THE SUCCESSFUL INTRODUCTION TO AUSTRALIA OF *DIVERSINERVUS* SP. NEAR *STRAMINEUS* COMPERE (HYMENOPTERA: ENCYRTIDAE), KENYAN PARASITOID OF GREEN COFFEE SCALE

D. Smith[1], D.Papacek[2] and C. Neale[3]

[1]Maroochy Research Station, Queensland Department of Primary Industries, Box 5083 SCMC, Nambour Qld. 4560, Australia
[2] Citrus Consultant, 28 Orton Street, Mundubbera Qld. 4626, Australia
[3]Queensland Department of Primary Industries and Fisheries, Meiers Road, Indooroopilly Qld. 4068, Australia
Email: Dan.Smith@dpi.qld.gov.au

Summary

The Encyrtid parasitoid *Diversinervus* sp. near *stramineus* Compere was introduced from coastal Kenya to Australia for the biological control of green coffee scale *Coccus viridis* (Green) infesting citrus, coffee and ornamentals. After quarantine testing against 16 other native and introduced insect scales and mealybugs, *D.* sp.nr *stramineus* was found to be specific to *C. viridis* and releases were made throughout Queensland and northern New South Wales. The parasitoid's effectiveness was assessed in three citrus blocks in southeast Queensland and in a coffee block in north Queensland over three to four years. The parasitoid established readily, becoming the dominant natural enemy within 12 months of release with parasitism levels of up to 80%. *D.* sp.nr *stramineus* has established throughout Queensland and northern New South Wales and together with the existing aphelinid parasitoids *Coccophagus ceroplastae* (Howard) and *Eurischomyia flavithorax* (Girault and Dodd) and the fungal pathogen *Verticillium lecanii* (Zimmerman), has reduced *C. viridis* to minor pest status. Three other parasitoids *Metaphycus baruensis* (Noyes), *Metaphycus stanleyi* Compere and *Coccophagus bogorensis* (Koningsberger) were introduced to quarantine but not successfully reared on *C. viridis*.

Keywords: citrus, green coffee scale, biological control, Queensland.

INTRODUCTION

Green coffee scale, *Coccus viridis* (Green) (Homoptera: Coccidae) is described as being nearly cosmopolitan in tropical and subtropical areas (Hamon and Williams 1984). In addition to Australia, it occurs in east Africa, Madagascar and islands of the Indian Ocean, Central and South America, Southeast Asia, Indonesia, Papua New Guinea, islands of the western Pacific, Hawaii and Florida (Ben-Dov 1993, Waterhouse and Sands 2001). Its centre of origin is most likely East Africa. In Australia, it infests the leaves and shoots of citrus, coffee and a range of ornamentals, producing copious honeydew and consequently sooty mould and attracting large numbers of ants. Ants interfere with natural enemies of the scale and may also reduce scale crawler deaths by preventing the accumulation of excess honeydew, moisture and pathogens. There is usually a serious problem with sooty mould on citrus fruit at harvest if 5% or more of green twigs are infested with scale (Smith *et al*. 1997). In Australia, *C. viridis* is attacked by the parasitic wasps, *Coccophagus ceroplastae* (Howard) and *Eurischomyia flavithorax* Girault and Dodd (Hymenoptera: Aphelinidae) and the ladybird *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) which preys on young scale. The fungus *Verticillium lecanii* (Zimmerman) can also cause up to 90% mortality of the scale following prolonged wet weather in summer-autumn. In spite of these natural enemies, serious infestations sporadically occur in citrus and coffee (Smith *et al*. 1997).

This paper describes the introduction, host specificity testing in quarantine, establishment and assessment of impact on *C. viridis* by *Diversinervus* sp. nr *stramineus* Compere (Hymenoptera: Encyrtidae) from Kenya. Brief reference is made to the attempted introduction of two other parasitoids – *Metaphycus baruensis* Noyes from Papua New Guinea (originally via Kenya) and *Metaphycus stanleyi* Compere from California and Kenya (Hymenoptera: Encyrtidae), and *Coccophagus bogorensis* (Koningsberger) (Hymenoptera: Aphelinidae) from Thailand.

