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Investigation on Host Finding Behavior of the Two Parasitoids of Potato Tuber Moth in a Flight Tunnel

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ABSTRACT

Laboratory experiments were conducted to understand the influence of host-finding behaviour of *Apanteles subandinus* Blanchard and *Orgilus lepidus* Muesebeck, two endoparasitoids of the potato tuber moth (PTM) *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), in a successful biological control of the pest. Responses of the two parasitoids to their host and to three host plants of the PTM were investigated in a wind tunnel individually. The results suggested that host-finding by both parasitoids is stimulated by a combination of chemicals. Females of both species discriminate between the volatiles of a mechanically damaged plant and those of PTM larvae-damaged plant. The combination of odours originating from plant host on which the host is feeding play a crucial role in the foraging behavior of these parasitoids. Developmental experience during larval stages and experience of adults to host plant influence their foraging for host. The implications of parasitoid response towards plant volatiles and their importance in biological control are discussed. Additional experiments are necessary to isolate and identify the nature of the volatile chemicals released from the host-plant complex and use them to improve the biological control of the pest.

Keywords: *Apanteles subandinus*, Host-finding, *Orgilus lepidus*, *Phthorimaea operculella*.

INTRODUCTION

The potato tuber moth (PTM), *Phthorimaea operculella* Zeller, is a major pest in many countries where potatoes, tomatoes and eggplants are grown. Control of this pest has been achieved until recently by the use of insecticides. Biological control of this pest has been undertaken in Australia since 1960 with parasitic wasps, including two braconid, solitary, larval endoparasitoids, *Apanteles subandinus* Blanchard and *Orgilus lepidus* Muesebeck (Horne, 1993). Recently Horne (unpublished data) reported an IPM program in Australia based on a biological control program to minimise application in the crop. Parasitism was high at unsprayed sites, reaching 95-100% at the critical stage before harvest.

Assessing the efficacy of a parasitoid is important in developing successful biological pest control. Host-finding by a parasitoid addressed that influence the ability of the parasitoids to control a pest. Host finding by a parasitoid depends on responses to cues from the host habitat and the host itself (Doutt, 1964; Vinson, 1975). According to Salt (1935), Doutt (1959) and Vinson (1975), host-finding by a parasitoid consists of five steps: 1) host habitat location, 2) host location, 3) host acceptance, 4) host suitability and 5) host regulation.

Knowledge of the factors that influence the searching behaviour of parasitoids has advanced substantially in recent years (see Vet and Dicke, 1992). There is considerable evidence that some parasitoids use chemical stimuli associated with interactions between

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the host and its food plant and respond to host herbivore-induced plant volatiles (Turlings *et al*., 1990 and 1991; Vet and Dicke, 1992; Tumlinson *et al.,* 1993; Keller and Horne, 1993; Steinberg *et al*., 1993; Takabayashi *et al*., 1994). Odours from hosts, host products or host plants can be important cues used by parasitoids to locate the habitats of their hosts and the hosts themselves (Keller and Lewis, 1989; Vinson, 1991; Vet and Dicke, 1992; Agelopoulos and Keller, 1994a&b; Kitt and Keller, 1998).

Little is known about the role of chemical cues in the foraging behaviour of the *A. subandinus* and *O. lepidus*. Hendry *et al*. (1973) reported that host-finding by female *O. lepidus* was mediated by two kairomones present in the frass of the host. They found that the active volatile component in the frass, heptanoic acid, normally functions to elicit only intense tapping by antennae and directed movements by the parasitoid females and that the probing response is mediated by the non-volatile component upon direct contact with the faeces. Keller and Horne (1993) studied searching and host-finding behaviours by *O. lepidus* in a wind tunnel and they found that, although female *O. lepidus* flew to both undamaged and mechanically damaged potatoes, more females flew to damaged plants when given a choice. Their research indicated that *O. lepidus* can discriminate between the volatiles of a mechanically damaged potato plant and those of a potato plant damaged by PTM larvae.

There are no reports on the foraging and host-finding behaviour of *A. subandinus*, nor is there any data available on the efficiency of *A. subandinus* and *O. lepidus* if reared on PTM fed on potato tuber, then exposed to hosts on other Solanaceous plants. Sheehan and Shelton (1989) tested *Diaeretiella rapae* (Hymenoptera: Aphidiidae) a parasitoid of aphids on cruciferous plants. They reared and experienced wasps on one host plant (collard) and then released them to another host plant (potato) infested with host. These researchers found that postemergence experience with potato did not increase orientation to potato, but postemergence experience with collard resulted in a trend of increased likelihood of flying towards collard odour.

