Host race pheromone polymorphism in the larch budmoth[1]

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Summary. Analysis of the sex gland content of *Zeiraphera diniana* females and field responses of males to synthetic compounds show that the sympatrically occurring food specialists on various coniferous hosts use different blends of *E*-9-dodecenyl acetate and *E*-11-tetradecenyl acetate as sex pheromones.

The larch budmoth, *Zeiraphera diniana* Guenée (Lepidoptera, Tortricidae, Olethreutinae) colonizes a number of host plants but derives its name form outbreaks on larch, *Larix decidua* Miller, in the European alps. The population dynamics of this insect have been studied most intensively in the Engadine valley in Switzerland[2]. Here, a 3×10^4 to 10^5-fold increase in population density leads to defoliation of larch forests at altitudes between 1700 and 1900 m at regular 9-year intervals[3]. Whereas the adults are monotypic, different larval types can be observed on the various hosts; dark larvae are predominantly associated with larch and light ones with cembran pine, *Pinus cembra* L.; a wide range of intermediate color types also occur[4]. This color polymorphism is closely linked with selective survival of larvae on the hosts[4].

In the first study of the sex pheromone of the species, *E*11-tetradecenyl acetate (*E*11–14:Ac) was identified as a male attractant based on the retention time of a biologically active fraction of a female sex gland extract on gas chromatographic columns (GC), the electroantennogram (EAG) response of the moth to synthetic compounds, and field activity[5]. In another study[6], EAG screening indicated that this compound is only effective for males developing on larch whereas for those on cembran pine, *E*-9-dodecenyl acetate (*E*9–12:Ac) is the most effective stimulus; consistently with this, only *E*11–14:Ac attracted males in a pure larch forest while *E*9–12:Ac was most attractive in a larch stand with cembran pine[6]. We report here the identification and proportions of these 2 compounds in the female sex glands of different color types and the occurrence of different male response types as evidenced by their attraction to traps baited with different doses and blends of *E*9–12:Ac and *E*11–14:Ac.

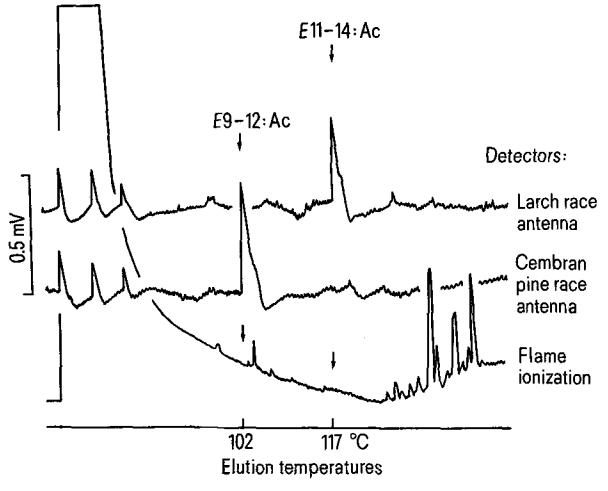

Figure 1. Gas chromatographic analysis of a sex gland extract from a female larch budmoth (intermediate color type) on a 25 m Silar 10C high resolution glass capillary column (splitless injection; temperature programmed 70 °C 2 min, 10 °C/min to 100 °C and 5 °C/min to 200 °C; He carrier gas). The column effluent was split 1:1 between electroantennographic and flame ionization detectors. Aliquots of the extract were analyzed separately with a larch and cembran pine race antenna sensitive to *E*11–14:Ac and *E*9–12:Ac, respectively.

