Effecten van het eilegremmend feromoon op het eileggedrag van het grote koolwitje, *Pieris brassicae*

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Oviposition behaviour as influenced by the oviposition deterring pheromone in the large white butterfly, *Pieris brassicae*

Proefschrift

ter verkrijging van de graad van
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CONTENTS

STELLINGEN

1. De opzet van de experimenten waaruit Jermy en Szentesi concluderen dat zintuiglijke informatie afkomstig van zintuigharen op de ovipositor van *Pieris brassicae* geen rol speelt in de keuze van ovipositiesubstraat, rechtvaardigt die conclusie niet.

   Jermy & Szentesi (1976), *Ent. exp. & appl. 24*: 258-271.

2. Het eilegremmend feromoon van het grote koolwitje kan ook als eilegremmend synomoon en als zoekgedrag stimulerend kairomoon betiteld worden.

   Dit proefschrift.

3. De wijze waarop Hebert veldgegevens over de larvale verspreiding van vlindersoorten hanteert als maat voor de verspreiding van eieren door adulte dieren is onjuist.

   Hebert, P.D.N., (1983), *Can. Ent. 115*: 1477-1481.

4. Studies naar het eetgedrag van fytofage rupsen zouden vaker aan 1e stadium larven verricht moeten worden.

5. Toepassing van gedragsmodificerende stoffen in de bestrijding van plaaginsekten zou de effektiviteit van bestaande bestrijdingsmethoden aanzienlijk kunnen verhogen.

6. Volgens Wemelsfelder zou gedragsonderzoek naar het welzijn van landbouwhuisdieren gebaseerd moeten zijn op een gevoelsmatige verbondenheid van de waarnemer met het dier. Deze benadering houdt het gevaar in dat het welzijn van dieren wordt overgeleverd aan de willekeur van individuele onderzoekers.

F. Wemelsfelder, *Landbouw & Onderzoek*, sept. 1984: 10-15.

7. Uitbreiding van het landbouwareaal wordt genoemd als één van de argumenten vóór inpoldering van de Markerwaard. De daaruit voortvloeiende produktieverhoging kan leiden tot een verhoging van de landbouwuitgaven van de Europese Gemeenschap waarmee in de kosten-baten analyse van inpoldering rekening gehouden zal moeten worden.

8. Officiële werkloosheidscijfers zeggen voortaan nog minder over de resultaten van het regeringsbeleid.

J.W. Klijnstra
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Wageningen, 13 maart 1985

Cover: Ad van Trier

GENERAL INTRODUCTION

In nature, many insect species are usually confronted with mixed vegetations containing (many) different plant species. In such situations, especially mono- and oligophagous insects (herbivorous insects that restrict their diet to only one or a few number of plant species) can only survive if they are able to differentiate between plant species and find their hostplants. In many insect species, newly emerged first instar larvae are small and usually incapable of moving large distances in search of food. This implicates that the food choice of young larvae is largely predetermined by the egglaying female at the time she selects a place to lay her eggs, as already pointed out by Dethier (1941).

Recognition of hostplants is mediated by the activity of the various sensory systems insects possess. Their sensory apparatus enables adult (and also larval) stages to perceive a variety of plant characteristics, more or less specific to certain plant species. Since the work of Verschaffelt (1911), who reported that *Pieris* caterpillars only feed on plants containing certain specific chemicals (the so-called mustard oil glucosides), much emphasis has been laid on such secondary plant substances, i.e. chemicals not participating in primary plant metabolism, as main determinants of hostplant selection behaviour (Fraenkel, 1959). At present, it is known that in several insect species also plant nutrients as chemical stimuli (Thorsteinson, 1960) as well as visual and tactile cues of plant origin may be involved in hostplant recognition (see reviews by Prokopy & Owens, 1983; Chew & Robbins, in press).

The process of hostplant selection behaviour of adult insects actually consists of a number of distinct behavioural steps, each of which may be influenced by various external and internal stimuli (Dethier, 1982). For example female butterflies, when ready to oviposit (internal signal), may be successively engaged in finding a habitat in which hostplants may occur, searching for hostplants within that habitat and finally examining hostplants for oviposition suitability (Feeny *et al.*, 1984). During the first two phases of oviposition behaviour, females orientate to visual and olfactory stimuli. During the third phase, females usually make contact with the plant and use additional gustatory and tactile cues to locate a suitable oviposition site. This chain of behavioural events usually leads to deposition of eggs on plants suitable for larval feeding, although oviposition "mistakes" sometimes may

occur (see Chew & Robbins, in press). If females fail to find a suitable plant, the result will be a reduction in survivorship of their larval offspring. Conversely, this means that a reduction of larval infestation levels of pest insects in crops perhaps can be achieved by interference with female oviposition behaviour. This idea forms the background of the present study.

The large white butterfly, *Pieris brassicae*, is quite common in many European (including the Netherlands) and Asian countries (Feltwell, 1982). The caterpillars of this species mainly feed on Cruciferous plants, however, with some preference for cultivated varieties of cabbage (Terofal, 1965). Feeding larvae may cause considerable economic damage which in some years amounts to millions of dollars world-wide (Feltwell, 1982). The larvae may recognize their hostplants by the presence of mustard oil glucosides (Verschaffelt, 1911; Terofal, 1965) which they can perceive by chemoreceptors on their mouthparts (Schoonhoven, 1967; Ma, 1972). Recognition of hostplants by egglaying females of *P. brassicae* is also mediated by these secondary plant substances (David & Gardiner, 1962). Chemoreceptors present on the legs of adult females are known to be specifically sensitive to these chemicals (Ma & Schoonhoven, 1973).

Some years ago, Rothschild & Schoonhoven (1977) discovered that ovipositing *P. brassicae* females can discriminate between cabbage plants with and without conspecific eggs. *P. brassicae* eggs release a chemical signal deterring other females from oviposition at that site. This chemical compound has been called the oviposition deterring pheromone (ODP). By modifying female behaviour, this pheromone may promote an even distribution of eggs over available oviposition sites. Consequently, this substance may be useful in the control of *Pieris brassicae*.

The present study was undertaken to investigate the prospects for application of this pheromone. Before field application can be attempted, however, basic knowledge is needed on several properties of this pheromone under standardized conditions. Therefore, most of the work described here has been done in the laboratory. The influence of the ODP on the distribution of eggs over available oviposition sites (the ultimate effect of the ODP) is investigated in Chapter 2. Chapter 3 provides an ethogram of oviposition behaviour of *P. brassicae* and a literature survey on the various sensory modalities which may be involved in egglaying behaviour of various insect species. Female responses to the ODP at the sensory level, in order to determine which chemoreceptors they may use in the perception of the pheromone, are described in Chapter 4. The next two Chapters (5 and 6) are concerned with an experimental

analysis of female pre-oviposition behaviour. During this behavioural phase, which ends at the moment of deposition of the first egg, females take the decision either or not to lay eggs. The detailed modifications in pre-oviposition behaviour induced by the presence of ODP are studied in intact females (Chapter 5) as well as in females with various sensory ablations (Chapter 6). In this way we were able to unravel the operating mechanism of the oviposition deterring pheromone. In Chapter 7, the results of small scale field experiments are reported to assess the potential of ODP as an agent in cabbage pest control. With respect to field application of the ODP, it is important to know whether this pheromone is species-specific in its effects or that perhaps other (lepidopterous) species are affected as well. Chapter 8 describes the behavioural responses of a related species, *Pieris rapae*, to the ODP of *Pieris brassicae*.

One aspect of the oviposition deterring pheromone will not be discussed here. One of the main objectives of this study, which started in 1980, was the chemical identification of the ODP. In close cooperation with the Netherlands Organization for Applied Scientific Research TNO where the chemical part of the work was conducted, an elaborate bioassay program was undertaken. At present, the isolation of the deterrent compound(s) is almost completed and identification of the pheromone seems close. Details on the isolation procedure and chemical properties will be published elsewhere.

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2 THE EFFECTS OF AN OVIPOSITION DETERRING PHEROMONE ON EGG-LAYING IN *PIERIS BRASSICAE*

ABSTRACT

A laboratory study was conducted in order to determine the influence of the oviposition deterring pheromone (ODP) upon the distribution of eggbatches and eggs by *Pieris brassicae* females. This pheromone is known to be associated with eggs. Butterflies were offered a choice between cabbage leaves treated in various ways with the ODP and control leaves. The presence of intact conspecific eggs on the treated leaf appeared to have a moderate deterrent effect upon oviposition. An aqueous solution of the ODP, obtained by washing eggs in distilled water was found to have a somewhat higher deterrent effect. Most effective in deterrence of oviposition, however, appeared to be a washing of *Pieris brassicae* eggs in methanol. Such a methanol solution can be stored at low temperatures for at least three years without loosing activity. Application of eggwash to either the upper or lower surface of the leaf does not make any difference to females. Percentage deterrence was found to increase with the concentration of eggwash. At very low concentrations, no significant difference could be observed anymore in the numbers of eggbatches and eggs laid on control and treated leaf. On the other hand, even very high concentrations of methanol eggwash do not fully protect cabbage leaves against oviposition. Washing *Pieris brassicae* eggs seven times consecutively in methanol, a series of pheromone solutions is obtained, all of them were found to possess a high deterrent activity. Although percentage deterrence slowly decreases in subsequent

washings, the seventh eggwash sprayed onto cabbage leaves, still resulted in less than one quarter of the total number of eggbatches and eggs being laid on the treated leaf. This suggests that *Pieris brassicae* eggs may contain a large amount of the oviposition deterring pheromone.

INTRODUCTION

Hostplant species utilized by *Pieris brassicae* L. are characterized by the presence of one or more so-called mustard oil glucosides(Verschaffelt, 1911). Most of these plant species belong to the family of Cruciferae, although exceptionally eggs and larvae have been found on plants from other families, e.g. Tropaeolacae and Resedacae (Terofal, 1965). Experiments conducted by David & Gardiner (1962), in which female *P. brassicae* were observed to oviposit on green paper cards treated with a sinigrin solution, and Ma & Schoonhoven (1972), who were able to induce oviposition on broad bean plants cultured in aqueous solutions of glucosinolates, exemplify the importance of these secondary plant substances.

Besides chemicals of plant origin, determining potential host plant suitability, oviposition of *Pieris brassicae* females is also influenced by a chemical signal related to host plant exploitation by conspecifics. Rothschild & Schoonhoven (1977) discovered that gravid females when having a choice between cabbage leaves with and without conspecific eggs, prefer to oviposit on the latter leaves. They found this deterrent effect of previously laid eggs to be mainly governed by a pheromone-like emanation associated in some way with the eggs. Behan & Schoonhoven (1978) demonstrated the deterrent compound(s) to be water soluble and suggested that females might perceive the pheromone by antennal olfactory hairs as well as by tarsal contact chemoreceptors. Schoonhoven *et al.* (1981) reported that in the laboratory an aqueous pheromone solution, when sprayed on a cabbage plant, retained its deterrent activity for at least 14 days. Furthermore, they found some indications that the pheromone has a low volatility and a high stability under laboratory conditions and that eggs may contain a considerable amount of this oviposition deterring pheromone (ODP). Such pheromones are known to play a role in oviposition behaviour of a growing number of phytophagous insects, at this time belonging to at least 6 orders and 16 families (Prokopy, 1981; Prokopy *et al.*, 1984). In a number of species, this epideictic (Corbet , 1971) pheromone appeared to be rather stable and water- (sometimes also methanol-) soluble, for example in various *Rhagoletis* species (Katsoyannos, 1975; Prokopy *et al.*, 1976), *Ceratitis capitata* Wiedemann (Prokopy *et al.*, 1978), *Anastrepha suspensa* Loew and *A. fraterculus* Wiedemann (Prokopy *et al.*, 1977; Prokopy *et al.*, 1982) and in *Agromyza frontella* Rondani (McNeil & Quiring, 1983). This similarity may indicate that we are dealing with an entirely new class of behaviour modifying chemicals.

The ecological consequence of the existence of these pheromones is that they promote a uniform distribution of eggs among available oviposition sites in nature. In a few species such a distribution of eggs has been reported (Remund *et al.*, 1980; Prokopy *et al.*,1982). These examples indicate that epideictic pheromones might be effective in the field and that they perhaps provide a new prospective tool in future pest control.

With regard to *Pieris brassicae*, however, we first need to know, besides the identity of the deterrent compund(s), more details on the biological activity of the pheromone under standardized laboratory conditions. In this paper, we will investigate the influence of various concentrations and types of pheromone solutions on the distribution of eggbatches and eggs.

MATERIALS AND METHODS

Animals

Pieris brassicae L. adults were obtained from a laboratory culture reared for many years on Brussels sprouts, *Brassica oleracaea* L. var. *gemmifera* D.C. In order to be assured of fertile females the whole year around, every 10 days freshly laid eggs were set apart and allowed to develop into a new generation of butterflies. The larvae were prevented from entering diapauze by growing them under a long day light regime (16 hours light; 8 hours dark). After emergence, butterflies were kept in large cages (80 x 100 x 80 cm), illuminated by mercury vapour lamps and additional daylight. The cages were provided with artificial flowers, containing a 10% sucrose solution on which the butterflies could feed ad libitum. The age of experimental females ranged from 4 to 14 days after eclosion.

Experiments

Females were given a choice between control leaves and ODP treated leaves to lay their eggs. For each experiment one pair of cabbage leaves, carefully matched for equal size, age and external appearance, was taken from the same plant. One of these leaves was treated with a solution of the oviposition deterring pheromone in the way described below and the other one served as the control leaf.

Pieris brassicae eggs were used as the source of the oviposition

deterring pheromone. Each day freshly laid eggs were removed from the plants by means of a drop of acetone and collected in a Petri dish. This Petri dish was stored in the refrigerator (4°C) untill, after about one month, 10-12 gr of eggs (being 50,000 - 60,000 eggs) was assembled. This amount of eggs was washed for 5 minutes in pure methanol or distilled water (depending on the experiment). The eggs were then removed by filtration (paper filter Schleicher & Schüll; no. 589) and the remaining solution was called methanol-eggwash or water-eggwash, respectively. Concentrations of eggwashes are expressed as egg-equivalents per ml solvent (ee/ml). The various concentrations of eggwash, which were used in a separate series of experiments (see Fig. 1), were all derived from one stock of eggs. In this case, a methanol-eggwash was made containing 1,024 ee/ml, and from this stock-solution all the other concentrations were prepared by a stepwise one-fold dilution with pure methanol.

In another series of experiments, subsequent eggwashes from one stock of eggs were tested. These eggwashes which we called methanol-eggwash I to VII, were made by simply washing these eggs several times in an equal amount of methanol. In this case, the first eggwash was made by rinsing 21 gr of eggs (approximately 105,000 eggs) in 210 ml pure methanol. The concentration of eggwash I is thus defined as 500 ee/ml. Immediately after filtration of the first washing 210 ml of methanol was added again to the same amount of eggs for the second washing and so on up to 7 times. The number of eggs involved in each of the 7 washings did not change, therefore the concentrations of subsequent eggwashes were also fixed at 500 ee/ml.

The treated leaves with a diameter of approximately 15 cm, were sprayed, by means of a Desaga° chromatography sprayer, with 1 ml of eggwash at the underside only, except in one series of experiments in which leaves sprayed at the upper- and underside were compared. In all experiments, the control leaves were sprayed similarly with 1 ml of the solvent only. Preliminary experiments had revealed that methanol alone did not have any effect upon oviposition. When applied in a fine spray, methanol did not harm the leaves either. The experiments were conducted in the large cages mentioned above with groups of butterflies (20-30 females and 20 males per cage). In each cage two pairs of leaves, with their petioles in water, were placed in a square with the two control leaves diagonally opposite to each other. The distance between the leaves was about 40 cm in all directions. Empirically, it was found that females lay most eggs during the morning hours, therefore all experiments were conducted between 9.00 h and 14.00 h. The temperature

in the cages during the experiments varied between 24° and 29°C and the relative humidity between 40 and 50%. Before an experiment, females were deprived of an oviposition substrate for about one hour. Two pairs of leaves were offered at the same time to the females in one cage in order to prevent too much interactions which might interfere with the choice behaviour of females.

After evaporation of the solvent, the leaves were exposed to the females for one hour. The number of eggbatches and eggs laid on the leaves during this period were counted. The activity of the oviposition deterring pheromone is expressed in terms of the percentage deterrence, calculated in the following way: % deterrence = (A-B) x 100/A+B, where A and B represent the numbers of eggbatches (or eggs) laid on control and treated leaves, respectively. A percentage deterrence of 100% indicates that females laid eggs only on the control leaf, whereas a percentage deterrence of 0% indicates no preference of females.

Table 1. Deterrent effects of various ODP sources upon *Pieris brassicae* oviposition in a choice situation. In addition, average batchsize and batchsize range are given for each type of leaf. VSOP: Very Superior Old Pheromone.

Type of choice experiment	no. exp.	no. eggbatches/eggs laid on leaves average batchsize batchsize range control	 treated	% deterrence
Blanc leaf		116/4454	62*/2119*	30.3/35.5 %
VV	20	38.4[a]	34.2[a]	
leaf with c. 250 eggs		3 - 121	3 - 101	
Distilled water		200/6002	87*/1803*	39.4/53.8 %
VV	30	30.0[a]	20.7[b]	
water-eggwash (250 ee/ml)		3 - 101	3 - 78	
Methanol		174/6103	31*/806*	69.8/76.7 %
VV	32	35.1[a]	26.0[a]	
Methanol-eggwash (250 ee/ml)		3 - 126	3 - 52	
Methanol		53/1402	54/1438	-0.9/-1.3 %
VV	16	26.5[a]	26.6[a]	
Methanol		3 - 78	3 - 93	
Methanol		293/10,307	54*/1433*	68.9/75.6 %
VV	36	35.2[a]	26.5[b]	
VSOP (250 ee/ml)		3 - 134	3 - 88	

* indicates significant difference in no. eggbatches/eggs between the leaves in the same row (sign test; $p < 0.01$). Values on mean batchsize in the same row followed by different letters are significantly different (chi-square test; df = 2; $p < 0.01$).

RESULTS

Female *Pieris brassicae* preferentially oviposit on cabbage leaves devoid of conspecific eggs, as can be seen in the first row of Table 1. The "treated" leaves in these 20 experiments all carried approximately 250 eggs distributed over 4-9 eggbatches, to allow a fair comparison with eggwashes of 250 ee/ml. An eggwash made in distilled water (250 ee/ml) has a similar deterrent effect upon oviposition: the far majority of eggbatches and eggs is laid on the control leaf (Table 1). Percentage deterrence has increased to 39.4% and 53.8% for the numbers of eggbatches and eggs, respectively. The latter value indicates that less than one quarter of the total number of eggs was laid on the treated leaf. A still stronger deterrent effect can be observed when cabbage leaves are sprayed with methanol-eggwash. This solution, at the same concentration of 250 ee/ml, was found to have a percentage deterrence of 69.8% and 76.7% for eggbatches and eggs, respectively. As a control of the experimental set up, we conducted a number of experiments in which females were offered a choice between two leaves both sprayed with methanol only. As can be seen in Table 1, females do not show any oviposition preference for one or the other leaf in this choice situation. The numbers of eggbatches and eggs deposited on both leaves were almost similar. To test whether and how long a pheromone solution in methanol could be preserved, females were offered the choice situation mentioned in the bottom row of Table 1. The abbreviation VSOP stands for Very Superior Old Pheromone, which consists of a mixture of 3 methanol-eggwashes made in April, May and June 1981, respectively. At the time this mixture (250 ee/ml) was tested, it had been stored in the refrigerator ($4^{o}C$) for more than three years. It is not the same methanol-eggwash mentioned earlier in Table 1. Nevertheless, the deterrent activity of this VSOP mixture lies at the same high level as that of an eggwash tested a few days after its preparation. Because of their high deterrent activity, only methanol-eggwashes were used in all further experiments.

At first, we examined whether it would make any difference to females when the pheromone is applied either to the upper or to the lower surface of the leaf. In these experiments, females were given simultaneously a control and treated leaf sprayed at the underside and a similar pair of cabbage leaves sprayed at the upperside. The results (Table 2) clearly indicate that the deterrent effect is not influenced by the way cabbage leaves are sprayed with the ODP. The percentage deterrence is almost equally high in both cases and

Table 2. Oviposition deterrence by methanol-eggwash (250 ee/ml) applied to upper or lower leaf surface. In addition, average batchsize and batchsize range are given for each type of leaf.

treated surface	no. exp.	no. eggbatches/eggs laid on leaves average batchsize batchsize range control	 treated	% deterrence
upper	21	117/4223	22*/627*	68.3/74.1 %
		36.1	28.5	
		3 - 107	3 - 77	
lower	21	109/4036	22*/763*	66.4/68.2 %
		37.0	34.7	
		3 - 131	5 - 89	

* indicates significant difference in no. eggbatches/eggs between the leaves (sign test; $p < 0.001$). Values on mean batchsize do not differ significantly between the leaves (chi-square test).

Table 3. Oviposition deterrence by various concentrations of methanol-eggwash. In addition, average batchsize on both types of leaves is given.

eggwash concentration (ee/ml)	no exp.	no. eggbatches/eggs laid on leaves average batchsize control	 treated	% deterrence
4	20	153/4870 (31.8)[a]	115[1]/3822 (33.2)[a]	14.1/12.1 %
8	20	93/4126 (44.4)[a]	74[1]/2643 (35.7)[a]	11.4/21.9 %
16	26	113/3692 (32.7)[a]	57[2]/1922[1] (33.7)[a]	32.9/31.5 %
32	23	149/4571 (30.7)[a]	71[2]/2159[2] (30.4)[b]	35.5/35.8 %
64	22	118/4606 (39.0)[a]	62[1]/1890[2] (30.5)[a]	31.1/41.8 %
128	37	274/10,223 (37.3)[a]	125[3]/3394[3] (27.2)[b]	37.3/50.2 %
256	30	167/5498 (32.9)[a]	27[3]/853[3] (31.6)[a]	72.2/73.1 %
512	36	318/10,237 (32.2)[a]	78[3]/1858[3] (23.8)[b]	60.6/69.3 %
1024	12	130/4348 (33.4)[a]	20[3]/426[3] (21.3)[a]	73.3/82.2 %

No. eggbatches/eggs differ significantly between the leaves at 1. 5% level; 2. 1% level; 3. 0.1% level (sign test). Values on mean batch size in the same row followed by different letters are significantly different (chi-square test; $df = 2$; $p < 0.05$).

comparable with the values given in Table 1.

Another series of choice experiments was carried out to determine female responses to various concentrations of methanol-eggwash. The numbers of eggbatches and eggs laid in these experiments are given in Table 3. At all concentrations of eggwash, the treated leaves received significantly lower numbers of eggbatches. The total numbers of eggs differed significantly between the two types of leaves from a concentration of 16 ee/ml. The deterrent activity of methanol-eggwash increases with the concentration. Fig. 1 displays the relation between the ^{2}log concentration of eggwash and the percentage deterrence. Plotted in this way, regression lines can be drawn which indicate an almost linear relationship between these two parameters for both the number of eggs ($r = 0.983$) and the number of eggbatches ($r = 0.925$).

Eggs may be washed with methanol several times consecutively. The seven washings we obtained in this way were all found to be highly deterrent to ovipositing *Pieris brassicae* females (Table 4). Cabbage leaves sprayed with the first or second eggwash are almost completely protected against oviposition. The percentage deterrence calculated for these two washings (500 ee/ml) is even higher than the % deterrence we found at 1,024 ee/ml in Table 3. In the third eggwash percentage deterrence is somewhat diminished but from this washing the deterrent activity remains at a constant (high) level up to and including the sixth washing. The percentage deterrence of eggwash VII is again slightly reduced in comparison to the four preceding pheromone solutions. But even in these experiments females laid less than one quarter of the total numbers of

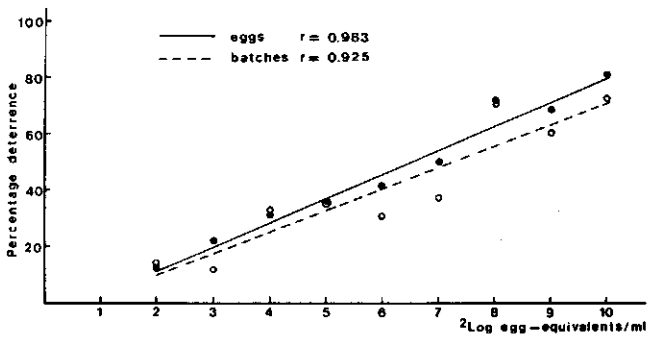

Fig. 1. Deterrent activity of methanol-eggwash (at concentrations 2^2 - 2^{10} ee/ml) on *Pieris brassicae* oviposition. Linear regression of percentage deterrence and ^{2}log eggwash concentration.

Table 4. Oviposition deterrence by subsequent methanol egg washings (500 ee/ml) from one stock of eggs.

serial number of eggwash tested	no. exp.	no eggbatches/eggs laid on leaves* control	treated	% deterrence
I	20	169/6874	4/67	95.4/98.1 %
II	20	119/4144	4/106	93.5/95.0 %
III	16	127/2662	18/276	75.2/81.2 %
IV	12	70/2463	11/261	72.8/80.4 %
V	16	104/2676	23/518	63.8/67.6 %
VI	14	80/2057	14/332	70.2/72.2 %
VII	25	188/4712	58/1222	52.8/58.8 %

* In each row the difference in no. eggbatches/eggs laid on the two types of leaves is highly significant (sign test; $p < 0.001$).

eggbatches and eggs on the treated leaves.

In Table 1-3 values are given on the average batchsize females laid on the two types of leaves. The differences which could be observed between the leaves, however, are not very consistent in the various experiments. Therefore, a possible effect of the ODP upon the size of eggbatches will be discussed in some more detail in the next section.

DISCUSSION

The deterrent effect of conspecific eggs on *Pieris brassicae* oviposition is clearly demonstrated in these experiments. When we compare our results with those reported by Rothschild & Schoonhoven (1977), however, a difference can be observed. In choice experiments (n = 45) conducted by the latter authors, females laid 6,913 eggs on the clean leaf and 1,129 eggs on the egg laden leaf. The percentage deterrence which can be calculated from these data (71.9%) is much higher than the 35.5% we found. This indicates that in their experiments egglaying was much stronger reduced by the presence of conspecific eggs as in our experiments. However, Rothschild & Schoonhoven (1977) do not mention the numbers of eggs by which the experimental leaves were laden. The higher deterrent effect they observed might very well be caused by a (much) higher number of eggs than we applied to the experimental leaves.

In comparison to intact eggs, eggwashes deter females (much) better from

oviposition. A reasonable explanation for this deterrence might be the uniform distribution of the pheromone which can be achieved by spraying a leaf with eggwash. With intact eggs, the pheromone possibly diffuses from eggbatches acting as a kind of point-sources. Comparing the activities of water- and methanol-eggwash, it can be seen that oviposition deterrence is much higher in the latter case. This indicates that the oviposition deterring pheromone of *Pieris brassicae* is more soluble in the organic solvent than in water. The pheromone does not loose any of its activity when it is stored in a methanol solution in the refrigerator even for periods up to several years. Although at this time, it seems premature to discuss practical application, the fact that this solution can be stored for a long time without apparent decay of the active component(s) reflects probably an attractive property.

Pieris brassicae females only very rarely oviposit on the upper side of a cabbage leaf. Eggbatches are usually deposited on the lower leaf surface. To match this situation, we sprayed the eggwash only on the lower surface of the leaf in almost all experiments. The results in Table 2, however, clearly indicate that it does not make any difference to females to which side the ODP is applied: percentage deterrence is equally high in both cases, so females perceive the pheromone equally well.

The dose-response curve displayed in Fig. 1 , demonstrates a very evident relationship between the concentration of eggwash and its deterrent activity. Percentage deterrence is calculated for both the number of eggbatches and the total numbers of eggs. Actually these two lines represent two different effects. The number of eggbatches as a measure of ODP activity only covers the decision of females whether to lay or not on the treated leaf. The percentage deterrence for the total numbers of eggs, however, additionally includes a possible effect of the ODP upon females after egglaying has started. The difference between the regression lines in fact represents the influence of the ODP on mean batchsize. Fig. 1 thus indicates that higher concentrations of ODP may induce females to lay smaller eggbatches. Whether this is really the case, will be discussed below.

Extrapolation of the regression lines reveals that at a concentration of 0 ee/ml, the percentage deterrence is also approximately 0%. No indications were found that low concentrations of eggwash act as oviposition attractants. Fig. 1 additionally indicates that the presence of 1,024 egg equivalents, which may be regarded as a very heavy eggload for one cabbage leaf, does not result in 100% deterrence. Apparently, some females sometimes ignore or do not perceive

this deterrent signal and lay eggs on the treated leaf. One may argue that this might be caused by the experimental design in which a limited number of leaves is presented to a group of females. At a certain moment, so many female might be ovipositing on the control leaf, that other gravid females simply can find a suitable spot on this leaf and thus select the treated leaf to deposit their eggs. However, experiments with single females offered a similar choice (see chapters 5 and 6), revealed that also in this situation the ODP treated leaf is occasionally preferred for oviposition. Relating these "deviant" ODP responses to the density of butterflies thus does not fully explain this phenomenon. Behan & Schoonhoven (1978) also tested various concentrations of eggwash, made in distilled water. In contrast to the results reported here, they found eggwashings from 1,000 ee/ml down to 60 ee/ml to have an absolute deterrent effect, whereas at 30 ee/ml this effect was lost. However, they conducted only one experiment with 10 gravid females at each concentration. Perhaps the different results are due to a difference in group size and to a difference in number of repetitions.