MATERIALS AND METHODS

Overseas surveys for parasitoids of C. viridis and their introduction to quarantine in Brisbane

Kenya: The Kenyan introductions followed a survey of *C. viridis* and its parasitoids on citrus in coastal Kenya (Varela *et al*. 1995). Interest in Kenya as a source of parasitoids for *C. viridis* followed previous surveys by Ingram (1983) and Murphy (1991, 1998). Coffee green scales *Coccus celatus* De Lotto, *Coccus alpinus* De Lotto (Hemiptera: Coccidae) and *C. viridis* (De Lotto 1960) were surveyed in Kenya during the 1980s for parasitoids suitable for introduction to coffee plantations in Papua New Guinea. *M. baruensis* was collected from *C. celatus* and then successfully established in the Papua New Guinea Highlands (Noyes 1988). Varela *et al*. (1995)

found that there were three major parasitoids in *C. celatus* and *C. alpinus* on coffee above 1000 m - *M. baruensis*, *M. stanleyi* and *D.* sp. nr *stramineus*. As *C. viridis* was scarce at this altitude and occurred mostly below 500 m, the survey for parasitoids in this scale was done on citrus, mostly in prison farms in the Coast Province where the most common parasitoid collected was *D.* sp. nr *stramineus*. Other primary parasitoids from *C. viridis* were *M. stanleyi*, two undescribed *Metaphycus spp*. and an *Aprostocetus sp*. (Hymenoptera: Eulophidae). *D.* sp.nr *stramineus* and *M. stanleyi* were imported into quarantine facilities in Brisbane in 1996.

Thailand: A survey was also made for parasitoids of *C. viridis* in Thailand on frangipanni at Chon Buri, and on *Ixora chinensis* and coffee at Chiang Mai. The main species collected, *C. bogoriensis*, was introduced into quarantine facilities in Brisbane (Smith and Papacek 1993a).

Papua New Guinea: The Coffee Research Institute in Papua New Guinea imported *M. baruensis* and *M. stanleyi* with the assistance of the International Institute for Biological Control at Nairobi in Kenya during the 1980s. *M. baruensis* was successfully established but *M. stanleyi* did not survive transit. Williams (1982, 1986) reported *M. baruensis* to be successfully established in both *C. celatus* and *C. viridis* in Papua New Guinea. A survey was made of coffee areas in highland Papua New Guinea in 1993 (Smith and Papacek 1993b, Smith 1998) and *M. baruensis* was imported to quarantine facilities in Brisbane. *M. stanleyi* was imported at the same time from Riverside in California.

Rearing of parasitoids in quarantine

C. viridis was reared on potted gardenia and coffee bushes 0.5-1.0 m high. *M. baruensis* showed no interest in *C. viridis* in the glasshouse and similar observations were made as to its host preferences in coffee and citrus in the field in Papua New Guinea. This parasitoid was, however, very active in *C. celatus* in Papua New Guinea giving 25-90% parasitism (Smith and Papacek 1993b, Smith 1998). It was attacked by at least one hyperparasitoid, *Cheiloneurus sp*. (Hymenoptera: Encyrtidae) in Papua New Guinea.

M. stanleyi reproduced on *C. viridis* in the glasshouse but this scale appeared not to be a preferred host and colonies died out after about 12 months.

C. bogoriensis was an active parasitoid on *C. viridis* in Thailand. However, only one consignment of the parasitoid could be collected and it was not successfully reared in quarantine at Brisbane. It is likely that the male is hyperparasitic on the female parasitoid and staggered introductions are necessary to establish a colony (G. Walter pers. comm. 1993).

