
     WRITELN(' (M) - Inspecting slit7 ');
     WRITELN(' (L) - Inspecting slit8 ');
     WRITELN(' (S) - Scraping ovipositor on pod ');
     WRITELN(' (H) - Scraping ovipositor on chamber ');
     WRITELN(' (E) - Laying an egg ');
     WRITELN(' (I) - Investigating own egg ');
     WRITELN(' (Q) - QUIT     ');
     WRITELN;
     WRITELN('Use the key as a toggle - Press once for ON');
     WRITELN('                        - Press again for OFF');
     WRITELN;
```

```
      WRITELN('N.B. Make sure Caps Lock is ON !!');
      WRITELN;
      WRITELN('Press a key.........:');
      WRITELN;
      REPEAT
        Key := UPCASE(READKEY);
        IF (Key IN ['1', '2', '3', '4', '5', 'Z', 'X', 'C', 'V',
        'B', 'N', 'M', 'L', S', 'H', 'E', 'I'])
          THEN
             BEGIN
                GETTIME(StartHour, StartMinute, StartSecond,
                Stopsec100);
                IF Key In ['1'] then WRITELN('Walking in
                chamber (1):');
                IF Key In ['2'] then WRITELN('Walking on pod
                (2):');
                IF Key In ['3'] then WRITELN('Stationary
                (3):');
                IF Key In ['4'] then WRITELN('Grooming
                (4):');
                IF Key In ['5'] then WRITELN('Inspecting pod
                (5):');
                IF Key In ['Z'] then WRITELN('Inspecting
                slit1 (Z):');
                IF Key In ['X'] then WRITELN('Inspecting
                slit2 (X):');
                IF Key In ['C'] then WRITELN('Inspecting
                slit3 (C):');
                IF Key In ['V'] then WRITELN('Inspecting
                slit4 (V):');
                IF Key In ['B'] then WRITELN('Inspecting
                slit5 (B):');
                IF Key In ['N'] then WRITELN('Inspecting
                slit6 (N):');
                IF Key In ['M'] then WRITELN('Inspecting
                slit7 (M):');
                IF Key In ['L'] then WRITELN('Inspecting
                slit8 (L):');
                IF Key In ['S'] then WRITELN('Scraping
                ovipositor on pod (S):');
                IF Key In ['H'] then WRITELN('Scraping
                ovipositor on chamber (H):');
                IF Key In ['E'] then WRITELN('Laying an egg
                (E):');
                IF Key In ['I'] then WRITELN('Investigating
                own egg (I):');
                Key := READKEY;
                GETTIME(StopHour, StopMinute,
                StopSecond,Stopsec100);
                ElapsedTime;
            END;
    UNTIL Key = 'Q';
End;


Procedure ElapsedTime;

   Procedure TimeDif(VAR Sec, Min:WORD);
```

```
   {This procedure calculates the time difference}
   {between Start and Stop and returns the answer as}
   {elapsed seconds to the calling line.}
   begin
   If StopSecond >= StartSecond then Sec := StopSecond -
   StartSecond
      Else Sec := 60-(StartSecond-StopSecond);
   If StopMinute >= StartMinute then Min := StopMinute -
   StartMinute
      Else Min := 60-(StartMinute-StopMinute);
   If StopSecond < StartSecond then Min := Min-1;
   end;  {Of TimeDif Procedure}

Begin    {of Procedure ElapsedTime}

 CASE Key OF

    '1': BEGIN
          TimeDif(WalkInChamSecond, WalkInChamMinute);
          WRITELN('Seconds ', WalkInChamSecond);
          TotalWalkInChamSecond := TotalWalkInChamSecond +
          WalkInChamSecond;
          If (TotalWalkInChamSecond > 60) then
            begin
              TotalWalkInChamSecond :=
              TotalWalkInChamSecond - 60;
              TotalWalkInChamMinute :=
              TotalWalkInChamMinute + 1
            end;
          WRITELN('Minutes ', WalkInChamMinute);
          TotalWalkInChamMinute := TotalWalkInChamMinute +
          WalkInChamMinute;
          WRITELN(Outfile, '1, ', WalkInChamMinute, ', ',
          WalkInChamSecond, ', ',
          TotalWalkInChamMinute, ', ',
          TotalWalkInChamSecond);
          WRITELN('TotalMinutes = ',
          TotalWalkInChamMinute);
          WRITELN('TotalSeconds = ',
          TotalWalkInChamSecond);
          WRITELN('-------------------------------------');
        END;

    '2': BEGIN
          TimeDif(WalkOnPodSecond, WalkOnPodMinute);
          WRITELN('Seconds ', WalkOnPodSecond);
          TotalWalkOnPodSecond := TotalWalkOnPodSecond +
          WalkOnPodSecond;
          If (TotalWalkOnPodSecond > 60) then
            begin
              TotalWalkOnPodSecond := TotalWalkOnPodSecond
              - 60;
              TotalWalkOnPodMinute := TotalWalkOnPodMinute
              + 1
            end;
          WRITELN('Minutes ', WalkOnPodMinute);
          TotalWalkOnPodMinute := TotalWalkOnPodMinute +
          WalkOnPodMinute;
          WRITELN(Outfile, '2, ', WalkOnPodMinute, ', ',
          WalkOnPodSecond, ', ',
```

```
          TotalWalkOnPodMinute, ', ',
          TotalWalkOnPodSecond);
          WRITELN('TotalMinutes = ', TotalWalkOnPodMinute);
          WRITELN('TotalSeconds = ', TotalWalkOnPodSecond);
          WRITELN('--------------------------------------')
        END;

