Both parameters of reproductive activity were lower in the SD, 26 °C group than in control females. In three other groups, the incidence of ovipositing females was 100% (or 97%) as in the control females, and the oviposition period lasted even longer than in the controls (table 2, fig. 3). The two SD groups differed markedly: in the 26 °C group, the primary diapause was induced in 76% of females, and in the 24% ovipositing females the secondary diapause was induced much more rapidly than in the 97% ovipositing females of the 15 °C group. The results indicate that 1) photoperiodic induction of diapause by SD is restrained at 15 °C, 2) feeding is not necessary for the photoperiodic induction of diapause at 26 °C, and 3) the induction of the primary diapause in starving females at SD, 26 °C is not a consequence of food deprivation, as starvation under the other three conditions does not induce diapause and the females oviposit when fed.

Discussion and conclusion. The adverse effect of 15 °C on the photoperiodic response cannot be explained by a shift in the critical daylength (as hypothesized in the previous chapter), since both the termination and induction of diapause were restrained. While the inhibition of photoperiodic activation by low temperature is a common phenomenon, the negative effect of low temperature on the induction of diapause is rather exceptional. There are, however, some data which are in concert with our results. Short days at 12 °C do not induce diapause in *Laspeyresia (Grapholita) molesta*[13]. A similar finding was reported for *Chloridea obsoleta*[12]. Induction of adult diapause was inhibited in *Drosophila testacea* when exposure to short days was associated with a decrease in temperature to 18 °C[14]. The exposure of diapausing *Chrysopa carnea* to short days at 7 °C did not maintain diapause; on the contrary, it potentiated a high subsequent reproductive activity[15]. Thus the diapause inducing and maintaining role of short days can be impaired by low temperature, at least in some insect species.

It is possible that in *P. apterus* the photoperiodic response is not completely restrained at 15 °C, but summation of much higher numbers of days is needed to obtain the same effect as at 26 °C. This contradicts the assumption that the number of days required for photoperiodic induction of diapause is similar at different temperatures[3]. The assumption is based on experiments with developing insects. Hence, the effects of temperature on the accumulation of photoperiodic signals and on the rate of development are not clearly separated. In the present experiments with *P. apterus* the hormonal activity necessary for vitellogenesis was inhibited during starvation at either temperature. Under these conditions, the summation of both diapause-promoting and diapause-terminating photoperiods was restrained at low temperature of 15 °C. Suppression of the photoperiodic timer at temperatures around 0 °C has been indicated for *Ostrinia nubilalis*[16] and *Megoura viciae*[17].

1 Beck, S.D., Insect Photoperiodism, 2nd edn. Academic Press, New York 1980.
2 Tauber, M.J., Tauber, C.A., and Masaki, S., Seasonal Adaptations of Insects. Oxford Univ. Press, New York Oxford 1986.
3 Saunders, D.S., Insect Clocks, 2nd edn. Pergamon, Oxford 1982.
4 Solbreck C., and Sillén-Tullberg, B., Oikos *36* (1981) 68.
5 Bell, C.H., J. Insect Physiol. *29* (1983) 485.
6 Hodek, I., Acta ent. bohem. *65* (1968) 422.
7 Hodková, M., Zool. Jb. Physiol. *86* (1982) 477.
8 Hodek, I., in: Diapause and Life Cycle Strategies in Insects, p. 33. Eds V.K. Brown and I. Hodek. Dr W. Junk, The Hague 1983.
9 Hodek, I., Věst. čsl. Spol. zool. *42* (1978) 172.
10 Hodek, I., and Hodková, M., J. Insect Physiol. *32* (1986) 615.
11 Lees, A.D., The Physiology of Diapause in Arthropods. Cambridge Univ. Press, Cambridge 1955.
12 Danilevsky, A.S., Photoperiodism and Seasonal Development of Insects, Engl. transl. Oliver & Boyd, London 1965.
13 Dickson, R.C., Ann. ent. Soc. Am. *42* (1949) 511.
14 Kimura, M.T., Experientia *38* (1982) 371.
15 Hodek, I., and Honěk, A., Oecologia (Berl.) *25* (1976) 309.
16 Beck, S.D., J. Insect Physiol. *31* (1985) 41.
17 Lees, A.D., J. Insect Physiol. *32* (1986) 79.