A better understanding of the host-finding behaviour of *O. lepidus* and *A. subandinus* may play an important part in developing a more effective biological control program for PTM. Chemical cues could be used to stimulate parasitoids' host-finding, e.g. in rearing or prior to the release of wasps. Therefore, this study examines the cues that lead *A. subandinus* to its host, and the responses of the two parasitoids to different host plants of PTM. Two series of experiments were conducted for the two parasitoids in the wind tunnel. Both were mainly concerned with step two of the host finding process, host location. The aims of this study were: 1) to determine which factor (s) female *A. subandinus* utilise when foraging for hosts and 2) to determine the role of host plants in affecting the behaviour of *O. lepidus* and *A. subandinus* in the process of host-finding.

MATERIALS AND METHODS

Parasitoids and their Release

The parasitoids host-finding behaviour was studied in a wind tunnel in Waite Campus, the University of Adelaide, Australia during 1994-98. Parasitoids of both species were 2-3-day-old females and reared on PTM larvae feeding on potato tubers in the insectary. Prior to their use in experiments, females were kept in cages with males where they had access to honey and water at all times.

A screened cage (15×15×20cm) containing potato leaves infested with ten PTM larvae was placed in the wind tunnel and five wasps (in each trial) were released in the cage for 30-60 minutes prior to release in the wind tunnel in order to gain experience with hosts plants and host insects. Thus, all parasitoids used in tests were 2-3-day-old and experienced before flying in the wind tunnel.

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All tests were carried out between 9am to 6pm.

The latency of flight (the time from wasp placement in the release vial to take off) and flying time were recorded during tests, as was the site of landing. The behaviours were recorded using an Observer Version 2 (Noldus, 1991) and recording started when each wasp oriented towards odours from the plants and finished when each wasp landed on one of the two plants.

Assays in a Wind Tunnel

The wind tunnel used in these experiments was a rectangular plexiglass chamber (65 x 65 x 160cm) described by Keller (1990). The environmental conditions in the wind tunnel were the same throughout the experiments: wind speed at 32.4cm/s, temperature at 24°C and light intensity at 4800 lux at the insect release site.

Individual wasps were released 25cm above the floor of the wind tunnel at the same height as the target plants. They were released from a glass tube 2.5cm in diameter and 13.5cm in length with two open ends. The vial was placed horizontally in the airstream on a wooden stand and positioned so that the odour plume could pass through. The distance from the releasing vial to the plants was 30cm. Each female had a time limit of five minutes to respond. Wasps that landed on the walls or ceiling were omitted from the analysis. The number of females that responded in each cohort and choice of landing site was recorded.

The plants (potato / tomato or potato / eggplant, damaged or undamaged) were placed 10cm apart (2cm between their lateral leaves). A smoke test using a mixture of acetic acid and diaminoethane showed that the two plants produced separate plumes.

Data Analysis

The numbers of females attracted to each of the paired plants were compared by a Binomial test (Zar, 1984), using a computer program written by M. Keller in 1997. In all tests $P< 0.05$ was used to determine significance.

First experiment: responses of *A. subandinus* to its host (PTM) and host plant (Potato)

The ability of *A. subandinus* females to discriminate between two types of damaged potato leaves was studied to determine if *A. subandinus* could distinguish between the plant-host complex and a mechanically damaged plant. In this experiment, the potato shoots with at least three matured leaves of the same size and as similar as possible in shape were cut with a surgical knife and fixed in 150ml containers containing tap water. To keep each shoot fresh for at least seven days, its stem was submerged in water through a hole (10mm in diameter) in the lid of the container. A small cotton ball was used to cushion the stem against the side of the hole and to hold the plant upright. In each test, two container plants were used, a potato infested with ten 48hr-old larval PTM and a mechanically damaged potato. The second plant was damaged just one hour prior to the test using a pin to pierce the leaves similar to the shape of damage caused by PTM.

Two series tests were conducted. In the first series, 51 female *A. subandinus* were released individually, each only once, in the wind tunnel. In the second, 65 female wasps were released individually twice. At the second release, the positions of the two plants were switched. The aim of the second experiment was to be confident that wasps responded to separate plumes not to one direction.

Second experiment: host plant preference by the two parasitoids

The aim of this study was to determine whether responses of *A. subandinus* and *O. lepidus* females are dependent on rearing conditions (on potato tuber). The information about the source of cues responsible for host-finding of the two parasitoids would be useful to delineate their role in the biological control of PTM.

