

Appendix 2:

Risks to non-target species from potential biological control agent *Cotesia urabae* against *Uraba lugens* in New Zealand

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Introduction

Biological control of insect pests is a sustainable approach to pest management that seeks to correct ecological imbalances that many new invaders create on arrival in a new country. This is done by introducing carefully selected natural enemies of the pest, usually from its native range. This method is the only means of establishing and maintaining self-sustaining control of pest insects and it is highly cost effective in the long term (Greathead, 1995). However there is considerable concern for the risk new biological control agents might pose to other species in the country of introduction, and most countries now have regulations to manage decisions on whether to allow biological control introductions (Sheppard et al., 2003).

A key component of the research required to gain approval to release a new agent is host range testing, to determine what level of risk the agent might pose to native and valued species in the country of introduction. Although methods for this are well developed for weed biological control, protocols are less established for arthropod biological control, and the challenges in conducting tests are greater (Van Driesche and Murray, 2004; van Lenteren et al., 2006; Withers and Browne, 2004). This is because insect ecology and taxonomy are relatively poorly understood, and there are many more species that need to be considered. Add to this the challenges of obtaining and identifying many species, as well as the difficulties of maintaining and coordinating colonies of non-target, target and agent to conduct tests (Kuhlmann et al., 2006). These challenges are particularly great in a country such as New Zealand, with a unique and relatively unstudied insect fauna, and the strictest regulatory process for biological control introductions (Sheppard et al., 2003) to help protect this unique fauna.

Host range in parasitic Hymenoptera

It is not possible to test all potential hosts when conducting host range testing of a proposed new biological control agent, so the first step is to select which non-target species to test. Non-target selection depends on the biology and known host range of the proposed biological control agent which can to some degree be predicted by its life history and known hosts. The proposed biological control agent in this study is a parasitic hymenopteran, a highly abundant and relatively unstudied group with huge diversity in life history and host range patterns (Godfray, 1994). Although host range among the parasitic Hymenoptera may be broad or narrow, the most important determinants of host range are taxonomic relatedness and shared ecology of the hosts (Godfray, 1994).

In this study, the proposed agent is *Cotesia urabae* Austin & Allen (Hymenoptera: Braconidae). This species is a koinobiont larval endoparasitoid, that develops inside the growing larvae of its lepidopteran host (as opposed to idiobiont ectoparasitoids, which paralyse their host to stop development and feed attached to the outside of the host). Koinobiont larval endoparasitoids have an intimate biochemical and physiological connection to their host, and thus have evolved mechanisms to combat the host's immune system (Godfray, 1994). These adaptations are often highly specific to one host species or a group of closely related host species.

Thus taxonomic relatedness to the host is the primary driver in the selection of non-target species for *C. urabae* host range testing. This species is only known to attack one host, *Uraba lugens*

Walker (Lepidoptera: Noctuidae: Nolinae) (Austin and Allen, 1989). *Uraba lugens* is taxonomically isolated, with only one congeneric species described in Australia (Edwards, 1996), and only one species in the same subfamily in New Zealand (none in the same genus) (Dugdale, 1988). These factors increase the likelihood that this species is truly host specific (Godfray, 1994) and thus make it a good candidate as a biological control agent.

Selecting non-target species to test

Kuhlmann et al. (2006) propose a method for non-target selection that accounts for the diversity of host range patterns found among parasitoids. This involves developing an initial testing list of non-target species based on phylogenetic or taxonomic affinities, ecological similarity to the target and socioeconomic considerations. This list is then filtered by spatial, temporal and morphological attributes that might make a species inaccessible to the agent, and by the feasibility of obtaining the non-target species for testing. During the process of host range testing, any new information gathered (such as attack by the proposed agent on one of the non-target species) might alter the type or extent of testing required, and potentially the final list of non-target species selected (Kuhlmann et al. 2006). This approach is similar to that proposed by Hoddle (2004). An initial non-target species list for host range testing of *C. urabae* has been developed following these principles (Berndt et al., 2009).

Parasitoid host location

Host range testing experiments aim to test what threat a parasitoid might pose to novel species in the country of introduction. In practice, the difficulty of replicating field conditions in the laboratory mean that experiments must be designed to test specific aspects of parasitoid host acceptance and physiological host range.

Parasitoids must locate hosts in a complicated and heterogeneous environment and on finding a possible host must make a range of reproductive decisions. Parasitoids have two phases of host location, long distance and close range. For long distance host location parasitoids often use cues associated with the host plants of the target insect, or the chemicals associated with specific insect damage to the host plant to guide it to the correct habitat and plant in order to locate its host (Godfray, 1994).

Once a parasitoid is in the near vicinity of a potential host, close range cues come into play. These may be chemical, visual or tactile, and may include features such as the movement of the potential host (Godfray, 1994). Acceptance of a potential host at close range may result in the parasitoid attacking the insect by stabbing it with its ovipositor, but this in itself does not mean that the insect is a true host. The parasitoid may use its ovipositor to detect further cues, and may decide not to deliver an egg. If she does oviposit onto or into that insect, that egg may not hatch or the larva may not be able to develop. A true host is one which:

1. the parasitoid can locate over a long distance in time and space
2. the parasitoid can locate at close range
3. the parasitoid can attack and successfully deposit an egg
4. the egg hatches and the parasitoid larvae develops, overcoming host immune responses
5. the parasitoid larvae emerges from the host and develops into an adult parasitoid
6. the parasitoid population is self perpetuating on that species

The physiological (or fundamental) host range is the suite of species for which the parasitoid can perform steps 3-5. There may be more species in the physiological host range than the parasitoid actually attacks in the wild (the ecological host range) (Haye et al., 2005). This is because a lack of ability in the parasitoid to locate the insect in the environment (step 1 and 2) or an inability to maintain a population in that species (step 6) may rule out species. The criteria that a parasitoid species has evolved for long distance host location, combined with close range cues and adaptation to the hosts immune system, define its ecological host range.

In a quarantine environment it is very difficult to design experiments that allow parasitoids to behave naturally, utilising long range host location mechanisms and thus testing the ecological host range. Non-target testing designs are generally most effective at examining physiological host range. This risks generating misleading false positive results, where a potential biological control agent is rejected due to attack on a non-target which would not happen in the field. Assessments of ecological host range can be done in the country of origin of the parasitoid, although not usually on the non-target species of interest, and this does not necessarily reflect the behaviour of the parasitoid in a novel environment (Cameron and Walker, 1997; Endersby and Cameron, 2004).

Designing host range tests

Host range testing methodology for arthropod biological control is under ongoing development to try and address the difficulties of dealing with parasitoid behaviour and assessing ecological host range (Andreassen et al., 2009; Haye et al., 2005; van Lenteren et al., 2006; Withers and Mansfield, 2005). Van Lenteren et al. (2006) proposed a sequential framework for testing starting with small arena no-choice tests with behavioural observations, to assess physiological host range. The sequence then progresses to large arena choice tests to increase ecological realism, followed by field tests if these can be done without risk of establishment. If non-target species are attacked in small arena no-choice tests, then the next stage in the sequence should be conducted. If consistent attack is found in large arena choice tests, or in the field then the risk to non-targets is likely to be significant.

Following these principles, we here describe host range testing for the parasitoid *Cotesia urabae* against *Uraba lugens* in New Zealand. *Uraba lugens* is an Australian eucalypt defoliator which is recently established in New Zealand (Kriticos et al., 2007), threatening the eucalypt industry as well as amenity trees. It also poses a threat to public health as larvae have stinging hairs which can cause a painful rash. It has 8-13 larval instars, with larvae feeding gregariously to around the 5th instar, after which they are largely solitary (Berndt and Allen, in review). In Australia, *U. lugens* is attacked by up to 21 primary parasitoid species (Allen, 1990c; Berndt and Allen, in review). Of these, *C. urabae* has been extensively studied and is believed to attack only *U. lugens* (Allen, 1989; Allen, 1990a; Allen, 1990b; Allen, 1990c; Allen, 1991). This is the first attempt at developing biological control for *U. lugens*.

