Physical and chemical plant characters inhibiting the searching behaviour of *Trichogramma chilonis*

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Abstract

Several plant characters are known to affect the searching behaviour and parasitization efficiency of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae). In this study, plant characters contributing to the low *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) egg parasitism levels on pigeonpea (*Cajanus cajan* (L.) Millspaugh) were investigated. The efficiency of *T. chilonis* on pigeonpea was dependent on the plant structure on which the host eggs were found. In a cage experiment, more than 55% of eggs placed on leaves were parasitized, while 1% of eggs on calyxes and no eggs on pods were parasitized. In a filter paper bioassay, parasitoids were deterred by acetone and hexane surface extracts from pigeonpea pods but showed no response to water extract. The searching behaviour of the parasitoids was not affected by different solvent extracts from the surface of pigeonpea leaves. In a four-armed airflow olfactometer, *T. chilonis* was repelled by volatiles from pigeonpea pods but showed no response to volatiles derived from hexane extract of pod surfaces. Volatile infochemicals and hexane surface extracts from pods of two wild *Cajanus* species, *C. scarabaeoides* (L.) Thours and *C. platycarpus* (Bentham) van der Maesen, were similarly deterrent to *T. chilonis*. The movement of the parasitoids on pigeonpea pods and calyxes was inhibited by long trichomes and wasps were trapped by sticky trichome exudates. Parasitoids walked significantly faster on leaves than on pods. The walking speed on both pods and leaves increased significantly after washing with hexane. The results presented in this paper show that the plant growth stage and the plant structures preferred by *H. armigera* for oviposition are the least suitable for *T. chilonis*, contributing to the low parasitoid efficiency on pigeonpea.

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is one of the major grain legumes of the tropics and subtropics with the largest production in India (Nene & Sheila, 1990). A key pest on pigeonpea throughout the Old World is the pod borer *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Lateef & Reed, 1990).

In India, high levels of egg parasitism, mainly caused by *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), have been reported from *H. armigera* eggs collected from some host plants such as sorghum, maize or cotton. But on pigeonpea, par- asitism levels remain low with less than 1% of eggs parasitized (Romeis & Shanower, 1996). This difference in the level of parasitism on different plants may be partly caused by plant volatiles. In an airflow olfactometer, female *T. chilonis* were repelled by volatiles emitted from pigeonpea plants in the reproductive growth stage while they were arrested by sorghum volatiles (Romeis et al., 1997a). This suggests that pigeonpea fields are less preferred by *Trichogramma* spp. searching for hosts.

When the parasitoids land on a plant, trichomes and their exudates may reduce the wasps' walking speed or even trap them, thus, inhibiting their ability

to locate a host (Rabb & Bradley, 1968; Treacy et al., 1985, 1986; Keller, 1987; Kauffman & Kennedy, 1989; Kashyap et al., 1991). Five trichome types have been identified from pigeonpea (Shanower et al., 1996). Type A is a glandular trichome which secretes a sticky exudate at the tip, while the Type B glandular trichome is a globular sac which releases its contents only after the cell wall is ruptured. Two unsegmented non-glandular trichomes (Types C and D), and a very small glandular trichome (Type E) with no visible secretion, have also been described. There are large differences in the distribution, density, length and orientation of trichomes between leaves and reproductive structures (pods, buds, and flower-calyxes) (Romeis et al., 1996a; J. Romeis, T. G. Shanower & A. J. Peter, unpubl.). On reproductive structures, trichomes are longer than on leaves, and the exudate-secreting Type A trichomes are much more common. Reproductive structures are preferred oviposition sites for *H. armigera* with less than 20% of eggs found on vegetative plant structures (Romeis, 1997).

All five trichome types have also been found on *C. platycarpus* (Bentham) van der Maesen and *C. scarabaeoides* (L.) Thours, wild relatives of pigeonpea (Shanower et al., 1996). Significant differences in the distribution, density and length of the trichome types have been detected between the three *Cajanus* species; the most important finding is the general absence of Type A trichomes on pods and calyxes of *C. scarabaeoides* (Shanower et al., 1996; J. Romeis, T. G. Shanower & A. J. Peter, unpubl.). These wild *Cajanus* species have been recognized as a potential source of resistance to *H. armigera* or diseases (Pundir & Singh, 1987; Saxena et al., 1990; Shanower et al., 1997).

The following study was conducted to identify the plant characters which inhibit the searching behaviour of *Trichogramma* egg parasitoids on pigeonpea and to determine if these characters are also present in wild *Cajanus* species.

