THE EFFECT OF HOST DENSITY AND PARASITOID INOCULUM SIZE ON THE MASS PRODUCTION OF *LEPTOMASTIX DACTYLOPII* HOWARD (HYMENOPTERA: ENCYRTIDAE) AND *APHYTIS LINGNANENSIS* COMPERE (HYMENOPTERA: APHELINIDAE) IN QUEENSLAND.

D. Smith[1], C. G. Freebairn[1] and D. F. Papacek [2]

[1] Queensland Department of Primary Industries, Nambour QLD 4560

[2] 'Bugs for Bugs', 28 Orton St, Mundubbera QLD 4626

Summary

Leptomastix dactylopii Howard and *Aphytis lingnanensis* Compere are mass reared for augmentative release in Queensland citrus in the spring - early summer against citrus mealybug *Planococcus citri* Risso and red scale *Aonidiella aurantii* (Maskell) respectively. This paper reports the results of studies on the effects of host density and inoculum size on parasitoid production in a system using *P. citri* as host for *L. dactylopii* and oleander scale *Aspidiotus nerii* Bouché for *A. lingnanensis*, both grown on butternut pumpkins, *Cucurbita moschata* (Duchesne ex Lam.) Duchesne ex Poiret (Cucurbitaceae).

For *L. dactylopii*, highest production was achieved when mealybugs (21 days old at 25°C) were exposed to a parasitoid inoculum of 3 per cm^2. A host density of 20 per cm^2 (6 000 per pumpkin) was most efficient because it resulted in higher levels of parasitism, produced numbers of parasitoids only slightly less than the higher density and suffered lower losses due to rotting of the pumpkins. *A. lingnanensis* production was highest when the density of oleander scale was 60 per cm^2 and the parasitoid inoculum 33 per cm^2.

Introduction

Leptomastix dactylopii Howard (Hymenoptera:Encyrtidae) was introduced to Queensland from California to control citrus mealybug, *Planococcus citri* Risso (Hemiptera:Pseudococcidae), in citrus and custard apple in October 1980 (Smith *et al.* 1988; Smith 1991). It is a solitary endoparasitoid of third instar *P. citri,* usually deposits a single egg (Fisher 1963, Lloyd 1958), has 4 larval stages and pupates within a brown cylindrical puparium-like structure composed of the integument of the host. The whole life cycle takes less than 3 weeks at 25°C.

Following its introduction *L. dactylopii* became the most common natural enemy of *P. citri* throughout south-eastern Queensland but parasitoid numbers were low during winter and spring. Augmentative releases of 5,000-10,000 parasitoids per ha in spring - early summer were shown to be beneficial (Smith *et al.* 1988) and the parasitoid is now commercially mass reared by 'Bugs for Bugs', Mundubbera, and released annually in 1,000 ha of Queensland citrus and 200 ha of custard apples.

For mass production of *L. dactylopii* in Queensland, its host, *P.citri,* is reared at 25°C on the butternut pumpkin, *Cucurbita moschata* (Duchesne ex Lam.) Duchesne ex Poiret (Cucurbitaceae). About 150 butternuts, each covered with first and some third instar mealybugs, are placed on a four shelved steel mesh rack (2 m x 2 m x 0.6m) covered with a fine mesh cloth cage. About 20 parasitoids per butternut are introduced to the cage. These parasitise the third instars and their progeny contribute to the additional inoculum of approximately 12,000 (80 per butternut) *L. dactylopii* (50% females, 50% males) which are introduced 17 days later when the bulk of the mealybugs, now 21 days old, have reached the third instar. Parasitism occurs during the next 7 - 14 days after which the mealybugs begin to produce eggs and become unsuitable as hosts. Emergence of the new generation of parasitoids begins 17 days after inoculation and continues for

about a week. Adults (100,000 - 200,000 per cage) attracted to the top of the cage by fluorescent lights are collected as they settle on white polystyrene lids (0.6 x 0.4m).