Behan & Schoonhoven (1978) and Schoonhoven *et al.* (1981) reported subsequent washings of *Pieris brassicae* eggs in distilled water to retain a high deterrent activity. Even a 1 ml eggwash of 125 eggs, previously rinsed with 300 ml of water was found to be highly active. Our experiments show that in subsequent methanol-washings of *Pieris brassicae* eggs the deterrent activity also diminishes only very gradually. Before the last eggwash was made, the eggs were already washed with a total volume of 1,260 ml of methanol, which is a better solvent for the ODP than water. In spite of this there is still enough pheromone left in the eggs to gain a seventh eggwash possessing a fairly high percentage deterrence. Schoonhoven *et al.* (1981) suggested that *Pieris brassicae* eggs probably contain a large amount of pheromone and that during washing the eggs release the pheromone only gradually. Our findings corroborate this idea. The deterrent activity of the first two washings in Table 4 is much higher than the percentage deterrence we found in the eggwash of comparable strength (512 ee/ml) in Table 3. This difference cannot be readily explained. The washings mentioned in Table 3 and 4 were derived from different stocks of eggs. The latter stock of eggs had been stored in the refrigerator for 3 months before they were used. This is about twice as long as the other stock and perhaps this difference has something to do with the observed difference in activity.

In a number of experiments a significant difference was found in the average batch size between control and ODP treated leaves (see Table 1 and 3).

This indicates that the oviposition deterring pheromone might influence female behaviour also after oviposition has started. However, if this effect indeed is induced by the ODP, it might be expected to occur in all experiments with at least the higher concentrations of pheromone. As can be seen in Table 1-3, this is not the case. An alternative explanation for the difference in batch size might be the following. When the leaves are introduced into the cage, most females will select the control leaf to deposit their eggs. For whatever reason some females, however, will start oviposition somewhat later than other ones. These females, possibly faced with a control leaf occupied by a large number of ovipositing butterflies, then perhaps select the treated leaf for oviposition. As explained above, this might be the reason that in our experiments always some eggs are laid on the treated leaves. Important at this time, however, is the point that egglaying on the treated leaf might start later in the experiment than oviposition on the control leaf. We do not have hard data available to prove this hypothesis, but preliminary behavioural observations indicate that this might happen. Both types of leaves are removed from the cage about one hour after introduction. This means that on the treated leaf, females had less time available to oviposit, thus resulting in smaller eggbatches (*Pieris brassicae* females usually oviposit at a very constant rate of 3 eggs/minute; personal observations). Indirectly, this hypothesis is supported by the figures on the range of batch sizes given in the Tables 1 and 2. On the control leaf, the upper limit of this range reaches a much higher value as on the treated leaf. Tentatively, it is concluded that the observed difference in batch size might be due to an experimental artefact rather than being induced by the oviposition deterring pheromone.

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3 EGG-LAYING BEHAVIOUR AND SENSORY MODALITIES INVOLVED IN OVIPOSITION SITE SELECTION OF *PIERIS BRASSICAE*

INTRODUCTION

The oviposition deterring pheromone of *Pieris brassicae* affects the distribution of eggs over potential hostplants or, in general terms, modifies female oviposition behaviour (Klijnstra, 1982). How and when female behaviour is influenced by this pheromone cannot be defined until normal behaviour, i.e. in the absence of the pheromone, has been analysed in some detail. Therefore, a qualitative description (ethogram) of oviposition behaviour of *Pieris brassicae* is given in this paper. This ethogram also provides information on the role of the various sensory systems involved. Details of the role of various sensory stimuli will be discussed in view of known data from the literature.

MATERIALS AND METHODS

Pieris brassicae females were obtained from a culture maintained for several generations on cabbage plants (*Brassica oleracea* L. var *gemmifera* D.C.). Butterflies were kept in large wooden cages (80x80x100 cm) with a glass roof and nylon gauze side walls. Cages were illuminated by mercury vapour lamps (from above) and additional daylight, entering from the backside of the cage. During the day temperature was maintained at ca. 24^{o}C and the butterflies were allowed free access to a 10% sucrose solution by means of artificial flowers.

Behavioural observations were carried out during the morning hours on single females (4-14 days old), which were transferred to an observation cage of the same size before the experiments. Females were offered one or more cabbage leaves, taken from plants which had never been in contact before with butterflies and the differential behavioural steps displayed by ovipositing females were closely examined.

RESULTS AND DISCUSSION

Egg-laying behaviour

Pieris brassicae females usually mate on the second or third day after emergence. Under our laboratory conditions mating takes about two hours (at

24°C). Females start egg-laying on the third or fourth day after eclosion by laying one or two small batches of 5-10 eggs. On the second day of the oviposition period batch size increases to an average of 40-60 eggs (cf. David & Gardiner, 1962). Batch size, however, is dependent on butterfly density. Although quantitative data are not available, we noticed a tendency of decreasing batch size with increasing numbers of butterflies per cage. Most eggs are laid during the morning hours. Female fecundity remains at a high level till 15 days after emergence. During this period females often mate for a second time around the 9th day of adult lifetime.

When oviposition behaviour is observed in the laboratory several behavioural steps or actions can be distinguished. Usually these steps are displayed in the sequence given in Table 1. *Approach* flight which is the first behavioural step in oviposition, can be distinguished from other flight behaviour by its larger wing-stroke amplitude and a reduced flight speed (cf. Terofal, 1965).

Landing usually occurs upon the upper side of the leaf. Alighting is often followed immediately by the characteristic *drumming* behaviour, described earlier by Ilse (1937) and Terofal (1965). This *drumming* behaviour has also been found in other Lepidoptera (Fox, 1966; Myers, 1969; Vaidya, 1969 a,b; Calvert, 1974; Ichinosé & Honda, 1978). In *P. brassicae* these alternating tapping movements of the fore-legs are always accompanied by fluttering movements of the wings.

Once landed on the upper side of the leaf, females gradually move to the margin of the leaf while *drumming* is continued. These first three steps actually form a continuum, whereas the switch from *drumming* to *curving*, the next step in the behavioural chain, is discrete. Before being able to bend the abdomen around the edge of the leaf, a female has to fold back her wings and thus she must stop wing fluttering. This short pause between *drumming* and *curving* separates two phases in oviposition behaviour:
1. before settlement (*approach; landing; drumming*); 2. after settlement (*curving; touching; oviposition*).

Females often break off the regular sequence between *curving* and *touching* or between *touching* and *oviposition*. This fact might indicate that also abdominal (chemo)receptors play a role in oviposition site selection. Therefore *curving* and *touching* are entered in table 1. as seperate steps.

In the literature little attention is paid to a discrimination of these behavioural steps after settlement. Saxena & Goyal (1978) mention abdominal

Table 1. Qualitative description of pre-oviposition behaviour of *Pieris brassicae* females in the laboratory.

ETHOGRAM		SENSES (POSSIBLY) INVOLVED
APPROACH	♀ flies in rather straight line to the leaf, sometimes interrupted by "turning" i.e. a sudden change in flight-direction (away from the leaf)	vision olfaction
▼		
LANDING	the first contact with the leaf by the tarsi, often immediately followed by	(olfaction) tarsal taste hairs
▼		
DRUMMING	alternate tapping movements on upper leaf surface with fore-tarsi, accompanied by wing fluttering	(olfaction) tarsal taste hairs
▼		
CURVING	♀ bends her abdomen around the edge of the leaf without touching lower surface	taste hairs on tarsi and ovipositor
▼		
TOUCHING	touching lower leaf surface with the extruded ovipositor	taste hairs on tarsi and ovipositor mechanoreceptors on ovipositor
▼		
OVIPOSITION	deposition of the eggs, one by one, in a batch	mechanoreceptors and taste hairs on ovipositor

curling in *Papilio demoleus*. Singer (1982) describes *curving* and *touching* in *Euphydryas editha* as the final stage of oviposition search behaviour, in which females extrude the ovipositor and probe the underside of the leaf.

The final step in Table 1. is *oviposition*. Eggs are deposited one by one in a cluster at a rather constant rate of 3 eggs per minute. However, females which have been deprived of cabbage plants for several hours, may start at a much higher rate of 6-8 eggs per minute, which reflects an urge to oviposit and thus a heavy eggload.

It should be noted here that the ethogram given in Table 1. is an abstraction of the behaviour actually displayed. Ovipositing females frequently perform parts of this sequence several times before deciding whether and where to lay eggs. Moreover, the variability in individual sequences as well as in duration of pre-oviposition behaviour is very large.

Sensory modalities involved in oviposition behaviour

Visual cues

Colour perception plays an important role in egg-laying behaviour of butterflies. *Pieris brassicae* females, when ready to oviposit, are attracted by the colour green (Ilse, 1937). David & Gardiner (1962) found the characteristic drumming movements to occur on any green plant species and even on

green cardboard paper. Our observations also indicate green as a primary attractive factor in oviposition behaviour. A similar response has been found in *Papilio demoleus* (Vaidya, 1969 a,b; Saxena & Goyal, 1978).

The colour yellow plays a role both in adult feeding behaviour (David & Gardiner, 1962) and in oviposition behaviour. *P. brassicae* eggs have a bright yellow colour: Rothschild & Schoonhoven (1977) found this colour to be partly responsible for the deterrent effect of previously laid eggs.

The shape of previously laid conspecific eggs is also an important visual cue in egglaying behaviour of pierids (Rothschild & Schoonhoven, 1977; Shapiro, 1981 a,b), heliconiines (Gilbert, 1975) and papilionids (Rausher, 1979). The shape and colour of plant structures which act as mimics of insect eggs inhibit oviposition in some other species (Shapiro, 1981 a,b; Williams & Gilbert, 1981).

In *Battus philenor*, females utilize leaf shape as primary factor in hostplant location (Rausher, 1978), which again reflects the importance of visual cues in oviposition behaviour of diurnal insects. However, acceptance (or rejection) of visually chosen plants occurs only after alighting. Stanton (1979, 1980) found similar responses in *Colias* butterflies.
In *Pieris brassicae*, there is no evidence that leaf shape is involved in hostplant selection.

Olfactory cues

In oviposition, orientation to potential hostplants is frequently mediated by olfactory cues. Although secondary plant substances are of main importance, also other volatiles take part in hostplant selection (e.g. oviposition deterring pheromone).

In *Pieris brassicae*, the role of volatile secondary plant substances in oviposition behaviour is still not fully understood. Cruciferous plants, as main hostplants of *P. brassicae*, are characterized by the presence of glucosinolates. These substances,however, are relatively non-volatile (see Vaughn et al., 1976), limiting their use in hostplant selection to contactchemoreception. Derivatives of glucosinolates like allylisothiocyanate (mustard oil) or allylnitrile, which are volatile, are more likely to be involved in distance perception of hostplants. Behavioural experiments, however, revealed somewhat contradictory results. David & Gardiner (1962) found plant odour to play little part in the attraction of ovipositing females. On the contrary, Terofal (1965) presumed the odour of mustard oils to elicit in females an

area-restricted search for potential hostplants. Antennae of female *P. brassicae* bear four types of sensilla, two of them are thought to have an olfactory function (Behan & Schoonhoven, 1978). Electrophysiological recordings revealed low responses of antennal olfactory hairs to isothiocyanates at EAG level (Behan & Schoonhoven, 1973) as well as at single cell level, where no specifically tuned cells to isothiocyanates were found (Den Otter et al., 1980). MacLeod (1976) has shown that allylnitrile can be released as the major derivative of sinigrin (a glucosinolate present in *Brassica oleracea*). Studying hostplant selection in *P. brassicae* under field conditions Mitchell (1977) found a clear preference for those plants performing the highest scores in the so-called picrate-test. This test is in part a measure of the release of allylnitriles. Therefore Mitchell assumes that these derivatives are involved in distance perception of cabbage plants.

The exact role and nature of volatile plant chemicals in hostplant selection of *P. brassicae* females thus needs further study. It is conceivable that hostplant odours only trigger female responses to other stimuli, for instance visual targets or wind direction. Such responses are known to occur in a number of insect species, for example, in the cabbage root fly, *Erioischia brassicae*, where gravid females perform an odour induced anemotaxis after stimulation with allylisothiocyanate (Hawkes & Coaker, 1976; see Kennedy, 1977 for further examples).

In the cabbage root fly, olfactory stimuli still affect female behaviour after alightment by acting as synergists in the stimulation by non-volatile glucosinolates (Traynier, 1965, 1967a, b; Zohren, 1968; Nair & McEwen, 1976). Such a phenomenon could also exist in *Pieris brassicae*. During *drumming* behaviour females perform apparent fluttering movements with their wings. This wing fluttering might evoke an airstream prompting the perception of volatile plant chemicals. Therefore olfaction is placed between parentheses behind *landing* and *drumming* in Table 1. On the other hand it could be argued that olfactory stimuli remain essential in the case of *E. brassicae* during egg-laying since this species is not in direct contact with its hostplant during egg-laying, in contrast to *Pieris*. As an alternative wing fluttering might serve to counterbalance a certain loss of grip during *drumming*. Of course the two explanations are not mutually exclusive.

In *Pieris brassicae*, complete removal of the antennae does not inhibit egg-laying (Ma & Schoonhoven, 1973; see chapter 6). Similar results are found in *Danaus gilippus* (Myers, 1969) and *Papilio protenor* (Ichinosé & Honda, 1978).

However, all these authors provide no figures on number of eggs laid after antennectomy. The presence of intact antennae might be essential for maintaining a normal oviposition level, as was found in *Chlosyne lacinia* (Calvert & Hanson, 1983).

Another volatile chemical involved in egg-laying behaviour of *P. brassicae* is the oviposition deterring pheromone, discovered by Rothschild & Schoonhoven (1977). Volatile components of this pheromone are probably perceived by antennal olfactory sensilla, although as yet no specifically tuned receptors have been found (Den Otter *et al.*, 1980). EAG recordings revealed a low but significant response to the odour of intact eggs (Behan & Schoonhoven, 1978). Further details on the perception of pheromone volatiles will be given in chapters 5 and 6, where the operating mechanism of this pheromone is discussed.

Contactchemosensory cues

Contactchemoreceptors start to play a role in hostplant selection once the female has alighted on the plant. In *Pieris brassicae* the importance of contactchemoreceptors in oviposition has been demonstrated before by David & Gardiner (1962) and Terofal (1965). They concluded that the ultimate decision of a female whether to accept or reject a plant is taken after physical contact with that plant. They also found a stimulating effect of sinigrin upon oviposition. This finding was corroborated by Ma & Schoonhoven (1973) who additionally established the presence of contactchemoreceptors on female fore-tarsi. Electrophysiological recordings revealed the existence of at least one particular sense cell sensitive to mustard oil glucosides.

In *Pieris brassicae* each group of chemosensory hairs on the 5th tarsomere is associated with a spine with its base on the 4th tarsomere.
Fox (1966) suggested that a combination of chemosensory hairs and leafabrading spines, which he found at the female fore-tarsi of several butterfly species, permits females to detect plant compounds present in the leaf surface.According to this *drumming* behaviour would improve the perception of plant chemicals. The occurrence of similar *drumming* movements in oviposition behaviour of other species (Fox, 1966; Myers, 1969; Vaidya, 1969; Calvert, 1974; Ichinosé & Honda, 1978) suggests that female butterflies mainly rely upon fore-tarsal chemoreceptors in hostplant selection. This is true in the case of *Papilio protenor demetrius*, where oviposition is suppressed completely after removal or coating of the female fore-tarsi (Ichinosé & Honda, 1978).

In *Pieris brassicae*, however, females still lay eggs when the forelegs are removed or when the fore-tarsal chemoreceptors are inactivated. Oviposition is prevented only after inactivation of all tarsal chemoreceptors (Ma & Schoonhoven, 1973). Myers (1969) obtained similar results for the tarsi of *Danaus gilippus berenice*. In the nymphalid *Chlosyne lacinia*, females deprived of their fore-tarsi maintain an almost normal oviposition level, but fail to discriminate between host- and non-hostplants (Calvert & Hanson, 1983). Removal of the antennae, on the other hand, causes a drastic reduction in the numbers of eggs laid. Therefore, they suggest ovipositional release and host discrimination to be separate functions mediated by different sense organs. Whether this is the case in *Pieris brassicae* will be discussed later (chapter 6).

Behavioural observations revealed that in *Pieris* settlement on a plant is not invariably followed by oviposition. A similar difference between the frequency of settlement and abdominal curving and the frequency of egg-delivery has been found in *Papilio demoleus* (Saxena & Goyal, 1978). Rejection of the plant as an oviposition substrate after settlement has taken place, might be caused by additional sensory input from (chemo)receptors, located at the ovipositor. In *Pieris*, the presence of abdominal contactchemosensory hairs was established by S.E.M.- and electrophysiological studies (Klijnstra, 1982; see chapter 4). Such hairs are also known to occur in some other lepidopteran species (Chadha &Roome, 1980; Valencia & Rice, 1982; Waladde, 1983).

Also other body parts such as antennae and proboscis might be provided with contactchemoreceptors. However, ovipositing *Pieris* females were hardly ever seen to touch the oviposition substrate nor with the antennae nor with the proboscis. Thus these receptors, if present, are probably not involved in oviposition site selection.

The perception of the oviposition deterring pheromone by contactchemoreceptors will be discussed in some detail in chapter 4.

Tactile cues

With respect to the possible role of tactile cues in oviposition site selection by *Pieris* females in the first place physical characteristics of the oviposition substrate would merit our attention. Female *Plutella maculipennis* are known to prefer substrates with small crevices and cavities to lay their eggs (Gupta & Thorsteinson, 1960). Ovipositing potato tuber moths (*Phthorimea operculella*) show a preference for surface depressions and in

addition for hairy substrates (Fenemore, 1978). The opposite reaction, i.e. inhibition of egg-laying by the presence of hairs, was found by Hagley *et al.* (1980) in the codling moth, *Cydia pomonella*. Probably such plant properties are perceived by mechanoreceptors, especially those located at the ovipositor. Such tactile hairs are known to occur in a number of lepidopteran species (Yamaoka *et al.*, 1971; Fenemore, 1978; Chadha & Roome, 1980) including *Pieris brassicae* (Klijnstra, 1982).

In the second place, abdominal mechanoreceptors may be involved in the formation of egg-batches. *Bombyx mori* females normally deposit compact, monolayered clusters of eggs. After destruction of mechano-sensory hairs on the anal papillae, Yamaoka *et al.* (1971) found eggs to be laid in disorganized piles. Chadha & Roome (1980) suggests a similar function for abdominal tactile hairs in *Chilo partellus*.

Returning to *Pieris brassicae*, no preference has been found for certain specific surface structures (eggs can be found on any part of the plant). Composition of egg-batches, however, is probably mediated by abdominal tactile cues, considering the apparent search for previously laid eggs by the extruded ovipositor.

CONCLUDING REMARKS

Pre-oviposition behaviour of *Pieris brassicae* consists of a series of distinct behavioural steps which, if performed in the proper sequence ultimately lead to the deposition of an egg-batch. Generally females follow the sequence given in Table 1, although frequencies and duration of the various actions may vary largely among individual females.

The various sensory modalities discussed above probably mediate different steps of the behavioural chain. *Approach* and *landing* may be initiated by visual (colour green) and olfactory cues (odour of cabbage plants). After alightment on a plant contactchemosensory cues further determine female behaviour. Perception of glucosinolates by tarsal contactchemoreceptors may prompt females to continue egg-laying behaviour with *curving* and *touching* whereas O.D.P. perception by tarsal and perhaps abdominal tastehairs may have the opposite effect, namely interruption of pre-oviposition behaviour. Mechanoreception as the fourth sensory modality seems to play an important role in egg-batch formation, which takes place during the final step of the behavioural

chain: the deposition of eggs.

However, this does not mean that pre-oviposition behaviour of *Pieris brassicae* is only the result of a sequence of successively operating sensory stimuli. An appropriate physiological condition and suitable climatological circumstances form important prerequisites for oviposition. In addition, it might very well be possible that females employ sensory information from more than one sense organ at a time during a particular behavioural step. For instance, the simultaneous occurrence of wing fluttering during *drumming* behaviour might indicate that, besides gustatory cues, females need olfactory input to continue egg laying.

In conclusion, we have seen that oviposition behaviour may be influenced by many environmental factors. Females have several sense organs at their disposal to perceive these stimuli but the question how females integrate sensory input in order to achieve an adaptive behavioural response under various circumstances needs some more study.

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4 PERCEPTION OF THE OVIPOSITION DETERRING PHEROMONE BY TARSAL AND ABDOMINAL CONTACTCHEMORECEPTORS IN *PIERIS BRASSICAE*

ABSTRACT

Perception of the oviposition deterring pheromone by contactchemosensory hairs in female *Pieris brassicae* was studied employing a tip recording technique. Electrophysiological responses of tarsal taste hairs to eggwash solutions show a marked increase in frequency of spikes merely originating from one sensory cell. This suggests that females in foretarsal taste hairs, apart from the glucosinolate-cells also possess sense cells specifically sensitive to the oviposition deterring pheromone.

Morphological studies by means of the scanning electron microscope revealed that the ovipositor of *Pieris brassicae* is provided with two groups of contactchemoreceptors. Electrophysiological recordings from these sensilla indicate the presence of at least three sensory cells, one of them being a mechanoreceptor. Stimulation with eggwash evokes a slight increase in spike frequency which cannot be ascribed to one particular sense cell. This indicates that abdominal taste hairs in some way may participate in the perception of the oviposition deterring pheromone. Responses to glucosinolates do not differ significantly from control stimulations. Therefore, it seems unlikely that these hairs are involved in hostplant recognition.

INTRODUCTION

Gravid *Pieris brassicae* females are deterred from oviposition by previously laid conspecific eggs. Besides the colour of eggs, a pheromone-like emanation associated with the eggs was found to be responsible for the deterrent effect (Rothschild & Schoonhoven, 1977). Their behavioural experiments have indicated that olfactory receptors as well as contactchemoreceptors might be involved in the perception of this pheromone. EAG-recordings, conducted by Behan & Schoonhoven (1978), revealed that olfactory sensilla on the female antennae can perceive the odour of eggs, although as yet in single-cell recordings specifically tuned cells were not found (Den Otter *et al.*, 1980).

In *Pieris brassicae*, the female fore-tarsi bear a large number of contact-chemosensory hairs (Ma & Schoonhoven, 1973). These tarsal B-hairs, as they were called, are innervated by four chemosensory cells and a mechanoreceptor. One of the chemosensory cells appeared to be specifically sensitive to mustard oil glucosides, whereas two other cells were slightly responsive to salt. The fifth cell could not be identified electrophysiologically. In behavioural experiments Ma & Schoonhoven (1973) established intact tarsal taste hairs to be indispensable for induction of oviposition. Preliminary experiments by Behan & Schoonhoven (1978) have indicated that the tarsal B-hairs might be sensitive to the oviposition deterring pheromone. We conducted a series of electrophysiological experiments, of which the results are presented here, to elucidate details of this sensitivity.

Behavioural observations of ovipositing *Pieris brassicae* females suggested the presence of chemoreceptors on the ovipositor (Klijnstra, 1982). The results of morphological studies of ovipositor preparations by means of a scanning electron microscope, which are reported here confirm the presence of contact-chemoreceptors on the ovipositor. Electrophysiological recordings were carried out in order to determine the sensitivity of these tastehairs to various chemicals. Finally, the possible role of abdominal chemoreceptors in oviposition behaviour is discussed.

MATERIALS & METHODS

Insects

Pieris brassicae females were obtained from a culture maintained for

several generations on cabbage plants. Rearing conditions were similar to those described by David & Gardiner (1962).

Morphology

Ovipositor preparations were made by cutting off the three terminal segments of the abdomen. The isolated abdominal tip was fixed by injecting a cold 7% glutaraldehyde solution (pH 7.0). Injection of the fixative improved the quality of the preparation and additionally caused extrusion of the ovipositor. After dehydration in a series of ethanol solutions the preparations were dried in a critical point drier, coated with a thin layer of gold and examined under a JEOL JSM 35C scanning electron microscope.

Electrophysiology

For electrophysiological experiments forelegs of 3-7 days old females were amputated proximal of the femur. Ovipositor preparations of 6-20 days old females were made as described above. Preparations were mounted on a silver-chloride coated silver wire which served as the recording electrode. A glass capillary, containing the stimulus solution and an AgCl-Ag wire, was applied to the tip of the sensillum and served as the indifferent electrode. The recording electrode was connected to a "femto-probe" (1x; input capacity in the order of femto Farads) and a "custom made" amplifier (100x). After passing a 50 Hz bandpass filter, the electrical signals were displayed on a Tektronix cathode ray oscilloscope and permanently recorded on an AKAI GX-215 D audio tape deck and a Siemens Oscillomink ink yet recorder.

The sensory hairs were stimulated with various concentrations of NaCl (0.05 M NaCl and 0.1 M NaCl served as control stimuli for the tarsal and abdominal preparations respectively). Other stimuli, dissolved in the corresponding control solution (to improve the conductivity of the stimulus solution), were: 0.02 M and 0.012 M glucotropaeolin tetra-ammonium salt (GTA; Carl Roth O.H.G.), 0.025 M and 0.02 M sinigrin (Aldrich Europe) and several concentrations of eggwash. Eggwashes, containing the oviposition deterring pheromone (O.D.P.) (Behan & Schoonhoven, 1978; Klijnstra, 1982) were prepared by washing *Pieris brassicae* eggs for 5 minutes in the control (salt) solution. Concentration of eggwash is expressed as egg equivalents per ml solvent (ee/ml).

During the experiments the glass capillaries were kept at a high relative humidity to prevent evaporation of the solvent and between the experiments the stimulus solutions were stored in the refrigerator.

Tarsal recordings were usually made from one sensillum located at the margin of a group of B-hairs on the 5th tarsomere, which could be traced relatively easily in all females. Before the test-solution was applied each hair was first stimulated with 0.05 M salt. Duration of stimulation was approximately 1 sec whereas stimulus intervals were at least 90 seconds. After each stimulation the hair was washed with aqua dest.

For abdominal hairs a different procedure was followed. After the preparation was mounted on the silver wire, a sketch was made of the positions of tastehairs on one valve. Then all sensilla were first stimulated with 0.1 M salt. After 5 minutes desadaptation the test solution was applied to the hairs in the same order and after another 5 minutes a second series of salt recordings was conducted. This procedure allowed us to obtain a complete picture of the response profile of the sensory equipment present on one valve of the ovipositor.

Recordings were analyzed by counting the number of spikes occurring in the first second (tarsal hairs) or the first 500 msec (abdominal hairs) of stimulation. In addition the amplitudes of spikes were measured in order to determine the number of responding sense-cells.

Fig. 1. Tarsal taste hair responses to (A) 0.05 M NaCl and (B) eggwash (250 ee/ml) dissolved in 0.05 M NaCl. Arrow indicates bending of the hair, showing stimulation of the mechanoreceptor. Calibration: 0.4 mV; 50 ms.

RESULTS

Tarsal contactchemoreceptors

Stimulating tarsal B-hairs with eggwash a marked increase in spike frequency can be observed as compared to the salt response (Fig. 1). This increase appeared to be highly significant for both concentrations of eggwash (250 ee/ml and 1000 ee/ml) tested. In order to determine the number of sense cells responding, spike amplitude histograms were made of all recordings. Figure 2 displays three representative recordings of one sensillum to NaCl, eggwash and glucotropaeolin respectively and the corresponding histograms are given in Fig. 3.

Spike amplitudes can be devided into four groups, indicated by the Roman numerals I to IV. The first group, having the smallest amplitude, represents the mechanosensory cell which could be traced in all three recordings. The response to salt (Fig. 2A) is characterized by a low and irregular spike frequency pattern. The amplitude histogram (Fig. 3A) indicates that in this case only one cell type (group IV) responded (cf. Ma & Schoonhoven, 1973).

The middle trace of Fig. 2 displays the response to eggwash (1000 ee/ml). In this recording the salt-sensitive cell is still present. The increase in spike frequency mentioned above, merely originates from another sensory cell (group III) as can be seen in Fig. 3B. Such a response to eggwash was found in 16 out of 22 recordings. This cell, which sometimes also responded to stimulation with salt, probably corresponds with what Ma & Schoonhoven (1973) called the S_2-cell.

Also glucotropaeolin evokes a marked increase in spike frequency, when compared to the salt stimulation (Fig. 2C). The amplitude of these spikes originating from the glucosinolate-cell (Ma & Schoonhoven, 1973) appeared to be somewhat smaller than those responding to eggwash (see Fig. 3C). In this recording the salt-sensitive cell has ceased from firing, although NaCl is still present in the stimulus solution.

