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PRACTICE ORIENTED RESULTS ON THE USE OF PLANT EXTRACTS AND PHEROMONES IN PEST CONTROL

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EXTRASOL - NEW PROMISING BIOINOCULANT FOR SUSTAIABLE AGRICULTURE

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Review

Throughout its history, the world population has constantly been facing a challenge of providing itself with food. Adverse weather conditions, diseases, and productivity of cultivated crops used as food are those factors which hamper the solution of this problem that remains urgent even today. The intensive agricultural methods, practised in the last century, helped to raise the fertility of soils and productivity of crops due to the use of fertilisers and chemicals for crop protection. It was, surely, a positive step toward the desired goal. However, there are certain negative aspects in such an approach as well. The use of chemicals results in environmental pollution that affects nature as a whole.

Today, the developing countries in Europe and America are witnessing overproduction of agricultural produce owing to the introduction of progressive farming methods, including a wide-scale use of chemicals both for protecting crops against pests and as growth stimulators. Unfortunately, the intensive use of chemical fertilisers has caused a situation when agricultural food products contain nitrates and pesticides in amounts frequently exceeding the level normally allowed and safe for human beings. Chemical fertilisers are also to a great extent responsible for the fertility and ecological state of soils. In this connection, the world has come to face an extremely urgent problem of a restrained and competent use of chemical fertilisers and pesticides so as not to endanger the environment. Of late, many of the developed countries have adopted legislative acts prohibiting or limiting the wide-scale use of chemicals in agriculture. Biological fertilisers and pesticides are gradually replacing these.

Extrasol

EXTRASOL is a preparation of plant growth-promoting and nitrogen-fixing bacteria inhabiting plant roots intended to improve nutrition of vegetable, grain and industrial crops, as well as to enhance their productivity.

EXTRASOL increases germinating power of seeds, improves the absorption of nutrient elements by plants, intensifies their development, and increases their resistance to the phytopathogens affection, all of which increases substantially the plants' productivity.

Bacteria, which naturally inhibit rhizosphere root surfaces of healthy plants, were isolated, selected and used for production of EXTRASOL preparation. Bacteria of genera *Pseudomonas, Bacillus, Azomonas, Agrobacterium, Flavobacterium, and Arthrobacter* are known to be superior in boosting crop yields and suppressing phytopathogenic microorganisms. Their inherent features are quick growth and the ability to colonize the roots or rhizosphere of cultivated plants, thus ousting the microorganisms inhibiting the plants' growth. All of the above bacteria are capable, to a greater or lesser extent, of synthesizing the growth hormones and fix molecular nitrogen, which affects the roots' absorbing capacity and the general productivity of agricultural crops. In the numerous field tests in Institute's Geographical Experimental Network the newly selected bacteria were comprehensively studied for being non-toxic for plants and for their ability to stimulate the growth, fix molecular nitrogen, and suppress phytopathogenic microorganisms. The results show that the new selected bacteria are harmless to human beings, animals, and insects, nor do they contribute to the environmental pollution. The bacteria selected from natural microbial populations are intended for production of biopreparations of EXTRASOL series, their total concentration not exceeding *1-2%* of the total number of soil and rhizosphere bacteria. Individual stocks or several species (associations) of bacteria are used to produce the specifications, depending on the type or variety of the plant. The selection of the optimal variant is done experimentally to suit regional plant varieties.

The final preparation is a pure bacteria culture (Arthrobacter mysorens 7, Flavobacterium sp. L-30, Agrobacterium radiobacter 204, Azomonas agilis 12, Bacillus subtilis 4-13, Pseudomonas fluorescens 2137, Azospirillum lipoferum 137) planted on sterile peat, or else obtained in a liquid form. Externally, EXTRASOL looks like a moistened peat mass or bacterial suspension. Moisture content of the peat preparation amounts to 45-60%. The proportion of bacterial cells in the preparation should be at least 0.1-2 billion/g depending on certain strain and the concentration of alien microorganisms in a freshly produced preparation should not exceed 1% of the number of cultivated bacteria, increasing to within 10% by the end of the warranty period (6-12 months). Rot fungi shall not be present except certain specially selected microscopic fungi (Trichoderma, Gliocladium, etc.).

EXTRASOL is supplied in polyethylene bags of 300-1000 g, or in sterile 1-20 1 containers, which should be stored in dry, cool room, separately from hazard chemicals at temperatures not exceeding 8-20°C. EXTRASOL is used for cultivation of grain, vegetable, and industrial crops. The preparation is applied to the seeds, put into holes in transplanting seedlings, which is preceded by wetting the seedlings' roots and also used for foliar application. The use of the preparation increases the crop yield by 20-40% and improves the absorption of nutrient elements by plants by 10-20%. EXTRASOL is easy to store, transport and apply.

Technical data on EXTRASOL preparation

1. Active bioagent or substance: rhizospherous, nitrogen-fixing bacteria and their metabolites.

2. Effect: improves mineral nutrition of plants, intensifies their development, increases yield of crops and improves their quality.

3. Form of output: loose mass with moisture content of 50-55% packed in polyethylene bags of 300-1000 g or liquid form in 1-20 1 containers.

4. Colour, odour: dark-brown or black odourless mass; light-brown or fawn odourless liquid.

5. Amount of bioagent: 0.1-2 billion bacteria per gram of preparation

6. Filler: peat

7. Emulsifier: nutritious medium residue and peat additives perform adhesive, emulsifying and stabilizing functions.

8. The preparation does not contain ultra-violet radiation protectants.

9. Proportion of nutrition residual matter in the preparation:35-40%.

10. Working suspension pH: 6.3 - 7.2.

11. Biological activity of the preparation is evaluated by determining its titration standard. Greenhouse or small-plot testing verifies the preparation's effect on crop yield.

12. Active shelf life: 6-24 months since release date.

13. EXTRASOL is recommended as a crop nutrient, as a preparation for pre-sowing treatment of seeds, or to be taken into soil during sowing, as well as for foliar nutrition of plants. The preparation is rather simple in handling - the main requirement calls for the preparation to be uniformly distributed along the entire layer of seeds and stay firmly on their surfaces. Many crops, especially the vegetable ones, are susceptible to foliar nutrition of vegetating crops with 0.5-2% liquid solution of EXTRASOL.

14. Application period: 1-2 months.

15. Phytotoxicity: none.

16. Effect on the homoiothermal: none.

17. Safety precautions in handling the preparation: same as usual requirements of personal hygiene, i.e. the operator should wear cotton overall or apron, also boots and a cotton wool-gauze mask. After work hands and face should be washed.

18. No detoxication or disinfection is practiced.

Application of extrasol preparation for treatment of crops

Grain crops (barley, rye, wheat, oats, sorghum, millet, buckwheat)

1. Pre-sowing treatment of seeds to suppress seeds infections (helminthosporiosis, fusarial wilt, etc.) and introduces useful bacteria on the seeds. Treatment with EXTRASOL preparation is carried out 1-60 days before sowing. <u>Consumption</u>: one litter per ton of seeds. Any kind of equipment for seed sterilisation can be used. EXTRASOL is compatible with all fungicides, herbicides and chemical fertilisers.

2. Treatment of crops during vegetation to stimulate plant growth and protect crops against fouler diseases (brown rust, powdery mildew, snow mould, etc.).
   EXTRASOL are to be used for the purpose.
   <u>Consumption:</u> 2-3 L per hectare. The preparation is compatible with herbicides, microelements, and chemical fertilisers. Two-stage treatment is recommended:
   a) During rooting phase
   b) During tailoring phase.
   Seeds are recommended to be treated before drying in order to suppress seed infections during storage period. Consumption of EXTRASOL : one litre per ton of seeds.
   Note: Drying and finishing of seeds do not affect the useful bacteria of the preparation, which remains on the seeds for 8-12 months, i.e. until they are sown.

Potato

1. Preplantng treatment of tubers to suppress seeds infections (rhizoctoniosis, phytophtorosis, etc.), stimulate tubers sprouting and introduce plant useful bacteria into the root system. Treatment with EXTRASOL preparation is carried out 1-5 days before or on the day of planting.
   <u>Consumption</u>: one litre per ton of tubers. EXTRASOL is compatible with all fungicides, herbicides and chemical fertilisers.

2. Treatment of crops during vegetation to protect plants against infections (phytophtorosis, rhizoctoniosis, and alternaria blight) and stimulates potato growth. EXTRASOL are used for the purpose.
   <u>Consumption</u>: 2-3 L per hectare. The preparation is compatible with microelements and herbicides. Two-stage treatment is recommended:
   a) 2-4 weeks after sprouting
   b) 5-6 weeks after sprouting
   <u>Note</u>: To protect potato crops from phytophtorosis, it is recommended to carry out treatments every 9-12 days, using 2-3 litres per hectare.

3. Treatment of tubers of food potato before storing in orders to protect from phytophtorosis, rhizoctoniosis, soft rots, and fusarial wilt.
   <u>Consumption</u>: one litre per ton of tubers.

White cabbages

1. Treatment of seeds. Seeds are wetted for 3-8 hours in a 1-% solution of EXTRASOL to suppress seed infections and introduce plant useful bacteria on the seeds.

2. Treatment of greenhouse substrate with a 0.1% solution of EXTRASOL at 3-5 days before sowing with a consumption of one litre per 100 m^2. Treatment of substrate with growing seedlings with a 0.1% solution of EXTRASOL at 3-5 days before transplanting to the field.
   Consumption: one litre per 100 m^2. Treatment of seeds and soil for seedlings is done to protect crops from soil-inhabiting phytopathogens and introduce plant useful microorganisms on the roots.

3. Treatment of crops during vegetation to stimulate their growth. EXTRASOL is used for the purpose.
   Consumption: 2-3 litres per hectare. The preparation is compatible with microelements. Two-stage treatment is recommended:
   a) 2-4 weeks after transplanting of seedlings
   b) 5-6 weeks after transplanting of seedlings

4. Treatment of finished food products with EXTRASOL before storage to prevent them from spoiling.
   Consumption: one litre per ton of produce (5-10% solution).

Carrots

1. Pre-sowing treatment of seeds to suppress seeds infections and introduces useful bacteria on the seeds. Treatment with EXTRASOL preparation is carried out 1-60 days before sowing.
   Consumption: one litre per ton of seeds, seeds should be dried before sowing. The preparation is compatible with disinfectants, herbicides and chemical fertilisers.

2. Treatment of crops during vegetation is carried out to stimulate plant growth. EXTRASOL used for the purpose.
   Consumption: 2-3 litres per hectare. EXTRASOL is recommended to be applied 2-5 days after using herbicides.