0014-4754/87/040454-03$1.50 + 0.20/0

The oak processionary caterpillar (*Thaumetopoea processionea* L.) an urticating caterpillar related to the pine processionary caterpillar (*Thaumetopoea pityocampa* Schiff.) (Lepidoptera, Thaumetopoeidae)

M. Lamy and F. Novak

Université de Bordeaux I, Laboratoire de Neuro-endocrinologie, U.A. 1138 du CNRS, Avenue des Facultés, F-33405 Talence cedex (France), 9 July 1986

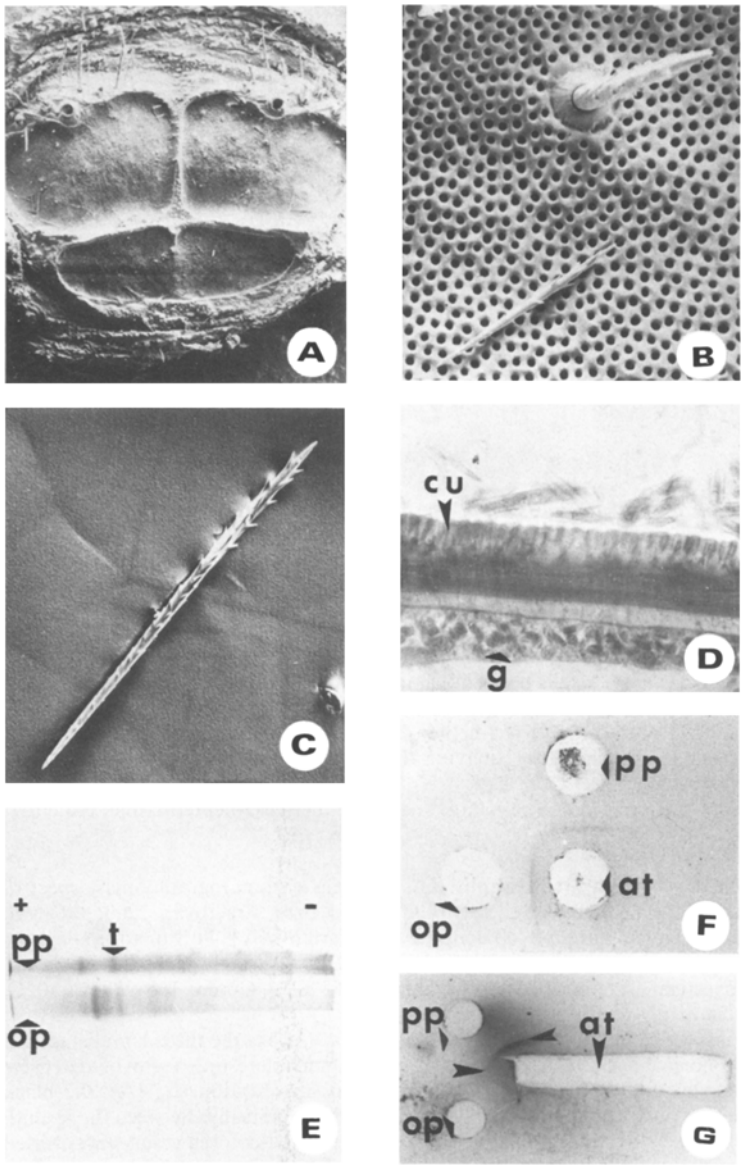
Summary. The hairs of *Thaumetopoea processionea* caterpillars (Lepidoptera) provoke a cutaneous reaction in man and animals. The urticating apparatus, the urticating gland which produces hairs, and the urticating hairs, are similar to those of the *T. pityocampa* caterpillar. The irritant fraction extracted from hairs contains soluble proteins; one of these shows immunological identity with thaumetopoein, the urticating protein of the *Th. pityocampa* caterpillar. This thaumetopoein-like protein is currently undergoing isolation and will be subjected to dermatological tests.