When a tarsal B-hair is stimulated with a mixture of sinigrin and eggwash, several groups of three different spike-amplitudes, apart from the mechanoreceptor, can be recognized. This in contrast to the responses to sinigrin and eggwash (both dissolved in 0.05 M NaCl) alone, in which only two spike-types were seen. The recordings shown in Fig. 4 illustrate this additional indication that the responses to glucosinolates and eggwash originate from different chemosensory cells.

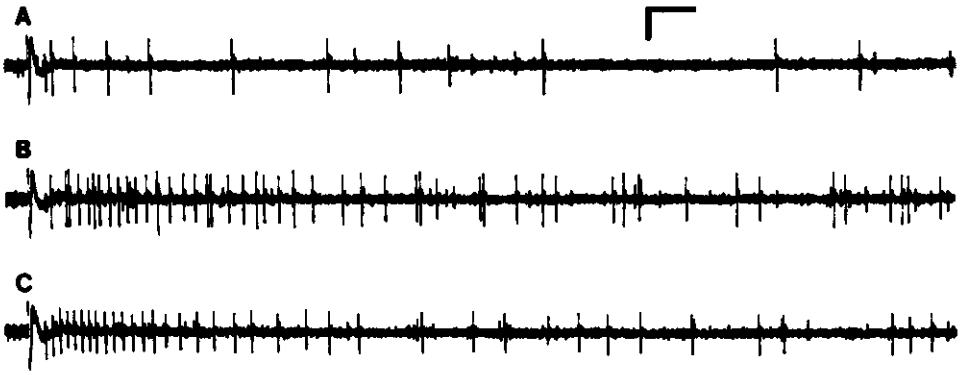

Fig. 2. Representative responses of one tarsal taste hair to (A) 0.05 NaCl, (B) eggwash (1000 ee/ml) and (C) 0.02 M glucotropaeolin. Stimuli B and C were dissolved in 0.05 M NaCl. Calibration: 0.8 mV; 50 ms.

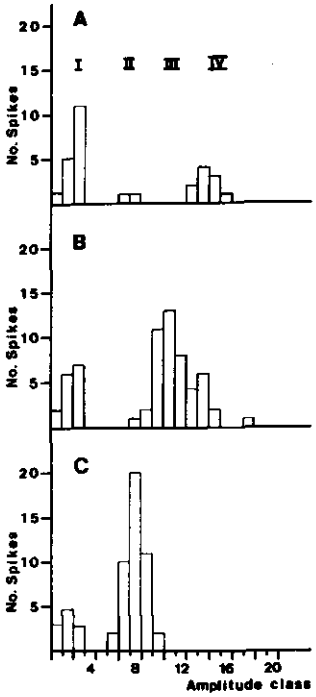

Fig. 3. Amplitude-histogram of spikes occurring in the initial second of the recordings displayed in Fig. 2. (A) salt; (B) eggwash; (C) glucotropaeolin. Roman numerals I to IV indicate four groups of amplitude classes. See text for further explanation.

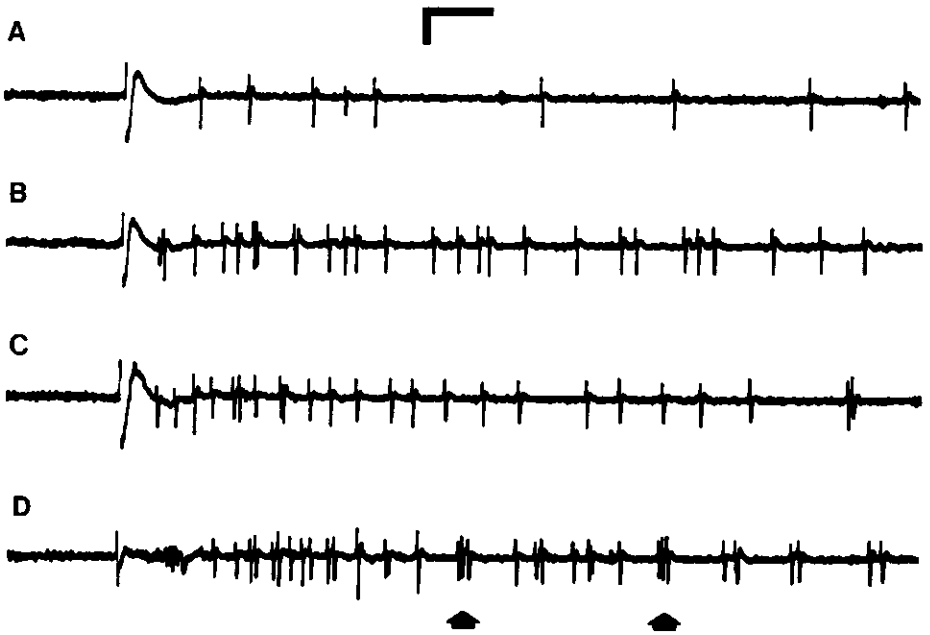

Fig. 4. Responses of one tarsal taste hair to (A) 0.05 M NaCl, (B) eggwash (250 ee/ml), (C) 0.025 M sinigrin and (D) a mixture of eggwash (250 ee/ml) and 0.025 M sinigrin. Stimuli B, C and D were dissolved in 0.05 M NaCl. Arrows indicate groups of three different spike-amplitudes. Calibration: 0.8 mV; 25 ms.

Abdominal contactchemoreceptors

Morphology

In *Pieris brassicae*, the ovipositor consists of two valves, one on each side of the ovipore, each bearing a large number of dissimilarly shaped hairs (Fig. 5A). On both valves we found one group of hairs, located on a papilla close to the inner edge of the valve, looking quite different from th surrounding hairs. This group consists of 10-17 straight and relatively short hairs (approximately 90 µm) which are placed on sockets (Fig. 5B) and possess a blunt tip with an evident porelike structure (Fig. 5C). The outer morphology of these hairs suggests them to be contactchemoreceptors (Slifer, 1970). The invagination at the top of the socket, at which the hair inserts, allows the hair to be movable which indicates the presence of a mechanoreceptor.

There exists a large variation in the shape of the papillae and the positions of hairs on the papillae among individual females. Even in a single female the number of hairs present on left and right papilla are not necessarily the same.

Fig. 5A. Caudal view at the ovipositor of *Pieris brassicae*. Arrow indicates one group of abdominal taste hairs. Bar = 100 µm.

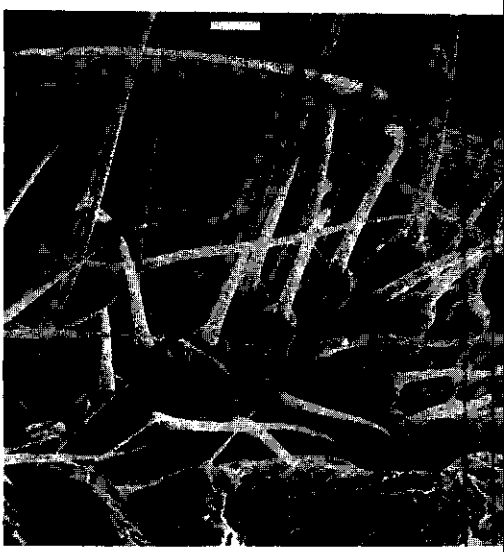

Fig. 5B. Details of one group of taste hairs. Bar = 20 µm.

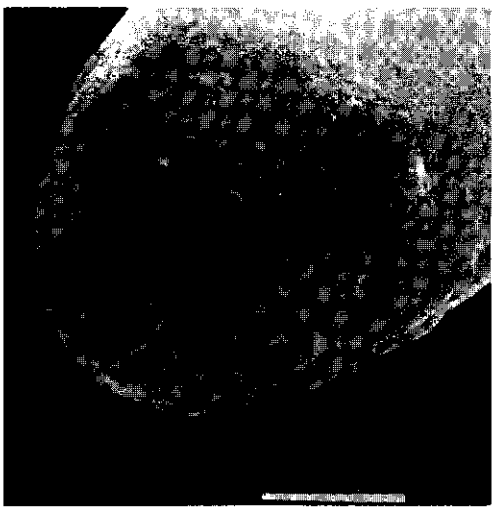

Fig. 5C. Top structure of one abdominal taste hair. Bar = 1 µm.

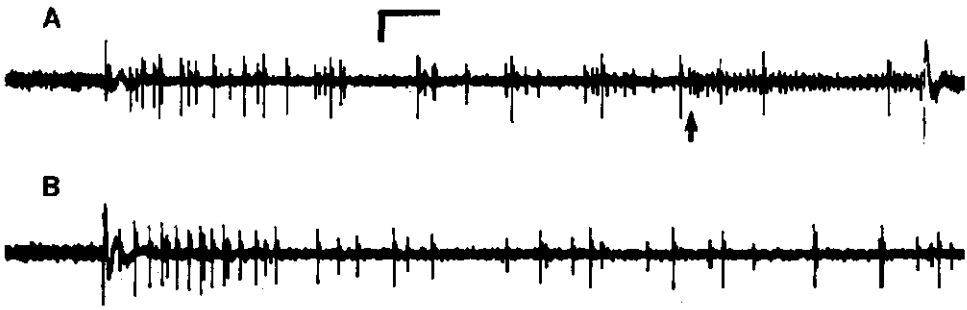

Fig. 6. Abdominal taste hair responses to (A) 0.1 M NaCl and (B) eggwash (500 ee/ml), dissolved in 0.1 M NaCl. Arrow indicates bending of the hair, inducing mechanoreceptor activity. Calibration: 0.5 mV; 50 ms.

Electrophysiology

Of all hairs present on the ovipositor only the short blunt tipped hairs show electrophysiological responses when contacted with a micropipette. The response to salt (Fig. 6) indicates that at least three receptor cells are present in these sensilla. Occasionally a fourth spike could be distinguished, but in most recordings the differences between the various spike amplitudes were too small to discriminate four distinct sense-cells. Bending the hair

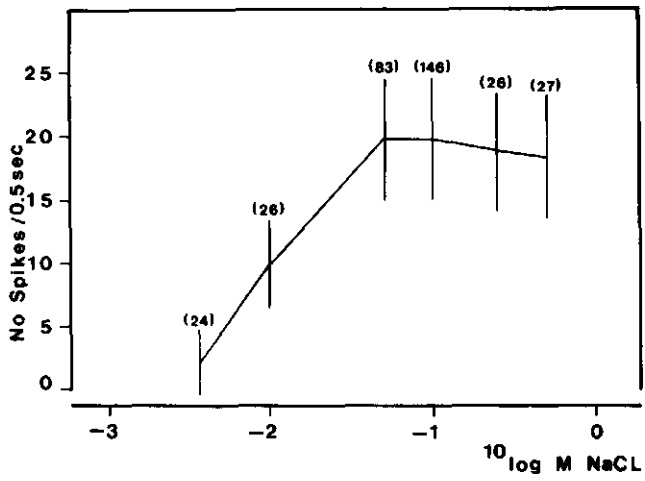

Fig. 7. Dose-response curve of abdominal taste hairs to NaCl. Ordinate displays the numbers of spikes occurring in the initial 500 ms of stimulation. Vertical bars indicate standard deviations. Between parentheses the numbers of recordings at each concentration are given.

Table 1. Mean no. spikes (± S.D.) in abdominal taste hairs during the first 500 ms of stimulation with various concentrations of eggwash. Control stimulations represent the response to 0.1 M NaCl. In each recording (hair) the response to eggwash was compared to the salt response and the differences were statistically tested by means of the sign-test.

	no. recordings	mean no. spikes ± S.D.	
Control		17.5 ± 5.2	
	48		$p < 0.005$
1000 ee/ml		22.0 ± 8.4	
Control		18.6 ± 4.4	
	27		$p < 0.005$
500 ee/ml		25.0 ± 6.7	
Control		19.8 ± 5.6	
	42		$p < 0.01$
250 ee/ml		22.6 ± 6.8	
Control		17.1 ± 4.7	
	76		N.S.
125 ee/ml		18.0 ± 4.5	

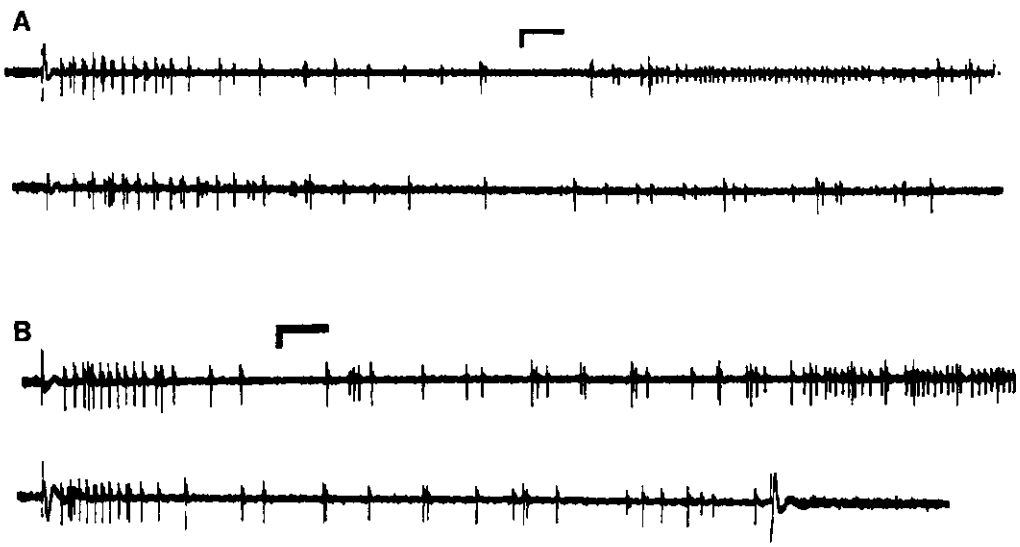

Fig. 8. Responses of abdominal taste hairs to glucosinolates. (A) upper trace: 0.1 M NaCl; lower trace: 0.02 M sinigrin in 0.1 M NaCl. (B) upper trace: 0.1 M NaCl; lower trace: 0.012 glucotropaeolin in 0.1 M NaCl. Calibration: 0.5 mV; 50 ms.

induces an increase in spike frequency of the smallest spike (arrow, Fig 6), indicating that this spike arises in a mechanosensory cell. The other spikes originate from chemosensory cells which show low and irregular frequencies in response to NaCl.

Responses to various concentrations of NaCl are given in Fig. 7. Spike-activity increases with stimulus concentration until at 0.05 M NaCl a maximum spike-frequency is reached. Beyond this concentration the response of the salt sensitive cells remains at a constant level.

Responses of these hairs to eggwash show a slight increase in total spike-frequency as compared to the salt response (Fig. 6). This increase, which appeared to be statistically significant for the three highest concentrations of eggwash tested (see Table 1), cannot be ascribed to increased activity of one particular sense cell.

Stimulation with solutions of sinigrin or glucotropaeolin evokes responses which do not differ from the corresponding control (salt) stimulations (Fig. 8). With respect to the stimuli mentioned above, we found no indication for the existence of different response-spectra among these abdominal taste hairs.

DISCUSSION

The responses of tarsal B-hairs we obtained after stimulation with salt and glucosinolates correspond quite well with the results published by Ma & Schoonhoven (1973). They also found a weak reaction of one or two sense cells (S_1 and S_2 cell) to salt and a very specific response, originating from one sensory cell (G-cell) to glucosinolates.

The finding of Behan & Schoonhoven (1978) that eggwash evokes a marked increase in spike frequency in the tarsal B-hairs is confirmed by our results. In addition we established the fact that in the majority of stimulations with eggwash only one cell type increased its activity. Spike-amplitude discrimination and especially the responses to mixtures of sinigrin and eggwash, clearly indicate that different sense cells are responsible for the sensitivity of tarsal B-hairs to eggwash and sinigrin. The chemosensory cell sensitive to eggwash probably corresponds with the cell Ma & Schoonhoven (1973) called the S_2 cell, indicating that the oviposition deterring pheromone rather than salt seems to be the adequate stimulus for this sense cell. It should be remembered,

however, that eggwash may contain besides the pheromone many other compounds which also might stimulate the chemosensory cells. It nevertheless seems likely that tarsal contactchemoreceptors play an important role in the perception of the oviposition deterring pheromone. This indicates that the pheromone contains at least one (relatively) non-volatile component.

The ovipositor of *Pieris brassicae* is provided with one paired group of chemosensory hairs located close to the ovipore in such a way that these hairs may contact the leaf surface each time an egg is laid. Electrophysiological recordings have shown that at least two chemosensory cells and a mechanosensory cell are present in these hairs, although additional ultrastructural studies are needed to determine the actual number of sense cells.

Abdominal sensory information may influence oviposition behaviour in several ways. The presence of mechanosensory cells enables females to assess physical characteristics of potential oviposition substrates. For instance, female potato tuber moth, *Phthorimea operculella* are known to possess abdominal mechanoreceptors and were found to prefer surface depressions and hairy substrates for oviposition (Fenemore, 1978). In *Pieris brassicae*, however, behavioural observations have shown that females are not so critical with respect to leaf texture and lay their eggs on almost any part of the plant.

Secondly, abdominal mechanoreceptors probably have a function in the formation of egg-batches. Deposition of compact monolayered clusters of eggs can only be accomplished when females gather sensory information on the positions of previously laid eggs. In *Bombyx mori*, tactile hairs on the anal papillae were found to be essential in egg-batch formation (Yamaoka *et al.*, 1971). Chadha & Roome (1980) suggest a similar function for abdominal tactile hairs in *Chilo partellus*. In *Pieris brassicae*, it can be observed that females, each time an egg is laid, first probe the leaf surface with the extruded ovipositor and, on finding previously laid eggs, readily oviposit adjacent to these eggs.

The presence of contactchemoreceptors on the female terminalia has been described in a number of other insect species. Some entomophagous hymenopterans utilize abdominal chemoreceptors to distinguish between parasitized and non-parasitized hosts (Vinson, 1972; Ganesalingam, 1974). In the desert locust, *Schistocerca gregaria*, oviposition is partly guided by sensory information on soil quality, acquired by chemosensilla located at the ovipositor (Norris, 1968).

Abdominal contactchemosensory hairs were also found in the migratory locust, *Locusta migratoria* (Rice & McRae, 1976),the flea beetle *Attica lythri*

(Phillips, 1978) and in the lepidopteran species *Chilo partellus*, *Spodoptera littoralis* (Chadha & Roome, 1980), *Phthorimea operculella* (Valencia & Rice, 1982) and *Eldana saccharina* (Waladde, 1983).

The ovipositor of the sheep blowfly, *Lucinia cuprina*, is provided with a number of chemosensilla containing at least three neurons sensitive to various chemicals (Rice, 1976). One of these neurons was found to be specifically sensitive to monovalent cations (Rice, 1977). Rice suggests that these receptors play a role in the selection of suitable egg-laying sites. Waladde (1983) found chemosensilla, present on the ovipositor of *Chilo partellus*, to be sensitive to various salt solutions. Stimulation with 0.05 M NaCl evoked complex spike-patterns from at least three chemosensory cells. The response to 0.005 M NaCl was characterized by the presence of a large number of small spikes, not occurring at higher concentrations, which he suggested to be the response of a water sensitive cell. In *Pieris brassicae*, at least two chemosensory cells can be recognized in the responses to salt. In contrast to Waladde we did not find different spike-amplitudes at low concentrations of NaCl. In addition, the dose-response curve (Fig. 7) shows that, in *Pieris*, the total spike frequency decreases with the concentration of salt. Therefore, we think that the ovipositor of *P. brassicae* is not provided with a water sensitive cell. The function of salt sensitive cells on the ovipositor of *Pieris brassicae* remains unclear. Perhaps they might act, as Chadha & Roome (1980) suggest in *Chilo partellus*, as a final defence against oviposition on surfaces, chemically harmful to eggs or young larvae.

The responses to glucosinolates clearly indicate that these taste hairs are not very likely to be involved in hostplant recognition. This finding supports the results of behavioural experiments, conducted by Jermy & Szentesi (1978), who found the role of abdominal sensory information in oviposition substrate selection of *P. brassicae* to be negligible. In addition Traynier (1979) documents that *Pieris rapae*, a related species with almost the same range of hostplants and in which we found similar chemoreceptors on the ovipositor (unpublished results), neither employs abdominal sensory information in hostplant selection.

Stimulating these hairs with eggwash, we only found a slight increase in total spike-frequency. The maximum increase, occurring at 500 ee/ml, was about 5 spikes in the first 500 msec. Still lower responses were found at the other concentrations of eggwash. This not very specific response might have been due to the nature of the stimulus. Perhaps eggwash, which may contain many other

compounds, is not the optimal formulation of the ODP to stimulate the abdominal taste hairs. This supposition is contradicted, however, by the tarsal responses we found to eggwash. The responses of abdominal sensilla to eggwash might have been influenced also by the relative high concentration of salt in which eggwashes were prepared. As can be seen in Fig. 7, this concentration (0.1 M) already lies beyond the concentration at which a maximum spike-frequency can be observed. If for example the ODP and NaCl stimulate the same sensory cell(s), such a high concentration of salt might interfere with a possible perception of the pheromone.

On the other hand one may argue that also small increases in spike-frequency might be sufficient to females. Convergence of sensory information may lead to amplification of the signal (van Drongelen, 1980). When this takes place from all 20-34 taste hairs present on the ovipositor of *P. brassicae*, such summed responses could easily influence female behaviour. Whether or not the increased spike frequency observed with eggwash is due to stimulation by the ODP remains for the time being unsettled.

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5 MODIFICATION OF PRE-OVIPOSITION BEHAVIOUR BY THE OVIPOSITION DETERRING PHEROMONE IN *PIERIS BRASSICAE*

ABSTRACT

Pre-oviposition behaviour of *Pieris brassicae* L. females was investigated in laboratory experiments. In order to determine the detailed effects of the oviposition deterring pheromone (ODP) of *Pieris brassicae* upon this behaviour we observed individual females in a choice situation with cabbage leaves treated with ODP and control leaves. The various behavioural components displayed by females were recorded separately on magnetic tape. Afterwards the behavioural protocols were analysed for several parameters including the sequence of behavioural acts. Our observations indicate that ODP at best induces only minor changes in pre-alighting behaviour of females and that there is hardly any distance effect of the pheromone. Most important differences between the two leaves were found during post-alighting behaviour. After landing on the treated leaf, the number of females that proceeded with *curving* after *drumming* was much lower as compared to the control leaf. Also the probability that a female will leave again after *drumming* was found to be significantly higher on the treated leaf. Among others, these findings implicate that females perceive ODP already during *drumming*. The behaviour displayed by females after *curving*, was also found to be different between the two leaves, suggesting that also during *curving* ODP may be perceived. Since during both steps females contact the leaf with their tarsi, it is concluded that the tarsal

contactchemoreceptors will mediate the behavioural response to ODP. The role of abdominal contactchemoreceptors in the perception of the pheromone could not be clarified in these experiments. Finally, we observed a reduced tendency of females to stay on or around the treated leaf, suggesting that, besides a reduction of oviposition, the ODP additionally may induce other dispersion effects in *Pieris brassicae* females.

INTRODUCTION

Distribution of eggs by *Pieris brassicae* L. females over potential host-plants is affected by previously laid eggs. Rothschild & Schoonhoven (1977) demonstrated in laboratory and greenhouse experiments that gravid females, when having a choice, prefer to lay their eggs on cabbage leaves devoid of conspecific eggs. Besides the colour of eggs, they found a chemical factor to be responsible for the deterrent effect. Behan & Schoonhoven (1978) demonstrated that this chemical factor, which was called the oviposition deterring pheromone (ODP), could be isolated by washing *Pieris brassicae* eggs in water. When sprayed onto cabbage leaves, this eggwash reduced egglaying in a similar way as intact eggs (see also Klijnstra, 1982). Thus in a choice situation with control leaves and leaves treated with eggwash, the gross effect of the oviposition deterring pheromone is a reduction in numbers of eggs laid on the treated leaves. More detailed effects of the pheromone upon the various behavioural components, which can be distinguished in pre-oviposition behaviour of *Pieris brassicae* females, are not known yet. Table 1 displays a qualitative description (ethogram) of this behaviour. Performed in the sequence indicated in Table 1, the various behavioural steps ultimately lead to the deposition of eggs. When as a general result, oviposition is reduced, ODP thus should affect in some way the occurrence and/or the appearance of one or more behavioural components.

Table 1. Qualitative description of pre-oviposition behaviour of *Pieris brassicae* females in the laboratory.

ETHOGRAM		SENSES (POSSIBLY) INVOLVED
APPROACH	♀ flies in rather straight line to the leaf, sometimes interrupted by "turning" i.e. a sudden change in flight-direction (away from the leaf)	vision olfaction
↓		
LANDING	the first contact with the leaf by the tarsi, often immediately followed by	(olfaction) tarsal taste hairs
↓		
DRUMMING	alternate tapping movements on upper leaf surface with fore-tarsi, accompanied by wing fluttering	(olfaction) tarsal taste hairs
↓		
CURVING	♀ bends her abdomen around the edge of the leaf without touching lower surface	taste hairs on tarsi and ovipositor
↓		
TOUCHING	touching lower leaf surface with the extruded ovipositor	taste hairs on tarsi and ovipositor mechanoreceptors on ovipositor
↓		
OVIPOSITION	deposition of the eggs, one by one, in a batch	mechanoreceptors and taste hairs on ovipositor

Table 1 additionally displays the (chemo) sensory systems that might mediate the various behavioural steps. Studying the detailed modifications of pre-oviposition behaviour induced by ODP, we might get inside in the mechanism of perception of the pheromone. Untill now, this mechanism is only partly understood. Electro-antennograms, conducted by Behan & Schoonhoven (1978) indicated that females may perceive the pheromone by antennal olfactory hairs but single-cell recordings on these hairs did not reveal the presence of specifically tuned sense cells to the odour of eggs (Den Otter *et al.*, 1980). Tarsal contactchemoreceptors also may be involved in the perception of the pheromone. In the so-called tarsal B-hairs, eggwash elicits a rather specific response which probably originates from one sensory cell (Klijnstra & Roessingh, submitted). In addition *Pieris brassicae* females possess a limited number of contact-chemosensory hairs on the ovipositor (Klijnstra, 1982). When stimulated with eggwash, a slight increase in total spike frequency could be observed in these sensilla (Klijnstra & Roessingh, submitted). This means that also abdominal chemoreceptors might be sensitive to the oviposition deterring pheromone. Having three chemoreceptory organs at their disposal which females may use to perceive the pheromone, the question arises which organs they actually employ in ODP perception. To answer this question we conducted a series of experiments in which pre-oviposition behaviour of individual females was observed in fine detail. For this we selected a choice situation with a control and a treated leaf as the experimental set-up, because in such a situation the deterrent effect of ODP is demonstrated at best. By comparing the behaviour displayed on a pheromone treated leaf with that performed on a control leaf, the difference(s) then might indicate the moment(s) at which ODP influences female behaviour and the way how females perceive the pheromone.

MATERIALS AND METHODS

Experimental animals

Pieris brassicae females were obtained from a culture reared in the laboratory for many years on *Brassica oleracaea* L. var. *gemmifera* D.C. Butterflies were kept in large cages (80 x 100 x 80 cm), illuminated by mercury vapour lamps and additional daylight, in which they were allowed to feed on artificial flowers containing a 10% sucrose solution. Experimental females ranged in age from 4 to 14 days after emergence.

Experimental design

Oviposition behaviour of females (N = 24) was observed in choice experiments with matched pairs of leaves taken from the same plant. Females were given a choice between a treated leaf, sprayed with a solution of the oviposition deterring pheromone (ODP) and a control leaf, sprayed with the solvent only. Solutions of ODP were prepared by washing *Pieris brassicae* eggs for 5 minutes in pure methanol. Concentration of eggwash used in these experiments was 250 egg equivalents/ml (ee/ml). Leaves were sprayed at the underside only with 1 ml of eggwash and methanol respectively, by means of a Desaga° chromatography sprayer. Experiments were conducted in similar large cages as mentioned above on single females between 9.00 and 13.00 hour. Leaves, with their petioles in water, were placed opposite to each other, with a distance between them of about 40 cm, along the longitudinal axis of the cage. Temperature in the cage varied between 24° and 29°C.

Females used in the experiments were deprived of an oviposition substrate for one hour before the experiment. Selection of motivated females took place on their response of approach to a cabbage leaf which was shortly introduced into the cage. The female chosen was put apart in the experimental cage 15 minutes before the start of the experiment. Some females were used more than once on subsequent days. Female behaviour was observed from the moment of introduction of the leaves into the cage. Registration of behaviour started when the female approached one of both leaves and was ended after deposition of the first egg or after 10 minutes.

Recording equipment

Female behaviour was recorded by means of a custom-made system consisting of a keyboard, an interface and a Philips Minilog° cassetterecorder. The keyboard was provided with eleven keys divided in two groups of 5 and 6 keys respectively, each key representing a particular behavioural act. The interface translated the signals from the keyboard into a binary code and sent that code to the Minilog° recorder which was used in the four channel mode. The interface was additionally provided with 11 LED´ s (Light Emitting Diodes), one for each behavioural code. As long as a key was depressed the corresponding LED alighted and the corresponding code was recorded on tape. During playback of the tape the LED´ s were used to get a first indication of the frequencies of the various acts in the recording.