3. Treatment of finished food products with EXTRASOL before storage to prevent them from spoiling.
   Consumption: one litre per ton of produce (5-10% solution).

Beets (sugar-beet, beet root, mangel-wurzel)

1. Pre-sowing treatment of seeds to suppress seeds infections and introduces useful bacteria on the seeds. Treatment with EXTRASOL preparation is carried out 1-60 days before sowing.
   Consumption: one litre per ton of seeds. The preparation is compatible with disinfectants, herbicides, and chemical fertilisers.

2. Treatment of crops during vegetation to protect plants against infections (cercosporosis, etc.) and stimulates beets growth. EXTRASOL is used for the purpose.
   Consumption: 2-3 litres per hectare. The preparation is compatible with microelements and herbicides. Two-stage treatment is recommended:
   - a) 2-3 weeks after sprouting
   - b) 5-6 weeks after sprouting.

Maize (grain, silage)

1. Pre-sowing treatment of seeds to suppress seeds infections and introduces useful bacteria on the seeds. Treatment with EXTRASOL (peat based or dried formulation) preparation is carried out 1-60 days before sowing.
   Consumption: one litre or kg per ton of seeds. EXTRASOL is compatible with all fungicides, herbicides, and chemical fertilisers.

2. Treatment of crops during vegetation to stimulate plants growth and protects against foliar diseases. EXTRASOL is used for the purpose.
   Consumption: 2-3 litres per hectare. The preparation is compatible with herbicides, microelements and chemical fertilisers. Two-stage treatment is recommended:
   - a) 2-4 weeks after sprouting
   - b) 5-6 weeks after sprouting.

   Before drying grain, it is recommended to treat kernels to suppress seed infections over the storage period.
   Consumption: one litre per ton of kernels.
   Note: Drying and finishing of kernels does not affect the useful microflora of the preparation, which remains on the kernels for 8-12 months, i.e. to the moment of planting.

Sunflower

1. Pre-sowing treatment of seeds to suppress seeds infections and introduces useful bacteria on the seeds. Treatment with EXTRASOL preparation is carried out 1-60 days before sowing.
   Consumption: one litre per ton of seeds. Any kind of equipment for seed disinfection can be used. EXTRASOL is compatible with all fungicides, herbicides, and chemical fertilizers.

2. Treatment of crops during vegetation to stimulate plant growth and protect crops from diseases (sclerotinia, phomopsis blight).
   Consumption: 2-3 litres per hectare. The preparation is compatible with herbicides; microelements and chemical fertilisers. Two-stage treatment is recommended:
   a) 2-4 weeks after sprouting
   b) 5-6 weeks after sprouting.

Before drying grain, it is recommended to treat seeds to suppress seed infections over the storage period.
Consumption: one litre per ton of seeds.
Note: Drying and finishing of seeds does not interfere with the useful microflora of the preparation which remains on seeds for 8-12 months, i.e. until the time of planting.

Grapes

1. Treatment of vine in the phase of sap rising and buds swelling with EXTRASOL, using 2 litres per hectare.

2. Treatment of vine in the phase of 2-4-leaf sprouts with EXTRASOL, using 2 litres per hectare.

3. Treatment of vine before florescence with EXTRASOL, using 2 litres per hectare.

4. Treatment of vines 2-3 weeks -after florescence with EXTRASOL, using 2 litres per hectare.

5. Treatment of vine 2-3 weeks after the last dressing with EXTRASOL, using 4-5 litres per hectare.

6. If cases of mildew and oydium are reported (or predicted by the Plant Protection Service), vine in the affected area should be treated with EXTRASOL, using 5-6 litres per hectare. The treatment is to be repeated, if necessary, after 10-15 days, using 5-6 litres per hectare of the affected area.

7. In order to protect the harvest of grapes from grey mould (based on the forecast of the Plant Protection Service), vine shall be treated with EXTRASOL 10-14 days before harvesting, using 5-6 litres per hectare.

8. Treatment of finished food products with EXTRASOL before transportation or storage to prevent them from spoiling.

Consumption: one litre per ton of produce (5-10% solution).

Tomatoes, paprika, egg-plants, tobacco, etc. (seedlings) as well as gourds and onions

1. Treatment of seeds. Seeds are wetted for 3-8 hours in a 1-% solution of EXTRASOL to suppress seed infections and introduce plant useful microflora on the seeds.

2. Treatment of substrate in greenhouse with a 0.1% solution of EXTRASOL at 3-5 days before sowing using one litre per 100 m^2. Treatment of substrate with seedlings with an O.I% solution of EXTRASOL at 3-5 days before transplanting seedlings, using one litre per 100 m^2. Treatment of seeds and soil for seedlings is done to protect plants against soil-borne phytopathogens and introduce plant useful microflora on the roots.

3. Treatment of crops during vegetation to stimulate plant growth. EXTRASOL is used for the purpose.

Consumption:2-3 litres per hectare. The preparation is compatible with microelements. Two-stage treatment is recommended:
a) 2-4 weeks after transplanting of seedlings
b) 5-6 weeks after transplanting of seedlings
Note: In order to protect crops of the nightshade family from phytophtorosis, it is recommended to treat them with EXTRASOL every 9-12 days, using 2-3 litres per hectare.

4. Treatment of finished food products with EXTRASOL before transportation (tomatoes, paprika etc.) to prevent them from spoiling.
   Consumption: one litre per ton of produce (5-10% solution).

Sheltered ground (cucumbers, tomatoes, paprika)

1. Treatment of seeds. Seeds are wetted for 3-8 hours in a 1-% solution of EXTRASOL.

2. Treatment of substrate in greenhouse with a 0.1% solution of EXTRASOL at 3-5 days before sowing, using one litre per 100 m^2. Treatment of seeds and substrate for seedlings should be done to protect plants against soil-borne phytopathogens and introduce plant useful microflora on the roots.

3. Treatment of crops during vegetation to protect them against diseases, as well as to stimulate plant growth. EXTRASOL is used for the purpose. Consumption: one litre per 100 m. The preparation is compatible with microelements
   Treatments are recommended to be carried out every two weeks.

Form of preparation

Liquid of yellow or dark-brown colour. The preparation does not block spraying devices. It is harmless for human beings, livestock, and plants

EXPERIENCE OF PINE AND SPRUCE NEEDLE'S EXTRACTIVES USING FOR PLANT PROTECTION

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Fitoekols-IF – a product with fungicidal and insecticidal properties was tested and is effective against different fungus species (Mildew, Grey root, Cladosporium, Botritis, Oidium, Fusarium, Phytophtora etc*.) and sucker-type pests (Aphis, Myzoides, Trialeurodes etc.).*

Fitorodents-RF is a product with repelling properties against hares and rodents and with fungicidal and insecticidal properties against wintering on the bark insects and diseases. Both products degrease damages of fungus and insects by 70–90%, like another biocides, and damages of hares and rodents decrease by 98–100%. Both of them are biodegradable under natural conditions and may be characterised as low-hazard substances falling in toxicity class 4. The products on the basis of coniferous needles extracts open new possibilities for developing a new line of environmentally friendly phytopesticides as well as complex utilisation of local forest plant biomass resources.

Key words: *Fitoekols-IF, Fitorodents-RF, fungicide, insecticide*

Introduction

A lot of information of chemical composition of coniferous tree foliage and biological activity of different extractive substances – terpenes, resin acids etc. shows high insecticidial, fungicidal and repelling properties of them. Commercial environment friendly preparations for plant protection are elaborated on the basis of long-term investigations.

The investigations about using possibilities of coniferous foliage extracts for plant protection was started at 1987 in the Latvian Forest research Institute " Silava" and in the Latvian Institute of Biology.

In the gardening the use of vegetable origin substances for suppressing pests and diseases is known since time immemorial. For example water extracts of garlic, marigold, tobacco e.t.c. are well known in plant protection folk traditions.

However, very few markets offer volume- produced plant protection agents having a vegetable-origin substances as the active ingredient, readily decomposable in biological processes. This is accounted for by the costs for cultivating the plants known for their fungicidal or insecticides properties prohibitively high, the manufacturing depending on the season of the year, the lack of science-based knowledge and the like. We were looking for cheap raw material for phytopesticides obtaining.

Results and discussion

It is well known the intensive secretion of resins is essential response of coniferous to outset of pests and diseases, and we selected the coniferous foliage as raw material for phytopesticides obtaining. Today we can say the selection was successful.

On the basis of lot of trials was elaborated phytopesticide " Fitoekols-IF" with fungicidal and insecticide properties. The active substance of preparation is mixture of sodium salts of resin and fatty acids. " Fitoekols-IF" is tested for suppressing different fungus species (Mildew, Grey root, *Cladosporium, Botritis, Oidium, Fusarium, Phytophtora etc*.) and different sucker-type pests (*Aphis, Myzoides, Trialenrodes, Heliotrips, Partheotrips etc.)* both under cover and in the open areas. The product decrease the damages of *Fungus* and insects by 70-90%, like another biocides, for example Neem tree extracts. The best results show the mixture of pine and spruce extractives.

The toxicological evaluation was done following the OECO Guidelines for testing of Chemicals (Paris,1984): 401 Acute Oral Toxicity, 405 Acute Eye Irritation, 404 Acute Dermal Irritation and 406 Skin Sensitisation. The product is classed as low hazard substance with no ill effect to man and environment, biodegradable in natural conditions.

The product is not showing negative influence to earth-warms, soil microflora, plant physiology (chlorophyll content, evaporation).

The more than 10 years tests show that insects are not developing resistance to "Fitoekols".

The precise effects of the " Fitoekols" on the insects and diseases are difficult to pinpoint and additive investigations are needed.

The pine needle extractives in the form of hexane solution under trade name " Fitorodents-RF" was elaborated for plant protection against hares and rodents in the wintertime. Using of " Fitorodents-RF" decrease the damages of fruit-trees by hares and rodents in the orchards by 95-100%. The product destroys 60-70% of pests and 50-60% of pathogenic fungus spores wintering on the bark of trees, suppresses the development of fruit tree scab and linchen too.

ALTERNATIVE VARROA-BEKÄMPFUNG - BEKÄMPFUNGSKONZEPT MIT THYMOVAR

S. GISLER, F. BOLLHALDER

Andermatt Biocontroll, Schweiz

Thymovar comes in wafers, each containing 15 g of Thymol a substance allowed in food-supplies. Through the evaporation of the volatile oil, Thymol, in the wafers, vapour concentrations in the hive result that are highly toxic for the varroa mites, but not for the bees.