   '3': BEGIN
          TimeDif(StatSecond, StatMinute);
          WRITELN('Seconds ', StatSecond);
          TotalStatSecond := TotalStatSecond + StatSecond;
          If (TotalStatSecond > 60) then
            begin
              TotalStatSecond := TotalStatSecond - 60;
              TotalStatMinute := TotalStatMinute + 1
            end;
          WRITELN('Minutes ', StatMinute);
          TotalStatMinute := TotalStatMinute + StatMinute;
          WRITELN(Outfile, '3, ', StatMinute, ', ',
          StatSecond, ', ',
          TotalStatMinute, ', ', TotalStatSecond);
          WRITELN('TotalMinutes = ', TotalStatMinute);
          WRITELN('TotalSeconds = ', TotalStatSecond);
          WRITELN('--------------------------------------')
        END;

   '4': BEGIN
          TimeDif(GroomSecond, GroomMinute);
          WRITELN('Seconds ', GroomSecond);
          TotalGroomSecond := TotalGroomSecond +
          GroomSecond;
          If (TotalGroomSecond > 60) then
            begin
              TotalGroomSecond := TotalGroomSecond - 60;
              TotalGroomMinute := TotalGroomMinute + 1
            end;
          WRITELN('Minutes ', Groomminute);
          TotalGroomMinute := TotalGroomMinute +
          GroomMinute;
          WRITELN(Outfile, '4, ', GroomMinute, ', ',
          GroomSecond, ', ',
          TotalGroomMinute, ', ', TotalGroomSecond);
          WRITELN('TotalMinutes = ', TotalGroomMinute);
          WRITELN('TotalSeconds = ', TotalGroomSecond);
          WRITELN('--------------------------------------');
        END;

   '5': BEGIN
          TimeDif(InspectSecond, InspectMinute);
          WRITELN('Seconds ', InspectSecond);
          TotalInspectSecond := TotalInspectSecond +
          InspectSecond;
          If (TotalInspectSecond > 60) then
            begin
              TotalInspectSecond := TotalInspectSecond -
              60;
              TotalInspectMinute := TotalInspectMinute + 1
            end;
          WRITELN('Minutes ', InspectMinute);
```

```
       TotalInspectMinute := TotalInspectMinute +
       InspectMinute;
       WRITELN(Outfile, '5, ', InspectMinute, ', ',
       InspectSecond, ', ',
       TotalInspectMinute, ', ', TotalInspectSecond);
       WRITELN('TotalMinutes = ', TotalInspectMinute);
       WRITELN('TotalSeconds = ', TotalInspectSecond);
       WRITELN('-----------------------------------');
     END;

'Z': BEGIN
       TimeDif(Slit1Second, Slit1Minute);
       WRITELN('Seconds ', Slit1Second);
       TotalSlit1Second := TotalSlit1Second +
       Slit1Second;
       If (TotalSlit1Second > 60) then
         begin
           TotalSlit1Second := TotalSlit1Second - 60;
           TotalSlit1Minute := TotalSlit1Minute + 1
         end;
       WRITELN('Minutes ', Slit1Minute);
       TotalSlit1Minute := TotalSlit1Minute +
       Slit1Minute;
       WRITELN(Outfile, 'Z, ', Slit1Minute, ', ',
       Slit1Second, ', ',
       TotalSlit1Minute, ', ', TotalSlit1Second);
       WRITELN('TotalMinutes = ', TotalSlit1Minute);
       WRITELN('TotalSeconds = ', TotalSlit1Second);
       WRITELN('-----------------------------------');
     END;

'X': BEGIN
       TimeDif(Slit2Second, Slit2Minute);
       WRITELN('Seconds ', Slit2Second);
       TotalSlit2Second := TotalSlit2Second +
       Slit2Second;
       If (TotalSlit2Second > 60) then
         begin
           TotalSlit2Second := TotalSlit2Second - 60;
           TotalSlit2Minute := TotalSlit2Minute + 1
         end;
       WRITELN('Minutes ', Slit2Minute);
       TotalSlit2Minute := TotalSlit2Minute +
       Slit2Minute;
       WRITELN(Outfile, 'X, ', Slit2Minute, ', ',
       Slit2Second, ', ',
       TotalSlit2Minute, ', ', TotalSlit2Second);
       WRITELN('TotalMinutes = ', TotalSlit2Minute);
       WRITELN('TotalSeconds = ', TotalSlit2Second);
       WRITELN('-----------------------------------');
     END;

'C': BEGIN
       TimeDif(Slit3Second, Slit3Minute);
       WRITELN('Seconds ', Slit3Second);
       TotalSlit3Second := TotalSlit3Second +
       Slit3Second;
       If (TotalSlit3Second > 60) then
         begin
           TotalSlit3Second := TotalSlit3Second - 60;
```

```
           TotalSlit3Minute := TotalSlit3Minute + 1
         end;
       WRITELN('Minutes ', Slit1Minute);
       TotalSlit3Minute := TotalSlit3Minute +
       Slit3Minute;
       WRITELN(Outfile, 'C, ', Slit3Minute, ', ',
       Slit3Second, ', ',
       TotalSlit3Minute, ', ', TotalSlit3Second);
       WRITELN('TotalMinutes = ', TotalSlit3Minute);
       WRITELN('TotalSeconds = ', TotalSlit3Second);
       WRITELN('-----------------------------------');
     END;

'V': BEGIN
       TimeDif(Slit4Second, Slit4Minute);
       WRITELN('Seconds ', Slit4Second);
       TotalSlit4Second := TotalSlit4Second +
       Slit4Second;
       If (TotalSlit4Second > 60) then
         begin
           TotalSlit4Second := TotalSlit4Second - 60;
           TotalSlit4Minute := TotalSlit4Minute + 1
         end;
       WRITELN('Minutes ', Slit4Minute);
       TotalSlit4Minute := TotalSlit4Minute +
       Slit4Minute;
       WRITELN(Outfile, 'V, ', Slit4Minute, ', ',
       Slit4Second, ', ',
       TotalSlit4Minute, ', ', TotalSlit4Second);
       WRITELN('TotalMinutes = ', TotalSlit4Minute);
       WRITELN('TotalSeconds = ', TotalSlit4Second);
       WRITELN('-----------------------------------');
     END;