For further processing behavioural data were introduced into a PDP 11

minicomputer which, by means of especially written software, added a timebase to each alteration of behaviour and stored these data onto a discette. Sampling frequency of this programme was 100 Hz. Behavioural data were analysed by means of a number of Fortran programmes which were run on a DEC-10 computer.

Registration of pre-oviposition behaviour

The behavioural components of pre-oviposition behaviour displayed by the females were recorded separately for the control leaf and the treated leaf by assigning one group of keys to each leaf. The various behavioural acts which were recorded and the code numbers by which they were registered are shown in Table 2. In comparison to Table 1, *landing* and *touching* are omitted and *leaving* is added as a separate act in Table 2. Preliminary observations have revealed that in most cases *landing* is immediately followed by *drumming*. This indicates that duration of *landing* does not seem to be an important parameter. And whereas the frequency of *landing* can be readily deduced from the frequency of "*not-landing*" (i.e. the transition *approach* to *leaving*), there is no necessity of assigning a key especially to *landing*. *Leaving* was registered as a separate behavioural act, because, in our opinion, it might be an important step indicative for the moment females perceive the ODP. The absence of *touching* in Table 2 is due to the limited number of keys that was available. *Curving* and *touching* were combined to one step, which was recorded as *curving*. *Rest on leaf* was encoded by the same key for both leaves. They could be easily separated, however, during analysis of the recordings by looking at the preceding behavioural acts.

Table 2. Components of pre-oviposition behaviour that were recorded and code numbers assigned to them.

Behavioural step	Code	
	Control leaf	Treated leaf
APPROACH	1	6
DRUMMING	2	7
CURVING	3	8
OVIPOSITION	4	9
LEAVING	5	10
REST ON LEAF	11	11
START/STOP	14	

A special combination of 2 keys depressed simultaneously, resulted in code 14, which was used to indicate the beginning and the end of a recording.

Analysis of pre-oviposition behaviour

We were interested in the moment(s) at which ODP has its influence on pre-oviposition behaviour. Therefore, a comparison was made between female behaviour displayed on the control leaf and the behaviour on or around the treated leaf. The following parameters were analysed in more detail: (1) The percentage responding, which represents the percentage of females that have displayed the various behavioural components, regardless of their frequency; (2) Leaf choice of females at which the first performance of various behavioural acts was observed; (3) The mean frequencies of acts; (4) The mean bout-lengths of acts, which were calculated per female per leaf by dividing the duration of a particular code by its frequency.

Furthermore, we considered the sequence of acts. For this, first order transitions between the various behavioural components were considered. This means that only the relation between two consecutive acts was studied. In the first place we compared the number of females that displayed similar transitions on control and treated leaf. This parameter was called (5) the percentage responding of transitions. In the calculation of these percentages only those females were included that had actually displayed the first behavioural component of the transition. In the second place, the sequence of pre-oviposition behaviour was analysed by calculating, per female, (6) the conditional probability of each transition. The mean conditional probability of a particular transition on the control leaf was then compared with that of the corresponding transition on the treated leaf. Differences were tested for significance by means of the Mann-Whitney U test and the values given for p are one-tailed probalities.

RESULTS

Percentage responding

Approach, as the first behavioural step, was found to be displayed on both leaves by approximately the same number of females (Fig. 1). When looking at *drumming* the same can be said, although the difference between control and

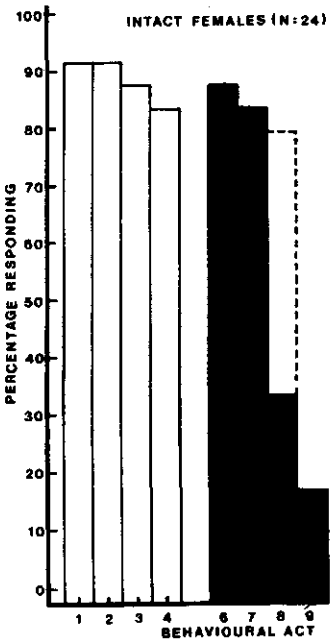

Fig. 1. Number of females (expressed as % responding) that displayed the various behavioural acts indicated at the abscissa. 1 and 6: *approach*; 2 and 7: *drumming*; 3 and 8: *curving*; 4 and 9: *oviposition*. White columns: control leaf; Black columns: treated leaf. Dotted line: see explanation in text.

treated leaf is somewhat larger. For *curving*, on the contrary, a large difference between the two leaves was found. On the control leaf, 21 females (87.5%) were observed to perform *curving* whereas only 8 females (33.3%) performed this step on the treated leaf. Compared to what was found on the control leaf, we expected for *curving* on the treated leaf a percentage responding of 79.2% (dotted line, Fig. 1). The number of females starting *oviposition* on the control leaf was 20 (83.3%), whereas the remaining 4 females (16.7%) laid their first egg on the treated leaf. This difference was statistically tested by means of the sign-test (which is allowed in this case, because the behavioural codes 4 and 9 are mutually exclusive) and appeared to be highly significant ($p = 0.001$).

Leaf choice of females for first performances

The figures on leaf choice of females for the first performance of several behavioural acts are given in Table 3. With regard to *approach* and *drumming* females exhibited no preference for one or the other leaf. The first bout of *curving*, however, is displayed more often on the control leaf (siginificant at 5% level). For *oviposition* the distribution of first bouts is already given

Table 3. Number of females that choose the control or treated leaf for the first performance of various behavioural acts.

Behavioural step	Control leaf	Treated leaf	
APPROACH	12	12	n.s.*
DRUMMING	13	11	n.s.*
CURVING	17	7	p=0.032*
OVIPOSITION	20	4	p=0.001*

*Sign test, one-tailed probabilities; n.s.: not significant

of course by the percentage responding (cf. Fig. 1).

Frequencies of acts

The mean frequencies of the various behavioural components displayed by the females are given in Table 4. For each female we first determined the difference in frequency of a particular behavioural act between control and treated leaf and then these differences were tested by means of the Wilcoxon matched-pairs signed-ranks test (Siegel, 1956). This procedure was followed because of the large variation we observed in the total duration of the protocols, and with it the frequency of acts, between individual females. The standard deviations given in Table 4 illustrate this variation. For all behavioural components, except for *oviposition*, the differences between control and treated

Table 4. Mean frequencies (±S.D.) of the behavioural components displayed by the females (n=24).

Behavioural step	Control leaf	Treated leaf	
APPROACH	8.46 ± 8.96	7.25 ± 9.19	n.s.**
DRUMMING	6.00 ± 6.67	4.79 ± 6.11	n.s.**
CURVING	1.25 ± 0.90	0.75 ± 1.22	n.s.**
OVIPOSITION	0.83	0.17	p=0.001*
LEAVING	7.63 ± 9.02	7.08 ± 9.06	n.s.**
REST ON LEAF	1.17 ± 1.24	0.75 ± 1.07	n.s.**

*Sign test, one-tailed probability

**Wilcoxon matched-pairs signed-ranks test; n.s.: not significant

Table 5. Mean bout-lengths (± S.D.) in seconds of some behavioural components displayed by (n) females on control- and treated leaf.

Behavioural step	Control leaf	Treated leaf	
APPROACH	1.01 ± 0.38 n = 22	0.83 ± 0.40 n = 21	p=0.0418*
DRUMMING	1.60 ± 0.68 n = 22	1.44 ± 0.80 n = 20	n.s.*
CURVING	7.89 ± 17.53 n = 21	4.32 ± 3.91 n = 8	n.s.*

*Mann-Whitney U test, one-tailed probability; n.s.: not significant

leaf were found to be not statistically significant. *Oviposition*, as the final act in pre-oviposition behaviour and in our recordings, could only be displayed once per female, either on the control leaf or on the treated leaf. Therefore, the figures for *oviposition* in Table 4 correspond exactly with the percentage responding given in Fig. 1.

Bout-lengths of acts

In Table 5 mean bout-lengths are given for *approach*, *drumming* and *curving*. Not all 24 females did perform these behavioural components on both leaves, therefore the number of females (n) for which bout-length could be computed is given in each compartment of Table 5. For this reason the Mann-Whitney U test was used in this case for testing the differences between control and treated leaf. Considering *approach* this difference appeared to be significant (at 5% level). When approaching the treated leaves, females thus interrupt this flight somewhat sooner as compared to approach-flight to the control leaf. The bout-length of *drumming* was about equal at both leaves. For *curving* we found a clear difference between control and treated leaf, but we also found a large standard deviation, especially on the control leaf. This variation merely originated from one female, which performed code 3 only 2 times with a total duration of 167.6 seconds. Therefore, the mean bout-length of *curving* was not found to be significantly longer on the control leaf.

Percentage responding of transitions

The numbers of females that displayed the various transitions are shown

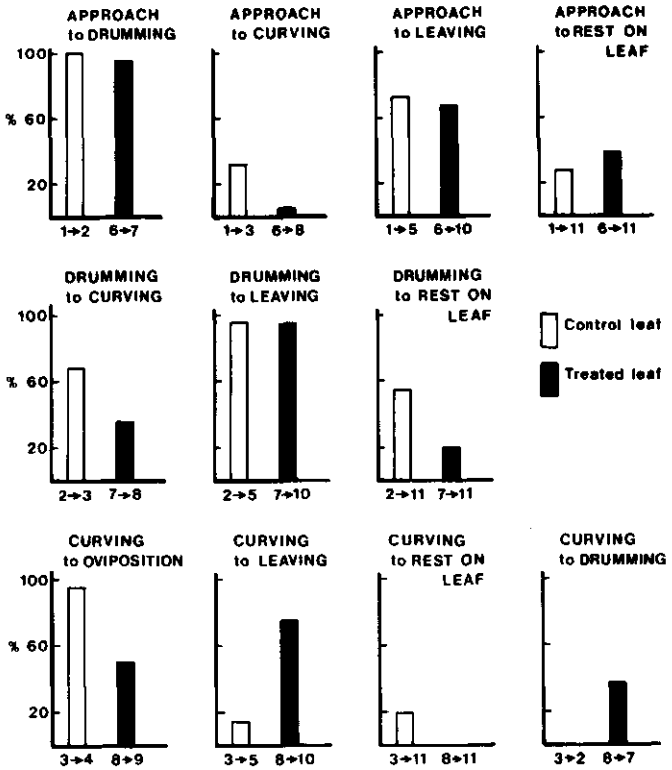

Fig. 2. Number of females (expressed as percentage responding) that have displayed the various behavioural transitions indicated at the abscissa. For each transition the number of females that corresponds with 100% responding is dependent on the number of females that displayed the first behavioural component of the transition (cf. fig. 1) and may therefore be different.

in Fig. 2. In this figure and in the following the notation A → B indicates the transition of behavioural code A to behavioural code B. The transition *approach* to *drumming* (1 → 2 on the control leaf and 6 → 7 on the treated leaf) was displayed by almost the same number of females. With regard to *approach* to *leaving* (1 → 5 vv 6 → 10) neither a substantial difference between the two leaves was found. When we look at *drumming* to *curving*, however, there is a marked difference. About twice as much females perform this transition on the control leaf. A similar difference between control and treated leaf was found for the transition *curving* to *oviposition*. On the treated leaf, a considerable number of females apparently decides after *drumming* not to continue with *curving*. And of those females that did perform *curving* on the treated leaf (only 8 females, see Fig. 1),50% did not continue with *oviposition*.

There are some more transitions for which marked differences between the two leaves can be observed. On the control leaf a larger number of females displayed *approach* to *curving*. For *curving* to *leaving*, on the other hand, far more females were seen to perform this transition on the treated leaf. The transition *curving* to *drumming*, a striking one because of its direction which is opposite to the other transitions, only occurred on the treated leaf. Furthermore, we found that after contact with the treated leaf, a smaller number of females continued with *rest on leaf*, as compared to the control leaf (transitions 2 → 11 vv 7 → 11 and 3 → 11 vv 8 → 11). No significant difference between the two leaves was observed in the number of females that displayed *leaving* after *drumming* (2 → 5 vv 7 → 10).

Conditional probabilities of transitions

A flow diagram of the behaviour of intact females in a laboratory choice situation is displayed in Fig. 3. Fifty % of the females start with *approach* on the control leaf and the other half on the treated leaf (as indicated already in Table 3). The probability that *approach* is followed by *drumming* is rather high on both leaves. A similar tendency can be observed when we look at the summed probabilities of *approach* to *drumming*, *approach* to *curving* and *approach* to *rest on leaf*. Thus the chance that *approach* will be followed by *landing* on the leaf is 78.0% on the control leaf and 74.0% on the treated leaf. After *drumming*, larger differences can be observed. The probability that a female continues with *leaving* after *drumming* is high on the control leaf, but still (and significantly) higher on the treated leaf($p = 0.0129$). For *drumming* to *rest on leaf*, on the other hand, the probability of 2 → 11 appeared to be significantly larger ($p = 0.0087$) than that of 7 → 11.

Only in about 1 out of 6 times *drumming* is followed by *curving*. For this transition we found no significant difference between the two leaves. When *curving* is displayed on the control leaf, there is a high probability that it will be followed by *oviposition* (82.1%). On the treated leaf, this probability is only 20.8% (highly significant, $p = 0.00011$). On the latter leaf, females more readily continue with *leaving* after *curving* as compared to the control leaf (also highly significant, $p = 0.0009$). Another striking difference between the two leaves which was already mentioned in the former section, is the observation that on the treated leaf females may perform *curving* to *drumming* with a mean conditional probability of 22.9%, whereas on the control leaf this transition does not occur at all.

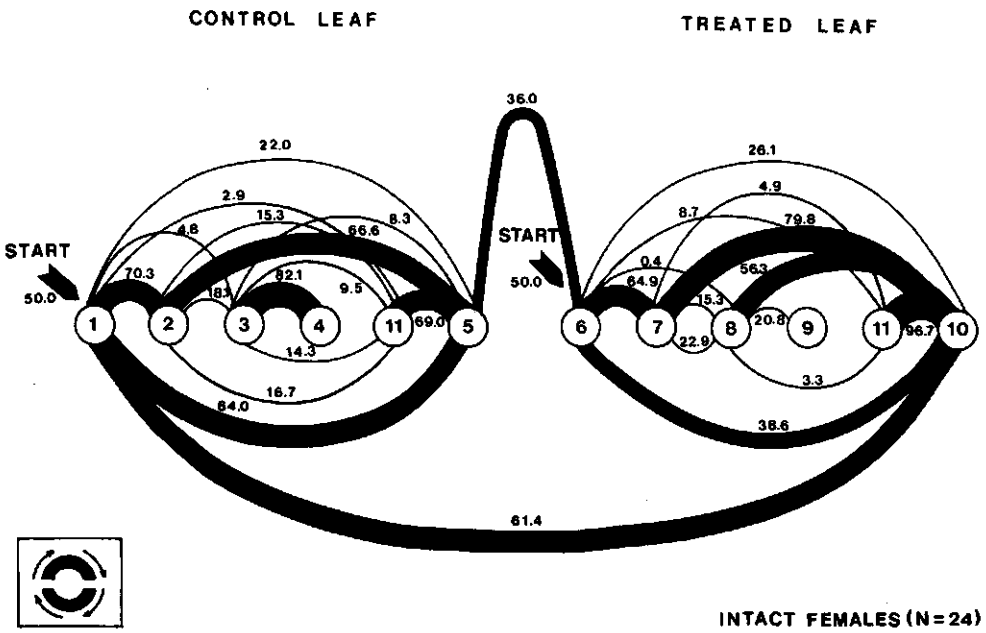

Fig. 3. Sequence of oviposition behaviour of intact *P. brassicae* females in a laboratory choice situation. The numbered circles represent the various behavioural components (cf. Table 2). Numbers along the bands are the mean conditional probabilities (%) of a particular transition between two behavioural codes. Probabilities > 30% are additionally depicted by the thickness of the band; below 30% only single lines are drawn. Behaviour flows from left to right in the upper bands and from right to left in the lower bands. START: percentage of females performing the first attempt of *approach* on control or treated leaf.

Females were also found to behave differently after *leaving*. A female that left the control leaf more readily performed 5 → 1 (64.0%) rather than 5 → 6 (36.0%). On the treated leaf, on the other hand, the probability of 10 → 1 (61.4%) appeared to be much higher than the probability of 10 → 6 (38.6%). Females thus perform a tendency to stay around or to return to the control leaf. When we compare the conditional probabilities of the transitions 5 → 1 vv 10 → 6 indeed a significant difference is found ($p = 0.0082$). Finally, we also observed significant differences between the conditional probabilities of 1 → 3 and 6 → 8 ($p = 0.0099$) and between 11 → 5 and 11 → 10 ($p = 0.0136$).

DISCUSSION

Structure of pre-oviposition behaviour

The left part of the sequence diagram, given in Fig. 3, illustrates how females behave in the absence of the oviposition deterring pheromone. When approaching the leaf, there is a high probability that females will land on that leaf and perform *drumming*. After *drumming*, however, females mostly continue with *leaving* and then *approach* the same leaf again. Females thus do not follow the sequence indicated in the ethogram (Table 1) linearly, but they rather probe the leaf a few times only until *drumming*, before continueing with further behavioural steps on the leaf. Drumming behaviour has been described in a number of other butterfly species (e.g. Fox, 1966; Calvert, 1974, see Chew & Robbinns, in press, for further references). Fox (1966) suggested that a combination of leaf-abrading spines and chemosensilla on female fore-tarsi, which also occurs in *Pieris brassicae* (Ma & Schoonhoven, 1973), permits females to detect plant compounds. The tarsal taste hairs in *Pieris brassicae* females are known to possess a number of sensory cells, specifically sensitive to mustard-oil glucosides (Ma & Schoonhoven, 1973). These secondary plant compounds (of Cruciferae), however, are mainly located within the plant tissue. The drumming movements of the fore-legs then might serve to improve the contact between leaf chemicals and chemoreceptors and thus facilitate the perception of plant compounds. The pattern of repeated *drumming* attempts we observed, perhaps indicates that one bout of *drumming* does not provide enough sensory information of a leaf's suitability as an oviposition substrate. Calvert & Hanson (1983) describe a similar repeated drumming behaviour in *Chlosyne lacinia* females.

The transition *drumming* to *curving* seems to be a crucial step in the behavioural sequence. Once this step is performed, the high probability of 3 → 4 indicates that in most cases oviposition will follow.

The behavioural pattern described above represents pre-oviposition behaviou of *Pieris brassicae* females in the laboratory. In the field it might very well be possible that females behave differently. For example, the observed recurrent *approach* of the same leaf might be induced by the fact that only a choice between two leaves was given to the females. In a field plot, where numerous plants may be available females perhaps do not return to the same plant. Until now, however, detailed behavioural observations in the field have not been conducted.

Pre-alighting effects of ODP upon pre-oviposition behaviour

The effects of the oviposition deterring pheromone upon pre-oviposition behaviour may be seperated in two parts: before and after contact with the leaf has been made. We will first consider possible distance effects of the pheromone. Our results indicate no clear distance effect of ODP. The number of females performing *approach* (Fig. 1) is about equal for both leaves. The percentage of females first approaching the treated leaf is exactly 50 percent and also the mean frequency of *approach* does not differ significantly between control and treated leaf. Considering the mean bout-length of *approach* a small significan difference was found. This finding, however, should be considered with some caution, because it sometimes appeared to be difficult to determine the beginning of *approach*. The duration of *approach* therefore, might have been influenced also by our own interpretation of female flight behaviour. The sequential parameters of pre-alighting behaviour support the previous findings. When looking at the number of females that did not land after *approach* (*approach* to *leaving*, Fig. 2) no difference between the two leaves was found. Also *approach* to *drumming* was performed by about the same number of females on both leaves. Only the number of females continueing with *curving* immediately after *approach* appeared to be substantially higher on the control leaf. Fig. 3 shows that also the conditional probabilities of transitions starting with *approach*, except for *approach* to *curving*, do not differ significantly. From the results described above, we thus may conclude that at best ODP induces only minor changes in pre-alighting behaviour and that a distance effect of the pheromone must be considered as doubtful.

Post-alighting effects of ODP upon pre-oviposition behaviour

The major differences in behaviour displayed on control and treated leaf respectively, were found after alighting. The low percentage of females that was observed to perform *curving* on the treated leaf and the clear preference of females to perform the first bout of *curving* on the control leaf suggest that females may perceive the pheromone already during *drumming*. The lower percentage responding of the transition *drumming* to *curving* (Fig. 2) we observed on the treated leaf agrees with this. For a large number of females, post-alighting behaviour on the treated leaf thus only consists of *drumming*. About one third of the females, however, still proceed with *curving* on the treated leaf. Perhaps those females did not perceive ODP well enough to

interrupt the behavioural chain at that time. Or perhaps they possessed a high egglaying motivation, overriding any sensory input evoked by the pheromone. The conditional probability of 7 → 8, given in Fig. 3, is of course only based on behavioural data from these females. Therefore, it does not surprise that the difference between the conditional probabilities of 2 → 3 and 7 → 8 was not found to be statistically significant. Another indication that most females perceive the pheromone during *drumming* is the higher probability of *drumming* to *leaving* we found on the treated leaf. This transition was displayed by a similar number of females on both leaves (Fig. 2).

Important differences in post-alighting behaviour were also observed after *curving*. Fig. 1 shows already that a distinct majority of females deposit their first egg on the control leaf. When we look at the sequential parameters, indeed a reduction in number of females performing 8 → 9 was found (Fig. 2). And if *curving* was displayed on the treated leaf, the probability of being followed by *oviposition* was only 20.8% (Fig. 3). Instead of *oviposition*, females mostly continued with *leaving*.

Whereas the most important behavioural differences were found after contact with the leaf, we may conclude that females employ contactchemoreceptors in ODP perception. During *drumming*, only the tarsal taste hairs are in contact with the leaf and, considering their significant sensitivity to eggwash (Klijnstra & Roessingh, submitted) they thus will mediate the behavioural response to the pheromone. The sensory systems involved with *curving* cannot be designated directly. When *curving* is displayed by females, chemoreceptors present on the ovipositor (Klijnstra, 1982) might come into play and then female behaviour might also be influenced by abdominal chemosensory input. However, electrophysiological recordings have shown that the abdominal taste hairs respond rather weakly when stimulated with eggwash (Klijnstra & Roessingh, submitted). Moreover, *curving* and *touching* (see Table 1) were not recorded separately, so we cannot determine the number of times females actually touched the treated leaf with the ovipositor. Although in the literature some examples of contactchemoreceptors responding to odours are known (Städler & Hanson, 1975), this probably does not apply here considering the low response already to contact stimulation with ODP and the low volatility of the pheromone. Therefore, we may say that contactchemoreceptors on the ovipositor are unlikely to be involved in the perception of the pheromone.

During *curving*, females hang around the edge of the leaf with their tarsi still in contact with that leaf. We therefore think that also during *curving*

tarsal chemosensory input determines the behavioural response to ODP. This input might originate from the fore-legs as well as from mid- and hind-legs. Ma & Schoonhoven (1973) already demonstrated the presence of similar B-hairs on mid- and hind-tarsi in *Pieris brassicae* females but until now, these hairs have not been studied electrophysiologically. That females indeed perform drumming movements with the fore-legs to enhance the perception of chemical cues may be illustrated by the occurrence of the transition *curving* to *drumming* on the treated leaf.

We observed other effects that may be ascribed to the oviposition deterring pheromone. Although the frequency of *rest on leaf* did not appear to be significantly higher on the control leaf (table 2), both the number of females performing 7 → 11 and the conditional probability of 7 → 11 were found to be lower than the corresponding parameters on the control leaf. It seems likely that females do not like to stay on the treated leaf. In addition, we observed a tendency of females to stay around or to return to the control leaf, as explained already in the results section. These results should be interpreted with caution, however, because they might have been induced by the experimental conditions in which females were observed. Additional experiments need to be done before we may conclude that ODP also stimulates dispersion.

There were four females (16.7%, Fig. 1) observed to oviposit on the treated leaf. Why did these females, despite the presence of ODP, select the treated leaf for their first egg? We can only guess to an answer. Two females did not perform any behavioural step on or around the control leaf. Perhaps they did not even notice the presence of another leaf. The rather short time they took to deposit their first egg on the treated leaf, however, suggests that they did not have many problems in accepting this leaf as an oviposition substrate. The other two females have contacted both leaves before they decided to oviposit on the treated leaf. Perhaps these four females were less sensitive to the oviposition deterring pheromone or perhaps some unknown leaf factors (in the control leaves) were responsible for this deviant behaviour. Deviant behaviour, as a matter of fact, has also been observed in laboratory bioassays with the pheromone, in which groups of females were offered a similar choice between control and treated leaves. Even a very high concentration of eggwash (1000 ee/ml) did not suppress oviposition on the treated leaf completely (Klijnstra, 1982). "Mistaken" choices of oviposition sites, in the sense that females select oviposition substrates that are not suitable somehow for larval development, are known to occur in a number of butterfly species (see references in Chew &

Robbins, in press). One example of such a "mistake" is that females may oviposi on suitable hostplants of insufficient size to support complete larval development (e.g. Dethier, 1959; Chew, 1977). Our observations of "deviant" behaviour described above might be classified in the same category. Whether one may consider this behaviour as a real mistake can of course only be established in follow-up studies on larval survival.

Among other insect species (see Prokopy, 1981 a for a comprehensive review the apple maggot fly *Rhagoletis pomonella* has also been reported to employ an oviposition deterring pheromone (Prokopy, 1972). Similar to the ODP in *Pieris brassicae*, this pheromone appeared to be water- and methanol-soluble and of low volatility (cf. Schoonhoven *et al.*, 1981; Prokopy, 1981 b). Ablation experiments by Prokopy & Spatcher (1977) have indicated that *Rhagoletis pomonella* females may perceive the pheromone only after contact, probably by fore-tarsal taste hairs. Electrophysiological recordings indeed established a significant sensitivity of these contactchemoreceptors to ODP solutions (Crnjar *et al.*, 197 So there exists a remarkable conformity between *Rhagoletis pomonella* and *Pieris brassicae* with regard to the properties and behavioural effects of their conspecific ODP's. Whether this means that both ODP's contain similar chemical compounds is not known. In both species the identification of the pheromone has not been succeeded yet.

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6 INACTIVATION OF CHEMORECEPTORS AND ITS EFFECTS ON THE PERCEPTION OF THE OVIPOSITION DETERRING PHEROMONE IN *PIERIS BRASSICAE* ADULTS

ABSTRACT

Pre-oviposition behaviour of *Pieris brassicae* L. females with various sensory ablations was investigated in the laboratory in order to determine the role of various chemoreceptors in oviposition and in the perception of the oviposition deterring pheromone (ODP). Individual females were observed in a choice situation with a cabbage leaf treated with ODP and a control leaf. The various behavioural components which can be distinguished in pre-oviposition behaviour were recorded separately on magnetic tape. The behaviour displayed by females on control and treated leaf was compared and analysed, employing a computer, for several parameters including the sequence of behavioural acts. The role of abdominal chemoreceptors in the perception of the ODP was investigated in another type of behavioural experiments.

When all six tarsi, whether or not in combination with the antennae, are inactivated (by HCl treatment), oviposition is almost completely suppressed. Further analysis of the behaviour of these females revealed that they do not discriminate between a control leaf and an ODP treated leaf. This indicates that sensory information on the suitability of a plant species as an oviposition substrate is most important to females.

In females with intact chemoreceptors on at least one pair of tarsi, oviposition was about normal and also discrimination between the two types of leaves was found to occur. The perception of the oviposition deterring

pheromone is not limited to one particular sensory system. Females may employ antennal olfactory hairs, fore-tarsal taste hairs as well as contactchemoreceptors on mid- and hind-tarsi to perceive this pheromone. In addition, we found that antennal chemoreception of the ODP does not only take place at a distance from the leaf, but also during post-alighting behaviour on the treated leaf. Contactchemoreceptors on the ovipositor of *Pieris brassicae* females are not involved in the perception of the oviposition deterring pheromone. Finally, it was demonstrated that females do not respond to the ODP anymore, once the first egg has been laid. The absence of a behavioural response is probably accomplished by a central inhibition, blocking the sensory input from peripheral chemoreceptors.

INTRODUCTION

The oviposition deterring pheromone (ODP) of *Pieris brassicae* L. influences female pre-oviposition behaviour in such a way that, in a choice situation with control and ODP treated cabbage leaves, as an overall result oviposition on the treated leaf is strongly reduced (Rothschild & Schoonhoven, 1977; Behan & Schoonhoven, 1978; Klijnstra, 1982). The detailed effects of the ODP upon the various behavioural components which can be distinguished in pre-oviposition behaviour (see Table 1), are discussed in the previous chapter. A comparison of female pre-oviposition behaviour on a control leaf and an ODP treated leaf respectively, revealed that most apparent differences in behaviour can be observed after alightment. On the treated leaf females were found to behave differently after *drumming* and after *curving*, as compared to the control leaf, indicating that the ODP may be perceived during these behavioural steps. In electrophysiological studies of the fore-tarsal contactchemoreceptors, indications were obtained for the presence of one sensory cell specifically sensitive to the oviposition deterring pheromone (Klijnstra & Roessingh, submitted). Therefore, it seems likely that during *drumming* these chemoreceptors are involved in the perception of ODP. However, the ODP treated leaf was sprayed with an eggwash solution at the underside only. And females

Table 1. Qualitative description of pre-oviposition behaviour of *Pieris brassicae* females in the laboratory.