D. sp. nr *stramineus* was obtained from the International Institute of Biological Control at Nairobi in Kenya in 1996. It established in the laboratory readily on *C. viridis* and was the only one of the four parasitoids to proceed to host specificity testing prior to release. This species was described by Compere (1938) and referred to by Prinsloo (1985). Noyes (1998) lists *C. celatus*, *C. alpinus* and *Saissetia persimilis* Newstead (Hemiptera; Coccidae) as hosts. The imported material was examined by both Prinsloo and Noyes (cited as pers. comm. in Malipatil *et al*. 2000). Prinsloo considered the *Diversinervus* to be possibly a new species and Noyes considered it *D. stramineus* with some minor geographic variations. Both males and females of the imported material are dark brown to black. Until the issue is clearly resolved it was considered appropriate to treat the species as 'near *stramineus*'.

Host specificity testing

The host species tested, the details of the testing and the results are listed in Table 1. Non-choice oviposition tests were conducted in maximum-security quarantine at the Alan Fletcher Research Station, Brisbane. Tests and controls were carried out in four-sided perspex boxes with a gauze sleeve at each end, or aluminium framed, fine gauze cages. The box or cage used depended on the size of the plant material needed for the test species. Boxes and cages used ranged in size up to 100 x 100 x 80 cm. In the majority of cases each species was tested at least three times. The exceptions were *Eriococcus tepperi* Maskell (Hemiptera: Eriococcidae) and *Phenacoccus parvus* Morrison (Hemiptera: Pseudococcidae) which were tested twice and *Coccus longulus* (Douglas) (Hemiptera: Coccidae) for which only one test was performed. A small piece of wax paper, finely smeared with honey, was attached to the side of the box or cage as a food source for the parasitoids. Development of *D.* sp. nr *stramineus* (from egg to adult) took 21 days at temperatures varying from 20°C at night to 30°C during the day.

Test scales or mealybugs of a range of instars on appropriate plant material were placed in the perspex box or cage. Controls consisted of a range of instars of *C. viridis* on a potted gardenia plant. One control was set up to correspond to all tests set up on a particular day. Approximately fifteen female and five

male *D.* sp. nr *stramineus* were used in each test and control. All parasitoids used in tests set up on a particular day were collected from the same colony at the same time and randomly placed in the tests and control. The test species and control were exposed to the parasitoids for seven days, after which all parasitoids were removed. Individual *D.* sp. nr *stramineus* were only used once. During the seven days, tests and control were checked every second day and parasitoid behaviour observed. Tests were checked regularly for any emerged parasitoids and continued until all parasitoids had emerged from the control. When parasitoids had finished emerging from the control, the plant material containing the test species was microscopically examined. Scales or mealybugs were counted and checked for any sign of parasitism. Numbers above 100 were rounded to the nearest ten. The gardenia plant from the control was also examined and parasitised and unparasitised *C. viridis* counted.

Release and establishment

Approval to release *D.* sp. nr *stramineus* was given in 1999. Initially the parasitoid was reared in the glasshouse on *C. viridis* on gardenia and coffee. Small colonies of between 50 and 100 adult parasitoids were released at several dozen sites, mostly in citrus throughout southeast Queensland – Nambour, Beerwah, Gayndah, Mundubbera and in coffee at Mareeba in north Queensland.

Assessment of effectiveness

D. sp. nr *stramineus* was released and studied in three citrus blocks - at Nambour and Beerwah (27°S, 153° E) and Mundubbera (26°S, 151°E) in southeast Queensland, and in one coffee block at Mareeba (16° S, 145° E) in north Queensland. At Nambour the study site was a 0.5 ha block of 20 year-old Late Valencia oranges. At Beerwah a 2 ha block of five year-old Late Valencia oranges was used and at Mundubbera, effectiveness was assessed in a 0.5 ha block of five year-old Washington Navel oranges. The coffee block at Mareeba was a 2 ha patch of 3 m high coffee. About 1000 parasitoids were released into each block between February and April 1999. One hundred scale-infested leaves were randomly sampled at about monthly intervals for two to three years. Assessment was then made of the percentage of live adult scales; parasitism by *D.* sp. nr *stramineus*, *C. ceroplastae* or *E. flavithorax*; and, scales affected by the pathogen *V. lecanii.*

