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Short communication/Kurze Mitteilung

A simple and effective bioassay to study the effect of plant surface chemicals on the behaviour of *Trichogramma* spp.

Ein einfacher Test zur Bestimmung der Einflüsse von Chemikalien auf der Pflanzenoberfläche auf das Verhalten von *Trichogramma* spp.

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Summary

A simple and effective filter paper test was developed to study the role of plant surface chemicals on the searching and parasitization behaviour of *Trichogramma* spp.. First results have shown that *Trichogramma chilonis* Ishii is repelled by hexane- and acetone-extracts of pigeonpea (*Cajanus cajan* (L.) Millsp.) leaf surface, a host plant on which parasitized eggs of a preferred host, *Helicoverpa armigera* (Hübner), are rarely found.

Key words: Plant surface chemicals; *Trichogramma chilonis*; parasitization behaviour; *Helicoverpa armigera*; bioassay

Zusammenfassung

Das Such- und Parasitierungsverhalten von *Trichogramma* spp. kann von Chemikalien auf der Pflanzenoberfläche beeinflußt werden. Ein einfacher und effektiver Filterpapier-Test kann den Einfluß solcher Chemikalien auf das Verhalten des Parasitoiden nachweisen. Ersten Versuchen zufolge wird *Trichogramma chilonis* Ishii von Hexan- und Aceton-Extrakten, gewonnen von der Blattoberfläche der Straucherbse (*Cajanus cajan* (L.) Millsp.) abgeschreckt. An dieser Wirtspflanze werden nur niedrige Parasitierungsraten an Eiern von *Helicoverpa armigera* (Hübner) gefunden, einem bevorzugten Wirt.

: Chemikalien der Pflanzenoberfläche; *Trichogramma chilonis*; Parasitierungsverhalten; *Helicoverpa armigera*; Bio-Test

1 Introduction

Trichogramma spp. (Hymenoptera: Trichogrammatidae) parasitizes eggs of a wide range of insect species on many different host plants. It has often been observed that parasitism levels on a specific host species vary widely, depending on the host plant on which the eggs are found (Keller et al. 1985; Noldus 1989). This appears to be associated with the speed at which the parasitoid can walk

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on the plant surface (BIGLER et al. 1988). At least three attributes of the plant surface could affect the walking speed and behaviour of *Trichogramma* spp.: the physical structure (e.g. trichomes), exudates of glandular trichomes and surface chemistry (KELLER 1987; KASHYAP et al. 1991). The latter has not been evaluated.

T. chilonis Ishii is native to India where it parasitizes *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) eggs on a wide range of plants. Only rarely (< 1 %) does it parasitize this host on pigeonpea (*Cajanus cajan* (L.) Millsp.), while on sorghum (*Sorghum bicolor* L.) up to 80 % of *H. armigera* eggs are parasitized (BHATNAGAR et al. 1982). The reasons for the low levels of parasitism on pigeonpea are unknown and form the basis of this study. In contrast to pigeonpea pods, leaves apparently do not possess exudate-secreting glandular trichomes and only short trichomes which are apressed to the surface (BISEN and SHELDRAKE 1981). *T. chilonis* walking behaviour is not apparently affected and the very low level of *H. armigera* parasitism may be due to plant surface chemicals. To test this hypothesis, we developed a filter paper test to study the impact of plant surface chemicals on the searching and parasitization behaviour of *T. chilonis* females.

2 Materials and methods

2.1 Parasitoid rearing

A *T. chilonis* strain was reared at ICRISAT at 26 ± 2 °C, 70 % RH under constant 16 : 8 light : dark photoperiod on eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). The female parasitoids used for the experiments were 1–2 d old, mated unexperienced and fed with honey agar.

2.2 Plant extracts

Water-, hexane- and acetone-extracts were made by dipping pigeonpea leaves into these solvents for approximately 15 sec. For all extracts, undamaged leaves from greenhouse grown pigeonpea (ICPL 87) were used. One set of leaves was washed in distilled water and after drying, washed in acetone. A second set (with the same total leaf area) was washed in hexane. The water extract was later freeze-dried to half of the initial volume to concentrate the solutes.

2.3 Bioassay

The bioassay was modified from the filter paper test of LEWIS et al. (1972). A circle (6 cm diameter) was drawn on a Whatman® No. 1 filter paper and divided into four quadrants. The plant extract was pipetted onto two diametric quadrants with an equal amount of pure solvent placed on the other two remaining quadrants as controls. Each of the four spots was 2 cm in diameter. After drying (30 min), three fresh *H. armigera* eggs (< 1 day old) were attached to each quadrant using a wet brush. The distance between the eggs within a quadrant was at least 5 mm, as *Trichogramma* spp. can visually recognize host eggs up to 4 mm (BRUINS et al. 1994). One *T. chilonis* female was released in the middle of a Petri dish (6 cm diameter) before placing it over the circle on the filter paper. This was then inverted, so that light came through the filter paper, attracting the parasitoid to it. Females had a choice between two treatments, therefore attractiveness as well as repellence could be measured. The wasp was removed after 30 min and 5 days later the black, parasitized eggs were counted. Each test was replicated at least 30 times. Differences in the number of eggs parasitized on the two treatments were compared using a X^2 test for a fixed-ratio hypothesis (GOMEZ and GOMEZ 1984).

3 Results and discussion

In the untreated control, all quadrants were equally preferred by the parasitoids (Table 1). None of the three solvents used in this study had an effect on the searching or parasitization behaviour of *T. chilonis* females.

Table 1. Number of *Helicoverpa armigera* eggs parasitized by *Trichogramma chilonis* on filter paper treated with different solvent extracts of pigeonpea leaves, pure solvents or untreated

Tab. 1. Anzahl an von *T. chilonis* parasitierten *H. armigera* Eiern auf Filter-Papieren, behandelt mit verschiedenen Lösungsmittel-Extrakten von Strauchersen-Blättern, reinen Lösungsmitteln oder unbehandelten Kontrollen

Treatment	Total number of eggs parasitized	p[a]	Mean number of eggs/ females/replicate (± SE)	n
Control	55			
vs.				
control	53			
Water	158			
vs.				
control	145			
Acetone	140			
vs.				
control	123			
Hexane	55			
vs.				
control	42			
Water-leaf extract	42			
vs.				
water	32			
Acetone-leaf extract	28			
vs.				
acetone	50			
Hexane-leaf extract	30			
vs.				
hexane	47			

[a] X^2 test for fixed ratio hypothesis

Acetone-extract of pigeonpea leaf surfaces was significantly repellent ($p < 0.05$). Fewer eggs were parasitized compared to the control on patches treated with hexane-extract, but the difference was not significant. The water-extract had no affect on behaviour. Therefore, chemicals on the surface of pigeonpea leaves are at least partly responsible for the unattractiveness of this host plant to *T. chilonis* females. These chemicals are not water soluble. Which specific chemicals are involved and their concentration and distribution on different pigeonpea plant parts is under investigation.

Our results have shown that this filter paper bioassay provides a fast and simple method for investigating the effect of plant surface chemicals on the searching and parasitization behaviour of *Trichogramma* spp.. The bioassay could be improved by using a light table, providing light from underneath and eliminating the need for inverting the Petri dish and filter paper.

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