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KAIROMONES FOR THE EGG PARASITE *Trichogramma evanescens* WESTWOOD[1] II. Effect of Contact Chemicals Produced by Two of Its Hosts, *Pieris brassicae* L.[2] and *Pieris rapae* L.[2]

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Abstract—In observation-cage experiments some new contact kairomones for the egg parasite *Trichogramma evanescens* Westwood are demonstrated. *T. evanescens* females search significantly longer on cabbage leaves treated with the wing scales of two hosts, *Pieris brassicae* L. and *P. rapae* L. Further, egg washes of *P. brassicae* containing an oviposition deterrent pheromone for the butterflies, were found to have a contact-kairomonal effect on the parasite. *T. evanescens* females search significantly longer on cabbage leaves sprayed with a methanol or water wash of *P. brassicae* eggs than on leaves treated with the solvent only.

Key Words—*Trichogramma evanescens,* Hymenoptera, Trichogrammatidae, *Pieris brassicae*, *Pieris rapae*, Lepidoptera, Pieridae, kairomone, oviposition deterring pheromone, accessory gland, egg parasite, tricosane.

INTRODUCTION

In a previous paper we discussed the significance of volatile kairomones for the egg parasite *Trichogramma evanescens* Westwood in host-habitat location (Noldus and van Lenteren, 1985). Now we present the results of experiments concerning the involvement of contact kairomones in host location by this parasite.

The first demonstration of contact-kairomonal effects for *Trichogramma* spp. occurred some 50 years ago. At that time, Laing (1937) found that female *T.*

[1] Hymenoptera: Trichogrammatidae.
[2] Lepidoptera: Pieridae.

evanescens reacted to an "odor trail" left at oviposition sites by adult *Sitotroga cerealella* (Olivier) moths. Contact with the host trail led to strong klinokinetic and klinotactic responses, the parasite often returning to the same place. Later, Lewis et al. (1971) showed a comparable effect with *Heliothis zea* (Boddie). They found that the wing scales were the source of the kairomone (Lewis et al., 1972) and that the main component was tricosane (Jones et al., 1973). *T. evanescens*, *T. pretiosum* Riley, and *T. achaeae* Nagaraja and Nagarkatti also responded to the kairomone with an intensified searching behavior (Lewis et al., 1975). Application of this substance in the field can lead to a considerable increase in the rate of parasitism of *H. zea* eggs, provided that a proper treatment pattern is used (Lewis et al., 1979; Gross, 1981). Recently, the same effect has been shown with the wing scales of *Mamestra brassicae* L., one of the main lepidopteran pests in cabbage fields in the Netherlands (Smits, 1982). Two other hosts of *T. evanescens* in cabbage are *Pieris brassicae* L. and *P. rapae* L. The effect of the wing scales of these species on the searching behavior of the parasite has not been investigated before and was the subject of the present study.

Besides wing scales, another possible source of kairomone has been tested. Rothschild and Schoonhoven (1977) have found that *P. brassicae* females deposit an oviposition-deterring pheromone on and around the eggs during oviposition. This pheromone probably originates from the accessory gland (Behan and Schoonhoven, 1978). It can be collected by washing eggs with methanol or water. *P. brassicae* lays significantly fewer eggs on leaves that have been treated with such an extract than on untreated leaves. The material acts mainly at contact and in a limited degree at a short distance (Klijnstra, 1982). We wondered whether this cue, informing the host about the presence of eggs of conspecifics, might also be used by the parasite; however, in the latter case, resulting not in emigration but in arrestment. Therefore, we examined the influence of a methanol and a water wash of *P. brassicae* eggs on the searching behavior of the parasite.

MATERIALS

The *T. evanescens* strain used for the experiments was the same as mentioned in the previous paper (Noldus and van Lenteren, 1985). It was collected in the Netherlands in 1981 from eggs of *M. brassicae* in cabbage, and has been reared in the laboratory since then on eggs of *Ephestia kuehniella* Zeller. Adult wasps were maintained on honey in glass tubes at 25°C. Only experienced female wasps with an age varying from 2 to 4 days were used (see Noldus and van Lenteren, 1985).

Pupae of *P. brassicae* and *P. rapae* were obtained from a laboratory rearing of the Department of Animal Physiology, Agricultural University, Wageningen. The adults were kept in Plexiglas cages at 20°C.

