Entomotropica
antes/formerly *Boletín de Entomología Venezolana*
Vol. 16(3): 149-155. Diciembre 2001

ISSN 1317-5262

Biology and laboratory culturing of the root-feeding flea beetle, *Longitarsus columbicus columbicus* Harold, 1876 (Chrysomelidae: Alticinae): a potential natural enemy of *Lantana camara* L. (Verbenaceae) in South Africa

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Resumen

BAARS JR 2001. Biología y cultivo en laboratorio del coquito pulga, *Longitarsus columbicus columbicus* Harold, 1876 (Chrysomelidae: Alticinae): un enemigo potencial natural de *Lantana camara* L. (Verbenaceae) en Sur Africa. Entomotropica 16(3):149-155.

La planta ornamental introducida, *Lantana camara* L. (Verbenaceae), es una de las peores malezas invasivas de Sur Africa. Esta especie ha sido objecto de programmas de biocontrol en las últimas cuatro décadas. Aunque se han reconocido algunos enemigos naturales, el nivel de control es considerado insatisfactorio y un número de biocontroladores potenciales están siendo evaluados. El coquito pulga *Longitarsus columbicus columbicus* Harold ha sido considerado como altamente destructivo, atacando las raíces de la *Lantana*, un nicho en el pasado ignorado en gran medida en los estudios de biocontroladores. Es poco lo que se sabe acerca de este potencial enemigo natural y los atributos de su historia de vida y modo de acción son discutidas. Los adultos se alimentan de hojas y depositan sus huevos en las hojas bajas, cercanas a la superficie del suelo. Las larvas penetran dentro del suelo, donde se alimentan externamente de las raíces secundarias. El tiempo de desarrollo toma alrededor de 60 días durante el verano con el potencial de 2 a 3 generaciones por año en el campo de Sur Africa. Se describen técnicas de cría, siendo notable el uso de una jaula modificada y se comparan los resultados con un medio alternativo de cultivo en cápsulas de petri. Se discuten las implicaciones de la especifidad con el hospedero de *L. columbicus columbicus*, de otras especies de *Longitarsus* y del coquito pulga en condiciones de laboratorio.

Palabras clave adicionales: Cariaquito, comedores de raíces, control biológico clásico, cría en laboratorio.

Abstract

BAARS JR 2001. Biology and laboratory culturing of the root-feeding flea beetle, *Longitarsus columbicus columbicus* Harold, 1876 (Chrysomelidae: Alticinae): a potential natural enemy of *Lantana camara* L. (Verbenaceae) in South Africa. Entomotropica 16(3):149-155.

The introduced ornamental plant, *Lantana camara* L. (Verbenaceae), is one of South Africa's worst invasive weeds. It has been the target of a biological control programme here for the past four decades. Although several natural enemies have been established, the level of control is considered unsatisfactory, and a number of new potential biocontrol agents are being evaluated. The flea beetle *Longitarsus columbicus columbicus* Harold is considered to be highly destructive, attacking the roots of lantana, a niche largely ignored by biocontrollers in the past. Little is known about this potential natural enemy, and attributes of its life history and mode of feeding are discussed. Adults feed on the leaves and deposit eggs in the leaf litter near the soil surface. The larvae burrow into the soil, where they feed externally on the secondary rootlets. Development takes about 60 days during summer, with the potential of 2-3 generations per annum in the field in South Africa. Rearing techniques are described, notably the use of a modified cage, and the results are compared with the alternative of rearing cultures in petri dishes. Implications for the host-specificity screening of *L. columbicus columbicus* and other *Longitarsus* and root-feeding flea beetle species under laboratory conditions are discussed.

Additional key words: External root-feeder, laboratory culture, classical biological control.

Introduction

Several natural enemies have been imported, evaluated and released as biological control agents against *Lantana camara* L. in South Africa (Baars and Neser 1999), and other countries worldwide (Julien and Griffiths 1998). The majority of the agents released are leaf- and flower-feeding and stem-attacking species. With the exception of two cerambycid beetles, *Plagiohammis spinipennis* (Thomson) which bores the stem and root crown, and *Paravander xanthomelas* (Guérin-Méneville) which bores into the stem and large roots, no other root-feeding natural enemies have been considered for release on *L. camara* worldwide.

