Oviposition by *Comperiella lemniscata* Compere and Annecke (Hymenoptera: Encyrtidae) in *Aonidiella orientalis* (Newstead) (Hemiptera: Diaspididae)

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ABSTRACT Both sexes of oriental scale, *Aonidiella orientalis*, were exposed to the parasitoid *Comperiella lemniscata* at the beginning of the first, second and third female instars. The parasitoid successfully oviposited and developed in second- and third-instar female scales, but male scales were used as hosts only in their second instar and at low levels. When given a choice, *C. lemniscata* oviposited only in third-instar female scales.

To produce large numbers of the parasitoid *Comperiella lemniscata* Compere and Annecke for biocontrol testing in the field, Elder and Smith (1995) developed a method of rearing oriental scale, *Aonidiella orientalis* (Newstead), in the laboratory on butternut gramma pumpkins (*Cucurbita moschata*). They showed that males took 19.5 ± 2.7 d (mean ± SD) and females 44.2 ± 2.4 d from the crawler stage to adult. The parasitoid was subsequently released to test its efficacy as a biological control agent against *A. orientalis*, which is a major and frequent pest of papaws (*Carica papaya*) (Elder and Smith 1994).

The present study was undertaken to determine the ovipositional preferences of the parasitoid in various scale instars and to increase efficiency of parasitoid production in the laboratory. The overall aim was to determine the most productive time to introduce adults of *C. lemniscata* into a culture of *A. orientalis* to maximise parasitoid production.

At least 1,000 *A. orientalis* crawlers were brushed onto each of 25 pumpkins on the same day. Three days later the 18 healthiest pumpkins were blocked into six groups of three on freshness and the amount of blemishes, i.e. the three freshest/least blemished were in block one and so on. An estimated 550 scales were left on each of the 18 pumpkins, the remainder being removed by wiping. Three treatments were allocated randomly to each group of three pumpkins giving six replicates of each treatment. Treatments were: 1. to introduce *C. lemniscata* 3 d after crawlers had been put on pumpkin; 2. to introduce *C. lemniscata* when 50 to 70% of scales were half way through enlargement of the waxy covering of the second growth phase (beginning of second instar, 14 d after crawlers had been brushed onto pumpkins); 3. to introduce *C. lemniscata* when 50 to 70% of scales were half way through enlargement of the waxy covering of the third growth phase (beginning of female third instar, 24 d after crawlers had been brushed onto pumpkins) (Elder and Smith 1995). In all treatments, the parasitoids were removed 5 d after introduction.

Pumpkins were placed on shredded paper in separate gauze-covered 9.5 L buckets and 22 female *C. lemniscata* up to 3 d after emerging as adults, were added when the treatments dictated (males of *C. lemniscata* do not occur or are rare). Each bucket had 5 cm^2 of finely honeyed paper to provide food for the parasitoids. The buckets were maintained at 25 ± 0.5 °C and 46 ± 4% RH under continuous light provided by fluorescent tubes.

The maximum diameter of female and length of male scales were measured under a binocular microscope with a micrometer eyepiece, 36-46 d after the crawlers were introduced. Scales from any one replication were all measured on the same day. Ten scales from each sex/replicate/treat ment/status (dead, live, parasitised) combination were measured where present. Up to 100 second, third female and male scales per pumpkin were examined for evidence of parasitism by lifting the scales with a fine needle. The percentage of parasitised and dead (unknown causes) scales was calculated. The total number of scales on each pumpkin was counted at this time.

An average of 8.8 ± 1.6 (mean ± SD), n = 18, individuals of *C. lemniscata* out of 22 introduced per container were retrieved 5 d after introduction. Only 7% of the retrieved parasitoids were alive.

Because of their very small size, it proved very difficult to reduce the number of scales accurately to 550, 3 d after brushing crawlers onto the pumpkin. There was an average of 649 ± 208, n = 18 scales per pumpkin at the end of the experiment. The average number of scales per introduced parasitoid was 29.5 ± 9.5, n = 18, i.e. a parasitoid/prey ratio of 1 to 29.5.

Analyses of variance were undertaken using the groups of pumpkins as a blocking term. Scale size was analysed for differences in parasitoid

introduction times and scale status, and parasitism data were analysed for differences in parasitoid introduction times only.

Separate analyses for females and males indicated that live female scales were significantly ($P < 0.05$) larger than parasitised and dead female scales, and parasitised larger than dead (Fig. 1). There were no differences between treatments. For male scales, live scales from treatment 1 and 3 were significantly ($P < 0.05$) larger than live scales from treatment 2. Similarly dead scales from treatment 1 and 3 were larger than dead scales from treatment 2. Parasitised scales from treatment 2 were significantly ($P < 0.05$) larger than all other treatment combinations. Otherwise for both sexes the differences were not significant ($P > 0.05$).