Aphytis lingnanensis Compere is an important ectoparasitoid of red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), particularly in coastal and sub-coastal Queensland (Smith 1978). Females oviposit in second instar and virgin adult females, and second instar or prepupal male scales. At 25°C and 60% RH the life cycle takes about 17 days. Host feeding, in which females aquire protein necessary for egg production, usually results in the death of the scale and can add up to 50% to the mortality due to parasitism alone (Rosen and DeBach 1979). *A. lingnanensis* has been commercially mass reared in Queensland by 'Bugs for Bugs' since 1978 on oleander scale, *Aspidiotus nerii* Bouché, reared on butternut pumpkins (Papacek and Smith 1985).

In mass production of the host mealybugs and scales in Queensland the aim is to produce uniform coverage at moderate densities on the pumpkins, however, in practice considerable variation in density occurred and parasitoid production was erratic. Variation in inoculum size was also expected to affect parasitoid production. The aim of this study was to investigate the effects of host density and parasitoid inoculum level on parasitoid production.

Materials and Methods

The experimental unit consisted of a single butternut pumpkins (750-1,000 g) with an estimated surface area of 300 cm^2 placed on an absorbent pad in a white polystyrene box (40 x 30 x 15 cm) with a fine cloth mesh window (30 x 20 cm) in the lid. Experiments were conducted at a constant temperature of 25°C and RH of 60%. The *L. dactylopii* and *A. lingnanensis* used in these experiments came from the 'Bugs for Bugs' colonies which are re-established annually with material collected in the field from their hosts, *P. citri* and *A. aurantii* respectively.

L. dactylopii:

Experiment 1. Individual butternut pumpkins carrying an estimated 12,000 (40 per cm^2) citrus mealybugs 21 day old were exposed to inoculums of 50, 100, 250, 500 and 1,000 parasitoids, replicated four times (total of 20 pumpkins). This is equivalent to inoculum levels of 0.17, 0.33, 0.85, 1.7 and 3.3 per cm^2. The proportion of parasitised mealybugs was determined for 3 randomly chosen 4 cm^2 sections of each pumpkin just prior to the start of parasitoid emergence. Parasitoids were collected 14 - 21 days after inoculation and counted using a stereo microscope.

Experiment 2. Individual butternut pumpkins each with approximately 6,000 (20 per cm^2) or 12,000 (40 per cm^2) *P. citri* were exposed to inoculums of 100, 250, 500 and 1,000 *L. dactylopii*, with each host density - inoculum size combination replicated 4 times (total of 32 pumpkins). Parasitoids were harvested and counted 14 - 21 days after inoculation. Boxes were prepared as above, except that the butternuts were placed on a 10 cm long piece of cardboard egg carton on top of absorbent tissue to minimise the crushing of mealybugs on the bottom of the pumpkins.

A. lingnanensis:

Experiment 3. Two butternut pumpkins with scale densities of approximately 80 per cm^2 (estimated by counting the scales in 10 randomly chosen 1 cm^2 sections) were exposed for 48 hours to *A. lingnanensis*, one to 5,000 (17 per cm^2), the other to 10,000 (33 per

cm^2) parasitoids. A 10 x 20cm piece of greaseproof paper covered with fine honey droplets was placed in each box to provide supplementary food. The proportion of live unparasitised scale, parasitised scale (comprising those with live parasitoids plus those from which parasitoids had emerged) and scale killed by host feeding was recorded from 20 randomly chosen 1 cm^2 sections 28 days after inoculation when most parasitoids had emerged. Parasitoids emerging 15 - 28 days after inoculation were collected and counted.

Experiment 4. Pumkins with scale densities estimated (from 10 randomly chosen 1 cm^2 sections) at 40, 60 and 100 per cm^2 were individually exposed to 5,000 (17 per cm^2) and 10,000 (33 per cm^2) parasitoids, with 4 replicates for each density-inoculum combination (total of 24 pumkins). The proportion of scales parasitised was recorded on day 13, and parasitoids collected and counted 14 - 21 days after inoculation.

Data Analysis

Means were subjected to analysis of variance and significant differences determined using LSD's.

Results

L. dactylopii:

Experiment 1. The mean number of *L. dactylopii* produced from pumpkins with 12,000 mealybugs (40 per cm^2) increased significantly with increasing inoculum from an average of 1,103 (inoculum 50) to 9,072 (inoculum 1,000) per butternut and there was a similar significant increase in the rate of parasitism of the host mealybugs (Table 1). Estimates of percentage parasitism of mealybugs from the number of parasitoids emerged were lower than those from pre-emergence samples, particularly at the lower inoculum levels, but the general trend of increasing parasitism with increasing inoculum size was evident.