Root-feeding flea beetles, in particular species of *Longitarsus*, may be able to supplement damage inflicted by the suite of agents already established on lantana.

Field surveys in the countries of origin of the *Lantana* complex have revealed several flea beetle species associated with the plants (Winder and Harley 1983; Palmer and Pullen 1995). The intensity of adult feeding damage noted in the field during exploratory surveys in USA (Florida), Mexico, Jamaica, Trinidad and Venezuela, has encouraged interest in the potential of various root-feeding flea beetles, including species in the genus *Longitarsus*, as biocontrol agents.

Although a few *Longitarsus* species are considered to be polyphagous, most are oligophagous and several have been used as biocontrol agents. Examples include *L. jacobeae* (Waterhouse) and *L. flavicornis* (Stephens) on *Senecio jacobeae* L. (Compositae) (Frick 1970; Frick and Johnson 1973), *L. albinaeus* (Foudras) on *Heliotropium europaeum* L. (Boraginaceae) (Delfosse and Cullen 1981; Huber 1981) and *L. aeneus* Kutschera and *L. echii* Koch on *Echium plantagineum* L. (Boraginaceae) (Wapshere 1982). Other *Longitarsus* species are under consideration as biocontrol agents, including *L. horni* Jacoby on *Chromolaena odorata* (Zachariades et al. 1999), and *L. quadriguttatus* Pont. on *Cynoglossum officinale* L. (Boraginaceae) (Jordon 1997). In addition, the host records of other species, such as *L. columbicus columbicus* Harold that has only been recorded from *Lantana* spp. in Venezuela (Bechyné 1997), indicate the potential of these species as biocontrol agents.

Little is known about the life history and host-range of *L. columbicus columbicus*, besides its association with the *Lantana* complex. This paper investigates the biology and laboratory culturing of this flea beetle, as the first step towards evaluating its potential for release as a natural enemy against *L. camara* in South Africa.

Materials and Methods

A laboratory culture was started with some 55 adults that were collected north of Maracay, along the road to Choroni (lat ° 34'45"N, long ° 34'67"W) in Venezuela, and imported into South Africa in October 1998. Field collections were made with a cupped beating tray, during the late afternoon. Identifications were confirmed by Prof. Vilma Savini, and voucher specimens of the beetles are deposited at the Museo del Instituto de Zología Agrícola (MIZA), Maracay, Venezuela. *Longitarsus columbicus columbicus* belongs to the tribe Longitarsini, and has also been collected from five other sites in Venezuela and one in Colombia (Bechyné 1997). The closely related *L. columbicus centroamericanus* Bechyné has been collected in Guatemala, El Salvador and Nicaragua (Bechyné 1997).

During culturing in quarantine (Pretoria, South Africa) field-collected adults were split into two groups. Twenty adults were exposed to shoot tip cuttings in 15 cm petri dishes, and the remaining 35 adults were exposed to whole plants placed in rearing cages (Figure 1). Each cage comprises a mild steel frame with psylla screen gauze (Climatex cc, South Africa) and is fitted over a plastic trough with drainage. In these cages, three small rooted lantana plants were transplanted into the plastic troughs 2 weeks prior to exposure to promote new root growth. Adults were removed after two weeks and placed into additional cages with fresh plants. Cages were maintained under controlled glasshouse conditions and watered (from above) so as to maintain fairly damp soil conditions. Temperatures ranged from 30°C to 20°C in a day/night cycle, under a natural summer photoperiod of about 14 hours. Emerging first generation adults were recorded and collected weekly, and exposed to similar rearing conditions.

Petri dish rearing trials were conducted under laboratory conditions, in a controlled temperature room at temperatures ranging from 29°C to 22°C in a day/night cycle. Experiments were conducted on laboratory benches under artificial light banks on a 13 hour photophase. Adults were exposed to fresh cuttings every two days. The number of eggs, size of egg clusters, oviposition site, and mode of feeding was recorded. These trials were repeated using newly emerged adults from the first laboratory generation when the preoviposition period was also measured. Eggs were maintained under moist conditions and the duration of embryonic development recorded. Measurements were made with the use of an ocular micrometer mounted on a Wild microscope. Illustrations by the author were made with the use of a *camera lucida*.