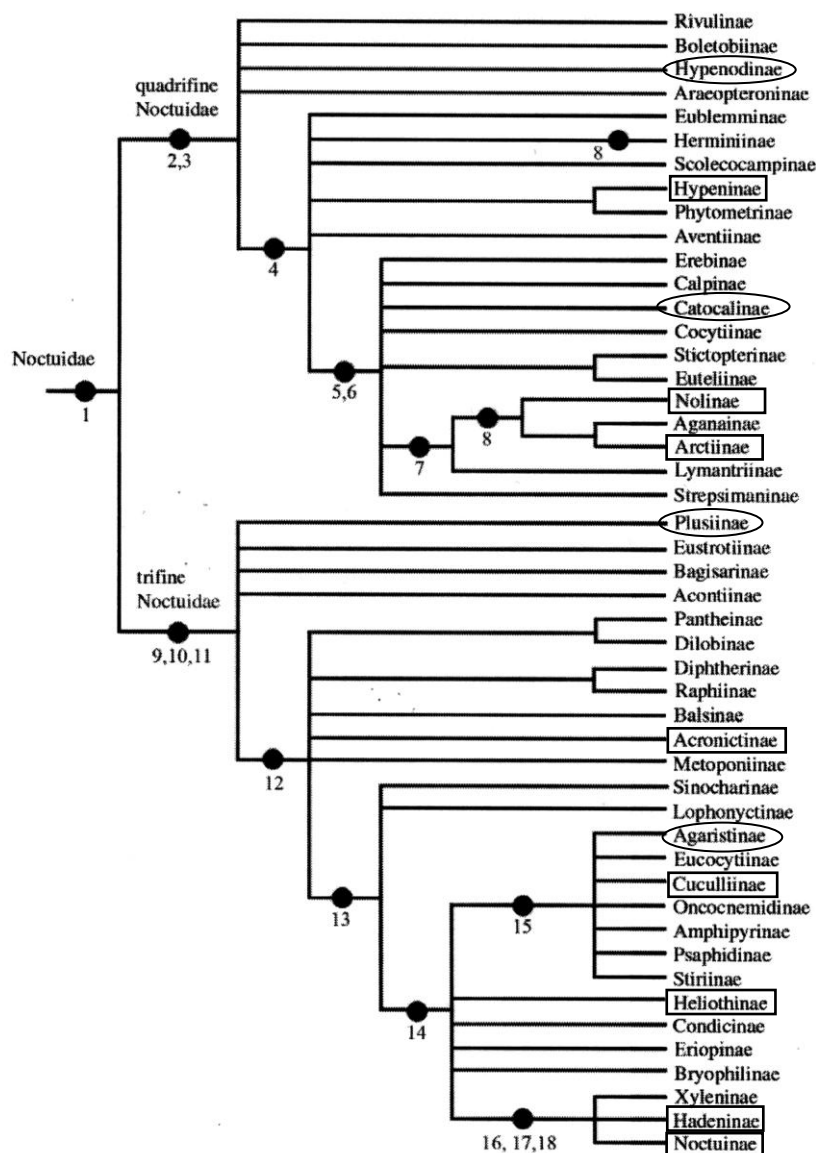
***Uraba lugens* taxonomy**

Various changes have been made to the taxonomic status of the Nolinae (to which *U. lugens* belongs) and related subfamilies, but there is as yet no consensus. Many authors (Allen, 1990c; Austin and Allen, 1989; Farr, 2002) place it as a subfamily within the noctuids following Edwards (1996) and Lafontaine and Fibiger (2006). However other authors (Berry and Mansfield, 2006; Kriticos et al., 2007; Suckling et al., 2005) follow Kitching and Rawlins (1998) and Mitchell et al. (2006) in assigning it family rank. Despite the inconsistencies with assigning subfamily or family rank, the relationships between groups are fairly consistent, with the nolines most closely related to the arctiines and lymantriines. In discussing the selection of non-target species to test, Berndt et al. (2009) follow Lafontaine and Fibiger (2006) as the primary authority on phylogeny on the advice of Robert Hoare (Landcare Research), but take into account the various schools of thought on phylogenetic relationships. We will follow this approach here. The phylogeny proposed by Lafontaine and Fibiger (2006) is reproduced in Figure 1, with subfamilies with New Zealand representatives highlighted.

Hypothesis

Based on current knowledge and theoretical predictions described above, our hypothesis is that *C. urabae* is highly specific in its host selection, and would only attack the target species *U. lugens* if the parasitoid were released in New Zealand. Initial tests established that representatives of the trifine noctuids (*Helicoverpa armigera* and *Spodoptera litura*) are not hosts for *C. urabae* (Berndt et al., 2007). Based on the prediction that phylogenetic relationships are the primary driver of host range for parasitoid with life histories such as *C. urabae* (Godfray, 1994), species for host range testing are thus selected from within the quadrifine noctuids (the group which includes *U. lugens*, Figure 1).

Figure 1: Phylogenetic tree of the Noctuidae, after Figure 28 in Lafontaine and Fibiger (2006). Subfamilies inclosed in a rectangle have New Zealand endemic representatives (Dugdale, 1988). Those enclosed with an oval have only exotic or cosmopolitan representatives in New Zealand (Dugdale, 1988). Numbers refer to the morphological features that define that clade. Refer to Lafontaine and Fibiger (2006) for further details.



Methods

Test species selection

A list of possible non-target species was compiled based on relatedness to the target host, endemism and value to New Zealand (Berndt et al., 2009). The Noctuidae is a very large family with 48 subfamilies worldwide (Lafontaine and Fibiger, 2006), and at least 174 species (138 of these endemic) present in New Zealand (Dugdale, 1988). In selecting species to test, endemic or valued species within the same subfamily, and in other subfamilies in the same phylogenetic clade as the Nolinae (clade 7 of Figure 28, Lafontaine and Fibiger (2006), reproduced here in Figure 1) were given highest priority. This included the only New Zealand representative of the Nolinae *Celama parvitis* (Howes) and all of the New Zealand resident Arctiinae (*Metacrias erichrysa*

Meyrick, *M. huttoni* (Butler), *M. strategica* (Hudson), *Nyctemera annulata* (Boisduval) and *Tyria jacobaeae* (L.)).

Three other subfamilies within the quadrifine Noctuidae (a subgroup of the noctuids defined according to wing venation patterns) have New Zealand representatives (Figure 1). The Catocalinae has 14 New Zealand representatives, but these are all exotic species and mostly occasional immigrants and so were not included on the test list (Dugdale, 1988). The Hypeninae has two described endemic species, both of which are common and widespread (Dugdale, 1988). One of these (*Rhapsa scotosialis* Walker) was included on the list as a representative (Berndt et al., 2009), but given low priority. Species in this subfamily are nocturnal litter feeders and thus do not inhabit the same niche as *U. lugens* or the New Zealand noline *C. parvitis* (J. S. Dugdale pers. comm. 2009). The Hypenodinae has one cosmopolitan representative in New Zealand and so was also excluded from the list on this basis. The Lymantriinae are also recorded as having a New Zealand representative (painted apple moth, *Teia anartoides* Walker) (Dugdale, 1988), but this exotic invader has now been eradicated (MAF Biosecurity New Zealand 2009, www.biosecurity.govt.nz/pests/painted-apple-moth).

Two outgroup species were selected for testing from the trifine noctuids (Berndt et al., 2007; Berndt et al., 2009). These were *Helicoverpa armigera* Walker (Noctuidae: Heliothinae) and *Spodoptera litura* (F.) (Noctuidae: Acronictinae). Host range testing methods and results for *H. armigera* and *S. litura* were described in (Berndt et al., 2007). These data have been re-analysed in this study for comparison with the other non-target species.

Test sequence

Host range tests were designed according to the testing sequence suggested by van Lenteren et al. (2006), taking into account the behaviour of the parasitoid. The testing sequence was designed to initially maximise the likelihood of attack to check if the most closely related non-target species were physiological hosts. This was done using sequential no-choice tests, as described by Berndt et al. (2007). If significant attack was observed, then choice tests and larger arena sizes were used to increase ecological realism (van Lenteren et al., 2006; Withers and Mansfield, 2005) using the following sequence:

1. Sequential no-choice test, small arena (9 cm Petri dish) – to determine attack behaviour and physiological host preferences of the parasitoid.
2. Choice test, medium arena (30 x 30 x 30 cm cage) – to examine if parasitoids will attack non-targets in the presence of the target host.
3. No-choice test, large arena (70 x 70 x 100 cm cage) – to increase ecological realism and number of larvae exposed to check for rare events.

Source and rearing of parasitoids

Cotesia urabae were reared from *U. lugens* larvae collected in Hobart, Tasmania, Australia, in November and December 2007 and 2008. Parasitoid cocoons were shipped to the Scion quarantine facility in Rotorua on 11 December 2007 and 13 December 2008. The *C. urabae* colony was maintained on second to fourth instar *U. lugens* larvae reared on *Eucalyptus nitens*. The 2007 colony was maintained through nine generations, and the 2008 colony through two generations. Females were individually paired for mating in a mesh sided vial one day after emergence. Those that were not observed to mate were transferred to a small cage with multiple females and males for 24 hours. Mating success in these cages was fairly low (19 %), and these females were then considered ‘possibly mated’.

Source of test insects

Non-target larvae were collected as eggs, larvae or adults from various field locations and reared on their host plant (or artificial diet for *H. armigera* and *S. litura*) to the appropriate stage larvae for experiments (Table 1). Larvae were maintained during experiments on the same rearing substrate. Rearing was conducted at 10 – 20°C, depending on requirements to coordinate the colonies with

parasitoid and *U. lugens* availability and the temperature requirements of the non-target species. Experiments were conducted in quarantine at 20°C, 65% RH, and 14L:10D light regime, over the summers of 2007-08 and 2008-09.

Table 1: Collection locations for target and non-target species in order of priority for host range testing.

