Available online at www.sciencedirect.com
ScienceDirect
C. R. Biologies 330 (2007) 49–54
COMPTES RENDUS BIOLOGIES
http://france.elsevier.com/direct/CRASS3/

Animal biology and pathology / Biologie et pathologie animales

Life cycle of the coleopter *Bruchidius raddianae* and the seed predation of the *Acacia tortilis Subsp. raddiana* in Tunisia

Salma Derbel [a], Zouhaier Noumi [a], Klaus Werner Anton [b], Mohamed Chaieb [a,*]

[a] *Department of Biology, Science Faculty of Sfax, BP 802, 3018 Sfax, Tunisia*
[b] *Grünewaldstraße 13, 79312 Emmendingen, Germany*

Received 15 May 2006; accepted after revision 29 August 2006

Available online 9 January 2007

Presented by Pierre Buser

Abstract

Seeds of *Acacia tortilis* (Forsskal) Hayne ssp. *raddiana* (Save) Brenan, a savannah tree of great potential for forestry and an excellent browse for game and livestock, is heavily infested by the bruchid beetle *Bruchidius* raddianae Anton & Delobel. The development from egg to beetle, and the various development stages were investigated using Scanning Electron Microscopy (SEM). The adult *B. raddianae* lays eggs on the green pod in the autumn. The first instar larva hatches from five to seven weeks and develops outside the host seed. From the second instar onwards development took place inside the host seed. Pupation takes about three weeks, late in the summer. The beetle of *B. raddianae* is univoltine and the newly emerged adult makes an exit hole to leave the seed. During the development from first instar lava to imago, all embryonic tissue is destroyed. This results in a weakness of its soil seed stock, which reduces the possibilities of natural regeneration of the species. ***To cite this article: S. Derbel et al., C. R. Biologies 330 (2007).***

Résumé

Cycle biologique de *Bruchidius raddianae* (*Coléoptère* : *Bruchidae*) et prédation des semences d'*Acacia tortilis Subsp. raddiana* en Tunisie. Les graines d'*Acacia tortilis* (Forsskal) Hayne ssp. *raddiana* (Save) Brenan sont attaquées par les bruches. Il en résulte un affaiblissement du stock de semences du sol, ce qui réduit les possibilités de régénération naturelle de l'espèce. L'identification de ce séminivore a été réalisée pour la première fois. Il s'agit de *Bruchidius raddianae* Anton & Delobel. Ce coléoptère n'a jusqu'ici fait l'objet d'aucune étude exhaustive et certaines particularités de sa biologie sont encore inconnues. Les différents stades larvaires ont été visualisés au microscope électronique à balayage. Il s'avère que le développement larvaire s'effectue en quatre stades et s'échelonne sur huit mois. Les divers échantillonnages ont permis de mettre en évidence une seule génération annuelle du ravageur. Ce *Bruchidius* est pourvu d'une aptitude colonisatrice et son cycle biologique est étroitement lié à celui de sa plante hôte. ***Pour citer cet article : S. Derbel et al., C. R. Biologies 330 (2007).***

Keywords: *Acacia tortilis raddiana*; *Bruchidius raddianae*; Bruchidae; Seed development; Plant–insect relationships; SEM

Mots-clés : *Acacia tortilis raddiana* ; *Bruchidius raddianae* ; Bruchidae ; Développement des semences ; Relations plante–insecte ; MEB

* Corresponding author.
E-mail address: mohamed.chaieb@gnet.tn (M. Chaieb).

1631-0691/$ – see front matter
doi:10.1016/j.crvi.2006.09.003

1. Introduction

Bruchids comprise a cosmopolitan family of chrysomeloid beetles, and their larvae develop in the seeds of plants, mainly legumes. Many species are of economic importance as pests of grain legumes, whilst others are of potential importance as natural enemies of invasive legumes such as *Acacias*.

Acacia species are socio-economically important. They are used for fence posts, firewood, as a livestock feed for tanning, and as an algicide and molluscicide [1,2]. *Acacia* is also valuable commercially [3] and medicinally [4]. Their particular value in arid zones lies in their extreme resistance to heat, drought, salinity and alkalinity, drifting sand, grazing and repeated cutting [5]. Owing to their socio-economical and commercial uses, *Acacia* trees are important re-afforestation species in Africa. Bruchid beetles constitute the major pests of *Acacia* seeds that can seriously hamper re-afforestation programs [6].