Eight dominant *L. camara* varieties from the Mpumalanga, Northern and KwaZulu-Natal Provinces of South Africa were used to rear the beetles in both petri dishes and cages. In the cages, different varieties were used to compare relative susceptibility for feeding and development, which was subjectively rated by the amount of shot hole damage to the leaves, and the number of progeny emerging from rearing cages respectively.

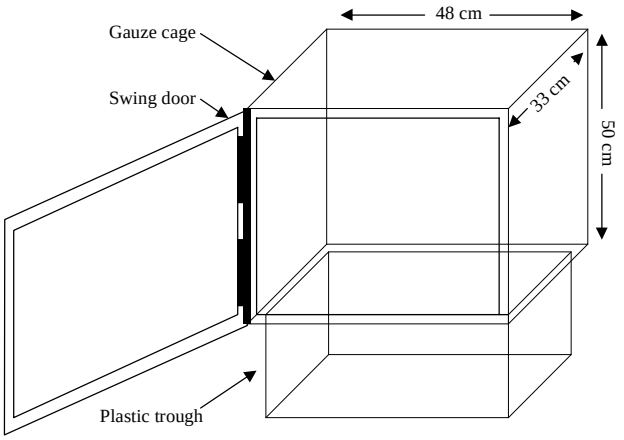

FIGURE 1. Design of cages used to rear *Longitarsus columbicus columbicus* on *Lantana camara* under quarantine glasshouse conditions.

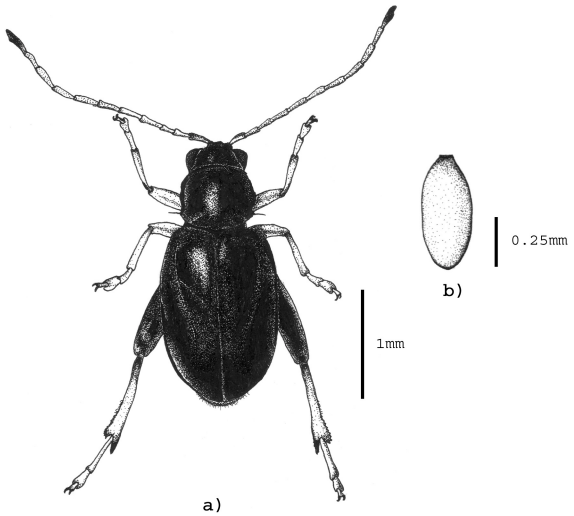

FIGURE 2. Some life stages of *Longitarsus columbicus columbicus*, reared on *Lantana camara* under laboratory conditions. **a)** female adult, **b**) egg.

Results

Biology

Adults of *Longitarsus columbicus columbicus* (Figure 2a) are small (about 2 mm in length) and have a dark copper-brown colour. Adults feed on the leaves causing small shot holes, leaving the upper epidermal layer intact. Field-collected adults survived for two months under laboratory conditions, and remained fertile during this period. Adults emerging from the laboratory cultures survived for up to three months, and displayed a preoviposition period of about 2 weeks.

Adult feeding damage in the field was severe, and was similar to damage observed for other unidentified species of flea beetles (including prob. *Longitarsus* spp.) in Florida and Trinidad in April 1996, Mexico in October 1998, and Jamaica in July 1999. Adults are active and jump readily, and peak activity appears to occur during the cooler hours of the late afternoon, or under cloudy conditions. Adults shelter in the lower parts of the lantana plants, and amongst the surrounding vegetation during the hotter periods of the day.

