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Determination of the most effective method for field establishment of biocontrol agents of the genus *Chilocorus* (Coleoptera: Coccinellidae)

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Abstract

The coccinellid biocontrol agent *Chilocorus nigritus* (Fabricius) can be established on the non-target scale *Asterolecanium* sp. (Hemiptera: Asterolecaniidae) on giant bamboo *Dendrocalamus giganteus*. When these sites are adjacent to citrus orchards, the coccinellid readily moves across to reduce population levels of the target prey, red scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae). *C. bipustulatus* (Linnaeus) and *C. infernalis* Mulsant are potential new biocontrol agents against *A. aurantii* in South Africa. Each life stage of *C. nigritus, C. bipustulatus* and *C. infernalis* has advantages and disadvantages for field introductions. This paper investigates different methods of introduction of the different life stages for field establishment. A number of synthetic materials were tested as egg pads for *C. infernalis* without success. Introduction of eggs adhering to stiff paper cards was unsuccessful and impractical for all species. Eggs, young larvae (first and second instars), older larvae (third and fourth instars) and adult *C. nigritus* were introduced into stands of bamboo. The adult was the most suitable life stage for establishment followed by older larvae, then younger larvae, with the least suitable being the egg stage. Adults of the three species were released on to scale-infested bamboo at two sites to evaluate the viability of such introductions and to compare their persistence at these sites. *C. nigritus* and *C. infernalis* persisted at one release site through the winter and the hottest period of the summer.

Introduction

Chilocorus nigritus (Fabricius) is an economically important biocontrol agent of red scale, *Aonidiella aurantii* (Maskell), on citrus in southern Africa (Samways, 1984, 1986, 1988). It has also been utilized by biocontrol specialists in several countries against various other scales on several crops (Samways, 1984). This predator was first observed in southern Africa in the 1970s, possibly having entered the region through dispersal from Réunion, Aldabra or Madagascar (Samways, 1989). *C. nigritus* successfully colonized major portions of the eastern region of southern Africa. This increase in distribution has been aided by releases of insectary-reared beetles (Samways, 1989).

C. bipustulatus (Linnaeus), indigenous to the Mediterranean region (Smith, 1915), is a well known predator of various armoured and unarmoured scale insects (Huffaker & Doutt, 1965; Smith, 1915). *C. bipustulatus* is considered a valuable biocontrol agent of *A. aurantii* on citrus in Israel (Nadel & Biron, 1964; Rosen & Gerson, 1965), and has become established in California where it preys on olive scale, *Parlatoria oleae* (Colvée) (Hemiptera: Diaspididae), (Gordon, 1965; Huffaker & Doutt, 1965). This predator has been imported into South Africa for *A. aurantii* control in hot and dry areas where *C. nigritus* has not been successful, and attempts at establishment are presently under way (M.M. Clark, pers. comm.). The cold tolerant

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C. infernalis Mulsant from the foothills of the Himalayas, is also being cultured in the insectary for release against *A. aurantii* in the colder regions of southern Africa.

The citrus orchard is a relatively hostile environment for the introduction of coccinellids. Spring insecticide applications and frequent crashes in prey population levels make direct establishment in the orchard difficult. However, it is now well known that an important alternative prey for *C. nigritus* is the scale *Asterolecanium* sp. on giant bamboo *Dendrocalamus giganteus* (Samways, 1984). Stands of this alien Asian plant support large populations of this scale insect throughout the year and are regularly seen along water courses in the eastern part of South Africa. This is also the country's major citrus producing area, and as the streams are of great importance for irrigation, many of the bamboo stands are close to the citrus orchards. *C. nigritus* regularly inhabits the bamboo stands and moves across to the citrus when *Aonidiella aurantii* levels begin to increase. This prey-alternating behaviour has enormous economic benefits, and additionally, provides considerable security that the biocontrol oriented pest management programme will be successful.