Bruchids initially attack fresh green *Acacia* pods on the tree; re-infestation following emergence may then occur on mature dry pods on the tree or ground [7,8]. Larvae of bruchid beetles may destroy large portions of the Acacia seeds, especially if conditions are benificial for reinfestation, such as in seeds that are not dispersed [9]. Attack rates can reach 99% in Africa and 70% in central America [10]; although lower attack rates are usually reported [11]. The bruchid larvae beetles develop inside seeds and can destroy 9–100% of the cotyledons [12]. Infested seeds are usually non-viable [11], although not exclusively so.

In the national park of Bou Hedma, *Acacia tortilis* (Forsskal) Hayne ssp. *raddiana* (Save) Brenan has undergone a progressive degradation owing to *Bruchidius* which poses a threat to its disappearance. To our knowledge, insects attacking the seeds are not yet identified and many particularities of its biology still unknown. The aim of this work was not only to identify this species and to determine its biological cycle, but also to appreciate the extent of its damage and to determine the rate of its infestation.

2. Materials and methods

2.1. Study area

Our investigations were carried out (from June 2001 to September 2002) in the Bou Hedma Natural park (34.39°N, 9.48°E), which follows the Saharian fringes of Tunisia. Nowadays, *Acacia tortilis* (Forsskal) Hayne ssp. *raddiana* (Save) Brenan, in following text named *A. raddiana*, is a species which exists only under the form of relics in this Park. The study site is characterized by an arid bioclimate and described fluently as for its edapho-climatic characteristics [13]. The reserve receives a mean annual rainfall of 303 mm. Temperature varies from 32 to 36 °C in summer and from 4 to 7 °C in winter.

2.2. *Development of* Acacia *seeds and life cycle of* Bruchidius

Flowering phenology and seed development were noted. Samples of pods were collected and examined for trace of damage and presence of eggs, larvae or emergence hole.

After emergence of the adult bruchid, we dissected the seed and pod to determine the location of the pupal chamber. The path of the larva through the seed, the number of seeds fed upon, the point of entry through the pod and seed coat, were also examined. Weights of seeds and insects were performed on a torsion balance recording to 0.05 mg.

The males and females of *Bruchidius raddianae* Anton & Delobel were placed into oviposition chambers each filled with 50 three year old, uninfested seeds of *A. raddiana*. Each chamber was a glass jar of 3 cm diameter by 4.5 cm high. After 15 days, when eggs were produced, all adult beetles were removed and the jar was stored at 20 °C and 60% of relative humidity. Development of eggs was observed under a binocular microscope.

The jars were controlled on hatched and non-hatched eggs, dead first instar larvae and seeds with entrance hole. The observation of the detailed morphology of eggs, fourth instar larvae and adult beetles, was made using scanning electron microscopy (Philips XL 30).

2.3. Degree of infestation

The pods of *A. raddiana* were collected from the National Park of Bou Hedma below 10 trees selected randomly. Pods of each tree were pooled. Seeds were removed and 350 seeds were examined for bruchids in order to establish differences in bruchid infestation between trees. Seeds predated by *B. raddianae* can be recognized by a round hole either with a diameter of less than 0.5 mm, as a sign of the first instar's entrance, or with a diameter of 1 to 3.5 mm, as sign of the adult beetle's departure.

The percentage of infestation is calculated of the following manner:

$$\%\text{ of infestation} = (\text{number of seed infested} / \text{number of seed examined}) \times 100$$

