Introduction and evaluation of *Cotesia rubecula*, a parasitoid of *Pieris rapae* in New Zealand

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Abstract

The host specificity of *Cotesia rubecula* (Marshall) was examined through literature searches, behavioural and ecological observations, and host specificity tests of a South Australian population. The results led to the importation and testing of *C. rubecula* in quarantine in New Zealand where its specificity to the genus *Pieris* was confirmed. Introduction of the parasitoid was approved and the species was first released in New Zealand for control of *Pieris rapae* (L.) in December 1993. It has now overwintered successfully and has parasitised 71–97% of larvae at study sites. Three paired comparisons of sites with and without the parasitoid showed that *C. rubecula* significantly reduced the survival of *P. rapae* to 5th instar, and also reduced parasitism by *Cotesia glomerata* (L.).

Key words: *Cotesia rubecula*, *Pieris rapae*, host specificity, introduction, impact.

Introduction

Pieris rapae (L.) (Lepidoptera: Pieridae), small white butterfly, is second only in importance to *Plutella xylostella* (L.), (Lepidoptera: Plutellidae), diamondback moth, as a pest of vegetable brassicas in New Zealand. The existing larval parasitoid, *Cotesia glomerata* (L.) is not well synchronised with its host and often provides insufficient control in the summer (Beck and Cameron, 1992). The introduction of *Cotesia rubecula* (Marshall) (Braconidae: Microgastrinae) was therefore proposed. Although *C. rubecula* partially displaces *C. glomerata* (Parker and Pinnell, 1972), the overall efficacy of parasitism is improved by the reduced feeding of larvae parasitised by *C. rubecula* (Parker and Pinnell, 1973). A further factor favouring approval of *C. rubecula* for introduction into New Zealand was its reputed specificity to the genus *Pieris*. Richards (1940) confirmed that *C. rubecula* is almost specific to *P. rapae* and noted that exceptionally it will attack *Pieris brassicae* (L.) In his description of *C. rubecula*, Wilkinson (1945) noted that other than *Pieris* spp., the only other recorded host was *P. xylostella*. Mustata (1992) also recorded *P. xylostella* as a rare host in Moldavia, although in Australia, *C. rubecula* has not been recovered from this species (Austin and Dangerfield, 1992; Goodwin, 1979). Approval for release in New Zealand required the verification of this specificity, which is documented in this paper. The second part of this paper reports on the release, dispersal and impact of *C. rubecula*.

Methods

Host specificity

Initial information on the host specificity of *C. rubecula* was based on the field collections of Dr M.A. Keller and G.J. Baker of *P. xylostella* and *Anaphaeis java* (Sparman) (Papilionoidea: Pieridae) in the Adelaide region of South Australia. In addition, *Bassaris itea* F. (Papilionoidea: Nymphalidae) larvae were collected (by PJC) from this region in 1992 and 1994. The acceptability of these three species and *P. rapae* to *C. rubecula* was also compared in flight tunnel tests at the University of Adelaide using the methods of Keller (1990). *A. java* was presented on *Capparis mitchelli* (Capparaceae), *B. itea* on nettle (*Urtica dioica*), and *P. xylostella* and *P. rapae* on cabbage. The preference of *C. rubecula* females was tested by presenting 3–6 larvae per leaf of each test species as a choice compared with *P. rapae* larvae. The choice by female parasitoids between plants, and any subsequent oviposition into larvae, was noted for each of five tests for each combination.

Confirmatory testing of oviposition responses was also carried out in quarantine at Crop & Food Research in Auckland, New Zealand. Individual mated female parasitoids were exposed in 10 x 2.5 cm glass tubes to single larvae of alternate species, each for a period of 5 minutes. Ten to 24 larvae, 3–10 mm in size were tested for each species. Oviposition responses were recorded and when any response occurred the larva was removed and reared individually on its usual host plant until parasitism was confirmed or the larvae pupated normally. To confirm that females were capable of oviposition, every second or third test larva was *P. rapae*. Larvae were either tested with no plant matter, or with their usual host plant, or after confinement with cabbage. In experiments over longer exposure periods, two mated female parasitoids were placed in 450 ml vented plastic containers for 16 h with 10 larvae on their original host plant, or cabbage. Voucher specimens were deposited in the New Zealand Arthropod Collection at Landcare Research in Auckland.