Potato tuber buds were planted instead of whole tubers. Buds (2×2cm) were cut from the tubers with a cork-borer; from one 100g tuber at least five buds could be obtained. Buds were placed in a solution of 2 ppm gibberelic acid water for 15 minutes. The buds were placed on tissue paper until dried and planted 1.5-2cm deep in small plastic pots (10cm in diameter). Potato buds and seedlings of tomato and eggplant were planted in black plastic pots 10cm in diameter. The plants for each test were approximately the same size (12-15cm) and as similar as possible in shape and the surface area of their foliage. Because of differences in the size of foliage of three plants with different morphological characteristics, the size of the plants, foliage was measured using a Paton Electronic planimeter (Pearcy *et al.*, 1989). Ten cuts of foliage from each plant were measured as representative samples for choosing the same approximate size of three plants to be infested with PTM.

Tests were conducted comparing the preference for potato versos tomato and potato versos eggplant. Each plant was infested with the same number of PTM larvae (10/plant) at the same time and each test was conducted individually for each parasitoid species. In each test, five female wasps were tested individually on the same set up plants. The number of female parasitoids released in different treatments were not similar.

RESULTS AND DISCUSSION

First Experiment

Female *A. subandinus* were strongly attracted to infested plants when given a choice between potato infested by PTM and potato mechanically damaged in both series of tests (Figure 1A & B). There were highly significant differences between the two groups of females ($P < 0.001$, binomial test) which had chosen infested plants with PTM and those that had chosen mechanically damaged plants. A small proportion (23% in the first series and 17% and 8% respectively in the 1st and 2nd release of the second series of tests) flew to the sides of the wind tunnel. The wasps walked around the inside and outside of the vial after having been placed into the releasing vial, then extended their antennae away from the face and raised them. After a period of standing and moving, they finally flew to the plants. On a few occasions some flew immediately to the top of the wind tunnel. This latency

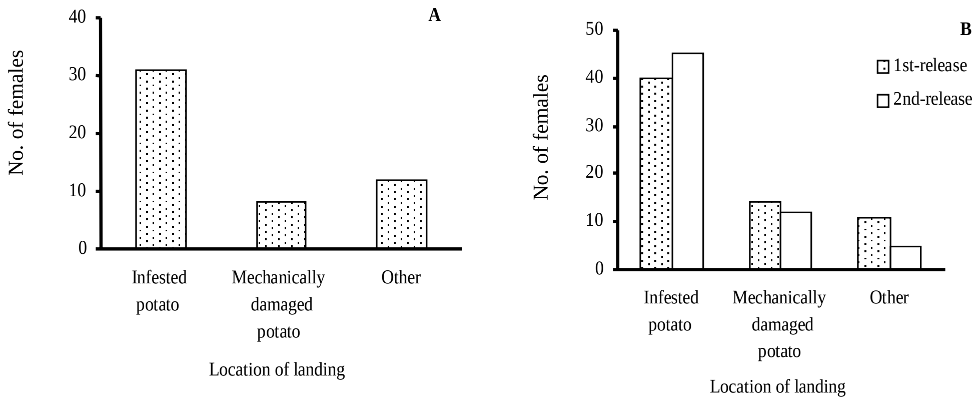

Figure 1. Landing sites of *A. subandinus* females when given a choice between potato plants infested by PTM and mechanically damaged potatos in the wind tunnel. “Other” includes walls of the wind tunnel. A) females were released once (n = 51, $P < 0.001$), and B) each female was released twice (n = 65, $P < 0.001$), Binomial test.

of flight averaged 104.2 ± 6.0s (n= 65, range, 30-320s). The duration of latency did not differ among wasps landing in different locations the duration of flight ranged between 1-10s and the mean was 2.5±0.23s. Flights were often direct and rapid towards the plants. The flight time decreased when females flew to the walls of the wind tunnel (1.7 ± 0.19s).

The results show that complex cues from the larvae mining inside the potato tissues stimulate searching by *A. subandinus* females. However, this study did not separate the volatile components which attract this species. Keller and Horne (1993), similarly found that *O. lepidus* females respond to odours caused by PTM larvae by landing preferentially on infested plants.

Female *A. subandinus* discriminate between the volatiles of a mechanically damaged plant and those of a PTM larvae-damaged plant. Mattiacci *et al*. (1994) found that artificially damaged plants produce fewer compounds and in lower amounts than plants damaged by a host larva. In the present study it is possible that differences in the release rates of compounds in potato plants damaged by host and those mechanically damaged may explain the differences in response. In addition, the variability in the mix of compounds in the odour blend may result in subtle differences in the behaviour of parasitoids (Mattiacci *et al*., 1994).