Materials and methods

Insect material. *Trichogramma chilonis* females were used in all experiments. The parasitoids were reared on eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) at 26 ± 2 °C, 50 ± 10% r.h., L16:D8. The female parasitoids used in the experiments were 1–2 d old, mated, inexperienced, and fed with honey agar. *Helicoverpa armigera* was reared on a chickpea-based diet at 22 ± 2 °C, 70 ± 10% r.h.

(Armes et al., 1992). Only fertile eggs, recognized by a horizontal brown ring which develops after approximately 12 h, were used. Eggs were ≤24 h old and killed with UV-irradiation (60W, 50 min, 30 cm distance) or untreated. This short UV-irradiation does not affect the acceptance and suitability of the eggs for *T. chilonis* (Romeis et al., 1997b). UV-irradiated eggs have the advantage that no larvae emerge from unparasitized eggs and destroy the experiment.

Plant material. Plant material was collected from greenhouse-grown, short-duration pigeonpeas (genotypes ICPL 87, ICPL 84052, ICPL 86012), *C. platycarpus* (accession ICPW 68) and *C. scarabaeoides* (accession ICPW 82).

Effect of host egg location on parasitization. This experiment was conducted to compare *T. chilonis* parasitization efficiency among *H. armigera* eggs laid on different pigeonpea plant structures. For each test, four pigeonpea (ICPL 87) plants were artificially infested with *H. armigera* eggs with a moistened brush. A replication consisted of 40 eggs on each of the following plant structures: pods, calyxes (buds and flower-calyxes), upper and lower leaf surfaces (10 eggs on each plant structure on each of the four plants). The four infested plants were kept in one cage (160 cm × 75 cm × 100 cm) in the greenhouse. Between 2000 and 3000 female *T. chilonis* were released per cage. After 24 h, the eggs were collected and kept in the *Trichogramma* rearing incubator until blackening of the parasitized eggs. The percentage of eggs parasitized on each plant structure was recorded. The experiment was replicated 10 times. The percentage of eggs parasitized was compared among the different plant structures using one-way analysis of variance (ANOVA) on the angular transformed percentage data. Means were compared using the least significant difference (LSD) at P=0.05.

Filter paper bioassay. This bioassay was designed to evaluate the impact of plant surface extracts on the searching behaviour of *T. chilonis*. A circle with a diameter of 6 cm was drawn on a Whatman® No. 1 filter paper and divided into four quadrants. Plant extract (50 μl) was pipetted onto two opposite quadrants, forming a patch of approximately 2 cm diameter (3.1 cm^2), and was allowed to dry completely for 30 min. An equal amount of pure solvent was placed on the two remaining quadrants as a control. Three *H. armigera* eggs separated by more than 5 mm were

attached to each patch using a moistened brush. One parasitoid was released in the middle of a petri-dish (6 cm dia.) which was then placed over the circle on the filter paper. This was then inverted so that light came through the filter paper, attracting the wasp to it. The parasitoid was removed after 30 min, and 5 days later, patches containing black, parasitized eggs were counted. The number of patches with parasitized eggs were compared between treatments using a χ^2-test with a null hypothesis of equal distribution. In contrast to the method used by Romeis et al. (1996b), the number of patches rather than the number of eggs parasitized per treatment was compared. *Trichogramma* spp. search in the vicinity of the host after parasitization, and therefore the chances of finding a second egg in the same patch are greater than locating one in another patch (Laing, 1937; Gardner & van Lenteren, 1986). Experiments were carried out in an incubator at $26 \pm 2\,°C$, $50 \pm 10\%$ r.h.

The following surface extracts were tested:

(A) Water, acetone and hexane extracts from pigeonpea (ICPL 87) pods and leaves.

(B) Hexane extract from pigeonpea pods, 1 and 2 days after treatment of the filter paper.

(C) Acetone extract from pods previously washed with hexane, and hexane extract from pods previously washed with water (after a drying period of 30 min).

(D) Hexane extracts from pods of other pigeonpea genotypes (ICPL 84052 and ICPL 86012), *C. platycarpus* and *C. scarabaeoides*.

The parasitoids do not respond to pure solvents (Romeis et al., 1996b). Each test was replicated a minimum of 30 times.

Surface extracts were made by dipping plant parts in the solvents for 15 s. The volume of the hexane and acetone extracts was reduced under vacuum, the water extracts were lyophilized. Extracts were used immediately or stored in a freezer at $-6\,°C$ for a maximum of 24 h. The concentrated extracts were diluted so that the amount of surface chemicals applied to the filter paper on the area of approximately $3.1\,cm^2$ was equal to (= natural concentration) or larger than the amount of chemicals washed from an equivalent plant surface area.

