Mass rearing *Pteromalus puparum* on *Pieris rapae* to assist eradication of *Pieris brassicae* from New Zealand

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Abstract *Pieris brassicae*, great white butterfly, was first detected in New Zealand in May 2010 in Nelson, and since November 2012 has been the target of a Department of Conservation eradication programme. Methods were available for killing *P. brassicae* eggs, larvae and adults, but not pupae. To assist eradication, a parasitoid of *P. brassicae* pupae, *Pteromalus puparum*, was mass reared at Lincoln and released at Nelson locations where there was a high probability of *P. brassicae* pupae being present. To avoid transporting *P. brassicae* from Nelson to Lincoln, *Pt. puparum* was reared on another of its hosts, *Pieris rapae*, which occurs throughout New Zealand. Between late February and early April 2015, an estimated 14,280 *Pt. puparum* adults from 837 parasitised *P. rapae* pupae were released in Nelson. However, parasitism of unparasitised *P. rapae* pupae, which were used as sentinels for monitoring parasitism rates, remained low. Although evidence of the releases being effective was weak, augmenting *Pt. puparum* populations remains the most practical method for increasing the mortality of *P. brassicae* pupae in Nelson.

Keywords butterfly eradication, great white butterfly, small white butterfly, parasitoid release, parasitoid augmentation, biosecurity

INTRODUCTION

Pieris brassicae L. (Lepidoptera: Pieridae), great white butterfly, was first detected in New Zealand in Nelson on 14 May 2010. It is listed as an 'Unwanted Organism' under the Biosecurity Act 1993 because it could damage New Zealand native, forage and vegetable brassicas (East 2013). Many of New Zealand's native brassicas are already threatened, thus the Department of Conservation (DOC) commenced an eradication programme in November 2012 (Green 2014).

DOC has controlled eggs, larvae and adults of *P. brassicae* through a combination of passive surveillance, active searching, host plant

control, insecticide sprays, capturing adults and releasing the parasitic wasp *Cotesia glomerata* L. (Hymenoptera: Braconidae) to parasitise *P. brassicae* larvae (Phillips et al. 2015). However, *P. brassicae* pupae proved difficult to detect and control because they can shelter in crevices on inanimate objects away from host plants (Phillips et al. 2014). Pupae comprise a major proportion of the *P. brassicae* population in Nelson during winter and summer (Kean & Phillips 2013), and could be inadvertently transported on inanimate objects to locations outside Nelson (Huszczynska-Sawicka & Phillips 2014).

Parasitic wasps can find their host insects

even when host population densities are low (Godfray 1994), thus they have the potential to assist eradication attempts. *Pteromalus puparum* L. (Hymenoptera: Pteromalidae) was introduced to New Zealand to control pupae of *Pieris rapae* (Muggeridge 1943), and it also parasitises pupae of *P. brassicae* and other species (Lasota & Kok 1986). This paper describes mass-rearing of *Pt. puparum* on *P. rapae* at Lincoln, and its release in Nelson, to assist eradication of *P. brassicae*.

MATERIALS AND METHODS

Laboratory rearing *Pieris rapae*

Two physically separated *P. rapae* colonies were established using adults captured in Canterbury during spring 2014. Adults were kept inside fabric or metal mesh cages, with 30–40 adults per cage, at 20°C and 16:8 h light:dark. To provide food, each cage contained three cotton dental wicks pushed halfway into the neck of a 30 ml glass container filled with sterilised water containing 10% sucrose and 0.15% yellow food colouring (attractant). Oviposition occurred on 1-month-old cabbage seedlings, and a fresh seedling was provided every 2–3 days, or whenever more than ca 30 eggs had been laid per plant. Cabbage seedlings with eggs were placed under lights to sustain plant growth (Sylvania GroLux 5' T8 Fluorescent Tubes 58W) at 20°C and 16:8 h light:dark. *Pieris rapae* larvae were provided with fresh plant material as required, and were kept until they reached the final (5th) instar.

