

MASS-REARING, ESTABLISHMENT AND DISPERSAL OF *PSEUDAPHYCUS MACULIPENNIS*, A BIOCONTROL AGENT FOR OBSCURE MEALYBUG

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ABSTRACT

Approximately 750,000 *P. maculipennis* (internal, facultatively gregarious, encyrtid parasitoids of the obscure mealybug (*Pseudococcus viburni*)) were released to 41 pipfruit orchards in Hawke's Bay, Nelson/Motueka and Auckland, and to the Wellington Botanic Gardens between 2001 and 2004. At least a year later, they were recovered from 83% of the Hawke's Bay, and 60% of the Nelson orchards, and from the Wellington Botanic Gardens, indicating that the parasitoid has a solid foothold in New Zealand. *Pseudaphycus maculipennis* have dispersed since their release at a natural rate of about 200 m/year. Laboratory, mass-rearing production techniques for *P. maculipennis* are described. No measures of the impact of *P. maculipennis* on obscure mealybug populations in orchards have yet been carried out

Keywords: biocontrol, obscure mealybug, *Pseudaphycus maculipennis*, mass-rearing, establishment.

INTRODUCTION

Pseudococcus viburni (Signoret 1875), the obscure mealybug, is a polyphagous, cosmopolitan pest (Ben-Dov 1994), probably introduced to New Zealand through commercial trade. It was first recorded in 1922 as *P. maritimus* (which does not exist in New Zealand) (Charles 1998) and is now one of the most common exotic mealybugs, occurring between Northland and mid-Canterbury. It has been an important pest of pipfruit in Hawke's Bay for at least 50 years (Charles 1989), but, until this programme, was the only major pest mealybug in New Zealand without any effective parasitoid (Charles 1993).

Pseudaphycus maculipennis (Mercet 1923) (Hym: Encyrtidae) is host-specific, and has reportedly provided good control of obscure mealybug in France and the Republic of Georgia. It is a facultatively gregarious, endoparasitoid; it is a koinobiont, ovipositing in one developmental stage of the mealybug (usually the third instar female, although second instars and adult females are also attacked) and emerging from the next (usually the adult). Male and female wasps often emerge from the same mealybug. The first releases into New Zealand were made in February 2001 when it became the first biocontrol agent to be released under the Hazardous Substances and New Organisms (HSNO) Act 1996 (Charles 2001).

This paper describes the rearing and release programme for establishing *P. maculipennis* in New Zealand. Estimates of the rate of natural dispersal are also provided.

METHODS

Rearing and quality control

Pseudaphycus maculipennis was introduced into New Zealand from Australia in December 1997 (Charles 2001). From an initial 50 individuals, a small colony was

maintained in quarantine until approval to release was given in September 2000. The colony was then expanded to provide sufficient numbers for release. Laboratory colonies of obscure mealybug and *P. maculipennis* were mass reared on sprouting potatoes, usually of the cultivars 'Desirée' or 'Ilam Hardy', at ca 22°C. The health and quality of both species were dependent on the quality of the potatoes. Great care was taken to ensure that the tubers were not treated with anti-sprouting chemicals, which significantly inhibited the development of mealybug crawlers. Seasonal changes in potato physiology also affected both the sprouting of tubers and mealybug growth-rate, but these factors were accommodated by the rearing programme. Obscure mealybugs were reared in 37 litre plastic bins on a single layer of about 30 potatoes placed on raised racks that allowed air circulation and hence prolonged the quality of the tubers. The bins were covered by nylon organza held in place by elastic bands. After 3 months (or about three mealybug generations), potatoes with *P. maculipennis* in mealybug mummies were added. After a further 25 days (about one *P. maculipennis* generation) each bin provided peak numbers of parasitoids for release or restarting the next rearing cycle.

The health of the parasitoid laboratory colony, and hence the 'quality' of the rearing programme and released parasitoids, was estimated periodically by collecting 50 mummified *P. viburni* into individual gelatine capsules, and recording the numbers and sex of the emerging parasitoids from each mealybug. It was hypothesised that any substantial changes in these parameters over succeeding generations would signal that the parasitoid population was stressed in some way, and that changes to the rearing programme might be required.