To determine the extent to which volatiles might be responsible for the observed effects of plant surface extracts on the behaviour of *T. chilonis*, the bioassay was slightly modified. Instead of treating the filter paper directly, treated paper discs with a diameter of 2 cm were placed on the filter paper. With this design, the edges of the treated patches were clearly visible to the observer. The experiment was conducted using the hexane extract from the surface of pigeonpea (ICPL 87) pods at the natural concentration. Paper discs treated with pure hexane were offered simultaneously as a control. One parasitoid was placed at the center of the filter paper and observed for 10 min. After 5 min, the filter paper was rotated. The number of contacts and acceptance (= walking onto the treated paper disc by one body length) was noted separately for test and control paper discs. A total of 48 parasitoids were tested for each of three treatments: paper discs dried for 40 min, 2 days and 4 days at room temperature. The filter paper and paper discs were changed after observing three parasitoids. Experiments were conducted at $24 \pm 2\,°C$, $50 \pm 10\%$ r.h.

To test if the plant extract affected the searching parasitoids before contact, the number of females making more contacts with the test discs than with the control discs was compared to the number of females making more contacts with the control discs than with the test discs using a χ^2-test. The acceptance/contact (a/c) ratio was calculated separately for test and control discs. A 2×2 contingency-table for the distribution of acceptance and contacts was constructed and differences in the a/c ratios were compared using a χ^2-test. The null hypothesis of equal distribution was used for both χ^2-tests.

Olfactometer bioassay. A four-armed airflow olfactometer was used in these experiments as described previously (Romeis et al., 1997a). The test material was presented to individual *T. chilonis* in two opposite treatment chambers.

The following quantities of each test material were placed in a single treatment chamber:

(A) Filter paper treated with hexane surface extract from 20 pigeonpea (ICPL 87) pods ($\sim 160\,cm^2$ pod surface).

(B) Filter paper treated with hexane surface extract from 40 pigeonpea (ICPL 87) pods ($\sim 320\,cm^2$ pod surface).

(C) Pods of ICPL 87, frozen, thawed and washed with hexane (20 pods; $\sim 160\,cm^2$ pod surface).

(D) Fresh pods of ICPL 87 after washing with hexane (20 pods; $\sim 160\,cm^2$ pod surface).

(E) Fresh pods of ICPL 86012 (20 pods; $\sim 160\,cm^2$ pod surface).

(F) Fresh pods of *C. platycarpus* (15 pods; $\sim 160\,cm^2$ pod surface).

(G) Fresh pods of *C. scarabaeoides* (50 pods; ~160 cm^2 pod surface).

The extracts used in tests A and B were collected as described for the filter paper bioassay. For tests A and B, filter paper treated with hexane was used as a control. For all other tests, air humidified with wet tissue paper (Kimwipes$^®$) was used as a control, since plant material increases the relative humidity in the treatment chambers and *T. chilonis* is arrested by humidified air (Romeis et al., 1997a).

Parasitoids were observed for 10 min beginning when the parasitoid crossed a line, 5 mm around the entry tube. The total time spent by a parasitoid in each of the four flow fields was recorded using the computer software package 'The Observer$^®$ ' (Noldus Information Technology, 1993).

After every two to three observations the exposure chamber was cleaned with 70% ethanol, and it was rotated after every five observations. The behaviour of at least 40 parasitoids was recorded for each test material. All experiments were carried out at 26 ± 2 °C and 50 ± 10% r.h.

The number of parasitoids which spent more than 50% of the observation time in the flow fields containing plant volatiles was compared to the number spending more than 50% of the observation time in the control fields using a χ^2-test with the null hypothesis of equal distribution.

Walking speed. The walking speed of *T. chilonis* was measured on freshly excised pigeonpea (ICPL 87) leaves (upper and lower surface) and pods. After placing an individual wasp on the plant structure it was covered with a glass petridish where the walking path of the parasitoid was followed with a marker pen. The time was stopped when the parasitoid reached the margin of the plant structure or after 1 min. Five wasps were observed individually on each plant structure. The pods and leaves were then washed with hexane, dried for 30 min, and walking speed was measured again using new parasitoids. A total of 60 females were observed on each, the upper and lower surface of 12 unwashed and hexane-washed leaves, and 50 females on 10 unwashed and hexane-washed pods. Fewer wasps were observed on pods as the wasps showed almost no movement. To evaluate the average walking speed, the path drawn on the petridish was copied on a paper, enlarged 4× and measured with a cartographic odometer. The experiment was carried out at 26 ± 2 °C and 60 ± 10% r.h. Walking speeds

on different plant structures, before and after hexane washing, were compared using Student's *t*-test.

