ROLE OF OLFACTION IN HOST SELECTION BY *PSEUDAPHYCUS MACULIPENNIS* (HYMENOPTERA: ENCYRTIDAE)

W.R.M. SANDANAYAKA, J.G. CHARLES and P. RAMANKUTTY

HortResearch, Private Bag 92169, Auckland, New Zealand

Corresponding author: msandanayaka@hortresearch.co.nz

ABSTRACT

The volatile stimuli for host finding by *Pseudaphycus maculipennis* were examined in a Y-tube olfactometer. The responses of naïve female parasitoids (2–3 days old) to two mealybug species, *Pseudococcus viburni* (obscure mealybug) and *Pseudococcus longispinus* (longtailed mealybug) on sprouting potato were measured. The percentage of parasitoids to choose the odour-laden arm compared to clean air was similar for both mealybug species on potato sprouts (80 to 82.5%). When given a choice between mealybug species on potato sprouts, the parasitoid preferred the obscure mealybug. The parasitoids also responded to an excised sprout from an uninfested potato when compared with clean air. Further choice tests showed that honeydew secreted by either mealybug species was similarly attractive, while honeydew was more attractive than the ovisacs from obscure mealybug. This work shows that *P. maculipennis* exhibits a strong response to the plant of their host and prefer obscure mealybug to longtailed mealybug despite a similar response to honeydew from both species.

Keywords: *Pseudaphycus maculipennis*, *Pseudococcus viburni*, *Pseudococcus longispinus*, host selection, olfaction.

INTRODUCTION

Pseudaphycus maculipennis Mercet is a host specific, internal parasitoid of *Pseudococcus viburni*, the obscure mealybug, and was reported to provide successful biological control of the mealybug in France and the Republic of Georgia (Panis & Brun 1971; Yasnosh & Mjavanadze 1983). Obscure mealybug has been the dominant mealybug pest of Hawke's Bay apple crops for many decades, but lacked any effective, host specific parasitoid (Charles 1993), until *P. maculipennis* was introduced to New Zealand from Australia in 2001. It has since established in Hawke's Bay, Nelson and Auckland pipfruit orchards (Charles et al. 2004).

Before release was approved, host-testing in quarantine indicated that *Pseudococcus longispinus* (longtailed mealybug) was occasionally attacked by *P. maculipennis*. In order to better understand these responses, a laboratory study of the olfactory responses of the parasitoid to mealybugs was initiated. Olfaction is an important mechanism involved in host location by insect parasitoids (Vet et al. 1983). A previous Y-tube olfactometer study showed that parasitoids can be attracted by odours produced by the food plant of their host (Sandanayaka & Charles 2004). This paper investigates olfactory stimuli that may be used by *P. maculipennis* in their search for host plants and mealybugs.

MATERIALS AND METHODS

Pseudaphycus maculipennis was reared from an obscure mealybug laboratory colony maintained on sprouting potatoes at 23±1°C and 16:8 h light:dark. Mummified mealybugs were held individually in gelatine capsules until the parasitoids emerged. Male and female parasitoids of the same age group were held in glass vials (7.5 x 2.5 cm diameter) with gauze-covered stoppers for mating and were fed with a honey-agar diet (D.J. Allan,

pers. com.). Females that were 2-3 days old and assumed to be mated, but with no prior experience in oviposition, were used in the experiments.

Obscure mealybug and longtailed mealybug colonies were reared on the same variety of potato (*Solanum tuberosum* L. cv. Desiree) in separate rooms maintained at $23 \pm 1^\circ\text{C}$ and 16:8 h light:dark).

The responses of female *P. maculipennis* to different odours were tested in a glass Y-tube olfactometer (1 cm internal diameter tubes), with an airflow of 75 ml/min (measured by soap bubble air meter, Pye Unichem® Ltd) through each arm. Filter paper discs (2 cm diameter) treated with distilled water were placed in both control and treatment arms to equalize humidity. Since *P. maculipennis* are positively phototactic, the olfactometer was centered directly under the only light in the room. The temperature in the test area was kept the same as the rearing rooms described above. The treatments used in the nine choice tests are shown in Table 1.

Prior to the experiment, a blank choice test (with no treatments) was run with 20 parasitoids to confirm that there was no detectable bias present in the system. Treatments 1, 2 and 3 used similar size potato sprouts excised from the potatoes in the mealybug laboratory colonies. The uninfested potato sprouts used in test 4 were obtained from the same variety of potatoes. In tests 5, 6 and 7, approximately equal amounts (ca 50 mg) of obscure mealybug and longtailed mealybug honeydew from the mealybug laboratory colonies were collected on to filter paper discs (2 cm diameter). Obscure mealybug ovisacs in tests 8 and 9 were held on nylon gauze mounted in a 4 cm diameter circular cardboard frame. Honeydew and mealybug larvae were avoided when the ovisacs were collected from the obscure mealybug colony. In each test, a single female parasitoid was released through a hole at the downwind end of the Y-tube and exposed to a mixture of the two airflows until it reached the junction of the arms of the Y-tube. Individual female parasitoids were used only once during the experiments. The responses of forty females were measured in each test. Ten replicates were considered as a block and the treatments were renewed for each block. The position of the Y-tube arms was reversed after the fifth replicate of each block. The proportion of parasitoids that reached the chambers in either side of the Y-tube and the time taken to enter the target zone (to the nearest second) were recorded.