To complete the Thymovar treatment of bee hives it is almost always necessary to apply a winter treatment (oxalic acid or Perizin) as soon as there is no brood in the colony. The winter treatment can only be omitted if within 14 days after Thymovar treatment less than one mite per day falls. This will ensure that Varroa-infestation does not exceed the threshold of damage.

Thymovar works best at maximum day temperatures 12 to 30°C, and so its use should be adapted to the season, the microclimate and the type of hive. If unfavorable conditions still curtail the efficiency of Thymovar, a correct winter treatment will guarantee that the Varroa-infestation can be reduced below 50 mites/hive. An additional effect of a winter treatment lies in the change in active substance, thus lowering a possible development of resistance against Thymovar.

Einleitung

Zur vollständigen Varroa-Behandlung mit Thymovar gehört eine Nachbehandlung im brutfreien Zustand, damit die Varroa-Milbenpopulation wirksam unter der Schadschwelle gehalten werden kann. Nur bei idealen Temperaturen (Tagesmaximaltemperatur zwischen 12 und 30 ^{o} C) verdampft das Thymol in der richtigen Menge. Deshalb sollte der Einsatz von Thymovar genau auf die Jahreszeit, das Mikroklima und das Beutesystem abgestimmt werden. Falls trotzdem ungünstige Bedingungen den Behandlungserfolg schmälerten, muss mit einer Nachbehandlung der Varroabefall unter 50 Milben reduziert werden. Der Einbezug eines zweiten Wirkstoffes bei der Nachbehandlung kann zudem die Wirkung der Behandlungsmittel langfristig aufrecht erhalten (Resistenzmanagement).

Bekämpfungsstrategie

Thymovar muss zweimal im August und September angewandt werden. Im November oder Dezember sollte unbedingt eine Nachbehandlung (Oxalsäure oder Perizin) folgen.

Vor, während und vor allem nach der Behandlung mit Thymovar ist der Milbenfall zu kontrollieren, um über die Vermilbung der Völker informiert zu sein. Falls während den zwei Wochen nach der zweiten Thymovar-Behandlung im Schnitt weniger als eine Milbe pro Tag ausgezählt wird, ist mit einer geringen Varroa-Population zu rechnen und **nur dann** kann auf die Nachbehandlung verzichtet werden. Mit diesem Vorgehen ist garantiert, dass der Varroa-Befall bis zum nächsten August unter der Schadschwelle bleibt, vorausgesetzt, es findet keine massive Rückinvasion statt.

Anpassen der Thymovar-Behandlung an das Mikroklima und den Beutetyp

Um eine optimale Wirkung von Thymovar zu erreichen, sollte die Tagesmaximaltemperatur zwischen 12 und 30 ^{o}C liegen. Zu tiefe Temperaturen ergeben eine zu niedrige Thymolkonzentration in der Stockluft. Das Thymol dampft zuwenig ab, und die Varroa-Milben werden nicht abgetötet. Deshalb ist es wichtig, dass mit der Thymovar-Behandlung nicht zu spät begonnen wird, und diese vor den kühleren Herbsttagen abgeschlossen ist. Hingegen lassen sehr hohe Temperaturen (> 30 ^{o}C) den Wirkstoff zu schnell verdunsten. Die Thymolkonzentration kann anfänglich über die Bienenverträglichkeit ansteigen und es wird zuviel Thymol verdampft.

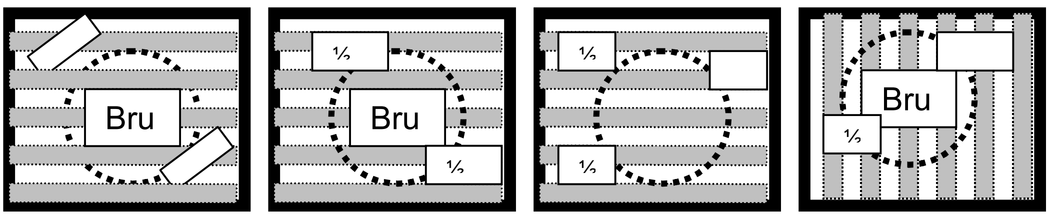
Mittlerweile haben zusätzliche Erfahrungen aus dem Ausland gezeigt, dass bei grösseren Beutetypen bis zu 2 Thymovar-Plättchen angewandt werden und richtig in die Beute eingelegt werden sollten (Graphik 1).

Tabelle1 und Graphik 1: Empfehlung zur Menge und Anordnung von Thymovar-Plättchen bei verschiedenen Beutetypen

Beutetyp	Thymovar- Plättchen*		Wirksamkeit
	1. Behandlung	**2. Behandlung**	
Kleine Ablegerkästchen	½	½	
Warmbau, Hinterbehandlung	1	1	>95 %
Magazin, 1 Zarge	½+½	½+½	>90 %
Magazin, 2 Zargen	1+1	1+1	>90 %
Dadant	1+½	1+½	>90 %

*Tagesmaximaltemperatur zwischen 12 und 30 ^{o}C

Magazin 2 Zargen | Magazin 1 Zarge | Dadant | Schweizer Kasten



Erfahrungen und Resistenzmanagement

Wird das Mikroklima und der Beutetyp berücksichtigt, zeigten die Erfahrungen der letzten drei Jahre, dass die Völker nach abgeschlossener Thymovar-Behandlung durchschnittlich noch mit 50-200 Milben befallen waren. Im schlechtesten Falle verblieben bei einem stark befallenen Volk (> 2500 Milben) nach der Thymovar-Bekämpfung max. 500 Milben im Bienenvolk. Diese Milben können aber mit einer korrekten Nachbehandlung im Winter auf weniger als 50 Milben/Volk reduziert werden.

Ein weiterer Vorteil der Nachbehandlung liegt darin, dass nach der Spätsommerbehandlung (Thymovar), ein Wirkstoffwechsel (z.B. Oxalsäure) im Winter vorgenommen werden kann. Dadurch wird einer allfälligen Resistenzentwicklung der Varroa-Milbe gegen Thymol vorbeugend entgegengewirkt. Eine Nachbehandlung wird deshalb dringend empfohlen und garantiert, dass die Völker mit einer geringen Varroa-Belastung überwintern und gesund in die neue Saison gehen.

AN ESTIMATION OF INFLUENCES EVOKED BY SOME NATURAL INSECTICIDES ON GREENHOUSE PEST-INSECTS AND MITES

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*In the present research we compared the effects of three commercial preparations of natural origin − Neko (Finnish firm OY Neko AB,), Neem (Scotts Celaflor GmbH and Co) and Phytoverm-0,2% (Russian PharmBioMed) on the greenhouse pests − greenhouse whitefly (*Trialeurodes vaporariorum*), two-spotted spider mite (*Tetranychus urticae*), and tobacco thrips (*Thrips tabaci*). Karate, a synthetic pyrethroid, served as a standard insecticide, whose efficacy was compared with the above-mentioned natural substances.*

All the natural preparations tested proved to be more effective against the eggs of two-spotted spider mite than the synthetic Karate, used as standard. About 90% of eggs perished when Neem was applied, followed by Neko (84%), Phytoverm (70%) and Karate (42%).

From all the preparations tested, the Neko proved to be the most effective against the larvae and pronymphs of greenhouse whitefly causing the death of 90% of the individuals. On eggs the effect was weaker than that of Neem, which possessed strong ovicidal properties. Phytoverm and Karate did not reveal any action on the eggs because of low temperature.

On the larvae and adults of tobacco thrips the effectiveness of Neko was on the same level with Karate used as standard. The efficacy of Phytoverm and Neem remained behind that of Neko and Karate. A shortcoming of Neko is its strong phytotoxicity to cucumber plants.

***Key words:** Celaflor® Schädligsfrei Neem, Neko, Phytoverm-0.2%, greenhouse whitefly, two-spotted spider mite, tobacco thrips.*

Introduction

All the problems, which have emerged in connection with the use of synthetic means of pest control, impel us to refer to nature for help. In the interests of sparing our health it is not recommended, or it is prohibited, to employ poisonous chemicals in greenhouses. At the same time, in a glass-covered area there are optimal conditions for the reproduction of pests. That is why there has occurred interest in new natural means of pest control. The insecticidal and acaricidal properties of a number of plants have been discovered long ago, and some of the plants can compete with synthetic means of control (Hedin, Hollingworth, 1997). Especially remarkable are tropical plants, from which hundreds of products of secondary metabolism with insecticidal properties have been extracted. Recently neem tree (*Azadirachta indica*) has received a lot of attention. Its refined seed extract is an insecticide of wide spectrum, containing several azadirachtins as active substances (Addor, 1994; Mordue (Lunz), 1979). Attention should also be given to the plants from our home country, which could prove useful in pest control, especially if they are used as mixtures of certain combinations. On the basis of such plants some firms have composed and produced commercial preparations which have passed necessary tests, and to which exploitation licences have been given. Nevertheless, further information about the effectiveness of the preparations is needed, because susceptibility to toxicants varies in different populations. Besides, there are other factors to be considered: climatic conditions, chemical means of control used earlier, and many other unpredictable circumstances that may influence the effectiveness of a certain preparation. Thus it is not surprising that evaluations given to a single preparation may sometimes be very different, or even contradictory. The goal of commercial enterprises is to expand the markets for their products while consumers are interested in effective nature-friendly means of pest control.

The aim of the present research is to compare the effects and efficacy of commercial preparations of natural origin produced by three different enterprises (OY Neko Ab, Scotts Celaflor GmbH and Co, PharmBioMed) to the most widely-spread and damaging greenhouse pests, the twospotted spider mite (*Tetranychus urticae*), tobacco thrips (*Thrips tabaci*) and greenhouse whitefly (*Trialeurodes vaporariorum*). These pests can be encountered in the greenhouses heated all year round, and it is difficult to control them. The mentioned pests have acquired resistency towards many toxins, besides, their biology and behaviour complicate the control. A layer of wax and honeydew protects greenhouse whitefly, while twospotted spider mite secretes a protective net cover. Tobacco thrips passes a part of its developmental cycle under another means of protection: eggs are laid into plant tissue, overwintering period is spent in soil or in plant buds.

The preparations were obtained directly from the representatives of the producer firms interested in the Estonian market.