Second Experiment:

There were no significant differences in the number of *A. subandinus* females landing on the two targets in choice tests between infested potato and infested tomato leaves (*P*= 0.419) or infested potato and infested eggplant leaves (*P*= 0.202) (Figure 2). Significantly more *O. lepidus* landed on infested potato than tomato (*P*= 0.030). In addition, a greater number of *O. lepidus* females landed on potato than on eggplant (*P*=0.037).

When given the choice between potato-host complex versos tomato-host or eggplant-host complex, *A. subandinus* females showed no preference for landing on potato plants (*P*= 0.419 and *P*= 0.202 respectively). Regardless of cues associated with plants, *A. subandinus* females landed on each plant with PTM larvae. This suggests that *A. subandinus* would move freely between these plants when searching for hosts, but experience over time may lead them to prefer some plants above others.

O. lepidus females were more attracted to the potato-host complex than to the tomato- and eggplant-host complexes. The prior experience of the wasps on potato may have affected their choice to fly to plants infested with PTM, as has been reported in several parasitoid-host communications (see Vet and Dicke, 1992 for a review). Possibly, the complex chemical compounds from potato plants and PTM larvae are more attractive to *O. lepidus* females than the chemical compounds from tomato- or eggplant-host complexes. This specificity suggests that the potato plant-host complex provides important cues to the searching *O. lepidus* females.

CONCLUSIONS

From the results of this investigation it is concluded that *A. subandinus* females were able to distinguish from a distance of 30cm, between infested and uninfested plants with PTM larvae. The host recognition infochemicals for *A. subandinus* females are present in host plants damaged by PTM larvae. An active series of physiological and biochemical processes occurs with host feeding on plant leading to quantitative or qualitative changes in the volatiles emitted (e.g. Du *et al*., 1996). Odours originating from infested plant foliage appear to be important attractive stimuli for female *A. subandinus*. When infested potatoes and mechanically damaged potato plants were introduced, *A. subandinus* females were attracted to the infested potato plant. When infested potato were introduced with infested tomato or eggplant, females were equally likely to land on either infested

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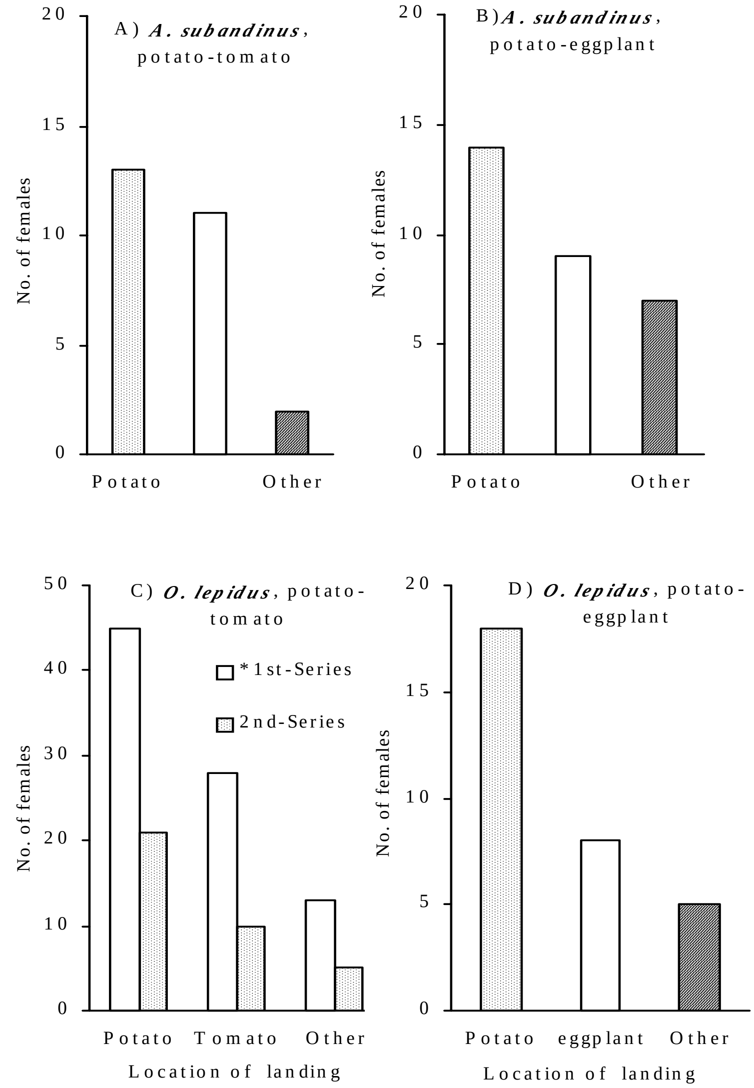