Release methods

The chances of establishing *P. maculipennis* were maximised by choosing obscure mealybug-infested apple and pear orchards in Hawke's Bay and Nelson/Motueka for parasitoid release. Orchards were selected, at least initially, through detailed examination of packhouse data from the previous harvest that helped to identify blocks and varieties with high numbers of mealybugs. These data provided a means to plan the distribution of parasitoids to obscure mealybug 'hotspots' within a district. Releases were timed to minimise any possible mortality from seasonal insecticide or other pesticide sprays, and hence were often made immediately before, or just after, harvest. Ideal blocks contained older trees that had many cracks and crevices to support mealybug populations. Many release blocks were of the apple variety 'Royal Gala', which characteristically grow large burr knots (incipient aerial roots) that provide ideal habitats for *P. viburni*. A list of release sites is given in Table 2.

About 3 weeks prior to a planned release date, large numbers of *P. maculipennis* from the rearing colony were introduced to bins of potatoes infested with *P. viburni* at ca 100/potato. The very first F₁ *P. maculipennis* emerged about 19 days after their parents were added to the mealybug colony. At 20 days, the potatoes and parasitised mealybugs (and any that had escaped parasitism) were transferred into onion bags for transport to release orchards. Each bag held 5–6 potatoes, depending on size. Abrasive damage to the fragile, mummified mealybugs was minimised by loading the potatoes into the bag through a ca 100 mm diameter tube. Numbers of emerging *P. maculipennis* varied considerably, but were estimated, through sub-sampling and experience, to be ca 500 per bag.

Once at a release site, one onion bag per tree was stapled to the underside of a branch, or to the trunk of a tree in such a way to maximise protection from weather and vertebrate predators. Where feasible, bags were stapled to every 5th tree in alternate rows through the block. The estimated numbers of parasitoids released per orchard varied from 13,000–20,000, and the release schedule ensured that large numbers of *P. maculipennis* emerged shortly after the potatoes were placed in the trees.

Establishment of *P. maculipennis*

Experiments to determine whether *P. maculipennis* had established at a release site were usually initiated from 12–17 months after release. Establishment was determined by the use of *P. viburni* infested 'trap' potatoes. They were placed in onion bags as above, but at two potatoes/bag. A bag was stapled to each of about 5 trees in each release

block for 10–14 days. During this period it was expected that *P. maculipennis*, if they were present, would find and parasitise the mealybugs. When the potatoes were retrieved, they were placed into sealed 'Click Clack[®]' plastic storage boxes with gauze-ventilated lids to prevent any parasitoid or mealybug from escaping, and returned to the laboratory to await parasitoid emergence. The presence of any *P. maculipennis* was taken as evidence of establishment at that orchard.

Dispersal of *P. maculipennis*

P. maculipennis dispersal was measured during February and March 2004 at three Hawke's Bay orchards in which the parasitoid was known to have been established for two years. A bag containing two *P. viburni* 'trap' potatoes was stapled to each of five apple trees along a ca 1.6 km, linear transect at increasing distances (nominally 50 m, 100 m, 200 m, 400 m and 800 m) from the centre of the original release block. All three transects extended through adjacent pipfruit properties, and were orientated approximately NE-SW. The orientation was chosen to provide the longest possible transect within an orchard environment, with no regard to prevailing wind direction, which was predominantly W-SW. One arm of each transect at orchard 2 and 3 was abbreviated at 630 m and 400 m by a ploughed field and an industrial area respectively. The potatoes were collected after 14 days as above, and returned to the laboratory for parasitoid rearing.

