

INTRODUCTION INTO NEW ZEALAND OF *BRACON PHYLACTEOPHAGUS*, A BIOCONTROL AGENT OF *PHYLACTEOPHAGA FROGGATTI*, EUCALYPTUS LEAF-MINING SAWFLY

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ABSTRACT

A braconid was imported into New Zealand from Australia to control the introduced Eucalyptus leaf-mining sawfly *Phylacteophaga froggatti* Riek (Hym. : Pergidae). Difficulties in rearing the parasite in quarantine facilities were overcome after it was discovered that the imported material included two closely related *Bracon* spp. with different mating requirements. The principal parasite (subsequently described as *Bracon phylacteophagus* Austin (Hym. : Braconidae)) was released and is now well established in some localities. Early results show a very rapid spread of the parasite with up to 98% parasitism and indicate that the sawfly population should be considerably reduced by late summer 1990.

Keywords: biocontrol; mating; laboratory rearing; *Bracon phylacteophagus*; *Phylacteophaga froggatti*.

INTRODUCTION

Phylacteophaga froggatti, an Australian leaf-mining sawfly which infests species of *Eucalyptus*, was first reported in New Zealand in March 1985 near Auckland International Airport. During the 1985–86 season the sawfly spread rapidly throughout the greater Auckland area and also became established in isolated areas in the northern half of the North Island (Nuttall 1985; Kay 1986). According to unpublished records of the Forest Research Institute (FRI), *P. froggatti* was established in most of the North Island north of Tokoroa and in the Hastings area by June 1989. Because of the absence of specific biocontrol agents in New Zealand, Kay (1986) considered this insect a potential threat to the establishment of eucalypt plantations and to the use of eucalypts as garden ornamentals.

The sawfly is of little commercial significance in Australia although some studies have been done (Riek 1955) and its attendant parasite complex is relatively well known. Farrell & New (1980) noted two species of parasites which were commonly bred during their study of *P. froggatti* and considered these identical to the two species associated with *Phylacteophaga* spp. which were recorded by both Riek (1955) and Moore (1966). The larger parasite, a braconid, was reared only from *Phylacteophaga*; the other, a species of *Cirrospilus* Westwood (Eulophidae), was both a primary parasite of *Phylacteophaga* and a hyperparasite of the braconid, and apparently identical with a species reared (as both a primary parasite and a hyperparasite) from representatives of several families of leaf-mining Lepidoptera. The

observations of Farrell & New (1980) also suggested that hyperparasitism is “an integral part of the *Cirrospilus* life cycle rather than a casual occurrence”.

In 1986 funds were contributed by the New Zealand Forest Service and the Forest Owners' Association to introduce the braconid into New Zealand as a biocontrol agent of *P. froggatti*. Consignments of this parasite were sent to New Zealand in December 1986 and January 1987 from Melbourne, Australia, and FRI staff tried to breed and rear this insect in the Department of Scientific and Industrial Research (DSIR) quarantine facilities in Auckland. From 488 females and 552 males imported, more than 600 adult progeny were reared, but this attempt was abandoned when it became obvious that no mating had occurred. Unmated braconid females produce only male progeny. Except for 15 females which were offspring of females collected in the field in Australia, all the reared adults were males. During December 1987 and January 1988 three consignments of the parasite were sent to the FRI and from these were obtained adults for rearing trials in the quarantine facilities. This paper gives an account of the importation, rearing, release, and establishment of the braconid (*Bracon phylacteophagus*) in New Zealand.

IMPORTATION

Except for a few field-collected females, the parasites consigned in 1987–88 were collected as cocoons in Australia and airfreighted to New Zealand, mostly as adults, individually in small clear plastic tubes inserted into polystyrene blocks. A small drop of honey as food was placed inside the lid of the tubes. Altogether 103 live males and 92 females were obtained for breeding. Of the females, 12 had been field collected, 22 had been allowed to fly free around the laboratory in Australia with males for 2–4 days, and the remainder had either emerged singly in tubes or with other adults in various-sized plastic containers.

REARING

Sawfly-infested foliage was collected from Tauranga and Hamilton. Young sawfly larvae are not attacked by the braconid (Farrell & New 1980) so during the summer only foliage with large mines was gathered. When material became scarce in winter all sizes of host were collected and small sawfly larvae grew to full size on infested leaves kept in sealed plastic bags at 22°C.