Materials and methods

Insecto-acaricidal commercial preparations. Firstly, we tested Celaflor® Schädligsfrei Neem (Neem) (Scotts Celaflor GmbH and Co) including 1-% azadirachtin of 4% of natural neem-seed extract Neem-Azal. The active agent substances in the preparation are different azadirachtins (from the group of limonoids), and other neem ingredients. In the preparative form liquid soap and vegetable oil are included. The preparations produced on the basis of neem have been exploited for a long time. The first commercial preparation from neem was registered under the name of Margosan-0 in America already in 1985 (Jacobson, 1989; Ascher, 1993). Initially its use was permitted only on technical cultures and, since 1993, on certain foodplants. In European countries the NeemAzal T/S (Trifolio GmB, Germany) was first registered in Switzerland in 1966, and two years later in Germany (Zuber, 1998). At present time the Neem-Azal is in use in a number of European countries, and the negotiations about the marketing of Neem products in other European countries have been set going. In many Asian countries the neem products have already been used during many centuries.

The Finnish firm OY Neko AB on the basis of a number of plant extracts produced the second preparation tested by us, Neko. The active substances of the preparation were pine-soap solution produced from pine – *Pinus sp*. oils (25g/l), tansy − *Tanacetum vulgare* (3.3 g/l), sage − *Salvia officinalis* (3.3 g/l), and pink hidcote lavender − *Lavendula angustifolia* (3.3g/l). The preparation has already been registered for use in Finland and Estonia. The plants included in the composition of the preparation contain different quantities of ether oils, bitter subtances, glycocides, tanning substances, resins, phenol acids, etc., due to which the plants posses insecticidal properties (Hiller, 1988).

The third preparation that went through our testing was Phytoverm-0.2% (Phytoverm), produced in Russia by PharmBioMed, 1 g of which contains 2 mg of aversectin C. The active substances of the preparation are a complex of natural avermectids produced by soil fungi – *Streptomyces avermitilis*. Since 1994, the preparation has passed an extensive approbation in Russia, and is primarily valued as an effective acaricide (Berezina et al., 1997).

Karate served as a synthetic standard, containing lambda-cyhalothrin as the active substance (50 g/1kg). Chemically Karate is a pyrethroid, and can be found in the list of the means of pest control used for controlling the wide range of agricultural pests both by contact action and by ingestion. Pyrethroids are nonsystemic insecticides and they do not possess any translaminar properties. They rapidly penetrate the insect cuticle, and are thus more active against insects when applied topically than orally. Pyrethroids affect every part of the insect´s nervous system, including central and peripheral nerves (Kornis, 1994). Neurosecretory cells are very susceptible targets for pyrethroids, and the disruption of the balance of the neurohormones within the insect body could be a major factor in the toxicity (Henrick, 1995).

Pest-insects and -mites. The tests with greenhouse whitefly were performed on evergreen decorative plants − tomato tree (*Cyphomandra crassicaulis*) and angel´s trumpet (*Datura arborea*) in the palm house of Tartu Botanical Gardens. In the palm house the temperature fluctuated from 14° to 22 °C, and additional light was used there during winter periods. The plants were heavily infested with all the instars of greenhouse whitefly. Periodically different insecticides were employed to control the pest, although during the testing no chemical control was carried out.

The trials with two-spotted spider mite and tobacco thrips were made in the Grüne Fee Eesti AS greenhouses located in Väike-Lohkva, Tartu County. The plants exploited were older, strongly damaged cucumber plants in their final fruit-bearing phase. In the greenhouses the temperature was 22° to 25 °C, relative air humidity ca 100%, and additional light was used continually. In the greenhouses of Grüne Fee no chemical means of pest control are employed at all. A predatory mite (*Phytoseiulus persimilis*) was used for biological control, but nevertheless, the expansion of the pest could not be fully restricted. The heavy damage on plants was local, and some of the leaves seemed paper-like. Tobacco thrips had spread unevenly throughout the whole greenhouse, but in some places the damage was very serious.

Test methods

The preparations tested were applied in the concentrations recommended in their instructions: Neem as 0.4% water emulsion, Phytoverm-0.2% as 0.5% water emulsion with the addition of 0.02% green soap, Neko in the original bottle as a ready-made emulsion, Karate as 0.15 % water emulsion. For the control leaves were sprayed with water. The freshly prepared solutions were sprayed evenly onto the leaves, and after that the leaves were isolated with bags made from agryl cover.

All the trials were performed in three replications, in each replication the growing plant had a bigger leaf, 22-27 cm in diameter. The tested cucumber leaves were heavily damaged by both two-spotted spider mite and tobacco thrips. Due to the uneven distribution of tobacco thrips the square-method could not be applied, thus the counting was carried out on a whole leaf. The leaves of tomato tree and angel´s trumpet were infested with all the instars of greenhouse whitefly. Fourty eight hours after spraying the plants were first observed, and the stage of potential phytotoxicity of the preparations was determined. The trial lasted three weeks altogether. During this period the number of living and dead individuals of the whitefly on 3-5 randomly selected squares on the plants was counted.

Results

Greenhouse whitefly. Leaves of tomato tree and angel´s trumpet from different height were selected for the test. On the top leaves of plants there were mainly eggs and adults, on the medium ones larvae of different ages, on the lower ones pronymphs. None of the preparations exerted a phytotoxic effect on the leaves of tomato tree and of angel´s trumpet.

The efficiency of the spraying on eggs could be determined only after two weeks, when the embryogenesis had ended, and in the control series all the larvae had emerged. Phytoverm and Karate did not reveal any action on the eggs of greenhouse whitefly, and there was no significant difference from the control (Fig. 1). In the series with Neko and Neem over 80% of the eggs perished. In case of Neko the larvae did not hatch, the eggs started to dry. In the Neem series the embryonic development took place, but the larvae died during the emergence from the eggshells.

The larvae of greenhouse whitefly were constantly observed during three weeks. When Karate or Phytoverm was used, over 90% of the larvae completed the metamorphosis, and normal adults emerged. There were no differences from the control (Fig. 1, Table 1). In the Neko series the results became evident already a couple of days after spraying, the larvae had

Table 1. The differences in efficacy of four natural insecticides by calculated P-values of student t-tests (data from Fig. 1-3). The differences are significant at $P < 0,05$ (asterisks).

Eggs (above black diagonal), larvae and adults (below black diagonal) of two-spotted spider mite, *Tetranychus ultrica*

	Neko original	Neem 0,4%	Karate 0,15%	Phytoverm 0,5%	Control
Neko original		0,72675	0,05898	0,44564	0,02017*
Neem 0,4%	0,11536		0,01871*	0,25443	0,00362*
Karate 0,15%	0,46192	0,20483		0,12414	0,02468*
Phytoverm 0,5%	0,19595	0,17759	0,32849		0,02347*
Control	0,01165*	0,00017*	0,00963*	0,00000*	

Larvae and adults of tobacco thrips, *Thrips tabaci* (above black diagonal), and eggs of greenhouse whitefly, *Trialeurodes vaporariorum*, (below black diagonal)

	Neko original	Neem 0,4%	Karate 0,15%	Phytoverm 0,5%	Control
Neko original		0,02529*	0,84818	0,13069	0,00080*
Neem 0,4%	0,61701		0,02984*	0,20195	0,12577
Karate 0,15%	0,00416*	0,00209*		0,15782	0,00628*
Phytoverm 0,5%	0,00622*	0,00298*	0,81577		0,00060*
Control	0,00000*	0,00229*	0,90733	0,85889	

Larvae (above black diagonal) and pronymphs (below black diagonal) of greenhouse whitefly, *Trialeurodes vaporariorum*

	Neko original	Neem 0,4%	Karate 0,15%	Phytoverm 0,5%	Control
Neko original		0,00074*	0,00030*	0,00030*	0,00029*
Neem 0,4%	0,03647*		0,00054*	0,00054*	0,00039*
Karate 0,15%	0,00119*	0,03640*		1,00000	0,55055
Phytoverm 0,5%	0,00003*	0,04404*	0,79097		0,55055
Control	0,00002*	0,03294*	0,31058	0,02552*	

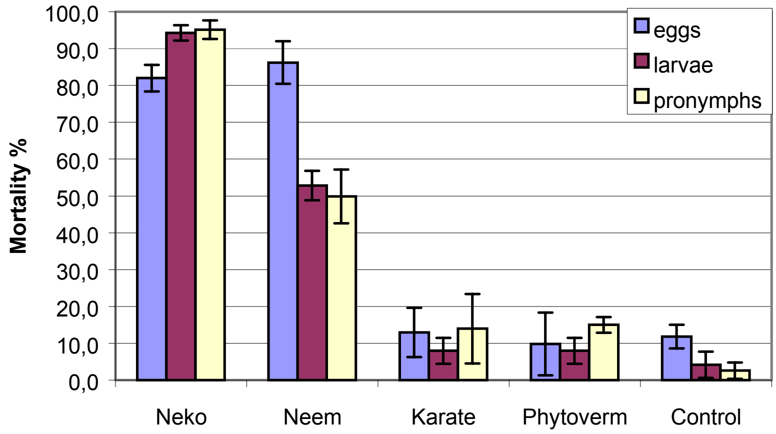

Fig. 1. The effects of different natural insecticides on the mortality rate of greenhouse whitefly, *Trialeurodes vaporariorum*

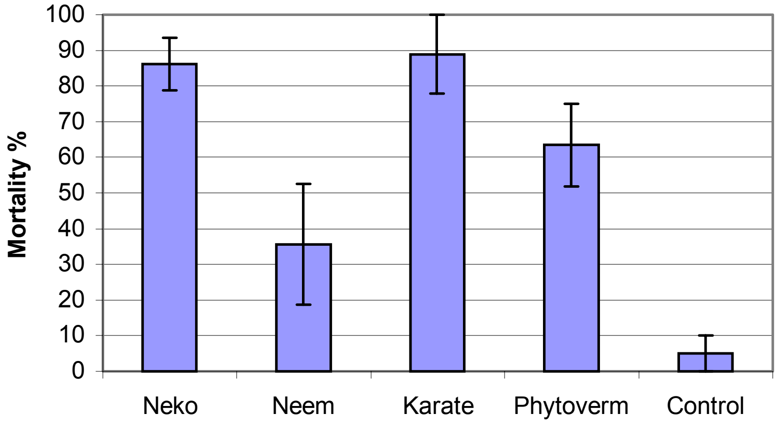

Fig. 2. The effects of different natural insecticides on the mortality rate of tobacco thrips, *Thrips tabaci*

turned brown, and haemolymph had flown out of their bodies. The death rate was over 90%. Only a very small number of larvae, found near leaf veins, developed into adults (Fig. 1). Evidently they were not in contact with the preparation as the microscopic observation of the leaves revealed no brownish traces of the dried preparation.