RESULTS AND DISCUSSION

Rearing and quality control

Hygiene, and both spatial and temporal separation between the mealybugs and parasitoids, were crucial factors in maintaining the colonies over such a long period. Bins were regularly cleaned and rotting or insect/mite infested potatoes removed. Preventing *P. maculipennis* from prematurely invading the obscure mealybug-rearing colony depended vitally on keeping the two colonies in separate rooms as far apart as possible, and ensuring that the mealybug colony was never visited after the parasitoid colony on the same day. When, for unknown reasons, the laboratory colony of *P. maculipennis* fell to very low numbers (possibly zero) in March 2002, parasitoids recovered from orchards in Hawke's Bay were used to re-invigorate the colony.

Six 'quality control' samples were analysed between March 1998 and August 2003, a period during which an estimated 40-70 overlapping parasitoid generations were reared. Both the mean number of *P. maculipennis* per *P. viburni* mummy and the sex ratio remained substantially the same (Table 1), providing some confidence that the parasitoid colony was healthy and that no significant "laboratory" traits were being selected for.

TABLE 1: Mean number of male and female *P. maculipennis* emerging from mealybug mummies sampled from 1998 – 2003.

Date sampled	No. mealybug mummies	Male <i>P. mac.</i> /mb	Female <i>P. mac.</i> /mb	Total <i>P. mac.</i> /mb	Sex ratio (M:F)
20-25 Mar 1998	348	0.65	2.44	3.09	1: 3.72
6 Jan 2000	50	2.30	3.42	5.72	1: 1.49
13 Feb 2001	45	0.53	1.44	1.98	1: 2.71
20 Mar 2003	43	1.02	2.67	3.70	1: 2.61
17 July 2003	39	0.85	2.44	3.28	1: 2.88
11 Aug 2003	50	0.56	2.04	2.60	1: 3.64

Releases

The establishment programme for *P. maculipennis* progressed largely according to plan, and within budgetary constraints. Releases were made from February 2001 until February 2004. During this period approximately 750,000 *P. maculipennis* were released to 42 sites, which were 23 pipfruit orchards in Hawke's Bay, 15 pipfruit orchards in the Nelson/Moutere area, 3 apple blocks in Auckland and the Wellington Botanical Gardens (Table 2).

TABLE 2: Releases and recoveries of *P. maculipennis* from February 2001–February 2004. ‘(+x)’ = the numbers of 2003–04 release orchards at which establishment will not be assessed until 2004–05. ‘-’ = release orchards where recovery has yet to be assessed. ‘0’ = search has so far failed to detect establishment.

Release region	Location	No. release orchards	No. recovery orchards	No. <i>P. mac</i> released
Auckland	Mt Albert	1	0	21,500
	Oratia	1	-	15,000
	Kumeu	1	-	15,000
Wellington	Botanic Gardens	1	1	4,000
Hawke's Bay	Hastings: Twyford	7 (+1)	6 (-)	127,000
	Hastings: Longlands	2 (+2)	1 (-)	88,000
	Hastings: Waiohiki	2	2	25,000
	Havelock North	5	5	88,500
	Pakowhai/Clive	2 (+1)	1 (-)	50,500
	Waipukurau	0 (+1)	(-)	18,000
Nelson	Richmond/Hope	5 (+4)	4 (-)	183,000
	Mapua/Moutere	5 (+1)	2 (-)	122,000
	Total	32 (+10)	22 (-)	757,500

Recoveries

By May 2004 *P. maculipennis* had been recovered from 22 of the 32 release sites sampled (Table 2). They were recovered from 83% (15/18) of the ‘release’ orchards in Hawke’s Bay, and 60% (6/10) of the ‘release’ orchards in Nelson. They had also been recovered from the Wellington Botanic Gardens. The high recovery rates the year after release (about 50%) followed by further recoveries in subsequent years (Fig. 1) indicated that the criteria for selecting release orchards and numbers of parasitoids for release were effective. The key orchard areas of Havelock North and Twyford in Hawke’s Bay, and Hope and Moutere in Nelson, can now be considered centres of *P. maculipennis* establishment. Establishment at 2 of the 3 Auckland release blocks has yet to be assessed.

