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Identification of pheromone of the greater wax moth *Galleria mellonella* from the different regions of Russia

K.V. Lebedeva, N.V. Vendilo, V.L. Ponomarev, V.A. Pletnev, D.B. Mitroshin

Institute of Plant Protection Chemicals 109088 Ugreshskaya 31, Moscow, Russia

Abstract: The composition of pheromone volatiles from calling males of the greater wax moth (GWM) *Galleria mellonella* from six regions of Russia was studied by GC-MS. The volatiles from calling males from all the regions contain nonanal and undecanal as the main components, but in different ratio for the males of GWM from different regions. Hexanal, heptanal, octanal, decanal, undecanol and 6,10,14 -trimethylpentadecanon-2 were found as minor components also in different combinations. The structure of the ketone was proved by the comparison its mass-spectrum with spectrum of synthetic ketone.

Key words: sex pheromone, male volatiles, *Galleria mellonella* , hexanal, heptanal, octanal, decanal, undecanol, 6,10,14-trimethylpentadecanon-2, Lepidoptera, Pyralidae.

Introduction

The greater wax moth (GWM) *Galleria mellonella* (Lepidoptera: Pyralidae) is an important pest of the honeybee *Apis mellifera*. The larval stage of the GWM feeds on the honey, pollen, and wax produced by honeybees. However, an effective method of control of this pest has not been developed. Physical, chemical, and biological methods are imperfect (Ali *et al*., 1973, Burges 1977, 1978, Cantwell and Smith 1970), and further studies are needed to find more effective control methods. Some studies have been conducted on the use of pheromone traps for capturing GWM. It was found that GWM male adults produce a sex pheromone in glands located on their forewings (Barth 1937, Roller *et al*., 1968). The pheromone was identified as a mixture of two aldehydes, nonanal and undecanal (7: 3) (Leyrer and Monroe 1973). However, the response of females to the synthetic bait in laboratory tests was not as high as their response to live males (Finn and Pyne 1977,), and in field tests this mixture was practically inactive (Flint and Merkle 1983). Two additional components,

nonanol and undecanol, were found among volatiles collected from GWM males from Canada during their calling period. The ratio of the main components, nonanal and undecanal, of this population was found 1: 3 (Romel et al. 1992). The ratio of these components in volatiles of GWM from USA was 7 : 3 (Leyrer and Monroe 1973).

In the work reports we analysed the volatiles of GWM calling males from four regions of Russia: the central part of Russia (Penza, Nizhnij Novgorod and Ivanovo), middle Ural (Ufa), Altai (Barnaul), and Far East to ascertain the differences in their pheromone compositions.

Material and methods

Insect source and collection of effluvia. The GWM larvae, collected from the domestical hives, were kept in 2 L glass containers (50-75 specimens) with frilles filter paper for pupation. Larvae, pupae, and adults were kept in darkness at 28-31° C and 60% humidity. Adults were obtained from the first, second, or third generation of laboratory colonies started from wild stock. Insects were reared either on comb wax from their native hives. Before using the comb wax was kept frozen at -15°C. After 5-7 days from the start of pupation the cocoons were dessected, and males were separated from females. Five to ten of 2- to 7-day-old GWM males were kept in a glass cylinder (60 x 230 mm) in the dark at 28-31°C for 1-3 days after exclosion. Effluvium of males was collected once a day (at 2-5 PM) for half an hour using a glass tube with a charcoal disk (d = 4 mm; l = 3 mm), which was connected to the narrow end (d = 5 mm) of cylinder and a vacuum source (20-30 cm/sec), using a Personal Air Sampler (PAS_1000, SUPELCO, USA). The mean amounts of components were obtained as the ratio of its absolute quantity to (number of males x number of collection periods).

Isolation and analysis of volatiles. The volatiles were rinsed from the charcoal disk with 300 µl methylene chloride and were first analyzed by GC-MC without evaporation of solvent to estimate the quantity and ratio of the main components; then they were concentrated to the desired volume for analysis of minor components. Capllary GC-MS analysis was performed on an LKB 2091 EI system at 70 eV, coupled with LKB-CLINICON (LKB Sweden) 2130-310 data system (PDP-11/05). A 30m x 0.25 mm SE-30 fused silica column was programmed 30 min at 40°C and then to 230°C at 4°/min. The column was operated in the splitless mode. Before analysis, 40 ng of nonadecan was added as standard to the samples for comparison of retention times and quantification of unknown substances. For identification of small amounts of minor components we used the reconstruction of total ion mass chromatogram by characteristic ions. Collection and identification of male volatiles were repeated two to four times for insect from each region.

