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Pheromone research in Estonia

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Abstract. The pheromone screening of nineteen insect species was conducted in different habitats and geographical regions of Estonia. The purpose of this screening was to measure the population density of selected insects to sort out species suitable for environmental monitoring. The other research objective was to standardize the pheromone materials for plant protection as alternative approaches to the use of conventional pesticides. To monitor the changes in environment *Archips podana, Hedya nubiferana, Choristoneura diversana, Enarmonia formosana, Tortrix viridana, Archips xylosteana* and *Cerapteryx graminis* showed great potential. In fruit gardens the pheromone kit for codling moth *Cydia pomonella* is recommended. The pheromone dispenser for Currant shoot borer, *Lampronia capitella,* is the first of its kind. in the world.

The old-fashioned Russian-made substrate was successfully replaced by Hevea's rubber- and silicone-based materials. Our laboratory is ready for lab-scale production of all tested dispensers.

Key words: pheromone, dispenser, pheromone screening, plant protection, environmental monitoring, *Archips podana, Hedya nubiferana, Choristoneura diversana, Enarmonia formosana, Tortrix viridana, Archips xylosteana, Cerapteryx graminis, Lampronia capitella*

INTRODUCTION

The aim of current plant protection is to guarantee effective pest management while avoiding damage to our food and environment. There are too many residues of plant protection chemicals in our food which, even in small doses, are dangerous for our health. Therefore considerable attention has been focused on finding alternative approaches to the use of conventional pesticides in the control of insect pests.

One of the important developments in this direction has been the evaluation of pheromones as the biologically active compounds modifying the behaviour of the insects in integrated pest management and organic farming. Pheromones which- are already active at a very low rate enable the protection of plants against insect damage without insecticides, or dramatically reduce the number of treatments and therefore the contamination of food and the environment.

A new approach is the monitoring of changes in the environment using pheromone traps, which act as specific and sensitive indicators.

In recent years our research has been focused on the standardization of ecologically important pheromone materials. Our main object has been the development of materials for screening and plant protection in gardens and organic farming areas.

MATERIALS AND METHODS

Increasing demand for a standardized, stabile and longer lasting pheromone dispenser has actuated the search for the new substrate. Pheromone components must be stable in the dispenser and guarantee required release rates.

For many years, two different types of apparatus, using pieces cut from red and black tubing, have been used as substrate for the pheromone dispenser. Many of the components of this old- fashioned substrate, produced in Russia, were rather unstable, resulting in decomposition and isomerization. Furthermore, the rubber cracked, increasing the evaporation rates.

We contacted Hevea Ltd. in Tallinn, a company specializing in rubber details. They were able to manufacture the rubber sheets and cut out tablet-shape dispensers. Besides traditional sulphur vulcanized rubber, they were also able to produce silicone rubbers and to vary the composition of materials according to our specifications.

After measuring the evaporation characteristics of the main pheromone components of moths important in Estonia from the new candidates for dispenser substrate,one red silicone substrate and one black rubber-type material were selected. The shape of the tablet was most convenient and economical.

For the standard analysis, the pheromone dispenser was cut into four equal pieces and put into a 5 ml vial. 0.5 ml of hexane was added along with a suitable amount of internal standard. The vial was closed tightly and kept at room temperature until reaching equilibrium, which took 2 hours. Analysis was performed on gas chromatograph Varian model 3900 equipped with flame ionization detection, helium as a carrier gas and capillary column CP Wax 52 CB 30 m x 0.25 mm and film thickness 0.25 µm.

We detected the sulphur compounds that cause the isomerization of the conjugated dienic pheromone components in both the rubber and silicone-based substrates. It was our supposition that these sulphur compounds had been used for manufacturing the substrate materials, and we found a method to remove these admixtures from the dispensers.

For the field screening experiments in April 2005, 19 different dispensers were prepared in our laboratory from red or black tablet-shaped substrate. The substrate was impregnated with a blend of up to 3 mg of pheromone in the old type of dispenser; new dispensers were loaded with 0.14-1.0 mg of pheromone, ca 0.5 mg, in most cases. We concluded that the new substrates are more economical than the old ones and provide a large economical advantage, as pheromone compounds are expensive.

