

A METHOD FOR HOST RANGE TESTING OF A BIOLOGICAL CONTROL AGENT FOR *URABA LUGENS*

L.A. BERNDT¹, S. MANSFIELD² and T.M. WITHERS¹

¹Ensis Forest Biosecurity and Protection, PO Box 3020, Rotorua, New Zealand

²Faculty of Agriculture, Food and Natural Resources, University of Sydney, NSW 2006, Australia

Corresponding author: lisa.berndt@ensisjv.com

ABSTRACT

Uraba lugens (gum leaf skeletoniser) is a serious pest of *Eucalyptus* spp. in Australia. It is now well established in the greater Auckland region, and is spreading. Two parasitoid species are under consideration as potential biological control agents of *U. lugens*. This paper describes host range testing methods developed using one of these species (*Cotesia urabae*) against two non-target species, *Helicoverpa armigera* and *Spodoptera litura*. Using sequential no-choice tests to test the response of mated *C. urabae* females, clear preferences were observed for *U. lugens* over both non-target test species. Some females did attempt to attack the non-target species, but no evidence of parasitism was observed when non-target hosts were reared or dissected. This method elucidated both behavioural responses and physiological development of *C. urabae*, and it is proposed to be a suitable host range testing method for full evaluation of this species.

Keywords: classical biological control, sequential no-choice test, parasitoid, Braconidae, Lepidoptera.

INTRODUCTION

Eucalyptus trees nationwide are under serious threat from *Uraba lugens* Walker (Lepidoptera: Nolidae; gum leaf skeletoniser) a significant pest of eucalypts in its native Australia. This pest was first found in Mt Maunganui in 1992, but was eradicated from that location. Another population was found in Auckland in 2001, and this continues to spread, threatening eucalypt plantations and amenity trees over much of the country (Berndt 2007). Research into biological control of *U. lugens* is underway, and permission was obtained in 2004 to import four Australian parasitoid species into quarantine in New Zealand for further study (Mansfield et al 2005). Two of these species (*Cotesia urabae* Austin & Allen and *Dolichogenideia eucalypti* Austin & Allen (both Hymenoptera: Braconidae)) have been reared in containment and remain in contention as potential biological control agents (Berndt 2007). These species are solitary larval endoparasitoids in the subfamily Microgastrinae, preferentially attacking gregarious 1-5th instar *U. lugens* larvae (Allen 1990).

A list of lepidopteran species appropriate for host range testing of potential *U. lugens* biological control agents includes *Celama parvitis* (Howes) (Nolidae), the only New Zealand endemic in the same family as *U. lugens*, as well as representatives of the related family Arctiidae (Withers 2003; Mansfield et al. 2005). Two species of Noctuidae, the pests *Helicoverpa armigera* (Hübner) and *Spodoptera litura* (F.), are also included as *U. lugens* was previously placed in this family (Withers 2003).

There is ongoing debate over what methods are appropriate to determine host range for arthropod biological control, and the biology of the agent and hosts must be taken into account in test design (Van Driesche & Murray 2004). No-choice test designs are currently favoured, with the parallel no-choice test format the most widely used (Barratt

2004; Withers & Mansfield 2005). In such a design, one replicate consists of parasitoids, randomly assigned to either target or non-target host. Initial observations of *C. urabae* indicated that 40% of females were not motivated to attack host larvae within an hour of exposure (L.A. Berndt, unpubl. data). Therefore the parallel no-choice test format would require high replication to overcome this known high variability in female responsiveness to hosts. Because the number of mated female *C. urabae* available for experiments was low, and many non-target species are difficult to obtain in large numbers (Mansfield et al. 2005), a more economical method was required.

A sequential no-choice design with an A-B-A sequence (where A is the target host and B is the non-target host) is described here, using *C. urabae* against the non-target species *H. armigera* and *S. litura* as a model. This method is controversial due to the potential confounding effects of time-dependent and experience-induced changes in parasitoid responsiveness to hosts (Barton Browne & Withers 2002; Withers & Mansfield 2005). However, the A-B-A sequential no-choice design openly controls for (and effectively excludes) those female parasitoids that for unknown reasons are not motivated to attack hosts. Therefore this design is more economical with rare parasitoids and hosts than the more widely used parallel no-choice test formats.