Collecting *Pteromalus puparum* from the field and rearing it in the laboratory

Methods for collecting wild *Pt. puparum* adults were based on Hardwick et al. (2016). From November 2014 to February 2015, a total of 36 cages, each containing ca 30 3-day-old *P. rapae* pupae (total of 1071 pupae) were placed in unsprayed brassica plots in Canterbury. Cages were 3 L or 5 L rectangular plastic boxes (Sistema Plastics, KLIP IT™ 1830 or 1850) fitted with mesh panels (900 µm) in the lids and sides. A soldering iron was used to make drainage holes in the bottom

of the cages to prevent flooding during rain. The mesh allowed entry of *Pt. puparum* adults, which were attracted to their hosts, but not larger predators such as earwigs. Adults that entered cages tended to remain beside pupae and were captured by gently drawing them into a plastic tube.

For laboratory rearing, the same cages were used and were placed inside a larger 7 L plastic container (Cuisine Queen Hardwares, FS337) fitted with a finer mesh panel (250 µm) in its lid to prevent *Pt. puparum* adults from escaping. Adult *Pt. puparum* were supplied 3-day-old lab-reared *P. rapae* pupae, which were maintained at 20°C and 16:8 h light:dark. Any *P. rapae* pupae that escaped parasitism and became adults (ca day 14) were removed. *Pteromalus puparum* adults that emerged (ca day 20) were fed for 2 days via dental wicks soaked in 20% honey solution or tap water, then provided with fresh *P. rapae* pupae to parasitise.

Releasing *Pteromalus puparum* in Nelson and monitoring parasitism using *Pieris rapae* as sentinels

Methods were based on Hardwick et al. (2016). Table 1 summarises the eight deliveries of *P. rapae* pupae that were couriered overnight from Lincoln

Figure 1 Schematic of a typical delivery from Lincoln to Nelson of parasitised and unparasitised *Pieris rapae* pupae to a release site, and unparasitised *Pieris rapae* pupae to a control site, with the anticipated outcome.

to Nelson between 23 February 2015 and 7 April 2015. Pupae were sent to Nelson in cages (previously described), with 30–40 pupae per cage.

In Nelson, cages were used in one of three ways (Figure 1). Those containing *P. rapae* pupae parasitised by *Pt. puparum* were placed at release sites where there was a high risk of *P. brassicae* pupae being present (identified by DOC). Those containing unparasitised *P. rapae* pupae were used

as sentinels, and were either placed at release sites, where they were situated within 2–3 m of a release cage to monitor *Pt. puparum* adult emergence from the release cage (release sentinels), or placed at least 200 m from a release cage to monitor for the presence of *Pt. puparum* adults in the absence of a release (control sentinels).

Pteromalus puparum larvae sent to Nelson were ca 18 days old to ensure they would

Table 1 Dates of Lincoln to Nelson deliveries of cages containing either parasitised, or unparasitised *Pieris rapae* pupae, and the Nelson sites they were allocated to.