In the Neem series, during the first days after spraying no external changes in larvae of greenhouse whitefly were seen, and the final result could be estimated only after three weeks, when the control larvae had hatched. By that time the larvae had reached pronymphal stage and ended their metamorphosis. Through the cracked pronymphal shell the body parts of the adult could be seen - the white wing-rudiments and antennae, however, more than half of them perished inside the shell instead of emerging (Fig.1).

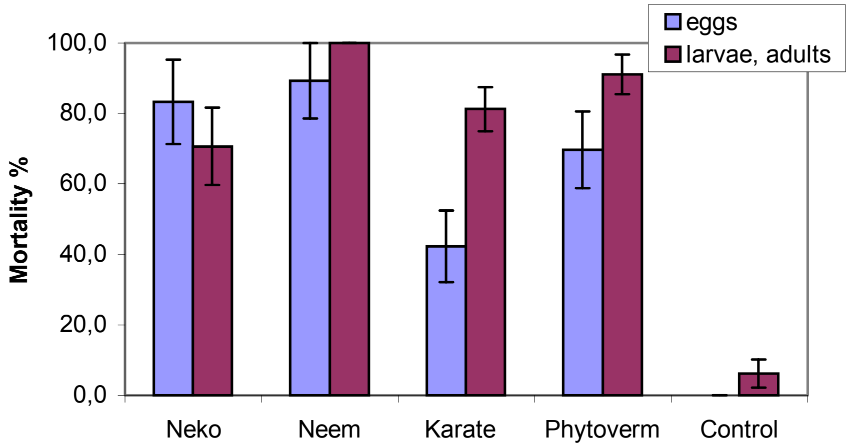

Fig. 3. The effects of different natural insecticides on the mortality rate of two-spotted spider mite, *Tetranychus urtica*

The pronymphs of greenhouse whitefly exhibited similar results. The efficacy of Karate and Phytoverm was low, the death rate was under 20%, and there were no differences from the control (Table 1). Neko exhibited direct toxic action, and the perished pronymphs could be noticed already after a few days, the death rate being over 90%. In the Neem series half of the individuals either died during the metamorphosis, or did not emerge from the nymphal shells after ending their metamorphosis (Fig. 1).

Tobacco thrips. The cucumber plants selected for the trial were seriously damaged by tobacco thrips. On the infected leaves there appeared a number of ´silver spots,´ marking the areas where the pest had sucked the plant cells empty. The action of different preparations on the plant and thrips was first estimated 48 hours after spraying.

Neko proved to be strongly phytotoxic for the cucumber leaves. Fair spots had appeared on the leaves, later the burned spots turned brown and dried. Phytoverm, Neem and Karate showed no phytotoxic effects on cucumber plants.

Phytoverm did not wet the whole surface of the leaf, the liquid sprayed gathered into bigger drops, while some parts remained dry. The webnet secreted by the mites obviously did not allow the preparation to spread evenly on the leaf surface.

During the microscopic observation carried out 48 hours after the spraying with Neko and Karate the larvae and adults of tobacco thrips did not respond to prodding. After some days they turned brownish and adhered to the leave surface. That is one of the reasons why the spraying with Karate and Neko resulted in 90% of mortality (Table 1, Fig. 2).

On the first days the results of treatments with Neem were unclear: the adults and larvae of tobacco thrips responded to prodding, however uncharacteristically of the species they were languid and inactive. The final estimation could be given only after a week had passed. In the Neem series the death rate of tobacco thrips was low, only 36% (Fig. 2). Evidently the webnet secreted by two-spotted spider mite provides good protection for tobacco thrips, and the preparation doses reaching the organism remained too low. In the Phytoverm series over a half of the individuals died (Fig. 2), but they were distributed unevenly on the plant leaf. It is beyond doubt that the reason was the uneven wetting of the leaf surface. In the control the death rate was under 10%. After two weeks freshly hatched larvae of the thrips were noticed in the control and in the test series. Probably the preparations did not cause toxic action on the eggs laid in leaf tissues, although this assertion requires special tests.

Two-spotted spider mite. All the instars of two-spotted spider mite were simultaneously on the cucumber leaves. On the first day after treatment it was difficult to determine the state of eggs, because initially there are no external changes to be seen. Since there were simultaneously eggs of different age on the leaves, the final counting was carried out only after one week. At that time the embryonic development in the control series had been completed, and the larvae had hatched from all the eggs.

All the natural preparations tested proved to be more effective against the eggs of two-spotted spider mite than the synthetic Karate, used as standard (Table 1). About 90% of eggs perished when Neem was applied, followed by Neko (84%), Phytoverm (70%) and Karate (42%) (Fig. 3). In the Phytoverm series the uneven distribution of the preparation on the leaf was noticed. In some places all the eggs had lost the ability to develop, at the same time in the neighbouring areas vital larvae hatched. Neko evidenced by extensive burned spots and mortified tissues seriously damaged cucumber leaves. On the mortified leaf parts the eggs dried, and no larvae did hatch.

For the first time the effect of the preparations on the larvae and adults of two-spotted spider mite was estimated two days after spraying, but then it was too early to determine whether the mites had perished or not. The final counting was performed two weeks after spraying, by that time the perished mites had turned brownish and dried. Neem and Phytoverm appeared to be highly effective. In both the series all the larvae and adults of two-spotted spider mite had perished (Fig. 3). The effects of Karate and Neko were weaker while Karate used as standard seemed to be slightly more effective than Neko, although statistically it was not proved (Table 1). In the Neko series almost all the larvae and adults had died on the leaf surfice, whereas along leaf veins there were many surviving individuals.

The number of predatory spider mites (*Phytoceiulus persimilis*) on cucumber leaves was small, and there was no separate counting carried out. However, in all the series dead larvae and adults of the spider mite were found while examining the treated leaves.

Discussion

Neko consists of several different plant extracts, such as pine, tansy, sage, lavender, all known for their insecticidal properties. The aim of combining plant extracts is to achieve greater effects than in their separate use. For example, in combining the extracts of pine shoots and absinth, synergizing effect on two-spotted spider mite has been obtained (Kuusik et al, 1999; 2000). Besides synergizing effects, there may occur also summarising or complementary effects (Hewlett, 1960). It is difficult to determine which of these effects has been achieved by means of the plants included in Neko. As a result of the tests carried out, the combination may be considered successful.

From all the preparations tested, the Neko proved to be the most effective against the larvae and pronymphs of greenhouse whitefly, causing the death of 90% of the individuals already a couple of days after the treatment. On eggs the effect was weaker than that of Neem, which possessed strong ovicidal properties (Table 1, Fig.

1). On the larvae and adults of tobacco thrips the effectiveness of Neko was on the same level with Karate used as standard. Neko killed only 50% of the larvae and adults of two-spotted spider mites, being thus in this case weaker than the other preparations. A shortcoming of Neko is its strong phytotoxicity to cucumber plants grown in artificial light conditions. There burnt spots appeared on the leaves already on the second day, later the tissues became mortified. Because of the phytotoxicity it was very difficult to estimate the direct action on the eggs of the pests. On the damaged leaves the eggs may have perished simply due to the loss of moisture. Consequently, the high death rate of the eggs may not have been the result of the direct action of the preparation. The high level of phytotoxicity found on the cucumber leaves shows the necessity for a cautious use of the preparation. But angel´s trumpet and tomato tree, whose textures are hardier, did not reveal any signs of phytotoxicity.

The Russian association "Pharmbiomed" had brought Phytoverm to the market of plant protection means already in 1994 (Berezina et al, 1997). The active substance of Phytoverm is Aversectin C, a mixture of 8 avermectins extracted from the biomass of *Streptomyces avermitilis*. Avermectins are specific natural neurotoxins, which through food or body surface may find their way into the organisms of arthropods, and damage their nervous system by blocking the transmission of nervous impulses (Kornis, 1994). Consequently, the insects lose their ability to move and perish. Phytoverm has been characterized as an insecto-acaricidal preparation active against spider mites, nemotodes, and other insects. Since temperatures lower than 20 °C reduce the efficacy of the preparation, in northern areas it can be considered useful mostly in warm greenhouses. In our tests between 22° and 24 °C, all the larvae and adults of red spider mite perished, but 30% of the eggs completed their development successfully. The relatively low efficacy of avermectins on the eggs of mites has been noted already earlier (Kornis, 1995). Nevertheless, there are data indicating that at higher temperatures (28°C) Phytoverm reveals also ovicidal action (Instruction in Russian). Taking into consideration the lesser sensitivity of the eggs of two-spotted spider mite to Phytoverm spraying should be repeated several times. Avermectins have repeatedly been tested on the species of thrips inhabiting greenhouses, but the results have not been satisfactory for commercial preparations (Kornis, 1995). Because avermectins lack a systematic effect the thrips leading half-covered life-styles may not happen to get in contact with the preparations sprayed on plants. We had relatively good results in tests with the early larval instars and adults, but the efficacy of Phytoverm remained behind that of the Neko and Karate (Fig. 1). In concentrations we used it, the Phytoverm did not affect the several stages of the greenhouse whitefly, suggestively due to insusceptibility of whitefly to Phytoverm, or due to relatively low temperatures during tests (14 ° to 22 °C).

The means of plant protection composed on the basis of neem ingredients have received a lot of attention. The present commercial preparation Celaflor(r) Schädlingsfrei Neem, based on the natural extract of neem seed, has been recommended for many pests, including those of greenhouse. Different insect species reveal considerable differences in their sensitivity to neem products, and for that reason there have been carried out very extensive laboratory and field trials with neem.

The main principles for the practical use of neem in plant protection have already been elaborated, and, first of all, they include the recommended concentrations and timing of treatments, taking into account the modes of action of neem and the appearance of the susceptible stages of pest insects. The modes of application may play also an essential role in the efficacy of Neem (Kleeberg, Hummel, present proceedings).

In case the pest insects are able to avoid contact with neem the results may be low rates of mortality. The plants infested by twospotted spider mite and tobacco thrips were simultaneously covered with cobweb offering a good protection for pests. Therefore, a low mortality was observed. In our earlier tests, when the solution was applied directly to the body surface with a syringe, the death rate achieved was nearly 90% (Hiiesaar et al., 1998). When the cucumber plants were heavily infested with tobacco thrips alone, and plants were not covered with a cobweb, we got entirely rid of tobacco thrips by spraying the plants with Neem (our unpublished data).

Some literature data exist about the possible phytotoxicity of Neem on certain plant species and/or cultivars, including the greenhouse plants with tender leaves (Schmutterer, 1995; Bettum, 1998). In our tests the Neem preparation did not cause any phytotoxicity.