'B': BEGIN
       TimeDif(Slit5Second, Slit5Minute);
       WRITELN('Seconds ', Slit5Second);
       TotalSlit5Second := TotalSlit5Second +
       Slit5Second;
       If (TotalSlit5Second > 60) then
         begin
           TotalSlit5Second := TotalSlit5Second - 60;
           TotalSlit5Minute := TotalSlit5Minute + 1
         end;
       WRITELN('Minutes ', Slit5Minute);
       TotalSlit5Minute := TotalSlit5Minute +
       Slit5Minute;
       WRITELN(Outfile, 'B, ', Slit5Minute, ', ',
       Slit5Second, ', ',
       TotalSlit5Minute, ', ', TotalSlit5Second);
       WRITELN('TotalMinutes = ', TotalSlit5Minute);
       WRITELN('TotalSeconds = ', TotalSlit5Second);
       WRITELN('-----------------------------------');
     END;

'N': BEGIN
       TimeDif(Slit6Second, Slit6Minute);
       WRITELN('Seconds ', Slit6Second);
       TotalSlit6Second := TotalSlit6Second +
       Slit6Second;
```

```
          If (TotalSlit6Second > 60) then
            begin
              TotalSlit6Second := TotalSlit6Second - 60;
              TotalSlit6Minute := TotalSlit6Minute + 1
            end;
          WRITELN('Minutes ', Slit6Minute);
          TotalSlit6Minute := TotalSlit6Minute +
          Slit6Minute;
          WRITELN(Outfile, 'N, ', Slit6Minute, ', ',
          Slit6Second, ', ',
          TotalSlit6Minute, ', ', TotalSlit6Second);
          WRITELN('TotalMinutes = ', TotalSlit6Minute);
          WRITELN('TotalSeconds = ', TotalSlit6Second);
          WRITELN('------------------------------------');
        END;

   'M': BEGIN
          TimeDif(Slit7Second, Slit7Minute);
          WRITELN('Seconds ', Slit7Second);
          TotalSlit7Second := TotalSlit7Second +
          Slit7Second;
          If (TotalSlit7Second > 60) then
            begin
              TotalSlit7Second := TotalSlit7Second - 60;
              TotalSlit7Minute := TotalSlit7Minute + 1
            end;
          WRITELN('Minutes ', Slit7Minute);
          TotalSlit7Minute := TotalSlit7Minute +
          Slit7Minute;
          WRITELN(Outfile, 'M, ', Slit7Minute, ', ',
          Slit7Second, ', ',
          TotalSlit7Minute, ', ', TotalSlit7Second);
          WRITELN('TotalMinutes = ', TotalSlit7Minute);
          WRITELN('TotalSeconds = ', TotalSlit7Second);
          WRITELN('------------------------------------');
        END;

   'L': BEGIN
          TimeDif(Slit8Second, Slit8Minute);
          WRITELN('Seconds ', Slit8Second);
          TotalSlit8Second := TotalSlit8Second +
          Slit8Second;
          If (TotalSlit8Second > 60) then
            begin
              TotalSlit8Second := TotalSlit8Second - 60;
              TotalSlit8Minute := TotalSlit8Minute + 1
            end;
          WRITELN('Minutes ', Slit8Minute);
          TotalSlit8Minute := TotalSlit8Minute +
          Slit8Minute;
          WRITELN(Outfile, 'L, ', Slit8Minute, ', ',
          Slit8Second, ', ',
          TotalSlit8Minute, ', ', TotalSlit8Second);
          WRITELN('TotalMinutes = ', TotalSlit8Minute);
          WRITELN('TotalSeconds = ', TotalSlit8Second);
          WRITELN('------------------------------------');
        END;
```

```
'S': BEGIN
           TimeDif(ScrapeSecond, ScrapeMinute);
           WRITELN('Seconds ', ScrapeSecond);
           TotalScrapeSecond := TotalScrapeSecond +
           ScrapeSecond;
           If (TotalScrapeSecond > 60) then
             begin
               TotalScrapeSecond := TotalScrapeSecond - 60;
               TotalScrapeMinute := TotalScrapeMinute + 1
             end;
           WRITELN('Minutes ', ScrapeMinute);
           TotalScrapeMinute := TotalScrapeMinute +
           ScrapeMinute;
           WRITELN(Outfile, 'S, ', ScrapeMinute, ', ',
           ScrapeSecond, ', ',
           TotalScrapeMinute, ', ', TotalScrapeSecond);
           WRITELN('TotalMinutes = ', TotalScrapeMinute);
           WRITELN('TotalSeconds = ', TotalScrapeSecond);
           WRITELN('------------------------------------');
        END;

   'H' : BEGIN
           TimeDif(ScrChamSecond, ScrChamMinute);
           WRITELN('Seconds ', ScrChamSecond);
           TotalScrChamSecond := TotalScrChamSecond +
           ScrChamSecond;
           If (TotalScrChamSecond > 60) then
             begin
               TotalScrChamSecond := TotalScrChamSecond -
               60;
               TotalScrChamMinute := TotalScrChamMinute + 1
             end;
           WRITELN('Minutes ', ScrChamMinute);
           TotalScrChamMinute := TotalScrChamMinute +
           ScrChamMinute;
           WRITELN(Outfile, 'H, ', ScrChamMinute, ', ',
           ScrChamSecond, ', ',
           TotalScrChamMinute, ', ', TotalScrChamSecond);
           WRITELN('TotalMinutes = ', TotalScrChamMinute);
           WRITELN('TotalSeconds = ', TotalScrChamSecond);
           WRITELN('------------------------------------');
        END;