METHODS

Cotesia urabae were reared from *U. lugens* larvae collected in Hobart, Tasmania, Australia, in November and December 2006. Parasitoid cocoons were shipped to the Ensis quarantine facility in Rotorua on 12 December 2006. The *C. urabae* colony was maintained on second to fourth instar *U. lugens* larvae reared on *Eucalyptus nitens*. Non-target larvae (*H. armigera* and *S. litura*) were reared in individual pottles on pinto bean diet (supplied by HortResearch, Auckland). Experiments were conducted in quarantine at 20°C, 65% RH, and a 14:10 h light:dark regime. Sequential no-choice experiments were conducted between 19 and 27 January 2007 using mated female *C. urabae* (1-4 days old) from the F1 generation.

One replicate consisted of one female parasitoid observed on target and non-target hosts in experimental arenas using an A-B-A sequence. Between six and eight replicates were conducted for each non-target species. A maximum of two replicates could be observed simultaneously, along with one negative control with host larvae but no parasitoids. Parasitoids were enclosed initially with the target host 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 each set of hosts.

Experimental arenas were 100 mm diameter glass Petri dishes, each with a piece of foliage, *E. nitens* for *U. lugens*, silverbeet (*Beta vulgaris* var. *cicla*) for *S. litura*, or clover (*Trifolium repens*) for *H. armigera*. The silverbeet and clover also had a ca 10 mm cube of pinto bean diet on the foliage, which was added as additional food for non-target larvae, because the non-target food plants desiccated quickly. Ten second or third instar *U. lugens* larvae were added to *E. nitens* in A1 and A2 arenas, and 10 non-target hosts of a similar physical size (3-10 mm) were added to the appropriate foliage in B arenas prior to the start of observations.

Each parasitoid was observed attacking target hosts for 10 minutes (A1 arena), then moved to a non-target B arena and observed for two consecutive 10 minute periods (B1 and B2). Parasitoids were then left in the non-target B arena for 24 hours before another observation of two consecutive 10 minute periods (B3 and B4). At the end of these observations, parasitoids were moved to new target hosts (A2 arena) and observed for a further 10 min. Behavioural observations and the A1 period began once the parasitoid first attacked the target host larvae. To eliminate parasitoids that were not ready to attack larvae, those that did not show interest in the target hosts (A1) within 30 min were excluded.

Behavioural observations consisted of total frequencies of the number of times parasitoids approached larvae, probed, and attacked larvae. Larval approach consisted

of the parasitoid moving close enough to a larva to touch it with its antennae. Probing behaviour consisted of the parasitoid stabbing with its ovipositor but not contacting a larva with it. Larval attack was recorded when the parasitoid successfully stabbed a larva with its ovipositor. Behavioural data were analysed using a log-linear model (GENMOD procedure, SAS Version 8.2). After the completion of the observations, target and non-target larvae were reared to parasitoid development or pupation. Three weeks after completion of the experiments, five non-target larvae from each replicate were frozen, dissected and examined for evidence of parasitoid attack at 8x, 25x and 50x magnification. Mortality of non-target larvae was analysed using Wilcoxon signed-rank test (NPAR1WAY procedure, SAS Version 8.2).

RESULTS AND DISCUSSION

In sequential no-choice tests, *C. urabae* approached *U. lugens* larvae significantly more than they did non-target larvae ($P < 0.005$, Fig. 1). No instances of probing behaviour were observed in response to non-target species, although this was a common behavioural response to *U. lugens* larvae and larval feeding damage on eucalypt leaves (Fig. 1). Larval attack behaviour was observed on both *S. litura* and *H. armigera* larvae, however the incidence of this was significantly lower than on *U. lugens* ($P < 0.0001$, Fig. 1). Parasitoids

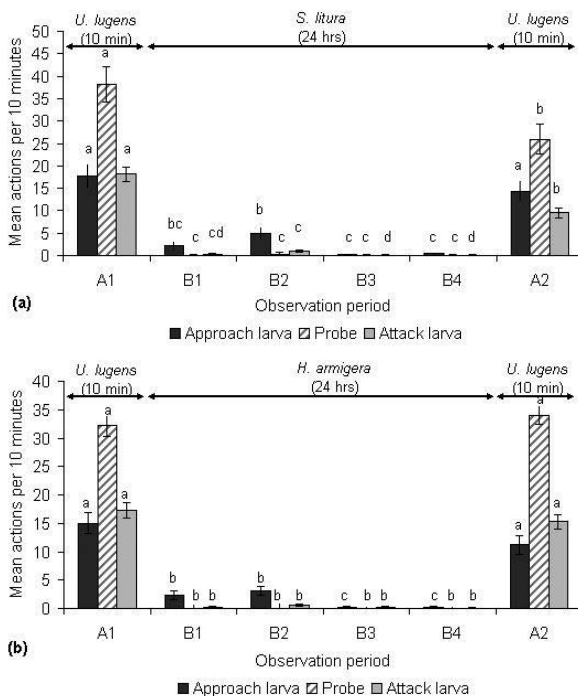


FIGURE 1: Mean (\pm SE) number of larval approaches, probes and larval attacks by *Cotesia urabae* on target (*U. lugens*) and non-target (a) *Spodoptera litura*, (b) *Helicoverpa armigera* larvae. Values are the mean per 10 min observation period in A-B-A sequential no-choice tests. Bars of one behaviour type sharing a letter do not differ significantly at $P < 0.05$. Means are back transformed.