RESULTS

Host specificity tests

After seven days, between 0 and 18 adult *D.* sp. nr *stramineus* remained alive in the tests and between 0 and 17 in the controls. During the seven days, in tests containing 11 of the test species, parasitoids were observed on the sides of the box or cage and on the plant material. There were no observations of any contact between parasitoids and test scale, or mealybugs. In the other five species parasitoids were only observed on the box or cage walls. In the controls, *D.* sp. nr *stramineus* were observed on the sides of the box and on leaves and stems of the gardenia. The results of the host specificity tests and controls are shown in Table 1. Parasitism occurred in all controls. There was no emergence of *D.* sp. nr *stramineus* from any of the test species.

Field effectiveness

Parasitism and disease levels at the four sites are shown in Figures 1-4.

At Nambour (Figure 1) *C. ceroplastae* dominated before release of *D.* sp. nr *stramineus* with 10-35% parasitism. *V. lecanii* killed about 10% of scales. Twelve months later in early 2000, *D.* sp. nr *stramineus* was well established throughout the block and peaked at 80% parasitism. The percentage of live scales dropped from 50-80% to below 10% and the scales became difficult to find.

At Beerwah (Figure 2), parasitism by *C. ceroplastae*, and scales killed by *V. lecannii* remained below 10% during the study. Within 12 months, parasitism by *D.* sp. nr *stramineus* rose to over 80%. The percentage of live scales dropped to below 10% and again scales became difficult to find.

At Mundubbera (Figure 3), parasitism by *C. ceroplastae* averaged 20% before release of *D.* sp. nr *stramineus*. A second parasitoid, *E. flavithorax* averaged 5-10% parasitism but there was little evidence (in this drier sub-coastal area) of *V. lecanii*. Parasitism by *D.* sp. nr *stramineus* rose to 80% within 12 months. The percentage of live scale dropped to 5-20% and again scales became difficult to find.

At Mareeba (Figure 4) in coffee, parasitism by *C. ceroplastae* was usually below 10% and increased to 30% only periodically. *V. lecanii* killed about 5% of scales. Parasitism by *D.* sp. nr *stramineus* reached approximately 70% after 12 months. The percentage of live scales dropped to below 10% and scales again became very scarce.