C. lemniscata preferred female scales in the second and third instars to first-instar female scales ($P < 0.05$), but parasitisation levels of second- and third-instar females were not significantly ($P > 0.05$) different. The parasitisation percentages for female scales were analysed. Treatment 1 was excluded, as the variance was significantly smaller than treatment 2 and 3 ($P < 0.001$ using Bartlett's test). There was a much lower level of parasitisation of second-instar male scales and first-instar female scales, with no parasitisation in first- and third-instar male scales (Fig. 2). Males showed much higher levels of mortality in all three treatments than females, with the highest levels in treatments 2 and 3. Treatments 2 and 3 were not significantly different ($P > 0.05$).

A separate scale (of mixed ages) and *C. lemniscata* culture was used to determine the time spent searching by the parasitoid prior to oviposition, oviposition, and from oviposition to parasitoid emergence. The age of the observed adult parasitoids was not known. The culture was maintained at 26 ± 0.9°C and 46 ± 2% RH under continuous light provided by fluorescent tubes. Scale-infested pumpkins were removed to a laboratory bench and the activity of adult parasitoids recorded. Scale into which oviposition was observed were immediately marked by drawing a circle around them on the pumpkin. Daily observations were made on parasitoid emergence.

The only data used for analysis were those which included observations of searching and oviposition, and successful emergence of an adult parasitoid (n = 30).

Female *C. lemniscata* searched for 181 ± 195 s (mean ± SD) (30 different parasitoids) before inserting their ovipositor into a scale. Oviposition (recorded as the period during which the ovipositor was inserted into a scale) lasted for 405

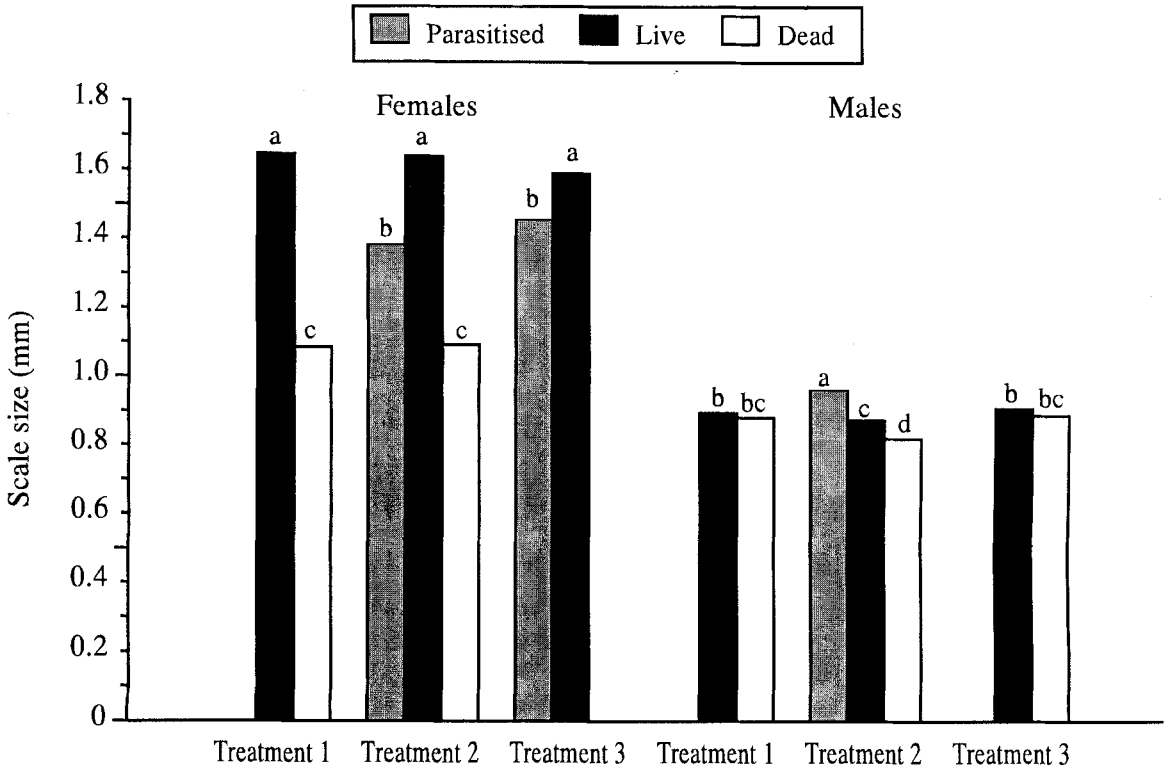

Fig. 1. Average size of female and male *A. orientalis* scales which were parasitised by *C. lemniscata*, measured a minimum of 36 d after the crawlers were brushed onto the pumpkins. Parasitoids were introduced at the start of each of the three female instars (Treatments 1, 2 and 3). Taking the sexes separately, bars with the same letter are not significantly different ($P > 0.05$).