Observations of T. chilonis *on pigeonpea plant structures.* The behaviour of *T. chilonis* was observed on single leaves, calyxes and flowers of pigeonpea (ICPL 87) plants under the microscope and on whole plants. Parasitoids ($n = 65$) were individually placed on pods and observed to see what effect exudates secreted by Type A glandular trichomes had on the wasps. Observations were made at 26 ± 2 °C and 60 ± 10% r.h.

Results

Effect of host egg location on parasitization. The percentage of *H. armigera* eggs parasitized by *T. chilonis* differed significantly among the pigeonpea plant structures on which the eggs were located (F = 91.3, P < 0.001). None of the eggs placed on pods were attacked and only 1% of the eggs on calyxes, while significantly higher parasitism levels were recorded on eggs placed on the upper and lower leaf surface (55.7 and 59.7%, respectively) (P < 0.05, LSD). The difference in the percentage of eggs parasitized on the two leaf surfaces was not significant (P > 0.05, LSD).

During the experiment, more eggs fell from leaves than from the reproductive structures. While, out of the 40 eggs exposed, 39.5 ± 0.19 (± s.e.) eggs were collected from pods and 37.4 ± 0.80 eggs were collected from calyxes, only 35.0 ± 0.96 eggs were found on the upper leaf surface and 33.5 ± 1.44 on the lower leaf surface.

Filter paper bioassay. The acetone and hexane extracts from the surface of pigeonpea (ICPL 87) pods were deterrent to *T. chilonis* (Table 1, test 2, 3). A similar response was observed for hexane extract from pods which had previously been washed with water (test 5). Water extract (test 1) and acetone extract from pods which had been washed in hexane earlier (test 4) did not elicit a response in the parasitoids. The hexane extract from pods (at natural concentration) continued to deter the parasitoids up to two days after application to the filter paper, though the effect became weaker (fresh extract, $n = 44$, $\chi^2 = 20.6$, P < 0.001; 1 day old extract, $n = 51$, $\chi^2 = 13.8$, P < 0.001; 2 day old extract, $n = 61$, $\chi^2 = 9.7$, P < 0.01). The different tests were not compared with each other as they were conducted on different days. In contrast

to pod extracts, leaf extracts did not significantly affect the searching behaviour of the parasitoids even at higher than natural concentrations (Table 2). Hexane pod extracts at natural concentration from two other pigeonpea genotypes (ICPL 86012, $n = 40$, $\chi^2 = 30.8$, P < 0.001; ICPL 84052, $n = 53$, $\chi^2 = 11.8$, P < 0.001) and the two wild *Cajanus* species (*C. scarabaeoides*, $n = 57$, $\chi^2 = 12.8$, P < 0.001; *C. platycarpus*, $n = 64$, $\chi^2 = 6.2$, P < 0.05) were also significantly deterrent to *T. chilonis*.

Filter paper discs freshly treated with hexane extract from pigeonpea (ICPL 87) pods were contacted by the parasitoids significantly less often than control discs (Table 3). Only 38% of the contacts with a test disc lead to an acceptance, while control discs were accepted to 71% after contact. When the paper discs were tested 2 or 4 days after treatment, there was no significant difference between test and control discs in the contact preference but the a/c ratio for test discs was still significantly smaller than for control discs. While the a/c ratio for control discs did not change over time, the ratio for test discs increased with time after treatment.

Olfactometer bioassay. *Trichogramma chilonis* did not respond to volatiles derived from filter papers treated with the hexane surface extracts from either 20 or 40 pigeonpea (ICPL 87) pods (Table 4, test 1, 2). Volatiles emitted by both frozen (test 3) and fresh (test 4) pigeonpea (ICPL 87) pods, although washed with hexane, were repellent to the parasitoids. *Trichogramma chilonis* were also significantly repelled by volatiles emitted from pods of pigeonpea (ICPL 86012) (test 5) and the two wild *Cajanus* species, *C. platycarpus* (test 6) and *C. scarabaeoides* (test 7).

Walking speed. Walking speed was significantly slower on pods than on leaves (Table 5). On unwashed leaves it was significantly slower on the lower leaf surface as compared to the upper leaf surface. On unwashed pods walking speed was almost zero as the parasitoids were trapped by the sticky trichome exudates.

Walking speed increased significantly, by 21% on the upper leaf surface and by 59% on the lower surface, after leaves were washed with hexane (Table 5). On pods walking speed increased more than 14 times after washing, but was still significantly slower than on leaves.