Although *Asterolecanium* sp. is a highly suitable alternative prey for *C. nigritus*, this does not appear to be the case for the larvae of *C. bipustulatus* and *C. infernalis* (Hattingh & Samways, unpublished). This suggested that the use of bamboo, for establishing and maintaining economically important populations of *C. bipustulatus* and *C. infernalis* in climatic areas unsuitable for *C. nigritus*, was not likely to be successful.

In the insectary, females of the three species lay eggs beneath covers of oleander scale, *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae), and *C. nigritus* and *C. bipustulatus* will also readily oviposit on synthetic egg pads. Large quantities of *C. nigritus* eggs are easily obtained within a few days by draping 0·01 m^2 strips of polyester fibre wadding over vegetables infested with *A. nerii*. *C. bipustulatus* oviposits between the frayed strands along the edges of strips of linen (Nadel & Biron, 1964). These pads are convenient for transport and introduction into the field (Samways & Mapp, 1983). They are also useful for separating eggs and the subsequent young larvae, from the adults in the insectary, thereby avoiding cannibalism. However, the ability of newly emerged larvae to escape from these pads was unknown. There appear to be no reports of a suitable artifical oviposition substrate for *C. infernalis*.

The use of egg pads was investigated, as was the viability of using cardboard cards on to which the eggs were glued. It was the commercial distribution of glasshouse whitefly 'pupae', (*Trialeurodes vaporariorum*, Westwood, Hemiptera: Aleyrodidae) parasitized by *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) glued on to cards (Scopes & Pickford, 1985) that stimulated this latter approach.

All three species have been successfully reared in the insectary on *A. nerii*. This scale is extensively used because of the ease with which it can be cultured (Samways & Tate, 1986) and its ready acceptance by the three beetle species. These insectary reared beetles have been released on to *A. aurantii* on citrus and *Asterolecanium* sp. on bamboo during field introductions. Samways & Wilson (1988) reported a reduction in feeding rate by *C. nigritus* larvae following a transfer from *A. nerii* to *A. auranti* in the laboratory. The deleterious effects of a change in prey type have been observed for larvae and adults ~~of~~ all three species, and appear to be more acute among the larvae than the adults (Hattingh & Samways, unpublished). This may influence the suitability of the various life stages reared on *A. nerii*, for field releases onto *A. aurantii* or *Asterolecanium*.

The introduction of eggs avoids the problem of a forced change in prey type. Many more eggs than adults can be introduced, with less insectary work required. However, the susceptibility of the eggs to the environmental extremes in the field is unknown. Also, the time required for larval development, during which *Chilocorus* spp. are particularly vulnerable, before reproduction can begin, is a disadvantage.

The introduction of larvae requires less insectary work than adults, and therefore more individuals can be released. However, the larvae are difficult to work with, each must be individually transferred with a soft paint brush. Additionally, they are apparently more susceptible than adults to the deleterious effects of a change in diet (Hattingh & Samways, unpublished). Samways & Wilson (1988) showed that the younger *C. nigritus* larvae are incapable of eating the mature *A. nerii* and *A. aurantii* females on which fourth instar larvae and adults can feed.

In contrast, with adults, after mortality due to the rearing process, only a limited number of individuals are available for release. Should these then disperse extensively on release, there is little chance of establishment. Hattingh & Samways (1990) showed that high density of *Chilocorus* spp. during transport and after intensive release did not result in greatly increased dispersal, owing to intraspecific interference. However, other factors such as the physical disturbance of handling, hunger levels, physical surroundings at the release site, and depletion of prey due to exploitation, are still possible causes of increased dispersal at the point of release.

The role of bamboo for field introductions of the three *Chilocorus* spp. was determined. The use of egg pads for insectary rearing and field introductions was investigated. The various life stages of *C. nigritus* for field releases were evaluated.