   'E': BEGIN
          TimeDif(EggLaySecond, EggLayMinute);
          WRITELN('Seconds ', EggLaySecond);
          TotalEggLaySecond := TotalEggLaySecond +
          EggLaySecond;
          If (TotalEggLaySecond > 60) then
            begin
              TotalEggLaySecond := TotalEggLaySecond - 60;
              TotalEggLayMinute := TotalEggLayMinute + 1
            end;
          WRITELN('Minutes ', EggLayMinute);
          TotalEggLayMinute := TotalEggLayMinute +
          EggLayMinute;
          WRITELN(Outfile, 'E, ', EggLayMinute, ', ',
          EggLaySecond, ', ',
          TotalEggLayMinute, ', ', TotalEggLaySecond);
          WRITELN('TotalMinutes = ', TotalEggLayMinute);
```

```
          WRITELN('TotalSeconds = ', TotalEggLaySecond);
          WRITELN('--------------------------------------')
        END;

     'I': BEGIN
          TimeDif(OwnEggSecond, OwnEggMinute);
          WRITELN('Seconds ', OwnEggSecond);
          TotalOwnEggSecond := TotalOwnEggSecond +
          OwnEggSecond;
          If (TotalOwnEggSecond > 60) then
            begin
              TotalOwnEggSecond := TotalOwnEggSecond - 60;
              TotalOwnEggMinute := TotalOwnEggMinute + 1
            end;
          WRITELN('Minutes ', OwnEggMinute);
          TotalOwnEggMinute := TotalOwnEggMinute +
           OwnEggMinute;
          WRITELN(Outfile, 'I, ', OwnEggMinute, ', ',
          OwnEggSecond, ', ',
          TotalOwnEggMinute, ', ', TotalOwnEggSecond);
          WRITELN('TotalMinutes = ', TotalOwnEggMinute);
          WRITELN('TotalSeconds = ', TotalOwnEggSecond);
          WRITELN('------------------------------------');
        END;


    END; { of CASE }

END; { of PROCEDURE ElapsedTime }


BEGIN { actual program }

   Initialise;
   Intro;
   CLOSE(OutFile);