Species	Location, Crosby region and altitude	Date	Stages collected	Host plant for rearing
<i>Uraba lugens</i>	Auckland Region, AK	various, 2007-2009	eggs, larvae	Shining gum (<i>Eucalyptus nitens</i>)
<i>Celama parvitis</i>	Dunedin, DN (sea level)	31 Dec 2007	adults	Niniaio (<i>Helichrysum lanceolatum</i>)
	Waihemo, DN (300m)	1&4 Jan 2008 3 Jan 2009	adults	
	Lake Wakatipu, OL (300m)	8 Jan 2008	adults	
	Danseys Pass, CO (400m)	20 Jan 2008	adults	
<i>Metacrias strategica</i>	Mt Bengier, CO (950-1150m)	Nov 2007	larvae	Catsear (<i>Hypochoeris radicata</i>)
	Tiwai Point, SL (sea level)	Sept & Oct 2008	larvae	
<i>Nyctemera annulata</i>	Rotorua, BP (300m)	summer, 2007-2009	eggs, larvae	Ragwort (<i>Jacobaea vulgaris</i>)
<i>Metacrias erichrysa</i>	Homer Tunnel, FD (945-1100m)	Nov 2007 Jan 2009	larvae	Catsear (<i>Hypochoeris radicata</i>)
<i>Metacrias huttoni</i>	Nevis, CO (770-1100m)	28 Oct 2007 Nov 2008	larvae	Catsear (<i>Hypochoeris radicata</i>)
	Mt Bengier, CO (1160m)	3 Nov 2007	larvae	
	Rock & Pillar Range, CO (1300m)	Nov 2008	larvae	
	Thomsons Gorge, CO (740m)	Nov 2008	pupae and larvae	
<i>Tyria jacobaeae</i>	Rotorua, BP (300m)	summer, 2007-2009	eggs, larvae	Ragwort (<i>Jacobaea vulgaris</i>)
<i>Spodoptera litura</i>	Lab colony, Plant and Food Research, Auckland		eggs	General purpose Lepidoptera diet
<i>Helicoverpa armigera</i>	Lab colony, Plant and Food Research, Auckland		eggs	General purpose Lepidoptera diet

Sequential no-choice tests

To examine the physiological host range of *C. urabae*, parasitoids were enclosed with larvae of each non-target species in Petri dishes for 24 hours. The use of this small (9 cm diameter) arena gave the parasitoids the maximum possible chance of encountering the non-target species. This design only tests the response of the parasitoid to the non-target species at close range.

Test design

In order for the tests to be valid, it was necessary to show that the parasitoids behaved normally. Thus parasitoids were exposed to the target host *U. lugens* before and after the exposure to the non-target in a sequential no-choice test design (Berndt et al., 2007). Parasitoids that did not attack *U. lugens* within 30 min of exposure were not used in the tests. To provide a comparison for the behaviour of the parasitoid on the non-target species over 24 hours, positive controls were conducted using the same design, but with *U. lugens* as the test species. Negative controls, without parasitoids present, were also conducted to provide a comparison for the survivorship of the non-target species. Two replicates were run simultaneously, along with one negative control with host larvae but no parasitoids, together making one experiment. Experiments were conducted when insects of the appropriate age/size were available, with 4-8 replicates per non-target species.

Parasitoids were enclosed initially with the target host *U. lugens* for 10 minutes (A1), then moved on to the non-target host for 24 hours (B), before being moved to another set of target hosts for 10 minutes (A2). Observations were made of parasitoid behaviour on all sets of hosts. The A1 and A2 parts of the design were intended as a control to check that the behaviour of the parasitoid was normal when on their natural host *U. lugens*. An additional experiment was conducted using *Tyria jacobaeae* to test if exposing the parasitoid to *U. lugens* before the exposure to the non-target species affected the parasitoids' behaviour. That experiment showed that the order of presentation, regardless of host species, did not affect parasitoid behaviour ($P > 0.15$).

Experimental arenas were 9 cm diameter glass Petri dishes, each containing a piece of appropriate foliage for both target and non-target hosts (Table 1). Ten second or third instar *U. lugens* larvae were added to *E. nitens* foliage in A1 and A2 arenas, and 10 non-target hosts of a similar size (3-10 mm) were added to the appropriate foliage in B arenas prior to the start of observations. Insufficient non-target larvae were available to obtain 10 per arena for some replicates of *C. parvitis*, *M. strategica* and *N. annulata*, and 5-8 larvae were used in these instances. One female *C. urabae* was used in each replicate. Parasitoids were 1-11 days old (mean 3.0 ± 0.3 days), and were mated or possibly mated and naïve (had not previously encountered hosts). These were mainly parasitoids from the generation that had been imported from Tasmania as cocoons, or progeny of imported parasitoids (i.e. the first lab generation).

A streak of honey was added to the inside lid of the dish as food for the parasitoid. The same experimental design and methodology was used to conduct positive controls, but with the target host *U. lugens* replacing the non-target host. One positive control experiment (two replicates) plus negative control was conducted for each parasitoid generation that was used for sequential no-choice tests.

Behavioural observations

Each parasitoid female was observed on *U. lugens* larvae for 10 minutes from the start of attack behaviour (A1 arena), then moved to the non-target species (B arena) for 24 hours. In the B arena parasitoids were observed on test larvae for two consecutive 10 minute periods (B1 and B2) at the start of the 24 hours. Parasitoids were then left in the B arena before another observation of two consecutive 10 minute periods (B3 and B4) at the end of the 24 hour period. Parasitoids were then moved to the second *U. lugens* arena (A2) and observed for a further 10 min to confirm that they were still motivated and able to parasitise their preferred host.

Behavioural observations consisted of continuous 10 min observations. Frequencies of four behavioural events were recorded by hand and are fully described for *H. armigera* and *S. litura* in Berndt et al. (2007). These behaviours were: parasitoid approaches to the leaf, parasitoid approaches to larvae, parasitoid probing, and parasitoid attacking larvae. The latter of these, larval attack, is the behaviour of most significance as a measure of impact on non-target species, and only this behaviour will be reported here. Larval attack was defined as when the parasitoid successfully stabbed a larva with its ovipositor. Successful deposition of an egg during larval attack could not be confirmed by observation. For analysis, behavioural data were transformed to normal by log ($x +$

0.5) and analysed using ANOVA (MIXED procedure in SAS v9.1), comparing data from the 24 hr exposure period of the sequence for target and non-target species.

Larval dissections and rearing

Larvae used in the sequential no-choice tests were either dissected or reared to measure the outcome of the observed *C. urabae* attack behaviour on them. This enabled assessment of the ability of the parasitoid to develop in the non-target species, and the impact of the presence of the parasitoid on larval mortality.

One week after the exposure of the parasitoid to the larvae, half of the surviving larvae from each replicate were frozen, dissected and examined at 8x, 25x and 50x magnification for the presence of parasitoid eggs or larvae. For larvae of *H. armigera* and *S. litura* this period was three weeks after the start of the experiment (Berndt et al., 2007). The remaining half of the larvae were reared on their usual diet (Table 1) until parasitoids completed development, or until larvae died or successfully pupated. For replicates where only five larvae were used, all larvae were reared, rather than dissected.

Dissection and rearing data from the 24hr exposure period were transformed using an arc sin transformation and compared using ANOVA (MIXED procedure in SAS v 9.1). Three levels of increasing parasitoid impact on non-target larvae were compared with *U. lugens* controls as defined by the following derived variables:

- 1) % successful attack = non-target larvae with parasitoid found on dissection / total number of larvae dissected * 100.
- 2) % parasitoids emerged = number of parasitoids emerged from larvae / total number of larvae reared * 100.
- 3) % adult parasitoids = number of adult parasitoids produced / total number of larvae reared * 100.

Note: The number of larvae reared, which is used in the latter two variables, excludes any missing larvae (possibly due to cannibalism) as well as those that died of other unknown causes.

The impact of parasitoid presence (in treatment arenas) and absence (in negative control arenas) on non-target larval mortality was compared with the impact on the target host *U. lugens*. This analysis did not include dissected larvae. Total mortality does include missing larvae, larvae that died from unknown causes, as well as larvae that died when the parasitoid emerged from them.

Choice tests

Choice tests were conducted to examine if *C. urabae* would attack non-target species in the presence of the target host *U. lugens*. A medium cage size was used to increase ecological realism of the arena. Choice tests were conducted on those species for which significant attack behaviour or successful attack was observed in the previously described sequential no-choice tests, and for which sufficient larvae were available. These species were:

- *Celama parvitis* (1 experiment, 8 larvae per cage),
- *Metacrias huttoni* (2 experiments, 10 larvae per cage),
- *Metacrias erichrysa* (1 experiment, 6 larvae per cage),
- *Nyctemera annulata* (2 experiments, 10 larvae per cage), and
- *Tyria jacobaeae* (2 experiments, 10 larvae per cage).

Experimental design

The design of this experiment follows that suggested by van Lenteren et al. (2006), and was intended to enable a comparison of analysis methods recommended by these authors (whereby the non-target in the choice cages are compared with the target in the no-choice cages) with a more commonly used method (comparing non-target and target species together within the choice cages). Choice tests were conducted in 30 x 30 x 30 cm plastic mesh cages ('Bugdorm', Bioquip). One experiment consisted of eight cages: 4 choice cages (CH) with target and non-target larvae and one

parasitoid; three no-choice (NC) positive controls with target larvae and one parasitoid; and one negative control (NEG) with target and non-target but no parasitoid (Table 2). Parasitoids used in choice tests were naïve 2-13 day old (mean 7 ± 0.4 days) mated or possibly mated females from the generation that had been imported from Tasmania as cocoons, or progeny of imported parasitoids.

Cages were set up with one sprig each of *E. nitens* and the non-target host plant (or two sprigs of *E. nitens* for the no-choice positive control) in flasks of distilled water sealed around the cut stem with Parafilm. The host plant used for rearing *Metacrias* spp. (*Hypochoeris radicata*) is an herbaceous rosette, and whole plants were dug up and placed with roots in distilled water in 500 ml lidded plastic cups (Lily). The lid of the cup provided support for the rosette leaves of the plant. All sprigs and plants were selected to be of similar size within one experiment, and were washed and checked for invertebrates prior to use. A smear of honey was added to the inside cage wall as food for the parasitoid.