No attempt was made to measure the quantitative impact of *P. maculipennis* on obscure mealybug populations during this stage of the biocontrol programme. Nevertheless, other mealybug natural enemies were also recovered from the trap potatoes in both Hawke’s Bay and Nelson, indicating that *P. maculipennis* was successfully integrating into the natural enemy communities within the orchards. The other species included *Ophelosia bifasciata* and *O. charlesi* (Hymenoptera: Pteromalidae), both of which are known egg predators and facultative parasitoids of obscure mealybug, and predatory larvae of *Cryptoscenea australiensis* (Neuroptera: Coniopterigidae) (Charles 1993).

Comparing mealybug rejection rates at packhouses over time is unlikely, by itself, to provide adequate evidence for the impact of *P. maculipennis*, because two other species of mealybugs are also often present in orchards. Mealybugs are rarely identified to species on rejected fruit, and only obscure mealybug is attacked by *P. maculipennis*. When experimental methods to measure the seasonal population impact of *P. maculipennis* in orchards are developed, they will also enable the impacts of other mealybug parasitoids on other mealybug species to be described, and provide a measure of the effects of different orchard management practices on mealybug pest status. The laboratory ‘quality control’ data may also provide a measure of the relative fitness over time of populations as they adapt to the wild.

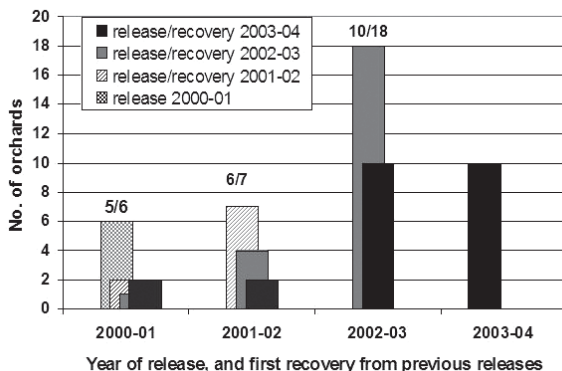


FIGURE 1: Total numbers of pipfruit orchards into which *P. maculipennis* was released each year from 2000-04. Recoveries from release orchards are denoted by overlapping columns, shaded by the year in which they were first found. For example, parasitoids were released into six orchards in 2000-01. They were recovered from two of the orchards in 2001-02, one in 2002-03 and two in 2003-04. Column labels give the total number of release orchards from which *P. maculipennis* had been recovered by April 2004.

Dispersal

At one orchard *P. maculipennis* was recovered only from one arm of the transect, at 100 m and 200 m from the release site. At the other two orchards, they were recovered from most of the trap potatoes up to 800 m along the transects (Table 3). When related to the dates of release, these data indicate that the natural spread of *P. maculipennis* was rather slow, at 100–300 m per year. The influence of wind on *P. maculipennis* has not been measured, but in these sheltered orchards over a two-year period wind was probably not a major component in dispersal. Successful biological control by parasitoids may be greatly influenced by habitat fragmentation (Tschamtkke & Kruess 1999; Tschamtkke et al. 2002). However, many of the pipfruit orchards in Hawke’s Bay and Nelson are contiguous, so even slow natural dispersal should ensure wide establishment of the parasitoid in these regions within a few years. *Pseudaphycus maculipennis* may disperse more rapidly to isolated orchards or to new regions by involuntary human transport, but the extent to which this may happen is unpredictable.

TABLE 3: Recovery in March 2004 of *P. maculipennis* along 1.6 km transects in three Hawke’s Bay orchards.

Orchard	Date transect established	Date <i>P. mac</i> released	Distance (m) from release point (2 sides of transect)											
			800	400	200	100	50	0	50	100	200	400	800	
1	Jan 04	Dec 01	X	X	✓	✓	X		X	X	X	X	X	
2	Apr 04	Dec 01 (630m)	✓	X	✓	✓	X		✓	✓	✓	✓	X	
3	Apr 04	May 01	✓	X	✓	X	✓		✓	✓	✓	X	ns	

✓=*P. maculipennis* recovered; X=*P. maculipennis* not recovered; ns=no sample taken.

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