that did attack non-target species tended to attack larvae that were roaming the arena and that spun down when encountered, ignoring those settled on the leaf or remaining motionless on encounter. This contrasted strongly with attack behaviour on *U. lugens*, where parasitoids repeatedly visited the leaf to attack the cluster of larvae. No evidence of parasitoid attack was found in either non-target species from rearing or dissection, compared to *U. lugens* parasitism of 65 to 92% (Table 1).

TABLE 1: Parasitism (%) of *Uraba lugens* and non-target (*Spodoptera litura* and *Helicoverpa armigera*) larvae by *Cotesia urabae* in A-B-A sequential no-choice tests. Also shown is the mean number of unexplained deaths among non-target larvae. Ten larvae were exposed to each parasitoid in each arena per replicate, n = number of replicates.

Non-target species	Mean \pm SE parasitism ¹ in no choice tests (n)			Mean \pm SE unexplained deaths among non-target larvae (n)	
	<i>U. lugens</i> (A1)(10 min)	Non-target (B) (24 hr)	<i>U. lugens</i> (A2) (10 min)	Exposed to parasitoid	Negative control
<i>S. litura</i>	92 \pm 6.2 (6)	0 \pm 0 (6)	74 \pm 10.6 (6)	1.2 \pm 0.48 (6)	2.0 \pm 1.15 (3)
<i>H. armigera</i>	82 \pm 6.5 (8)	0 \pm 0 (8)	65 \pm 6.4 (7)	0.5 \pm 0.33 (8)	0.3 \pm 0.25 (6)

¹Percent parasitism = larvae with evidence of parasitism from rearing or dissection / number of larvae reared or dissected (excluding unexplained deaths) \times 100.

Non-target attack occurred in the first 20 minutes of non-target exposure (B1 and B2), with only one exception (Fig. 1), suggesting that the parasitoids' response to these species was in some way a central excitatory state caused by immediate prior contact with the target host (Withers & Mansfield 2005). This priming could result in false positive results (i.e. parasitoids attacking hosts they would not normally attack), potentially removing a valuable biological control agent from contention (Coombs 2004). However, host attack is not evidence of impact on the host unless parasitoid offspring are produced, or host fitness is reduced (Van Driesche & Murray 2004). No evidence of successful non-target parasitism was found, and exposure to parasitoids did not significantly increase the mortality rate of larvae in this study (*S. litura*: $P=0.596$, *H. armigera*: $P=0.911$, Table 1).

Arguments against the use of sequential no-choice tests centre on the risk of obtaining false negative results (Withers & Mansfield 2005). These can occur if the period of access to non-target hosts is not long enough to allow time dependent changes in parasitoid responsiveness to become apparent (Barton Browne & Withers 2002). In the current experiments, both non-target species were attacked early in the 24 h period of non-target exposure when observations were being made. This design therefore maximised the chance of non-target attack, as advocated by Withers & Mansfield (2005). The addition of a positive control, effectively A-A-A, at the same durations as A-B-A, would be beneficial to confirm that the durations chosen are appropriate for the normal host attack behaviour of *C. urabae* (Withers & Mansfield 2005). The addition of one positive control test for each non-target host species would minimise the need for extra parasitoids, hosts and observation time to conduct these tests.

The non-target attacks observed here permitted testing of the physiological ability of *C. urabae* to develop in *S. litura* and *H. armigera*, which is the ultimate test of host suitability, but is often difficult to test in endoparasitoids (Withers & Barton Browne 2004; Barratt et al. 2007). Therefore the host range testing methodology proposed here was successful for testing *C. urabae* and will be used against the remainder of the proposed non-target species. The suitability of the duration of the exposure will be further confirmed with positive controls and would remain the same across different non-target hosts. If

a similar response can be demonstrated for *D. eucalypti*, the other potential biological control agent against *U. lugens*, then the method proposed here will also be used for that species, with exposure durations adjusted according to its host attack behaviour.

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