Site	Street	Dis-patch	Date	Type ¹	Dis-patch	Date	Type ¹
1	Halifax St, Nelson	1	23/02/15	R, RS	5	23/03/15	R, RS
2	Green St (site a), Tahunanui	1	23/02/15	R, RS	5	23/03/15	R, RS
3	William St, Richmond	1	23/02/15	R, RS	5	23/03/15	R, RS
4	Thompson Tce, Nelson	1	23/02/15	CS	3	11/03/15	R, RS
5	Milton St, Nelson	1	23/02/15	CS	3	11/03/15	R, RS
6	Muritai St, Tahunanui	1	23/02/15	CS	3	11/03/15	R, RS
7	Cleveland Tce, Nelson	2	02/03/15	R, RS			
8	Washington Rd, Nelson	2	02/03/15	R, RS			
9	Rocks Road, Tahunanui	2	02/03/15	R, RS			
10	Todd Bush Rd, Atawhai	2	02/03/15	CS	4	16/03/15	R, RS
11	Bolt Rd, Tahunanui	2	02/03/15	CS	4	16/03/15	R, RS
12	Green St, Tahunanui	2	02/03/15	CS	4	16/03/15	R, RS
13	Campbell St, Nelson	3	11/03/15	CS	6	25/03/15	R, RS
14	Black St, Stoke	3	11/03/15	CS	6	25/03/15	R, RS
15	Green St (site b), Tahunanui	3	11/03/15	CS			
16	Westerham Pl, Nelson	4	16/03/15	CS	7	30/03/15	R, RS
17	Airlie St, Glenduan	4	16/03/15	CS	7	30/03/15	R, RS
18	Washington Rd, Nelson	4	16/03/15	CS	7	30/03/15	R, RS
19	Stanley Cres (site a), Nelson	5	23/03/15	CS	8	07/04/15	R, RS
20	Stanley Cres (site b), Nelson	5	23/03/15	CS	8	07/04/15	R, RS
21	Green St, Nelson	5	23/03/15	CS	8	07/04/15	R, RS
22	Brougham St, Nelson	7	30/03/15	CS			
23	Kawai St South, Nelson	7	30/03/15	CS			
24	Tui Glen Rd, Atawhai	7	30/03/15	CS			
25	Frost Rd, Lud Valley	8	07/04/15	CS			
26	Dodson Valley Rd, Atawhai	8	07/04/15	CS			
27	Toi Toi St, Nelson	8	07/04/15	CS			

¹Cage types: R=release; RS=Release Sentinel; CS=Control Sentinel

become adults within 1–2 days of being placed at a release site, and were prepared as follows. Eighteen days before delivery, 3–5 *Pt. puparum* adults were transferred to a cage containing 40 *P. rapae* pupae (< 3-days-old), and kept at 20°C and 16:8 h light:dark. Parasitoid adults were removed after 1 week, and any adult *P. rapae* that developed from unparasitised pupae were removed after 2 weeks. On day 18, the remaining pupae were checked for signs of parasitism (parasitised pupae become hard and brown), and any diseased pupae (which become soft) were discarded.

Sentinel *P. rapae* pupae sent to Nelson were < 3-days-old to optimise their suitability for parasitism by *Pt. puparum*, and were prepared as follows. Five to 7 days before delivery, 30 final instar *P. rapae* larvae were transferred to a cage containing several cabbage seedling leaves. The larvae usually completed feeding within 4 days and pupated on the cage sides and lids, which reduced disease incidence caused by moisture on cage floors during heavy rain.

The eight deliveries were allocated to 27 Nelson sites (Table 1). Twenty sites received releases and release sentinels (Table 1). Releases comprised a total of 837 *P. rapae* pupae split between 23 cages, and release sentinels comprised 689 pupae, also split between 23 cages. In order to evaluate the effect of releases on *Pt. puparum* adult abundance, 14 sites initially received a control sentinel cage, then 2 weeks later received a release cage (Table 1). Three sites were treated twice (Table 1, sites 1–3). Control sentinels comprised a total of 630 *P. rapae* pupae split between 21 cages, and were allocated to 21 sites (Table 1). The three control sentinel cages delivered on 23 February 2015 served as controls for three releases made on 23 February 2015 and an additional two on 25 February 2015. Seven sites only received control sentinels (Table 1).

RESULTS AND DISCUSSION

Laboratory rearing of *Pieris rapae*

At 20°C and 16:8 h light:dark, *P. rapae* took 6 weeks to develop from egg to adult, with pupation starting at ca 4 weeks. Development of 4th and 5th

instar larvae could be slowed by maintaining them at 10°C, but not of earlier instars because their survival rates declined. *Pieris rapae* was reared for five generations before the production of viable eggs abruptly ceased in late March 2015, probably due to a microsporidian disease infecting the colony, which is prevalent in autumn (G. Walker, Plant & Food Research, personal communication).