END. { of PROGRAM }
```

APPENDIX 4.
INTERSPECIFIC INTERACTIONS BETWEEN *N. ARIZONENSIS* AND *ALGAROBIUS PROSOPIS*

This appendix presents a preliminary investigation into the determination of whether the ovipositional behaviour of *N. arizonensis* relates to the survival of offspring in the presence of conspecifics and *A. prosopis*, under laboratory conditions that were deemed to be optimal for oviposition by *N. arizonensis*. This was undertaken by examining the outcome of the mixing of *N. arizonensis* and *A. prosopis* females in different proportions and in different sequences on the survival of offspring, as measured by the proportions of adult progeny that emerged. Although the experiments were preliminary and artificial, the results may have some relevance in terms of the relationship between *N. arizonensis* and *A. prosopis* under natural conditions.

METHODS

Damaged vs. undamaged pods

Several *Prosopis* pods that had been gathered in the field had much of the exocarp stripped away by wheat crickets, *Acanthoproctus* sp. (Hetrodinae), so that the mesocarp and endocarp were exposed. Some bruchids prefer to oviposit on the exposed seeds of pods that have dehisced, whereas others prefer intact pods (Messina, 1984; Pimbert and Pierre, 1983). Initially, it was necessary to determine whether damaged or undamaged pods were more suitable for either *N. arizonensis* or *A. prosopis*, so that conditions that were optimal for *N. arizonensis* could be provided in subsequent experiments. The emergence of adult offspring was utilised as an indicator of pod suitability.

Fifteen *N. arizonensis* females, newly-emerged from pods, were provided with 100g of damaged and 100g of undamaged 'mottled-purple' pods. In a separate container, 15 *A. prosopis* females, recently emerged from pods, were provided access to the same amount and variety of pods. All females were at peak oviposition age and were provided with mates and a diet of pollen before the experiment. Oviposition in *A. prosopis* peaks between 10 to 20 days after females emerge from pods (Hoffmann *et al.*, 1993a). After seven days the bruchids were removed and damaged and undamaged pods were separated. Subsequent adult emergence from the pods was observed for each species for 73 days. A Chi^2 test determined whether there was a difference within each species between the number of males and females that emerged from pods. Thereafter, a

Chi^2 test examined whether the number of each species that emerged from pods, differed significantly for each pod type.

Interspecific interactions

The seed resources (oviposition sites and larval food supply) that were provided were deliberately limiting, to examine the impact on the emergence of *A. prosopis* and *N. arizonensis* offspring. The mass of mottled purple pods of various sizes, totalling 1000 'seeds', was determined to be 365g. Each experiment was set up with 365g undamaged mottled purple pods in plastic containers of dimensions 35 x 15 x 12 cm. Females were provided with mates and food before the experiments and were utilised during their peak oviposition period. A diet of pollen and water was provided at the start of the experiment and renewed every two days.

To assess the effect of the timing of access to pods on the emergence of first generation offspring of *A. prosopis* and *N. arizonensis* from pods, two basic designs were followed: (a) *N. arizonensis* and *A. prosopis* females were provided simultaneous access to pods and the ratio of species was manipulated in three ways, each of which was replicated five times; (b) *N. arizonensis* and *A. prosopis* females were provided sequential access to pods, with an alternation of which species had access first, each of which was replicated five times.

(a) Simultaneous access to pods

Emergence of first generation offspring from pods was investigated under three conditions:

(i) Twenty five *N. arizonensis* females and 25 *A. prosopis* females were simultaneously placed in each of five containers with pods. The beetles were removed after five days.

(ii) Twelve *N. arizonensis* females and 38 *A. prosopis* females, resulting in a 1:3 ratio in favour of *A. prosopis*, were simultaneously placed in each of five containers for five days.

(iii) Thirty eight *N. arizonensis* and 12 *A. prosopis* females, resulting in a 1:3 ratio in favour of *N. arizonensis*, were simultaneously placed in each of five containers for five days.

(b) Sequential access to pods

Hypothetically, a species that obtains earlier access to pods may be at an advantage to a species that has access to the same pods at a later stage. The sequential-access experiments used a 1:1 ratio with 25 females of each species. Emergence of first generation offspring from pods was investigated under two conditions:

(i) Twenty five *N. arizonensis* females were placed in each of five containers with pods for five days, after which they were removed and replaced with 25 *A. prosopis* females for a further five days.

(ii) Twenty five *A. prosopis* females were placed in each of five containers with pods for five days, after which they were removed and replaced with 25 *N. arizonensis* females for a further five days.

The emergence of adult male and female offspring from pods was monitored for 85 days to ensure that all adults had emerged from the pods, but emergence is displayed for only 70 days in Figs. 12 and 13. Data on the number of eggs laid and the proportion of eggs that hatched as larvae and emerged as adults from pods, could not be obtained because destructive techniques are necessary for collection of those data.