Release and recovery

Cultures of *C. rubecula* in New Zealand were based on four shipments totalling 383 *C. rubecula* cocoons in 1993/94 (Cameron *et al.* 1995) and one shipment of 85 cocoons in 1994/95, all supplied by Dr M.A.

Keller of the University of Adelaide in South Australia. Females from the source culture were mated with field-collected males in Australia to maintain genetic variability in the shipments. In New Zealand, all importations were reared separately for at least two generations to ensure each shipment contributed parasitoids for release. A disease-free culture of *P. rapae* was established on cabbage to provide small larvae for parasitism. The resulting cocoons of *C. rubecula* were harvested for release, placed in 20 x 5 cm cardboard tubes (100–200/tube) with a small exit hole at one end, and supplied with honey-agar-sugar as food for emerging adults. These release containers were placed in small shelters at canopy height in brassica field sites where larvae of *P. rapae* were present. Sites were located at research stations or organic gardens where a succession of unsprayed brassica crops were planted. A total of 9456 parasitoids were released in 1993/94, and 20167 in 1994/95 *(Table 1)*.

Release sites near Auckland were monitored every 1–2 weeks, and parasitism was assessed by collecting and rearing a minimum of 40 larvae. To confirm over-wintering, surveys were undertaken the following spring or summer after initial releases. Estimates of levels of parasitism were also gained from these collections, and parasitism by *C. glomerata* and the occurrence of hyperparasitoids were recorded from all collections. The dispersal of *C. rubecula* from a release site was monitored weekly from 30 January to 7 March 1996 in the Pukekohe (South Auckland) vegetable-growing region by placing trap plants (cabbages with 30–50 small larvae) at approximately 0.5–1.0 km spacings out from the release site. Plants were placed in pairs (about 10 m apart) at the edge of vegetable brassica crops or near road-side brassica weeds, left in the field for two days, and then collected. Larvae were then reared in the laboratory to determine the extent of parasitism. If *C. rubecula* was recovered, plants were located further away from the original site on the next test occasion until no further parasitism was detected.

Impact

The impact of *C. rubecula* on *P. rapae* was evaluated near Auckland on three occasions at pairs of experimental sites separated by 2–10 km. One site in each pair was an earlier release site for *C. rubecula* and the other site was in the same growing region, but without the new parasitoid. This comparison was performed on cabbage in the 1993/94 and 1994/95 summer seasons, and on broccoli in the 1995/96 summer. The sites consisted of at least 400 plants of similar age that had received no insecticide applications. Size distribution of *P. rapae* larvae was assessed by recording the number of larvae in each instar in weekly samples from 20–40 randomly selected plants. Rates of parasitism were monitored by collecting and rearing the first 30–60 large 1st to 3rd instar larvae encountered during this weekly sampling. Percent parasitism was calculated as the number of parasitised larvae compared with the number of survivors plus parasitised larvae.

Results and Discussion

Host specificity

In the Adelaide region of South Australia, investigations of host specificity were focused on near relatives of *P. rapae*. The closest relative of *P. rapae* that occurred close to mixed cropping areas where *C. rubecula* was present was the pierid, *A. java*. These larvae were common on *C. mitchelli* in the grounds of the Waite Campus of the University of Adelaide, but were not parasitised by *C. rubecula* (Austin and Dangerfield, 1992; M.A. Keller, pers. comm.). *B. itea*, the yellow admiral, occurs on nettle *(U. dioica)* in both Australia and New Zealand. As New Zealand has few attractive butterflies there is interest in ensuring its conservation. Approximately 132 larvae of this species were collected from six locations around Adelaide over two summers. None were parasitised. Previous extensive collections of *P. xylostella* from vegetable and wild brassicas by M.A. Keller and G.J. Baker confirmed that *C. rubecula* was not a parasitoid of this species in the Adelaide region.

In flight tunnel experiments, *C. rubecula* was attracted to and oviposited in *P. rapae*, but females were not attracted to either *A. java* or *B. itea*. Any females that alighted on *Capparis/A. java* or nettle/*B. itea* immediately took flight and often moved to cabbage. In the comparison of *P. rapae* with *P. xylostella*, female parasitoids flew equally to either plant, but oviposition responses were directed only at *P. rapae*. These observations are consistent with those of Agelopoulos and Keller (1994).