For added security against parasitoid escape in quarantine, experimental cages were housed in four ventilated clear Perspex cages (100 x 70 x 70 cm) for the duration of the experiment. Experimental cages were randomly assigned a location in the Perspex cages. Six to 10 larvae of each species were placed on the appropriate host plant prior to introduction of the parasitoid (Table 2). In the no-choice positive controls, 12-20 *U. lugens* larvae were split equally across the two *E. nitens* sprigs and reared as one group after exposure to the parasitoids. One female *C. urabae* was randomly assigned to each experimental cage for 24 hours, after which larvae were removed to rearing containers. After seven days, half of the surviving larvae in *M. huttoni*, *N. annulata*, and *T. jacobaeae* experiments were killed for dissection, and the remainder reared to completion. Experiments were ended after approximately 60 days and any surviving larvae were considered to have been successfully reared without parasitism. For *C. parvitis* and *M. erichrysa* experiments there were insufficient surviving larvae to warrant killing any for dissection, and all larvae were reared to completion.

Data analysis

On analysis, the comparison between non-target and target species within the choice cages was found to be more robust than the comparison between non-target and target species in choice and no choice respectively, as suggested by van Lenteren et al. (2006). In the interests of simplicity, only those results from the former comparison are presented here. The methodological comparison will be explored elsewhere. Thus dissection and rearing data from non-target and target species in the choice (CH) cages were compared using ANOVA (MIXED procedure in SAS v9.1) on angular transformed data with cage as a blocking effect. The same analysis method was used to compare mortality in choice cages (CH) with the parasitoid and negative control cages (NEG) without the parasitoid.

Table 2: Choice test design. For *C. parvitis* and *M. erichrysa*, insufficient non-target larvae were available to obtain 10 per cage as indicated, and eight and six larvae respectively were used per cage. *Uraba lugens* numbers were adjusted accordingly. ‘A’ = target species *Uraba lugens*, ‘B’ = non-target species. ‘Euc’ = *Eucalyptus nitens* foliage.

Cage	Treatment	Larvae	Host plants	Parasitoid females
1 CH	choice	10A, 10B	euc + non-target	1
2 CH	choice	10A, 10B	euc + non-target	1
3 CH	choice	10A, 10B	euc + non-target	1
4 CH	choice	10A, 10B	euc + non-target	1
5 NC	no-choice A	20A	euc + euc	1
6 NC	no-choice A	20A	euc + euc	1
7 NC	no-choice A	20A	euc + euc	1
8 NEG	negative control	10A, 10B	euc + non-target	0

No-choice large cage tests

No-choice large cage tests were conducted in an attempt to increase the space available to the parasitoids as much as possible under quarantine conditions, and thus the ecological realism of its interaction with the target and non-target hosts. These tests also aimed to increase the number of non-target larvae exposed to parasitoids to explore the risk of rare events. Tests were conducted in clear Perspex cages (100 x 70 x 70 cm) with mesh inserts for ventilation. They were conducted when sufficient larvae were available after other tests had been completed, on species for which successful parasitoid attack had been recorded. Because of limited larval availability they were not replicated, and thus provide anecdotal evidence only.

In February 2008, one cage was set up with 49 *T. jacobaeae* on ragwort. Five mated or possibly mated *C. urabae* females were added to the cage and left for five days. Larvae were then removed and reared to completion. In January 2009, four cages were set up with 100 larvae in each on the appropriate host plants: two cages of *M. huttoni*, and one cage each of *N. annulata* and *U. lugens* (positive control). Three possible mated, 15-17 day old female *C. urabae* were added to each cage. These females had previously been exposed to *N. annulata* in another experiment, and were used because of a lack of other available parasitoids. After addition of the parasitoids to each cage, behavioural observations were made for 10 minutes on each cage sequentially. Larvae were exposed to parasitoids for 24 hours, with another behavioural observation for the last 10 minutes of the exposure period. Parasitoid approaches to and attack on larvae were recorded. After the exposure period, larvae were reared to completion.

Results

Sequential no-choice tests

Behaviour

Cotesia urabae exhibited attack behaviour on all of the non-target species presented within these small glass Petri dishes (Figure 2). However the parasitoid exhibited significantly less attack behaviour towards *C. parvitis*, all three *Metacrias* spp., *H. armigera* and *S. litura* than it did towards its host *U. lugens* ($P < 0.05$) (Figure 2). Attack behaviour rates on *N. annulata* and *T. jacobaeae* did not differ from *U. lugens*. The rate of attack behaviour differed significantly over the duration of the exposure only for *U. lugens*, with fewer attacks at the end of the 24 hour exposure period than at the beginning.

Rearing and dissections

Although attack behaviour was recorded on all eight non-target species tested (Figure 2), only five species (*C. parvitis*, *M. erichrysa*, *M. huttoni*, *N. annulata*, *T. jacobaeae*) were found to contain parasitoids within them upon dissection (successful attack) (Figure 3a). A significantly smaller proportion of *Tyria jacobaeae* than the target *U. lugens* were successfully attacked ($P < 0.05$). However the proportion of *C. parvitis*, *M. erichrysa*, *M. huttoni* and *N. annulata* larvae successfully attacked did not differ significantly from *U. lugens* ($P > 0.05$) (Figure 3a).

The results from reared larvae (Figure 3b) were different from dissections (Figure 3a) suggesting there was a high failure rate for parasitoid larvae attempting to complete development in the non-target species. Parasitoids emerged from larvae and produced cocoons from only two species of non-targets: *M. erichrysa* (2 cocoons) and *M. huttoni* (3 cocoons). By comparison, 16 cocoons were reared from *U. lugens* in the same tests. However, due to very low success at rearing larvae to completion in the laboratory for these two non-target species, there was no significant difference in % parasitoids emerged compared to *U. lugens* ($P > 0.05$). No other species had any parasitoids emerge from them, although no *C. parvitis* were successfully reared to completion within the laboratory, thus no analysis could be conducted on that species (Figure 3b).

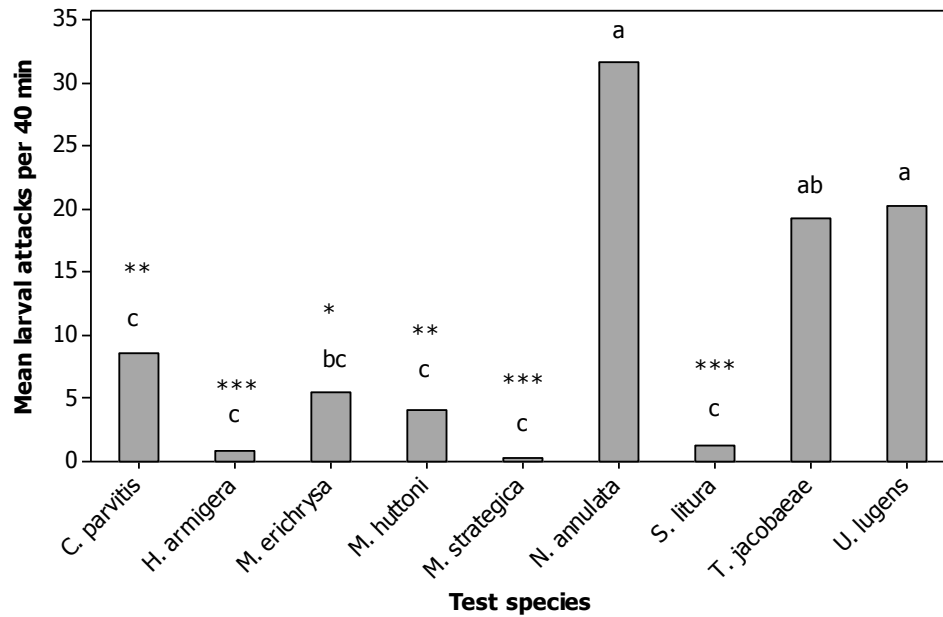


Figure 2: Mean attacks by *Cotesia urabae* on larvae of non-target and target Lepidoptera species during 40 min of observation. Two 10 minute observation periods at the start, and two 10 min observation periods at the end of a 24 hour exposure have been pooled. Bars sharing the same letter do not differ significantly. Asterisks indicate significant difference from target host *U. lugens*: * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$.

None of the parasitoid cocoons that developed from the non-target species listed above successfully developed to adult *C. urabae* (Figure 3c). All non-target species except *C. parvitis* were significantly lower in % adult parasitoids compared to *U. lugens*, although only *M. erichrysa* and *M. huttoni* produced parasitoid cocoons that could have developed to adult parasitoids. As mentioned above no *C. parvitis* larvae were successfully reared thus no analysis could be conducted on that species (Figure 3c).

The presence of *C. urabae* had a significant effect on the mortality of *M. huttoni* ($P = 0.0225$), and on the target species *U. lugens* ($P = 0.0005$) (Figure 4). However mortality of *M. huttoni* directly attributable to the parasitoid was only 7.5%, compared to 39.2% for *U. lugens* ($P = 0.0007$). The mortality due to parasitism for *M. erichrysa*, the other species with successful parasitism, was also significantly lower than that for *U. lugens* ($P = 0.0017$). This comparison of mortality in the presence and absence of the parasitoid *C. urabae* (Figure 4) illustrates the very high background mortality that commonly occurs when attempting to rear many non-target species in the laboratory. This was particularly a problem for *C. parvitis*, *M. erichrysa*, and *N. annulata* in this study.