ETHOGRAM		SENSES (POSSIBLY) INVOLVED
APPROACH ▼	♀ flies in rather straight line to the leaf, sometimes interrupted by "turning" i.e. a sudden change in flight-direction (away from the leaf)	vision olfaction
LANDING ▼	the first contact with the leaf by the tarsi, often immediately followed by	(olfaction) tarsal taste hairs
DRUMMING ▼	alternate tapping movements on upper leaf surface with fore-tarsi, accompanied by wing fluttering	(olfaction) tarsal taste hairs
CURVING ▼	♀ bends her abdomen around the edge of the leaf without touching lower surface	taste hairs on tarsi and ovipositor
TOUCHING ▼	touching lower leaf surface with the extruded ovipositor	taste hairs on tarsi and ovipositor mechanoreceptors on ovipositor
OVIPOSITION	deposition of the eggs, one by one, in a batch	mechanoreceptors and taste hairs on ovipositor

usually perform *drumming* at the upperside of the leaf. Further investigations are thus needed for a fully understanding of the mechanism of ODP perception during this stage of pre-oviposition behaviour.

In addition to this, we would like to know more details on a possible role of mid- and hindtarsi in the perception of the pheromone. On meso- and metathoracic tarsi female *Pieris brassicae* also possess contactchemoreceptors, similar to those on the fore-tarsi (Ma & Schoonhoven, 1973). The electrophysiological sensitivity of these sensilla to various stimuli has not yet been investigated, but it could very well be that these hairs are also sensitive to the ODP. Therefore, it was hypothesized in the previous chapter, that taste hairs on the mid- and hindtarsi are involved in the perception of the ODP durir *curving*. This suggestion requires additional experimental evidence.

To clarify the questions mentioned above, pre-oviposition behaviour of females with various sensory ablations was observed. The experimental set-up was identical to that described in chapter 5, i.e.: single females were offered a choice between a control and an ODP treated leaf and the behaviour of females was recorded on magnetic tape. First, we will determine if females with a particular ablation still can discriminate between a control and a treated leaf. If so, a detailed comparison of the behaviour displayed on control and treated leaf respectively, should reveal the moment(s) in the behavioural chair at which females recognize the treated leaf and thus perceive the pheromone. From these findings the sensory system(s) involved in the perception of the oviposition deterring pheromone might be deduced.

Another interesting point which needs further investigation is the possible role of abdominal chemoreceptors in female responses to the ODP. Contactchemoreceptors, present on the ovipositor of *Pieris brassicae* females, were found to be slightly sensitive to eggwash solutions, containing the oviposition deterring pheromone (Klijnstra & Roessingh, submitted). The electrophysiological findings alone do not provide sufficient evidence to allow the conclusion that females also employ these chemosensilla in ODP perception. Additional behavioural evidence is required. Ablation of the abdominal chemoreceptors was not attempted because this might interfere too much with oviposition. Another type of behavioural experiments, called the dual leaf tests, was conducted to establish the role of chemoreceptors on the ovipositor in the response of females to the oviposition deterring pheromone.

MATERIALS AND METHODS

Experimental animals

Pieris brassicae females were obtained from a culture reared in the laboratory for many years on *Brassica oleracaea* L. var. *gemmifera* D.C. Butterflies were kept in large cages (80 x 100 x 80 cm), illuminated by mercury vapour lamps and additional daylight, in which they were allowed to feed on artificial flowers containing a 10% sucrose solution. Experimental females ranged in age from 4 to 14 days after emergence. Females with several sensory ablations were used. Preliminary experiments revealed that cutting off the antennae or one or more legs, interfered too much with the locomotory abilities of females. Therefore, ablations were carried out by dipping the various chemosensory structures for 40 seconds into a Pasteur-pipette containing a 5 M HCl solution. In this way, seven groups of females were prepared for the behavioural experiments, namely: 1) females in which the antennae were treated with HCl (A-females); 2) females with inactivated fore-tarsi (FT-females); 3) females with both the antennae and fore-tarsi inactivated (AFT-females); 4) females in which the antennae, mid- and hindtarsi were treated with HCl (AMH-females); 5) females with all six tarsi inactivated (T-females); 6) females in which the antennae and all six tarsi were inactivated (AT-females); 7) females in which both antennae and the six tarsi were treated with distilled water. This group of females served as controls (CON-females). In the first set of experiments (see below) a group of intact females was also included.

After the behavioural experiments, a (small) number of females from several groups was submitted to an electrophysiological check of the ablation. For this, we used the tip-recording technique as described in Klijnstra & Roessingh (submitted). In the (relatively small) number of sensilla tested of all females with a particular sensory ablation, no electrophysiological responses were found after stimulation with sinigrin. In the control females, the response to sinigrin (10^{-4}M) appeared to be normal (cf. Klijnstra & Roessingh, submitted). In addition to this, the electrophysiological tests revealed that the mechanosensory cells, which are present in many chemosensilla, were not inactivated by the HCl treatment.

Ablation experiments

Two types of ablation experiments were carried out. In the first place,

groups of females with a particular sensory ablation were offered a choice between a control leaf and an ODP treated leaf in order to determine egglaying activity of these females. Both cabbage leaves were taken from the same plant and sprayed at the underside only with 1 ml of methanol and eggwash respectively by means of a Desaga° chromatography sprayer. Eggwash solutions, containing the oviposition deterring pheromone, were prepared by washing *Pieris brassicae* eggs for 5 minutes in pure methanol. Concentration of eggwash used in the experiments was 250 egg-equivalents per ml solvent. Experiments were conducted in similarly large cages as mentioned above during the morning hours. Leaves, with their petioles in water, were placed opposite to each other, with a distance between them of 40 cm, along the longitudinal axis of the cage and were exposed to the females for one hour. The temperature in the cage varied between 25^0 and 29^0 C. After one hour, the number of eggbatches and eggs laid on both leaves were counted and egglaying activity determined. The behavioural activity of eggwash was expressed in terms of percentage deterrence calculated in the following way: % deterrence = (A-B)x100/A+B, where A and B represent the numbers of eggbatches (or eggs) laid on control and treated leaves, respectively. This percentage deterrence was determined in order to get a first impression on the discriminatory abilities of the various groups of females.

In the second place, pre-oviposition behaviour of individual females was observed in similar choice experiments with control and ODP treated cabbage leaves. Females used in these experiments were deprived of an oviposition substrate for one hour before the experiment. Selection of motivated females took

Table 2. Components of pre-oviposition behaviour that were recorded and code numbers assigned to them.

Behavioural step	Code	
	Control leaf	Treated leaf
APPROACH	1	6
DRUMMING	2	7
CURVING	3	8
OVIPOSITION	4	9
LEAVING	5	10
REST ON LEAF	11	11
START/STOP	14	

place on their response of approach to a cabbage leaf, which was shortly introduced into a cage containing a group of females with a particular ablation. The female chosen was put apart in the experimental cage 15 minutes before the start of an experiment. In these single trials, each female was used only once. Female behaviour was observed from the moment of introduction of the leaves into the cage. Registration of behaviour started when the female approached one of both leaves and was ended after deposition of the first egg or after 10 minutes.

Registration of pre-oviposition behaviour

Female behaviour was recorded by means of a custom-made system consisting of a keyboard, an interface and a Philips Minilog° cassetterecorder. The keyboard was provided with eleven keys, divided in two groups of 5 and 6 keys respectively, each key representing a particular behavioural act. For a detailed description of the recording equipment see chapter 5.

The behavioural components of pre-oviposition behaviour displayed by the females were recorded separately for the control leaf and the treated leaf by assigning one group of keys to each leaf. The various behavioural acts which were recorded and the code numbers by which they were registered are shown in Table 2.

Analysis of pre-oviposition behaviour of individual females

We were interested in the abilities of the various groups of females to discriminate between a control leaf and an ODP treated leaf. Therefore, a comparison was made between female behaviour displayed on the control leaf and the behaviour on or around the treated leaf. The following parameters were analysed in more detail: (1) The percentage responding, which represents the percentage of females that has displayed the various behavioural components, regardless of their frequency; (2) The mean frequencies of acts. For each female we first determined the difference in frequency of a particular behavioural act between control and treated leaf and then these differences were tested statistically by means of the Wilcoxon matched-pairs signed-ranks test (Siegel, 1956). Between groups of females the mean frequencies of acts were compared employing the Student's T-test; (3) The sequence in which the behavioural steps were displayed by females. For this, first order transitions between the various behavioural components were considered. This means that only the relation between two consecutive acts was studied. By means of a Fortran

computer-programme we first determined the frequencies of the various first order transitions per female and from this the conditional probabilities were calculated for each transition. In the calculation of these probabilities only those females were included that had actually displayed the first behavioural component of the transition. The mean conditional probability of a particular transition on the control leaf was then compared with that of the corresponding transition on the treated leaf. Differences between the two leaves were tested for significance by means of the Mann-Whitney U test. Between the various groups of females the conditional probabilities were also compared using the Mann-Whitney U test.

Dual leaf tests

These experiments were also conducted in large cages, now containing only one blanc cabbage leaf standing in a glas vial filled with water. Single females were released in the cage and allowed to land on that leaf. At the moment the female started to perform *curving*, a small cabbage leaflet was manipulated between the blanc leaf and the extruded ovipositor. In this way, females performed *touching* (see Table 1) on the leaflet, while all the tarsi were still in contact with the blanc leaf. Spraying the leaflet with eggwash allowed us to stimulate exclusively the abdominal taste hairs with ODP. The leaflets were sprayed at upper- and lowerside with in total 0.3 ml methanol eggwash, containing 500 ee/ml. Control leaflets were sprayed with 0.3 ml methanol. When *touching* occurred, we called this one oviposition attempt and from this moment the behavioural responses of females were observed. Selection of motivated females was done in the same way described above. Most females were allowed to perform several attempts of oviposition.

RESULTS

Egg-laying activity

Egg-laying activity of ablated females was determined in choice experiments with groups of females, having a choice between a control and an ODP treated lea The results of these experiments are displayed in Table 3. Expressed as number of eggbatches and eggs per female, a large reduction in oviposition was found when all six tarsi, whether or not in combination with the antennae, were inactivated. Females with intact fore-tarsi only (AMH-females) and females with

Table 3. Egglaying responses of *P. brassicae* females with various sensory ablations in choice experiments with control and ODP-treated cabbage leaves. Abbreviations of female groups: see materials and methods.

Females	no. ♀♀ per exp.	no. exp.	no. eggbatches/eggs laid on leaves		no. eggbatches/eggs per ♀ per exp.	% deterrence
			control	treated		
INTACT	20	14	73/2856	15/340	0.31/11.4	65.9/78.7
CON	9	6	28/860	5/120	0.61/18.1	69.7/75.5
A	6	5	11/332	4/90	0.50/14.1	46.7/57.3
FT	6	4	13/367	0/0	0.54/15.3	100 / 100
AMH	6	4	8/379	0/0	0.33/15.8	100 / 100
T	6	9	3/180	0/0	0.06/ 3.3	100 / 100
AT	9	9	4/148	2/57	0.07/ 2.3	33.3/44.4

inactivated antennae or fore-tarsi (A-females and FT-females respectively) laid comparable numbers of eggbatches and eggs as intact and control females. From Table 3, it might be deduced that females perform a tendency of laying smaller eggbatches on ODP treated leaves. Possible ODP effects on the size of eggbatches, however, have been discussed in chapter 2 and will not be included in this paper which is concerned with the influence of ODP on pre-oviposition behaviour.

Discriminatory ability

The percentage deterrence given in the last column of Table 3, reflects the ability of females to discriminate between the two leaves and thus the ability to perceive the oviposition deterring pheromone. Percentages found in intact and control females are about equal and correspond very well with those found in earlier experiments (see chapter 2). The discriminatory ability of females seems to diminish somewhat when the antennae are inactivated. A further reduction of the percentage deterrence was found in the AT-females. In contrast to this, females with inactivated tarsi only (T-females) seem to discriminate much better between the two leaves, even better than the intact and CON-females. In both T- and AT-females, however, egglaying activity is much lower as compared to the other groups. A direct comparison of the percentages deterrence

found in these two groups, to those of the other groups therefore is difficult.

FT- and AMH-females are each antipoles with regard to the chemosensory equipment they have available. Nevertheless, percentage deterrence was found to be the same in both groups and, in addition, very high, indicating that females with these sensory ablations may discriminate even better than intact and control females.

The general conclusion which can be drawn from Table 3, is that all groups of females more or less show a positive discrimination with respect to the control leaf. The observed differences in percentage deterrence do not indicate a particular sensory structure to be indispensable for the perception of the pheromone. Percentages deterrence, however, only provide a global insight into the capabilities of females to discriminate between the two leaves. The distribution of eggbatches over the two leaves, from which percentage deterrence is inferred, only represents the ultimate result of female choice behaviour. More detailed information on female choice behaviour and the ability to discriminate is obtained by observing pre-oviposition behaviour of single ablated females.

Percentage responding

The first parameter determined is the number of females in all groups that have displayed the various behavioural components, i.e. the percentage respondin (Fig. 1). In the control females a large difference was found in the number of females performing corresponding behavioural steps on control and treated leaf. *Approach* and *drumming* already were less often observed on the treated leaf but especially for *curving* and *oviposition* the difference is very clear. On the control leaf, the difference in number of females performing *drumming* (code 2) and *curving* (code 3) respectively is much smaller than the difference between *drumming* (7) and *curving* (8) on the treated leaf. *Oviposition* could be observed on the control leaf only.

The A-females show, when compared to the control females, less pronounced differences between the two leaves. The number of females approaching the control and treated leaf is exactly the same. The number of females performing *drumming* is slightly lower at the treated leaf. A large difference, although not as large as in the CON-females, was again found for *curving*, behavioural codes 3 and 8 respectively. In this group, 2 females (10,5%) were observed to start oviposition on the treated leaf.

The number of FT-females performing the various behavioural steps do not

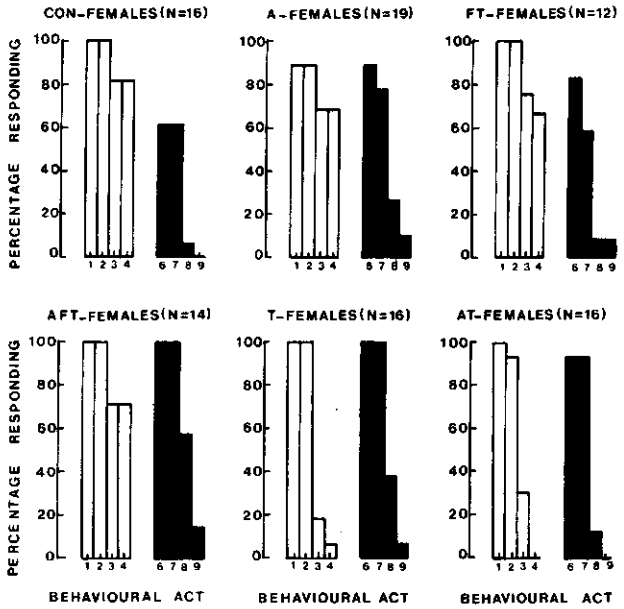


Fig. 1. Number of females (expressed as percentage responding) in each group that displayed the various behavioural acts indicated at the abscissa. 1 and 6: *approach*; 2 and 7: *drumming*; 3 and 8: *curving*; 4 and 9: *oviposition*. White columns: control leaf; Black columns: ODP treated leaf. Abbreviations of female groups: see Materials and Methods.

differ very much from those in the preceding groups. The percentage of females performing *drumming* on the treated leaf is slightly lower than that for *approach* to the same leaf. On the control leaf, we found these percentages to be equal. Again the number of females that displayed *curving* on the treated leaf is very low and in this group only 1 out of 12 females laid her first egg on the treated leaf.

In the AFT-females some differences to the former groups were found. Both leaves were approached by the same number of females (100%) and also *drumming* was displayed by all females on both the control and the treated leaf. We found a high percentage of females (57,1%) performing *curving* on the treated leaf. For this behavioural step, the difference between control and treated leaf is much smaller than those found in the other groups. With regard to *oviposition*, however, the AFT-females behave similarly as the other groups: the majority of females prefers to oviposit on the control leaf.

Females with all tarsi inactivated seem to make little difference between the two leaves. Except for *curving*, all behavioural steps are displayed by the same number of females on both leaves. On the control leaf, only 3 females (18,8%) were seen to perform *curving*: a very low percentage when compared to the preceding groups. The number of females that ultimately laid an egg was, as expected already, also very low: one female started ovipostion on the control leaf, another female on the treated leaf.

In the AT-females, an almost similar pattern can be observed. In this group, the number of females performing *curving* on the control leaf is somewhat higher than the percentage found on the treated leaf. But there still is a large difference between *drumming* and *curving* on the control leaf. In these individual trials, none of the AT-females was seen to lay an egg.

Frequencies of acts

The mean frequencies of the various behavioural components displayed by the females are given in Table 4. The control females perform the various behavioural steps more often on the control leaf. The variation in frequency for a particular step between individual females was very large, as illustrated by the standard deviations given in Table 4. Despite this large variation, the differences between the two leaves were found to be statistically significant for all behavioural steps, except *rest on leaf*.

In the A-females the differences in frequencies observed on the control and the treated leaf are smaller than those found in the CON-females. All behavioural components were displayed more often on the control leaf, but significant differences between the two leaves were only found for *curving* and *oviposition*. The frequencies with which the various behavioural components were displayed by A-females, however, do not differ significantly from those observed in the CON-females.

The mean frequencies of acts performed by females with inactivated fore-tarsi neither are significantly different from those in the control females. With regard to the differences between the corresponding behaviours on control and treated leaf respectively, FT-females show a similar pattern as the A-females: all behavioural acts were less often observed on the treated leaf but only for *curving* and *oviposition* significant differences were found.

When in addition to the fore-tarsi, the antennae are inactivated (AFT-females), the frequencies observed for several behavioural steps differ in some more respects. In contrast to the former groups, *approach*, *drumming* and

Table 4. Mean frequencies of acts (± S.D.) displayed by the various groups of females on both types of leaves. Figures 1 to 5 and 11 C represent the control leaf; 6 to 10 and 11 T the ODP-treated leaf.

	APPROACH		DRUMMING		CURVING		OVIPOSITION		REST ON LEAF		LEAVING	
	1	6	2	7	3	8	4	9	11 C	11 T	5	10
CON	8.69	⁑ 4.56	8.06	⁑ 3.31	1.50	⁑ 0.07	0.81	⁑ 0	0.88	0.50	7.88	⁑ 4.50
(N=16)	±9.12	±5.11	±7.02	±3.38	±1.46	±0.25	±0.40		±1.67	±1.15	±9.26	±5.09
A	8.45	6.89	5.68	4.58	1.32	⁑ 0.32	0.68	⁑ 0.11	0.89	0.89	7.74	6.79
(N=19)	±9.66	±7.87	±6.29	±5.08	±1.53	±0.58	±0.48	±0.32	±1.37	±1.63	±9.47	±7.91
FT	7.83	5.25	6.25	3.33	1.33	⁑ 0.17	0.67	⁑ 0.08	1.67	1.50	7.17	5.00
(N=12)	±6.77	±6.44	±4.49	±4.74	±1.07	±0.58	±0.49	±0.29	±2.02	±3.00	±7.04	±6.08
AFT	7.93	8.57+	5.36	7.14+	1.29	1.57+	0.71	⁑ 0.14	1.36	1.21	7.21	8.36
(N=14)	±5.78	±4.67	±4.03	±6.14	±1.13	±2.21	±0.47	±0.36	±2.56	±1.97	±5.77	±4.55
T	15.31	17.13+	12.38	15.63+	1.13	1.31	0.07+	0.07	2.63	3.63	15.06	16.75
(N=16)	±11.63	±11.59	±8.48	±11.56	±2.83	±3.00	±0.25	±0.25	±1.75	±2.53	±11.58	±11.45
AT	17.38	14.13+	14.50	11.25+	1.56	0.31	0+	0	2.63	2.00	16.94	14.06
(N=16)	±16.75	±11.64	±16.69	±10.23	±3.16	±1.01			±3.10	±1.93	±16.68	±11.69

⁑ indicates a significant difference in mean frequency of a particular behavioural component between control and treated leaf (Wilcoxon matched-pairs signed-ranks test; two tailed probability < 0.05).

+ significantly different from the value in CON-females in the same column (Student's T-test; two tailed probability < 0.05).

curving were all three more often displayed on the treated leaf, so the significant difference between codes 3 and 8 (*curving*) which is still present in both A- and FT-females, has disappeared in this group. Only *oviposition* happened to occur significantly more often on the control leaf. Another striking difference is found when we compare the AFT-females to the control females. As indicated in Table 4 the frequencies of the behavioural codes 6, 7 and 8 in AFT-females are significantly higher than those in the CON-females.

Inactivating all six tarsi of the females, we see a large increase in mean frequency, and also of the standard deviation, of *approach* and *drumming* on both leaves. Comparing the frequencies of these acts between the two leaves, the differences are not statistically significant. A comparison to the CON-females, however, reveals that such a difference is absent on the control leaf but present on the treated leaf for both *approach* and *drumming*. For *curving* on the treated leaf, there is a large difference between the T-females and the CON-females but, because of the large variation and the low number of females performing *curving* (see Fig. 1), not statistically significant. The frequency of *oviposition*, which in fact equals the number of females performing *oviposition* (see Fig. 1), is very low on both leaves. So, for *oviposition* there exists a significant difference between T- and CON-females for the control leaf.

The AT-females, finally, perform the various behavioural steps in similar frequencies as the T-females. Between the two leaves, no significant differences were observed and when we compare the AT-females with the control females, we find statistically significant differences for the same behavioural codes as in the group of females with inactivated tarsi only.

To summarize the results with respect to the mean frequencies of acts displayed by all groups of females: it seems as if the T- and AT-females do not sense any difference between the two leaves. The AFT-females only show a significant difference for *oviposition*, which suggests that these females may perceive the pheromone during *curving*. On the same line of reasoning then, the A- and FT-females, in which significant differences between the two leaves were found for both *curving* and *oviposition*, might perceive the pheromone already during *drumming*. This argument does not fit, however, on the behaviour displayed by the control-females. Here it should lead to the conclusion that the CON-females perceive the pheromone already before starting *approach*. Therefore a further analysis of the behavioural data is needed to determine the point at which the various groups of females perceive the pheromone.

Conditional probabilities of behavioural transitions

The conditional probability of a particular transition reflects the chance (in this case expressed as a percentage) that once the first behavioural step is displayed, the second step will follow. The mean conditional probabilities of all possible behavioural transitions in all groups of females are summarized in Table 5. In this table and in the following the notation A → B indicates the transition of behavioural step A to behavioural step B.

CON-females

The sequence of pre-oviposition behaviour of the control females is visualized in the flow diagram displayed in Fig. 2. Table 5 indicates that in the CON-females significant differences between the two leaves were found for several transitions. After *approach*, landing more readily occurs on the control leaf (1 → 2) as on the treated leaf (6 → 7). *Approach* to *leaving* (1 → 5 vv 6 → 10), as the complementary transition, is more likely to occur on the treated leaf. A similar pattern was observed for the transitions *drumming* to *curving* (2 → 3 vv 7 → 8) and *drumming* to *leaving* (2 → 5 vv 7 → 10). On the control leaf, CON-females more readily stay on the leaf and continue with post-alighting behaviour, wheras on the treated leaf females are more likely to leave again. The probabilities of the transitions *curving* to *oviposition* (3 → 4 vv 8 → 9) and *curving* to *leaving* (3 → 5 vv 8 → 10) differ largely between the two leaves. However, there was only one female observed to perform *curving* on the treated leaf (see Fig. 1), which is too low to allow a statistical test. Females were also found to behave differently after *leaving*. *Approach* of the same leaf again has a larger probability on the control leaf than on the treated leaf (5 → 1 vv 10 → 6). This of course implies that the difference in the probabilities of *leaving* to *approach* to the opposite leaf is also statistically significant. Thus, these females prefer to stay around or return to the control leaf. The results described above indicate that the control females may discriminate between the two leaves at least during *approach* and during *drumming*. Whether discrimination takes place during *curving* is difficult to say, because only few females perform this step on the treated leaf.

A-females

A flow diagram of pre-oviposition behaviour of these females is given in Fig. 3. In the A-females the probabilities of transitions starting with *approach* do not differ significantly between the two leaves (Table 5). This implies that females with inactivated antennae do not discriminate between both leaves from

Table 5. Mean conditional probabilities of first order transitions observed in pre-oviposition behaviour of P. brassicae females with various sensory ablations. Behavioural transitions, represented by figure codes (see Table 2), are divided into five groups according to the first behavioural component of the transition. Within a group a subdivision is made into pairs of corresponding transitions on control and treated leaf. The number of females that has performed the first behavioural step of a particular transition is given between parentheses only in the first two rows in each group but it also applies to subsequent rows in the same group.

	FEMALES					
	CON (N=16)	A (N=19)	FT (N=12)	AFT (N=14)	T (N=16)	AT (N=16)
1 → 2	0.916 * (16)	0.756 (17)	0.827 * (12)	0.660 (14)	0.806 (16)	0.670 (16)
6 → 7	0.718 * (11)	0.602 (17)	0.450 * (10)	0.695 (14)	0.850 (16)	0.726 (15)
1 → 3	0	0.022	0	0.024	0	0
6 → 8	0	0	0	0	0	0
1 → 5	0.084 *	0.220	0.173 *	0.266	0.152	0.264
6 → 10	0.282	0.373	0.541	0.294	0.099	0.237
1 → 11	0	0.002	0	0.050	0.043	0.066
6 → 11	0	0.025	0.009	0.011	0.050	0.038
2 → 3	0.303 * (16)	0.263 * (17)	0.341 * (12)	0.285 (14)	0.062 (16)	0.052 (15)
7 → 8	0.018 * (11)	0.083 (17)	0.048 (7)	0.120 (14)	0.076 (16)	0.017 (15)
2 → 5	0.620 *	0.596 *	0.435	0.534	0.649	0.676
7 → 10	0.847	0.792	0.701	0.674	0.640	0.647
2 → 11	0.078	0.141	0.224	0.182	0.290	0.272
7 → 11	0.135	0.126	0.251	0.206	0.284	0.336

3 → 2	0.206	(13)	0.031	(13)	0.241	(9)	0.150	(10)	0.433	(3)	0.160	(5)
8 → 7	0	(1)	0	(5)	0.500	(1)	0.510	(8)	0.472	(6)	0.500	(2)
3 → 4	0.778		0.749		0.630		0.700		<u>0.162</u>		<u>0</u>	
8 → 9	0		0.400		0.500		0.141	*	0.056		0	
3 → 5	0.015		0.167		0.074		0.150		<u>0.400</u>		<u>0.840</u>	
8 → 10	1.000		0.600		0		0.349		0.472		0.500	
3 → 11	0		0.054		0.056		0		0		0	
8 → 11	0		0		0		0		0		0	
11 → 2	0.200	(5)	0.025	* (8)	0	(8)	0.067	(5)	0.105	(13)	0.065	(9)
11 → 7	0.167	(3)	0.321	(7)	0.083	(4)	0.040	(5)	0.089	(14)	0.121	(11)
11 → 3	0		0.188		0.042		0.200		0		0	
11 → 8	0		0		0		0.200		0		0	
11 → 5	0.800		0.788		0.958		0.733		0.894		0.935	
11 → 10	0.833		0.679		0.917		0.760		0.911		0.879	
5 → 1	0.844	* (14)	0.651	(17)	<u>0.607</u>	(11)	<u>0.569</u>	(13)	<u>0.614</u>	(16)	<u>0.569</u>	(15)
10 → 6	0.583	(11)	0.489	(17)	<u>0.203</u>	* (10)	0.511	(14)	0.611	(16)	0.558	(15)
5 → 6	0.156	*	0.351		<u>0.393</u>	*	<u>0.431</u>		<u>0.386</u>		<u>0.431</u>	
10 → 1	0.417		0.511		<u>0.797</u>		0.489		0.389		0.442	

* indicates a significant difference between two corresponding transitions (between the two leaves) within the group of females. (Mann Whitney U test; two tailed probability < 0.05).