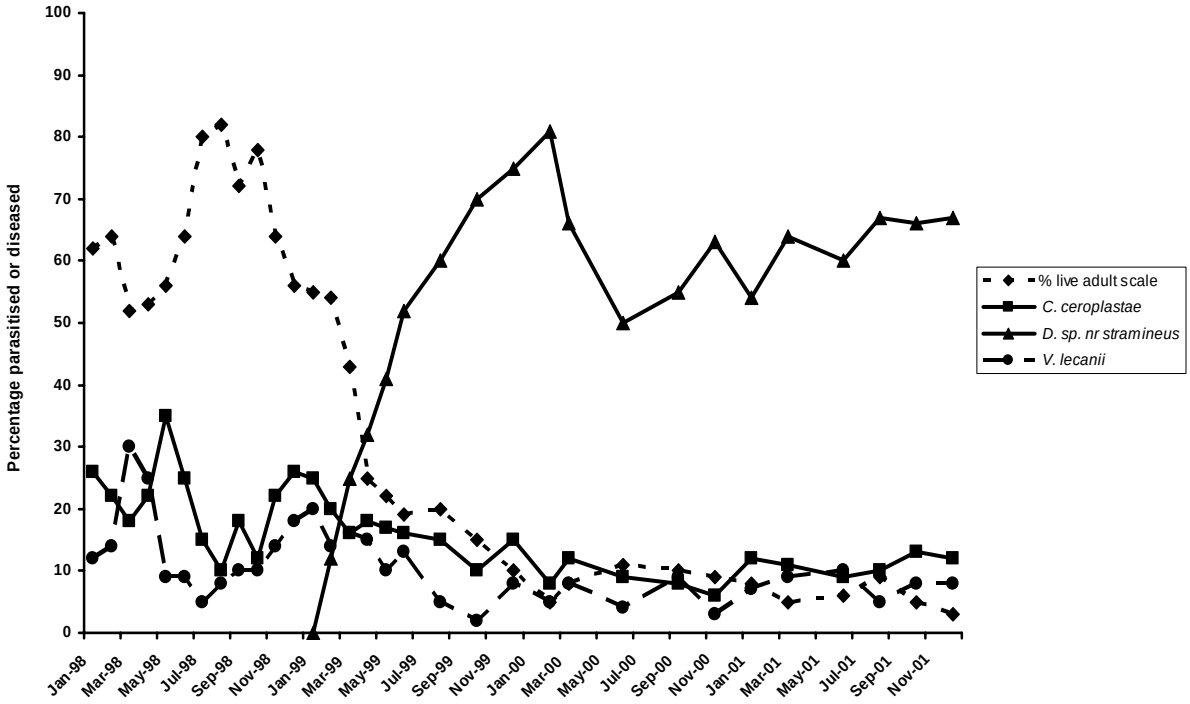

Figure 1. Parasitism and disease levels in *Coccus viridis* in citrus at Nambour 1998-2001.

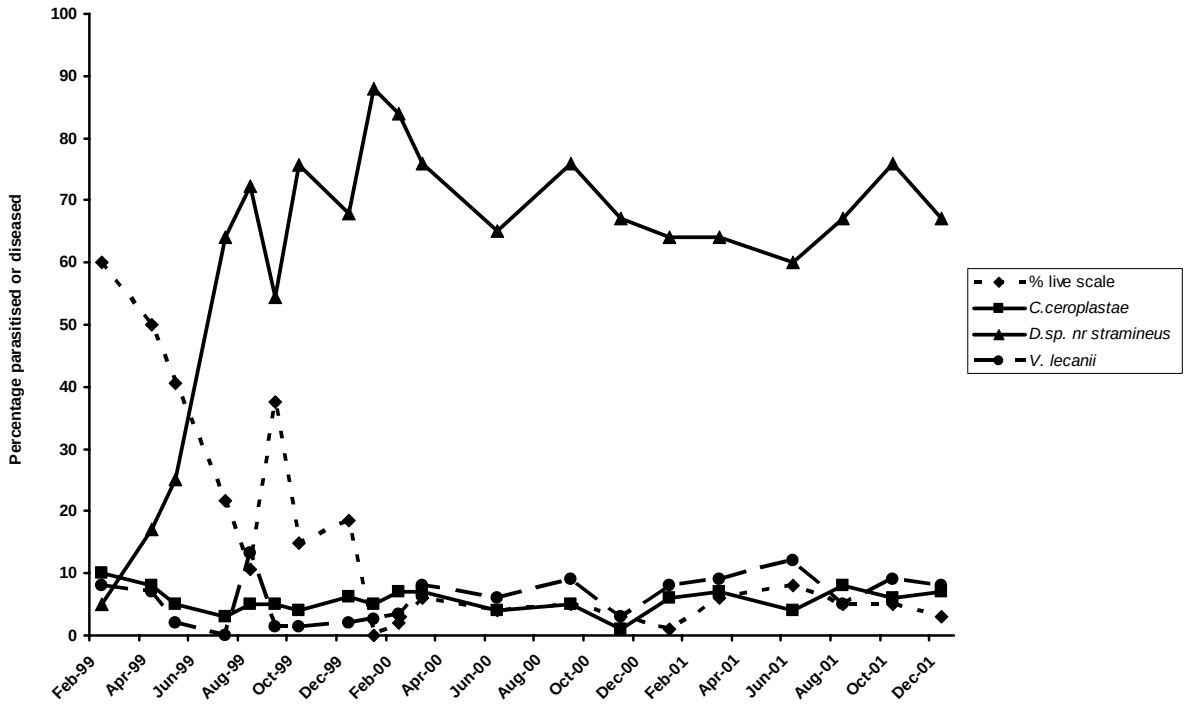

Figure 2. Parasitism and disease levels in *Coccus viridis* in citrus at Beerwah 1999-2001.