Observations of T. chilonis *on pigeonpea plant structures.* On leaves, parasitoids walked preferably on the margins and on the major veins on the lower leaf surface. On whole plants, parasitoids easily walked from leaf to leaf but were never observed walking onto reproductive structures. When placed on a flower petal, wasps walked around, turning whenever they touched the calyx and then flew away. As on pods, the parasitoids had difficulties in walking on calyxes due to the sticky trichome exudates. Out of the 65 parasitoids placed on pods, 51 (78.5%) were trapped and killed by the exudates.

Discussion

Several pigeonpea plant characters were found to affect different stages of the host location process of *T. chilonis*. Plant volatiles were repellent, and trichomes and trichome exudates inhibited the movements of the wasps on the plant. In addition, searching parasitoids were deterred by surface chemicals.

Trichogramma chilonis was repelled by volatile infochemicals emitted by pods of pigeonpea (ICPL 86012) and the two wild *Cajanus* species. Earlier, it has been shown for pigeonpea (ICPL 87) that the repellent infochemicals are only emitted by plants in the reproductive growth stage and pods alone (Romeis et al., 1997a). It is unlikely that the volatiles derive from the exudates secreted by the Type A trichomes as fresh and frozen pigeonpea pods remained repellent after the exudates had been washed off with hexane. Frozen pods are considered to be physiologically inactive so that no new exudates could have been produced. Also, volatiles from *C. scarabaeoides* pods which do not possess the exudate secreting Type A trichomes (Shanower et al., 1996) were repellent to the parasitoids in the olfactometer.

It has been suggested that surface chemicals can mediate the behaviour of parasitoids and predators of herbivores (Eigenbrode & Espelie, 1995) but to our knowledge no such example has so far been reported. Keller (1987) first discussed the possible impact of surface chemicals on *Trichogramma* spp. behaviour. The surface extracts from pods of all three *Cajanus* species tested contained chemicals which were deterrent to *T. chilonis* in a filter paper bioassay. The chemicals on the surface of pigeonpea pods are soluble in hexane and acetone but not in water, suggesting that they are apolar. No deterrent chemicals were detected in surface extracts from pigeonpea leaves. The deter-

Table 1. Number of patches containing eggs parasitized by *Trichogramma chilonis* on filter paper treated with different surface extracts from pigeonpea (ICPL 87) pods or pure solvent

Test	Treatment	n	Concentration[a]	Patches with ≥ 1 parasitized eggs	χ^2	P[b]
1	Water-extract			25		
		30	1.5		0.6	n.s.
	Water			20		
2	Acetone-extract			7		
		36	1.0		16.0	P < 0.001
	Acetone			32		
3	Hexane-extract			7		
		30	1.0		19.6	P < 0.001
	Hexane			36		
4	Acetone-extract (after washing with hexane)			42		
		44	1.0		0.0	n.s.
	Acetone			42		
5	Hexane-extract (after washing with water)			6		
		44	1.0		32.7	P < 0.001
	Hexane			48		

[a] Natural concentration = 1.0 (see text)
[b] n.s. – not significant (P > 0.05).

Table 2. Number of patches containing eggs parasitized by *Trichogramma chilonis* on filter paper treated with different surface extracts from pigeonpea (ICPL 87) leaves or pure solvent

Test	Treatment	n	Concentration[a]	Patches with ≥ 1 parasitized eggs	χ^2	P[b]
1	Water-extract			21		
		30	1.5		0.2	n.s.
	Water			18		
2	Acetone-extract			42		
		65	1.0		1.1	n.s.
	Acetone			52		
3	Hexane-extract			27		
		47	1.0		3.3	n.s.
	Hexane			42		
4	Hexane-extract			31		
		48	1.5		3.3	n.s.
	Hexane			47		
5	Hexane-extract			33		
		60	3.0		3.5	n.s
	Hexane			50		

[a] Natural concentration = 1.0 (see text)
[b] n.s. – not significant (P > 0.05).

Table 3. Number of contacts and acceptance of female *Trichogramma chilonis* with filter paper discs treated with hexane surface extract from pigeonpea (ICPL 87) pods or pure hexane ($n = 48$)

Test	Treatment	Contact preference[a]	Number of contacts (c)	Discs accepted (a)	a/c ratio	χ^2	P
1	Fresh extract	9	325	124	0.38		
						86.2	P < 0.001
	Hexane	35*	464	331	0.71		
2	2 d old extract	22	473	220	0.47		
						95.2	P < 0.001
	Hexane	19[n.s.]	456	354	0.78		
3	4 d old extract	22	519	302	0.58		
						29.2	P < 0.001
	Hexane	20[n.s.]	517	383	0.74		

[a] Number of parasitoids making more contacts with either the test discs or control discs. Numbers are compared using a χ^2-test (* P < 0.001; n.s. P > 0.05).