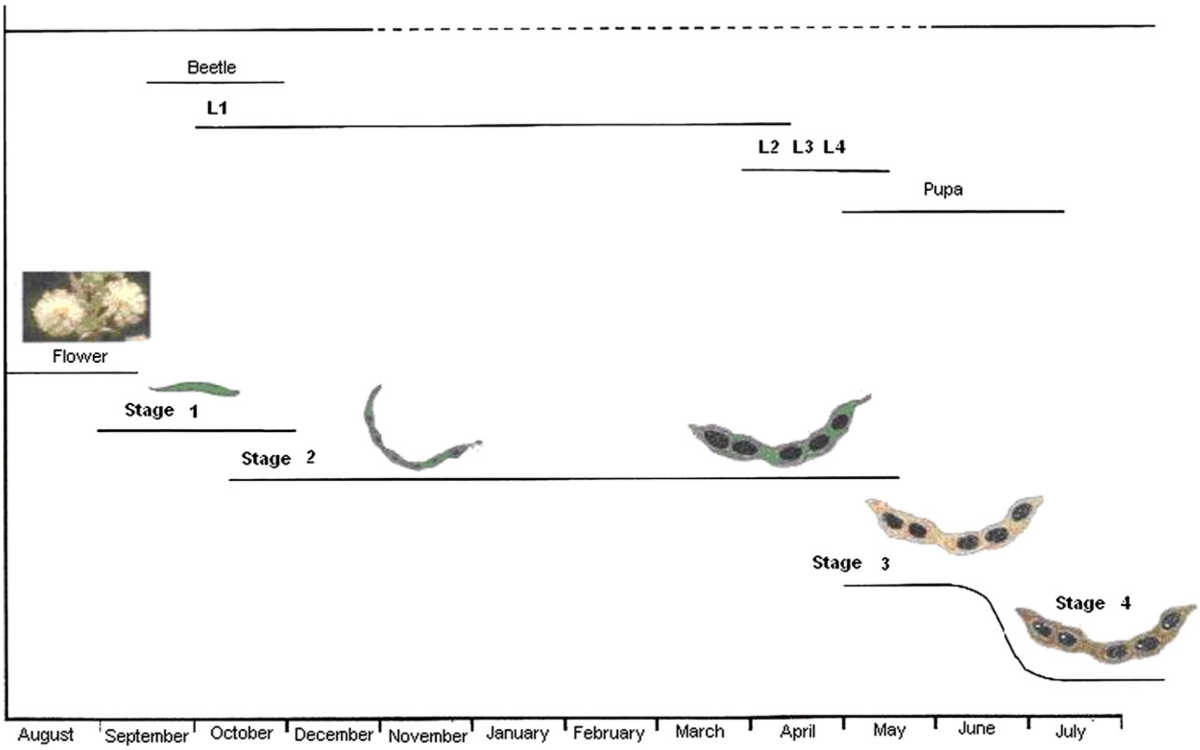

Fig. 1. Relation between cycle of *Bruchidius* and phenology of *Acacia*. L1, L2, L3 and L4: the four larvae stages of *B. raddianae*; Stage 1: green pods which seeds of which are not yet formed; Stage 2: green pods with visible seeds during growth; Stage 3: yellow pods with dehydrated pericarp; Stage 4: dry pods with mature seeds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results and discussion

3.1. Bruchidius *identification*

The species attacking seeds of *A. raddiana*, has been identified **by one of us**. It consists of *Bruchidius raddianae* Anton & Delobel **2003**. It is a small-sized species with rather variable color of adults, from almost uniformly light yellowish to light reddish-brown with slightly darker and paler spots to distinctly chequered with blackish-brownish and whitish spots.

B. raddianae is distinguished from the closely related *B. centromaculatus* by having stronger remote eyes, weaker teeth at the base of elytra, complete shiny margin surrounding deep foveae of female pygidium, shorter median lobe [14]. The name refers to larval development in seeds of *Acacia tortilis* ssp *raddiana*. Other host plants mentioned by various authors include *A. ehrenbergiana*, *A. farnesiana, A. gerrardii, A. nilotica, A. Senegal, A. seyal and A. sieberiana*. This insect has not been, so far, the object of exhaustive survey, and some details of its biology are still unknown.

3.2. Development of Acacia *seeds and life cycle of* Bruchidius

The following description is dealing with *Acacia raddiana* and *Bruchidius raddianae*, as it is the most extensively examined interaction, but appears to be very typical of all combinations found in this survey. Fig. 1 is based on bulked information acquired during the period of study.