Key words. Thaumetopoea processionea; oak processionary caterpillar; erucism; urticating apparatus; venom; thaumetopoein-like protein.

In France, and particularly in the Poitou-Charentes region, the oak processionary caterpillar (*Thaumetopoea processionea* L.), in association with another Lepidoptera, *Lymantria dispar*, causes considerable damage to oak forests. The hairs of *Th. processionea* caterpillars are responsible for provoking a cutaneous reaction in man, known as erucism, which is similar to that produced by the hairs of the pine processionary caterpillar (*Th. pityocampa* Schiff.)[1]. We have shown that the hairs of this latter species cause a cutaneous reaction in man and animals via the discharge of a toxic substance. The irritant fraction extracted from hairs contains soluble proteins. One 28,000 dalton protein is hair-specific and causes a reaction in pig skin identical to that produced by crude hair extract[2]. It is therefore an urticating protein, and we have named it thaumetopoein[3,4]. This urticating protein is produced by an urticating gland[5].

In this study, the urticating apparatus and urticating hairs of the oak processionary caterpillar have been studied by morphological, histological and biochemical techniques and compared to those of the pine processionary caterpillar. Last instar larvae of *Th. processionea* collected on oak trees near Fouras, France* were used.

A morphological study of the urticant apparatus of this caterpillar was undertaken using the scanning electron microscope with conditions described elsewhere[6,7]. The apparatus is quite similar



Morphological, anatomical and biochemical studies of *Thaumetopoea processionea* venom apparatus: *A, B, C* Scanning electron microscopy of caterpillar at last larval instar: *A* a 'mirror' without urticating hairs (×45). *B* 'mirror' with sites of hair implantation and a non-urticating hair (×1000). *C* an urticating hair (×650). *D* Section through the abdominal urticating gland (×100). cu = cuticle; g = gland. *E* Polyacrylamide gel electrophoresis (7.5%) of urticating hair extract. pp = pine processionary (*Thaumetopoea pityocampa*); op = oak processionary (*Thaumetopoea processionea*); The arrow indicates the urticating protein (thaumetopoein = t). *F* Ouchterlony double diffusion pattern of urticating hair extract. pp = pine processionary (*Thaumetopoea pityocampa*); op = oak processionary (*Thaumetopoea processionea*); at = anti-thaumetopoein immune serum. *G* Immunoelectrophoretic pattern of urticating hair extract (Short-trough technique): pp = pine processionary (*Thaumetopoea pityocampa*); op = oak processionary (*Thaumetopoea processionea*); at = anti-thaumetopoein immune serum. Arrows indicate the continuous band.

to that of the pine processionary. Hairs are implanted in cuticular holes of the 'mirror' (Demolin's terminology)[8] and are identical in size and form to those of the pine processionary (fig., A–C). Histological methods[5] show that the urticating apparatus is not different from that of the pine processionary caterpillar. Section through an abdominal 'mirror' (fig., D) indicates that the hypoderm which produces urticating hairs is formed from a polylayer of trichogen cells. The trichogen cells are smaller than the cuticular cells. A similar cell cluster of trichogen cells forms the urticating gland of the *Th. pityocampa* caterpillar[5].

For biochemical and immunological study hairs were broken by liquid nitrogen treatment[9] and crushed in Laemmli buffer. The suspension was mixed for 48 h at 4 °C, then centrifuged. The clear supernatant was employed either fresh or frozen at −20 °C. Hair extract was submitted to electrophoresis in 7.5% polyacrylamide gels and Tris-glycine buffer (pH 8.6) (ddp: 150 V – 5 h)[10], to immunodiffusion tests[11] and immunoelectrophoresis[12]. For the two latter techniques, the antiserum used was an antiserum against hair proteins of the *Th. pityocampa* caterpillar adsorbed against cuticular extract. It is therefore an anti-thaumetopoein immune-serum[3,4]. A total of 17 soluble proteins were detected in the hair extract by electrophoresis. Six fractions with high molecular weight were at low concentration. Other fractions stained more intensely with Coomassie brillant blue. One of them is a major protein. Comparative studies with *Th. pityocampa* hair proteins indicate that the proteins present some similarities (fig., E). This was confirmed by Ouchterlony's double diffusion tests and immunoelectrophoresis (fig., F and G). Ouchterlony's double diffusion test with anti-thaumetopoein serum produced a continuous band between the wells containing *Th. pityocampa* hair extracts and those with *Th. processionea* hair extracts. Immunoelectrophoresis confirms this result: short-trough techniques confirmed that one band was common to the hair extracts from both species.