Experiment 2. Host density had no significant effect on mean parasitoid production for any inoculum level except 1,000 (3.3 per cm^2) where the lower density (20 per cm^2, 6 000 per pumpkin) produced significantly more parasitoids (Table 2a). Inoculum size had a significant effect on parasitoid production at the lower host density, with numbers increasing with inoculum size to a maximum of 6,178 at inoculum size 1,000 (3.3 per cm^2), but no effect at

TABLE 1

Experiment 1: Effect of five inoculum sizes on mean number of *L. dactylopii* produced and % parasitism for butternut pumpkins each with 12,000 (40 per cm^2) *P. citri*. Pre-emergence % parasitism determined from 3 randomly chosen 4 cm sections of each pumpkin, emerged % based on mean No. emerged as a proportion of 12,000 hosts per pumpkin.

Inoculum size (No. per cm^2)	Mean No. *L. dactylopii*	% parasitism Pre-emergence	Emerged
50 (0.17)	1,103 d	31.6 d	9.2
100 (0.33)	2,233 cd	49.9 cd	18.6
250 (0.85)	4,199 bc	65.3 bc	35.0
500 (1.70)	5,476 b	77.7 ab	45.6
1 000 (3.30)	9,072 a	96.3 a	75.6
LSD (P = 0.05)	2,315	23.8	

Means within columns followed by different letters are significantly different (P = 0.05).

the higher host density for inoculum sizes greater than 250 (0.85 per cm^2). Pooling the data by density showed that pumpkins with 6,000 *P. citri* produced significantly more *L. dactylopii* (4,312) than those with 12,000 (3,246).

Rotting of the pumpkins caused significant losses of production in this experiment, particularly at the higher mealybug density. The lost production was determined by estimating the proportion of the surface area of each pumpkin lost to rotting. A mean % loss was derived for each inoculum level - host density combination and corrected mean production figures calculated by the formula, corrected mean = observed mean /(100 - % rotten) x 100 (Table 2b). When the LSD for the observed means was applied to the corrected means 3 changes in significant differences resulted; 1) for inoclum 500, parasitoid production was significantly greater for higher host density pumpkins (compared with no significant difference for observed means), 2) there was no significant difference due to mealybug density at inoculum 1,000 (compared with significantly more at the lower density for observed means), and 3) for the lower host density pumpkins there was no significant difference between inoculums of 500 and 1,000 (compared with significantly more at 1,000 for observed means).

A. lingnanensis:

Experiment 3. The mean scale density of the two butternuts was similar at 79.1 and 83.5 per cm^2, giving an estimated total scale population of 23,730 and 25,050 respectively (mean surface area per pumpkin of 300 cm^2). The higher inoculum level produced 1.5 times more *A. lingnanensis* (12,840) than the lower level (8,740) (Table 3).

The proportion of parasitised scale was greater at the higher inoculum level (65.0%) than the lower level (51.4%) and host feeding rates were similar at 22 - 23%. Thus utilisation of the host scale was better at the higher inoculum level. The proportion of parasitoids failing to emerge was low (1.8 to 2.5%) for both inoculum sizes.

Experiment 4. At inoculum size 10,000 (33 per cm^2) significantly more *A. lingnanensis* were produced than at 5,000 (17 per cm^2) for all scale densities (Table 4). The increase in numbers of parasitoids was proportional to the difference in inoculum size with the higher level producing 1.9 - 2.6 times as many as the lower level.

Pumpkins with scale densities of 60 and 100 per cm^2 produced numbers of parasitoids not significantly different from each other, but both produced significantly more than pumpkins with 40 per cm^2.

The proportion of scales parasitised was not significantly affected by inoculum size except at the density of 100 per cm^2 where it was greater for the higher inoculum size. The effect of scale density on the proportion parasitised did not follow a consistent trend and when data were pooled across inoculum sizes there was no significant difference between densities.