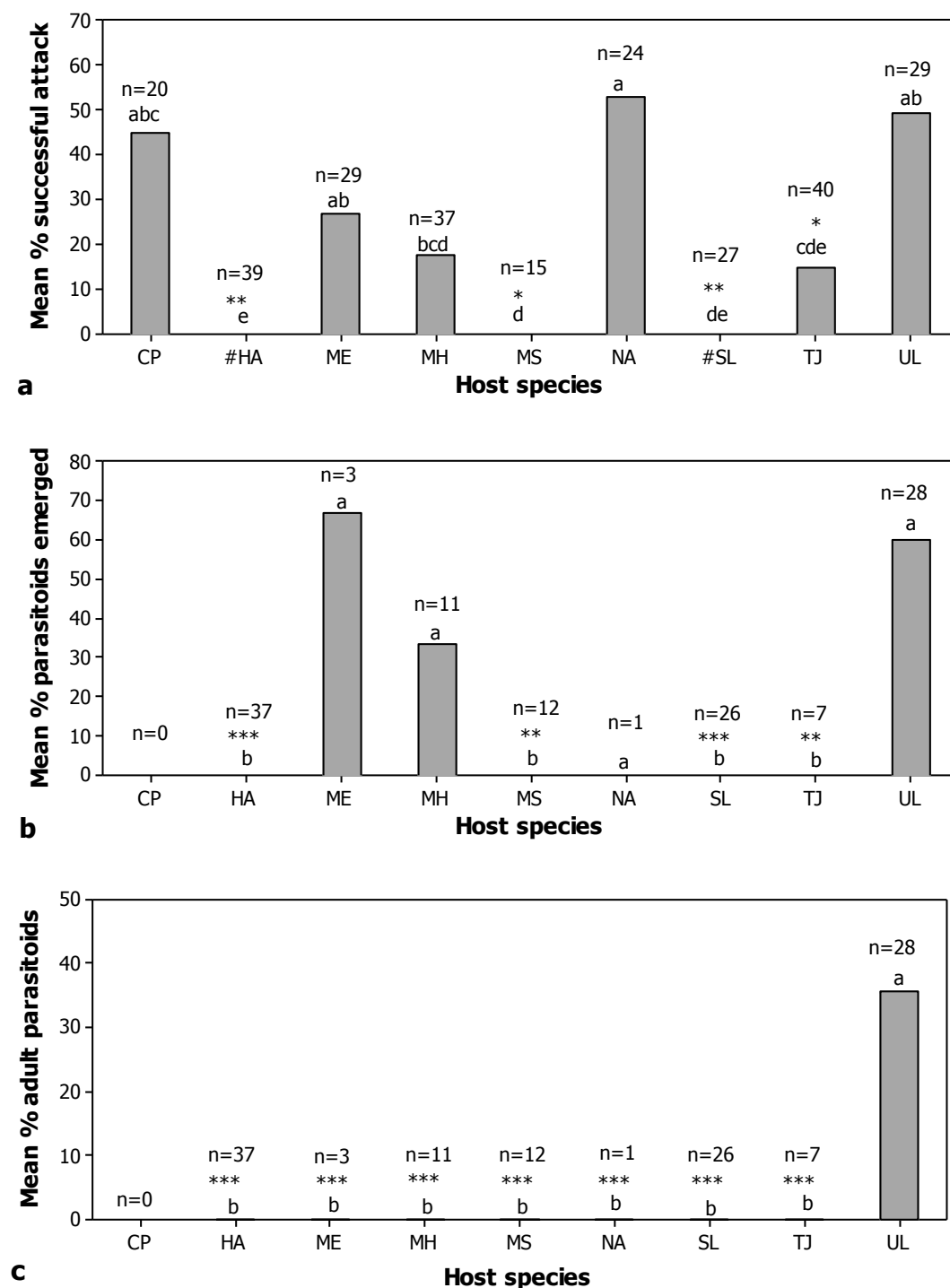


Figure 3: Outcome of sequential no-choice tests for non-target species compared with target species *U. lugens* for (a) % successful attack, as revealed by dissections after one week (or three weeks for species indicated by #), (b) % parasitoids emerged from target and non-target larvae, and (c) % adult parasitoids produced. Bars sharing a letter do not differ significantly ($P < 0.05$). Asterisks indicate significant difference from the target host *U. lugens*: * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$. n = total number of larvae reared or dissected. CP = *Celama parvitis*, HA = *Helicoverpa armigera*, ME = *Metacrias erichrysa*, MH = *M. huttoni*, MS = *M. strategica*, NA = *Nyctemera annulata*, SL = *Spodoptera litura*, TJ = *Tyria jacobaeae*, UL = *Uraba lugens* (target species).

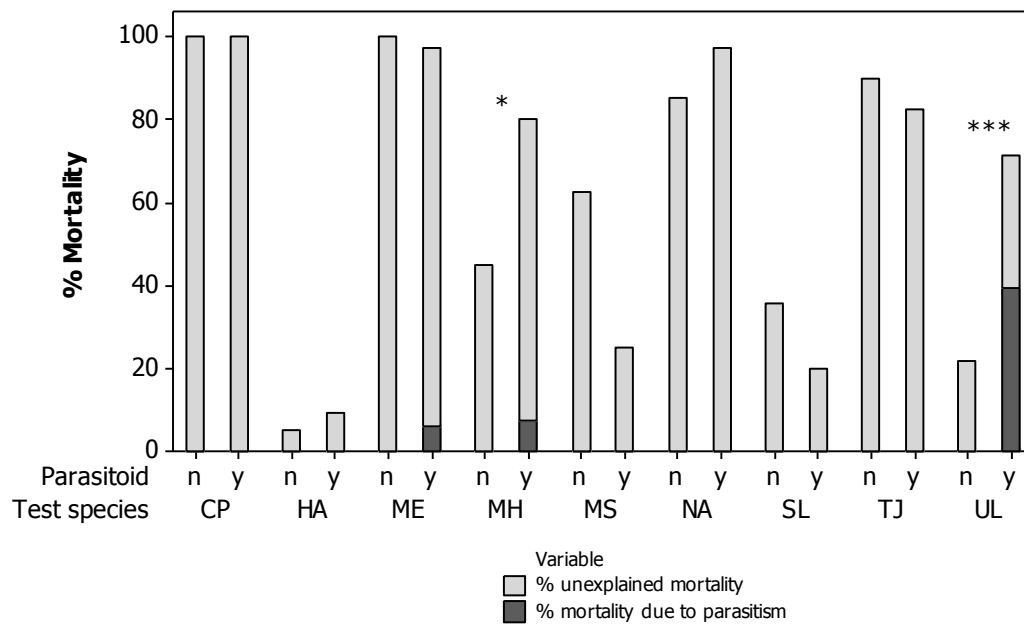


Figure 4: Mean unexplained mortality and mortality due to parasitism of non-target and target species in the presence (y) and absence (n) of the parasitoid *C. urabae* within the laboratory. Asterisks indicate significant difference between treatments at the following levels: * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$. CP = *Celama parvitis*, HA = *Helicoverpa armigera*, ME = *Metacrias erichrysa*, MH = *M. huttoni*, MS = *M. strategica*, NA = *Nyctemera annulata*, SL = *Spodoptera litura*, TJ = *Tyria jacobaeae*, UL = *Uraba lugens* (target species).

Choice tests

Dissections were conducted on larvae of three of the non-target species presented to *C. urabae* in choice tests. Parasitoid larvae were found in two of these species (*M. huttoni* and *N. annulata*), indicating successful attack (Figure 5a). For both these species, the proportion of larvae successfully attacked was less than half that of the target host *U. lugens* although this difference in attack rate was only significant for *N. annulata* ($P = 0.0131$). *Tyria jacobaeae* did not show any evidence of successful attack ($P = 0.0005$).

Only one *M. huttoni* larva had a parasitoid emerge from the cadaver on rearing, out of all five non-target species exposed to *C. urabae* in choice tests. The mean percent of *M. huttoni* larvae with parasitoids emerging did not differ significantly from *U. lugens* however ($P > 0.05$) (Figure 5b). No parasitoids emerged from any other non-target species, although the difference from *U. lugens* controls was only significant for *T. jacobaeae* ($P = 0.0001$) due to poor rearing success for other species. The single parasitoid cocoon that developed from the non-target *M. huttoni*, did not develop into an adult parasitoid, although between 13 and 25% of *U. lugens* larvae produced adult parasitoids (Figure 5c).