Results and discussion

The main and some minor male pheromone components of *Galleria mellonella* from different regions of Russia were identified before. The structure of C_6-C_{11} aldehydes and C_{10} alcohol was determined by their mass-spectra (Ponomarev et al. 1997). Mass-spectrum of another minor component, 6,10,14-trimethylpentadecanon-2, confirms its structure (Fig. 1). The compound of similar structure, 6,10,14-trimethylpentadecanol-2, was discovered in female pheromone of *Corcyra cephalonica* (Pyralidae) (Hall *et al*., 1987). The volatiles of *Galleria mellonella* males from Ivanovo (Table 1) do not include this ketone, hexanal and undecanol. The ratio of main components, nonanal and undecanal, is various for the populations from different regions: 100: 50 (Ufa), 100: 60 (Ivanovo), 100: 70 (Nizhniy Novgorod), 100: 80 (Far East), 100: 100 (Barnaul) and 80: 100 (Penza).

Table. Ratio of the components in the volatiles of calling males *Galleria mellonella* from different regions.

Compound	Ivanovo	N.Novgorod	Penza	Ufa	Barnaul	Far East
Nonanal	100	100	80	100	100	100
Undecanal	60	70	100	50	100	80
Hexanal	-	1	0.2	1	1	1
Heptanal	2	5	1	3	20	7
Octanal	12	18	4	6	40	20
Decanal	6	6	5	3	30	20
Undecanol	-	0.1	2	0.3	2	0.1
6,10,14,Trimethyl-pentadecanol –2	-	3	2	1	7	4

The ratio of minor components in volatiles of GWM males from different area is various substantially. Only the ratio of all components (exept undecanol) in male volatiles from N.Novgorod is similar to that in GWM from Far East.The application of mixture nonanal: undecanal (3: 1) for the protection of 173 hives in Ivanovo resulted twofold reduction of damage caused by *Galleria mellonella*.

Conclusion

The volatiles of male pheromone of Galleria mellonella from six different area consist of similar components in different ratio of main and minor compounds.

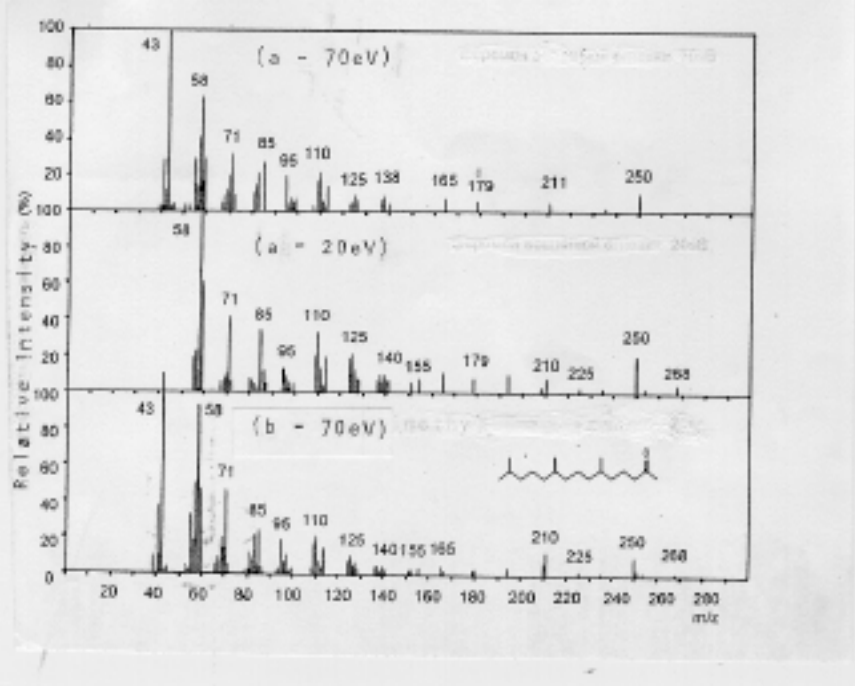

Figure. Mass-spectra of minor component of male *Galleria mellonella* volatiles (a) and 6,10,14-trimethylpentadecanon-2 (b)

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