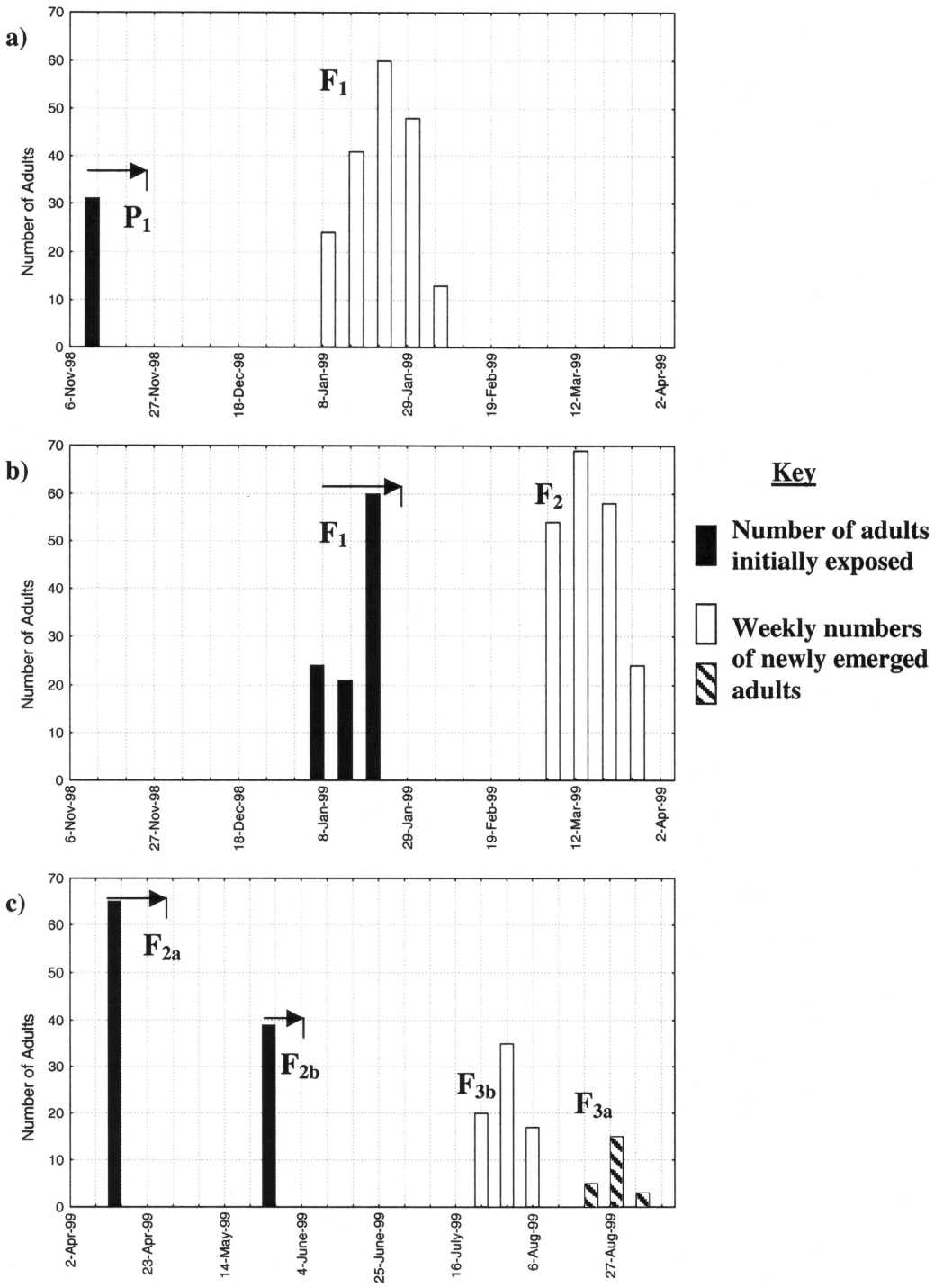

FIGURE 3. **(a)** First, and **(b)** second generations of *Longitarsus columbicus columbicus*, maintained in gauze cages, kept in quarantine glasshouses during summer conditions. **(c)** Third generation *L. columbicus columbicus*, kept in a glasshouse (F_{2a} & F_{3a}), and laboratory (F_{2b} & F_{3b}) during winter conditions. P_1 – field-collected adults; F_1, F_2 & F_3 – First, second and third generation adults; arrows indicate length of adult exposure.

