Journal of Chemical Ecology, Vol. 16, No. 5, 1990

DOES THE IMPORTED CABBAGEWORM, *Pieris rapae*, USE AN OVIPOSITION DETERRING PHEROMONE?

L.M. SCHOONHOVEN, E.A.M. BEERLING, R. BRAAKSMA, and
Y. VAN VUGT

Department of Entomology
Agricultural University
P.O. Box 8031, Wageningen, The Netherlands

(Received May 25, 1989; accepted September 20, 1989)

Abstract—Eggs of *Pieris rapae* (L.) contain a water- and methanol-soluble oviposition-deterring pheromone (ODP) that is avoided by ovipositing females offered treated and untreated cabbage leaves in a choice situation. Water extracts of female accessory glands also deter oviposition. Egg washes and gland extracts stimulate a contact chemoreceptor in sensilla on the fore tarsi. Electroantennogram (EAG) recordings show that antennal receptors respond to volatiles from conspecific eggs, but not to volatiles from eggs of *Mamestra brassicae.* No differences were detected between North American and European insects with regard to production and perception of ODP. These results differ from conclusions in the literature based upon field observations. It is concluded that this discrepancy in due to methodological differences.

Key Words—*Pieris rapae,* Lepidoptera, Pieridae, imported cabbageworm, oviposition deterring pheromone, egg dispersion, chemoreception.

INTRODUCTION

Previous studies provide conflicting evidence about whether the imported cabbageworm, *Pieris rapae* (L.)(Lepidoptera; Pieridae) marks its eggs with an oviposition-deterrent pheromone (ODP) and thereby promotes an even distribution of eggs among available host plants. Rothschild and Schoonhoven (1977) concluded from preliminary experiments under laboratory conditions that *P. rapae* discriminates between a cabbage leaf from which conspecific eggs had been removed and a "clean" control leaf. Klijnstra (1985a, p. 230), on the basis of 17 laboratory experiments, concluded somewhat equivocally that "females may tend to lay fewer eggs on egg laden plants." However, several other studies

0098-0331/90/0500-1649$06.00/0

based on field (Kobayashi, 1965; Ives, 1977; Root and Kareiva, 1984) or laboratory (Traynier, 1979) observations failed to show discrimination against egg-containing plants. In some cases even a certain degree of clumping has been reported (Harcourt, 1961; Jones, 1977).

These conflicting conclusions may be caused by any of the following reasons. (1) The preliminary results used to infer the presence of an ODP in *P. rapae* (Rothschild and Schoonhoven, 1977; Klijnstra, 1985a) may misrepresent reality. (2) Geographical differences may exist between the insects used in different studies. (3) Methodological differences may have caused different results. To identify the cause of the discrepancy, we have conducted a more detailed study of whether *P. rapae* employs an ODP and thus deters conspecific females from ovipositing in the neighborhood of previously laid eggs. Additionally, oviposition responses between European and American insects were compared to detect possible geographical differences in the use of an ODP.

METHODS AND MATERIALS

Insects. A colony of *P. rapae*, established from insects caught in the wild in the Netherlands, was kept for about 25 generations in a greenhouse (conditions: 19–25°C, photoperiod light-dark = 16–8, relative humidity: 65–80%) on cabbage (*Brassica oleracea gemmifera* cv. titurel). Insects of American origin were kindly provided by Dr. J.A.A. Renwick from a culture derived from females collected near Ithaca, New York, and kept for about 20 generations in a greenhouse on cabbage (*B. oleraccea gemmifera* cv. Golden Acre). All butterflies were fed on artificial flowers containing 10% sugar solution.

Egg Washes. Eggs laid by European or American insects were carefully brushed off the leaf surface and shaken for 5 min with methanol or water (500 eggs/1 ml solvent). Egg washes were stored at 3°C for one to eight days before use.

Accessory Gland Extracts. Water extracts of female accessory glands were obtained by bathing two pairs of excised glands in 1 ml water for 2 hr. Washes from comparable amounts of female fat body were used as a control. For further details see Behan and Schoonhoven (1978).

Behavioral Tests. Two cabbage leaves (10–15 cm diam.) picked from the same plant were offered in an 80 × 100 × 100-cm cage to 4–10 butterflies, 2–12 days old. One leaf was sprayed with 1 ml egg wash or accessory gland extract, and the other was treated with solvent only (control). To avoid position effects, leaves were transported periodically. In addition to natural light each cage was illuminated from above by a mercury vapor lamp. Each test lasted from 10:00 AM until 2:00 PM. ODP indexes were calculated using the formula: % deterrence = $[(A - B) \times 100]/(A + B)$, where A and B are the numbers of eggs laid on control and treated leaves, respectively. An ODP index of 100% indicates that butterflies laid all eggs on the control leaf, whereas 0% indicates no preference.