The cabbage plants, necessary for the experiments and for collecting eggs, originated from R. Zwaan B.V., De Lier, and from Duphar B.V., 's Graveland.

The egg washes of *P. brassicae* were obtained from J.W. Klijnstra, Department of Animal Physiology, Agricultural University, Wageningen. The methanol solution had a concentration of 500 EE/ml (EE = egg equivalent, see Klijnstra, 1982). The concentration of the water solution was 250 EE/ml.

METHODS

In order to determine whether a substance had a contact-kairomonal effect on *T. evanescens*, the searching time spent on a cabbage leaf treated with the substance to be tested was compared to the searching time spent on an untreated leaf. A Plexiglas observation cage with a green rear side and an open front side was used (Figure 1). In the cage two glass tubes with cabbage leaves (about 25 cm^2) were placed so that the leaves were hanging above a third tube from which wasps could be released. Stimulated by the light of a fluorescent tube, hanging above the set-up, the wasps flew up and could land on a leaf. From that moment onwards the wasps were observed until they left the leaf by flying up or walking away along the petiole, and the time spent searching was recorded. The observations were eased by a mirror placed behind the three tubes. To compensate for a possible effect of asymmetrical influences from outside the set-up, the position of the leaves was alternated at the midpoint of a series of observations.

The experiments were carried out in a climate room at a temperature of 20°C. Possible differences in average searching time were tested for significance by means of a Mann-Whitney U test (α = 0.05).

For the treatment of a cabbage leaf with host scales, a clean paintbrush

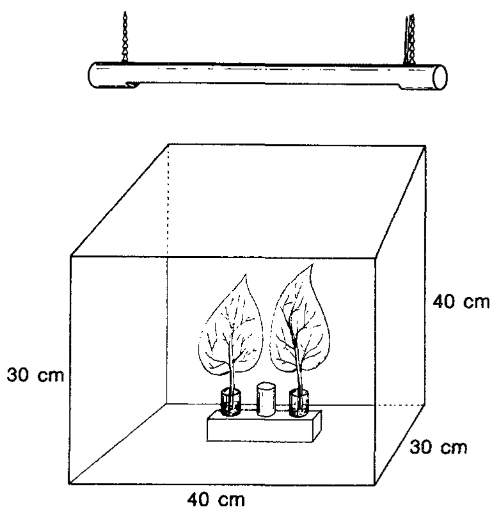

FIG. 1. Plexiglas observation cage. Wasps are released from the vial placed between the two leaves.

was gently moved over the fore and hind wings of an adult female butterfly. The scales collected in this way were spread out over the upper and lower surface of a clean cabbage leaf. This was repeated until both sides were uniformly covered with scales. The control leaf was not treated.

In the case of the egg wash of *P. brassicae*, a cabbage leaf was covered with a very thin layer of the extract by means of a Desaga® chromatographic sprayer. This treatment resulted in a large number of tiny droplets on the waxy leaf surface. One millileter of the methanol solution was used; 2 ml of the water solution was necessary for an equal treatment. The control leaves received a similar amount of the solvent only. The experiments were started as soon as the solvent had evaporated.

RESULTS

Host Wing Scales. The results of the experiment on the kairomonal effect of wing scales of the host are shown in Table 1. To obtain an impression of the responsiveness to kairomones of the *T. evanescens* strain used, first a short series of observations with the scales of *Mamestra brassicae* was made ($N = 5$). The kairomonal effect of *M. brassicae* scales on *T. evanescens* has already been demonstrated by Smits (1982), who worked with a strain collected in Moldavia (U.S.S.R.).

For all three species, we note significantly longer searching times on treated leaves. So the wing scales of *M. brassicae*, *P. brassicae*, and *P. rapae* contain a contact kairomone leading to arrestment of the parasite.

During the first part of each experiment (about 40 landings), the number of landings turned out to be equally distributed over both leaves, which indicates that no long-range attraction occurs. Therefore the observations for each experiment were finished as soon as 25 landings had occurred on each leaf.

Egg wash of P. brassicae. Table 1 also lists the results of the bioassays with the egg wash of *P. brassicae*. The methanol as well as the water solution, led to a significant arrestment of the parasite compared with the control leaves. So the egg wash of *P. brassicae*, which contains an oviposition-deterring pheromone for the butterflies, also contains a contact kairomone for *T. evanescens*.