Discussion

L. dactylopii:

In the first experiment the effect of inoculum level on *L. dactylopii* production for pumpkins with 12,000 mealybugs (40 per cm^2) was clear; higher inoculum levels resulted in significantly higher rates of parasitism and larger numbers of parasitoids. As a general rule, to produce significantly larger numbers of parasitoids, or increases in the proportion of mealybugs parasitised, required 4 fold increases in inoculum level (Table 1).

In the second experiment, where host densities were the same and 50% of those in experiment 1, there was a significant effect of inoculum levels above 250 (0.85 per cm^2) for the lower host density (20 per cm^2), but not for the higher host density (40 per cm^2), on observed parasitoid production (Table 2a). This caused the lower density to appear to be the more productive. However, when means were corrected for lost production caused by rotting of the pumpkins, which on average was worse at the higher mealybug density (33.1% compared with 18.8%), the higher host density pumpkins were more productive overall because they produced significantly more parasitoids at inoculum 500 and similar numbers at inoculum 1,000, compared with similar numbers at 500 and significantly less at 1,000 for observed means (Table 2b). In addition, for corrected means, the significant increase in observed production for the inoculum of 1,000 at the lower density was no longer apparent; inoculum levels above 250 did not result in significant increases in parasitoid production, regardless of host density.

Lloyd (1958) found that 10 female *L. dactylopii* parasitised an average of 392 mealybugs over 7 days, approximately 40 per female. Based on these figures, the expected rates of parasitism were calculated and compared with the observed or corrected rates for our results which were derived by calculating the mean number of parasitoids emerged as a percentage of the number of hosts per pumpkin (Table 5). For experiments with the higher host density (40 per cm^2), estimates of % parasitism were similar to the expected values for inoculums up to 250, but lower for inoculums of 500 and 1,000, whereas the pre-emergence sample estimates were higher at the lower inoculum levels and similar at 500 and 1,000. The rates based on parasitoids emerged were lower for all inoculum levels than those from the pre-emergence samples. At the lower host density (20 per cm^2), corrected means were similar to expected values, rates of parasitism were consistently higher than for the higher host density and the decline in emergence compared with expected values at high inoculum levels did not occcur. This suggests that significant numbers of parasitoids may have failed to emerge at the higher host density.

For efficient production of *L. dactylopii* it is important that inoculum levels are high enough for most hosts to be parasitised within 7 days of inoculation. After this, egg and meal production interferes with parasitism. The experiments reported here indicate that inoculum densities of 3.3 per cm^2 (1,000 per pumpkin) and a host density of 20 per cm^2 (6,000 per pumpkin) resulted in higher levels of parasitism, produced numbers of parasitoids only slightly less than the higher density and suffered lower losses due to rotting of the pumpkins.

A. lingnanensis:

Production of *A. lingnanensis* was greater at the inoculum density of 33 per cm^2 (10,000 per pumpkin) than at 17 per cm^2 (5,000 per pumpkin) for all scale densities tested, 40, 60, 80 and 100 per cm^2 (Tables 3 & 4a). In experiment 3 this could be attributed to increased rates of parasitism (Table 3), however, in experiment 4, rates of parasitism, whilst they were higher, were significantly so only at 100 scales per cm^2 (Table 4b). A scale density of 60 per cm^2 was best as it produced significantly more parasitoids than 40 per cm^2. Increasing scale density to 100 scales per cm^2 did not lead to increased parasitoid production.

DeBach and White (1960) consider the ideal scale density for *Aphytis* production to be 30-50 scales per cm^2 but they obtained good production from an oleander scale density of 70 - 79 per cm^2 on banana squash with an inoculum averaging 17.5 females per cm^2, similar to our higher inoculum of 33 per cm^2 (~ 17 females at a 1 : 1 sex ratio). They also reported good production from combinations of 15 females to 25 scales, 20 to 35, 20 to 45 and 30 to 35.

TABLE 2a

Experiment 2: Effect of four inoculum levels and two *P. citri* densities on mean number of *L. dactylopii* emerged per butternut.

Inoculum size	*P. citri* density (No. per butternut) 20 per cm^2 (6,000)	40 per cm^2 (12,000)	Total
100	2,033 c	935 c	1,484 b
250	4,140 b	4,170 b	4,350 a
500	3,577 b	4,367 b	4,120 a
1,000	6,178 a	4,145 b	5,161 a
Total	4,312 a	3,246 b	-

LSD's (P = 0.05) for: treatments 1,423; totals by inoculum size 1,266; totals by *P. citri* density 896. Means within the body of the table followed by different letters are significantly different, as are those of totals by inoculum size and by *P. citri* density.