Electrophysiology. Forelegs of 1- to 4-day-old female were cut at the middle of the tibia and a silver wire (ϕ 30 μm) was pushed into the distal part of the tibia and connected to the input of a high input impedance preamplifier (Hodgson et al., 1955; Drongelen, 1979). The stimulus capillary contained 3 mM NaCl (control) supplemented by either egg water wash (conc.: 500 eggs/ml) or 10 mM singrin, or 30 mM sucrose. The indifferent electrode was in contact with the stimulus solution. Tarsal B-hairs were stimulated by positioning the stimulus capillary over the tip of a sensillum for 1.5–2 sec. For further details see Ma and Schoonhoven (1973).

Electroantennograms (EAGs) were registered from excised antennae positioned between two micropipets and exposed to a constant airstream (1800 ml air/min, 20°C, humidified). The stimulus consisted of a 1 ml/sec airstream lasting 2 sec, which has passed along a piece of filter paper containing either 25 μl 0.1% *cis*-3-hexen-1-ol in paraffin oil (standard), or 0.2 g *P. rapae* eggs, or 0.4 g *Mamestra brassicae* eggs. Responses to eggs were expressed as percentages of responses to standard stimuli.

RESULTS

Behavioral Experiments. When butterflies were offered cabbage leaves treated with egg wash, together with "clean" control leaves, they showed a strong oviposition preference for control leaves (Table 1). Thus, ovipositing females apparently are able to detect the presence of a chemical extracted from

TABLE 1. CHOICE BEHAVIOR IN OVIPOSITING BUTTERFLIES WHEN EXPOSED TO ODP EXTRACTS

Stimulus	Replicates (*N*)	Total eggs (*N*) Treated leaves	Total eggs (*N*) Control leaves	ODP deterrence (%)
U.S.A. insects				
Egg wash U.S.A.	12	185	956	68[a]
Wageningen insects				
Egg wash Wageningen	9	249	1453	71[a]
Accessory gland extract	10	870	1302	20[a]
Fat body extract	4	318	268	−8 (N.S.)

[a] Different from 0, $P < 0.01$ (chi-square test).

the eggs. Insects originating from the United States showed the same responses as insects collected in Europe. A water extract from female accessory glands was weakly, although significantly, deterrent, whereas a fat body extract, which served as the control, did not significantly affect female behavior.

Electrophysiological Experiments. Sensitivity to water solutions containing ODP has been observed in the tarsal sensilla of the large white butterfly, *Pieris brassicae,* (Behan and Schoonhoven, 1978), the apple maggot fly (Crnjar and Prokopy, 1982), and the European cherry fruit fly (Hurter et al., 1987). In this study, the tarsal sensilla of *P. rapae* (Wageningen culture) displayed an intense electrophysiological response to egg washes; this response arose predominantly in one chemoreceptive neuron (Table 2). The same cell also exhibited a strong, albeit slightly lower, response to an accessory gland extract. This lower response may indicate that the gland extract (prepared from two paired glands per milliliter of solvent) contained less ODP than the egg wash (obtained from 500 eggs/ml).

Behavioral observations on *P. brassicae* females suggested that the ODP was perceived from some distance (Rothschild and Schoonhoven, 1977; Klijnstra, 1985b), indicating the presence of airborne cues. In a previous study, EAG recordings revealed that the antennae of *P. brassicae* females are stimulated by egg volatiles (Behan and Schoonhaven, 1978). To determine whether the antennae of *P. rapae* show a similar response, EAGs were recorded using intact egg odors and pieces of filter paper with egg wash. Eggs of *P. rapae* clearly evoked antennal responses, which were for European insects on average 10.3% (± 0.9; $N = 21$) of the response elicited by the (highly stimulatory) standard stimulus (hexenol, see Methods and Materials), and 11.4% (± 1.0; $N = 22$) for American insects. In contrast, neither eggs of *M. brassicae* (0.1% ± 0.1; $N = 8$) nor washes of *P. rapae* eggs produced a reaction in *P. rapae* antennae. Thus no differences in antennal responses occur between American and European insects.

TABLE 2. MEAN NUMBER OF IMPULSES (±SEM) IN FEMALE TARSAL SENSILLA DURING FIRST SECOND OF STIMULATION WITH VARIOUS EXTRACTS[a]

Stimulus	Impulse frequency ±SE
Egg wash	83.5 ± 9.3a
Accessory gland extract	53.9 ± 5.6b
Fat body extract	14.9 ± 2.6c
10 mM NaCl	15.1 ± 2.6c

[a] Legs and stimuli were derived from the Wageningen colony. Means with different letters are significantly different ($P \leq 0.01$), $N = 30$.