A list of dispensers, insects, loaded amounts and compositions is presented in Table 1.

Monoenic pheromone components were brought from Flora, and dienic compounds were synthesized at the Royal Institute of Technology, Stockholm, Sweden. All compounds were purified by medium pressure liquid chromatography on silica gel or silver nitrate-impregnated silica gel. Isomeric purity of monoenic compounds was above 99.5 %; of dienic compounds, above 98 %.

Table 1. Pheromone dispensers tested in Estonia in 2005.

INSECT	CODE	COMPONENTS	AMOUNT (mg/dispenser)	%
Archips podana	APS	14Z11Ac, 14E11Ac	1.2 + 0.8	60 + 40
Archips rosana	ARS	14Z11Ac, 14Z11OH	0.45 + 0.05	90 + 10
Agrotis exclamationis	AGF	14Z9Ac, 14Z5Ac	0.05 + 0.45	10 + 90
Croesia holmiana	CRS	14E11Ac	1.0	100
Archips xylosteana	AXS	14Z11Ac, 14E11Ac, 14Z11OH	0.28 + 0.02 + 0.2	56+4+40
Cydia nigricana	HMA	12E10Ac, 12Ac	0.8 + 1.2	40 + 60
Cydia pomonella	KMM	12OH, 8E10E12OH	0.2 + 0.6	24 + 76
Choristoneura diversana	CDM	14Z11Ac, 12Ac	0.3 + 1.0	24 + 76
Mamestra brassica	MBS	16Z11Ac	0.5	100
Argyresthia conjugella	ACO	18Z13Ac, 16Z11Ac	0.25 + 0.5	34 + 66
Cerapteryx graminis	CGS	16Z11Ald, 16Z11Ac	0.7 + 0.3	63 + 37
Plutella xylostella	PXS	16Z11Ald, 16Z11Ac, 16Z11OH	0.04 + 0.04 + 0.005	50+48+2
Enarmonia formosana	EA91	12Z9Ac, 12E9Ac	0.6 + 0.4	60 + 40
Aphelia paleana	APM	14E11Ac, 14Z11Ac	0.1 + 0.9	10 + 90
Tortric viridana	TVM	14Z11Ac	1.0	100
Dichrorampa petiverella	DPS5c	14E11Ac, 14Z11Ac	0.1 + 0.9	10 + 90
Hedya nubiferana	HNO	8E10E12Ac, 12Z8Ac	0.6 + 0.4	60 + 40
Yponomeuta malinellus	YM	12Z9Ac, 14Z11OH	0.2 + 0.8	20 + 80
Yponomeuta spp.	Y	14Z11Ac	1.0	100
Lampronia capitella	NKS	9Z11Z14OH, 9Z11Z14Ald, 9Z11Z14Ac	0.1 + 0.013 + 0.026	72+9+19
Rhobobota naevana	RHM	14Z11Ac, 14Z11OH, 12Z9Ac	0.6 + 0.2 + 0.05	65+30+5

Pheromone screening experiments were conducted in different habitats and geographical regions in Estonia from June to August 2005 at the following locations: Tartu: Raja garden, Tamme Puiestee; Ida-Viru: oak forest at Jõhvi; Lääne-Viru: oak forest at Tammiku; Tartu county: apple garden at Rõngu, red currant plantation at Vasula, black currant plantation at Kambja, home garden and alley at Kambja; Viljandi county: black currant plantation at Polli; Rapla county: home garden at Juuru, mixed forest near Juuru, swamp near Juuru at Atla; Harju county: home garden at Saue, Jõgeva county: field of peas.

Red Atracon A delta traps were used everywhere. Insects were caught with the entomological glue Pestifix. Traps were hung up from 1 to 2 m high in trees or bushes at the sunny side of the plant. Five replicates were used in each test site. Dispensers were not tested for all species in each test location.