Materials and methods. Larvae were collected in 1982 on larch and cembran pine in Ardez, Sils and Pontresina in the Engadine, segregated according to color type[4] and reared through to adulthood. Pupae and adults were maintained in a 16:8 h photoperiod. Conditions were 24 °C, 50% relative humidity and 3000–6000 lux in the photophase and 18 °C, 85% relative humidity and 1 lux in the scotophase. Extracts were made routinely at the start of the scotophase. The pheromone was obtained by squeezing the abdomen with forceps to evert the ovipositor and removing the terminal abdominal segments, which incorporate the sex gland, with a scalpel blade. Batches of 5–20 glands of each color type were extracted for a few minutes in 20 µl n-hexane for GC-MS and singly in 10 µl aliquots of the solvent for electroantennographic detection (EAD)[7]. Prior to analysis, the batch extracts were concentrated to circa 2 µl by evaporation of the solvent at room temperature.

Mass fragmentograms were obtained using a Finnigan 4000 quadrupole GC-mass spectrometer with a 6115 data system operating in the EI mode. Between 5 and 10 female equivalents and 300 pg of the standards were injected on a 25 m SE54 high-resolution glass capillary column (0.3 mm inside diameter; 80 °C for 2 min, 20 °C/min to 120 °C and 4 °C/min to 200 °C) monitoring m/z 166 (M^+-CH_3COOH) for dodecenyl and m/z 194 (M^+–CH_3COOH) for tetradecenyl acetates. Complete mass spectra (m/z 35 to 435) were obtained by injecting some 10–20 female equivalents and 1 ng of the standards on a 50 m SP1000 high-resolution glass capillary column (0.4 mm inside diameter; 60 °C for 2 min, 10 °C/min to 80 °C and 5 °C/min to 240 °C). *E*9–12:Ac and *E*11–14:Ac were separable from all other geometric and positional isomers on this phase. Because of the small amount of compound present in individual sex glands, characterization of the pheromone in single females was made by GC-EAD. The method has a sensitivity over 10 times higher than single ion monitoring. For this, the voltage across a male antenna placed in an air stream mixed with the GC effluent was continuously recorded[7]. Quantitation by GC-EAD is rendered somewhat difficult due to the facts

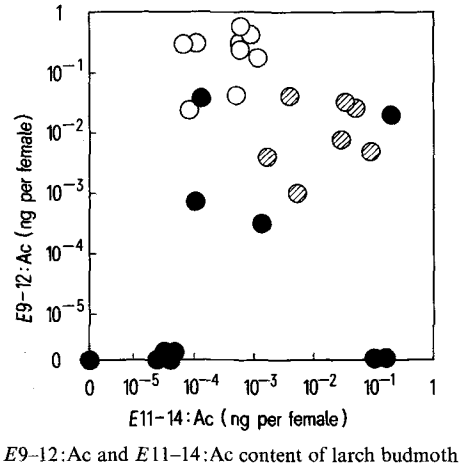

Figure 2. *E*9–12:Ac and *E*11–14:Ac content of larch budmoth females. Individuals arising from larvae of light (○), intermediate (⊘) and dark types (●).

that the antennal response follows a log function of the dose of stimulus delivered and declines with aging of the preparation. However, estimations to within a factor of 3 can be made by repeated calibration. In this manner the porportion of *E*9–12:Ac and *E*11–14:Ac in individuals of the different color types was established by analysing extracts with male antennae sensitive to each compound (fig. 1).

For field tests the sex attractants were applied in hexane into the hollows of red rubber septa and the solvent allowed to evaporate. Tetra traps[8] were placed at hand height on trees at God-God, S-chanf at a distance of some 30–40 m apart; catches were recorded at daily or shorter intervals as warranted.

Results. GC-mass spectrometry of pooled female glands using single ion monitoring revealed that the pheromone components co-eluted with *E*9–12:Ac and *E*11–14:Ac and that females of the light type contained, as main component, approximately 500 pg *E*9–12:Ac. The dark type individuals contained only traces of *E*9–12:Ac.The main pheromone component in the dark type individuals was *E*11–14:Ac; the amount present, however, was less than $1/10$ of the amount of pheromone found in the light type. Extracts of the light phenotype also contained some *E*11–14:Ac. Analysis of the intermediate color type indicated that the 2 products were present at approximately 20 pg each in sex glands. Mass spectra and elution temperatures of the natural products matched those of synthetic standards. In addition to dodecyl and tetradecyl acetate, early eluting components of all extracts were 2-hexanone, 3-hexanol, 2-hexanol and nonanal.

