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SEVEN-WEEK PERSISTENCE OF AN OVIPOSITION-DETERRENT PHEROMONE

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Abstract—Cabbage leaves sprayed with a water solution of the oviposition-deterrent pheromone of *Pieris brassicae* remain deterrent to ovipositing females for at least 14 days. Under laboratory conditions, the pheromone, when dried on a glass surface, retains activity for a period of at least 7 weeks. After 7 days under vacuum conditions, some pheromone is still present, indicating a low volatility and/or high stability of (an active fraction of) the pheromone. After 125 eggs are slowly rinsed with 300 ml water, they still release detectable quantities of the pheromone.

Key Words—Oviposition, deterrent pheromone, *Pieris brassicae*, Lepidoptera, Pieridae.

INTRODUCTION

Pheromones conveying messages to conspecifics often have a short news value. The physicochemical properties of such pheromones fullfil, among other requisites, the temporal aspects of the message (Wilson and Bossert, 1963). Thus, the alarm pheromone of aphids dissipates within 30–60 min (Nault et al., 1973), and the substance which signals to nectar collecting bees that a flower has been visited recently also disappears within about 10 min (Frankie and Vinson, 1977). On the other hand, trail pheromones in social Hymenoptera may last up to 3 days (Jander and Jander, 1979), and territorial flags in this insect group remain active for at least 12 days (Hölldobler and Wilson, 1978).

Oviposition-deterrent pheromones, which promote an even spatial distribution of eggs in many insect species (Prokopy, 1980), may also be

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expected to remain active for days rather than minutes. The volatile oviposition deterrent indicating the occupancy of a *Melandrium* flower by a conspecific egg of the moth *Hadena bicruris* lasts for only one day. This suffices, however, since the ovipositing females also show a clear preference for 1-day-old flowers over those of 2 days old (Brantjes, 1976). The marking pheromone produced by the apple maggot fly *Rhagoletis pomonella* lasts for at least 4 days (Prokopy, 1972). The solitary larvae of *H. bicruris* and *R. pomonella* protect their limited food supply by cannibalistic behavior or some other unknown mechanism to younger conspecifics (Prokopy, 1980). Likewise, the oviposition-deterrent pheromone of the sorghum shoot fly, *Atherigona soccata*, remains active for at least 5 days (Raina, 1980).

Pieris brassicae L. (Lepidoptera: Pieridae) butterflies produce batches of 20–100 eggs, to which during the process of egg-laying an oviposition-deterrent pheromone is added (Rothschild and Schoonhoven, 1977). Since, under natural conditions, egg incubation periods may vary between 5 and 15 days and the gregarious larvae in this case do not kill conspecifics, a higher degree of persistence of the oviposition-deterrent pheromone than observed in other insects seems important. This paper describes the minimum period during which the oviposition deterrent pheromone of *P. brassicae* remains biologically active. Since a study on persistence or decay of a pheromone requires some idea of the quantities involved, we also report some preliminary data on quantities of pheromone present in *P. brassicae* eggs.

METHODS AND MATERIALS

P. brassicae females were obtained from a lab culture on cabbage plants, started by Drs. W.A.L. David and B.O.C. Gardiner in 1953. Tests involving 8–12 females, 3–5 days old, were conducted in cages 70 × 90 × 70 cm. under artificial or natural light conditions. Pheromone solutions were prepared by carefully shaking 125 or 250 eggs (3–27 hr after oviposition) with 1 ml water for 5 min in a small test tube. The egg wash was painted with a soft brush or sprayed with a perfume vaporizer onto both sides of a cabbage leaf about 30 min before the start of an experiment. A control leaf, carefully matched for equal size and age and taken from the same plant, was treated with distilled water. The experimental and control leaves, with their petioles in water, were simultaneously offered at a 45° angle to the butterflies for 4–6 hr. Every 30 min the leaves were switched to alleviate position effects. All experiments were conducted under room conditions (20–28° C).

To determine pheromone persistence, 1 ml of egg wash was put into a 3-cm-diam Petri dish and air dried. It was stored uncovered, but protected from falling dust under room conditions or at reduced pressure (15 mm Hg) in a desiccator. After the storage period, the remaining pheromone was dissolved in 1 ml water and applied to the experimental cabbage leaf to be tested.

To determine the quantity of pheromone present, either 125 eggs were shaken with 1 ml water for 5 min, repeating this procedure 8–10 times and using fresh water every time, or 125 eggs were put on a filter paper in a funnel and rinsed with 300 ml water in 30 min or 1000 ml in 90 min. After being rinsed in either of the two ways, the eggs were shaken with 1 ml water for 5 min. The latter wash was tested.

RESULTS

Egg wash applied to leaves of intact cabbage plants renders these leaves almost completely deterrent to ovipositing females for a period of 3 days (Table 1). Thereafter, deterrency diminishes somewhat, although marked effects remain discernable for 14 days or longer.