The quarantine room is approximately 3 × 2.5 m, without windows. Simulated daylight with a strong UV component was provided from 1800 h to 800 h. Rearing work was done after 800 h under normal fluorescent light. Rearing temperature was 21–22°C with approximately 40% RH.

Imported Adults

Treatment

The imported adults were kept separate according to their pre-freight experience (i.e., field collected, laboratory flown) and released into three types of container in the quarantine room as follows:

- (1) Gauze cages — 600 × 500 × 500-mm wooden-framed gauze-covered cages containing small branches of sawfly-infested foliage placed in conical flasks filled with water. Dilute honey soaked into cellosene in a petri dish lid was provided as food.

- (2) Perspex containers — 250 × 110 × 160-mm clear perspex containers with sawfly-infested leaves taped to the inside with masking tape. Dilute honey (as for 1) was supplied as food.
- (3) Plastic cups — 95-mm-diameter × 110-mm-high translucent plastic cups. The lid was a clear plastic 90-mm petri dish lid with a gauze-covered 25-mm-diameter hole for ventilation. Sawfly-infested leaves were taped to the inside. A small pad of cellosene soaked in dilute honey was placed on the gauze and a small drop of pure honey under the gauze.

Every 2 days the leaves were removed from the containers into 200 × 250-mm clear plastic bags sealed with a rubber band. Fresh sawfly-infested leaves were then added.

Emerging adults were collected daily from the plastic bags, females being stored individually in tubes and males in wooden boxes with a glass top; each box was stocked with 300+ males. Liquid honey was supplied regularly as food.

Results

Most sawfly larvae in the plastic cups were paralysed after 2 days' exposure to the parasites and oviposition also occurred, but to a much lesser degree, in the cages and perspex containers.

Seventy-one females and approximately 1800 male F1 adults were reared. Seven of the females were progeny of the field-collected adults and 23 of a female seen to mate in a perspex container in the quarantine room. The remaining 41 were from three females in plastic cups and one in a gauze cage, all of which had been allowed to fly free with males in the laboratory at Melbourne for 4 days. Mating of these four females could have occurred in the rearing containers. However, in view of the previous year's experience it was considered more likely that mating had taken place in the laboratory in Australia indicating that natural light, space, or both were necessary for mating.

F1 Adults

Treatment

Five treatments to produce mated females were tried as follows:

- (1) Four females with many males in a gauze cage in the quarantine room (artificial light).
- (2) Nine females with many males in a gauze cage in the quarantine room with the perspex front of the cage upwards and facing the light source.
- (3) Seven females with many males in an outside insectary with 1-m-high *Eucalyptus botryoides* Sm. in pots.
- (4) Three females with many males in a 350 × 250 × 150-mm wooden box with a glass top in a room with natural light.
- (5) Fourteen females plus males in an insect-rearing room with natural light (window).

After exposure times of 2–4 days to these treatments, the females were removed into individual plastic cups with sawfly-infested leaves in the quarantine room.

Results

Only four F1 females produced female progeny. Three of these were from 4 days' exposure to treatment 5 and one from treatment 4. Altogether 41 female and approx.

500 male F2 adults were reared. Thirty-two of the females were offspring from one adult (hereafter referred to as No. 76). The only common factor in the two treatments which produced mated females was natural light.

F2–F5+ Adults

Treatment

F1 adults could be separated into two groups based on slight colour differences and this was at first thought to represent intraspecific variation. However, when differences in behaviour were discovered which also gave the same grouping, and mating trials demonstrated that no mating would take place between individuals in the different groups, the possibility of there being two species was considered. The presence of two distinct species of *Bracon* was confirmed and these have been subsequently described as *Bracon phylacteophagus* Austin and *Bracon confusus* Austin (Austin & Faulds 1989). A check of preserved specimens retained from the 1986–87 imports showed that both species had also been imported in that season. *Bracon phylacteophagus* is the principal parasite of *P. froggatti* as *B. confusus* made up only approx. 2.5% of the imports in both 1986–87 and 1987–88. The fact that there were two species explained much of the contradictory and confusing information concerning mating requirements. When the history of all previously mated females was checked it was found that, while these species are remarkably similar in appearance, *B. phylacteophagus* had mated only when free-flown in a room with natural light but *B. confusus* could mate in small containers under artificial light. Further mating trials then confirmed this. Rearing of *B. confusus* was discontinued in order to concentrate on *B. phylacteophagus* the principal parasite, and a simple procedure to breed and rear *B. phylacteophagus* was quickly identified. This consisted of releasing recently emerged females and males into an insect-rearing room with a window to admit natural light. Drops of liquid honey and water were placed on the window daily and a few leaves with sawfly mines were taped to the window frame. A 1-m-high eucalypt was placed near the window to provide a landing platform.