Figure 2. Number of *A. subandinus* and *O. lepidus* females landing on targets in two-choice experiments in the wind tunnel. A) *A. subandinus* in a choice between potato and tomato, n= 26, *P* = 0.419 and B) in a choice between potato and eggplant, n= 30, *P* = 0.202. C) *O. lepidus* in a choice between potato and tomato, n= 122, *P* = 0.003 and D) in a choice between potato and eggplant, n= 31, *P* = 0.037. The p-value is based on Binomial test (Zar, 1984), using a computer program written by Keller (1997).

plant.

The attraction of *A. subandinus* to plants infested with PTM show that females discriminated between the volatiles of mechanically damaged plants and those of host damaged plants as previously has been demonstrated for natural enemies (Vet and Dicke, 1992; Turlings *et al.*, 1990; Dicke, 1994). The weak responses observed to mechanically damaged plants show that chemicals emitted from host plant sources alone are not efficient cues for attraction of this wasp. *O. lepidus* females were more attracted to potato plants with active PTM larvae than to tomato and eggplant. Female wasps preferred infested potato plants with PTM larvae to uninfested plants or those with mechanical damage and the importance of this has already been well documented (Keller and Horne, 1993). It is not clear whether the prior rearing and experience on potato infested with hosts simply enhanced the response of *O. lepidus* to hosts on potato plants or not. The greater numbers of *O. lepidus* orientated to infested potato versus infested tomato or eggplant foliage implies that odours released from host-potato plant complex had a more attractive blend of volatiles that attracted *O. lepidus* females.

The results suggested host finding by both parasitoids is stimulated by a combination of chemicals. More information is needed about the types of stimuli which attract the wasps and how their previous experience influences responses to these stimuli. In addition, to isolation and identification of the volatile chemicals released from the host-plant complex additional experiments are necessary to elucidate (1) sources of attractive chemicals, (2) responses to them by both flying and walking wasps, and (3) the effects of the previous experiences during development and adult life. Such research could shed light on how foraging behaviour influences the searching efficiency of the parasitoids.

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بررسی رفتار میزبان‌یابی دو گونه از پارازیتوئیدهای بید سیب زمینی در یک تونل پرواز

لطیف صالحی و ام. آ. کلر

چکیده

آزمایشات آزمایشگاهی به منظور مشخص نمودن رفتار میزبان‌یابی زنبورهای *Apanteles subandinus* Blanchard و *Orgilus lepidus* Muesebck دو پارازیتوئید داخلی بید سیب زمینی، *Phthorimaea operculella* Zeller (Lep: Gelechiidae) در موفقیت مبارزه بیولوژیکی انجام گردید. عکس‌العمل‌های دو پارازیتوئید به میزبان و به سه نوع گیاه میزبان بطور جداگانه در یک تونل پرواز بررسی شد. نتایج نشان دادند که میزبان یابی توسط دو پارازیتوئید تحت تاثیر تلفیقی از مواد شیمیایی قرار دارد. ماده‌های هر دو گونه توانستند بوهای ناشی از ضایعه مکانیکی را از بوهای حاصل از تغذیه لارو بید سیب زمینی مجزا کنند. بوهای شیمیایی حاصل از گیاهی که میزبان روی آن فعالیت دارد، نقش اساسی در رفتار جستجوگری این پارازیتوئیدها دارد. تجربه پارازیتوئیدها در حین رشد ونمو مرحله لاروی و کسب تجربه حشرات کامل روی گیاه میزبان، در جستجوی آنها برای میزبان تاثیر دارد. نقش قاطع بوهای شیمیایی گیاه در گرایش پارازیتوئید به طرف آن و اهمیت آنها در مبارزه بیولوژیکی با آفات مورد بحث قرار گرفته است. به منظور افزایش کارایی این پارازیتوئیدها در مبارزه بیولوژیکی با آفت، لازم است آزمایشات بیشتری انجام گیرد تا مواد شیمیایی بو دار حاصل از مجموعه میزبان و گیاه جداسازی گردند و تشخیص داده شوند.