Figures underlined are significantly different from the value in CON-females in the same row (Mann Whitney U test; two tailed probability < 0.05).

a distance in contrast to control females. In the A-females, discrimination seems to take place after alightment, considering the significant differences between the transitions 2 → 3 and 7 → 8 and also between 2 → 5 and 7 → 10. After *drumming*, females more often continue with *curving* on the control leaf, whereas on the treated leaf a higher probability was found for *leaving*. When *curving* is displayed by A-females, there is a large difference between the two leaves in the probability that *oviposition* will follow (3 → 4 vv 8 → 9). However, because of the large variation we observed on the treated leaf, this difference is not statistically significant. A comparison with the control females reveals that on the treated leaf A-females more readily continue with *oviposition*, whereas on the control leaf the probability of *curving* to *oviposition* is about equal in both groups.

In A-females the transition *rest on leaf* to *drumming* is more likely to occur on the treated leaf. This difference was absent in the CON-females. Another interesting difference between the A-females and the control females is found when we compare the various transitions starting with *leaving*. The probability that the same leaf will be approached again (5 → 1 and 10 → 6) is lower on both leaves in the A-females and, consequently, the probability that an A-female will *approach* the opposite leaf is increased. The significant difference between the control and the treated leaf, which we found in the control females, has disappeared in this group.

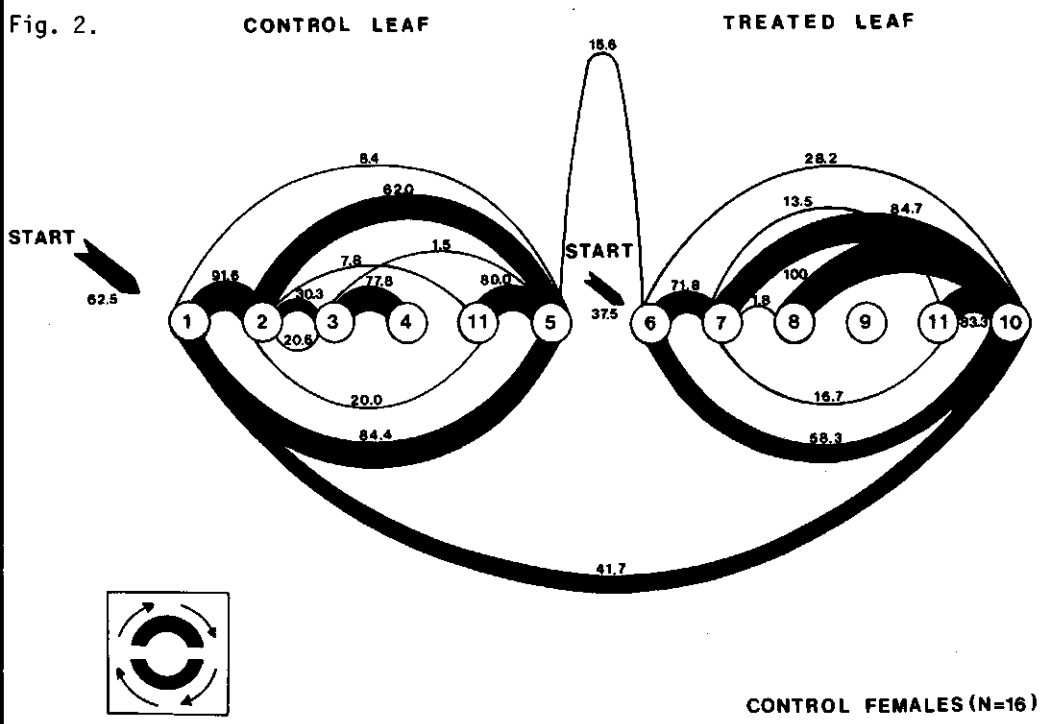
As we have seen in Table 3 already A-females may discriminate very well between the two leaves. The sequential analysis of the behaviour of these females described above suggests that discrimination will take place during *drumming*.

FT-females

Pre-oviposition behaviour of FT-females (flow diagram, see Fig. 4) shows

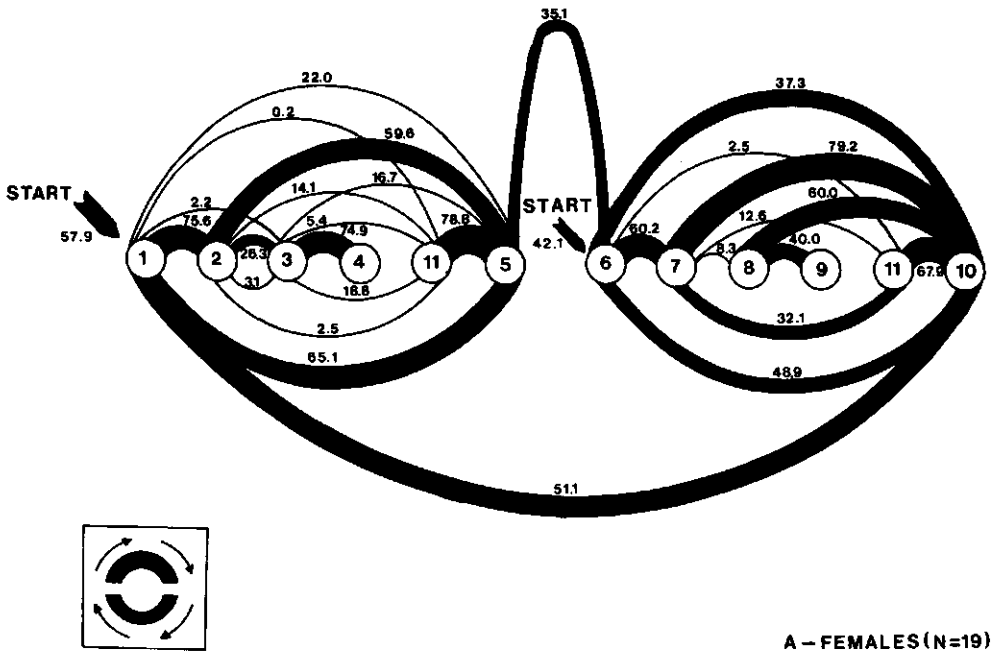
Fig. 2-7 (see next three pages). Sequence of pre-oviposition behaviour of *Pieris brassicae* females with various sensory ablations in a laboratory choice situation. The numbered circles represent the various behavioural components (cf. Table 2). Numbers along the bands are the mean conditional probabilities (%) of a particular transition between two behavioural codes. Probabilities > 30% are additionally depicted by the thickness of the band; below 30% only single lines are drawn. Behaviour flows from left to right in the upper bands and from right to left in the lower bands. START: percentage of females performing the first attempt of *approach* on control or treated leaf.

Fig. 2.



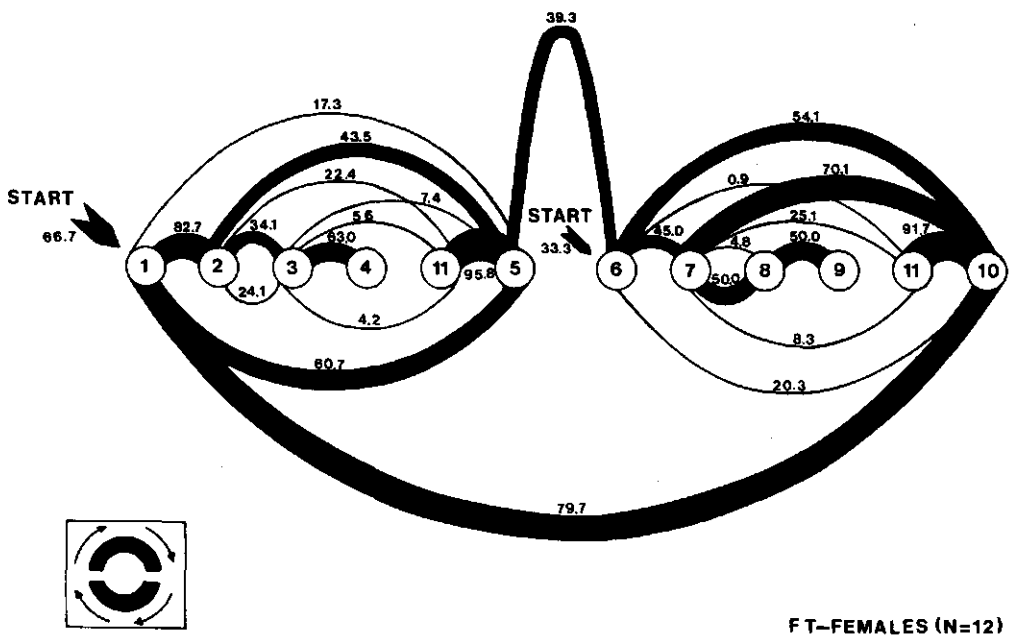
CONTROL FEMALES (N=16)

Fig. 3.



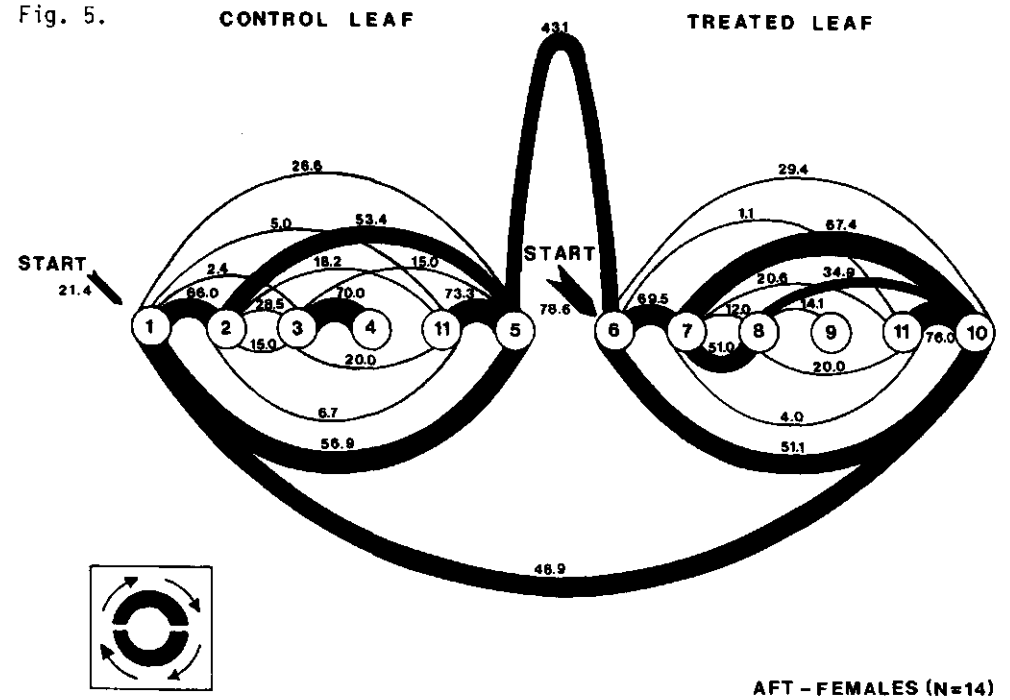
A – FEMALES (N=19)

Fig. 4.



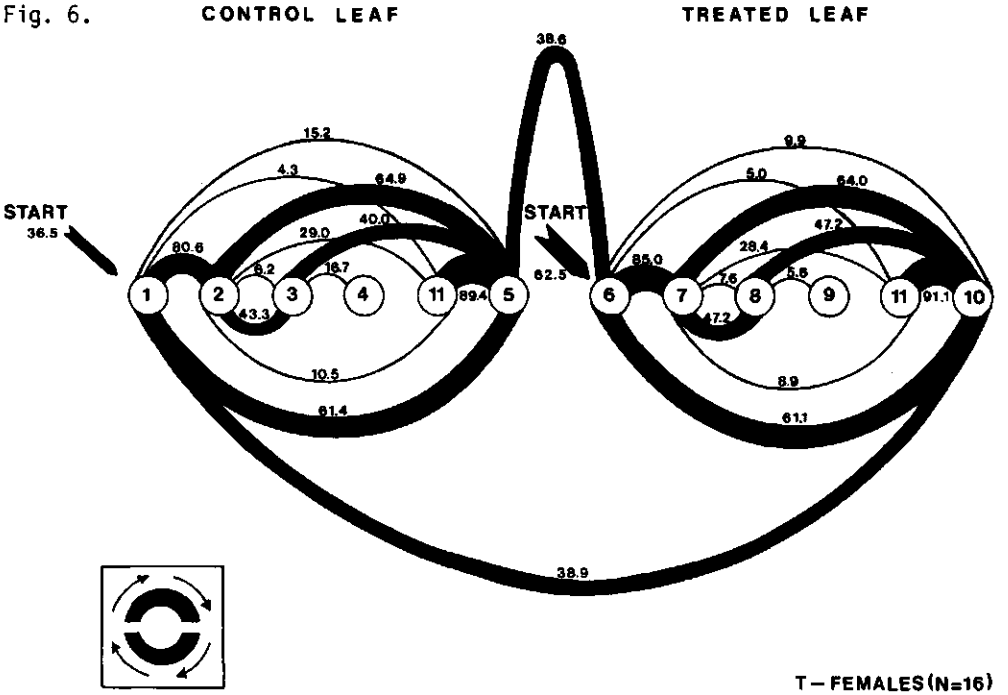
FT–FEMALES (N=12)

Fig. 5.



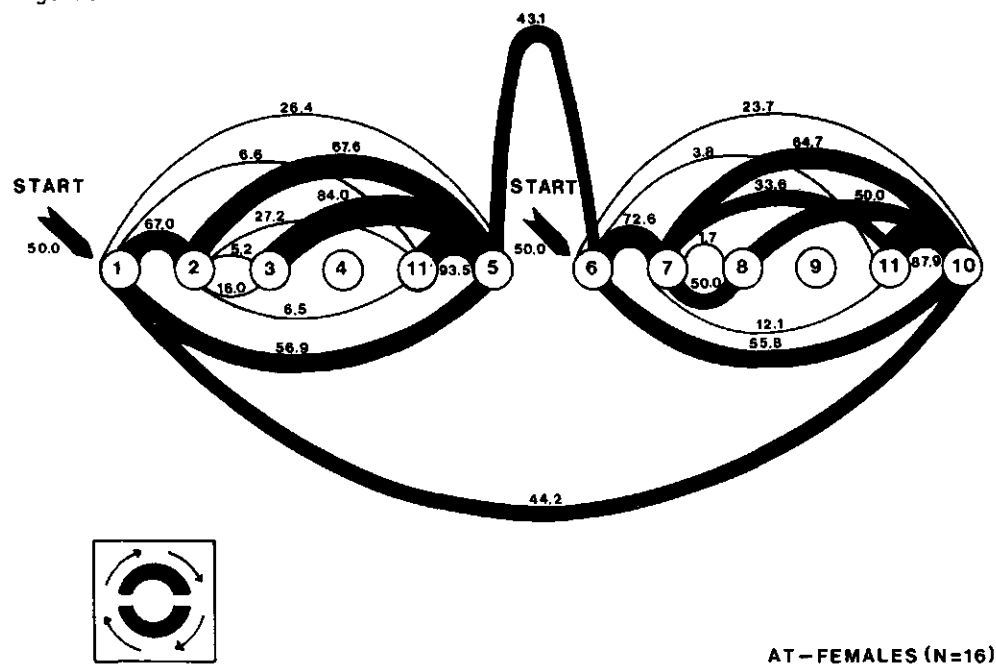
AFT - FEMALES (N=14)

Fig. 6.



T – FEMALES (N=16)

Fig. 7.



AT – FEMALES (N=16)

some similarity with the behaviour of the control females. In the first place, these females seem already to discriminate between the two leaves during *approach*. The probability that after *approach* landing will occur is much higher on the control leaf, thus implicating an equally large, but opposite, difference in the probability of *approach* to *leaving* between both leaves. As compared to the control females we even found a much lower probability of *approach* to *drumming* on the treated leaf.

Also during *drumming* discrimination seems to take place considering the difference between the transitions 2 → 3 and 7 → 8 in these females. The other transitions starting with *drumming* do not differ significantly in their probabilities between the two leaves. With regard to *curving*, the same problem was met as in the control females: the number of FT-females performing *curving* on the treated leaf (only one, see Fig. 1) is too low for any statistical test. This only female displayed behavioural code 8 two times: the first time she continued with *drumming* and the second time it was followed by *oviposition* on the treated leaf.

In this group of females, the behaviour displayed after *leaving* was again different between the two leaves. *Leaving* of the treated leaf was more often succeeded by *approach* of the opposite (control) leaf, whereas on the control leaf females were more likely to return to the same leaf again.

AFT-females

The behavioural steps displayed by AFT-females (flow diagram, Fig. 5) after *approach*, were found to have similar probabilities on both leaves. Like the A-females, these females apparently do not discriminate between the two leaves before alightment. An important difference with all three fore-going groups of females, however, is that AFT-females perceive no difference between both leaves during *drumming*. As can be seen in Table 5, the mean conditional probabilities of corresponding transitions on control and treated leaf respectively, do not differ significantly anymore. Considering the transition *drumming* to *curving*, the lack of such a difference is not due to a lower probability of 2 → 3 but, as compared to the fore-going groups, to an increase in the probability of *drumming* to *curving* on the treated leaf.

Table 5 indicates that AFT-females probably discriminate between the two leaves during *curving*. The probability that *curving* is followed by *oviposotion* is significantly lower on the treated leaf. On the control leaf, this probability lies at the same level as in the three fore-going groups. In AFT-females, approach of one or the other leaf after *leaving* has approximately the same

probability.

T-females

The sequence of pre-oviposition behaviour of T-females (see flow diagram, Fig. 6) on both leaves suggests, just like the data in Table 4 on the frequencies of the various behavioural codes, that females with all six tarsi inactivated do not make any difference between the two leaves. None of the transitions, listed in Table 5, appeared to be significantly different between the leaves with respect to their probabilities. In fact, the probabilities of corresponding transitions on control and treated leaf are all approximately equal.

When compared to the fore-going groups of females a number of differences can be observed. The probability of *approach* to *leaving* in T-females on the treated leaf is reduced in comparison to the other groups. The transition *drumming* to *curving* on the control leaf (2 → 3) has a far lower probability in T-females. And when *curving* is displayed on the control leaf, the probability that *oviposition* will follow (3 → 4) is again much lower as in the other groups. When this last transition is not very likely to occur in T-females it can be expected that other transitions starting with *curving* will have higher probabilities. As can be seen in Table 5, this is the case for both *curving* to *drumming* (3 → 2) and *curving* to *leaving* on the control leaf.

AT-females

In general, pre-oviposition behaviour of AT-females (flowdiagram: Fig. 7) is similar to the behaviour of T-females in that both groups apparently do not discriminate between control and ODP treated leaves. In this group of females we neither found any transition which showed a significant difference in the conditional probabilities between the two leaves. Comparing the last two groups in more detail, however, some differences can be observed. For example, the probability of *approach* followed by *leaving* is higher in AT-females on both leaves. The transition *curving* to *drumming* on the control leaf (3 → 2) has a lower probability in AT-females whereas on the other hand, the transition *curving* to *leaving* is more likely to occur in AT-females.

Most important differences between the AT-females and the first four groups of females were found, as in the T-females, in the transitions *drumming* to *curving* on the control leaf (2 → 3) and *curving* to *ovipostion* on this leaf (3 → 4). When *curving* was displayed on the control leaf, most AT-females continued with *leaving* (3 → 5).

Table 6. Oviposition responses of *P. brassicae* females in dual leaf tests. Oviposition frequency in last column indicates the number (and percentage) of attempts resulting in deposition of the first egg.

	leaflet	no. females tested	no. ovipositing females	no. oviposition attempts	oviposition frequency
INTACT FEMALES	methanol	21	20 (95.2%)	41	32 (78.0%)
	methanol-eggwash	29	5 (17.2%)	58	6 (10.3%)
A-FEMALES	methanol	8	8 (100%)	14	9 (64.3%)
	methanol-eggwash	14	12 (85.7%)	32	20 (62.5%)

Dual leaf tests

The results of these experiments are given in Table 6. Presenting the abdominal taste hairs a leaflet sprayed with methanol, we see that 20 out of 21 (95.2%) intact females deposit an egg on this leaflet. Out of the 41 attempts performed by these females, 32 (78.0%) resulted in oviposition. When the leaflet is sprayed with methanol eggwash, the percentage of females laying an egg on this leaflet in only 17.2%. The percentage of successful oviposition attempts is still further reduced to 10.3%. In the majority of oviposition attempts on the ODP treated leaflet, females decided to leave the blanc leaf and tried it again, usually at an other spot on the blanc leaf. From these results it seems very likely that females perceive the ODP via abdominal chemoreceptors which results in an interruption of pre-oviposition behaviour.

However, to exclude any possibility of olfactory perception of the ODP, we repeated these experiments with females in which the antennae were inactivated. To the control leaflet, we found similar responses as in intact females but to the ODP treated leaflet, A-females responded quite different. As indicate in Table 6, 85.7% of the females laid an egg on this leaflet and out of 32 attempts, 20 (62.5%) resulted in oviposition. Apparently, A-females do not sense any difference anymore between a control and an ODP treated leaflet. As a consequence we must conclude that in the first set of experiments antennal olfactory receptors instead of abdominal taste hairs probably were responsible for ODP perception. This conclusion will be discussed in relation to the results of the other experiments in the next section.

Another interesting phenomenon was observed in these experiments. When

intact females were allowed to lay their first egg on the blanc leaf and the ODP treated leaflet was then manipulated between the blanc leaf and the ovipositor, females continued oviposition without hesitation on the treated leaflet. Pushing gently the leaflet against the hind-tarsi in such a way that chemoreceptors on these legs came into contact with the ODP, neither interrupted the process of egglaying. We could even manipulate the leaflet in such a way that females stepped over and contacted the ODP leaflet with all six tarsi, without disturbing oviposition. Apparently, female behaviour is not influenced anymore by the oviposition deterring pheromone, once oviposition has started.

DISCUSSION

General structure of pre-oviposition behaviour

At first sight, the numerous differences in the various behavioural parameters described above, make it difficult to compare the behaviour displayed by the various groups of females. Such a comparison in which the main differences in pre-oviposition behaviour are analysed, is needed to elucidate the role of various sensory structures in ODP perception, as stated in the introduction already. The flow diagrams displayed in Fig. 2 to Fig. 7 help visualize the data of Table 5 and make a comparison somewhat easier. Before discussing the differences, however, some comments can be made on the general structure of the behavioural sequences of various groups of females. The transitions *approach* to *drumming*, *drumming* to *curving* and *curving* to *oviposition* may be regarded as the most important steps in the behavioural chain. On the control leaf, the conditional probabilities of these transitions show a similar pattern in the first four groups of females. Namely, *approach* is very likely to be followed by landing and *drumming*. *Drumming* on its turn, has a rather low probability of being followed by *curving*. This transition (2 → 3) seems to be a crucial step in the behavioural sequence of CON-, A-, FT- and AFT-females, because once *curving* is displayed, the high probability of 3 → 4 indicates that in most cases these females continue with *oviposition*. This pattern, which also was found in intact females (see chapter 5), thus is not influenced by inactivation of the antennae and/or the fore-tarsi.

Another pattern which could be observed in pre-oviposition behaviour of the first four groups of females is that, like in intact females (chapter 5),

drumming is mostly followed by *leaving*. Females apparently probe the leaf a few times only until *drumming*, before deciding to oviposit on the leaf. The figures 2 to 5 clearly illustrate this behavioural loop.

The structure of pre-oviposition behaviour of T- and AT-females after alightment on the control leaf is markedly different from the other groups. Fig. 1 shows that the number of T- and AT-females performing *curving* on the control leaf is very low in comparison to the other groups. Also the conditional probability of *drumming* to *curving* (2 → 3) was found to be very low. Instead of this transition, females were very often observed to continue with *leaving* (2 → 5) or *rest on leaf* (2 → 11) after *drumming*. *Rest on leaf* on its turn was again very likely to be followed by *leaving* (11 → 5) in both groups of females. Furthermore, it can be noticed from Table 4 that T- and AT-females, in comparison to females with other ablations, perform a very high frequency of *approach* and *drumming* on the control leaf. (Also on the treated leaf, as a matter of fact). This means that the behavioural loop described above is still more manifest in T- and AT-females. In fact, pre-oviposition behaviour of females in which at least all six tarsi are inactivated, mainly consists of this loop of behavioural components, only sometimes interrupted by *rest on leaf*.

The behavioural sequence of T- and AT-females deviates in another important respect from that of the other females. Whereas in the first four groups of females the transition *drumming* to *curving* was found to be the decisive step, a second "bottleneck", in addition to this step, can be observed in the sequence displayed by T- and AT-females, namely the transition *curving* to *oviposition*. The probability that T-females continue with *oviposition* after *curving* is only 16.7% and AT-females did not perform this transition at all. (This last observation only refers to the individual experiments. Table 3 indicates that also AT-females occasionally may perform *oviposition*. Principally this can only be achieved when females have displayed the transition *curving* to *oviposition*). At *curving*, T-females more often decide to continue with *drumming* (3 → 2) or *leaving* (3 → 5) and AT-females are most likely to leave the control leaf again. The HCl treatment to which all six tarsi of these females are subjected, prevents any perception of chemical stimuli by tarsal chemoreceptors. The drastic changes which we observed in pre-oviposition behaviour of T- and AT-females on the control leaf are probably caused by the inactivation of the so-called glucosinolate cells (Ma & Schoonhoven, 1973). These females simply do not recognize the cabbage leaf as a host plant anymore. Ma & Schoonhoven (1973) already suggested that host plant recognition may take place when at least one pair

of tarsi is left intact. In *Danaus gilippus berenice* a similar phenomenon has been observed: the presence of only one pair of intact tarsi is already sufficient to induce oviposition (Myers, 1969). *Papilio protenor demetrius* females, on the contrary, mainly rely on fore-tarsal chemoreceptors. Structural ablation of the fore-tarsi resulted in a dramatic reduction of oviposition (Ichinosé & Honda, 1978). A different situation exists in the nymphalid *Chlosyne lacinia* (Calvert & Hanson, 1983). In this species, the release of oviposition and discrimination of hostplants are presumed to be governed by separate sensory systems. Antennectomy causes a drastic reduction in egglaying activity whereas females deprived of their fore-tarsi maintain a normal oviposition level but fail to discriminate between hostplant species.

The structure of pre-oviposition behaviour of the various groups of females on the control and treated leaf respectively, may be compared by calculating the compound conditional probabilities of *approach* → *drumming* → *curving* → *oviposition* on each leaf. The values derived from these multiplications represent the probability that females will perform this "idealized" sequence when starting with *approach*. Table 7 provides a synopsis of these probabilities in which the general effect of the ODP upon pre-oviposition

Table 7. Compound conditional probability of the behavioural sequence *approach* → → → *oviposition* in the various groups of females (data extracted from Table 5).

Females	leaf	*approach* to *drumming*	*drumming* to *curving*	*curving* to *oviposition*	compound conditional probability (%)
CON	control	91.6	30.3	77.8	21.6
	treated	71.8	1.8	0	0
A	control	75.6	26.3	74.9	14.9
	treated	60.2	8.3	40.0	2.0
FT	control	82.7	34.1	63.0	17.8
	treated	45.0	4.8	50.0	1.1
AFT	control	66.0	28.5	70.0	13.2
	treated	69.5	12.0	14.1	1.2
T	control	80.6	6.2	16.7	0.8
	treated	85.0	7.6	5.6	0.4
AT	control	67.0	5.2	0	0
	treated	72.6	1.7	0	0

behaviour is clearly recognized. The detailed behavioural differences between control and treated leaf are discussed below. Table 7 reveals that in the first four groups of females, the probability of one attempt of *approach* ultimately followed by *oviposition* on the control leaf varies between 13.2% and 21.6%. This indicates that females perform several landings before they decide to oviposit. It is interesting to note that such behavioural patterns may also occur under field conditions. Stanton & Cook (1984), observing *Colias philodice eriphyle* butterflies in the field, found that (first-brood) females on the average laid eggs after only 21% of their hostplant contacts.

Chemosensory organs employed in ODP-perception

In the results section the behavioural differences between control and treated leaf within groups of females are described. From this, the moments at which discrimination between the two leaves, and thus perception of the pheromone, takes place are inferred. The next step, which will be executed in this paragraph, is to determine which chemoreceptors the various groups of females employ in the perception of the ODP.

The CON-females may discriminate between the two leaves during at least two phases of pre-oviposition behaviour namely, during *approach* and during *drumming*. Before landing on the treated leaf, only antennal olfactory hairs can be assumed to be involved in ODP perception, whereas the difference in post-alightment behaviour of the control females is probably mediated by sensory input from tarsal contactchemoreceptors. At first sight, the very obvious differences between the two leaves in the conditional probabilities of transitions starting with *curving* (Table 5) indicate that also during this behavioural step females might perceive the pheromone. It should be remembered, however, that these conditional probabilities (and also the frequency of behavioural code 8, Table 4) are based on only one female (see Fig. 1). For the present, this only observation does not validate the conclusion that also durin *curving* ODP is perceived. In chapter 5, the results of similar behavioural experiments with untreated females were described. In contrast to the control females, we did not find a clear distance effect of the pheromone upon the behaviour of non-treated females. This discrepancy might be caused by the water treatment to which CON-females were subjected. Perhaps the perception of ODP was improved by "washing" the antennal olfactory hairs with distilled water. In addition, CON-females were handled for quite a long time (both antenna and all six tarsi were each treated for 40 seconds with aqua dest), which might

have influenced female behaviour as well. In any case, it seems as if control females discriminate better between the two leaves than untreated females.