We have demonstrated previously[13] that the hemolymph proteins of *Th. pityocampa* and *Th. processionea* show a large degree of antigenic homology. The two urticating species also show similarity in urticating apparatus morphology and anatomy, in the size and form of urticating hairs[14] and glands and in the protein constitution of hair extract. Separation of the thaumetopoein-like protein of the *Th. processionea* caterpillar is in progress. The effect of this fraction will be assessed on guinea pig skin and mouse mast cells[2] to demonstrate its urticating properties.

*We thank Mr Alvere (D.D.A. of la Rochelle, France) for collecting urticating caterpillars.

1 Touraine, A., Thomas, J., and Caldera, R., Bull. Soc. Derm. Syph. *3* (1947) 202.
2 Lamy, M., Vincendeau, Ph., Ducombs, G., and Pastureaud, M.H., Experientia *39* (1983) 299.
3 Lamy, M., Pastureaud, M.H., and Ducombs, G., C.r. Acad. Sci. *301*, série III (1985) 173.
4 Lamy, M., Pastureaud, M.H., Novak, F., Ducombs, G., Vincendeau, Ph., Maleville, J., and Texier, L., Toxicon *24* (1986) 347.
5 Novak, L., and Lamy, M., Int. J. Insect Morph. Embryol. *15* (1986) 27.
6 Ducombs, G., Lamy, M., Bergaud, J.J., Tamisier, J.M., Gervais, C., and Texier, L., Annls Dermat. Venereol. *106* (1979) 769.
7 Lamy, M., Ducombs, G., Pastureaud, M.H., and Vincendeau, Ph., Bull. Soc. zool. Fr. *107* (1982) 515.
8 Demolin, G., Rev. Zool. agric. appl. *10–12* (1963) 107.
9 Papillon, M., and Cassier, P., Experientia *38* (1982) 1476.
10 Laemmli, U.K., Nature *227* (1970) 680.
11 Ouchterlony, O., in: Handbook of Experimental Immunology, p. 655. 1967.
12 Grabar, P., and Williams, C.A., Biochim. biophys. Acta *10* (1953) 193.
13 Lamy, M., C.r. Acad. Sci. *277*, série D (1973) 2557.
14 Sellier, R., Mathieu, N., and Angebault, J.Y., Bull. Soc. Sci. nat. Ouest France *73* (1975) 29.

0014-4754/87/040456-03$1.50 + 0.20/0

Chemical defence in the larvae of the leaf beetle *Gonioctena viminalis* L. (Coleoptera: Chrysomelidae)

K. Dettner* and G. Schwinger

Institute of Biology II (Zoology), RWTH Aachen, D-5100 Aachen (Federal Republic of Germany), and Institute of Chemistry, University of Hohenheim, D-7000 Stuttgart 70 (Federal Republic of Germany), 23 December 1985

Summary. The leaf beetle larva of *Gonioctena (Phytodecta) viminalis* L. has been shown to produce five volatile constituents within its paired abdominal defensive gland reservoirs. It is the first time that these compounds have been reported to occur in coleopteran defensive glands (linalool, phenylethanol) and Chrysomelidae larvae (6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, 2-hexenal). In addition to the gross morphology of the *Gonioctena* gland and its discharge behavior, the natural products found are discussed in terms of chemotaxonomy.
Key words. Defensive secretions; glands; Chrysomelidae; Coleoptera; phenylethanol; linalool; 6-methyl-5-hepten-2-one; 6-methyl-5-hepten-2-ol; 2-hexenal; chemotaxonomy.