Flower buds develop in racemes for several months before flowering in August and September, with variation in peak flowering of up to several weeks between conspecific trees. Young soft pods are evident from early August. They enlarge considerably until April. Generally, *Bruchidae* species lay eggs on pods of defined phonological stages: dry pods as the case of *Acanthoscelides obtectus* (Say), green pods as the case of *Bruchus pisorum* [15]. As for the case of *Bruchidius atrolineatus* harmful to *Vigna unguiculata*, females lay eggs on green pods and then on dry pods [16]. In September, females of *B. raddianae* oviposit on the epicarp of green pods, seeds are not yet formed. Eggs are laid singly, on the surface of young pods (Fig. 2a). The egg

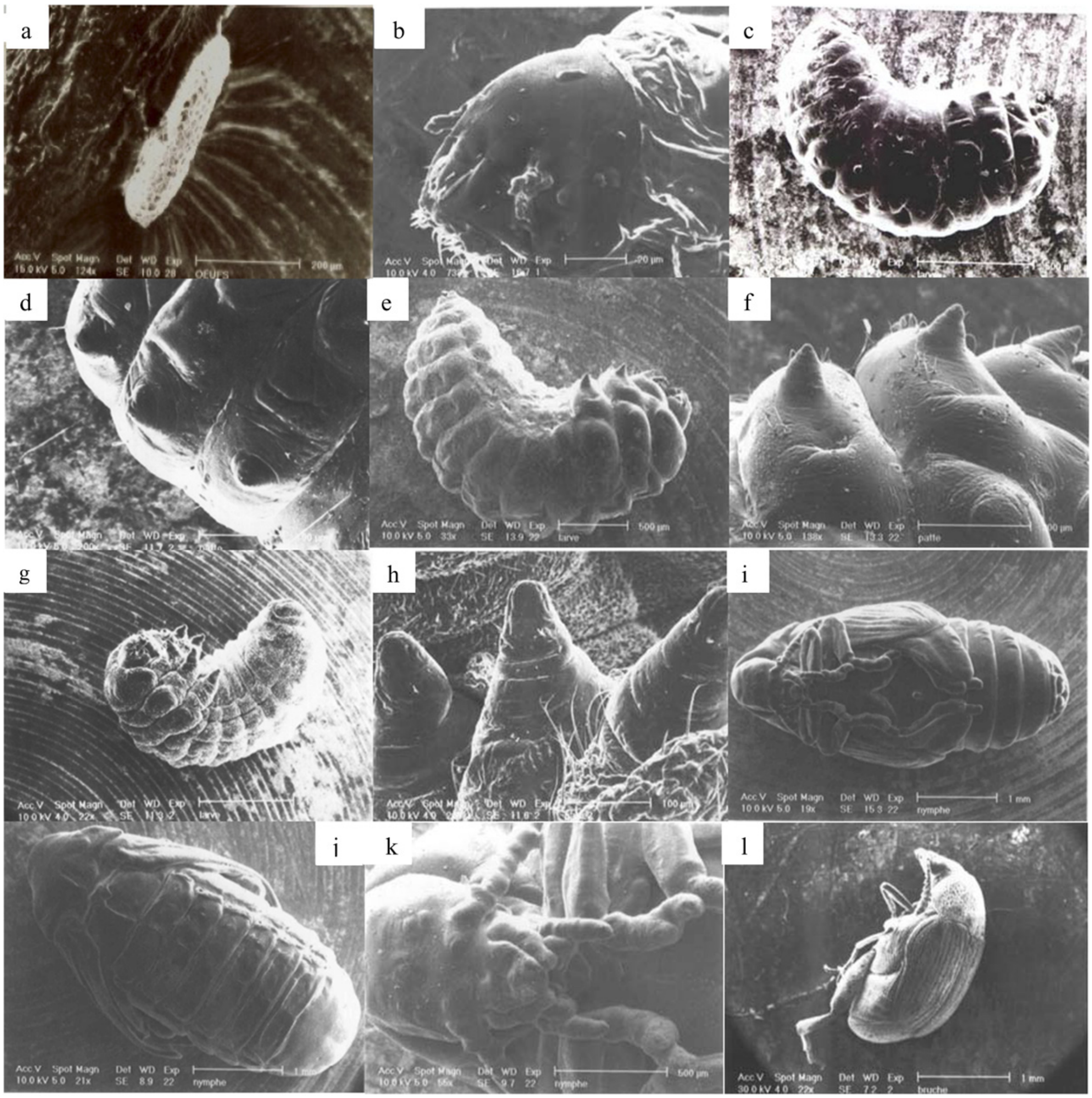

Fig. 2. (**a**) Egg of *Bruchidius raddianae* (bar = 200 µm). (**b**) First instar larva (bar = 20 µm). (**c**) Second instar larva (bar = 500 µm). (**d**) Locomotion bulges of second instar larva (bar = 50 µm). (**e**) Third instar larva (bar = 500 µm). (**f**) Locomotion bulges of third instar larva (bar = 200 µm). (**g**) Fourth instar larva (bar = 1 mm). (**h**) Locomotion bulges of fourth instar larva (bar = 100 µm). (**i**) Ventral face of the nymph (bar = 1 mm). (**j**) Dorsal face of the nymph (bar = 1 mm). (**k**) Head of the nymph (bar = 500 µm). (**l**) Beetle (bar = 1 mm).

had a mean dry mass of 8.6 ± 3.5 µg, with a water content of 50.1 ± 6% in the fresh egg. They enlarge considerably during October, with seeds swelling noticeably by the end of that month. First instar larvae (800 ± 1.5 µm; 1.5 µg) (Fig. 2b) have to fulfill several activities: cutting a hatching window out of the chorion, departure from the chorion and it attacks directly the partition of the pod. It takes 7 h thereabouts to cross this pod while letting adhere to its surface the cockle of the egg empty and transparent, and digging a tunnel gallery in the cloth of the pod.