Eggs were deposited under moist filter paper and cotton wool in petri dishes, but are normally deposited under moist leaf litter in cages. The eggs are minute, measuring 0.58±0.01mm (mean ± SE) by 0.25±0.02x10^{-1}mm (n=63) (Figure 2b), and were deposited in groups averaging about 3.2±0.5 eggs (n=26) in the petri dishes. Eggs took 12.0±0.2 days (n=18) to hatch and the emerging larvae are highly active and mobile, but suffer high mortality under dry soil conditions in the laboratory. First instar larvae enter the soil in search of secondary rootlets on which they feed externally. After exposure to a culture of *L. columbicus columbicus* plants were found to be largely devoid of secondary rootlets. Late instar larvae pupate in a hardened soil capsule near the soil surface. Newly emerged adults are active and highly mobile.

The entire life cycle (egg-laying to adult eclosion) took about 60 days in summer. The parent:progeny ratios of cultures in glasshouses, for the first two generations (Figure 3a & b) were high, P:F_1 (35:186) and F_1:F_2 (105:205). Concurrent F_1: F_2 cultures showed similar ratios of 132:215, and 106:201. The third generation development (Figure 3c) varied with the culturing conditions, with a ratio of 65:23 (F_{2a}:F_{3a}) exposed to shorter day lengths during the natural winter conditions in a glasshouse, and 39:79 (F_{2b}:F_{3b}) exposed in a laboratory (as described in the Materials and Methods). Under natural winter conditions adults emerged after about 126 days (Figure 3c F_{2a}:F_{3a}), whereas those under laboratory conditions emerged after about 60 days (Figure 3c F_{2b}:F_{3b}).

Rearing techniques

Adults reared on lantana shoot tips in petri dishes, fed and oviposited readily but experienced high mortality of up to 100% within 2 weeks. Adults were noted to jump readily when disturbed under these confined conditions, notably during the renewal of shoots. Eggs deposited under the moist filter paper were transferred to containers with moist cotton wool and the emerging larvae were transferred to the stem bases of lantana plants grown in 15cm pots. However, these plants periodically dried out and wilted, and no adult emergence occurred under these conditions. By contrast adults survived better under the large cage conditions, with larger numbers of progeny emerging (Figure 3a, b & c). However, plants in cage troughs were prone to invasion by ants, and in these instances larvae and pupae suffered extensively from ant predation.

The intensity of adult feeding damage, and the number of progeny emerging in the first and second generations were noted to be similar on the eight lantana varieties that were exposed in both petri dish and cage experiments. The varieties of *L. camara* used, including orange, pink and white flowering forms, differed in morphological features like leaf hairiness and toughness, factors which seemingly had no noticeable effect on rearing success.

Discussion

Previous attempts to culture an unidentified ?*Longitarsus* species on lantana from Mexico, during the winter period, have proved unsuccessful (Baars and Neser 1999). In this study, a culture of *L. columbicus columbicus* was successfully manipulated to maintain egg laying and immature development under artificial light and temperature regimes. Cultures reared in cages as described in this paper, were maintained under natural winter photo periods in glasshouses. The delayed emergence of the F_3 progeny of *L. columbicus columbicus* exposed to natural winter conditions, suggest a significantly slow developmental rate or a state of diapause was induced. An immature stage probably enters a state of oligopause, as described by Mansingh (1971), which may be induced by a gradual change in photoperiod and/or plant physiology. Presumably, field populations enter a similar state to overcome the drier conditions and plant dormancy in Venezuela.

Laboratory cultures of *L. columbicus columbicus* are best maintained under the caged conditions described above. Under these conditions, adults should be exposed to the plants for short periods (about 2 weeks) to reduce the egg load per cage. Plants with larvae should be carefully watered to maintain intermediate soil moisture levels, in order to promote secondary root development and reduce larval mortality. Although laboratory rearing techniques are largely successful, mass-collection of insects in the country of origin for use in host-specificity tests may provide an efficient alternative. This method has been used for other *Longitarsus* species (Wapshere 1982; Jordon 1997), especially univoltine species, which take too long to culture.