The unity of males:females was tested with Chi^2. Thereafter, Chi^2 tests were conducted separately for each experiment to examine whether the observed ratio of adult offspring of each species differed from the original ratio of the female parents of each species.

Mites, accompanying *Prosopis* pods collected in the field, caused the mortality of *A. prosopis* and *N. arizonensis* adults in some of the experiments and affected some emergence results.

RESULTS

Damaged vs. undamaged pods

Table 9: Number of offspring which emerged as adults from damaged and undamaged pods on which eggs had been laid by 15 *N. arizonensis* females and 15 *A. prosopis* females.

Pod type	Number of emerged adults	
	N. arizonensis	*A.prosopis*
Damaged	34	186
Undamaged	77	31

An equivalent number of males and females of each species was expected to emerge from the pods provided. However, the ratio of *N. arizonensis* males to females differed significantly (Chi^2 = 7.157, $p < 0.01$) from 1:1; more females emerged from pods than males, but the deviation from the expected 1:1 ratio was the same for both types of pods (Chi^2 = 0.336, n.s.). The ratio of *A. prosopis* males to females also differed significantly from unity (Chi^2 = 7.000, $p < 0.01$); more females emerged from pods than males, but there was no significant difference in the deviation from unity between pod types (Chi^2 = 0.418, n.s.).

There was a significant difference (Chi^2 = 103.401, $p < 0.005$) in the number of *N. arizonensis* and *A. prosopis* offspring that emerged as adults from damaged and undamaged pods (Table 9). About 69% of the *N. arizonensis* offspring that emerged as adults from all pods, emerged from pods with undamaged surfaces. About 86% of the *A. prosopis* offspring that emerged as adults, emerged from pods with damaged, stripped surfaces. To create optimal conditions for *N. arizonensis* oviposition, all subsequent experiments utilised undamaged pods.

Interspecific interactions

There was no significant difference in the number of *N. arizonensis* males and females ($\mathrm{Chi}^2 = 3.678$, n.s.) or *A. prosopis* males and females ($\mathrm{Chi}^2 = 5.718$, n.s.) that emerged from pods. Therefore, it was not necessary to consider each sex individually when comparing the number of adults of each species or the number of adults that emerged per treatment.

(a) Simultaneous access to pods

Table 10: Number of *A. prosopis* and *N. arizonensis* offspring that emerged as adults from pods on which females of both species had simultaneously oviposited. The results of Chi^2 tests between the parent and offspring ratio are shown as ** = $p < 0.01$, n.s. = no significant difference. Na = *N. arizonensis*, Ap = *A. prosopis*.

Treatment	*N. arizonensis*	*A. prosopis*	
5 x 25 Na:25 Ap (1:1)	518	539	$\mathrm{Chi}^2 = 0.171$, n.s.
5 x12 Na:38 Ap (1:3)	204	784	$\mathrm{Chi}^2 = 3.011$, n.s.
5 x 38 Na:12 Ap (3:1)	701	495	$\mathrm{Chi}^2 = 81.375$, **

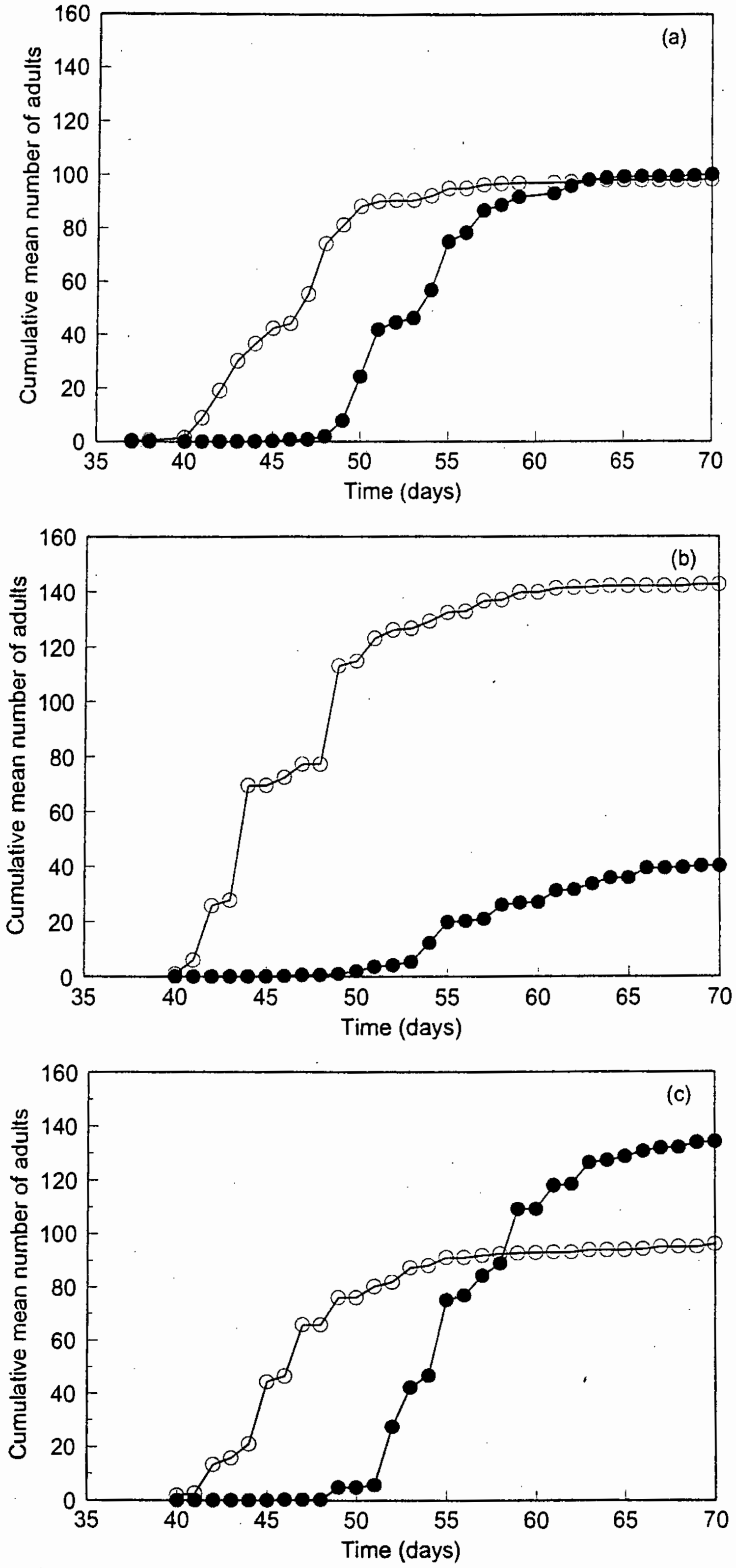

Fig. 12: Cumulative mean emergence of adult offspring from pods to which *N. arizonensis* and *A. prosopis* females had simultaneous access: **(a)** mean number of adult offspring from 25 *N. arizonensis* and 25 *A. prosopis* females (i.e. 1:1) (n = 5); **(b)** mean number of adult offspring from 12 *N. arizonensis* and 38 *A. prosopis* females (i.e. 1:3) (n = 5); **(c)** mean number of adult offspring from 38 *N. arizonensis* and 12 *A. prosopis* females (i.e. 3:1) (n = 5). O= *A. prosopis*, ●= *N. arizonensis*.

Although *A. prosopis* and *N. arizonensis* females had simultaneous access to pods, *A. prosopis* offspring emerged from pods about eight days before *N. arizonensis* (Figs. 12a-12c). The rates of pod emergence of the two species, indicated by the slopes of the curves in Figs. 12a-12c, were similar. There was about a two week emergence period for each species, with most emergence from pods occurring during the first 10 days. Few *A. prosopis* adults emerged from pods after day 55, while *N. arizonensis* adults emerged from pods until day 63.

(i) There was no significant difference in the number of *N. arizonensis* and *A. prosopis* offspring that emerged as adults from pods when equal numbers of females of each species had simultaneous access to pods (Table 10; Fig. 12a). An average of 4.3 offspring per *A. prosopis* female and 4.1 offspring per *N. arizonensis* female, emerged from pods.

(ii) When the ratio of females was 12 *N. arizonensis* to 38 *A. prosopis*, many more *A. prosopis* progeny emerged from pods than *N. arizonensis* (Table 10; Fig. 12b). Although the number of *N. arizonensis* adults and *A. prosopis* adults was slightly lower than expected (based on the results of the 1:1 situation), the ratio of *N. arizonensis* to *A. prosopis* did not differ significantly from the 1:3 ratio of the female parents that were originally placed in the boxes. An average of 4.1 *A. prosopis* offspring per female and 3.4 *N. arizonensis* offspring per female emerged from pods.

(iii) When 38 *N. arizonensis*:12 *A. prosopis* females were placed in the breeding boxes, proportionately fewer *N. arizonensis* offspring emerged from pods than had been anticipated (Table 10; Fig. 12c), while a greater proportion of *A. prosopis* offspring emerged from pods and there were almost double (8.3 progeny per female) the number of offspring than were produced in the 1:1 experiment. The reason for the disproportionate emergence of *A. prosopis* is not known. The ratio of *N. arizonensis* to *A. prosopis* offspring differed significantly from the 3:1 ratio of female parents that were originally placed in the boxes.