The growth and elongation of pods continues until the April to attain their sizes and their definitive structures. Finally, the first instar larva enters the seed. Each larva develops inside a single seed. The neonate larva molt while giving birth to the last three larval stadiums (L2: 1.5 ± 0.5 mm, 1 mg (Fig. 2c and d); L3: 2.6 ± 0.9 mm, 2.5 mg (Fig. 2e and f); L4: 2.9 ± 0.3 mm,

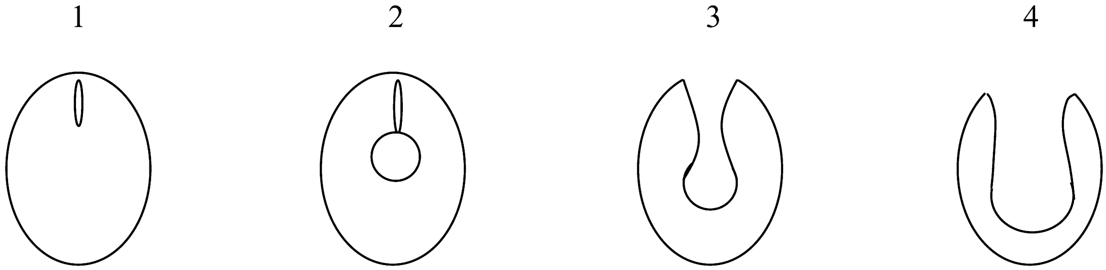

Fig. 3. Feeding pattern of larva of *Bruchidius raddianae* in seeds of *Acacia raddiana*. The testa of the seeds is removed for the demonstration of the damage. Stage 1 → first- instar larva, Stage 2 → second instar larva, Stage 3 → third instar larva, Stage 4 → fourth instar larva.

3 mg) (Fig. 2g and h). During May, pods become yellow with dehydrated pericarps. Before changing into the nymph, the larvae of the last stage go through a prenymphal stage. Nymphosis occur into a hull. This lodge is prepared by the larvae at the end of its latest larvae stage. This behaviour was also observed in the case of *Acanthoscelide obtectus* and *Bruchus pisorum*. Nymph (3.5 ± 0.3 mm, 5 mg) (Fig. 2i–k) changes into adult. The latter is soft with yellow color: After 5 days, it acquires its definitive aspect (Fig. 2l). From June, the seeds harden, pods become dry and the newly emerged adult of *B. raddianae* makes an exit hole. *Bruchidius raddianae* is univoltin species that is it has only one generation per year. The study of *Bruchidius raddianae* larvae stages in *Acacia* seeds allowed us to specify the temporal distribution of larvae as follows:

- larvae of the first stage L1 and that of the second stage L2 are totally absent in dry seeds;
- the third larvae stage can be observed in dry seeds but at low percentage;
- the fourth larvae stage L4 can be developed in dry seeds.