The A-females only discriminate between a control and an ODP treated leaf after landing. Most evident behavioural differences between the two leaves were found after *drumming*. This implicates that A-females employ tarsal chemoreceptors to perceive the ODP.

Females with inactivated fore-tarsi still may perceive the oviposition deterring pheromone very well. In the first place, females make use of antennal olfactory hairs, considering the behavioural differences before landing. After *drumming*, the differences in conditional probabilities of corresponding transitions indicate that also during this behavioural step ODP is perceived. Whereas the fore-tarsal chemosensilla are eliminated, contactchemoreceptors on mid- and hind-tarsi are likely to be involved, but we cannot exclude the possibility that the antennae still play a role during *drumming*. (In fact, this might be the case in the control females too). In the next paragraph, however, we will discuss the role of antennal chemoreceptors, in conjunction with the results of the dual leaf tests, in more detail. Whether ODP perception takes place during *curving* is difficult to say because of the low number of FT-females that displayed behavioural code 8 (see Fig. 1).

In AFT-females the number of behavioural differences between the leaves has decreased in comparison to the fore-going groups. The only difference in percentage responding (Fig. 1) was found for *oviposition*. This finding suggests already that the pheromone may be perceived only during *curving*. The conditional probabilities observed for several transitions confirm this supposition: the only transition in which a significant difference was found is *curving* to *oviposition*. So in this group of females, we have found clear evidence that contactchemoreceptors on mid- and hindtarsi participate in the perception of the oviposition deterring pheromone.

In T- and AT-females, pre-oviposition behaviour was not influenced by the oviposition deterring pheromone. The presence of intact antennal chemoreceptors in the T-females, potentially enabled females to perceive the pheromone. But even during *approach* no behavioural differences between the leaves were observed, so apparently this did not happen. The inability of females to perceive host plant specific stimuli probably predominated in such a way that the ODP, as a behaviour modifying stimulus, did not influence females anymore. In AT-females, the absence of host plant specific sensory information affects female behaviour in a similar way.

The results of the dual leaf tests clearly indicate that females do not employ abdominal taste hairs in the perception of the oviposition deterring pheromone. So, there exists some discrepancy between the results of electrophysiological experiments, which indicate a slight sensitivity of these taste hairs to eggwash (Klijnstra & Roessingh, submitted) and the behavioural results reported here. For the present, this contradiction cannot be readily explained. Perhaps the electrophysiological responses of abdominal taste hairs were due to stimulation by other components in eggwash than ODP. In conclusion of this paragraph, we have seen that the various groups of females employ several chemosensory structures (antennae; fore-tarsi; mid- and hindtarsi) to perceive the oviposition deterring pheromone. Intact females thus are not dependent on only one receptor system but may rely on all structures mentioned above, perhaps to perceive ODP under variable circumstances. The apple maggot fly, *Rhagoletis pomonella* is also known to possess an oviposition deterring pheromone (Prokopy, 1972). In structural ablation experiments Prokopy & Spatcher (1977) demonstrated that female *Rhagoletis pomonella* perceive this pheromone mainly by fore-tarsal chemoreceptors but that additional chemoreceptors may be present on mid- and hindtarsi. Electrophysiological studies conducted by Crnjar & Prokopy (1982) have confirmed these behavioural findings. In contrast to *Pieris* antennal chemoreceptors do not seem to be involved in the perception of the ODP in *Rhagoletis pomonella* (Prokopy & Spatcher, 1977).

The role of antennal chemoreceptors in ODP perception

The results of the dual leaf tests led us to reconsider the role of antennal olfactory hairs in the perception of the ODP. If *Pieris brassicae* females employ antennal olfactory hairs to perceive the pheromone during *touching*, which has been clearly demonstrated in these experiments, this might be the case during other stages of post-alighting behaviour too. This means that in the control females as well as in the FT-females, which both were found to discriminate between the two leaves during *drumming*, besides tarsal information also antennal sensory input might have been responsible for the interruption of pre-oviposition behaviour on the treated leaf. In these females, however, a possible antennal mediated effect upon the behaviour cannot be separated from the effect elicited by tarsal chemoreceptors.

The importance of intact antennal chemoreceptors in ODP perception after alightment, may be stressed by the fact that in the A-females the probability of *curving* to *oviposition* on the treated leaf (8 → 9) was found to be relatively high.

A similar response then might be expected to occur in the AFT-females, which neither possess intact olfactory hairs. In this group, however, the difference between the conditional probabilities of 3 → 4 and 8 → 9 is again very large. Therefore, the extent to which antennal olfactory hairs contribute to ODP perception during post-alighting behaviour remains difficult to assess. In any case, however, we may conclude that the various chemosensory structures involved in pre-oviposition behaviour do not operate as a chain of receptors each successively influencing only one particular behavioural step. At this point, an answer might be given to the first question raised in the Introduction. During *drumming* on the upper leaf surface of a cabbage leaf sprayed with eggwash at the lower side only, intact females might be able to perceive the pheromone by antennal olfactory hairs. This perception may be improved by an airstream along the leaf, evoked by the apparent wingfluttering which females perform during *drumming*.

Additional effects of the ODP upon female behaviour

The dual leaf tests also revealed that female behaviour is not influenced anymore by the oviposition deterring pheromone after the first egg is deposited. Adaptation of peripheral chemoreceptors cannot be the cause of this, because the tarsal taste hairs were not stimulated before by the ODP. Instead of this, we may suppose a central inhibition, blocking the sensory input on the ODP, to be responsible for the absence of a behavioural response to the pheromone once oviposition has started. *Pieris brassicae* females are known to lay eggs in compact clusters and the eggs contain the oviposition deterring pheromone. Assuming such a central inhibition might be a reasonable explanation for the fact that females do not interrupt egg-batch formation already after a few eggs have been laid.

Finally, some remarks should be made on the behaviour displayed by females after *leaving*. Only the CON- and FT-females perform an apparent tendency to stay around or return to the control leaf. This behaviour is possibly mediated by antennal olfactory perception of the ODP, just after *leaving*. Only these two groups of females possess intact olfactory hairs (when we leave the T-females out of consideration) and may perceive the pheromone already at a distance.

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7 EFFECTIVENESS AND PERSISTENCE OF THE OVIPOSITION DETERRING PHEROMONE OF *PIERIS BRASSICAE* IN THE FIELD

ABSTRACT

Small scale field experiments are described with the oviposition deterring pheromone (ODP) of *Pieris brassicae* in order to investigate its potency as a pest control agent. In a field cage, containing control and ODP treated cabbage plants, single females were released and their behaviour observed until females had selected a suitable oviposition site. In preliminary control experiments, without ODP treated plants, the plants being chosen for oviposition were distributed rather evenly over the cage. In the presence of the pheromone, oviposition sites selected by females were concentrated on plants along the sides of the cage, which suggests that the ODP may induce dispersal flight in female *P. brassicae*. The distribution of oviposition attempts over control and treated plants in the pheromone experiments, however, was not significantly different from that in the control experiments. This means that under these field conditions the effectiveness of the pheromone is very low.

The duration of female pre-oviposition behaviour and the mean number of landings during this site selection process were both found to be significantly increased in the presence of the pheromone. The ODP also affected the number of landings on control plants, being proportionally higher in the pheromone runs than in the control runs. Laboratory experiments with leaves of treated field plants tentatively indicated the persistence of the pheromone to be at least 3-5 days. It is concluded that in the field, the ODP influences pre-oviposition behaviour of *P. brassicae* females, but application in pest control should wait until further investigations have revealed whether the effectiveness of the pheromone can be improved.

INTRODUCTION

The oviposition deterring pheromone (ODP) of *Pieris brassicae* L. is known to be associated with the eggs (Rothschild & Schoonhoven, 1977; Behan & Schoonhoven, 1978). The presence of intact conspecific eggs on a cabbage leaf was found to have a moderate deterrent effect on ovipositing *P. brassicae* females (chapter 2). Egg-washings in distilled water and especially in methanol sprayed onto cabbage leaves appeared to be much more deterrent to females. A high concentration of methanol-eggwash protected in some cases cabbage leaves to *P. brassicae* oviposition almost completely (chapter 2). These and other (Schoonhoven et al., 1981) results of laboratory experiments indicate that the oviposition deterring pheromone of *P. brassicae* may be regarded as a potential pest control agent with perhaps realistic prospects for successful application. In this paper the first field experiments with this pheromone are described. The amount of pheromone available for these experiments was limited, because until now, the only way to obtain pheromone is to collect a (very) large number of *P. brassicae* eggs, from which it can be extracted. Therefore, only small scale field experiments could be conducted in a field cage with a relatively small number of cabbage plants.

One way of doing such field tests is to release a group of female butterflies in the cage with ODP treated plants and count the number of egg-batches and eggs laid on the various plants on subsequent days after spraying. In *Pieris*, however, this approach has two major disadvantages: 1. egg-batches laid by females during such an experiment release additional amounts of pheromone, disturbing the initial set up of the experiments to an unknown extent; 2. the results of such experiments (in the form of distribution of egg-batches and eggs) do not provide any information on the dynamics of female responses to the pheromone during pre-oviposition behaviour. Our field tests therefore were designed in such a way that pre-oviposition behaviour could be observed of singly released females in both the absence and presence of the pheromone.

The effectivity of the pheromone might be influenced by the way cabbage plants are treated with it. Preliminary experiments in the laboratory (reported in chapter 2) revealed that spraying cabbage leaves with ODP at either the upper or lower leaf surface did not make any difference to females.

In all laboratory experiments described in the previous chapters and in the literature, the effects of the oviposition deterring pheromone were demonstrated in choice situations with control and ODP treated leaves in a one to one ratio. For field application, such a proportion of course would be

unrealistic. *P. brassicae* females confronted with only ODP treated leaves, however, are not prevented from egglaying on those leaves (unpublished results), thus making it impossible to determine the deterrent activity of the pheromone in such a situation. In the field therefore, an intermediate between these two situations was chosen, which will be described in the next section.

MATERIALS & METHODS

Experimental animals

P. brassicae females were obtained from a laboratory culture maintained for many years on *Brassica oleracaea* L. var. *gemmifera* D.C. plants. Females were allowed to mate in the laboratory before they were used in the field. Age of experimental females ranged from 4 to 14 days after eclosion. Between the experiments (during evenings and nights) butterflies were kept in large cages containing a cabbage plant and an artificial flower filled with a 10% sucrose solution. Females were marked individually allowing each female to be used only once on the same day. On subsequent days, however, several females have been used a second or third time.

Field cage

Experiments were conducted in a cage, with a ground surface of 6 x 6 m and a maximum height of 2.4 m, covered by a nylon net with a mesh diameter of 0.5 cm. This cage contained 56 cabbage plants (*Brassica oleracaea* L. var. *gemmifera* D.C. cv Rampard), planted when they were 12 weeks old on May 28, 1984 in a rectangle with interplant distances of about 70 cm. Each row in the east-west direction consisted of 8 plants, whereas 7 plants were present in each north-south row. The diagrams displayed in Fig. 1, in which the latter rows are called columns, illustrate the plant setting. Plants were numbered with a two digit code according to their row-column position (see Fig. 1). By the time the first control runs (see below) were started two plants, 6-1 and 6-4, were found to be dead (because of uncautious weeding) and removed from the cage. Among the remaining 54 plants there were some differences in size and external appearance, but they all seemed to grow very well. Besides plant 2-8, which was infested by an aphid colony (*Brevicoryne brassicae* L.), there were no plants showing feeding damage of any importance. Before the experiments started and occasionally between the various experiments, plants were stripped of the

oldest leaves, in order to get plants with approximately the same number of leaves. Moreover, it facilitated the observation of female behaviour and recognition of the separate plants.

Experiments

Two sets of experiments were carried out each consisting of a number of runs in which single females were released in the cage and their behaviour observed until the first egg was laid. The first set consisted of 66 control runs with unsprayed plants, allowing the observation of female pre-oviposition behaviour in the field in the absence of the oviposition deterring pheromone. These control runs were conducted on various, inconsecutive days under comparable weather conditions (see below). The pheromone runs (n = 77), as the second set of experiments, were necessarily conducted, after completion of the control runs, on five subsequent days. Weather conditions on these days are described below. In these runs females were offered a choice situation with 44 ODP treated plants and 10 control plants. This experimental setting was selected as an intermediate between the one to one choice (one control leaf vv one treated leaf) usually applied in laboratory experiments and a no choice situation with only ODP treated plants, in which the pheromonal effect can hardly be demonstrated. The distribution of control plants in the field plot (see Fig. 1) was chosen in such a way that in all (wind) directions females would have a reasonable chance to find an ODP-free plant.

Plants were sprayed by means of a plant sprayer during the morning hours of day 1 in calm weather. The control plants received 30 ml of methanol, evenly distributed over both sides of all leaves. The pheromone plants were sprayed with 30 ml methanol eggwash, at a concentration of 500 egg-equivalents per ml methanol, thus resulting in a total amount of 15,000 egg equivalents present on each plant. With approximately 30 leaves per plant, this means that each leaf received ca. 1 ml of egg-wash. During spraying of a particular plant the surrounding plants were protected by cardboard plates. The pheromone solution used in these experiments was derived from a mixture of 7 subsequent washings of *P. brassicae* eggs in methanol. Laboratory experiments have shown that repeated washings retain a high deterrent activity (see chapter 2) and also this ODP field-blend was found to be very active in the lab.

Experiments were conducted between 10.00 and 16.00 h and females were deprived of an oviposition substrate for one to four hours. In each run one female was used. She was released from the center of the cage (see Fig. 1) at

approx. 2 m above the ground. The behaviour displayed by females was spoken into a portable cassette recorder. The flight path of females could be traced by mentioning the plant numbers approached or visited and components of post-alighting behaviour (see chapter 5) were scored separately. Once a female had made her choice and laid the first egg on a particular plant, the time of oviposition was recorded and the female was removed from the plant. This was done to prevent any disturbance of the experimental situation by egg-batches releasing additional amounts of pheromone. In both control and pheromone runs, some females were unable to find a suitable oviposition site within 15 minutes after release. These females were discarded. In the analysis of the distribution of plants selected for oviposition in control and pheromone runs respectively, we made a subdivision in "control" and "treated" plants also in the control runs. The same plants serving as controls in the pheromone runs (see Fig. 1) were also treated as control plants in the control runs. During the course of the pheromone runs, it appeared that spraying cabbage plants with a methanol solution eventually may cause some damage to the leaves. Whereas this pertained only to a small number of leaves of both control and treated plants, an effect on the results is presumably negligible.

In addition to the pheromone runs, a small number of laboratory experiments was conducted to obtain some information on the persistence of the pheromone under field conditions. On various days we picked one or more pairs of leaves from the field plants and introduced them into a laboratory cage containing a group of butterflies. The two leaves within a pair, although necessarily picked from two different plants (control and treated), were matched as far as possible for age, size and appearance. The leaves were exposed to the butterflies for one hour and afterwards the numbers of egg-batches and eggs laid on both leaves were counted. The deterrent activity of the egg-wash solution sprayed on the pheromone plants was determined in the following way: % deterrence = (C-T) x 100 / C+T, where C and T represent the numbers of egg-batches (or eggs) laid on control and treated leaves, respectively. On each of the first three days of the pheromone runs, only one pair of leaves was collected in order to minimize changes in the field situation.

Weather conditions during the field experiments

Ambient temperature and rainfall during and between the experiments were measured within the cage. The control runs were conducted on five, inconsecutive days with approximately similar weather conditions. These conditions were:

windforce c. 2 Beaufort from varying directions (SW, NW and NE); periods of sunshine, sometimes interrupted by clouds and a temperature in the cage varying between 22°C to 32°C. During the pheromone runs weather conditions varied somewhat more. On day 1, during the morning hours when the plants were sprayed, there was almost no wind, the sun was shining and the temperature was about 23°C. During the afternoon, the windforce rose to c. 4 Beaufort from NE. On the second day, it was calm weather with the sun shining through a thin veil of clouds, air temperature 26°C. Because of cloudy and cool weather (temperature 20°C) only some experiments were done on the third day. During the night between day 3 and 4 it rained slightly (0.5 mm). On day 4 and 5 the windforce was 2-3 Beaufort from NE to NW and from southern directions, respectively. Long periods of sunshine raised the temperature in the cage to approximately 28°C.

RESULTS

The distribution of field plants chosen by females for oviposition in the control series and in the test series is given in the diagrams of Fig. 1. In the control runs (Fig. 1A) first eggs were laid on 34 different plants, rather evenly distributed over the field plot. Only two plants, 1-2 and 1-7, were found to be selected more often than other plants: each of these two plants received 6 eggs.

Fig. 1B displays the results of the pheromone runs. Pooled for all days, the distribution of plants which were selected for oviposition is less uniform than in the control runs. The number of different plants on which oviposition was observed was 27. From these 27, five plants were seen to be selected by five or more females, and two plants (1-7 and 6-8) were even chosen ten times. Statistical analysis of the distribution of oviposition attempts on control and treated plants, however, revealed no significant difference between the control and pheromone runs, nor on the various days after ODP application nor in total (Table 1). In other words, in the pheromone runs the control plants are not chosen significantly more often than the corresponding plants in the control runs.

However, a striking difference can be observed in the general distribution of oviposition attempts between control and pheromone runs. In the presence of the pheromone (Fig. 1B) females seem to lay eggs more readily on plants located at the edge of the plot, whereas plants in the center of the cage are selected

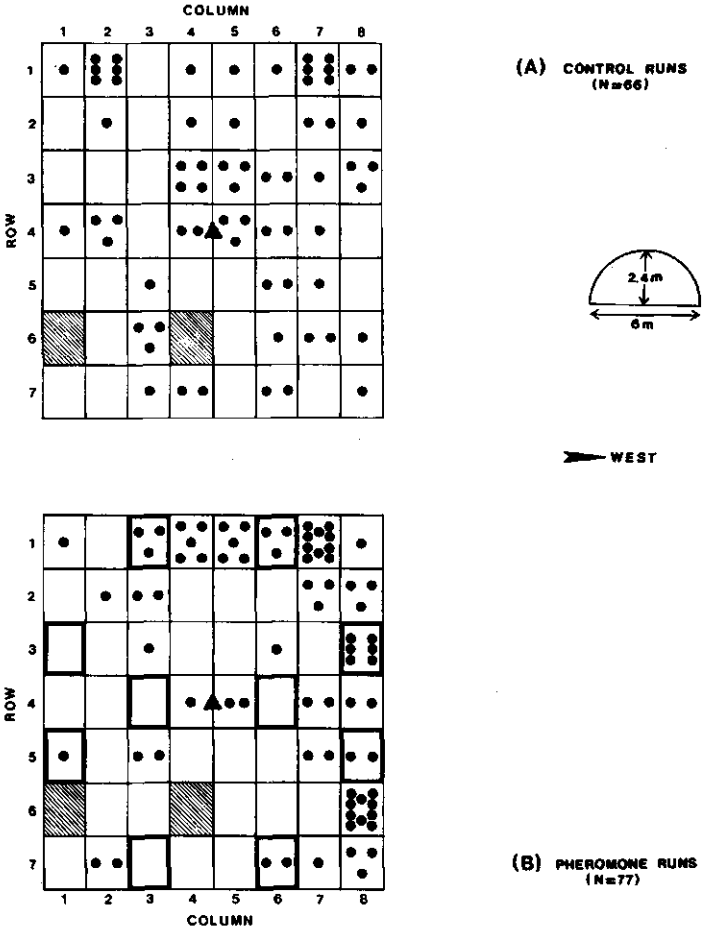

Fig. 1. Diagrams showing the experimental set-up and the results of field experiments with the oviposition deterring pheromone (ODP) of *P. brassicae*. The large squares represent the field cage which contained 54 cabbage plants. Each rectangle in the cage indicates one plant encoded by its row-column number. Positions 6-1 and 6-4 (shaded rectangles) were not occupied by plants. The triangle in the center of the cage indicates the point at which females were released. In (A) the results of the control runs (n = 66) are given. Each dot represents the choice of one female (= one run) to oviposit on that plant. In (B) the results of the pheromone runs (n = 77) are displayed. The thick-lined rectangles indicate the positions of control plants sprayed with methanol. The remaining 44 cabbage plants were sprayed with the ODP. The small diagram besides the upper square displays a side-view at the cage from the west.

Table 1. Effect of the ODP upon oviposition site selection of single *P. brassicae* females in a field cage. Figures represent the number of runs (one run = one female) in which a control or treated plant was chosen for oviposition on subsequent days after ODP application (Plants were sprayed on day 1). Totals given in the bottom row and the results of control runs are extracted from Fig. 1. In the control runs, with plants not being sprayed, a hypothetical distinction was made in "control" and "treated" plants corresponding to the experimental situation in the pheromone runs (see Fig. 1). The ratios of control and treated plants being chosen for oviposition on the various days and in total do not differ significantly from the ratio in control runs (chi-square test).

	Female oviposition choice		
	control plants(10)	treated plants(44)	no. runs
CONTROL RUNS	9 (13.6%)	57 (86.4%)	66
PHEROMONE RUNS			
Day 1	4 (25.0%)	12 (75.0%)	16
Day 2	5 (21.7%)	18 (78.3%)	23
Day 3	2 (33.3%)	4 (66.7%)	6
Day 4	4 (18.2%)	18 (81.8%)	22
Day 5	2 (20.0%)	8 (80.0%)	10
Total	17 (22.1%)	60 (77.9%)	77

less often. Therefore, a comparison was made between edge plants (n = 25) and central plants (n = 29) with regard to the numbers of eggs they received. Under the nil-hypothesis that edge plants and central plants will receive eggs proportionately to their distribution in the cage, the expected numbers of oviposition attempts on edge plants and central plants in the control runs were 30.6 and 35.4, respectively. The observed distribution (see Fig. 1A) was 30 attempts on edge plants and 36 on central plants which obviously does not differ significantly from expectation (chi-square one sample test, df = 1, $X^2 = 0.022$). In the pheromone runs (Fig. 1B), 60 females were observed to start oviposition on an edge plant whereas only 17 females selected a central plant for oviposition. These values differ very significantly from the expected values which were 35.7 and 41.3 for edge plants and central plants, respectively (chi-square one sample test, df = 1, $X^2 = 30.838$, $p < 0.001$). A direct comparison between control and pheromone runs also revealed a highly significant difference in the proportion of edge plants and central plants chosen for

oviposition (chi-square test, df = 1, X^2 = 14.698, $p < 0.001$). This means that in the pheromone runs females indeed lay eggs more readily on plants located at the edge of the plot and that central plants are selected less often as compared to the control runs.

Other parameters of female pre-oviposition behaviour which were determined in these field experiments are given in Table 2. The mean duration of the oviposition site selection process was found to be significantly longer in the pheromone runs. In the presence of the oviposition deterring pheromone females needed on the average 1.7 minutes more to decide where to oviposit.

A very significant difference was also found in the mean number of landings between control and pheromone runs (Table 2). The total number of landings in all 66 control runs was 1,185, whereas in the 77 pheromone runs 2,383 landings were observed. From these totals, the fraction of landings on control

Table 2. The influence of the ODP upon duration of pre-oviposition behaviour, the number of landings and the distribution of landings on control and treated plants displayed by *P. brassicae* females in the field.

	CONTROL RUNS (N = 66)		PHEROMONE RUNS (N = 77)
mean duration ± S.D. (sec) of pre-oviposition behaviour	193.2 ± 205.9		294.2 ± 224.7
Mann Whitney U test	z = 3.64	p(one tailed)	< 0.0005
mean no. landings ± S.D. per run	17.9 ± 21.6		30.9 ± 28.8
Mann Whitney U test	z = 3.91	p(one tailed)	< 0.0001
no. landings on			
control plants	143 (12.1%)		458 (19.2%)
treated plants	1042 (87.9%)		1925 (80.8%)
Total	1185		2383
chi-square test on ratio of no. landings	X^2 = 28.39	df = 1	p(one tailed) < 0.001

Table 3. Distribution of eggbatches/eggs by *P. brassicae* females over control and ODP treated cabbage leaves gathered from field plants on subsequent days after ODP application in laboratory choice experiments. Plants were sprayed on day 1. N indicates the number of pairs of leaves tested and % deterrence reflects the deterrent activity of the ODP. The low numbers of pairs of leaves tested prevent a statistical analysis of these data.

Day	N	No. eggbatches/eggs laid on leaves		% deterrence
		control	treated	
1	1	17/578	1/29	88.9/90.4%
2	1	13/378	1/30	85.7/85.3%
3	1	14/337	5/143	47.4/40.4%
4	5	64/1940	49/1263	13.3/21.1%
5	4	48/1764	25/642	31.5/46.6%
7	4	30/816	32/1033	-3.2/-11.7%
8	4	36/619	27/557	14.3/ 5.3%
9	4	45/792	38/749	8.4/ 2.8%
16	2	20/471	4/53	66.7/79.6%

plants was 12.1% in the control runs and 19,2% in the pheromone runs (see Table 2). This increase also appeared to be significant.

During the course of the pheromone runs on the first day already, the impression arose that the ODP did not affect female behaviour in the way it was expected namely, deterrence of oviposition on pheromone treated plants. To check whether this was due to the field conditions or to the egg-wash solution applied in the field, some leaves from the field plants were collected and tested in the laboratory with the same females. This test revealed that the amount of pheromone present on the treated leaf strongly reduced oviposition on that leaf (see Table 3). Similar tests were then conducted on subsequent days in order to obtain some information on the persistence of the ODP under field conditions. The results, given in Table 3, indicate that the pheromone is present during the first two days. Thereafter the deterrent activity seems to decrease, but not in a regular fashion. Although low on day 4 a higher percentage deterrence is measured on the fifth day. On the seventh to ninth day one can hardly speak of any deterrence, but then after 16 days again a high percentage deterrence was found. These results should be interpreted with caution, because the very low numbers of repetitions prevent any statistical analysis of the data.

DISCUSSION

The behavioural response of *P. brassicae* to the oviposition deterring pheromone under field conditions as described in this chapter, differs greatly from the response observed under laboratory circumstances. Whereas in laboratory choice experiments with control and ODP treated cabbage leaves a pheromone solution of 500 ee/ml was found to be highly deterrent to ovipositing females (see chapter 2), in the field plot a similar amount of pheromone per leaf on intact plants reduced egglaying on these plants only slightly. The ratio of control and ODP treated plants chosen for oviposition in the pheromone runs was found to be 17 to 60 (Table 1) or 1 to 3.5. In the field plot control and treated plants were present at a ratio of 1 to 4.4. This ratio might be used as an estimate of the expected ratio of oviposition attempts on control and treated plants. However, it does not take into account possible differences between individual plant characteristics which also may influence female choice behaviour (see Mitchell, 1977). A better estimate of this ratio, although still not perfect, can be derived from the results in the control runs which show on the average a proportion of one "control" plant to 6.3 "treated" plants being chosen for oviposition. Comparing the ratios observed in control and pheromone runs, it thus can be seen that in the presence of the pheromone females relatively more often select a control plant to lay their eggs but, as Table 1 reveals, the difference is not statistically significant.

The conclusion, however, that the oviposition deterring pheromone does not work in the field is incorrect. The number of oviposition attempts on edge plants is much larger, both in absolute sense and in proportion, in the pheromone runs as in the control runs. Furthermore, Table 2 shows that in the presence of the ODP, females need some more time to take a decision and that during this time also the number of landings increases. In addition, it can be seen that in the pheromone runs females land significantly more often on the control plants as in the control runs. So, under these field conditions female pre-oviposition behaviour certainly is influenced by the ODP, but apparently this does not result in a significant decrease of oviposition on ODP treated plants. A possible explanation for the absence of a clear deterrent effect might be sought in the experimental set up of these field experiments. In laboratory experiments, in which the deterrent effect of the ODP was found to be very reproducible (see chapter 2), control and ODP treated leaves were always

offered in a one to one ratio. As described above, in the field plot the proportion of control and treated plants was only 1 to 4.4, which perhaps hindered females in locating control plants. Out of the 60 females starting oviposition on an ODP treated plant (Table 1), 17 were never observed to land on a control plant. The remaining 43 females, however, had contacted at least one control plant during pre-oviposition behaviour and they thus have been in a position to oviposit on such a plant.

With regard to a possible application of this pheromone in the control of *P. brassicae*, it is important to note that the ODP apparently does not act as an absolute protecting agent against *P. brassicae* oviposition, but rather should be considered as a modifier of female pre-oviposition behaviour. These field experiments indicate that in the presence of the ODP the pre-oviposition period is extended and that oviposition initiatives of females are concentrated on plants along the sides of the cage. The oviposition deterring pheromone apparently induces females to follow a flight course leading away from the releasing point at the center of the plot (a centrifugal effect). In this cage, females were stopped by the net, and one may only guess what would happen when the experiments had been conducted in an open field plot. Induction of dispersal flight in *P. brassicae* females by the oviposition deterring pheromone might cause a substantial reduction of oviposition on plants in central areas of the field.