Table 4. Response of female *Trichogramma chilonis* to volatiles deriving from hexane surface extracts from pigeonpea (ICPL 87) pods, pods after washing with hexane, and pods from pigeonpea (ICPL 86012) and two wild *Cajanus* species in a four-armed olfactometer

Test	Test material	n	Longest time in flow fields (no.)[a]	χ^2	P[b]
1	Hexane pod extract (20 pods)		25		
		46		0.3	n.s.
	Hexane		21		
2	Hexane pod extract (40 pods)		21		
		40		0.1	n.s.
	Hexane		19		
3	Pods (frozen) washed		11		
		44		11.0	P < 0.001
	Humidified air		33		
4	Pods (fresh) washed		12		
		40		6.4	P < 0.05
	Humidified air		28		
5	Pigeonpea (ICPL 86012) pods		16		
		50		6.5	P < 0.05
	Humidified air		34		
6	*C. platycarpus* pods		17		
		50		5.1	P < 0.05
	Humidified air		33		
7	*C. scarabaeoides* pods		12		
		50		13.5	P < 0.001
	Humidified air		38		

[a] Number of parasitoids which spent more than 50% of the observation time in the two test fields or in the two control fields. Numbers were analyzed using a χ^2-test.
[b] n.s. – not significant (P > 0.05).

Table 5. Walking speed (mm/s) of female *Trichogramma chilonis* on pigeonpea (ICPL 87) leaves and pods, washed with hexane and unwashed ($\bar{x} \pm$ s.e.)

| | Leaf (60 females) | | Pod (50 females) |
	Upper surface	Lower surface	
Unwashed	2.4 ± 0.14 a	1.7 ± 0.12 b	0.04 ± 0.01 c
Hexane washed	2.9 ± 0.15 a*	2.7 ± 0.11 a*	0.58 ± 0.04 b*

Means followed by different letters within the same row are significantly different ($P < 0.05$; Student's *t*-test). Means followed by * within the same column are significantly different ($P < 0.05$; Student's *t*-test).

rence of pigeonpea pod surface extracts to *T. chilonis* was partly due to volatiles, as filter paper discs treated with hexane pod extract were less often contacted than discs treated with pure hexane. However, in the olfactometer, the parasitoids did not respond to volatiles derived from the hexane extract. Therefore, it is possible that the pigeonpea pod volatiles which repel *T. chilonis* in the olfactometer are different from the ones deterring the parasitoids in the filter paper bioassay. The latter may be less volatile than the ones emitted by the pods. The contact chemicals from the pigeonpea pod surface seemed to undergo a degradation process as the acceptance of treated filter paper discs increased over time.

The surface chemicals which affect the parasitoids' behaviour are presently unknown. Plant surfaces contain epicuticular waxes, chemicals produced internally and leached into the leaf wax, and compounds actively exuded to the plant surface including trichome exudates (Eigenbrode & Espelie, 1995). The chemicals responsible for *T. chilonis* deterrence are unlikely to be present in the exudates secreted by the Type A trichomes, as the surface extract from *C. scarabaeoides* pods, generally lacking this trichome type, was also deterrent. The contents of the Type B glandular trichomes are also unlikely to be responsible for the observed effect as these trichomes were not visibly damaged by the solvents. Type B trichomes are also common on leaves and leaf surface extracts were not found to deter *T. chilonis*. Surface chemicals from pods of all three *Cajanus* species also affect *H. armigera* larvae. Shanower et al. (1997) reported that acetone extracts from pigeonpea and *C. platycarpus*, but not *C. scarabaeoides*, had a feeding stimulant effect on *H. armigera* larvae while water extracts from *C. scarabaeoides* contained an antifeedant. A better knowledge of the surface chemistry will help to understand interactions between *Cajanus* spp., *H. armigera* and *T. chilonis*.

Trichomes and trichome exudates are known to inhibit the movement of small bodied insects (Obrycki, 1986). The different walking speeds of *T. chilonis* recorded on the upper and lower leaf surface and pods of pigeonpea are due to differences in the types, orientation and density of trichomes on these structures. Leaves mainly possess short and erect non-glandular trichomes which are more densely spaced on the lower than on the upper surface (see electron micrographs in Romeis et al., 1996a). The parasitoids prefer to walk on the leaf margins and on the major veins of the lower leaf surface. In contrast to the interveinal areas, veins and margins are covered with long non-glandular trichomes which are appressed to the surface (Romeis et al., 1996a), making walking easier on these structures. A similar preference by *Trichogramma* spp. has been observed on Brussels sprouts (Noldus et al., 1991) and maize (Gass, 1988; Suverkropp, 1997), and has also been reported for other arthropods (Evans, 1976; Ayal, 1987).