TABLE 2b

Experiment 2: Mean *L. dactylopii* produced per butternut pumpkin corrected for estimated % of surface area rotten.

Inoculum size	*P. citri* density (No. per butternut) 20 per cm^2 (6,000) % rotten	Corrected mean	40 per cm^2 (12,000) % rotten	Corrected mean
100	15.0	2392 c	27.5	1290 c
250	27.5	5710 b	30.0	5957 ab
500	30.0	5110 b	40.0	7278 a
1,000	2.5	6336 ab	35.0	6337 ab
Mean	18.8		33.1	

LSD's (P = 0.05) as for Table 2a, 1,423. Means followed by different letters are significantly different (P = 0.05).

TABLE 3

Experiment 3: Effect of two inoculum densities on utilisation of *A. nerii* and mean number of *A. lingnanensis* produced from two butternut pumpkins with scale densities of ~ 80 per cm^2.

Percent (%)	Inoculum density (No. per pumpkin) 17 per cm^2 (5,000)	33 per cm^2 (10,000)
Unparasitised	24.7	9.6
Parasitised	51.4	65.0
Host fed	22.1	22.9
Dead before emergence	1.8	2.5
Parasitoids emerged	8,740	12,840

TABLE 4a

Experiment 4: Effect of two inoculum levels and three *A. nerii* densities on mean number of *A. lingnanensis* produced per butternut.

A. nerii density	Inoculum density (No. per pumpkin) 17 per cm^2 (5,000)	33 per cm^2 (10,000)	Total
40	1,372 c	3,245 b	2,308 b
60	2,356 bc	6,075 a	4,215 a
100	2,640 b	4,973 a	3,806 a
Total	2,122 b	4,764 a	-

LSD's (P = 0.05) for: treatments 1,242; totals by *A. nerii* density 513; totals by inoculum size 419. Means within body of table followed by different letters are significantly different, as are those of totals by *A. nerii* density and by inoculum level (P = 0.05).

TABLE 4b

Experiment 4: Effect of two *A. lingnanensis* inoculum levels and three *A. nerii* densities on mean % *A. nerii* parasitised.

A. nerii density	Inoculum density (No. per pumpkin) 17 per cm^2 (5,000)	33 per cm^2 (10,000)	Total
40	21.0 cd	25.7 bc	23.6 a
60	24.2 bc	31.0 ab	27.9 a
100	15.3 d	37.5 a	26.4 a
Total	20.9 b	31.6 a	-

LSD's (P = 0.05) for: treatments 7.3; totals by *A. nerii* density 2.9; totals by inoculum size 5.8. Means within body of table followed by different letters are significantly different as are of totals by *A. nerii* density and by inoculum level (P = 0.05).

TABLE 5

Comparison of expected mean % parasitism of *P. citri* by *L. dactylopii* for 5 inoculum sizes and two host densities with the results of experiments 1 and 2. 'Expected' means are based on 40 eggs per female (Lloyd 1958). 'Observed' and 'Corrected' means from experiment 2 are calculated from numbers of parasitoids produced (data from Tables 2a & 2b) as a % of hosts per pumpkin, as are 'Emerged' means for experiment 1 (data from Table 1). 'Pre-em' refers to the % parasitised prior to emergence in 3 randomly chosen 4 cm^2 sections of pumpkin (from Table 1)

	P. citri density							
	20 per cm^2 (6,000)			40 per cm^2 (12,000)				
		Experiment 2			Experiment 1		Experiment 2	
Inoculum size	Expected	Observed	Corrected	Expected	Pre-em	Emerged	Observed	Corrected
50	-	-	-	8.3	31.6	9.2	-	-
100	33.2	33.9	39.9	16.6	49.9	18.6	7.8	10.8
250	83.7	69.0	95.2	41.7	65.3	35.0	34.8	49.6
500	>100	59.6	85.2	83.3	77.7	45.6	36.4	60.7
1000	>100	103.0	106.0	>100	96.3	75.6	34.5	53.1

Fernando (1993) found that *A. lingnanensis* from 'Bugs for Bugs' colonies (after a pre-oviposition period of 2-8 hours) laid an average of 191 ± 3.16 eggs, with 6.5 eggs laid on day one and 8.5 on day two. Thus each female could be expected to lay up to 15 eggs over the first 48 hours and at inoculums of 17 and 33 per cm^2 (assuming a 1 : 1 sex ratio) should produce 37,500 and 75,000 eggs, more than enough to parasitise the average 30,000 scales per butternut at our highest density of 100 per cm^2.