Fortunately, when the problem was elucidated there were 12 recently emerged *B. phylacteophagus* females (progeny from No. 76) still alive and these, plus males, were immediately released into the rearing room. Eight days later eight females were recovered, transferred into individual plastic cups in the quarantine room, and treated as described earlier. One slight modification was that the cellosene pad was soaked in water instead of a dilute honey solution.

By July 1988 there was a considerable overlap of generations and it was not possible to keep rearing records of separate generations beyond F5.

Some F3 females and most F4 and F5 cocoons were kept in cool storage at 4 °C. Freshly emerged females were kept individually in small tubes and fed honey for 2 days before going into storage. They were removed from the cool store for 1 day every 3 weeks and fed. Leaves with cocoons were stored in 200 × 250-mm plastic bags for 2 months.

Results

A summary of the total *Bracon* spp. reared to 31 August is given in Table 1. Approximately 25% of females died during 2 months in cool storage and those

remaining alive were less active than fresh unstored females. Adults emerged from 55% of the cocoons removed from the cool store; however, these adults were also not very active and many died within 1 week of emergence.

TABLE 1—Total *Bracon* spp. reared to 31 August 1988

Generation	Number reared
Males	
F1-F5	4224 (includes both species)
Females	
F1	71
F2	42
F3	99 <i>B. phylacteophagus</i> + 15 <i>B. confusus</i>
*F4	45 <i>B. phylacteophagus</i> + 81 <i>B. confusus</i>
*F5	22 <i>B. phylacteophagus</i> + 8 <i>B. confusus</i>

* Most developing *Bracon* spp. kept in cool storage in cocoon stage

Rearing After 31 August 1989

Treatment

All virgin females were placed with males in an insect-rearing room with natural light for up to 11 days before removal into the quarantine room.

Results

Most of the females mated (Table 2). Between 1 September 1988 and when rearing ceased on 6 April 1989, 1640 females and 11 072 males were reared. Time from oviposition to emergence was 14–22 days and varied with temperature. For example, developing *B. phylacteophagus* kept in plastic bags on the floor took 2–3 days longer to emergence than those on the bench in the same room where the temperature was 1–2 °C higher. The biotic potential as represented by the figures in Table 3 shows the

TABLE 2—Number and percentage of females mated

Date in room	Days in rearing room	Females recovered from rearing room	Females mated (No.)	Females mated (%)
22.10.88	8	18	13	72
30.10.88	9–10	17	14	82
23.11.88	7–8	12	8	67
2.12.88	8	7	7	100
10.12.88	9	10	8	80
19.12.88	9–11	31	29	93
7.1.89	7–12	18	12	67
20.1.89	7	11	8	73
27.1.89	8–10	21	14	67
8.2.89	5	4	3	75
13.2.89	7–8	17	15	88
21.2.89	6–7	15	13	87
Totals		181	144	80

progeny produced by 13 females which stayed alive 46 days or more in the plastic cups. The average progeny produced was 92 ($n = 13$; range 52–131).

The ratio of the total male to female progeny (Table 3) was 1.0:4.6. This ratio was not improved by constant contact of mated females with males.