Materials and methods

Egg pads

Materials were tested as egg pads for *C. infernalis* by placing 10 pads of each in a rearing cage with approximately 200 adults of all ages. The pads were inspected for eggs daily on 10 consecutive days. Materials tested were polyester fibre wadding as used for *C. nigritus*, frayed linen as used for *C. bipustulatus*, double layered paper towelling, cotton wool, surgical gauze and open cell sponge.

We attempted to remove *C. nigritus* and *C. bipustulatus* eggs from the pads for attachment to cards with various adhesives. The pads were gently teased with a soft paint brush and the polyester fibre pads were stretched and compressed to release the eggs.

Experimental sites

Our ability to judge the level of scale infestation on the bamboo stems by observation was quantified. Three

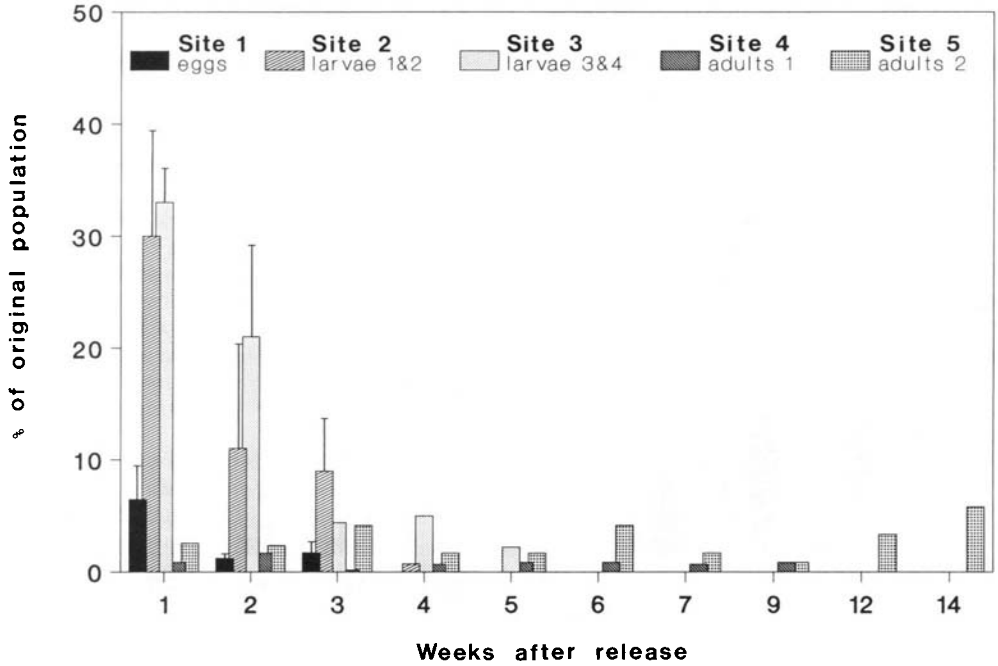

Fig. 1, Average percentages + 1SE and percentages of numbers released on 12 December 1988, observed during subsequent sampling at release sites, plotted against weeks after release, for various life stages of *Chilocorus nigritus*, 6 replicates for each immature life stage

sampling units were obtained from each of three of the release sites and two sampling units from an additional stand. Each sampling unit was a sliver of bamboo stem, and all were judged to be equally infested with all life stages present. A cardboard sheet with a rectangular window 40 mm × 10 mm, was attached to the surface of each sampling unit. The numbers of live and dead mature *Asterolecanium* sp. were counted using a dissecting microscope with 40 × magnification.

All life stages of the scale insects can be found on bamboo in the Pietermaritzburg area throughout the year provided that extreme heat, cold or drought are not experienced. Qualitative observations were made of the scale population levels throughout the experimental period.

Five sites for evaluating the life stages of released *C. nigritus* were selected in parks and open plots in and around Pietermaritzburg, Natal, 29°35'S, 30°25'E. These sites were stands of bamboo with similar levels of *Asterolecanium.* infestation and of approximately equal proportions; being the density of the leaf canopy, the surface area covered (95 m²), the number of stems (120), and the height of the stems (15 m). The persistence of adults of the three species at the release sites were compared at two localities. These were Site 5, used in the evaluation of life stages and Site 6, 800 m west of Sites 2 and 3 and complying with the standards set for the other sites.