In phytophageous Chrysomelidae or leaf beetles, each developmental stage, namely eggs[1], larvae[2], pupae[3] and adults[2], may be chemically protected. Up till now the paired eversible defensive glands which are located on meso- and metathorax and abdominal segments have been the subject of various investigations both with respect to gland morphology and the chemistry of the secretions[2]. But a considerable number of leaf beetle larvae which belong to Paropsina, Gonioctenina, Doryphorina and Chrysolinina are characterized by having only one pair of abdominal defensive glands which substitute for the serial defensive glands. These single paired defensive glands have not been studied morphologically and chemical data are only available from the larvae of the Australian, eucalyptus-defoliating species *Paropsis atomaria* Ol.; they secrete hydrogen cyanide, benzaldehyde, glucose[4] and mandelonitrile which are derived from prunasin[5] which is probably biosynthesized by the beetle, because it does not occur in the larval food plant. We now present, for the first time, a European species, the *Salix*-feeding *Gonioctena (Phytodecta) viminalis*, which possesses single paired larval defensive glands. We describe the gland structure and the chemical composition of the *Gonioctena viminalis* larval secretion, and want to place special emphasis on the chemotaxonomic data.

Materials and methods. Gregarious feeding larvae of *Gonioctena (Phytodecta) viminalis* L. were caught on the leaf surface of *Salix* trees in the bog area 'Hohes Venn' (Belgium). The identity of the species was confirmed by breeding. While being molested by carefully touching with forceps the *Gonioctena* larva was induced to push its abdominal tip on to a minute moist triangle of filter paper. The cooled filter paper triangle with the adhering defensive secretion was inserted into the groove of a movable wire plunger of a 0.1 µl mini-injector (Precision Sampling Corporation) and injected into a gas chromatograph[6]. This method allowed splitless injection of the larval secretion without using any solvent. The secretions from five to ten third stage *Gonioctena viminalis* larvae had to be collected in order to obtain one gas chromatogram. The secretions were analyzed by both gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) (GC: Carlo Erba Fractovap 2900; GC-MS: Varian 3700 coupled to a Varian MAT 44 S quadrupole mass spectrometer, 80 eV; 8 m CW 20 M glass capillary column, temperature program: 65–165 °C: 5 °C/min; 165–220 °C: 10 °C/min).

Results. When feeding on *Salix* leaves the three larval stages of *Gonioctena viminalis* exhibit an interesting form of defensive behavior. Following vigorous molestation (fig. 1A), the black larva suddenly bends its hindbody dorsally. Between the seventh and eighth abdominal tergit, two red-colored vesicles are everted for a short time; these bear droplets of a refreshingly smelling defensive secretion. Simultaneously a soft, bright red-colored membrane surrounding the anal region is everted.

The abdominal vesicles show a roughly sculptured surface compared with the relatively smooth intersegmental membrane (fig. 1B, C) which is characteristic for membranes where volatile compounds evaporate[7]. The everted, boot-shaped vesicles (fig. 1B) represent glandular reservoirs, apically supplied with numerous ovoid glandular units per vesicle. Like the larval segmental gland cells of Chrysomelina and Phyllodectina, the voluminous gland cells of *Gonioctena* are part of the reservoir epithelium. Preliminary results reveal the presence of a curved chitinous channel through which the secretion reaches the reservoir. The retraction of both vesicles after molestation is effected by at least four muscles whose exact structure has not yet been studied in detail.

The intensive-smelling droplet of the defensive secretion of *Gonioctena viminalis* contains a major component **2** with a molecular mass of 126 (fig. 2). Both the EI-mass spectral pattern of constituent **2** (fragments at m/z 126 M^+, 111, 108, 93, 83, 71, 69, 58, 55, 43, 41) and the comparison of retention values with an authentic sample of 6-methyl-5-hepten-2-one indicate that **2** represents 6-methyl-5-hepten-2-one. This ketone, which comprises 90–98% of the total peak area per larva, is accompanied by traces of the biogenetically related 6-methyl-5-hepten-2-ol (com-