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BIOLOGICAL PREPARATION AND OTHER ORGANIC METHODS IN PRACTICE OF ELKANA

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Introduction

Georgia is an agrarian country and agriculture has a leading position among other activity fields. However, agriculture in Georgia is in a crucial state at present, due to hard economic situation of the farmers and because of bad climate conditions in recent years.

In order to development sustainable agriculture we consider it essential that along with the existing conventional farming biological farming sold be promoted. The ideas of biological or organic farming are propagated in Georgia by association of biological farms Elkana, the main objectives of which are:

to assist the sustainable development of Georgian agriculture;

to promote the creation of social-economically sustainable organic farms in Georgia;

to improve living conditions in rural areas by stimulating self-assistance processes in Georgian villages.

In order to achieve these objectives, Elkana developed special services, namely, Extension Service for Organic Farmers, Extension Service for Rural Development, and Public Relations and Lobbying Service. Furthermore, Elkana supports the protection and sustainable use of local agrobiodiversity. Elkana has developed organic farming standards for Georgian conditions. With the aim to support organic farmers, Elkana intends to establish certification of organic products. A specially created Marketing Service is already working on this issue.

Results and discussion

The association Elkana unites about 200 organic farmers in various parts of Georgia. This itself is already a precondition for diversity of produced organic food.

Georgia is a southern country and, naturally, many pests and diseases are widely spead there. With the help of Elkana extension workers the farmers are using various biological means to control somehow these problems. Namely, we have tested various plant extracts and infusions on our trial plot and then distributed them to our organic farmers, for example, henbane (*Hyosciamus niger*) extract controls well the cabbage white butterfly (*Pieris brassicae*) and cabbage moth (*Mamestra brassicae*). Henbane extract and infusion with addition of 0.4% soup are efficient against cabbage flea beetles (*Phyllotreta* sp.) and mites (*Acari*). Horseradish extract (*Armoracia lapathifolia*) is efficiently used against stinking smut (*Tilletia levisi, Tilletia tritici*). In our practice we use extracts and infusions of Caucasian pyrethrum (*Pyrethrum*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*), garlic (*Allium* *sativum*), onion (*Allium cepa*), potato (*Solanum tuberosum*), clematis (*Clematis vitalba*), elder (*Sambucus ebulus*), common wormwood (*Artemisia absinthium*). Beside the extracts we use widely various bio-preparations, namely, Phytoverm, Bitoxibacylline, SBM, neem and others.

We also pay great attention to collecting the folk traditions and apply them into practice. Thus, we try to use to the maximum the knowledge accumulated by our ancestors, reviving the forgotten experience.

HEAVY METAL CONTENT IN FLUVIAL DRIFTS OF THE CITY POTI

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Environmental pollution by chemicals of technological origin is a significant problem of modern industrial cities. To plan remedial measures against the mentioned situation information is needed on soil pollution levels by heavy metals for all industrial and communal units, adjacent territories, parks, and city lawns.

Key words: *heavy metal pollution, fluvial drift.*

Introduction

The first one, which takes the technological “attack” on itself, is the most fertile upper humus layer of soil, where major portion of plant root system is developed. Heavy metals washout balance shows that upper layers of soil are globally polluted by anthropogenic factors. Micro and Macro-elements and other toxic substances are intensively accumulated there. Toxic compounds of pesticides, mineral and organic fertilizers are systematically added to the substances brought through air and water (Dobrovolski, 1983).

Materials and Methods

Investigation field works for different soil types of the Black Sea coastal zone were conducted in West Georgia during 1988-93. Agrochemical indices, heavy metals (Pb, Cu, Co, Zn; Ni) have been investigated in fluvial drifts of the city Poti. Soils had been investigated around the territories of different industrial units – 50, 100, 150, and 300 meters far from each unit. Soil samples were taken 0-20 and 20-40 cm. deep. 20 complete soil sections (profile) were made. Levels of soil pollution had been evaluated by several gradations. Particularly, by regional background indices (according to Vinogradov), local background indices calculated taking into account concrete element and soil type and concentration factor of Clark. For this reason during field works 50 mixed samples were taken 0-20cm. deep from 600 points and local background indices had been developed on the basis of obtained results. Content of heavy metals had been estimated through a method of atom-absorptive spectroscopy.

Results and discussion

According to our results in the fluvial drifts of the city Poti significant increase of concentration of heavy metals: lead (Pb), copper (Cu), cobalt (Co), and zinc (Zn) are found. For example, in fluvial drifts of tangerine and orange plantations of the Poti branch of the Research-Industrial Union for Tea and Subtropical Crops lead, zinc and cobalt concentrations are increased 0-20; 20-40 cm. deep. Concentration factors are variable within 2-10: Zn K_c=2-6; Co K_c=3-3,5; Cu K_c=2-5. With respect to concentration factors toxic elements create the following descending row: 0-20 cm. deep Co>Pb>Zn>Cu, and 20-40 cm. deep Pb>Zn>Co>Cu. According to summation indices of concentration factors (Z_c=7) soil pollution in the upper layer is low, while 20-40 cm. deep it is middle-level (Z_c=20). However, it should be taken into consideration that summation index of concentration factors 20-40 cm. deep is mainly increased due to Pb concentration (K_c=10), which is on the first position. In comparison with local background indices the given concentration of Pb is included in the III group of pollution, i.e. middle-level pollution index is registered.

In fluvial drifts on the territory of the factory of hydro-mechanisms maximal value of lead concentration factor is Pb K_c=8; Co K_c=9; Zn K_c=5. In this case moveable form of cobalt reaches its critical limits, about 2,8 mg/kg, what is the indication for attribution to the VI group of pollution (very high pollution), summation index of its concentration factors, which is equal to 9, is also high. Concentration of nickel that 0-20 cm. deep makes up 8,1 mg/kg, is high as well, and in comparison with local background indices is attributed to the V group (high pollution). In the complete soil section (profile) cobalt is on the first position in the descending row of element concentration indices Co> Pb>Ni >Zn>Cu.

The complete soil section (profile) 0-20; 20-40 cm. deep on the territory of the factory of comets shows very high concentration of moveable cobalt, respectively 2,8 mg/kg and 2,6 mg/kg (in comparison with local background indices is attributed to the VI group of pollution – very high pollution). According to summation indices of concentration factors the 0-20 cm. deep soil layer is highly polluted, while the 20-40 cm. deep soil layer shows middle-level pollution. In this case elements create the following descending row Co> Pb>Ni >Zn>Cu.

Soils of the territory of the secondary school N 1 in Poti show middle-level pollution. Summation indices of concentration factors are within 12-14. Here in the soil profile lead is on the first position in the descending row of concentration indices Pb>Ni >Co> Zn>Cu.

Conclusions

So far heavy metal pollution more or the less has been found in soils of other units investigated by us. The main sources of heavy metal pollution in the city Poti are big industrial units, such as factory of comets, shipyard, factory of hydro-mechanisms, “Potielectroapparatus” plant. The city traffic, communal service units, and various small-scale industrial works also contribute to heavy metal pollution of the city, while mineral fertilizers and utilized pesticides play a certain role in pollution of suburban agricultural fields. It should be mentioned that formerly most of the above-mentioned factories were closed military units and great deal of information was kept in secrecy. Even today we face difficulties during the investigation of these territories.

Table 1. Moveable Forms of Heavy Metals (mg/kg) in Fluvial Drifts of the City Poti

Object	Depth cm.	Pb	Zn	Co	Ni	Cu
F_1 Branch of the Union	0-20	1.9	18	1	6.2	2.9
	20-40	1.7	15	0.8	6	2.6
F_2 Factory of Hydro-Mechanisms	1-20	3.8	25.1	2.8	8.1	3.7
	20-40	2.9	23.7	2.1	7.3	2.9
F_3 Factory of Hydro-Mechanisms, 150 m far from the main workshop	0-20	3.4	20	2.3	7	3.4
	20-40	2.9	18.9	2.1	6.8	2.9
F_4 Factory of Comets	0-20	3.5	24.1	2.8	6.1	3.5
	20-40	2.5	21.9	2.4	5.8	2.8
F_5 School N1	0-20	1.9	18.3	1.9	6.1	3.1
	20-40	1.7	17.6	1.8	5.9	2.9
F_6 Factory Potielectro-apparatus	0-20	3.3	26.1	2.5	7.9	4.5
	20-40	3.2	24.0	2.0	7.4	4.1
F_7 "Shavgele" (Feijoa plantation)	0-20	2.0	16.9	1.9	5.3	3.6
	20-40	2.0	16.5	1.8	4.6	2.6

Table 2. Local Background of Moveable Forms of Heavy Metals (mg/kg)

Element	Background Clark	Soil groups and pollution level indices (md/kg)					
		1	2	3	4	5	6
Zinc	7	7	14	21	28	35	42
Nickel	1.55	1.55	3.1	4.65	6.2	7.75	9.3
Copper	1.5	1.5					
Lead	0.9	0.9	1.8	2.7	3.6	4.5	5.4
Cobalt	0.4	0.4	0.8	1.2	1.6	2.0	2.4

Comment: 0 Group – Background, Clark
1 Group – Weak pollution
2 Group – Light pollution
3 Group – Middle-level pollution
4 Group – Increased pollution
5 Group – High-level pollution
6 Group – Very high-level pollution

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OPTIMIZATION OF NEEM-INGREDIENTS FOR INSECT PEST CONTROL: PHYSICO-CHEMICAL AND BIOLOGICAL PROPERTIES AND THEIR CONSEQUENCES

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Modern products for pest control have to be efficient, specific and risks for the users, consumers as well as for the environment have to be minimized by law. Due to these increasingly higher pretensions the R&D cost for safe pest control products reduces the number of new developments.

Azadirachtins, a group of limonoids of the tropical Neem tree (*Azadirachta indica* A. Juss), fulfil even highest safety demands due to their specific action on the hormonal system of insects. The technical active ingredient „NeemAzal“ which is extracted from the seed kernels of the Neem tree can be formulated to a storage stable EC called NeemAzal-T/S with additives of plant origin. Efficacy of applications of NeemAzal-T/S have been investigated for more than 110 pest insects, 75% of which can be controlled successfully.

Knowledge of the composition of Neem-extracts helps to identify and enrich the active substance(s). By the analytical control of formulations and residues an increase in storage stability may be achieved and the mode of action understood better. This serves the identification of different possibilities of application and their optimisation.

The choice of proper formulation additives may influence among others the uptake of Azadirachtins by plants and insects.