RESULTS AND DISCUSSION

Results of the pheromone screening in 2005 are presented in the Table 2.

Table 2. Sum of trap catches of 5 pheromone traps in summer 2005.

Code	Forest Juuru	Atla	Home Juuru	Raja	Kambja	Jõge-va	Jõhvi	Tam-miku	Rõngu
CRS	1	12		26					
APM	8	23							
AXS	4	2			1				
HNO	78	130	101	236					25
AGF	9	21					7	0	
APS	75	64	38	105			57	126	22
TVM	3	0			0		60	23	
CDM	16	7	8	16					
CGS	50	48							
EA		17	25	663					
YM		4	3		0				
KMM		14	77	64	119				14
ARS		11	12	11					
HMA			12			180			
PXS			4						
MBS			10						
RHM			26						
Y			78						
ACO			2	5	2				

Test results show that the most numerous insects were polyphagous insects such as the large fruit tree tortrix *Archips podana* (APS) and *Hedya nubiferana* (HNO). The *Hedya nubiferana* pheromone dispenser was highly species-specific whereas the species specificity of *Archips podana* dispenser was not as high. *Choristoneura diversana* (CDM) was also found in each test location.

Despite the poor crop of apples in 2004, the codling moth, *Cydia pomonella* (KMM) was trapped everywhere. At the same time the damage by *Cydia pomonella* was very small, almost absent in 2005. Codling moth was present throughout Estonia until the beginning of August. There was high trap catch in some traps at Raja in the middle of August, suggesting a second generation of *Cydia pomonella,* due to the warm weather.

We found very few *Argyrestia conjugella* (ACO) moths in the traps. (There is no reason to indict a bad dispenser because the damage made by the apple fruit moth was not found).

The pheromone dispenser of *Enarmonia formosana* (EA) was very species-specific. This insect was found both in gardens and forest, and was especially dense at Raja garden. (The population density was higher at Raja for all tested insects compared with the same species at the other sites. A probable cause is the status of the garden: apple trees were neither cared for nor chemically treated for many years. Furthermore, we found very few birds that can regulate the number of insects).

The number of *Archips rosana* (ARS) was sufficient for environmental screening, but the species specificity of this dispenser was not satisfactory.

As tremendous damage to oak trees by mothswas reported, the dispensers of green oak moth *Tortrix viridana* (TVM) and brown oak moth *Archips xylosteana* (AXS) were tested. There were no green oak moths in the traps at Kambja and Juuru, but many at Jõhvi and Tammiku, 60 and 23 individuals respectively. *Archips xylosteana* was found in all locations.

The table of results also shows that the dispenser of antler moth, *Cerapteryx graminis* (CGS) was also working satisfactorily, and can be used for the screening the status of the grassland.

A new pest - *Lampronia capitella* (NKS) - has appeared in black and red currant plantations and shows a tendency towards increasing density. *Lampronia capitella* was tested in a home garden in Juuru, in a well cared for black currant plantation in Polli and in two dormant plantations at Vasula and Kambja. It appears that the number of moths in currant crops reflects the status of its surroundings: the number of these insects was relatively small in well-cared for plantations. Results are presented in Table 3.

Table 3. Sum of *Lampronia capitella* males trapped by 5 traps in different plantations in June 2005.

Dispenser	Juuru home	Polli	Vasula	Kambja
NKS	17	34	345	445

SUMMARY

We consider introduction of a new pheromone dispenser substrate a very important result of our latest research in the field of developing standardized pheromone kits. Fieldtests confirmed evaporation rates and trapping properties predicted from the laboratory measurements. The aim of our future research isthe regulation of evaporation rates from dispensers.

Farmers dealing with organic production and enthusiasts with small gardens are in need of alternative tools to control pest insects without insecticides. We expect them to be especially interested in the practical use of pheromones. Whereas pheromone dispensers for many insect species are now available in Estonia, we feel obligated to promote their use.

Work is still needed to determine the methods and materials for the monitoring of the status of the environment based on the interpretation of trap catches.

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