In experiment 4 half as many parasitoids were produced as in experiment 3 (Table 4a), the best being 6,075 in the treatment with 60 scale per cm^2 and an inoculum of 33 per cm^2, and the level of parasitism was also low (Table 4b), the best being 37.5% in experiment 4 compared to 65.0% in experiment 3. The parasitoids used in this experiment appeared to be less fit and suffered considerable mortality during the 48 hours of inoculation. The results, however, support the observation of the first experiment that high scale densities and low inoculum rates result in low production.

At high scale densities low rates of parasitism result probably because the surface of the butternut becomes a continuous mat of scales which may interfere with the complex process of scale examination and oviposition of *Aphytis* described by Rosen and DeBach (1979).

In Queensland, butternuts were infested by brushing or flicking crawlers on to them each morning for 4-5 days until they appeared to be well covered. This resulted in variable scale densities (eg experiment 3 the density averaged 83.5 and 79.1 per cm^2 but ranged from 30 to 150 per cm^2) with the density on one side of the pumpkin often much higher than the other. Less variable coverage was achieved by closely observing crawler density and rotating the butternuts 2-3 times daily, and more recently by placing crawler-producing butternuts inside fine organdie mesh bags and allowing the crawlers to fall through to a layer of fresh butternut pumpkins below.

Acknowledgments

This research was done with the assistance of two students from Burnside High School, Nambour - B. Diferdinando and K. Bowden.

References

DeBach, P. and White, E.B. (1960). Commercial mass culture of the California red scale parasite *Aphytis lingnanensis*. *Calif. Agr. Expt. Sta. Bull.* **770**: 58 pp.

Fernando, L.C.P. (1993). Ovarian status, activity patterns and ecology of *Aphytis lingnanensis* Compere in Queensland citrus. Thesis submitted for the degree of Doctor of Philosophy, University of Queensland. 250 pp.

Fisher, T.W. (1963). Mass culture of *Cryptolaemus* and *Leptomastix* - natural enemies of citrus mealybug. *Division of Agricultural Sciences, University of California Bulletin.* **797**: 37 pp.

Lloyd, D.C. (1958). Studies of parasite oviposition behaviour II. *Leptomastix dactylopii* Howard (Hymenoptera:Encyrtidae). *The Canadian Entomologist*, **90**: 450-461.

Papacek, D.F. and Smith, D. (1985). *Aphytis lingnanensis*. In *Handbook of Insect Rearing*, Vol 1. Pritam Singh & R.F. Moore (Eds). Elsevier Science Publishers B.V. , Amerstdam.

Rosen, D. & De Bach, P. (1979). *Species of Aphytis of the World.* W. Junk B.V. The Hague, pp17-48.

Smith, D. (1978). Biological control of scale insects on citrus in South Eastern Queensland. 1 Control of Red Scale *Aonidella aurantii* (Maskell). *Journal of the Australian Entomological Society,* **17**: 367-317.

Smith, D. (1991). The use of *Leptomastix dactylopii* Howard (Hymenoptera:Encyrtidae) to control *Planococcus citri* (Risso) (Hemiptera:Pseudococcidae) in custard apples in Queensland. *General and Applied Entomology*, **23**: 3-8.

Smith, D. and Papacek, D.F. (1985). Integrated pest management in Queensland citrus. *Queensland Agricultural Journal.* **111**: 249-259.

Smith, D. Papacek, D.F. and Murray, D.A.H. (1988). The use of *Leptomastix dactylopii* Howard (Hymenoptera:Encyrtidae) to control *Planococcus citri* (Risso) (Hemiptera:Pseudococcidae) in Queensland citrus orchards.*Queensland Journal of Agricultural and Animal Sciences.* **45**: 147-164.