Evaluation of life stages

The arenas for the introduction of *C. nigritus* larvae and eggs were 2 m sections of bamboo stems with similar levels of *Asterolecanium* sp. infestation. Six stems per site were chosen, and care was taken to obtain similar distributions of the stems within each stand. The arena was enclosed by two barriers, each consisting of a double layer of 'Bidim fibre' grade U 24 (used in the manufacture of ant bands to prevent access of ants to the canopy of citrus trees) wrapped around the stem. The lower barriers were 0.3 to 1 m above the ground. All side branches and branches from other plants in contact with the arenas were cut away to prevent the larvae from escaping.

Adult *Chilocorus* spp. for release were transferred from the rearing cages well stocked with *Aspidiotus nerii.* They were transported in brown paper bags 200 mm × 120 mm × 60 mm and released within one hour. The larvae were transported on the surface of *A. nerii* infested butternuts (*Cucurbita moschata* cultivar), and transferred to the bamboo stems with a soft paint brush. Adults, larvae and eggs were introduced into the bamboo stand on 12 December 1988.

At Site 1, 100 *C. nigritus* eggs in polyester fibre pads were pinned to the stem in each of the six arenas. Site 2 was 2 km north of Site 1, and here 50 first and second instar *C. nigritus* larvae were released per arena. Site 3 was a separate stand of bamboo 5 m from Site 2. Here 30 third and fourth instar *C. nigritus* larvae were released per arena. At Site 4, which was 5 km east of Sites 2 and 3, 120 *C. nigritus* adults were released from six brown paper bags. Each bag was opened carefully and pinned to the base of a bamboo stem in the centre of the stand. Site 5 was 600 m from Site 4, and here 120 adult *C. nigritus* were gently brushed out of brown paper bags on to the foliage in the centre of the stand of bamboo.

The number of coccinellid larvae in the arenas at Sites 1, 2 and 3 were monitored weekly. For the first seven weeks the adult release sites 4 and 5 were sampled weekly and thereafter every two or three weeks for the next 42 weeks. Sites 4 and 5 were sampled by spending 20 min searching each stand. The stems were not