TABLE 3—Progeny produced by *B. phylacteophagus*

Female No.	Days alive in cup	Progeny		
		Male	Female	Total
1	72	77	19	96
2	58	104	19	123
3	50	74	21	95
4	46	64	8	72
5	79	103	28	131
6	51	59	17	76
7	60	66	6	72
8	60	89	13	102
9	72	102	24	126
10	75	85	17	102
11	72	61	30	91
12	62	56	3	59
13	57	42	10	52
Total		982	215	1197

FIELD RELEASES OF *B. PHYLACTEOPHAGUS*

Because the male to female ratio of emergences from Australian field-collected cocoons (1:1 in 1986–87 and 1.5:1.0 in 1987–88) indicated that mating is common in the field, any progeny surplus to rearing requirements were released as soon as possible.

TABLE 4—Releases of *B. phylacteophagus* adults in 1988

Date released	Locality	Number released		Generation
		Males	Females	
19 Feb	Maungatapu (site 1)	300+	12	F1
22 Feb	Maungatapu (site 1)	200+	5	F1
25 Feb	Maungatapu (site 2)	200+	5	F1
29 Mar	Maungatapu (site 1)	20+		
6 Apr	Maungatapu (site 1)	50+		
15 Apr	Maungatapu (site 1)	50+		
25 Apr	Maungatapu (site 1)	100+		
29 Apr	Maungatapu (site 1)	50+		
17 May	Maungatapu (site 1)	150+		
8 Jun	Maungatapu (site 1)	100+		
16 Jun	Te Maunga	60	4	F3–4
28 Jun	Te Maunga	80	12	F3–4
19 Jul	Te Maunga	60+	20*	F3–4

*Of these females, 18 had been in cool storage for 6–8 weeks before release and appeared unhealthy.

N.B.: Most female emergences between 22 and 25 February were later known to have been *B. confusus* and it could have been *B. confusus* females which were released on 25 February at site 2.

The first release of F1 adults was made on 19 February 1988 at Maungatapu near Tauranga into a group of approximately 30 trees, 2–3 m high, which were heavily infested with sawfly. Releases were made by simply letting the insects fly out of the storage containers. Details of 1988 releases are presented in Table 4. Some of these releases were made in mid-winter and probably had little chance of establishment.

In 1989 releases were made in many areas (Fig. 1 and Table 5). Some of these releases were for experimental purposes.

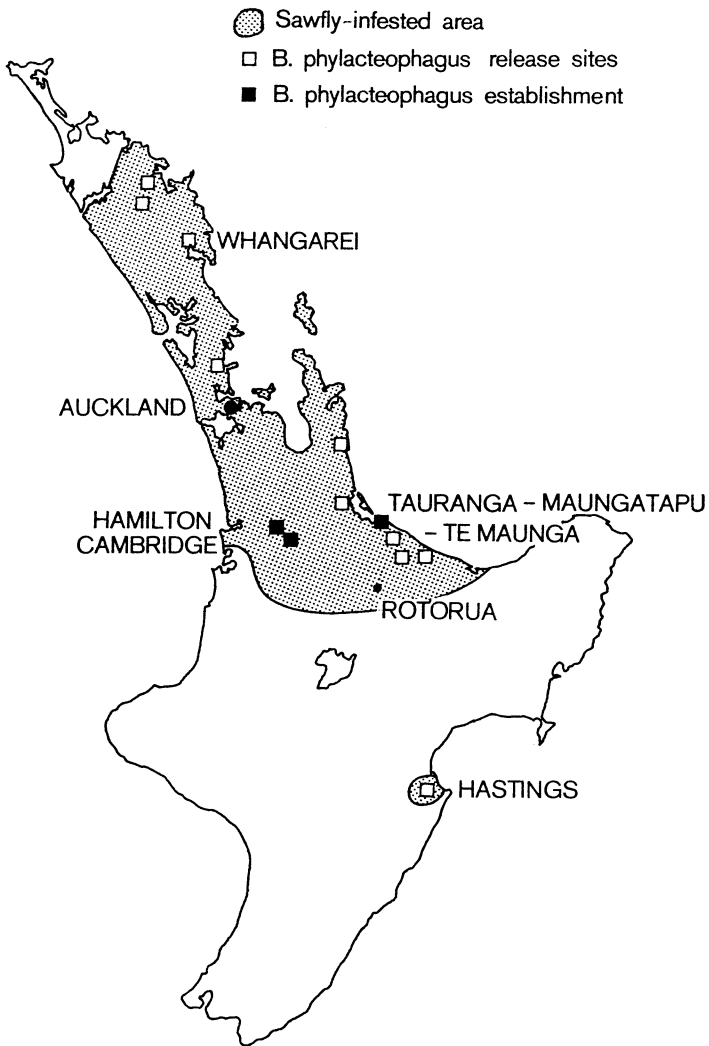


FIG. 1 — Sawfly-infested area as at July 1989 and *Bracon phylacteophagus* release and establishment sites in the North Island of New Zealand.