In *Rhagoletis pomonella* flies, which also respond to an oviposition deterring pheromone (Prokopy, 1972), it was found that ovipositing females rapidly emigrated from trees carrying fruits marked with ODP (Roitberg *et al.*, 1982). Roitberg *et al.* (1984) demonstrated that female flies were more likely to fly long distances after contact with ODP marked fruits than after encountering clean fruits.

If *P. brassicae* likewise increases its dispersal activity when perceiving ODP, our results on egg distribution may be explained by assuming that the butterflies have a preference to fly in a south-west direction (light and/or wind may be orienting factors). When arriving close to the barrier the urge to oviposit possibly overrides the ODP stimuli on the plants along the net, and these plants then appear to be sufficiently acceptable. This would result in an oviposition pattern as figured in Fig. 1B.

The data in Table 3 provide only a first indication on the persistence of the pheromone under field conditions. In the laboratory, cabbage leaves sprayed with a pheromone solution remain deterrent to ovipositing females for at least

14 days (Schoonhoven *et al.*, 1981), which is much longer than the period of 3-5 days suggested in Table 3. However, definite conclusions cannot be based upon these few experimental data and the results on day 16 indicate that also in the field a higher persistence cannot be excluded.

Many insect species have been reported to possess a spacing or epideictic pheromone (see Prokopy, 1981). Application of such pheromones to host plants in order to investigate their potency as pest control agents, has been tried in two other groups of pest insects: *Dendroctonus* bark beetles and *Rhagoletis* fruit flies. In *D. pseudotsugae*, aggregation behaviour of adults is influenced by a number of pheromones, one of them being 3,2-methyl-cyclohexenone (3,2-MCH) (Birch, 1984). When released at a high concentration 3,2-MCH appeared to inhibit landing of approaching males and females, thereby acting as a spacing pheromone (Rudinsky & Ryker, 1977). Field experiments with wild populations of *D. pseudotsugae* revealed that optimal concentrations of 3,2-MCH, diffused from liquid dispensers, could effect a more than 90% reduction in adult infestation and number of progeny in freshly felled Douglas fir trees (Furniss *et al.*, 1972, 1974).

A second example has been reported by Katsoyannos & Boller (1976, 1980) in *Rhagoletis cerasi*. Female *R. cerasi* deposit single eggs in cherry fruits and subsequently mark the surface of the fruit with a pheromone, deterring other females from oviposition in that fruit (Katsoyannos, 1975). In field tests during a wet summer, Katsoyannos & Boller (1976) sprayed entire cherry trees with a solution of this pheromone and found a 77% reduction in number of infested fruits as compared to untreated controls. A second test (Katsoyannos & Boller, 1980) revealed that in dry weather the effectiveness of pheromone sprays on entire cherry trees might even increase to 90% reduction in larval infestation of fruits. Thus in both *Dendroctonus* and *Rhagoletis*, the effectiveness levels of the treatments appeared to be very high. The effectiveness of the oviposition deterring pheromone of *P. brassicae* under our field conditions stays far behind this level. Further investigations under other field conditions and perhaps with other formulations of the pheromone are needed to determine whether the effectiveness can be improved.

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8 INTERSPECIFIC EGG LOAD ASSESSMENT OF HOSTPLANTS BY *PIERIS RAPAE* BUTTERFLIES

ABSTRACT

Egglaying responses of *Pieris rapae* butterflies to the oviposition deterring pheromone (ODP) of *Pieris brassicae* were studied in the laboratory. Choice experiments with ODP treated leaves and control leaves revealed that females perform a strong preference to lay their eggs on the control leaves. This preference is maintained even when during the experiment the control leaf becomes covered with a large number of conspecific eggs. Choice experiments with cabbage leaves with and without *P. rapae* eggs, seem to indicate the absence of intraspecific egg load assessment of hostplants in *P. rapae*. The deterrent effect of the ODP of *P. brassicae* to *P. rapae* females persists for at least 8 days. Behavioural observations suggest olfactory hairs as well as gustatory hairs to be involved in the perception of the ODP but electrophysiological recordings of the various chemoreceptors are necessary to confirm this. Finally the prospects of application of this pheromone/kairomone in cabbage pest control are discussed.

INTRODUCTION

In *Pieris rapae* L. the limited mobility of the first instar larvae puts the major burden of hostplant selection on the adult female. Selection of suitable larval foodplant species is guided by glucosinolates, which are known to be necessary for both oviposition and larval feeding (Hovanitz & Chang, 1963, 1964). Factors determining selection of hostplant individuals are less well known.

Harcourt (1961) studied spatial distribution of *Pieris rapae* eggs on foodplants in agricultural plots and found clumped patterns which could be fitted quite well to the negative binomial distribution. These clumped distributions, characterized by the presence of an excess of both uninfested plants and heavily infested plants over the expected number according to random distribution, should arise in some way from female behaviour. Jones (1977), in modelling oviposition behaviour of *Pieris rapae*, showed that egg distribution in agricultural plots for this species can be explained as a statistical result of female movement patterns. Hostplant factors that might elicit or affect these behavioural patterns are age and nutritive quality (Jones & Ives, 1979), water content (Wolfson, 1980), distinct morphological features (Ives, 1978) as well as spatial distribution of hostplants.

Secondly, assessment of egg load of a hostplant may also influence female movement patterns and thus egg distributions. Rothschild & Schoonhoven (1977) supplied laboratory evidence for the existence of an oviposition deterring pheromone (ODP) in (European) *Pieris rapae*, similar to what was found for *Pieris brassicae* L. However, Harcourt (1961) and Jones (1977) found in the field that, in Australian and Canadian populations of *Pieris rapae*, eggs were distributed in clumped patterns, which is not to be expected when egg load assessment is involved, resulting in regular egg distributions. Also Ives (1978) found no discrimination between plants with and without eggs in the latter populations of *Pieris rapae*.

Egg load assessment of plants is not necessarily limited to conspecific eggs: also interspecific interactions may occur (see Shapiro, 1980, 1981). The presence of *Pieris brassicae* larvae on a plant might be detrimental to developing *Pieris rapae* larvae. Therefore egglaying responses of *Pieris rapae* females to the oviposition deterring pheromone of *Pieris brassicae* (see Rothschild & Schoonhoven, 1977; Klijnstra, 1982) were studied.

MATERIALS & METHODS

Pieris rapae adults were obtained from a laboratory culture (introduced from the field in 1981) reared on *Brassica oleracea* L. var. *gemmifera* D.C. Females used in this study were inbred for about 10 generations. After emergence butterflies were allowed to mate in large cages (80x80x100 cm), illuminated by mercury-vapour lamps and additional daylight. Temperatures fluctuated between 20^{0} an 26^{0}C and the butterflies had free access to a 10% sucrose solution. Experiments were conducted between 9.00 h and 14.00 h. In order to intensify egglaying mercury-vapour lamps were only switched on during the experiments. In this light regime female fecundity remained high during at least 25 days. Experimental females ranged in age from 2 to 24 days after eclosion and were used several times.

Behavioural experiments are based on choice experiments with matched pairs of cabbage leaves, taken from the same plant. Solutions of the oviposition deterring pheromone of *Pieris brassicae* were prepared by washing *P. brassicae* eggs for 5 min in pure methanol. Both sides of the leaves were sprayed with 1 ml of eggwash, containing 250 egg-equivalents (treated leaf) or 1 ml of methanol (control leaf), except for the leaves in the second experiment (Table 2), where only one side was treated with 1 ml of eggwash (500 egg-equivalents) and 1 ml of methanol, respectively. So in all experiments the treated leafs were sprayed with in total 500 egg-equivalents. Preliminary choice experiments with blanc leaves and leaves treated with methanol revealed no significant effect of methanol upon oviposition.

After evaporation of methanol the leaves, with their petioles in water were exposed simultaneously to the butterflies for 4 hours. The number of butterflies in the cage varied between 10 and 25, but the number of females actually laying eggs was not recorded. Every 30 minutes numbers of eggs laid on both leaves were counted and positions of leaves were interchanged. The activity of ODP is expressed in terms of percentage deterrence, calculated in the following way: % deterrence = (A-B) x 100/ A + B, where A and B are the numbers of eggs laid on control and treated leaves, respectively.

In a similar set up *Pieris rapae* females were given a choice between cabbage leaves with and without conspecific eggs, in order to investigate the existence of intraspecific egg load assessment in *Pieris rapae*. In the various experiments (N=17), the numbers of *Pieris rapae* eggs present on the experimental leaves ranged from 62 to 199.

RESULTS

In general, pre-oviposition behaviour of *Pieris rapae* females closely resembles that of *Pieris brassicae* (cf. Klijnstra, 1982). The main difference in oviposition behaviour between these two species is that *Pieris rapae* lays its eggs singly whereas *Pieris brassicae* is laying eggs in clusters. Laying single eggs means for each egg females pass through the whole pre-oviposition sequence again, although a few females were occasionally seen to deposit more than one egg without any intervenient flight or locomotion.

The following behavioural steps can be distinguished in pre-oviposition behaviour of *Pieris rapae*: 1. *approach* flight (hardly distinguishable from other flight behaviour); 2. *landing* and *drumming* i.e. alternating tapping movements of the fore-legs on the leaf surface; 3. *curving*, that is bending of the abdomen around the edge of the leaf, often started already during *drumming*. Both *drumming* and *curving* are usually accompanied by wing fluttering; 4. *touching* where the lower leaf surface is contacted by the extruded ovipositor. This step only occurs after *drumming* and wing fluttering have stopped; 5. *oviposition*, the final step after which the whole sequence may start again. The sequence given here is an abstraction of the behaviour actually displayed by females and does not imply that each behavioural step is invariably followed by the next one. Females may interrupt the behavioural chain at each step.

Table 1. Egglaying responses of *Pieris rapae* to ODP of *P. brassicae* during 8 successive days. Leaves treated at day 1. N: number of experiments.

			no. eggs on leaves		
	Day	N	Control	Experimental	% deterrence
Methanol VV Methanol	1	4	213	207	1.4
Methanol VV Pheromone	1	9	358	30	84.5
	2	8	512	31	88.6
	3	9	793	58	86.4
	4	11	1276	158	78.0
	5	7	637	31	90.7
	6	5	559	33	88.9
	7	3	267	50	68.5
	8	6	528	41	85.6

Table 2. *Pieris rapae* oviposition on cabbage leaves, treated in various ways with ODP of *Pieris brassicae*. N: number of experiments.

Treated surface	N	leaves		% deterrence*
		control	treated	
upper and lower	9	358	30	84.5
upper	15	447	70	72.9
lower	4	159	32	66.5

* no significant difference between the three treatments (Kruskal Wallis one way analysis of variance)

When observing ovipositing females during choice experiments, no qualitative differences were found in behaviour displayed at the control leaf and the treated leaf, respectively. Quantitative data are not available but several observations suggest lower-frequencies of both *landing* and *touching* as well as a shorter duration of *drumming* on the treated leaf.

Egglaying responses of *Pieris rapae* females to ODP of *Pieris brassicae* are shown in Table 1. In the control experiments eggs are equally distributed on the two leaves, whereas in the other experiments the large majority of eggs is laid on the control leaf. The deterrent activity of ODP, indicated by the percentage deterrence, remains high during at least 7 days after application. Preliminary experiments even suggest a persistence of 14 days under laboratory conditions.

Percentage deterrence does not decrease significantly when, instead of both, either the upper or lower leaf surface is treated with ODP (Table 2).

Table 3 gives some figures on the numbers of eggs laid in 4 successive periods in a number of choice experiments with ODP. During all successive periods in these trials, the number of eggs laid on the control leaf far exceeds the number laid on the treated leaf. In spite of the increasing egg load on the control leaf, females maintain a strong oviposition preference

Table 3. No. eggs laid by *Pieris rapae* in successive periods during 6 choice experiments (pooled results) with ODP of *Pieris brassicae*.
Period I: 10.00-10.30; II: 10.30-11.00; III: 11.00-11.30; IV: 11.30-12.00
V: 12.00-14.00 h.

Leaves	PERIOD					Total
	I	II	III	IV	V	
control	83	143	100	120	251	697
treated	20	6	6	2	15	49

Table 4. *Pieris rapae* oviposition in choice experiments with cabbage leaves with and without conspecific eggs. N: Number of experiments

N	blanc	egg laden	% deterrence
17	2289	1702	14.7

for this control leaf throughout the whole experiment.

The presence of *Pieris rapae* eggs on cabbage leaves indeed is not very deterrent to ovipositing *Pieris rapae* females (Table 4). In this Table only the pooled results are given because a detailed analysis of the data revealed that the percentage deterrence found in a particular experiment did not show any systematic relation with the number of eggs previously laid on the experimental leaf. As can be seen in Table 4, the total number of eggs laid on the egg laden leaves is only slightly lower as on the blanc leaves, resulting in a percentage deterrence of 14.7%. However, in only 4 out of the 17 experiments more eggs were laid on the experimental leaves. This indicates that females may tend to lay fewer eggs on egg laden leaves.

DISCUSSION

Rothschild & Schoonhoven (1977), when first reporting on the existence of an ODP in *Pieris brassicae* , already suggested an interspecific effect of this pheromone upon *Pieris rapae* oviposition. The results of the choice experiments described here clearly demonstrate the ability of *Pieris rapae* females to perceive ODP of *Pieris brassicae* . Cabbage leaves sprayed with ODP remain deterrent to ovipositing *Pieris rapae* females for at least 8 days. This agrees quite well with the persistence found in *Pieris brassicae* which lasted for 14 days and longer (Schoonhoven et al., 1981).

Pieris brassicae females can perceive ODP via three chemoreceptory organs located at the antennae, tarsi and ovipositor respectively (Behan & Schoonhoven, 1978; Klijnstra, 1982). Behavioural observations of *Pieris rapae* females suggest olfactory hairs as well as gustatory hairs (at the fore-tarsi) to be involved. From Table 2 it seems evident that cabbage leaves treated with ODP only at the lower side are equally well recognized as unsuitable oviposition substrates. *Pieris rapae* females possess a limited number of contactchemo-

receptors on the ovipositor (Klijnstra, unpublished). Whether these chemoreceptors participate in ODP perception is unclear. During *curving* and *touching* females often hang at the edge of the leaf thereby contacting the lower surface with mid- and/or hind-tarsi. Therefore, it cannot be excluded that also in this case tarsal taste hairs mediated the behavioural response. To understand the mechanism of ODP perception by *Pieris rapae* females, more detailed behavioural observations as well as electrophysiological recordings of various chemoreceptors are needed.

The oviposition deterring pheromone of *Pieris brassicae* in the first place will serve a reduction of intraspecific larval competition in *Pieris brassicae*. In the second place, *Pieris rapae* females apparently may perceive the information on the degree of occupation of a potential hostplant by *Pieris brassicae* eggs. The possible reduction of interspecific larval competition between *Pieris brassicae* and *Pieris rapae* resulting from this, might favour both species, however, the major advantage is probably for the single egg laying species *Pieris rapae*. According to Nordlund (1981) the ODP of *Pieris brassicae* now likewise may be defined as a synomone, however, with a distinct kairomonal character.

The response of *Pieris rapae* females to the ODP of *Pieris brassicae* may be called adaptive only when field observations have established the existence of larval competition between the two species. The only facts we know at this time is that females of both species may use the same host plant species as oviposition substrates and that their distributions partly coincide in time (Terofal, 1965). In the literature no reports were found, however, on the coexistence of larvae of both butterfly species on the same plant. Interspecific interactions are known to occur in two other pierid species. Shapiro (1981) reported *Pieris protodice* and *Euchloe ausonides* occasionally to be engaged in interspecific egg load assessment, possibly guided by visual cues. Whether an allelochemical, similar to that of *Pieris brassicae*, is involved is not known.

Kairomonal effects of epideictic pheromones, such as ODP of *Pieris brassicae*, are not necessarily limited to deterrence of oviposition by other butterfly species. Prokopy (1981) describes several examples of such pheromones to be utilized by parasitoids as host finding cues. This might as well be the case in *Pieris brassicae*. Preliminary experiments by Noldus & van Lenteren (1983) indicate ODP of *Pieris brassicae* to act as a contact kairomone for *Trichogramma evanescens* WESTWOOD, leading to arrestment of the parasite. The

aforementioned kairomonal effects of ODP - deterrence of oviposition in *Pieris rapae* females and arrestment of *T. evanescens* parasites - thus far have only been demonstrated in the laboratory. Whether these effects play a significant role in *Pieris rapae* egg distribution or searching behaviour of *T. evanescens* under natural conditions has to be proven in field experiments. Simultaneous deterrence of oviposition in both *Pieris brassicae* and *Pieris rapae* as well as stimulation of parasite searching behaviour might improve the prospects for field application of ODP in cabbage pest control.

Rothschild & Schoonhoven (1977) supposed that besides *Pieris brassicae*, *Pieris rapae* also employs intraspecific egg load assessment. There is contrary evidence, however, that the presence of conspecific eggs on a particular plant does not influence further oviposition in *Pieris rapae* (Jones, 1977; Ives, 1978 Traynier, 1979).

The data in Table 4 do not provide conclusive evidence on the existence of intraspecific egg load assessment in *Pieris rapae*. On the one hand, the deterrent activity of previously laid eggs was found to be very low. On the other hand, the observation that in most experiments fewer eggs were laid on the experimental leaves, possibly suggests the presence of such a mechanism in *Pieris rapae*. Whether such a response, if present, is mediated by a conspecific pheromone, cannot be concluded from these experiments. The experimental leaves were carrying intact *Pieris rapae* eggs, which implicates that also visual or tactile cues might have been involved.

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SUMMARY

This thesis deals with a detailed analysis of egglaying behaviour of adult females of the Large White Butterfly, *Pieris brassicae*, and the way this behaviour is influenced by the oviposition deterring pheromone (ODP) in order to investigate the prospects for field application of this pheromone in cabbage pest control.

The study begins with a short introduction on the role of oviposition behaviour in the relationship between herbivorous insects and plants (Chapter 1).

In Chapter 2 the ultimate effect of the ODP on egglaying behaviour of *Pieris brassicae* was demonstrated. In a choice situation with control leaves and ODP treated cabbage leaves females performed a strong preference to lay their eggs on the control leaves. The pheromone, which is associated with the eggs, appeared to be water- and methanol-soluble and could be stored for more than three years without loosing activity. In addition we found some evidence that eggs may contain large amounts of pheromone. The existence of this oviposition deterring pheromone in *P. brassicae* might contribute to a regular distribution of eggs over available hostplants.

In Chapter 3, a detailed description is given of egglaying behaviour of *P. brassicae* in the absence of the pheromone. This description formed the ethological framework on which further investigations of the behavioural effects of the ODP were based. In addition to this ethogram, the various sensory cues which may be involved in oviposition behaviour are discussed.

Perception of the oviposition deterring pheromone by *P. brassicae* females was studied in some detail in Chapter 4. Electrophysiological experiments indicated that females possess sense cells specifically sensitive to the ODP in their fore-tarsal taste hairs. In morphological studies, by means of the scanning electron microscope, and by electrophysiological recordings we established the presence of contactchemoreceptors on the ovipositor of *P. brassicae* females. Stimulations with ODP solutions indicated that to some extent these taste hairs might be involved in ODP perception. Responses to hostplant chemicals revealed that hostplant recognition is not mediated by sensory input from these hairs.

Chapters 5 and 6 are concerned with an experimental analysis of female preoviposition behaviour in order to determine the detailed behavioural effects of the ODP and the chemoreceptors females actually employ in ODP perception. The experimental set-up was identical in both chapters with single females offered

a choice between a control and an ODP treated leaf. The behaviour of females was recorded by means of a keyboard system and afterwards analysed for several parameters, including the sequence of behavioural acts.

In Chapter 5 the results with untreated females are described. Our observations indicated that the ODP induces only minor changes in pre-alighting behaviour of these females, suggesting that the pheromone is not very volatile. Most obvious behavioural differences between the two leaves were observed after landing, which indicate that tarsal contactchemoreceptors probably mediate the behavioural response to ODP. In addition, females were observed to perform a reduced tendency to stay on or around the treated leaf. This observation is congruent with the idea that the ODP induces *P. brassicae* females to disperse.

In Chapter 6, these experiments were extended to six groups of females with various sensory ablations. When all six tarsi, whether or not in combination with the antennae, were inactivated (by HCl treatment), oviposition was almost completely suppressed and females did not discriminate anymore between the two types of leaves. In females with intact chemoreceptors on at least one pair of tarsi egglaying activity was about normal and also discrimination was found to occur. Behavioural evidence was presented that females may employ antennal olfactory hairs, fore-tarsal taste hairs, as well as taste hairs on mid- and hind-tarsi to perceive the pheromone. Chemoreceptors on the ovipositor were not found to be involved in ODP perception. Finally, it was observed that females did not respond to the ODP anymore once the first egg had been laid.

In Chapter 7, the first field tests with the ODP are described. In control experiments without pheromone, the plants chosen for oviposition by *P. brassicae* females were distributed rather evenly over the cage. When the majority of plants was sprayed with ODP, oviposition attempts were mainly observed on plants along the sides of the cage. This strongly suggests that the ODP indeed evokes females to disperse from the field. The presence of the pheromone did not result, however, in a significant decrease of oviposition on treated plants. This might have been due to our experimental conditions.

In Chapter 8, behavioural responses of *Pieris rapae* butterflies to the ODP of *P. brassicae* are investigated. ODP treated cabbage leaves remained deterrent to ovipositing *P. rapae* females for at least 8 days. A conspecific ODP in *P. rapae* could not be demonstrated. It is concluded that this interspecific effect of the ODP enhances the prospects for field application of this pheromone in cabbage pest control.

SAMENVATTING

Dit proefschrift beschrijft het eileggedrag van vrouwtjes van het grote koolwitje, *Pieris brassicae*, en de wijze waarop dat gedrag beïnvloed wordt door het zogenoemde eilegremmend feromoon teneinde de mogelijkheden voor toepassing van dit feromoon in de bestrijding van het koolwitje te onderzoeken.

In de inleiding (Hoofdstuk 1) wordt in het kort de relatie tussen herbivore insekten en planten besproken en de rol die het eileggedrag van adulte insekten daarin speelt.

Het uiteindelijke effect van het eilegremmend feromoon op het eileggedrag van koolwitjes wordt beschreven in Hoofdstuk 2. In een keuzesituatie met controle- en behandelde bladeren bleken eierleggende vrouwtjes een sterke voorkeur te vertonen voor het controleblad. Het feromoon, dat afkomstig is uit de eieren van *P. brassicae*, bleek oplosbaar te zijn in water en methanol en kon, zonder verlies aan activiteit, zelfs meer dan 3 jaar lang bewaard worden. Bovendien werden aanwijzingen verkregen dat de eieren mogelijk een grote hoeveelheid feromoon bevatten. De hier gevonden resultaten wijzen erop dat vrouwtjes van het grote koolwitje, via dit feromoon, in staat zijn hun nakomelingen evenwichtig te verdelen over beschikbare voedselplanten.

In Hoofdstuk 3 wordt een uitvoerige beschrijving van het eileggedrag van vrouwtjes in afwezigheid van het feromoon gegeven. Op deze beschrijving is het verdere onderzoek naar de precieze gedragseffecten van het feromoon gebaseerd. Daarnaast worden ook de verschillende zintuiglijke prikkels die een rol kunnen spelen in het eileggedrag besproken.

In Hoofdstuk 4 is de wijze waarop vrouwtjes het eilegremmend feromoon kunnen waarnemen bestudeerd. Elektrofysiologische experimenten toonden aan dat smaakharen op de voorpoten van vrouwtjes zeer waarschijnlijk zintuigcellen bevatten die specifiek gevoelig zijn voor het feromoon. In rasterelektronenmicroscopisch en elektrofysiologisch onderzoek is aangetoond dat de ovipositor van *P. brassicae* vrouwtjes ook voorzien is van een aantal smaakharen. De reacties op verschillende chemische prikkels gaven aan dat deze smaakharen mogelijk betrokken zijn in de perceptie van het feromoon, maar geen rol spelen in de waardplantherkenning door *P. brassicae* vrouwtjes.

In de Hoofdstukken 5 en 6 is een experimentele analyse gemaakt van het pre-ovipositiegedrag van vrouwtjes teneinde de precieze gedragseffecten van het feromoon en de chemoreceptoren die vrouwtjes daadwerkelijk gebruiken om het feromoon waar te nemen, te bepalen. In de experimenten beschreven in deze

hoofdstukken, werd aan afzonderlijke vrouwtjes een keuzesituatie met één controleblad en één behandeld blad aangeboden. Het gedrag van de vrouwtjes werd met behulp van een toetsenbord geregistreerd om naderhand verschillende parameters, waaronder de sequentie van gedragscomponenten, te kunnen analyseren.

Bij onbehandelde vrouwtjes (Hoofdstuk 5) werd gevonden dat het gedrag voorafgaand aan landen op het blad slechts in geringe mate beinvloed werd door het feromoon, hetgeen suggereert dat het feromoon niet erg vluchtig is. Na contact met het blad waren de verschillen in gedrag tussen de beide bladeren veel groter. Dit betekent dat contactchemoreceptoren op de poten van vrouwtjes waarschijnlijk verantwoordelijk zijn voor de gedragsreactie op het feromoon. Verder bleek dat vrouwtjes na contact met het behandelde blad minder de neiging hadden terug te keren naar dat blad dan op het controleblad het geval was. Dit duidt erop dat het feromoon de vrouwtjes aanzet tot dispersie.

In Hoofdstuk 6 werd het gedrag van zes groepen vrouwtjes met verschillende zintuiglijke ablaties bestudeerd. Wanneer chemoreceptoren op alle zes poten, al of niet in combinatie met die op de antennes, waren uitgeschakeld (na behandeling met HCl), bleken de vrouwtjes nauwelijks nog eieren te leggen en was er gedragsmatig geen verschil meer te zien tussen de twee typen bladeren. In vrouwtjes met intacte chemoreceptoren op tenminste 1 paar poten lag de eilegactiviteit vrijwel op het normale niveau en bleek ook het onderscheidingsvermogen van de vrouwtjes nauwelijks te zijn aangetast. Via deze gedragsexperimenten is aangetoond dat de vrouwtjes zowel reukharen op de antennes als smaakharen op de voor-, midden- en achterpoten kunnen gebruiken om het feromoon waar te nemen. Gedragsmatig werd ook vastgesteld dat de chemoreceptoren op de ovipositor geen rol spelen in de perceptie van het feromoon. Verder is gebleken dat vrouwtjes, als ze eenmaal begonnen zijn met het leggen van een eipakket, niet meer op het feromoon reageren.

In Hoofdstuk 7 worden de eerste veldexperimenten met het eilegremmend feromoon beschreven. De planten die door vrouwtjes gekozen werden als oviositiesubstraat waren, in afwezigheid van het feromoon, tamelijk evenwichtig over de veldkooi verdeeld. Na behandeling van een groot deel van de planten met het eilegremmend feromoon, bleken de vrouwtjes voornamelijk planten langs de randen van de kooi te kiezen. Dit zou erop kunnen duiden dat de vrouwtjes door het feromoon inderdaad worden aangezet tot het verlaten van het veld. Op met feromoon behandelde planten werd echter niet duidelijk minder gelegd dan op controleplanten. Dit is mogelijk een gevolg geweest van de gekozen experimentele opzet.

In Hoofdstuk 8 werden de gedragsreacties van het kleine koolwitje, *Pieris rapae*, op het eilegremmend feromoon van *P. brassicae* onderzocht. De eileg van *P. rapae* vrouwtjes bleek ook sterk geremd te worden door dit feromoon. Zelfs 8 dagen na bespuiting was nog een duidelijk remmend effect waar te nemen. *P. rapae* vlinders hebben vermoedelijk niet de beschikking over een soortseigen eilegremmend feromoon. De vooruitzichten op toepassing van het eilegremmend feromoon van *P. brassicae* in de bestrijding van koolwitjes worden door dit interspecifieke effect aanzienlijk verbeterd.

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CURRICULUM VITAE

Jacob Wieger Klijnstra werd op 16 mei 1954 te Sassenheim geboren. In 1972 behaalde hij het eindexamen HBS-B aan het Bogerman College te Sneek. In het zelfde jaar begon hij zijn studie Biologie aan de Rijksuniversiteit van Groningen. Het kandidaatsexamen (B-1) legde hij af in december 1976. In januari 1980 studeerde hij af met als hoofdvak zintuigfysiologie en als bijvakken neurofysiologie en vergelijkende endocrinologie (afgelegd aan de Vrije Universiteit van Amsterdam). Tevens behaalde hij zijn eerste graads onderwijsbevoegdheid voor biologie. Van 15 februari 1980 tot 15 februari 1983 was hij als promotie-assistent aangesteld bij de vakgroep Dierfysiologie van de Landbouwhogeschool te Wageningen, waar het in dit proefschrift beschreven onderzoek werd verricht. Nadien was hij tijdelijk (1 september 1983 - 1 september 1984) als wetenschappelijk ambtenaar aangesteld bij deze vakgroep.