DISCUSSION AND CONCLUSIONS

The behavioral evidence presented unequivocally demonstrates that *P. rapae* butterflies avoid laying eggs on plants that have been treated with ODP containing egg washes. This phenomenon has been described before for *P. brassicae* (Rothschild and Schoonhoven, 1977; Klijnstra, 1985b), which lays its eggs in batches of up to 100 eggs. *P. rapae,* however, oviposits single eggs. Despite a lower risk of conspecific food competition, it appears that *P. rapae* also has developed a chemical signal that aids the ovipositing female to avoid laying eggs on occupied plants.

The behavioral evidence for the presence of an ODP in *P. rapae* is corroborated by the finding that egg washes of this species stimulate a chemoreceptor in the female's tarsal sensilla. The same cell responds, although at a lower intensity, to extracts from the female's accessory glands. These extracts also inhibit oviposition to a certain degree, and therefore the glands likely contain the production sites and/or storage places of ODP.

Antennal responses measured by EAGs indicated that *P. rapae* possesses olfactory cells that can be stimulated by volatiles associated with conspecific eggs, but that are insensitive to odors of eggs from *M. brassicae,* a noctuid also occurring on cabbage plants. Presumably the ability to perceive airborne chemicals liberated from conspecific eggs plays an additional role in increasing the dispersion of *P. rapae* eggs.

Given that *P. rapae* employs oviposition-deterring chemicals, why have some previous studies failed to detect an ODP? Since the reports cited were all based on observations of *P. rapae* in North America and Australia, where it was introduced more than 100 years ago (Scudder, 1887; Elton, 1958) and where *P. brassicae* does not occur, these populations might have lost their ability to perceive conspecific eggs. It could be argued that, although during evolution ODPs were primarily developed to reduce oviposition by an insect near its own previously laid eggs (Roitberg and Prokopy, 1987) the deposition and perception of ODP by *P. rapae* is maintained mainly because it reduces competition with *P. brassicae.* The fact that ovipositing *P. brassicae* females avoid washes of *P. rapae* eggs, and vice versa (Schoonhoven et al., 1989), is in agreement with the supposition of a heterospecific function of the ODP. However, the similarity in response of the Dutch and American populations to egg washes argues strongly against this explanation.

It must be concluded, therefore, that the incongruity between some literature reports and the results from the present study are due to methodological differences. The conclusions reached by Harcourt (1961), Jones (1977), Ives (1978), and Root and Kareiva (1984) were all based on observations under natural conditions. Although ODPs in some other insects have been found to act under field conditions, for instance by increasing dispersal activity (Roitberg et al., 1984; Klijnstra and Schoonhoven, 1987), it may be difficult in the complexity of a natural situation to detect such activity statistically, especially when egg densities are relatively low. The observation by Kobayashi (1965), that at low densities *P. rapae crucivora* egg distributions show some degree of aggregation, whereas with the increase of density eggs are laid more evenly and extensively, agrees with the idea that egg density affects distribution pattern. Other environmental factors, such as host distribution patterns (Singer, 1986) and variations in host quality (Averill and Prokopy, 1989), also may override ODP effects. Kellogg (1985) found that *Pieris sisymbrii* possesses egg-avoiding behavior, which, however, may be easily masked by variation in host encounters. Additionally, unequivocal evidence for egg avoidance has been observed to operate in nature in two other pierid species that lay their eggs singly (Wiklund and Åhrberg, 1978; Shapiro, 1980). The present study, utilizing laboratory observations under highly standardized conditions and using large amounts of ODP, may have been able to detect behavioral responses to ODP that are relatively invisible in a complex and variable natural situation.

Acknowledgments—We thank Dr. J.A.A. Renwick for providing a culture from insects collected in the United States, Ms. B. Nübel for technical assistance, Drs. D.N. Karowe and B.D. Roitberg for helpful discussions, and D.N. Karowe for impoving the manuscript.

REFERENCES

AVERILL, A.L., and PROKOPY, R.J. 1989. Distribution patterns of *Rhagoletis pomonella* (Diptera: Tephritidae) eggs in hawthorn. *Ann. Entomol. Soc. Am.* 82:38–44.

BEHAN, M., and SCHOONHOVEN, L.M. 1978. Chemoreception of an oviposition deterrent associated with eggs in *Pieris brassicae*. *Entomol. Exp. Appl.* 24:163–179.

CRNJAR, R.M., and PROKOPY, R.J. 1982. Morphological and electrophysiological mapping of tarsal chemoreceptors of oviposition deterring pheromone in *Rhagoletis pomonella* flies. *J. Insect Physiol.* 28:393-400.

DRONGELEN, W. VAN, 1979. Contact chemoreception of host plant specific chemicals in larvae of various *Yponomeuta* species (Lepidoptera). *J. Comp. Physiol.* 134:265–279.