GC-EAD demonstrated that most of the single individuals examined contained both pheromone products with a tendency for the light form to produce a higher *E*9–12:Ac/*E*11–14:Ac ratio than the intermediate (fig. 2). On the other hand, the dark form was the only one in which a high proportion of females were found to produce *E*11–14:Ac alone, sometimes in very low amounts. Distinct EAD responses were consistently recorded from larch and cembran pine race antennae to components of the sex gland extracts eluting within and on the tail of the solvent peak (fig. 1). The identity of *E*9–12:Ac was confirmed in batch extracts of the 3 color types using the antenna of *Sparganothis pilleriana* Schiff. (Lepidoptera, Tortricidae, Sparganothinae) as the GC detector. On the other hand, *E*-11-tetradecenal for which receptors are found on antennae of both light and dark color types (E. Priesner, personal communication), was not found in the batch extracts employing the antenna of *Gracillaria syringella* Fab. (Lepidoptera, Gracillariidae, Gracillariinae) as the compound specific GC detector (detection limit < 1 pg).

The occurrence of 2 pheromone response types among males of the larch budmoth was confirmed with trap experiments in a number of forests in the Engadine valley. Records from a mixed larch-cembran pine forest are shown (fig. 3). In early September the highest catches were recorded in traps with *E*9–12:Ac alone; captures were inhibited with increasing doses of *E*11–14:Ac. Few insects flew during the latter part of September but when flight resumed in October the principal catches were made in traps baited with *E*11–14:Ac alone; increasing doses of *E*9–12:Ac were inhibitory. While the response pattern (fig. 3) is illustrative it presents a somewhat simplified picture since the flight of the 2 forms was separated only because of prevailing weather conditions. At many other locations where the same trap series was tested, and the flight-periods coincided, distinct peaks in male attraction were also recorded in traps baited mainly with *E*9–12:Ac or *E*11–14:Ac[9].

Complementary body length measurements[9] and EAG analysis[10] with the captured males confirmed that the 2 clearly segregated peaks in male capture represent attraction of extreme though otherwise morphologically indistinguishable phenotypes: smaller males sensitive to *E*9–12:Ac and larger ones more sensitive to *E*11–14:Ac. When a similar test was made in a pure larch forest supporting only a population of the dark type, the highest catch was made in traps baited with *E*11–14:Ac alone[11] and consisted of predominantly large individuals[9].

Discussion. While this study was made at peak densities of the budmoth cycle on larch, populations have since started to decline and several years will elapse before the involvement of pheromonal polymorphism in the cyclicity of populations can be examined again. Nevertheless, the present report accounts for pheromone polymorphism in the larch budmoth as revealed by analyses of female sex gland composition and different male response types to sex pheromone traps. Pheromone polymorphism has already been established in the Lepidoptera, i.e. *Ostrinia nubilalis* Hübner[12]. The facts reported here on *Zeiraphera diniana,* allied with the other known polymorphic traits of the species[13,14], permits us to propose the following hypothesis for the possible functioning of the 9-year population cycle on larch. Namely, that a correlation between colormorph and pheromone type provides the basis for assortative mating between the different fitness types which occur at different stages of the cycle. Previous studies have established that at highest population densities on larch, when the dark phenotype predominates, feeding results in complete defoliation of the forest and this leads to a pronounced deterioration