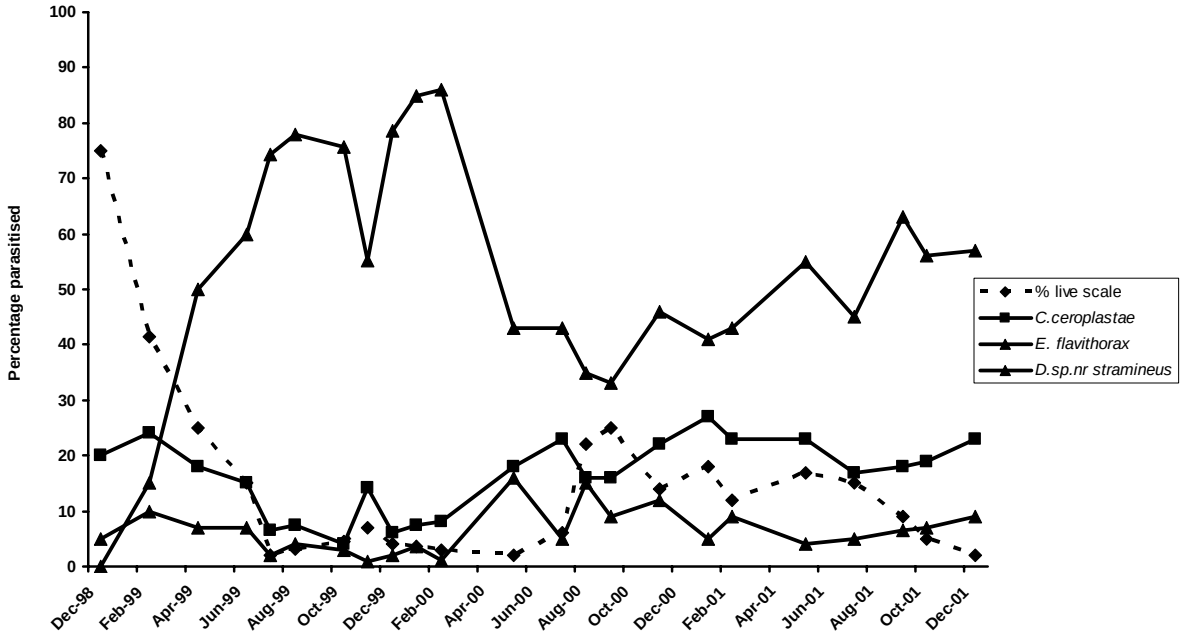

Figure 3. Parasitism in *Coccus viridis* in citrus at Mundubbera 1998-2001.