(b) Sequential access to pods

Table 11: Number of *A. prosopis* and *N. arizonensis* offspring which emerged from all pods on which females of both species had oviposited sequentially. The results of Chi^2 tests between the parent and offspring ratio are displayed, ** = $p < 0.01$, n.s. = no significant difference.

Treatment	*N. arizonensis*	*A. prosopis*	
A. prosopis followed by *N. arizonensis*	267	573	$Chi^2 = 56.897$, **
N. arizonensis followed by *A. prosopis*	273	260	$Chi^2 = 0.211$, n.s.

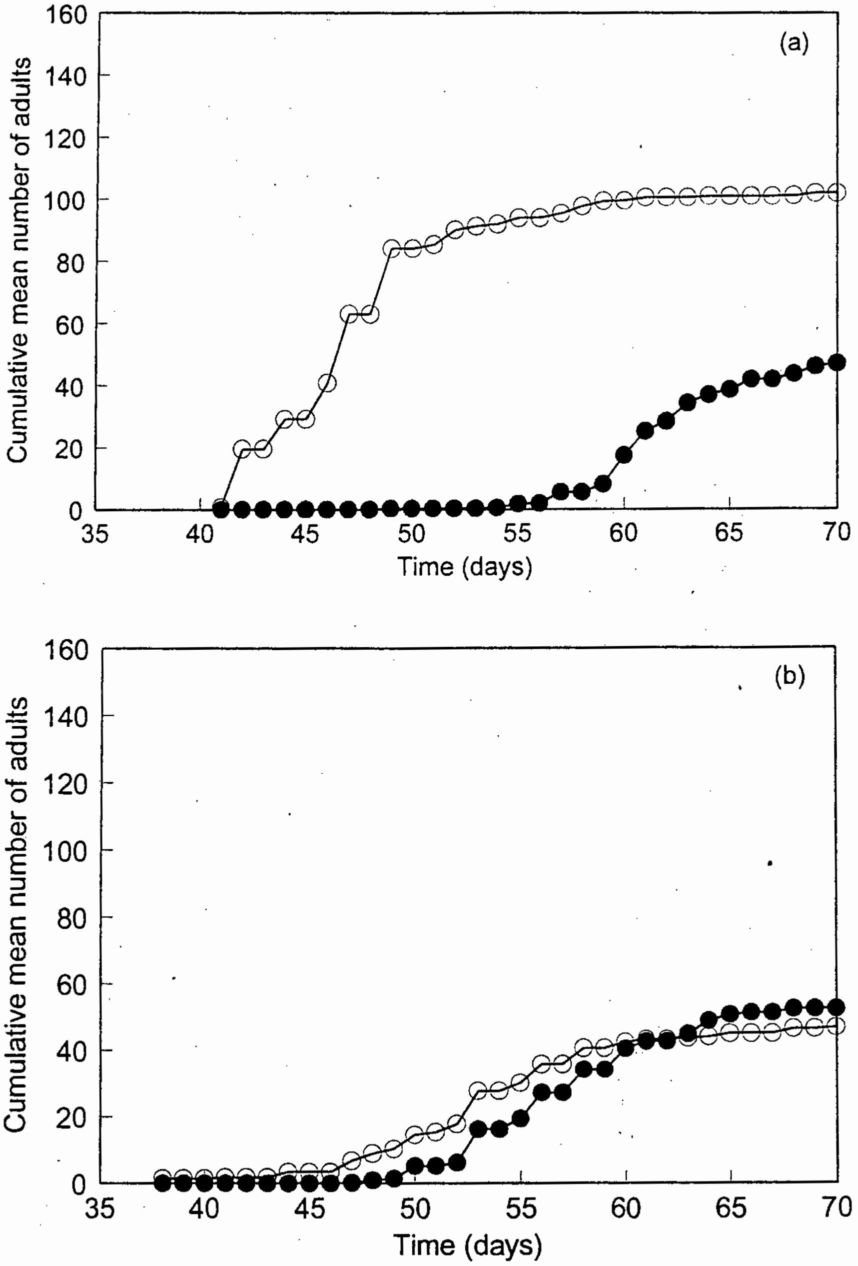

Fig. 13: Cumulative mean emergence of adult offspring from pods to which *N. arizonensis* and *A. prosopis* had sequential access: (**a**) mean number of adult offspring after 25 *A. prosopis* females had first access, followed after five days by 25 *N. arizonensis* females (n = 5); (**b**) mean number of adult offspring after 25 *N.arizonensis* females had first access, followed after five days by 25 *A. prosopis* females (n = 5). O = *A. prosopis*, ●= *N. arizonensis*.

If no advantage was gained from earlier access to pods, equal numbers of offspring of each species should have emerged from pods.

(i) The ratio of species differed significantly from unity when *A. prosopis* had access to pods first (Table 11), because twice as many *A. prosopis* offspring as *N. arizonensis* offspring emerged as adults from pods. The average number of *A. prosopis* offspring per female was similar to that measured in the experiments where females of both species were placed simultaneously in equal numbers in the breeding boxes (refer to Table 10), while the average number of offspring (2.1) per *N. arizonensis* female was half of that observed in the same experiments.

The earlier access of *A. prosopis* to pods and their more rapid development rate resulted in the start of the emergence of *A. prosopis* adult offspring from pods two weeks before that of *N. arizonensis* adult offspring (Fig. 13a).

(ii) The ratio of adult offspring that emerged from pods did not differ significantly from unity when *N. arizonensis* had access to pods before *A. prosopis* (Table 11). However, about 50% fewer offspring of each species emerged in total from pods - 2.1 offspring per *A. prosopis* female and 2.2 offspring per *N. arizonensis* female - than in the experiments where equivalent numbers of each species had simultaneous access to pods. Parasitism of adults by mites may have caused the death of some of the adult bruchids before they had emerged from the pods, resulting in low emergence.

Although *N. arizonensis* females had access to pods five days before *A. prosopis*, the timing of the start of emergence of the two species was similar because of the more rapid development of *A. prosopis* (Fig. 13b).

DISCUSSION

As expected, undamaged pods were more suitable for *N. arizonensis*, presumably as the pods offered suitable surfaces for the attachment of eggs. In contrast, damaged pods were more suitable for *A. prosopis*, because areas where the exocarp had been stripped away provided numerous oviposition sites that were ideal for the concealment of eggs. Eggs of both species were probably also deposited on the less suited pod type due to opportunism and an inadequate supply of oviposition sites. The *Prosopis* pods that were provided during the study of interspecific interactions were better suited to *N. arizonensis* than to *A. prosopis*.

Although not usual in bruchids, biased sex ratios are observed in some species due to differential mortality during development, possibly as a result of competition for resources (Cipollini, 1991; Ishihara and Shimada, 1993). This study demonstrated that the number of male and female *A. prosopis* and *N. arizonensis* offspring that emerged as adults from pods differed significantly from unity in both species in the damaged versus undamaged pods experiment but not in the interspecific interactions experiments.

The development period of *A. prosopis* is between 35 and 45 days (Conway, 1980; Hoffmann *et al.*, 1993a). In this study, the period from egg deposition to adult emergence from pods was a minimum of 39 days for *A. prosopis* and 47 days for *N. arizonensis*, with the majority of emergence from pods occurring within 10 days of the start of emergence of each species from pods. *Algarobius prosopis* first-instar larvae emerge from their egg cases about five days after oviposition, whereas *N. arizonensis* first-instar larvae remain in their egg cases for a longer period, tunnelling into the pod about eight days after oviposition (personal observation). *Neltumius arizonensis* also spent about five days longer within pods than *A. prosopis*, so that the total development duration of *N. arizonensis* was eight days longer than that of *A. prosopis*. Egg development in other bruchids is about five days, and the duration of development from oviposition to adult emergence from pods ranges from 29 to 63 days, depending on the temperature and the bruchid species (Brindley, 1933 in Smith, 1992; Parnell, 1966; Mitchell, 1975; Dick and Credland, 1984; Shimada, 1988 in Shimada and Ishihara, 1991; Moller et al., 1989a).