Collecting *Pteromalus puparum* from the field and rearing it in the laboratory

The first *Pt. puparum* adult was captured on 2 February 2015 and a further 40 were captured by 18 February; these were used to parasitise the first three batches of laboratory-reared *P. rapae* pupae. We observed high rates of parasitism in laboratory-reared pupae less than 4-days-old (ca 98%, n=900) and noted that parasitism levels decreased as the age of the pupae exceeded 4 days (ca 65% for 1 week old pupae, n=20). *Pt. puparum* has been reported to oviposit in *P. rapae* pupae of any age except those about to emerge (Muggeridge 1943). On 19 February 2015, the first *Pt. puparum* adults developed from eggs laid by parasitoids captured in early February, and henceforth became the main source of adults used to parasitise *P. rapae* pupae. At 20°C and 16:8 h light:dark, *Pt. puparum* eggs took ca 20 days to become adults, and adults lived ca 4 weeks.

Releasing *Pteromalus puparum* in Nelson and monitoring parasitism using *Pieris rapae* as sentinels

Table 2 summarises the results from the release cages. With the first two releases, flooding of cages by rain caused 39% mortality of pupae, and small predators – perhaps ants – were probably responsible for the disappearance of another 26% (Table 2). These issues were resolved in subsequent dispatches by improving drainage in cages, and retrieving cages after 2 weeks rather than 3–4 weeks. Disease incidence in parasitised pupae ranged from 0–39%. Eighty five percent of parasitised *P. rapae* pupae (n=837) produced parasitoids (Table 2). These pupae

Table 2 Fate of *Pieris rapae* pupae parasitised by *Pteromalus puparum*.

Date of dispatch	n parasitised pupae	Release site ID	Pupae that produced adult parasitoids n (%)	Estimated n parasitoids released	<i>P. rapae</i> adults n (%)	Diseased pupae n (%)	Missing pupae n (%)
23/02/15	90	1,2,3	61 (68)	1,220	6 (7)	0	23 (26)
2/03/15	118	7,8,9	58 (49)	1,160	14 (12)	46 (39)	0
11/03/15	120	4,5,6	111 (93)	2,220	2 (2)	5 (4)	0
16/03/15	108	10,11,12	104 (96)	2,080	0	4 (4)	2 (2)
23/03/15	98	1,2,3	94 (96)	1,880	0	4 (4)	0
25/03/15	64	13,14	64 (100)	1,280	0	0	0
30/03/15	120	16, 17, 18	112 (93)	2,240	0	8 (7)	0
07/04/15	119	19, 20, 21	110 (92)	2,200	0	9 (8)	0
Total (%)	837	20	714 (85)	14,280	22 (3)	76 (9)	25 (3)

Table 3 Fate of unparasitised release sentinel *Pieris rapae* pupae.

Date of dispatch	n sentinels at release sites	Release site ID	<i>P. rapae</i> adults n (%)	Diseased pupae n (%)	Missing pupae n (%)	Parasitised pupae n (%)
23/02/15	89	1,2,3	85 (96)	4 (5)	0	0
2/03/15	90	7,8,9	53 (59)	18 (20)	19 (21)	0
11/03/15	90	4,5,6	87 (97)	3 (3)	0	0
16/03/15	90	10,11,12	90 (100)	0	0	0
23/03/15	90	1,2,3	85 (94)	5 (6)	0	0
25/03/15	60	13,14	43 (72)	17 (28)	0	0
30/03/15	90	16, 17, 18	74 (82)	16 (18)	0	0
07/04/15	90	19, 20, 21	68 (76)	16 (18)	0	6 (7)
Total (%)	689	20	587 (85)	77 (11)	19 (3)	6 (1)

produced an average of 20 parasitoids each (range 1-60, n=96 pupae), which was consistent with Muggeridge (1943). Thus, ca 14,280 *Pt. puparum* adults were released in Nelson.