With NeemAzal-T/S free feeding as well as sucking insects and mites can be controlled due to the systemic transport. Since Azadirachtins degrade rapidly in the field special care has to be taken for the choice of the optimum time of application; usually this is in the early stages of the development of the infestation (young larvae, colony formation, appearance of fundatrices). Typical effects of the specific mode of action are: rapid feeding inhibition (some hours), reduction of moulting (after some days), fecundity reduction (after days, weeks). Thus assessments should be done with respect to the decrease in damage of the target crop rather than the mortality of the insects.

Azadirachtins degrade in aqueous systems with a half life of a few days. In addition to the very favourable toxicological properties the rapid degradation of the active ingredient assures the safety of the consumer due to lack of residues; the slow components of the mode of action guarantee a sufficient time of protection; thus the proper timing of the application is important for a high bioefficacy in the field. Still the protection of textiles for several months may be possible by the choice of proper formulations and mode of applications.

The high ecotoxicological safety of NeemAzal-T/S is due to the intrinsically ecofriendly (non-toxic to micro-organisms, aquatic organisms, beneficials, warm blooded animals etc.) properties as well as the rapid thermal, hydrolytic and microbiological degradation pathways.

WIRKSAMKEIT DES NEEM-PRODUKTES NEEMAZAL-T/S AUF SCHADINSEKTEN

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NeemAzal-T/S, ein Emulsionskonzentrat des Wirkstoffes NeemAzal™ aus den Kernen des tropischen Neem-Baumes ist seit 1996 in der Schweiz und seit 1998 in Deutschland zugelassen. Die sehr gute Wirksamkeit von NeemAzal-T/S gegen saugende und beissende Schadinsekten mit freier Lebensweise wurde in zahlreichen Versuchen bestätigt. Die Zulassung von NeemAzal-T/S in Deutschland erfolgte zunächst für den Zierpflanzenbau unter Glas gegen saugende Insekten, Weiße Fliegen, Minierfliegen und Spinnmilben und wird für Freilandanwendungen (wie für den Obst- und Kartoffelbau) im Laufe des Jahres 1999 erwartet.

Die Wirkung von NeemAzal wird praktisch ausschließlich durch die Substanzgruppe der Azadirachtine hervorgerufen. Diese Limonoide führen nach oraler Aufnahme des Wirkstoffes zu Störungen in der Produktion des Häutungshormons Ecdyson bzw. der Juvenilhormone; dies führt zu Phänomenen wie: Fraßstop, Inaktivität, Häutungshemmung, und Mortalität.

Die Inaktivität bzw. Fraßeinschränkung wurde im Freiland (Hummel, 1996) und Labor mit Kartoffelkäfern (*Leptinotatsa decemlineata,* Col., Chrysomelidae) und Kohleulen *(Mamestra brassicae,* Lep., Noctuidae) untersucht. In Tests mit Kartoffelkäferlarven (L III) wurden mit Neem-Azal-T/S (0,5%) behandelte Kartoffelblätter für 6 bis 26 Stunden lang angeboten. Schon wenigen Stunden nach dem Umsetzen der Larven auf unbehandelte Kartoffelblätter wurde zunehmende Inaktivität der Larven beobachtet, die sich in verringerter Blattmasseaufnahme und Gewichtszunahme wiederspiegelte. Während Larven von behandelten Blättern ihr Gewicht 2 bis 10 Tage nach dem Umsetzen nur etwa verdoppelten, wogen die Kontrolltiere bei der nach ca. 10 Tagen erfolgenden Verpuppung das 7-fache. Ähnliche Ergebnisse wurden im Versuch mit Kohleulenlarven erzielt. Das Gewicht der Larven (L II bis L IV), die 72 Stunden auf behandelten Blättern lebten, erhöhte sich innerhalb von 14 Tagen im Durchschnitt von 0,006 auf 0,03 g/Larve; die Kontrolltiere nahmen im gleichen Zeitraum von 0,005 auf 0,7 g/Larve zu; ab Tag 19. fand die Verpuppung statt.

Die Häutungshemmung kann sehr deutlich im Versuch mit Kartoffelkäfern (L III) beobachtet werden. Von jeweils 40 Larven konnte sich bei 20- bis 26-stündigem Fraß auf behandelten Blättern nur eine Larve und bei 6- bzw. 9-stündigem Fraß nur acht Larven einmal innerhalb von 10 Tagen häuten. Die 20 Kontrolltiere haben in dieser Zeit 17 Häutungshäute abgestreift.

Junge Larvenstadien reagieren besonders empfindlich auf die Aufnahme von NeemAzal-T/S, deshalb wird schon 2-3 Tage nach der Behandlung hohe Mortalität beobachtet. In einem Feldversuch mit Kartoffelkäfern (1998) waren 5 Testpflanzen vor der Applikation mit NeemAzal-T/S (2,5 l/ha) mit 99 Larven besiedelt (davon 12 im L I, 48 im L II und 39 im L III-Stadium). Nach 2 Tagen waren nur noch 28 Larven zu finden, davon 4 im L I und L II und 24 im L III und L IV Stadium. 7 Tage später waren auf den Pflanzen nur 4 Larven im L IV-Stadium vorhanden. Die Kontrollpopulation auf mit Wasser behandelten Pflanzen betrug sowohl vor als auch nach der Behandlung 132 Larven; die Larven haben sich normal entwickelt und gehäutet.

Die Aufnahme von NeemAzal durch Schadinsekten (besonders in späten Larvenstadien oder bei adulten Käfern) führt zusätzlich noch zu Fertilitätsreduktion.

Inaktivität, Fraßeinschränkung und Fertilitätreduktion wurde in Praxisversuchen mit Maikäfern (*Melolontha hippcastani,* Col., Scarabaeidae) nachgewiesen (Rohde, 1997). Im Freiland wurde NeemAzal-T/S (2,5 l/ha, 50 l Wasser, Luftapplikation) auf Fraßbäume von Käfern (bei Flugbeginn: Weibchen zu Männchen = 1:1) ausgebracht. Schon einige Tage nach der Behandlung ist die Wirkung deutlich: die Käfer werden träge, nehmen nur wenig Blattmasse auf. Nach 2 Wochen wurden sie im Labor weiter beobachtet, wobei die Eiablage der Weibchen und die Schlupfrate der Larven besonders kontrolliert wurde. Die Ergebnisse zeigen, dass die Weibchen nach NeemAzal-T/S-Behandlung viel weniger Nachkommen produzieren (3,9 Eier/Weibchen, 15% Schlupfrate) als in der unbehandelten Kontrolle (14,9 Eier/Weibchen; 53% Schlupfrate). Damit sind die Ergebnisse mit NeemAzal-T/S denjenigen von Rubitox und Decis überlegen.

Durch die in zahlreichen Versuchen nachgewiesenen systemischen Eigenschaften von NeemAzal-T/S können auch Schädlinge beeinflusst werden, die nicht direkt an behandelten Blättern oder Blatteilen fressen oder saugen.

Aufgrund der langsamen Wirkungsweise sind bei NeemAzal-T/S keine „Sofort-Effekte“ zu erwarten. In der Praxis ist eine endgültige Wirkungsbeurteilung erst 7-10 Tage nach der Behandlung sinnvoll. Dabei sollten die pflanzenschützenden Kriterien (Blattmasseverlust, Honigtaubildung, allgemeiner Zustand der Pflanze und der Schädlingspopulation, Ertrag, Nützlingsschonung usw.) im Vordergrund stehen und nicht die Anzahl toter Schädlinge. Der Behandlungserfolg ist vom Verlauf des Schädlingsbefalls abhängig. Bei zeitlich begrenztem Befall und synchroner Populationsentwicklung (Stammütter der Mehligen Apfelblattlaus *Dysaphis plantaginea,* erste geflügelte Holunderblattläuse *Aphis sambuci* (Hom., Aphididae), erste Junglarven von Kartoffelkäfern *L. decemlineata*, Flugbeginn von Maikäfern *Melolontha* sp.) reicht normalerweise eine Anwendung; bei wiederkehrendem oder kontinuierlichem Befall (z. B.: Thripse, Weisse Fliegen, Blattläuse, Spinnmilben) sind meist mehrere Applikationen erforderlich.

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EXPERIENCES WITH NEEMAZAL™-T/S IN 1994 - 2000

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*During the period 1994-2000 we have optimized the commercial formulation NeemAzal™-T/S with respect to its application by field and laboratory trials. The efficacy was tested against more 140 species of mites (*Acari*) and insects from* Coleoptera, Diptera, Heteroptera, Homoptera, Hymenoptera, Lepidoptera *and* Thysanoptera. *The results show, that NeemAzal-T/S is effective against a large variety of free feeding sucking and biting pests. In the phytotoxiticy experiments it was observed, that the formulation may be toxic to certain varieties of pear-trees and certain varieties of ornamentals in greenhouses. In order to obtain reliable results with respect to efficacy and phytotoxicity many tests have to be performed under various practical conditions.*

Key words: *NeemAzal-T/S effect, mites, insects.*

Introduction

In October 1998 we obtained the registration for the use of NeemAzal-T/S in Germany for the control of sucking insects, white flies, leaf miners and spider mites ornamentals in greenhouses and in 2000 for Rosy Apple Aphid, Colorado Potato Beetle, Winter Moth and other pest for field-application.

All results show that the application of NeemAzal-T/S is efficient with respect to the registered target pests and does not bear special risks to humans and the environment.

Due to these favourable properties work for the expansion of the registration for plant protection is in progress. Formulations which may efficiently be used for the control of human or animal ectoparasites have been developed and are currently tested.