Walking on pods is almost completely inhibited by the glandular and non-glandular trichomes which are much longer than similar types found on leaves (Romeis et al., 1996a, unpubl.). *Trichogramma chilonis* walking on the surface of pigeonpea pods and calyxes are trapped by sticky exudates. The parasitoids are smaller than the Type A trichomes on pods which reach an average length of 557 μm (J. Romeis, T. G. Shanower & A. J. Peter, unpubl.). Therefore, the exudates easily stick to the wings and the wasps cannot clean themselves. The detrimental effect of trichome exudates on trichogrammatids has been reported from other plants such as tobacco (Rabb & Bradley, 1968) and tomato (Kashyap et al., 1991). But the trichome exudates may benefit *H. armigera* as eggs were found to adhere better on the reproductive structures than on leaves. After pods and leaves were washed with hexane, *T. chilonis* walking speed increased significantly. On pods, this was mainly due to the removal of the sticky exudates from Type A trichomes. But

the increase in walking speed may also be due to the removal of deterrent chemicals in the hexane wash. It is unclear why the walking speed increased on leaves after washing with hexane. No deterrent effect was observed to hexane extracts from the leaf surface, and Type A trichomes have only been found in very low densities on the upper leaf surface (Romeis et al., 1996a). The orientation of the trichomes was not visibly altered, but washing with hexane may have caused changes in the surface structure of the cuticula. Though walking speed on pods increased after washing, it was still significantly slower than walking speed on any of the leaf surfaces, indicating that the trichomes act as a mechanical barrier for the searching parasitoids.

The results presented in this paper show that several physical and chemical factors hinder *T. chilonis* from finding and parasitizing host eggs on the reproductive structures of pigeonpea, while eggs are readily parasitized when located on leaves. These results help to explain the low egg parasitism levels reported from pigeonpea, as in the field *H. armigera* preferably oviposits on the reproductive structures (King, 1994; Romeis, 1997). Trichomes and sticky trichome exudates may also be responsible for the overall low number of efficient natural enemies of *H. armigera* on pigeonpea (Romeis & Shanower, 1996). The wild *Cajanus* species used in this study possess the same characters negatively affecting *T. chilonis* except for *C. scarabaeoides* which generally lack Type A trichomes on pods and calyxes. Therefore, they may be of limited use in breeding for a parasitoid-friendly pigeonpea genotype.

Acknowledgements

References

Armes, N. J., G. S. Bond & R. J. Cooter, 1992. The laboratory culture and development of *Helicoverpa armigera*. Natural Resources Institute Bulletin 57. Chatham, UK.

Ayal, Y., 1987. The foraging strategy of *Diaeretiella rapae*. Journal of Animal Ecology 56: 1057–1068.

Eigenbrode, S. D. & K. E. Espelie, 1995. Effects of plant epicuticular lipids on insect herbivores. Annual Review of Entomology 40: 171–194.

Evans, H. F., 1976. The searching behaviour of *Anthocoris confusus* (Reuter) in relation to prey density and plant surface topography. Ecological Entomology 1: 163–169.

Gardner, S. M. & J. C. van Lenteren, 1986. Characterisation of the arrestment response of *Trichogramma evanescens*. Oecologia 68: 265–270.

Gass, T., 1988. Einfluss der Temperatur auf die Bewegungsaktivität und die Parasitierungsleistung von *Trichogramma maidis* Pint. & Voeg. Diplomarbeit, Institut für Pflanzenwissenschaften, Eidgenössische Technische Hochschule Zürich, Switzerland.

Kashyap, R. K., G. G. Kennedy & R. R. Farrar, Jr., 1991. Behavioral response of *Trichogramma pretiosum* Riley and *Telenomus sphingis* (Ashmead) to trichome/methyl ketone mediated resistance in tomato. Journal of Chemical Ecology 17: 543–556.

Kauffman, W. C. & G. G. Kennedy, 1989. Relationship between trichome density in tomato and parasitism of *Heliothis* spp. (Lepidoptera: Noctuidae) eggs by *Trichogramma* spp. (Hymenoptera: Trichogrammatidae). Environmental Entomology 18: 698–704.

Keller, M. A., 1987. Influence of leaf surfaces on movements by the hymenopterous parasitoid *Trichogramma exiguum*. Entomologia Experimentalis et Applicata 43: 55–59.

King, A. B. S., 1994. *Heliothis/Helicoverpa* (Lepidoptera: Noctuidae). In: G. A. Matthews & J. P. Tunstall (eds), Insect Pests of Cotton. CAB International, Wallingford, pp. 39–106.