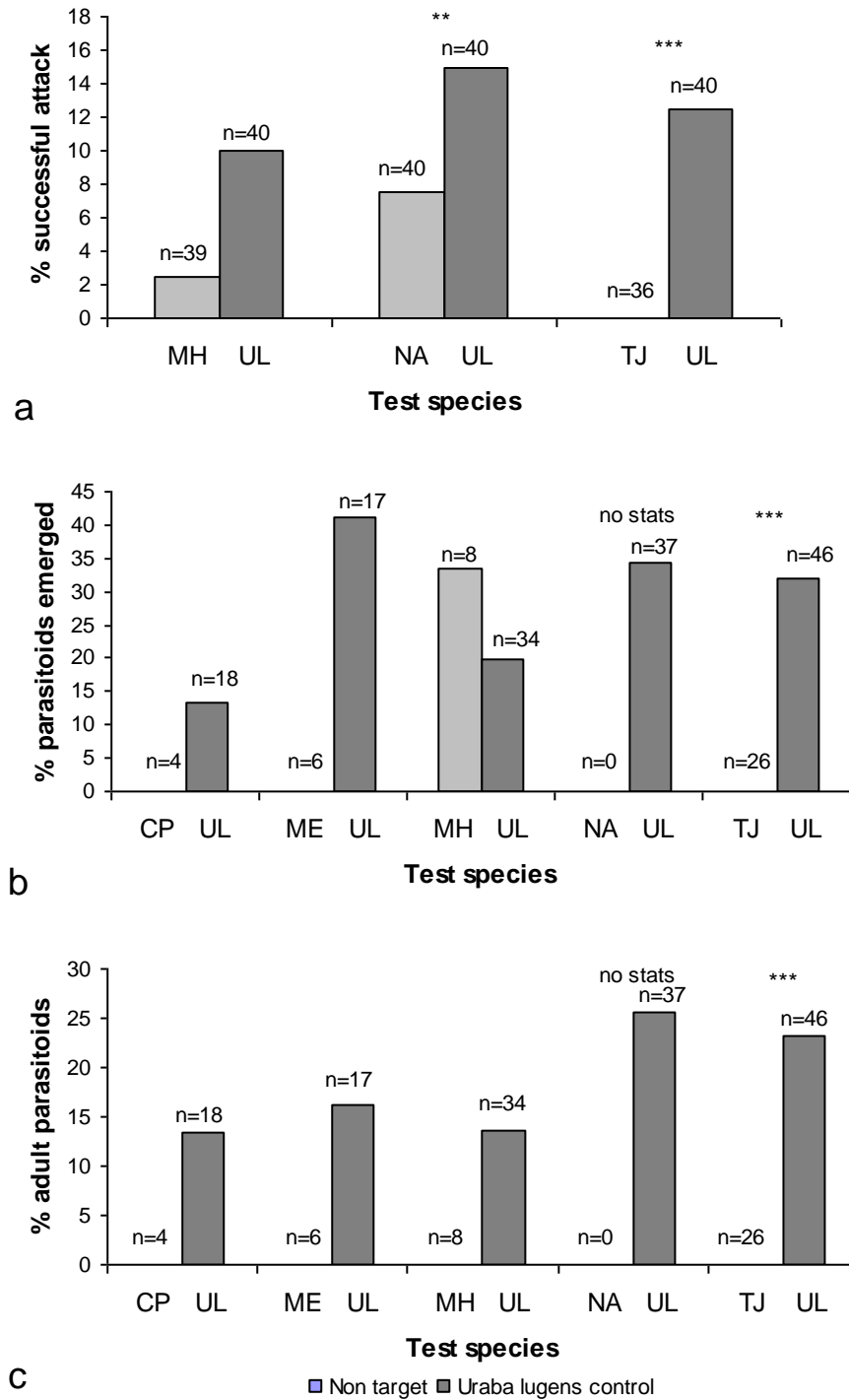


Figure 5: Outcome of choice tests for non-target species compared with target species *U. lugens* for (a) % successful attack, (b) % parasitoids emerged, and (c) % adult parasitoids. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$; n = number of larvae dissected (a) or reared (b and c). CP = *Celama parvitis*, ME = *Metacrias erichrysa*, MH = *M. huttoni*, NA = *Nyctemera annulata*, TJ = *Tyria jacobaeae*, UL = *Uraba lugens* (target species).

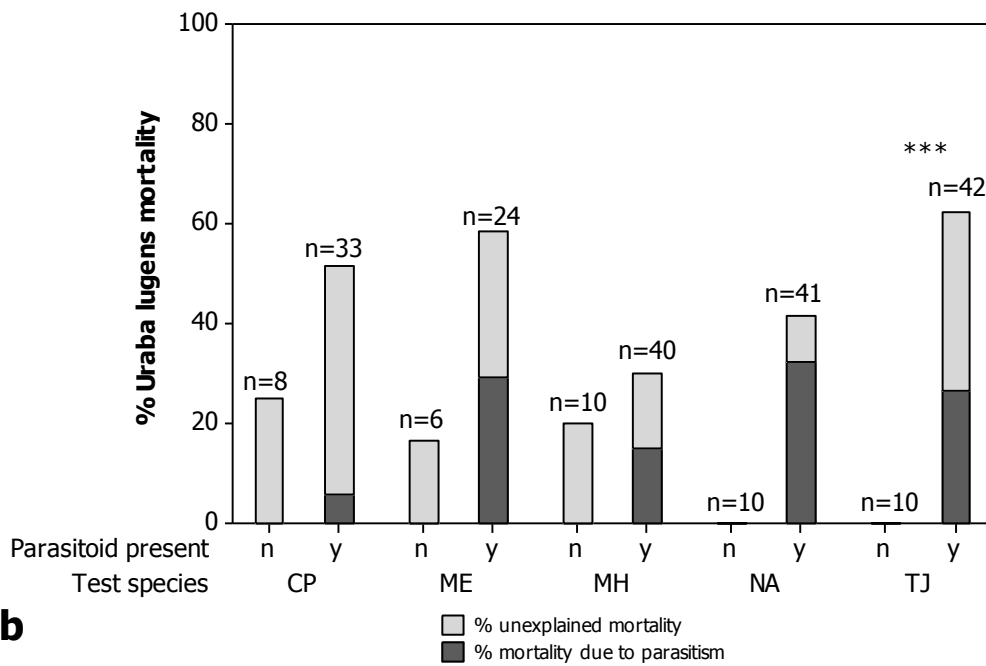
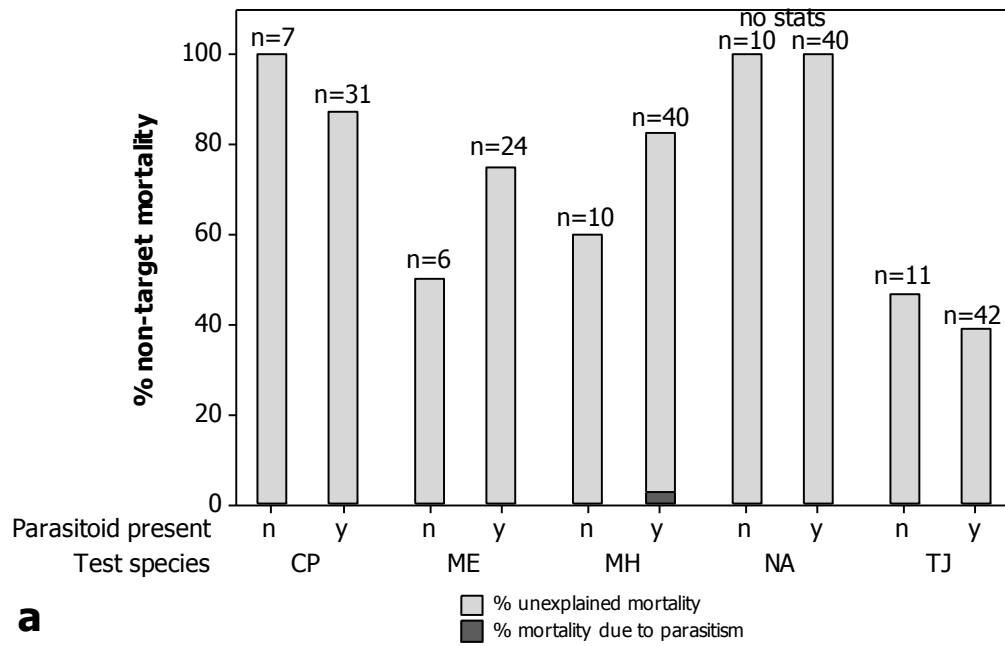


Figure 6: Mortality of (a) non-target larvae and (b) *U. lugens* in no-choice cages, in the absence (n, NEG treatment) or presence (y, CH treatment) of the parasitoid *C. urabae*. Asterisks indicate significant difference between treatments: *** $P < 0.001$; n = total number of larvae. CP = *Celama parvitis*, ME = *Metacrias erichrysa*, MH = *M. huttoni*, NA = *Nyctemera annulata*, TJ = *Tyria jacobaeae*.

No significant differences ($P > 0.05$) were found in the mortality of non-target larvae in the presence (CH treatment) or absence (NEG treatment) of the parasitoid (Figure 6a), although only one replicate (for *C. parvitis* and *M. erichrysa*) or two replicates (all other species) were conducted for the negative control (NEG) treatments. Larval mortality in both treatments was very high particularly for *C. parvitis* and *N. annulata*. For *U. lugens* in the same choice tests (Figure 6b), mortality was consistently higher with the parasitoid (CH) than without (NEG), although this was significant ($P < 0.001$) only for the *T. jacobaeae* test.

In an effort to obtain more robust data on mortality, an additional series of no-choice tests were conducted on *M. huttoni* and *N. annulata*. Unfortunately no parasitoids were successfully reared from positive (*U. lugens*) controls in this experiment, indicating that the parasitoids used were not behaving appropriately (for instance they may have been too old). None of the non-target species were parasitised either, and there were no apparent differences in non-target mortality in the presence of the parasitoid. However, because of the lack of successful attack in the controls, no conclusions can be drawn from these results, and the experiment is not fully described here.

Large cage no choice tests

One *C. urabae* cocoon developed from the non-target *M. huttoni*, out of the 200 larvae exposed. The cocoon did not develop to an adult parasitoid. Seven *C. urabae* cocoons were reared from 100 larvae of the target *U. lugens*. No parasitoids developed from *N. annulata* (100 larvae) or *T. jacobaeae* (49 larvae). In the *M. huttoni* cage that produced a parasitoid cocoon, parasitoids were seen attacking larvae of this species on two occasions during the 20 minutes of observation. No attacks were observed in the other *M. huttoni* cage. One attack was observed in the *N. annulata* cage. No attacks were observed in the *U. lugens* cage, despite the production of parasitoid cocoons from this cage. Parasitoids tended to stay on the walls and floor of the cages, rather than on the foliage. The larvae of all the non-target species in these tests are highly mobile and tended to wander or fall off the provided foliage and move on the walls and floor of the cage making interaction with the parasitoid more likely. *Uraba lugens*, however, tended to remain on the foliage provided.