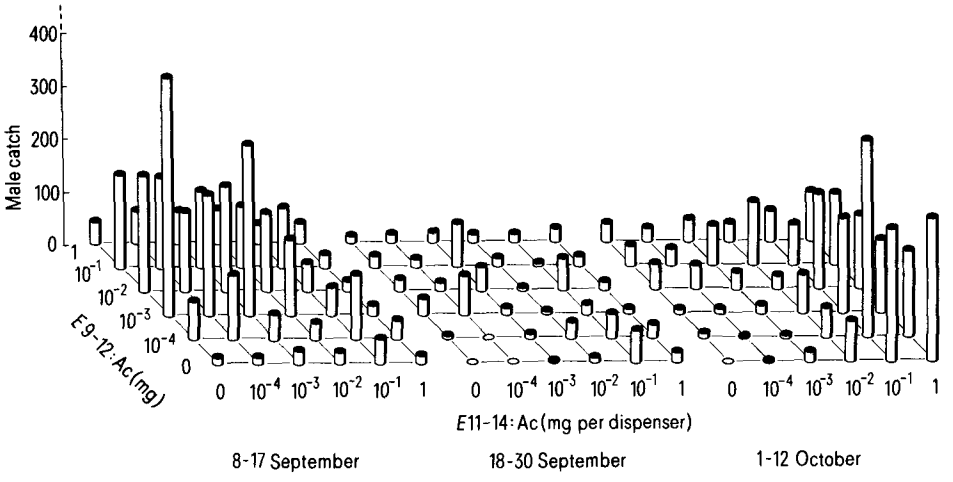

Figure 3. Sequential appearance of larch budmoth pheromone types in a mixed larch-cembran pine forest in the Engadine valley, Switzerland as indicated by their attraction to different doses and blends of *E*9–12:Ac and *E*11–14:Ac.

in food quality in subsequent years[15]. This in turn causes heavy larval mortality in early instars of the dark colormorph which is most vulnerable to nutritional stress[13]. During the subsequent decline in population density the proportion of the more robust intermediate color type increases until it predominates at lowest population densities, some 4 years after defoliation[14]. After relaxation of the selection pressure against the dark fitness type, coinciding with the recovery of the larch tree, assortative mating by the small proportion of the true-breeding dark food specialists remaining at minimum density of the cycle might represent the important driving mechanism which contributes to the rapid increase in its frequency in the population, and subsequent proliferation[14]. Pheromone polymorphism could thus be considered an important fitness characteristic of the larch budmoth permitting it to exploit continuously an inconsistent food resource.

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2 Baltensweiler, W., Benz, G., Bovey, P., and Delucchi, V., A. Rev. Ent. *22* (1977) 79.
3 Baltensweiler, W., Can. Ent. *96* (1964) 792.
4 Baltensweiler, W., Mitt. schweiz. ent. Ges. *50* (1977) 15.
5 Roelofs, W.L., Cardé, R., Benz, G., and von Salis, G., Experientia *27* (1971) 1438.
6 Baltensweiler, W., Priesner, E., Arn, H., and Delucchi, V., Mitt. schweiz. ent. Ges. *51* (1978) 133.
7 Arn, H., Städler, E., and Rauscher, S., Z. Naturf. *30c* (1975) 722.
8 Arn, H., Rauscher, S., and Schmid, A. Mitt. schweiz. ent. Ges. *52* (1979) 49.
9 Baltensweiler, W., in preparation.
10 Priesner, E., and Baltensweiler, W., in preparation.
11 Arn, H., Baltensweiler, W., Bues, R., Buser, H.R., Esbjerg, P., Guerin, P., Mani, E., Rauscher, S. Szőcs, G., and Tóth, M., in: Les Médiateurs Chimiques, p. 261. INRA, Versailles 1982.
12 Klun, J.A., and Maini, S., Envir. Ent. *8* (1979) 423.
13 Day, K.R., and Baltensweiler, W., Entomologia exp. appl. *15* (1972) 287.
14 Baltensweiler, W., in: Dynamics of Numbers in Populations, p. 208. Eds P.J. den Boer and G.R. Gradwell. PUDOC, Wageningen 1971.
15 Benz, G., Z. angew. Ent. *76* (1974) 196.