In this case also no long-range attraction occurred, and the experiments were finished after 25 landings on each leaf.

DISCUSSION

This study has revealed the existence of some new kairomone sources for *Trichogramma evanescens*.

First, the fact that wing scales of the host contain a contact kairomone for

TABLE 1. AVERAGE SEARCHING TIME OF FEMALE *Trichogramma evanescens* ON CABBAGE LEAVES TREATED WITH SUBSTANCES ORIGINATING FROM THE HOST AND ON CONTROL LEAVES

				Test leaves[a]		Control leaves		
Material tested	Host	Solvent	N	$\bar{t}$	r	$\bar{t}$	r	P
Wing scales	*Mamestra brassicae*		5	1327	634–2143	265	24–920	0.008
	Pieris brassicae		25	543	29–1780	264	10–899	0.0128
	Pieris rapae		25	712	40–3197	221	30–448	0.00046
Egg wash containing an oviposition-deterring pheromone	*Pieris brassicae*	Methanol	25	177	38–480	95	18–574	0.0020
		Water	25	130	60–271	83	14–171	0.0082

[a]($\bar{t}$ = average searching time (sec), r = range).
[b]In wing scale experiment control leaves were untreated; in egg wash experiment control leaves were treated with the solvent only.

the parasite, as shown for *Heliothis zea* (Lewis et al., 1972) and *Mamestra brassicae* (Smits, 1982), turns out to apply to *Pieris brassicae* and *P. rapae* too.

The possibility exists that also in this case tricosane, the most active component of the scales of *H. zea* (Jones et al., 1973), is responsible for the kairomonal effect. One might, however, wonder what the function of this substance can be in the field. Saturated long-chain hydrocarbons like tricosane are very common in surface lipids of insects (Nelson, 1978). Arrestment of a parasite after contact with the trail of any other insect is not likely to increase the searching efficiency. It has therefore been suggested that more host-specific components are present in the scales (Lewis, personal communication); kairomonal activity of components other than the four alkanes mentioned by Jones et al. (1973) has, however, not yet been reported.

Smits (1982), who demonstrated the contact-kairomonal effect of the scales of *M. brassicae* for *T. evanescens*, observed a significantly higher number of landings on treated leaves. However, we found an equal number of landings on both leaves. This difference may have been caused by the way the leaves were treated. In our study leaves were treated by moving a scale-covered brush over them, to make sure that only scales were put on the leaf. Smits exposed the plants to living butterflies and let them walk over the leaves, so that other substances may have been present on the surface.

The material collected by washing *P. brassicae* eggs, and which can act as an oviposition deterrent for the butterflies, can also act as a contact kairomone for the parasite. The only other case in which such a double effect is known is *Rhagoletis pomonella* (Walsh) and its larval parasite *Opius lectus* Gahan (Prokopy and Webster, 1978). The pheromonal and the kairomonal effect might be caused by the same compound. However, this can only be verified after the chemical analysis of the *P. brassicae* egg wash has been completed. Another example of the kairomonal effect of host accessory-gland material for an egg parasite has been reported by Strand and Vinson (1982, 1983). They demonstrated that two large proteins, originating from the accessory gland of *Heliothis virescens* F. and present on the chorion of the eggs, serve as an egg-recognition kairomone for *Telenomus heliothidis* Ashmead. They suggest that the proteins might function as an adhesive for the eggs; they do not mention the possibility of a pheromonal use as an oviposition deterrent. Oviposition-deterring pheromones have been demonstrated for two other hosts of *T. evanescens: Trichoplusia ni* (Hübner) (Renwick and Radke, 1980) and *Ostrinia nubilalis* (Hübner) (Dittrick et al., 1983). These, however, originate from larval frass and kairomonal effects are not known.

The adaptive value for an egg parasite of searching longer or more intensively in areas where host eggs have been deposited seems clear. Hence, the accessory-gland material offers interesting possibilities for application in pest control programs. It may have a double effect by deterring the oviposition of *P. brassicae* and enhancing the parasitization activity of *T. evanescens*. However, before large-scale experiments can be carried out, careful attention will have to be paid to ecological effects of application of such substances. A wrong treatment pattern might result in the same complications as have arisen with the application of moth scale extracts (Lewis et al., 1979). Studies will be necessary to find out which spraying dosage will yield a maximal joint phenomonal-kairomonal effect of the egg wash.

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