TABLE 5—Releases of *B. phylacteophagus* in 1989

Date released	Locality	Number released	
		Males*	Females
11 Jan	Cobham Drive Reserve, Hamilton	500	90
17 Jan	Bell Road, Papamoa	500	66 experimental release
19 Jan	Tairua Forest	350	49
25 Jan	Waitangi Forest	250	45
27 Jan	Maketu	20	2 experimental release
6 Feb	Forrest Road, Cambridge	200	57 experimental release
8 Feb	NZFP Absil's, near Kaikohe	250	47
9 Feb	Matata	200	20
14 Feb	Te Maunga, near Papamoa	200	29
16 Feb	Rodney County Council	200	45
19 Feb	Willoughby Park, Hamilton	75	17
28 Feb	Rotoehu Forest	400	77
3 Mar	Rotoehu Forest	300	26
6 Mar	Near Katikati		13 experimental release
13 Mar	Near Mt Maunganui	300	30
13 Mar	Near Paengaroa	300	34
13 Mar	Tauranga-Mt Maunganui highway	300	34
20 Mar	Melville, Hamilton	250	20
23 Mar	Whangarei City Council	450	128
30 Mar	Paengaroa	250	52
31 Mar	Near Hastings	250	45
6 Apr	Matata	50	24
	Total	5595	950

* Approximate numbers

RECOVERY AND ESTABLISHMENT

The first recovery from Maungatapu was on 19 March 1988 when a sawfly mine containing a *B. phylacteophagus* larva was found. A female parasite emerged on 1 April. On 25 March a cocoon was collected from which a female emerged 2 days later. These female offspring proved that the parasites released in February had mated in the field. Further cocoons and adults were seen regularly at this site during 1988 until 9 August, and when a fresh cocoon was collected on 5 January 1989 it was known that the parasite had successfully overwintered. Examination of field-collected cocoons during the winter of 1989 revealed diapause in the mature larval stage. At the end of January 1989, 29 of 30 sawfly mines examined had parasites present and parasite cocoons were found 2 km away from the release site. The release site at Maungatapu had been chosen in 1988 because of an extremely heavy sawfly infestation on the trees that year. In comparison, by mid-March 1989, only a few sawfly mines were present and, except for the very small mines, all contained a parasite. A survey for *B. phylacteophagus* in the Tauranga district on 3 May detected its presence throughout the area to 5 km to the west and 1 km to the east from the release site.

The localities where *B. phylacteophagus* has been recovered, and the stage recovered are shown in Table 6. Recovery of females (Table 6 and Fig. 1) indicates probable establishment.

TABLE 6—Recovery and establishment of *B. phylacteophagus*

Date recovered	Locality	Stage	Establishment (i.e., females found)
1988–89 March and May 1989	Maungatapu and Tauranga District	Larvae, cocoons, adults	Yes
April 1989	Cobham Drive Reserve, Hamilton	Cocoons	Yes
May 1989	Forrest Road, Cambridge	Adult, cocoons	Yes
May 1989	Te Maunga, near Papamoa	Adult, cocoons	Yes
May 1989	Maketu	Cocoon	
May 1989	Bell Road, Papamoa	Cocoon	
June 1989	NZFP Absil's Block, near Kaikohe	Cocoons	

Pediobius bruchicida (Eulophidae) and a species belonging to the family Platygastriidae emerged from *B. phylacteophagus* cocoons collected at Maungatapu in September 1989. Another species of eulophid (*Proacrius* sp.) was recorded as “possibly emerging from *B. phylacteophagus* cocoons” in June.

DISCUSSION

Initially this was a very difficult project because of the confusion caused by the presence of two species of *Bracon*. Only one species of *Bracon* was recorded by Farrell & New (1980) as a parasite of *P. froggatti* in Australia and there was no reason to suspect that there were two species in the imported material.