ELTON, C.S 1958. The Ecology of Invasions by Animals and Plants. Methuen, London.

HARCOURT, D.G. 1961. Spatial pattern of the imported cabbage worm, *Pieris rapae* (L.)(Lepid.: Pieridae), on cultivated cruciferae. *Can. Entomol.* 93:945–951.

HODGSON, E.S., LETTVIN, J.Y., and ROEDER, K.D. 1955. Physiology of a primary chemoreceptor unit. *Science* 122:417–418.

HURTER, J., BOLLER, E.F., STÄDLER, E. BLATTMANN, B., BUSER, H.R., BOSSHARD, N.U., DAMM, L., KOZLOWSKI, M.W., SCHÖNI, R., RASCHDORF, F., DAHINDEN, R., SCHLUMP, E., FRITZ, H., RICHTER, W.J., and SCHREIBER, J. 1987. Oviposition-deterring pheromone in *Rhagoletis ceras* L.: Purification and identification of the chemical constitution. *Experientia* 43:157–164.

IVES, P.M. 1978. How discriminating are cabbage butterflies? *Aust. J. Ecol.* 3:261–276.

JONES, R.E. 1977. Movement patterns and egg distributions in cabbage butterflies. *J. Anim. Ecol.* 46:195–212.

KELLOGG, T. 1985. Egg dispersion patterns and egg avoidance behavior in the butterfly *Pieris sisymbrii* Bdv. (Pieridae). *J. Lepid. Soc.* 39:268–275.

KLIJNSTRA, J.W. 1985a. Interspecific egg load assessment of host plants by *Pieris rapae* butterflies. *Entomol. Exp. Appl.* 38:227–231.

KLIJNSTRA, J.W. 1985b. Oviposition behaviour as influenced by the oviposition deterring pheromone in the large white butterfly, *Pieris brassicae.* PhD thesis. Wageningen Agricultural University, Wageningen.

KLIJNSTRA, J.W., and SCHOONHOVEN, L.M. 1987. Effectiveness and persistence of the oviposition deterring pheromone of *Pieris brassicae* in the field. *Entomol. Exp. Appl.* 45:227–235.

KOBAYASHI, S. 1965. Influence of parental density on the distribution pattern of eggs in the common cabbage butterfly *Pieris rapae crucivora. Res. Popul. Ecol.* 7:109–117.

MA, W.-C., and SCHOONHOVEN, L.M. 1973. Tarsal contact chemosensory hairs of the large white butterfly *Pieris brassicae* and their possible role in oviposition behaviour. *Entomol. Exp. Appl.* 16:343–357.

ROITBERG, B.D. and PROKOPY, R.J. 1987. Insects that mark host plants. An ecological, evolutionary perspective on host-marking chemicals. *BioScience* 37:400–406.

ROITBERG, B.D., CAIRL, R.S., and PROKOPY, R.J. 1984. Oviposition deterring pheromone influences dispersal distance in tephritid fruit flies. *Entomol. Exp. Appl.* 35:217–220.

ROOT, R.B., and KAREIVA, P.M. 1984. The search for resources by cabbage butterflies (*Pieris rapae*): ecological consequences and adaptive significance of Markovian movements in a patchy environment. *Ecology* 65:147–165.

ROTHSCHILD, M., and SCHOONHOVEN, L.M. 1977. Assessment of egg-load by *Pieris brassicae* (Lepidoptera: Pieridae). *Nature* 266:352–355.

SCHOONHOVEN, L.M., BEERLING, E.A.M., KLIJNSTRA, J.W., and VUGT, Y. VAN. 1990. Two related butterfly species avoid oviposition near each other's eggs. *Experientia* 46 (in press).

SCUDDER, S.H. 1887. The introduction and spread of *Pieris rapae* in North America 1860–1886. With a map. *Mem. Boston Soc. Nat. Hist.* 4:53–69.

SHAPIRO, A.M. 1980. Egg-load assessment and carry over diapause in *Anthocaris* (Pieridae). *J. Lepid. Soc.* 34:307–315.

SINGER, M.C. 1986. The definition and measurement of oviposition preference in plant-feeding insects, pp. 65–94, *in* J.R. Miller and T.A. Miller (eds.). Insect–Plant Interactions. Springer-Verlag, New York.

TRAYNIER, R.M.M. 1979. Long-term changes in the oviposition behaviour of the cabbage butterfly, *Pieris rapae,* induced by contact with plants. *Physiol. Entomol.* 4:87–96.

WIKLUND, C., and ÅHRBERG. 1978. Host plants, nectar source plants, and habitat selection of males and females of *Anthocaris cardamines* (Lepidoptera). *Oikos* 31:169–183.