Egg wash (1 ml from 250 eggs), air dried in a Petri dish, retains high deterrency for a least 7 weeks (Table 2). It also retains high activity for at least 7 days after exposure to low air-pressure conditions (Table 3).

An approximate estimation of the amount of the pheromone present in an egg batch can be obtained by rinsing eggs with various amounts of water. After shaking 125 eggs 8–10 times with 1 ml water or rinsing 125 eggs with 300 ml water, a deterrent effect of the next rinse wash can still be observed (Table 4). When the eggs are rinsed with 1000 ml water, the concentration of the inhibiting factor becomes too low to be detectable in our tests (Table 4).

TABLE 1. PHEROMONE ACTIVITY ON LEAVES OF INTACT CABBAGE PLANTS AFTER VARIOUS PHEROMONE EXPOSURE PERIODS TO ROOM CONDITIONS[a]

Exposure period (days)	*N*	Control leaf	Treated leaf
0	2	5/95[b]	0/0[b]
1	1	3/84	4/4
2	1	3/59	0/0
3	1	7/232	0/0
4	1	6/200	3/70
6	1	7/230	2/38
6*	1	7/98	1/2
8	1	8/250	2/40
8	1	19/386	0/0
12	1	12/390	3/80
14	1	6/160	2/34

[a] All experimental leaves were sprayed with 1 ml water wash from 125 eggs except those marked *, which were sprayed with 1 ml wash from 250 eggs. The pheromone-treated leaves remained attached to the plants until they were tested, 0–14 days after spraying. *N* = number of replicates.
[b] Number of egg batches/total number of eggs.

TABLE 2. PHEROMONE ACTIVITY AFTER VARIOUS EXPOSURE PERIODS TO ROOM CONDITIONS IN OPEN PETRI DISHES[a]

Exposure period (days)	N	Control leaf	Treated leaf
14	1	7/190[b]	1/4[b]
40	3	17/585	2/22
49	2	13/406	3/5

[a] 1 ml from 250 eggs.
[b] Number of egg batches/total number of eggs.

DISCUSSION

Behavioral evidence (Rothschild and Schoonhoven, 1977) as well as electrophysiological results (Behan and Schoonhoven, 1978) indicate that *P. brassicae* females detect conspecific eggs via olfactory as well as contact chemoreceptors. Either the same compound stimulates both receptor types or the pheromone is composed of volatile and nonvolatile factors. The evidence presented here indicates the presence of one (or more) highly stable component(s) with low vapor pressure, since activity is maintained when exposed for 7 weeks to air in Petri dishes or for 1 week under vacuum conditions. When applied to plant leaf surfaces, the pheromone is still relatively durable, although after 3 days its activity decreases somewhat, presumably owing to biological factors such as leaf growth. Even under these circumstances, however, an oviposition inhibiting effect is still detectable after 14 days.

The stability of the oviposition-deterring pheromone of *P. brassicae* seems unusually high compared to examples cited in the literature (see

TABLE 3. PHEROMONE ACTIVITY AFTER EXPOSING AIR-DRIED EGG WASH (1 ml from 250 eggs) FOR VARIOUS PERIODS TO LOW AIR PRESSURE AT ROOM-TEMPERATURE

Exposure period (days)	N	Control leaf	Treated leaf
3	4	6/91[a]	0/0[a]
5	2	15/355	2/29
6	1	7/160	6/6
7	1	5/191	0/0

[a] Number of egg batches/total number of eggs.

TABLE 4. ACTIVITY OF EGG WASH (1 ml of 125 eggs) AFTER EGGS RECEIVED VARIOUS PRETREATMENTS

Pretreatment	N	Control leaf	Treated leaf
Eggs washed			
8× with 1 ml water	2	24/460[a]	5/87[a]
10× with 1 ml water	1	8/140	0/0
Eggs rinsed			
With 300 ml water	5	42/1280	10/241
With 1000 ml water	2	11/530	8/330

[a] Number of egg batches/total number of eggs.

Introduction). Conceivably, however, the amount of pheromone present in the wash of 125 eggs is very large and some evaporation or degradation of the compound would then escape our attention. Some preliminary experiments suggest that the amount present in an egg batch is considerable when tested with the pheromone distributed evenly over a whole leaf surface. A rinse with 300 ml water still leaves enough pheromone to be demonstrated in our oviposition test. Apparently the eggs release the pheromone only gradually.

It is concluded that the oviposition-inhibiting pheromone of *P. brassicae* shows a high degree of stability. The apparent stability may, to a certain extent, be somewhat exaggerated due to the probably high concentration of the compound tested. Nonetheless, it remains an example of a highly persistent insect pheromone.

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