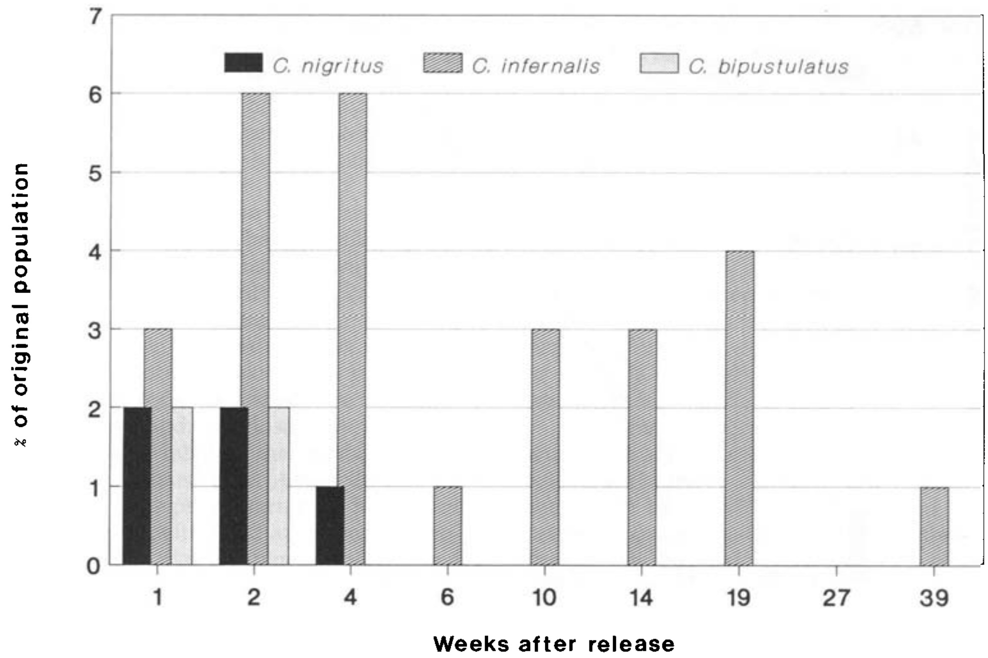

Fig. 2, Percentages of numbers of *Chilocorus bipustulatus*, *C. infernalis* and *C. nigritus* adults released at Site 5 on 10 April 1989, observed during subsequent sampling, plotted against weeks after release.

climbed, and only those coccinellids positively identified were recorded. Recognition of *C. nigritus* from more than 2 m was impossible since they could no longer be distinguished from the plentiful coccinellid *Exochomus flavipes* (Thunberg). None of the three *Chilocorus* spp. was present at the experimental sites prior to commencement of the trials, and there had been no previous reports of their occurrence in this area.

Species comparison

At Site 6, 50 adults of each species were released from brown paper bags in the centre of the stand on 10 April 1989. At Site 5, 100 adults of each species were released in the same way on the same day. The *C. nigritus* adults released at Site 5 were marked with a dot of white 'Tippex' (typing correction fluid) on one elytron. This was done to distinguish the adults in this release from those already on the bamboo as a result of the previous release during evaluation of the life stages. No apparent deleterious effects of such markings were observed in the insectary. These sites were sampled twice during the first two weeks, and then twice at two-weekly intervals, and thereafter monthly.

Voucher specimens of the coccinellids are located in the insect collection, South African Museum, Cape Town.

Results

Egg pads

None of the materials tested was accepted by *C. infernalis* as an oviposition substrate. *C. nigritus* and *C. bipustulatus* eggs could not be removed from the polyester fibre and frayed linen pads respectively, as the eggs were securely cemented to the fibres. Those eggs which did come free had damaged choria and soon became dehydrated. *C. nigritus* and *C. bipustulatus* larvae hatching from eggs in pads did not have difficulty in escaping from the tangled fibres. No larvae were found trapped or dead in any of the egg pads used in insectary rearing.

Experimental sites

The mean number of live mature *Asterolecanium* sp. per 400 mm^2 was 256 (n = 11, 1 SE = 42), and the mean number of dead scales was 149 (n = 11, 1 SE = 43). The small SE indicates that our judgement of the level of infestation was satisfactory.

Sites 1, 2, 3, 4, and 6 were on the banks of continuously flowing water courses and were therefore not exposed to drought. The temperatures during the experimental period were not extreme for this region. Regular qualitative observations showed that the scale population remained stable with all life stages present throughout the experimental period at Sites 1, 2, 3, 4 and 6. There was a slight increase in numbers of young scale in spring with a population peak in mid to late summer. At Site 5 the scale population was similar to the other sites for the duration of the life stage evaluation experiment. The plants at this site showed signs of water stress from 19 to 29 weeks after release in the species comparison experiment. This resulted in the loss of all the leaves from the plants and it became extremely difficult to find live scale insects.

Evaluation of life stages

At Site 1, the highest count of larvae, from the 600 introduced eggs, was one week after introduction, when 30 larvae were observed (fig. 1). Only five of these attained a size indicative of the fourth instar. None of these larvae pupated, and after three weeks no more live individuals were found.

The introduction of first and second instar larvae at Site 2 was more successful (fig. 1). The mortality rate was still very high with only 9% of the larvae surviving the first three weeks to pupation. After four weeks, only two adults were found and thereafter no live individuals were recorded. SE measurements (fig. 1) were possible for larval counts only since adults were not restricted to the arenas and thereby replication was lost. At Site 3, 21% of the released third and fourth instar larvae had pupated by the end of the second week. The maximum count of adults at Site 3 was 5% of the original number after four weeks. No individuals were recovered after six weeks.