During development from egg to imago, the dry weight of the bruchid increase from 1.5 µg (first instar larva) to a mean of nearly 5 mg. The only food available to the larva is that of the embryo of *A. raddiana*. After the first instar larva has bored through the seed coat they start to feed in center of the embryo, i.e., in the great cotyledons by first creating a hole (Fig. 3). During the further development of the larvae, they attack the embryonic axis, destroying the plumula and the adaxial part of the radicula. As soon the main axis is nevermore have germination success, thus safeguarding the development of the larva.

3.3. Bruchid infestation between different trees

The infestation size of *A. raddiana* seeds by bruchids varies from one year to another according to the humidity, temperature and the rainfall [17,18]. In this current study, Bruchid infestation of seeds varies significantly between *A. raddiana* trees (one-way ANOVA, $F = 0.082$; $P < 0.999$, Table 1). The high rates of bruchid infestation (from 25.9 to 79.9%), may be attributed to the high density and condensation distribution of this species at the park.

Table 1
The mean (±SE) percentage (%) of bruchid infested *A. raddiana* on different trees. 350 seeds of each trees were sampled

Tree	Mean (±SE) % seeds infested
1	43 ± 5.6
2	56 ± 7.3
3	78.2 ± 4.3
4	25.9 ± 13.3
5	69.7 ± 8.6
6	55.1 ± 10.8
7	85.6 ± 7.8
8	35.7 ± 15.4
9	79.9 ± 11.2
10	68.5 ± 9.3

Very high infestation rates have been found in other Acacia species for seeds, where reinfestation of the seeds occur, but the rates for seeds from fresh pods are generally lower [9,11]. Differences in bruchid infestation between trees has been widely reported [18–20]. This may relate to the size of the seed crop and tree density since the proportion of a seed crop destroyed and its size are often inversely related and infestation may be higher at low tree densities [21]. There may also be differences in seed protection against bruchids; for example, some trees of *Acacia raddiana* produce gum from their seed pods following the penetration of the first larva from egg mass; the immature seeds remain very small throughout the year and then abruptly grow to maturity just before being dispersed. Several species with swollen seed pods have developed a defense strategy against bruchids: thorn acacias flake off the surface of their pods, and this removes the eggs laid on that surface [22]. The seeds of *Cassia siamea* are such that bruchids cannot mature in them [23]. Concerning *Bauhinia monandra*, there is a layer of material on the seed surface that swells when the pod opens and detaches the attached eggs [23]. In *Dehiscence* (*Leucaena*), fragmentation (*Mimosa*) or explosion (*Canavalia*) of pods, scatters the seeds to allow them to escape from larvae coming through the pod walls and from ovipositing females [24]. It is to be noted that inter tree variation in attack rate, and generalizations on infestation levels based on samples from one or a few trees may be grossly misleading, a conclusion in accord with janzen data on bruchid infestation of *A. farnesiana* in Costa Rica.

Seed predation of *Acacia tortilis raddiana* in the national park of Bou Hedma raises a real problem which makes a threat to the presence of this species, not only in Tunisia, but also in Asia and sub-Saharan Africa, as reported by Anton et al. [14]. However, in Tunisia the presence of *Bruchidius raddianae* seems to be very dangerous, since our observations revealed the pod infestation from the beginning of its formation, where a majority of produced seeds were infested by this species. In addition, neonate larvae hollow out a gallery in pod tissues and reach the nearly seed. During its growth phase in the seed, the entire cotyledon is devoured. The seeds obtained are dangerously infested and germination is inhibited. Recent field observations (spring 2006) in the Bou Hedma Natural Park revealed: (*i*) a high tendency of seeds predation; and (*ii*) a low density of *A. raddiana* seedlings, thus supposing a high rate Bruchid infestation. Therefore, preventive measures have to be recommended (biological and integrated fight) in order to restrict seed predation and ensuring the germination within the Bou Hedma Park, considered, by UNESCO, as a biosphere reserve. In these conditions, seed germination increases species density in the park and contributes to the preservation of forest strata in this pre-Saharan ecosystem.

Acknowledgements

The authors are indebted to Professors Zouhaier Fakhfakh and Olfa for their able technical assistance at the SEM laboratory, Faculty of Sciences Sfax, Tunisia.