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Chronic active hepatitis in mice induced by 3-hydroxy-4-pyrone[1]

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Summary. Chronic active hepatitis was selectively induced in mice by the feeding of a diet containing 3-hydroxy-4-pyrone (0.5% by weight) for periods of 6 weeks and longer. This model should be of particular value in elucidating the pathogenesis of drug-induced forms of chronic active hepatitis. Maltol (3-hydroxy-2-methyl-4-pyrone) did not produce any liver lesion.

Chronic active hepatitis (CAH), an inflammation of the liver continuing without improvement for at least 6 months, is a pattern of progressive hepatocellular damage rather than a single disease and has several known causes in humans, including autoimmunity, chronic viral hepatitis (types B and non-A, non-B), alpha-1-antitrypsin deficiency, Wilson's disease, alcoholism and a few therapeutic drugs[3,4]. Whatever the cause, its diagnosis depends on the identification of the characteristic histological lesion consisting of periportal piecemeal necrosis and a dense infiltrate of lymphocytes and plasma cells and in severer cases on the presence of the additional features of portal-to-portal and portal-to-central bridging necrosis and fibrosis with isolation of groups of liver cells within the cellular infiltrates. The pathogenesis of drug-induced CAH is unknown and an experimental model is lacking. A liver lesion resembling CAH was observed in an earlier study of Swiss mice fed 3-hydroxy-4-pyrone, a known inhibitor of catechol methyl transferase[5] and of thyroid peroxidase and other peroxidases[6]. Here we report studies on the nature and progression of the lesion induced in mice by chronic feeding of 3-hydroxy-4-pyrone and on the effects of subsequent withdrawal of the substance from the feed. Maltol (3-hydroxy-2-methyl-4-pyrone), a commonly used flavoring agent in foods, was fed to mice in a parallel study but did not produce any liver lesions.

Materials and methods. Female Swiss mice, 20–25 g, were placed in separate cages in groups of eight. Food and water were available ad libitum. Mice were weighed weekly. 3-Hydroxy-4-pyrone was prepared as previously described[6]. Maltol was a gift from the Chemical Division, Pfizer, N.Y. Each compound (0.5% wt/wt) was added to ground mouse pellets as described in detail elsewhere[7]. The mouse pellets used (Clark King GR2 pellets, Victorian Wheatgrowers Corporation, Melbourne) had the following composition (manufacturer's analysis): protein 20%, fat 3.0%, fiber 3.0%, calcium 0.7%, phosphorus 0.6%, iodine 0.6 ppm, manganese 115 ppm, zinc 90 ppm, iron 100 ppm, copper 10 ppm.

Mice were killed by exsanguination under ether anesthesia at the times shown below. Livers were weighed and separate portions of the left and median lobes were fixed in phosphate-buffered 10% formalin and processed to paraffin sections which were stained with H&E and by the Gordon and Sweets method for reticulin. Other tissues processed similarly included the brain, skeletal muscle, heart, lungs, gut, kidneys, thymus, lymph nodes, spleen and bone marrow. The criteria used for the diagnosis of CAH were strictly histological and no time factor comparable to that required in human cases was taken into consideration[3,4].

The number of mice used was determined by the limited supply of the test compounds. Each test diet was fed to 24 mice. An equal number were fed normal mouse pellets. In the case of 3-hydroxy-4-pyrone, 4 test and 2 control mice were killed at 3, 6, 9 and 12 weeks and 2 test and 2 control mice were killed at 16 and 21 weeks. At 21 weeks the 4 remaining mice were returned to normal pellets to study the effect of withdrawal of the test compound. Two of these mice were killed at 10 and 20 weeks after removal of the test compound from the diet, i.e. 31 and 41 weeks after the start of the original experiment.

Results. The control and test groups of mice gained weight progressively and looked healthy. None died. The liver to body weight ratio remained constant. The livers of control mice