The external mode of larval feeding appears to expose the immature stages of *L. columbicus columbicus* to high rates of mortality during excessive soil desiccation, and also probably excessive soil saturation. Areas infested with *L. camara* in South Africa are generally subject to summer rainfall and dry winters. During winters the topsoil dries excessively forcing the lantana plants into a state of dormancy. As a result natural enemies attacking this niche have to be adapted to cope with these extreme winter conditions. For biocontrol

agents to successfully suppress lantana in the drier areas of South Africa, insect populations must survive the winter period in sufficient numbers to maintain levels of damage on plants during the spring regrowth, as was the case for *L. albineus* on *H. europaeum* (Huber 1981). By means of diapause the pupae of *L. columbicus columbicus* may thus provide a resistant life stage, which is able to cope with the unfavourable winter conditions. Wapshere (1983) discussed the effectiveness of biotypes of *L. jacobaeae*, originating from different geographical regions in Europe, as biocontrol agents in Australia, highlighting the importance of environmental synchrony.

Adults of root-feeding *Longitarsus* spp. that have been employed as biocontrol agents either feed externally, like *L. aeneus* on *Echium plantagineum* (Wapshere 1982) and *L. albineus* on *H. europaeum* (Delfosse and Cullen 1981; Huber 1981), or internally in the root crown, like *L. echii* on *Echium plantagineum* (Wapshere 1982), and *L. jacobaeae* on *S. jacobaeae* (Frick and Johnson 1973). Through resource partitioning, Wapshere (1982) argued that the combined impact of the two *Longitarsus* species on *Echium plantagineum* improved the prospects for control. Similarly the biocontrol programme against *L. camara* in South Africa may benefit if priority be given to determine whether there are *Longitarsus* species, or other root-feeding flea beetles, which feed internally to avoid dry soil conditions. Surveys for other root-feeding agents have so far been conducted in Mexico and Jamaica, in 1998 and 1999 respectively (Baars unpubl.). The Mexican survey, conducted in the northern coastal areas of the Yucatan, Tabasco and Veracruz provinces, resulted in the collection of an unidentified flea beetle (Accession number: AcSN 2431, National Collection of Insects, ARC- Plant Protection Research Institute, Pretoria, South Africa) that has a similar life cycle to *L. columbicus columbicus* except that the larvae feed internally in the cortex of the primary roots.

Lantana camara in South Africa is a highly variable entity with numerous varieties differing in morphology, physiology and genotype (Baars and Neser 1999). Varietal resistance is deemed to have contributed to natural enemies either failing to establish or performing poorly on certain varieties (Cilliers and Neser 1991). It is thus important to expose natural enemies under evaluation to the main varieties of lantana, which have invaded large areas of South Africa. Feeding intensity and progeny development of *L. columbicus columbicus* appeared not to be influenced by the lantana varieties exposed, indicating its potential to cope with this diverse weed.

Conclusions

Field and laboratory observations indicate that *L. columbicus columbicus* is a potentially damaging natural enemy that warrants more intensive host-specificity evaluation in South Africa. *Longitarsus columbicus columbicus* and other root-feeding flea beetles constitute a specialized guild that has not been represented in the biocontrol programme against *Lantana camara* worldwide. The rearing methods discussed are able to supply healthy laboratory cultures for host-specificity screening, but alternative methods such as mass-collections in the country of origin may also prove effective. The seasonal synchrony between *L. camara* and *Longitarsus* species will be an important factor influencing establishment and population increases in South Africa. With reference to the dry winter conditions, further exploration for other internal root-feeding flea beetle species remains a priority.

Acknowledgements

I thank my colleague Dr Costas Zachariades for accompanying me on the survey in Venezuela. Thanks are due to Fritz Heystek for his continual assistance during laboratory culturing. I also thank Beth Grobbelar of the National Collection of Insects (ARC-Plant Protection Research Institute, Pretoria, South Africa) and Prof. Vilma Savini (Universidad Central de Venezuela, Maracay) for their identification support and services, respectively. I would like to thank Profs Carlos Julio Rosales (Universidad Central de Venezuela, Maracay), and Vilma Savini for their assistance during our visits, and the Venezuelan Entomological Society for the opportunity to submit this manuscript. I thank Drs Martin Hill, Terry Olckers and Costas Zachariades for their very valuable comments on the manuscript. This research would not have been possible without the financial support of the National Department of Agriculture, Department of Water Affairs and Forestry and the Agricultural Research Council of South Africa.

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Recibido: 28-vii-2000
Aceptado: 06-viii-2001
Correcciones devueltas por el autor: 29-xi-2001