Laing, J., 1937. Host-finding by insect parasites. 1. Observations on the finding of hosts by *Alysia manducator*, *Mormoniella vitripennis* and *Trichogramma evanescens*. Journal of Animal Ecology 6: 298–317.

Lateef S. S. & W. Reed, 1990. Insect pests of pigeonpea. In: S. R. Singh (ed), Insect Pests of Tropical Food Legumes. John Wiley & Sons, New York, pp. 193–242.

Nene Y. L. & V. Sheila, 1990. Geography and importance. In: Y. L. Nene, S. D. Hall & V. K. Sheila (eds), The Pigeonpea. CAB International, Wallingford, pp. 1–14.

Noldus Information Technology, 1993. The Observer, Base Package for DOS. Reference Manual, Version 3.0. Wageningen, The Netherlands.

Noldus, L. P. J. J., R. P. J. Potting & H. E. Barendregt, 1991. Moth sex pheromone adsorption to leaf surface: bridge in time for chemical spies. Physiological Entomology 16: 329–344.

Obrycki, J. J., 1986. The influence of foliar pubescence on entomophagous species. In: D. Boethel & R. D. Eikenbary (eds), Interactions of Plant Resistance and Parasitoids and Predators of Insects. John Wiley and Sons, New York, pp. 61–83.

Pundir, R. P. S. & R. B. Singh, 1987. Possibility of genetic improvement of pigeonpea (*Cajanus cajan* (L.) Millsp.) utilizing wild gene sources. Euphytica 36: 33–37.

Rabb, R. L. & J. R. Bradley, 1968. The influence of host plants on parasitism of the eggs of the tobacco budworm. Journal of Economic Entomology 61: 1249–1252.

Romeis, J., 1997. Impact of plant characters and cropping systems on the searching behavior and parasitization efficiency of *Trichogramma* spp. egg parasitoids of *Helicoverpa armigera*. Ph.D. Thesis, University of Hohenheim, Germany.

Romeis, J. & T. G. Shanower, 1996. Arthropod natural enemies of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in India. Biocontrol Science and Technology 6: 481–508.

Romeis, J., T. G. Shanower & A. J. Peter, 1996a. Type and distribution of trichomes on pigeonpea leaves. International Chickpea and Pigeonpea Newsletter 3: 101–102.

Romeis, J., T. G. Shanower & C. P. W. Zebitz, 1996b. A simple and effective bioassay to study the effect of plant surface chemicals on the behaviour of *Trichogramma* spp. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 103: 213–216.

Romeis, J., T. G. Shanower & C. P. W. Zebitz, 1997a. Volatile plant infochemicals mediate plant preference of *Trichogramma chilonis*. Journal of Chemical Ecology 23: 2455–2465.

Romeis, J., T. G. Shanower & C. P. W. Zebitz, 1997b. Acceptance and suitability of ultraviolet-irradiated *Helicoverpa armigera* (Hübner) (Lep., Noctuidae) eggs for *Trichogramma chilonis* Ishii (Hym., Trichogrammatidae). Journal of Applied Entomology 121: 441–446.

Saxena, K. B., L. Singh, M. V. Reddy, U. Singh, S. S. Lateef, S. B. Sharma & P. Remanandan, 1990. Intra species variation in *Atylosia scarabaeoides* (L.) Benth., a wild relative of pigeonpea (*Cajanus cajan* (L.) Millsp.). Euphytica 49: 185–191.

Shanower, T. G., J. Romeis & A. J. Peter, 1996. Pigeonpea plant trichomes: Multiple trophic level interactions. In: T. N. Ananthakrishnan (ed), Biotechnological Perspectives in Chemical Ecology of Insects. Oxford & IBH, New Delhi, pp. 76–88.

Shanower, T. G., M. Yoshida & A. J. Peter, 1997. Survival, fecundity and behavior of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on pigeonpea and two wild *Cajanus* species. Journal of Economic Entomology 90: 837–841.

Suverkropp, B. P., 1997. Host-finding behaviour of *Trichogramma brassicae* in maize. Ph. D. Thesis Dissertation, Wageningen Agricultural University, The Netherlands.

Treacy, M. F., J. H. Benedict, J. C. Segers, R. K. Morrison & J. D. Lopez, 1986. Role of cotton trichome density in bollworm (Lepidoptera: Noctuidae) egg parasitism. Environmental Entomology 15: 365–368.

Treacy, M. F., G. R. Zummo & J. H. Benedict, 1985. Interactions of host-plant resistance in cotton with predators and parasites. Agriculture, Ecosystems and Environment 13: 151–157.