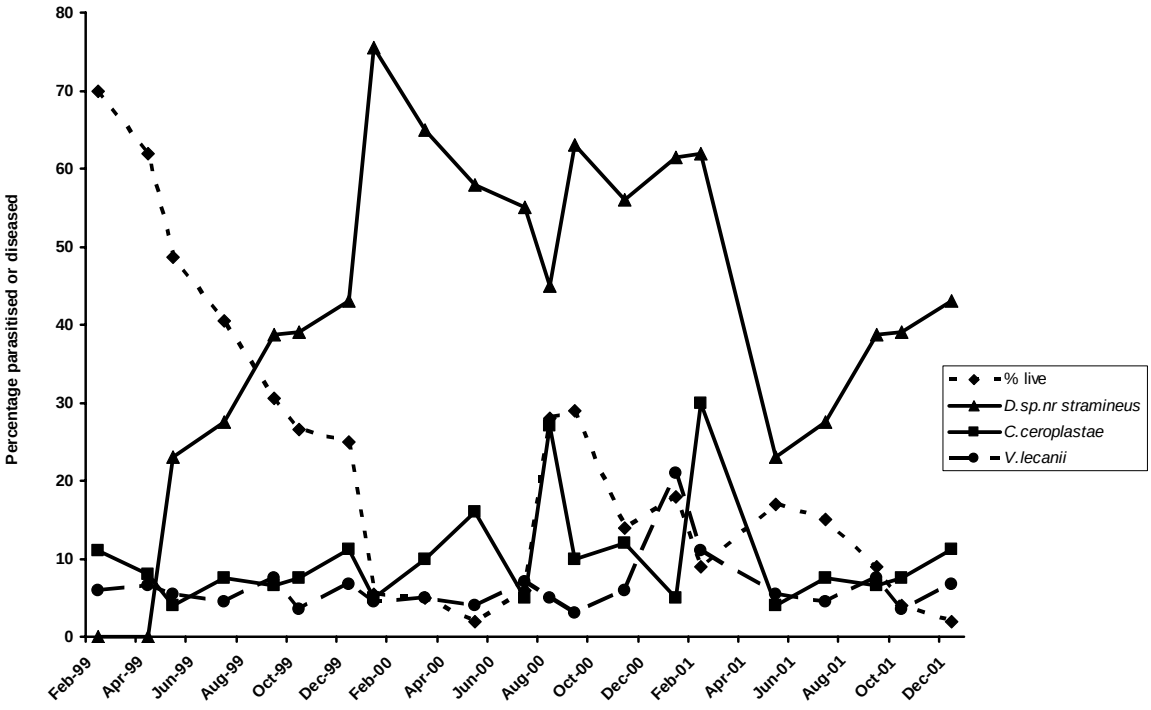

Figure 4. Parasitism and disease levels in *Coccus viridis* in coffee at Mareeba 1999-2001.

Table 1. Results of host specificity tests and controls for *D.* sp. nr *stramineus.*

Species tested	No. of tests	No. of test individuals exposed	Mean no. exposed/test	Total no. *D.* sp. nr *stramineus* emerged	Mean no. *D.* sp. nr *stramineus* emerged/test
*Coccus viridis**	15	4480	298.7	1864	124.3
Pulvinaria urbicola	4	320	80	0	0
Pulvinaria psidii	5	370	120	0	0
Pulvinaria dodonaeae	3	1440	480	0	0
Saissetia mirifica	3	305	101.7	0	0
Saissetia coffeae	4	565	141.3	0	0
Cryptes baccatus	3	230	76.7	0	0
Coccus longulus	1	150	150	0	0
Ceroplastes rubens	3	440	146.7	0	0
Eriococcus coriaceus	3	160	53.3	0	0
Eriococcus tepperi	2	185	92.5	0	0
Lindingaspis rossi (on Pandanus)	3	660	220	0	0
Lindingaspis rossi (on sedge)	3	890	296.7	0	0
Abgrallaspis cyanophylli	3	330	110	0	0
Gymnaspis aechmeae	3	3650	1216.7	0	0
Hemiberlesia palmae	3	265	88.3	0	0
Hypogeococcus festerianus	3	280	93.3	0	0
Phenacoccus parvus	2	170	85	0	0

* Controls

DISCUSSION

D. sp. nr *stramineus* is specific to *C. viridis*. It is the dominant parasitoid in lowland Kenya and established very easily in Queensland from release colonies with as few as 50 individuals. It became the dominant parasitoid of *C. viridis* in the four trials (three in citrus and one in coffee), reaching parasitism levels of up to 80% and reducing troublesome infestations to low levels within 12 months. Within two to three years the parasitoid became established throughout southeast Queensland, coastal north Queensland and coastal northeast New South Wales wherever *C. viridis* infestations occurred. It has reduced the pest status of *C. viridis* in citrus in Queensland to minor importance.

C. viridis was a significant pest of coffee in north Queensland, causing heavy deposits of sticky black mould and honeydew over foliage and berries and proving impractical to control with sprays. The scale's importance is now minor following the establishment of *D.* sp. nr *stramineus*.

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