Appendix 2

Table 4: Summary of results of host range testing for non-target species identified for testing by Berndt et al. (2009), in decreasing order of priority. Shaded cells indicate no significant difference from the target *U. lugens* in at least one test. Superscript letters indicate experiment in which positive result was found. Asterisks indicate the result was significantly lower than *U. lugens* (for the first four categories), or a significant effect of parasitoid on mortality was found. The potential for overlap between non-target and *U. lugens* parasitoids in distribution, habitat type and phenology is given.

Species (# exposed to parasitoids)	Subfamily	Larval attack behaviour	Successful attack (from dissection)	Parasitoids emerged from larvae	Adult parasitoids	Mortality affected by parasitoid	% Total parasitoid cocoons produced (cocoons/ larvae reared)	Niche overlap (exposed leaf feeders)	Distribution and habitat overlap	Phenology overlap
<i>Uraba lugens</i> (980) abcd	Nolinae	Yes ^a	Yes ^{ab}	Yes ^{abd}	Yes ^{abd}	Yes ^{ab***}	33 (156/469)			
<i>Celama parvitis</i> (71) ab	Nolinae	Yes ^{a**}	Yes ^a	No	NA	Insufficient evidence	0 (0/4)	Yes	Yes	Winter only
<i>Metacrias strategica</i> (31) ^a	Arctiinae	Yes ^{a***}	No [*]	No ^{**}	NA	No	0 (0/12)	Yes	Yes	Summer only
<i>Nyctemera annulata</i> (239) ^{abc}	Arctiinae	Yes ^{ad}	Yes ^{ab}	No ^a	NA	No	0 (0/1)	Yes	Yes	Yes
<i>Metacrias huttoni</i> (359) ^{abc}	Arctiinae	Yes ^{a**}	Yes ^{ab}	Yes ^{abd}	No ^{***}	Yes ^{ab*}	24 (5/21)	Yes	No	Summer only
<i>Metacrias erichrysa</i> (84) ^{ab}	Arctiinae	Yes ^{a*}	Yes ^a	Yes ^a	No ^{***}	Yes ^a	22 (2/9)	Yes	No	Summer only
<i>Tyria jacobaeae</i> (270) ^{abcd}	Arctiinae	Yes ^a	Yes ^{a*}	No ^{***}	NA	No	0 (0/91)	Yes	Yes	Summer only
<i>Rhaphs scotosialis</i> (0)	Hypeninae	Not tested						No	Yes	Yes
<i>Spodoptera litura</i> (60) ^a	Acronictinae	Yes ^{a***}	No ^{**}	No ^{***}	NA	No	0 (0/26)	Yes	Yes	Summer only
<i>Helicoverpa armigera</i> (80) ^a	Heliothinae	Yes ^{a***}	No ^{**}	No ^{***}	NA	No	0 (0/37)	Yes	Yes	Summer only

^a small arena sequential no-choice tests; ^b medium arena choice test; ^c large cage no choice tests (unreplicated), ^d order-of-presentation test

* $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$

Discussion

Rearing of the endemic non-target species for host range testing proved to be challenging, with very high mortality for some species during the tests. For many Lepidoptera, rearing is a process of trial and error, and requires considerable practice on each species to develop the required experience to know when conditions need to be adapted to keep larvae alive. For *C. parvitis* and the endemic arctiines in this study, we had the benefit of the experience of Brian Patrick (NZ Butterfly Enterprises Ltd.), one of very few people to have tried rearing endemic arctiines, and the only person to our knowledge to have reared *C. parvitis*. However the conditions and protocols he uses to rear these were different from what we were able to provide for host range tests in quarantine.

To coordinate species and conduct the greatest number of replicates possible, we needed to slow the development of the larvae at certain times, and speed it up at others using controlled temperature rooms. For the long lived endemic species, some of which are from alpine areas, this was not necessarily optimal for their development. For host range tests, we needed to rear very small numbers of larvae on cut foliage in small containers, a much less successful strategy than rearing larger numbers in larger containers (L.A. Berndt pers. obs.). To set up host range tests we had to move larvae from their feeding positions in the colony to cages and Petri dishes. This would have changed the microclimate and host plant quality, which for *C. parvitis* in particular often proved lethal. Disease was also a problem, probably transferred from cut foliage used to feed larvae in the laboratory. A key method for disease management in rearing Lepidoptera is to discard the entire group of larvae if one becomes infected to prevent spread of the disease. This was not possible when such small numbers of larvae were available.

Coordinating the rearing of similar sized larvae of the non-targets with *U. lugens*, and with parasitoid females of a suitable age was challenging, and resulted in variation in the size of larvae presented to the parasitoids in different replicates. This could have affected the observed attack rates on *U. lugens* controls in particular, as there is variation in the behaviour and performance of *C. urabae* on different life stages of its host (Allen, 1990a). To match *U. lugens* larval sizes to the available non-targets and because of larval availability, large larvae were used in some experiments possibly reducing attack success. *Uraba lugens* larvae are aggressive (thrashing, biting and regurgitating) and are well defended with stinging hairs. Younger larvae are gregarious, which is also a defensive strategy (Allen, 1990a). By comparison, the non-target species were solitary and showed minimal defensive behaviours. *Cotesia urabae* are most successful attacking small (1st instar) *U. lugens* larvae and least successful on large larvae (6th -7th instar) (Allen, 1990a). Female *C. urabae* have a high motivation to attack *U. lugens*, but on medium and large larvae, many of their approaches to the larvae are aborted (Allen, 1990a, L.A. Berndt, pers. obs.). In this study, females were seen preening and scraping their abdomen over the substrate after contacting *U. lugens* larvae in an apparent attempt remove irritants such as regurgitate and stinging hairs (L.A. Berndt, pers. obs.). This was not seen when females attacked non-target larvae.

The behaviour of some the non-target larvae may also have made them more likely to come into contact with *C. urabae*. The five arctiid species are highly mobile as larvae, and tended to wander or fall off the host plants provided and move around the cages during the experiments. In contrast, *U. lugens* almost always remained on the foliage. The parasitoids tended to move mainly on the walls and floor of the medium and large cage types and around the perimeter of the Petri dishes, often putting them in direct accidental contact with non-target larvae. This may have biased the results in favour of the non-target species. However in a field situation, where parasitoids seek out their host on its host plant using long distance cues, these differences in behaviour of the species tested would not increase the likelihood of the parasitoid encountering and attacking non-target species.

Despite these experimental difficulties, results indicate that *U. lugens* larvae proved to be better hosts than larvae of related Lepidoptera and indicated that the parasitoid is host-specific to this pest.

Selection of species to test

In testing the hypothesis that *C. urabae* is host specific to *U. lugens* we first tested two species from the trifine noctuid group (Figure 1) that are more distantly related to the nolines (Berndt et al., 2007). These two species, *S. litura* and *H. armigera*, are cosmopolitan crop pests that were also easy to obtain and rear for use in developing host range testing protocols (Berndt et al. 2007), unlike most of the other non-target species. The Department of Conservation (P. Craddock, pers. comm.) also indicated that *H. armigera* can act as a proxy for the threatened species *Australothys volatilis* Matthews & Patrick, the only New Zealand endemic representative of the Heliiothinae (Matthews and Patrick, 1998). The results of host range testing of these species were reported in Berndt et al. (2007), and re-analysed here for comparison with the other species. Although *C. urabae* females showed some attack behaviour on both species when confined in a Petri dish, the incidence of this was significantly lower than on *U. lugens* and no other indications of successful attack were observed from dissections or rearing of the larvae (Berndt et al. 2007, Table 4). We thus concluded that these trifine noctuids are outside the physiological host range of *C. urabae*, and narrowed our focus for host range to within the quadrifine noctuid group (Figure 1).

Within the New Zealand quadrifine noctuids, clade 7 (Figure 1) groups the nolines with the arctiines and lymantriids. Outside this clade, New Zealand has representatives in the Catocalinae and Hypenodinae (Dugdale, 1988), but species in these groups are all exotic or cosmopolitan, and testing of these species was considered a low priority compared to native species. The Hypeninae has at least two New Zealand endemic species, *R. scotosialis* and *Trigonistis anticlina* Meyrick, and the first of these was suggested as for testing a representative of the subfamily by Berndt et al. (2007). *Rhaphsa scotosialis* is common and widespread in forests and gardens but is a nocturnal litter feeder and thus does not occur in the same feeding niche as *U. lugens*. Shared feeding niche is a strong predictor of shared hosts for parasitoids (Godfray, 1994), thus *R. scotosialis* was considered of low risk and thus lower priority for host range testing. *Trigonistis anticlina* are found in native forests and are suspected to also feed on litter or possibly fungi, although this has not been confirmed (J. Dugdale pers. comm. 2009). The hypenine larvae are very different physically than the nolines and arctiines, being slim and hairless, rather than plump and hairy, which may make them unrecognisable as a host by the parasitoid *C. urabae* (J. Dugdale pers. comm. 2009).