The two response variables (proportion and time) in each test were analysed using GenStat Release 8.1. For each test, the 'proportion' data were compared by One-Sample Binomial analysis to the proportion expected by chance (50% to each target). Each block was analysed separately and jointly. The time to reach the chosen target (TRT) data was analysed by Residual Maximum Likelihood (REML) for all 9 experiments together, with target within experiment as the fixed effect and block as the random effect. REML assumes that the error terms are normally and independently distributed with zero mean and constant variances. The time-to-target data were transformed using the square root prior to analysis to ensure that these assumptions were met. Mean separation tests were carried out for different levels of the treatment factors using Fisher's Protected LSD at $P=0.05$.

RESULTS

A few female parasitoids moved directly from the release point to the target, but most spent some time walking around the junction of the two arms of the Y-tube before choosing one arm to walk down. Table 1 shows the proportion of parasitoids attracted to each of the treatments in the nine choice tests, and the average time taken to reach the chosen target. Parasitoids were attracted significantly more to either obscure mealybug (test 1) or longtailed mealybug (test 2) than to clean air. However, when given a choice between the mealybug species, parasitoids were attracted significantly more to the odours of the obscure mealybug colony than to longtailed mealybug (test 3). Positive responses to uninfested potato sprouts (test 4) demonstrated that *P. maculipennis* was also attracted to plant cues alone.

Parasitoids were equally attracted to both obscure mealybug and longtailed mealybug honeydew (test 7, Table 1). The initial response of parasitoids reaching the honeydew discs was to stop and touch the honeydew-treated area with their antennae and mouthparts.

This behaviour was similar for both obscure mealybug and longtailed mealybug honeydew. The response to the obscure mealybug ovisacs was not different from the response to clean air (test 8), and obscure mealybug honeydew was more attractive to *P. maculipennis* than obscure mealybug ovisacs.

TABLE 1: The proportion of female *P. maculipennis* moving towards the odour of treatments tested in the Y tube olfactometer. Mealybug colonies were on a potato sprout, honeydew was placed on filter paper and ovisacs were held on nylon gauze. The values presented are for the pooled data over four blocks.

Test	Treatments	Proportion reaching target (n=40)	Average time to reach target (min)
1	Obscure mealybug colony	0.83* ¹	5.7
	Clean air	0.17*	5.5
2	Longtailed mealybug colony	0.80*	4.8
	Clean air	0.20*	6.7
3	Obscure mealybug colony	0.73*	3.7*
	Longtailed mealybug colony	0.28*	5.4*
4	Uninfested potato sprout	0.83*	4.5
	Clean air	0.18*	5.8
5	Obscure mealybug honeydew	0.88*	5.4*
	Clean air	0.13*	2.7*
6	Longtailed mealybug honeydew	0.80*	5.7
	Clean air	0.20*	6.2
7	Obscure mealybug honeydew	0.48	3.9
	Longtailed mealybug honeydew	0.53	3.7
8	Obscure mealybug ovisacs	0.50	5.0
	Clean air	0.50	4.8
9	Obscure mealybug ovisacs	0.25*	6.5
	Obscure mealybug honeydew	0.75*	5.1

¹* indicates that the two means in the test are statistically different at $P=0.05$.

The times taken by the parasitoids to reach the chosen target were not significantly different, except in tests 3 and 5 (Table 1). In test 3, parasitoids reached the obscure mealybug colony more quickly (3.7 min) than the longtailed mealybug colony (5.4 min) ($P=0.05$). In test 5, 3 out of the 5 parasitoids that chose the control arm reached the target in less than a minute, resulting in a significantly shorter mean time to target than that for the majority of parasitoids that chose the obscure mealybug honeydew. Although more parasitoids were attracted to one side of the Y-tube in tests 1, 2, 4, 6 and 9, the time taken to reach the targets in both sides were not significantly different ($P=0.05$) from each other.

DISCUSSION

These experiments have extended our understanding of host selection behaviour of *P. maculipennis*. Although *P. maculipennis* responded equally to either obscure or longtailed mealybug in isolation, they differentiated between the two species. Neither the ovisacs of obscure mealybug nor the eggs within them were attractive to *P. maculipennis* females. Preliminary experiments have since indicated that the parasitoids were responding to the obscure mealybug sex pheromone. Such a response to sex pheromone has been recorded for *Allotropa* sp. and *Anagyrus subalbipes* (also Encyrtidae), two parasitoids of *Pseudococcus cryptus* Hempel in Japan (Arai 2003). Further studies are underway to determine the ability of *P. maculipennis* to resolve pheromone cues from host and non-host species in the mixed mealybug colonies typically found in New Zealand fruit crops.

The parasitoids also responded to uninfested potato sprouts, demonstrating the role that host plants may play in the parasitoids' search for mealybug hosts. Olfactory responses of encyrtid parasitoids to plant volatiles have also been reported (Souissi et al. 1998; Bertschy et al. 2001). Further research is planned to study the impact of different host plants on host selection by *P. maculipennis*.

The response of *P. maculipennis* females to honeydew appeared to be unaffected by the mealybug host from which it was collected. The fact that parasitoids responded equally to honeydew of the two mealybug species suggested that they regarded honeydew simply as a food source. Similar behaviour by *Aphidius nigripes* (Hymenoptera: Aphididae) on honeydew from host and non-host aphids was reported by Bouchard & Cloutier (1984). Nevertheless, honeydew may be an important component in habitat location in the field (Khandakar & Jahan 1993), and identification of the kairomones in honeydew could lead to its use for enhancement of mealybug parasitism. Hence the positive response to the longtailed mealybug colony in test 2 may have resulted from attraction to the honeydew in addition to the plant cues.

The present study clearly showed that once in the general habitat of their potential host, *P. maculipennis* females have the searching capacity to locate obscure mealybugs via olfactory cues. Whether these cues are sufficient by themselves to provide long-range attraction of the parasitoids to obscure mealybugs, or whether other environmental cues are also important, remain to be determined.

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