Counts of 0 to 2 *C. nigritus* adults were obtained at the adult release Site 4 for the first nine weeks, and thereafter none was observed (fig. 1). The number of positive identifications of *C. nigritus* adults at Site 5 remained low at 1 to 9 per sample. However, 49 weeks after release, adults and larvae were still present at this site. This colony had survived through the summer and the coldest part of winter.

Species comparison

The release of adults of the three species at Site 6 was not successful. One week after release, no *C. nigritus* or *C. infernalis* were found, and only one *C. bipustulatus* was sighted. Two weeks after release, one *C. bipustulatus* and one *C. infernalis* were found and thereafter no more specimens were observed.

The release of adults of the three species at Site 5 led to *C. bipustulatus* adults persisting at the site for two weeks (fig. 2). Marked *C. nigritus* adults were present for four weeks, which could possibly have been long enough to produce a second generation. Unfortunately, individuals of the new generation could not be distinguished from those of the previous release and their offspring.

C. infernalis were present at Site 5 in every sample for the first 19 weeks (fig. 2). However, there was a qualitatively observed gradual reduction with time in the size of adults observed. The mean weight of *C. infernalis* adults reared in the insectary on *Aspidiotus nerii*, and used for releases on bamboo was 12.1 mg, ($n = 40$, 1SD = 1.6). The specimen located at the release site at 39 weeks after release was captured and weighed 6.9 mg. After 19 weeks a very dry period was experienced, the bamboo lost all leaves and live scale insects could not be found. In spite of this stressful period of 10 weeks, *C. infernalis* adults were still present 39 weeks after release. This population had therefore survived the whole winter and the severest part of the summer.

Discussion

The low percentage recovery of first instar larvae from eggs and the poor survival of larvae, particularly the earlier instars, make eggs laid in pads unsuitable for field introductions. These pads nevertheless remain useful for harvesting eggs in the insectary. This is necessary to culture these *Chilocorus* spp. efficiently, as the rate of egg cannibalism by adults and larvae can be high (Hattingh, unpublished). Adults are the most suitable life stage for field introductions. Introduction of *C. nigritus* adults on to bamboo to obtain establishment, before natural dispersal and colonization of the citrus orchard, can be recommended. Field introduction of *C. bipustulatus* on to bamboo in the Natal midlands region is less likely to result in establishment than similar introductions of the other two species.

The prolonged survival of the *C. infernalis* colony on bamboo was surprising, since the *Asterolecanium* sp. is considered a sub–optimal diet for the larval development of this ladybird (Hattingh & Samways, unpublished). They found that *C. infernalis* larvae reared on *Asterolecanium* sp. in the laboratory produced adults which were significantly smaller than those obtained with a suitable larval diet such as *Aspidiotus nerii*. The gradual reduction with time in the size of *C. infernalis* adults observed in the stand of bamboo, was probably due to this dietary deficiency. In spite of an unsuitable prey type and the drought, *C. infernalis* survived through the severest part of the summer and winter. This suggests that *C. infernalis* is a good biocontrol candidate for survival in the Natal midlands region, but field introduction of this species on to bamboo is not advisable, making introduction directly into the citrus orchard necessary.

The variable results obtained with comparable releases in separate but very similar sites, demonstrates that introduction into an orchard without establishment does not indicate that an introduction into another nearby orchard will not be successful. The only observed difference between Sites 4, 5 & 6 at which adults were released, was the vegetational surroundings. The most successful site, No. 5, was in the middle of a lawn with the nearest shrub or tree 50 m from the stand. At Sites 4 and 6 many large trees grew within 5 m of the stands. The persistence of adults at Site 5 could be due to less dispersal from the release site. There was no large barrier of open space surrounding Sites 4 and 6, and more dispersal into the adjacent trees could be expected.

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