New Zealand species within clade 7 (i.e. the nolines, arctiines and lymantriines) are closely related to each other, and thus to *U. lugens*. In the selection of species to test, the lymantriines were excluded, as the sole established New Zealand species *Teia anartoides* (painted apple moth) (Dugdale, 1988) is an exotic invader and has now been eradicated (MAF Biosecurity New Zealand 2009, www.biosecurity.govt.nz/pests/painted-apple-moth). Within the arctiines, four species are New Zealand endemics (the tiger moths *Metacrias erichrysa*, *M. huttoni*, *M. strategica*, and magpie moth *Nyctemera annulata*) and one is an introduced biological control agent cinnabar moth (*Tyria jacobaeae*). All of these were tested and the results are discussed below. Two other species are occasional immigrants and were thus not included in the testing list (Berndt et al., 2009; Dugdale, 1988). New Zealand has only one species, the endemic *Celama parvitis*, in the Nolinae, and this was highest priority to test (Berndt et al., 2009) but was also difficult to obtain (B.H. Patrick, NZ Butterfly Enterprises Ltd., pers. comm.).

Arctiinae

In this study, *C. urabae* showed attack behaviour on all three *Metacrias* species when at close quarters in a Petri dish, but significantly less attack behaviour was observed than on the target

species *U. lugens* (Table 4). The larvae (woolly bear caterpillars) of all three species are highly mobile, and feed exposed during the day on native and exotic grasses and herbs. Larvae are present from mid summer through to when they enter obligatory larval diapause over winter (Gibbs, 1962). *Metacrias* spp. larvae could thus overlap with the presence of the parasitoid, but would not be available for parasitoid attack over winter. *Cotesia urabae* has two generations per one generation of its host *U. lugens*, which in New Zealand is bivoltine with a summer and a winter generation.

No parasitoid development was found in *M. strategica*, the only species whose habitat overlaps with the potential distribution of *U. lugens* (Table 4). This species occurs in coastal regions of Canterbury, Otago and Southland in the South Island (Gibbs, 1962). Its habitats range from coastal to forest-edge sites, up to the montane zone and include native grassland and exotic pastures (Gibbs, 1962; Patrick et al., 2003), thus could occur in the same environment as eucalypt trees supporting *U. lugens*.

Parasitoid larvae were found in *M. erichrysa* and *M. huttoni* and a small number of *C. urabae* larvae emerged to form cocoons from these species, but no adult parasitoids developed (Table 4). A significant effect of the parasitoid on larval mortality was found for *M. huttoni*. On the basis of these results alone, one could conclude that these species are not physiological hosts for the parasitoid, but we cannot rule out the possibility that greater replication could have resulted in the emergence of fertile adult parasitoids. Nevertheless, even when encounters with these species were forced in the laboratory, these were no more than marginally acceptable hosts. The likelihood that *Metacrias* species could be adequate field hosts is further reduced by ecological isolation. *Metacrias erichrysa* and *M. huttoni* occur in open herb and tussock fields at altitudes of 900 to 1200 m, predominantly in the South Island (Gibbs, 1962). Thus these species are unlikely to overlap in habitat with *U. lugens*, which is not predicted to occur above 600 m (Kriticos et al., 2007).

The magpie moth *Nyctemera annulata* is common throughout New Zealand on native and exotic herbs and shrubs in the tribe Senecioneae (Asteraceae) (Singh and Mabbett, 1976), including the common weed *Jacobaea vulgaris* (ragwort). As an endemic member of the Arctiinae it was a high priority for host range testing (Berndt et al., 2009). In this study, parasitoids did attack larvae of this species, and parasitoid larvae were found on dissection (Table 4). Although no parasitoid larvae emerged to form cocoons, this result was not significantly different from *U. lugens*. Larval mortality from unknown causes was extremely high in both treatment and control for this species, making it difficult to draw strong conclusions on this species, although a large number of larvae were presented to the parasitoids over all the experiment types. There are at least two generations per year of *N. annulata* and in the North Island breeding may be continuous so that larvae can be found all year round. Thus this species would overlap with all generations of *C. urabae*, and its host plants are likely to occur under eucalypt trees hosting *U. lugens*. We cannot rule out the possibility that further replication would result in the development of parasitoid cocoons, and possibly adult parasitoids from *N. annulata*, but we consider this highly unlikely. Although mortality of larvae in the host range tests was high, many of these larvae died well after the time that a parasitoid would be expected to emerge from a host, although the data gathered could not quantify this (L. A. Berndt pers. obs.). Even if *N. annulata* was an occasional host for *C. urabae*, the threat posed to this species would be minimal as it is abundant and widespread.

The cinnabar moth *Tyria jacobaeae* is an arctiine native to England, Ireland and Europe and was introduced into New Zealand as a biological control agent of *Jacobaea vulgaris* (ragwort) (Syrett et al., 1991). *Tyria jacobaeae* is established at sites throughout New Zealand, however it not considered a very successful biological control agent, and ragwort has now been brought under control in most areas by another agent, the ragwort flea beetle (*Longitarsus jacobaeae* Waterhouse) (Syrett et al., 1991). In this study, attack behaviour by *C. urabae* on

this species did not differ from that on *U. lugens*. Parasitoid larvae were also found in *T. jacobaeae* larvae on dissection, but at a rate significantly lower than in *U. lugens*, and none developed successfully to cocoon or emerged as an adult parasitoid (Table 4). The parasitoid had no effect on the mortality of *T. jacobaeae* larvae. Larvae are present from September to February, potentially overlapping with summer generations of *C. urabae*. As with *N. annulata*, the host plant of *T. jacobaeae* often occurs in the same habitat as eucalypt trees. However, host range testing data for *T. jacobaeae* was robust, and there is a very low likelihood that this species is a physiological host for *C. urabae*, and thus the risk to this species in the field is extremely low.

Nolinae

The endemic species *Celama parvitis* is the sole New Zealand member of the Nolinae (Dugdale, 1988), and thus was the highest priority for host range testing (Berndt et al., 2009). The cryptic larvae are solitary, feeding on the leaves of the shrub *Helichrysum lanceolatum* (Asteraceae) which is widespread in New Zealand growing in disturbed habitats (Smitsen et al., 2006). *Celama parvitis* has been found in Nelson, Lake Wakatipu and on the Otago Peninsula in the South Island (Hudson, 1928), as well as the Wairarapa coast (B.H. Patrick, NZ Butterfly Enterprises Ltd., pers. comm.) and Lake Waikaremoana (NZAC Collection specimens) in the North Island. Populations of *C. parvitis* can be locally common in the eastern and central South Island, but are scattered and difficult to locate, although they are not considered at risk of extinction (B.H. Patrick, NZ Butterfly Enterprises Ltd., pers. comm.). They are rarely found at the North Island localities. The distribution of *C. parvitis* does not overlap with the current distribution of *U. lugens*, but is within the potential distribution of this pest (Kriticos et al., 2007), and its host plants could occur in the same habitat as *Eucalyptus* species supporting *U. lugens*.

Celama parvitis larvae develop slowly over winter from April to November (B.H. Patrick, NZ Butterfly Enterprises Ltd., pers. comm.) so would overlap with winter generations of *U. lugens* and *C. urabae*. In this study, the parasitoid *C. urabae* showed attack behaviour on *C. parvitis* when at close quarters in a Petri dish, but significantly less than on the target species *U. lugens*. Parasitoid larvae were found inside *C. parvitis* on dissection but no parasitoid cocoons developed (Table 4). Because of difficulties rearing larvae of this species most larvae died from unknown causes and the results of rearing, and the impact of *C. urabae* on mortality was not able to be analysed. Larvae of *C. parvitis* grow to 7 mm in length (Hudson, 1950), about half the size of fully grown *U. lugens*. This could affect the ability of the parasitoid to develop in this host, although this has not been tested. This species could not support self sustaining populations of *C. urabae*, having only one winter generation per year. Although we cannot rule out the possibility that this species could be an occasional physiological host for *C. urabae*, it is highly unlikely that *C. parvitis* could support ongoing populations of the parasitoid.

Field experimentation in the country of origin

In this study, attack behaviour was observed on all the non-target species – for some to the same degree as on the target host. To assist in interpreting these results, an Honours project was initiated at the University of Tasmania, aiming to better understand the links between lab and field behaviour of *C. urabae*. Part of this project involves replicating the sequential no-choice test design used in this study with *Nyctemera amica* (White) (Lepidoptera: Acrtiidae). This Australian acrtiid is very closely related to the New Zealand species *N. annulata*, with the two species hybridising when occasional immigrants of *N. amica* to New Zealand establish (Kay, 1980). In Tasmania, *N. amica* are common on cotton fire weed (*Senecio quadridentatus*) growing under eucalypts bearing *U. lugens* and *C. urabae*. The behavioural response of *C. urabae* to *N. amica* in the Petri dish arena was similar to that found for *N. annulata* in this study, with larval attack not differing from that on *U. lugens* (R. Parr, unpublished data, 2009). Subsequent field collections of wild *N. amica* and exposures of sentinel larvae at locations with known populations of *C. urabae* and *U. lugens* found no

evidence of attack by *C. urabae*. Wild collections of 153 *N. amica* from December 2008 – September 2009 resulted in 32% parasitism, with none of the parasitoids being *C. urabae* (R. Parr, unpublished data, 2009). In March 2009, 160 larvae and 325 eggs of *N. amica* were released on potted host plants for four weeks, with 29 re-collected and none of these parasitised (R. Parr, unpublished data, 2009). This work was preliminary because of time and resource constraints, and more robust results may be produced by the conclusion of the thesis in April 2010.

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