References

[1] J. Timberlake, Handbook of Botswana Acacias, Ministry of Agriculture, Gaborone (Botswana), 1980.
[2] S.M.H. Ayoub, L.K. Yankor, Field trials for the evaluation of the molluscidal activity of Acacia nilotica, Fitoterapia 55 (1984) 305–307.
[3] D.M.W. Anderson, Chemotaxonomic aspects of the chemistry of Acacia Gum exudates, Kew Bull. 32 (1978) 529–536.
[4] L. Boulos, Medicinal Plants of North Africa, Reference Publications Inc. Algonac, Michigan, USA, 1983.
[5] C.W. Fagg, J.L. Stewart, The value of *Acacia* and *Prosopis* in arid and semi-arid environments, J. Arid Environ. 27 (1994) 3–25.
[6] S. Haoua, H. Mourier, Non-Poisonous Methods of Pest Control During Forest Seed Collection, Handling, Storage and Transfer, CNSF Ouagadougou, Burkina Faso, 1990.
[7] B.J. Southgate, Univoltine and multivoltine cycles: their significance, in: V. Labeyrie (Ed.), The Ecology of Bruchids Attacking Legumes (Pulses), Junk, The Hague, The Netherlands, 1981, pp. 17–22.
[8] M.F. Miller, M. Coe, Is it advantageous for *Acacia* seeds to be eaten by ungulates?, Oikos 66 (1993) 364–368.
[9] K. Tybirk, Regeneration of Woody Legumes in Sahel, in: AAU Report, vol. 27, Aarhus Univ. Press, Aarhus, Denmark, 1991, pp. 1–81.
[10] D.H. Janzen, Specificity of seed-attacking beetles in a Costa Rican deciduous forest, J. Ecol. 68 (1980) 929–952.
[11] M. Coe, C. Coe, Large herbivores, acacias trees and bruchid beetles, S. Afr. J. Sci. 83 (1987) 624–635.
[12] W.H.O. Ernst, Nutritional aspects in the development of *Bruchidius sahlbergi* (Coleoptera ∼ *Bruchidae*) in seeds of *Acacia erioloba*, J. Insect Physiol. 38 (11) (1992) 831–838.
[13] M. Chaïeb, Influences de réserves hydriques du sol sur le comportement comparé de quelques espèces végétales de la zone aride tunisienne, thèse, université des sciences et techniques du Languedoc, Montpellier, 1989 (292 p.).
[14] K.W. Anton, A. Delobel, African species of the *Bruchidius centromaculatus* group with eyed female pygidium (Coleoptera: Bruchidae: Bruchinae), Genus 14 (2) (2003) 159–190.
[15] A.S. Balachowsky, in : Entomologie appliquée a l'agriculture traitée, Tome I, Masson et C^{ie} éditeurs, 1962, pp. 434–469.
[16] J. Huignard, D. Rojas-Rousse, I. Alzouma, L'activité reproductrice de *Bruchidius atrolineatus* en zone sahélienne. Mise en évidence d'une diapause imaginale, Insect Trop. Sci. Appl. 5 (1) (1984) 41–63.
[17] G. Halevy, Effects of gazelle and seed beetles on germination and establishment of *Acacia* species, Isr. J. Bot. 23 (1974) 120–126.
[18] W.H.O. Ernst, J.E. Decelle, D.J. Tolsma, Predation of seeds of *Acacia tortilis* by insects, Oikos 54 (1989) 294–301.
[19] M.T. Hoffmann, R.M. Cowling, C. Douie, S.M. Pierce, Seed predation and germination of *Acacia erioloba* in the Kuiseb River Valley, Namib Desert, S. Afr. J. Bot. 5 (1989) 103–106.
[20] M.F. Miller, *Acacia* seed predation by bruchids in an African savanna ecosystem, J. Appl. Ecol. 33 (1996) 1137–1144.
[21] M.J. Mattson, Relationship between cone crop size and cone damage by insects in red seed production areas, Can. Entomol. 103 (1971) 617–621.
[22] D.H. Janzen, Seed-eaters versus seed size, number, toxicity and dispersal, Evolution 23 (1969) 1–27.
[23] J.C. Bridwell, Notes on the Bruchidae and their parasites in the Hawaiian Islands, Proc. Hawaii Entomol. Soc. 3 (1918) 465–505.
[24] R.A. Cushman, Notes on the host plants and parasites of some North American *Bruchidae*, J. Econ. Entomol. 4 (1911) 489–510.