± 172 s. Emergence of the resultant adult parasitoid took 22.93 ± 0.98 d. A further 46 searches and apparently successful ovipositions were observed, but no subsequent emergence of a parasitoid occurred. While the parasitoid occasionally oviposited into second-instar scale (3 times out of a total of 76), successful development of parasitoids did not occur. All successful emergences occurred from scales which were in the third instar when oviposition took place. This was in contrast to the oviposition experiment above, where second-instar female scales were utilised when they were the only development stage available.

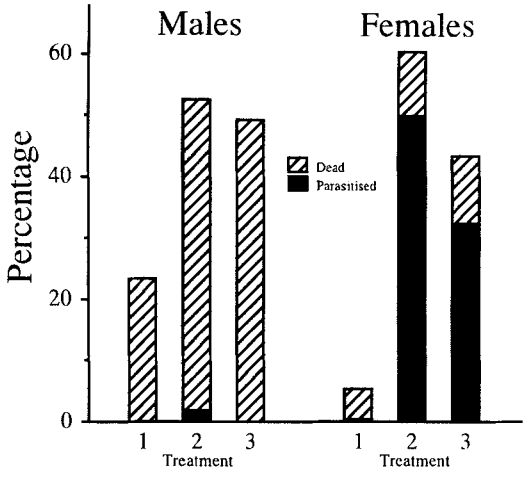

Fig. 2. Per cent male and female *A. orientalis* scales which were dead or parasitised by *C. lemniscata*, counted a minimum of 36 d after the crawlers were brushed onto the pumpkins. Parasitoids were introduced at the start of each of the three female instars (Treatments 1, 2 and 3).

C. lemniscata was not seen feeding on any exuded scale contents (host feeding) in spite of close observations during 27 h spread over 10 d. Mating was never observed, either during these observation periods or when undertaking culture maintenance. Smith, D. (unpub. data) was unable to find male *C. lemniscata* and the failure to observe mating (coupling) in this study supports the idea that males do not occur or are uncommon. *Comperiella pia* (Girault), one of the two other *Comperiella* spp. which occur in Australia reproduces parthenogenetically (Sands and Snowball 1980) and uniparental reproduction is well-known in other encyrtids (Noyes 1990).

Female scales reached the same size in treatments 2 and 3 in spite of the parasitoid being introduced in the second and third instars, respectively. This suggests that the parasitoid does not develop, or only partially develops, until the scale reaches some unknown stage of development, i.e. the wasp is an endoparasitic koinobiont.

Based on 49% parasitisation of female scales, a 1:6 male:female ratio for the scales (ratios of 1.6 and 1.9 were obtained by Elder and Smith (1995)) and 649 scales per pumpkin, each female *C. lemniscata* produced an average of 8.9 parasitoids.

We conclude that maximum production of parasitoids will be obtained if adult females are introduced into a uniform oriental scale culture when the scales are at the beginning of the second instar, i.e. at the second scale-enlargement stage (Elder and Smith 1995). Some parasitisation of male scales will also occur at this time. However, if parasitoid introduction is delayed until the beginning of the third female growth stage, many of the males will have emerged and will not be available to the parasitoids. Whether the early introduction of the parasitoid reduces the time to produce the next generation of parasitoids requires further study. It may not reduce the production time as this study showed that the parasitised scales develop to the same size no matter whether the parasitoid is introduced at the beginning of the second or third instar (Fig. 1). Also, Flanders (1944) found that the rate of development of *Comperiella bifasciata* Howard was fastest in third-instar female *Aonidiella aurantii* (Maskell) and this may also be the case for *C. lemniscata.*

At a temperature of 25 ± 0.5 °C and 46 ± 4% RH, the appropriate time for introducing *C. lemniscata* at the scale second-instar stage, is therefore 12 to 14 d after the transfer of the crawlers. Although mean parasitisation levels for female scales of 49% (Fig. 2) were achieved in this study, higher levels might be obtained by not removing the parasitoid after 5 d and also by introducing more parasitoids for a given number of scales. While the recovery rate for parasitoids was disappointing, some were still alive after 5 d. Previous observations by one of us (D. Smith, unpub. data) indicated that the parasitoids can survive for up 2 weeks.

Acknowledgments

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