The advantage to *A. prosopis* of a shorter development period than *N. arizonensis* is that larvae will be stronger and better developed by the time *N. arizonensis* larvae enter the pods. In addition, *A. prosopis* larvae have the potential to select the largest, most nutritious seeds. Although it is not known to what extent *A. prosopis* females select oviposition sites, female offspring that emerge earlier are able to select the best oviposition sites before females of the other species emerge. In all of the experiments, except where *N. arizonensis* had access to pods first, *A. prosopis* must have been more fully developed than *N. arizonensis* at any point in time. The difference between the stage of development between the two species will be cumulative with time so that *A. prosopis* will derive an increasing competitive advantage over successive generations.

Algarobius prosopis is more fecund than *N. arizonensis*. With unrestricted access to mates, an *A. prosopis* female lays about 200 eggs in her lifetime, 40-50 of which are laid during the first half of the peak oviposition period (Hoffmann *et al.*, 1993a), whereas a *N. arizonensis* female only laid about 80 eggs in her lifetime, with just less than 30 laid during the peak period of oviposition (Chapter 2). Due to their disruption of oviposition, no males were provided during the study of interspecific interactions, so fecundity will have been reduced by about half. As a result of the greater fecundity of *A. prosopis* and their high survival rate (Hoffmann *et al.*, 1993a), a greater number of

A. prosopis than *N. arizonensis* offspring should have emerged per female parent. However, there were some cases where the number of offspring of each species that emerged per female from pods was equal. The reason for this may have been either that *A. prosopis* females laid fewer eggs than usual because of unsuitable oviposition sites, or that oviposition was as per normal but development was reduced, perhaps because of interactions with *N. arizonensis*. It is more likely that unsuitable oviposition sites were responsible because when first instar larvae of both species were placed on the same seed, *N. arizonensis* was outcompeted by *A. prosopis* (F. Impson, personal communication).

Regardless of this, it is encouraging that *N. arizonensis* was able to produce as many offspring per female as *A. prosopis*, especially when three times as many *A. prosopis* as *N. arizonensis* females were present. Surprisingly, *A. prosopis* produced the greatest number of offspring per female when three times as many *N. arizonensis* as *A. prosopis* females had simultaneous access to pods, although the reason for this is not known. The results of competition experiments between other bruchid species determined that regardless of whether the weaker species began with a numerical advantage, it was eventually outcompeted, but the period of co-existence was longer than if the two species were numerically equivalent (Yoshida, 1957 in Fujii, 1967; Fujii, 1967).

Although it was expected that a competitive advantage would be gained from obtaining access to pods before another species, neither *A. prosopis* nor *N. arizonensis* emergence from pods was improved by gaining access to pods before the other species. Low emergence from pods could have resulted from inhibition of oviposition because of the presence of other bruchid eggs or females. However, there were a number of problems with this study, which could be improved upon in further investigations. The problem of bruchid mortality caused by predacious mites has already been discussed. It was not possible to monitor the proportion of eggs that successfully developed into larvae and emerged as adults from pods, and this aspect merits investigation because females may have deposited larger numbers of eggs than the number of offspring that emerged from pods, but mortality may have occurred as a result of density-related factors (e.g. crowding) or density-independent factors (e.g. an inability to tunnel through the seed coat) (Nwanze and Horber, 1976; Stamopoulos and Desroches, 1981; Parker and Courtney, 1984).

Interspecific competition is often regarded as being caused by exploitation of limiting resources, by direct density effects, by toxin production and by various combinations of these mechanisms (Tilman, 1987). Tilman (1987) suggested that a study of competition required a multifaceted approach. Information on which resources are limiting, density-manipulation experiments, testing of the effect of chemicals of one species on another, and information on behaviour, physiology and morphology should be obtained before conclusions can be drawn (Tilman, 1987). Short-term experiments are useful but multiple-generation experiments provide information on the process of competition as well as the final results (Toquenaga and Fujii, 1991a). Therefore, a combination of short- and long-term experiments and population models of varying complexity are best to determine the outcome of interspecific interactions (Bellows and Hassell, 1984). Further investigation into the processes that are involved in determining the outcome of interactions between *N. arizonensis* and *A. prosopis* is required.

Although the effect of one species on another could not be accurately determined because of predation by mites and because of the preliminary nature of this study, the results are still of interest. It appears that the oviposition behaviour of *N. arizonensis* imparts a high probability of survival in competitive situations, but the greater fecundity and shorter development period of *A. prosopis* may enable it to be competitively superior to *N. arizonensis* in the long-term.