Table 3 summarises results from release sentinels. The only parasitism of sentinel pupae ever recorded in this study occurred in association with the last release on 7 April 2015 (Table 3). This parasitism first became apparent on 21 May 2015, 6 weeks after the release. The 6 weeks for *Pt. puparum* to develop in the field, compared

to 3 weeks in the laboratory, was probably due to cooler field temperatures, and was consistent with unparasitised *P. rapae* pupae in the 7 April 2015 release taking 3 weeks to become adults compared to 2 weeks in the laboratory. The remaining 28 apparently healthy sentinel pupae from the 7 April 2015 release were returned to Lincoln on 26 May 2015, and maintained at 20°C and 16:8 h light:dark to monitor if they were parasitised. Between 26 May 2015 and 20 August 2015, parasitoids emerged from a further five sentinel pupae, and the other pupae

Table 4 Fate of unparasitised control sentinel *Pieris rapae* pupae.

Date of dispatch	n sentinels at control sites	Control site ID	<i>P. rapae</i> adults n (%)	Diseased pupae n (%)	Missing pupae n (%)	Parasitised pupae n (%)
23/02/15	90	4,5,6	76 (84)	14 (16)	0	0
2/03/15	90	10, 11, 12	64 (71)	23 (26)	3 (3)	0
11/03/15	90	13, 14, 15	90 (100)	0	0	0
16/03/15	90	16, 17, 18	90 (100)	0	0	0
23/03/15	90	19, 20, 21	67 (74)	23 (26)	0	0
25/03/15 ¹	n/a	n/a	n/a	n/a	n/a	n/a
30/03/15	90	22, 23, 24	80 (89)	10 (11)	0	0
07/04/15	90	25, 26, 27	78 (87)	12 (13)	0	0
Total (%)	630	21	545 (87)	82 (13)	3 (1)	0 (0)

¹Used control sentinels from batch 5, 23/03/15

either became adults or exhibited disease signs.

Although the release sentinels provided only very weak evidence of an effect of the releases on *Pt. puparum* abundance, it is possible wild *Pt. puparum* had just begun to increase in response to the releases when fresh sentinels became unavailable due to disease in the laboratory culture. It is also possible that released *Pt. puparum* overwintered in wild, rather than sentinel, pupae. If so, some would have emerged in Nelson in spring 2015 to increase parasitism-pressure on any *P. brassicae* pupae that remained.

Table 4 summarises results from control sentinels. No control sentinels, which were all >200 m away from releases sites, became parasitised. This was surprising because at ca 90% of the control sites, cages were placed within 1-3 m of host plants, where there was evidence of *Pieris* spp. feeding damage and *P. rapae* adults were often observed. At the remaining ca 10% of sites, *P. brassicae* had recently been present, and adjacent properties had host plants. By comparison, at Lincoln, ca 30% of 528 sentinel *P. rapae* pupae were parasitised by *Pt. puparum* during mid-January to mid-February, and 70% of 30 sentinel pupae were parasitised by *Pt. puparum* during late March and early April. At Lincoln, sentinel pupae that became parasitised were placed in densely-planted brassica plots, which contrast with the smaller mixed vegetable gardens that characterised

the Nelson sites. The difficulty measuring responses to parasitoid augmentation have been experienced in other studies (Avila et al. 2013).

Although evidence of the releases being effective was weak, augmenting *Pt. puparum* populations in Nelson remains the sole practical method for increasing the mortality of *P. brassicae* pupae in Nelson. Given the extremely low population densities of *P. brassicae* present in Nelson at the time of the *Pt. puparum* releases (Phillips et al. 2015), and the difficulty of parasitising the few remaining individuals in any eradication programme, eliminating just one or a few *P. brassicae* pupae by the released parasitoids could have provided significant benefits to the eradication programme.

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