Table 1. Releases and recoveries of *Cotesia rubecula* to April 1996, showing overwintering success and peak parasitism

Region	Release sites	First release	Number released	Recovery sites	Over-wintered (sites)	Peak parasitism (%)
Northland	11	Feb. 1994	5 328	4	2	61
Auckland	14	Dec. 1993	13 227	7	5	97
Feilding	3	Dec. 1994	3 374	3	2	14
Levin	3	Mar. 1994	2 711	3	1	88
Canterbury	2	Mar. 1994	3 267	2	1	71
Gore	1	Mar. 1994	1 816	1	0	52
Total	34		29 723	20	11	

Following the apparent specificity suggested by these tests, *C. rubecula* was imported into quarantine in New Zealand for further oviposition tests with relatives of *P. rapae* and other Lepidoptera found on brassicas, including native species *(Table 2)*. These tests attempted to force oviposition, or ovipositional errors, by artificially associating the parasitoid and test larvae. In addition, test larvae occasionally found on brassicas in the field were fed on cabbage prior to testing, thus generating potential oviposition stimuli in the form of frass volatiles. Oviposition was obtained only with *P. rapae*, but occasional oviposition-like probing was observed with *Graphania mutans* Walker (Noctuidae) and *P. xylostella (Table 2)*. Rearing and dissection of probed individuals detected no eggs or larvae, and no parasitoid cocoons were formed. All parasitoid stages were detected in *P. rapae* control insects. Choice experiments with *P. rapae* and *G. mutans* or *P. xylostella* showed that parasitoids would walk over the alternate species to selectively oviposit in adjacent *P. rapae*.

It was concluded that *C. rubecula* from the Australian source was specific to the genus *Pieris*. As there are no other Pieridae in New Zealand, the parasitoid was considered to be safe to import. Following public consultation, permission to release was obtained and the parasitoid was first released in December 1993.

Establishment and dispersal

At frequently sampled experimental sites in the Auckland region, parasitised larvae were recovered within 2–4 weeks of release, and in the first season parasitism reached 25–97% in five geographic regions (Cameron *et al.*, 1995). Overwinter survival was not recorded in all regions after one season of releases, but after two seasons of releases the parasitoid was considered to be established in all but one region *(Table 1)*. No geographic or climatic limitations to the establishment of the parasitoid were detected. The few failures to persist were attributed to low host populations rather than any biological limitations of *C. rubecula*.

Dispersal from release sites has so far been comparatively slow. In the first four months following release in the Pukekohe vegetable-growing region, parasitoids were detected 2.1 km from the release point. By March 1996, two years and three months after release, *C. rubecula* had spread approximately 12 km. By contrast, *Cotesia kazak* Telenga (Braconidae: Microgastrinae), a parasitoid of *Helicoverpa armigera* Hubner (Noctuidae), spread approximately 100 km in one year from the same Pukekohe release site (Cameron and Valentine, 1985).

Impact

The release of *C. rubecula* at experimental sites and its relatively slow dispersal allowed the comparison of sites with and without natural parasitoid populations. Weekly sampling of paired sites showed that as populations of *P. rapae* developed on brassica crops, fewer larvae survived to reach 5th instar where *C. rubecula* was present (*Figures 1a* and *b)*. In the cabbage trial at Kumeu in 1994/95, parasitism at the release site ranged from 71 to 77%. Just prior to harvest, no 5th instar larvae were found on 20 sample plants at the parasitoid release site, whereas an average of 1.95 large 5th instar larvae/plant were found at the site without *C. rubecula (Table 3)*. A comparison of the instar distribution of these larvae *(Fig. 1a)* showed that parasitism caused a high level of mortality in 4th instar *P. rapae*.

Similar results were obtained in the broccoli trial in 1995/96 where parasitism at the release site ranged from 71 to 93% *(Figure 2a)*. Although *P. rapae* populations were more dense than at the control site, very few larvae survived to enter the 5th instar. At the control site, *C. rubecula* was absent until one individual appeared in each of the last two sampling occasions, demonstrating successful dispersal from the release site *(Figure 2b)*. As the population at the control site developed, large 5th instar larvae became common on the plants. Just prior to harvest , 80% of the broccoli heads were infested with an average of 2 large larvae/plant. Comparison of the instar distribution of larvae at sites with and without *C. rubecula* showed high

Table 2. Oviposition responses by *C. rubecula* to test larvae related to *P. rapae*, and larvae associated with brassicas