Bracon phylacteophagus did not mate in the quarantine room with artificial light. The only way to mate imported adults was to allow males and females to free fly for several days in a room with natural light in Australia before they were sent to New Zealand. F1 adults, not subject to quarantine, were mated in this manner in New Zealand. Without the ‘free flown’ imports rearing would have failed. The fact that *B. confusus* mated without natural light showed that even closely related species can require markedly different mating conditions. These results indicate that unless mating requirements which can be satisfied in quarantine facilities are known, imported hymenopterous parasites should be mated in their country of origin where there are no such constraints.

Some of the behavioural differences which helped to separate the species have been documented by Austin & Faulds (1989). The two most outstanding of these are the different mating conditions required and the habit of female *B. phylacteophagus* of feeding on host haemolymph, whereas *B. confusus* does not. However, there were other less-precise differences. For example, when the leaves in the plastic cups were changed, disturbed *B. confusus* females tended to fly upwards while *B. phylacteophagus* flew to the bottom of the cup. Generally, *B. phylacteophagus* was more active, a condition which Austin & Faulds (1989) have suggested is correlated to that species feeding on host haemolymph. The absence of feeding by *B. confusus* on *P. froggatti* haemolymph, and the fact that only 2.5% of the imported parasites were *B. confusus*, could mean that in Australia this species will normally be found parasitising a host other than *P. froggatti*.

In addition to the failure to mate adults, in 1986–87 the number of adults reared was below replacement level. That season sawfly-infested leaves were exposed to the parasite in sealed plastic bags. This produced conditions of high humidity, and condensation was commonly seen inside the bags. Similar conditions arose in 1987–88 with imported adults placed in the practically airtight perspex containers, when small branches of sawfly-infested foliage, with their stems in tubes of water, were used instead of individual leaves taped to the sides. This procedure was quickly discontinued when the parasites became inactive and did not oviposit. A possible conclusion is that poor oviposition in 1986–87 was due to high humidity. Females do, however, seem to require frequent access to free water as more females died than was usual immediately after both times the cellosene pads on the plastic cups were not moistened daily.

There is no one clear reason to explain the 1.0:4.6 male to female sex ratio of progeny of laboratory-mated females compared with the 1:1 and 1.0:1.5 ratios found in Australian field populations. Of all the factors noted by King (1987) which can affect offspring sex ratios in parasitoid wasps only two, genetic and perhaps to some extent photoperiodic, were constant and might be relevant. With *B. phylacteophagus*, the sex ratio may be determined by the number of times a female mates. Females in the field have the opportunity to mate often as oviposition progresses. In contrast, because mating did not occur under artificial light conditions, once the laboratory females were moved from the mating room into the plastic cups there were no further mating opportunities. It was impractical to return females to the mating room. Although King found no evidence that multiple mating will result in more female progeny and cited one instance where in fact the opposite happened, multiple mating to give increased numbers of female offspring does seem a logical way to adjust sex ratios. For example, the presence of few males could mean no or few matings for the female and more male offspring. Conversely, many males could give multiple mating and more female offspring.

Considering that the establishment of *B. phylacteophagus* at Maungatapu-Tauranga was from as few as 17 females plus males, spread has been spectacular. This spread was through an urban area where there are only scattered individual or small groups of infested host trees. As most of the spread was against the prevailing wind a host-emitted attractant might be involved. The high percentage of sawfly parasitised is also very encouraging. No doubt this can be attributed to the absence of both competition and hyperparasitism by *Cirrospilus* sp. *Pediobius bruchicida*, which emerged from *B. phylacteophagus* cocoons, has previously been recorded as both a hyperparasite associated with braconids and a tertiary parasite (Valentine & Walker in press), but it is hoped neither this species nor the platygastriid which also emerged from *B. phylacteophagus* cocoons will have a great impact on the *B. phylacteophagus* population. If the results from the Maungatapu release are indicative of what will happen at the 1989 release sites, *B. phylacteophagus* should be widespread and may dramatically reduce the sawfly population by late summer 1990.

There are no plans to rear the parasite in the laboratory in future. Adults required for further releases will be obtained from field-collected cocoons.

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