Test larvae	Possible oviposition	Parasitoid development	Number of observations
Pieris rapae (L.) (Papilionoidea: Pieridae)	149	145	149
Related species			
Bassaris itea F. (Papilionoidea: Nymphalidae), native	0	–	18
Danaus plexippus L. (Papilionoidea: Nymphalidae)	0	–	20
Zizina labradus Godart (Papilionoidea: Lycaenidae), native	0	–	18
Species from brassicas			
Agrotis ipsilon (Hufnagel) (Noctuidae)	0	–	18
Epiphyas postvittana Walker (Tortricidae)	0	–	18
Graphania mutans Walker (Noctuidae), native	7	0	68
Helicoverpa armigera (Hubner) (Noctuidae)	0	–	24
Phrissogonus laticostatus Walker (Geometridae)	0	–	10
Plutella xylostella (L.) (Plutellidae)	3	0	76
Thysanoplusia orichalcea F. (Noctuidae)	0	–	23

Table 3. Mean number of 5th instar larvae/plant (n=20), two to three weeks prior to harvest

Site, year and crop	Mean number/plant (±SE)	
	With *C. rubecula*	Without *C. rubecula*
Pukekohe, 1993/94, cabbage	0.05 ± 0.05	1.65 ± 0.35
Kumeu, 1994/95, cabbage	0	1.95 ± 0.37
Pukekohe, 1995/96, broccoli	0.25 ± 0.12	2.05 ± 0.42

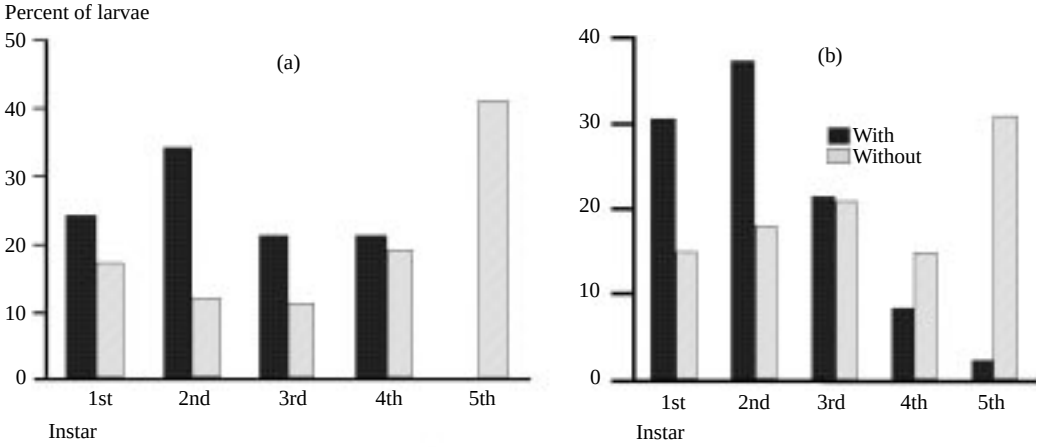

Figure 1. Instar distribution of P. rapae populations with or without C. rubecula at (a) paired cabbage sites at Kumeu, 1994/95, and (b) paired broccoli sites at Pukekohe, 1995/96

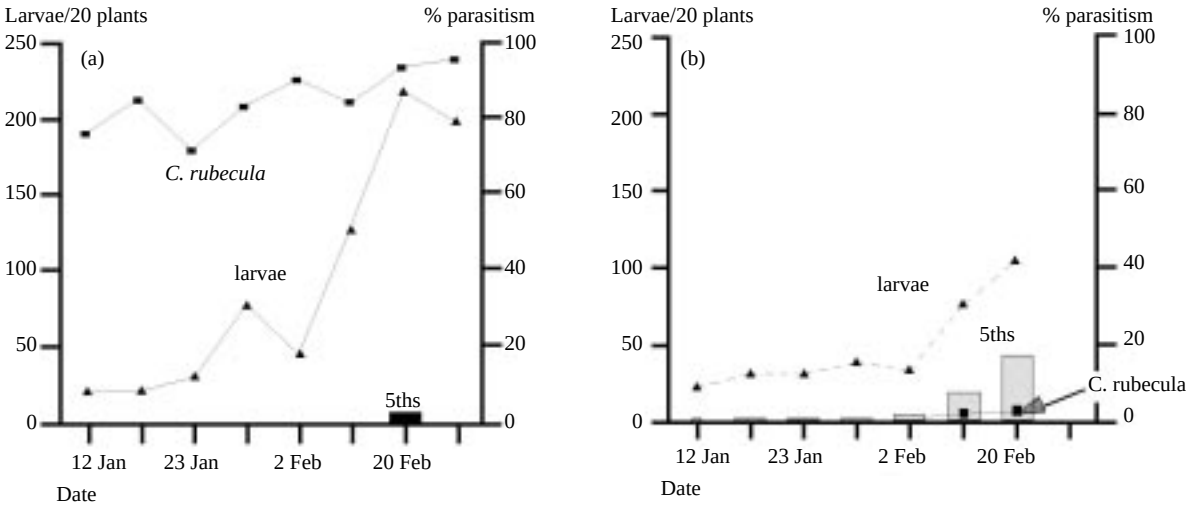

Figure 2. Development of P. rapae larval populations and 5th instar larvae, and parasitism by C. rubecula at (a) a previous release site for C. rubecula, and (b) a control site; Pukekohe, 1995/96

mortality from parasitoids emerging from 4th instar larvae *(Figure 1b)*.

Estimates of parasitism by *C. rubecula* and the multiple parasitoid *C. glomerata* showed the dominance of *C. rubecula*. Without *C. rubecula* at the broccoli control site in 1996, parasitism rates for *C. glomerata* reached 50% *(Figure 3a)*. Where both parasitoids were present, *C. glomerata* parasitism remained less than 10% *(Figure 3b)*. This finding is consistent with field observations by Parker and Pinnell (1972) and laboratory experiments by Laing and Corrigan (1987). Parker and Pinnell (1973) also demonstrated that partial displacement of *C. glomerata* was not detrimental to contol of *P. rapae* because larvae parasitised by *C. rubecula* are killed in the 4th instar and eat significantly less than those parasitised by *C. glomerata*.

Estimates of the infestation of plants by 5th instar larvae provided a summary of the impact of *C. rubecula* in all the experimental comparisons *(Table 3)*. As the paired sites were planted at similar dates and were within 10 km of each other, the seasonal or climatic differences between sites were minimised. All sites were free of insecticide applications, therefore natural enemies other than *C. rubecula* were also abundant and contributed to mortality. However, consistent differences between the size distribution of *P. rapae* larvae with and without *C. rubecula*, together with the high rates of parasitism, indicated that *C. rubecula* was a major factor in reducing the populations of large, damaging *P. rapae* larvae. Although these differences were reflected in reduced crop damage by *P. rapae* at sites where *C. rubecula* was present, damage from *P. xylostella* continued to

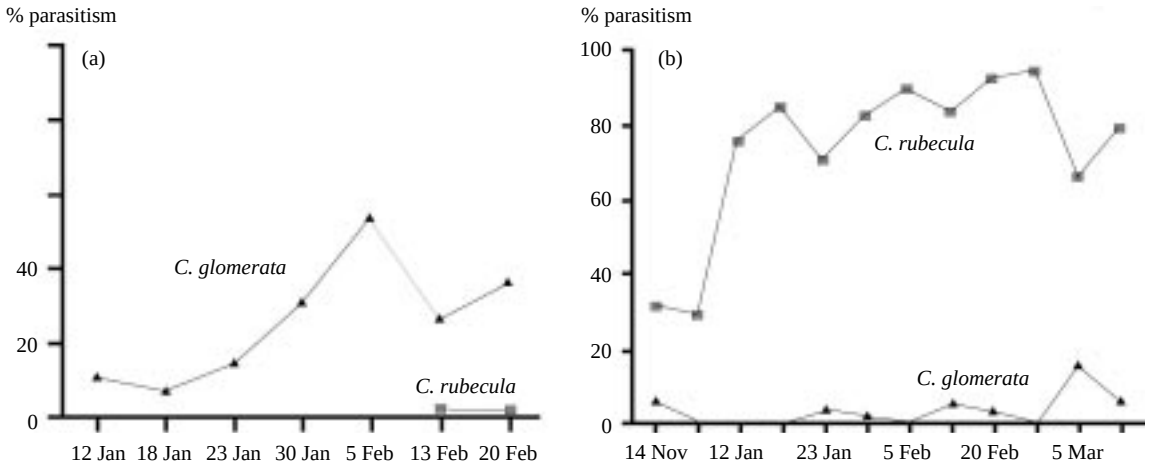

Figure 3. Parasitism of P. rapae by C. rubecula and C. glomerata at (a) a previous release site for C. rubecula and (b) a control site; Pukekohe, 1995/96

be the dominant problem at two of the three sites. Research to improve biological control of *P. xylostella* is the subject of an additional research programme also reported (see Cameron *et al.*, 1997) in these proceedings.

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