From the various results the effects of the NeemAzal application can be summarized in the term **„Insectistatic“.** The main aspects of this mode of action are:

Phenomenon	Timing	Description	Assessment
Feeding inhibition	after hours	reduced food uptake	reduction of: plant damage, faeces, honey dew
Inactivity	after days to 1-2 weeks	over all reduction of fitness, molting inhibition, starvation	mortality
Fertility reduction	after weeks (next generation)	reduction of progeny	reduction of the next population

Results

Ind.	Species	Pest	Number of results:		
			positive	undeci-sive	negative
	Acari, Tetrapodili				
0	**Eriophyes rubi**	Blackberr. Gallmite	-	-	1
	Acari, Tetranychidae				
+++	**Oliconychus coffeae**	Red Spider Mite	10	-	-
+++	*Panonychus ulmi*	-	2	-	-
+++	*Tetranychus cinnabarinus*	Spider Mite	1	-	-
+++	*Tetranychus urticae*	Spider Mite	5	2	1
	Coleoptera				
0	*Raphidodopalpa foveicollis*	-	2	-	-
	Coleoptera, Chrysomelidae				
0	**Galerucella nymphaeae**	-	1	-	-
0	*Dicladispa armigera*	Rice Hispa	2	2	1
0	*Gastroidae viridula*	-	-	1	-
+++	*Leptinotarsa decemlineata*	Color. Pot. Beetle	20	2	-
+++	*Oulema melanopus*	-	1	-	-
+++	*Phaedon cochleariae*	-	1	-	-
0	*Phyllotreta sp.*	-	-	2	1
0	*Psylliodes od. Phyllotreta sp.*	-	-	2	-
	Coleoptera, Coccinellidae				
++	**Epilachna vagintioctopunctata**	Epilachna Beetle	1	-	-
++	*Henosepilachna vigintioctp. .*	Afr. Mel. Ladybird	3	1	1
	Coleoptera, Curculionidae				
0	*Anthonomus pomorum*	-	1	-	1
-	*Ceuthorrhynchus assimilis*	-	-	-	1
-	*Ceuthorrhynchus napi*	-	-	-	2
-	*Ceuthorrhynchus quadridens*	-	-	-	1

Ind.	Species	Pest	Number of results: positive	undeci-sive	negative
0	**Coenorhinus aeguatus**	-	-	1	-
+	*Hylobius abietis*	-	1	-	-
0	*Otiorhynchus sulcatus*	-	1	1	-
0	*Phyllobius sp.*	-	-	-	1.
	Coleoptera, Nitidulidae				
0	*Meligethes aeneus*	-	-	-	1
	Coleoptera, Scarabaeidae				
+++	*Melolontha hippocastani*	Cockchafer	2	-	-
+++	*Melolontha melolontha*	Cockchafer	4	-	-

Ind.	Species	Pest	Number of results:		
			positi ve	undeci - sive	negati ve
	Diptera				
+++	**Agromyzidae**	-	2		
0	**Sciaridae**	-	1	1	-
	Diptera, Agromyzidae				
++	**Liriomyza huidrobrensis**	-	2	-	-
++	*Liriomyza sp.*	Leaf Miner	-	1	-
++	*Liriomyza trifolii*	-	1	-	-
++	*Phytomyza sp.*	-	1	-	-
	Diptera, Anthomyiidae				
0	**Delia brassicae**	Cabbage Maggot	1	-	1
	Diptera, Cecidomyiidae				
0	**Contarinia tritici**	-	-	-	1
0	*Dasineura brassicae*	-	-	-	1
0	*Orseolia oryzae*	Rice Gall Midge	1	-	-
	Diptera, Muscidae				
0	**Musca domestica**	-	1	-	-
	Diptera, Trypetidae				
0	*Platyparea poeciloptera*	-	-	-	1
0	*Rhagoletis cerasi*	Cherry Fly	-	3	-
	Heteroptera, Pentatomidae				

Ind.	Species	Pest	Number of results: positive	undeci-sive	negative
0	*Antestiopsis orbitalis*	Coffee Bug	-	1	-
	Heteroptera, Miridae				
0	*Lygus pabulinus*	-	1	1	-
0	*Plesiocoris rugicollis*	-	1	1	-
	Homoptera				
++	*Coccidae*	Mealy Bug	2	-	-
	Homoptera, Adelgidae				
0	*Pineus pini/orientalis*	-	-	1	-
	Homoptera, Aleyrodidae				
+++	*Aleurocanthus spiniferus*	White Fly	1	-	-
+++	*Aleurothrixus floccosus*	White Fly	-	1	-
+++	*Bemisia tabaci*	Cotton White Fly	12	3	-
+++	*Dialeurodes kirkaldyi*	White Fly	1	-	-.
+++	*Trialeurodes vaporariorum*	White Fly	8	4	-
	Homoptera, Aphididae				
++	*Acyrthosiphon pisum*	Green Pea Aphid	3	-	-
++	*Aphis fabae*	-	7	-	-
+	*Aphis gossypii*	Cotton Aphid	9	5	2

Ind.	Species	Pest	Number of results:		
			positi ve	undeci - sive	negati ve
++	**Aphis nasturtii**	-	2	-	-
0	*Aphis pomi*	Green Apple Aphid	1	3	2
+++	*Aphis sambuci*	Elder Bush Aphid	5	-	1
+++	*Aulacorthum circumflexum*	-	2	-	-
+++	*Aulacorthum solani*	-	3	-	-
0	*Brachycaudus helichrysi*	-	1	1	-
+	*Brevicoryne brassicae*	Cabbage Aphid	4	8	1
+	*Cavariella aegopodii*	-	2	-	-
+	*Cryptomyzus ribis*	-	1	-	-
0	*Drepanosiphum platanoides*	-	1	-	-.
++	*Dysaphis devecta*	-	2	1	-
+++	**Dysaphis plantaginea**	Rosy Apple Aphid	32	-	-
++	*Dysaphis pyri*	-	1	-	-
++	*Dysaphis sp.*	Rosy Apple Aphids	2	1	-

0	*Hyalopterus pruni*	-	-	1	-
+++	*Macrosiphoniella sanborni*	Black Aphid	2	1	-
+++	*Macrosiphum euphorbiae*	-	5	-	1
+++	*Macrosiphum rosae*	Green Rose Aphid	5	-	1
0	*Macrosiphum rosaeformis*	Aphid	1	-	-
++	*Megoura viciae*	Vetch Aphid	2	-	-
0	**Metopolophium dirhodum**	Rosy Grain Aphid	1	-	-
0	*Myzus nicotianae*	Aphid	1	-	-
+++	*Myzus persicae*	-	5	-	-
0	*Nasonovia ribisnigri*	-	-	-	1
++	*Phorodon humuli*	Hop Aphid	4	-	1
0	*Rhopalosiphum insertum*	-	-	2	4
	Homoptera, Chaitophoridae				
0	*Chaitophorus capreae*	-	1	-	-
	Homoptera, Cicadoidae				
++	*Amrasca biguttula*	Leaf Hopper	9	2	2
++	*Empoasca flavescens (vitis)*	Grape Leaf Hopper	-	2	1
++	*Idiocerus niveosparsus*	Mango Hopper	1	-	-
++	*Nephotettix virescens*	Green Leaf Hopper	2	1	-
++	*Sogatella fucifera*	White Pl. Hopper	1	-	-
	Homoptera, Coccidae				
+	*Coccus hesperidium*	-	-	2	-
	Homoptera, Delphacidae				
++	*Nilaparvata lugens*	Brown Pl. Hopper	6	1	-

Ind.	Species	Pest	Number of results:		
			positive	undeci-sive	negative
	Homoptera, Diaspididae				
0	*Lepidosaphes ulmi*	-	-	1	-
	Homoptera, Lachnidae				
0	*Eulachnus agilis*	-	-	-	1
0	*Schizolachnus obscurus*	-	-	1	-

	Homoptera, Phylloxeridaea				
+	*Dactulosphaera vitifoliae*	-	2	-	-
	Homoptera, Pseudococcidae				
0	*Planococcus citri*	Citrus Mealy Bug	-	1	-
0	*Planococcus lilacinus*	Mealy Bug	-	2	-
0	*Pseudococcus longispinus*	Mealy Bug	-	1	-
0	*Pseudococcus sp.*	Mealy Bug	1	1	-
	Hymenoptera, Diprionidae				
0	*Diprion sp.*	-	1	-	-
	Hymenoptera, Tenthredinidae				
0	*Hoplocampa testudinea*	-	-	-	1
	Lepidoptera				
0	*Achaea janata*	Rose L. Caterpillar	1	-	-
0	*Ascotis selenaria*	Giant Looper	-	-	1
0	*Hellula sp.*	Cab. Head Borer	2	-	-
0	*Hymenia recurvalis*	Leaf Caterpillar	1	-	-
0	*Leucinodes orbonalis*	Shoot&Fruit Borer	2	1	4
	Lepidoptera, Arctiidae				
0	*Hyphantria cunea*	Fall Webworm	1	-	-
	Lepidoptera, Gelechiidae				
0	*Phthorimaea operculella*	Potato Tuber Moth	-	1	-
	Lepidoptera, Geometridae				
+++	*Operophthera brumata*	Winter Moth	12	-	-
	Lepidoptera, Gracillariidae				
0	*Phyllocnistis citrella*	-	-	1	-
	Lepidoptera, Lasiocampidae				
0	*Dendrolimus pini*	-	1	-	-
	Lepidoptera, Lymantriidae				
0	*Euproctis chrysorrhoea*	Brown Tail Moth	-	-	1
+++	*Lymantria dispar.*	Gypsy Moth	3	-	-
+++	*Lymantria monacha*	Nun Moth	1	-	-
	Lepidoptera,				

Ind.	Species	Pest	Number of results:		
			positive	undecisive	negative
	Lyonetiidae				
0	*Leucoptera malifoliella*	-	-	1	-
	Lepidoptera, Noctuidae				
++	*Earias vittella*	Fruit & Shoot Borer	1	-	-
++	*Heliothis armigera*	„Amer.“ Bullworm	12	4	-
++	*Mamestra brassicae*	Cab. Army Worm	6	-	-
++	*Mythimna albistigma*	Cutworm	1	-	-
++	*Spodoptera littoralis*	Eg. Cott. Leafworm	1	-	-
+	*Spodoptera litura*	Leaf Catapillar	4	5	-
	Lepidoptera, Pieridae				
+	*Eurema blanda*	-	1	-	-
+	*Pieris brassicae*	Cabb. Butterfly	1	-	1
+	*Pieris rapae*	Cabb. Butterfly	2	-	1
	Lepidoptera, Pyralidae				
0	*Cnaphalocrocis medinalis*	Rice Leaf Folder	3	5	-
0	*Scirpophaga incertulus*	Yellow Stem Borer	2	-	-
-	*Tryporiza incertulus*	Rice Stem Borer	1	6	5
	Lepidoptera, Tineidae				
++	*Tineola bisselliella*	Cloth Moth	3	-	-
	Lepidoptera, Thaumetopoeidae				
++	*Thaumetopoea processionea*	Brown Tail Moth	4	1	-
	Lepidoptera, Tortricidae				
+	*Adoxophyes orana*	Sum. Tortrix Moth	3	2	1
0	**Cydia leucostoma**	Tea Flushworm	1	-	-
-	*Cydia pomonella*	Codling Moth	-	-	2
0	*Eupoecilia ambiguella*	Grape Berry Moth	-	1	-
0	*Lobesia botrana*	Europ. Grape Moth	-	1	3
0	*Pandemis heperana*	Sommer Fruit Moth	1	-	-
0	*Tortrix viridana*	Green Oak Moth	-	1	-

	Lepidoptera, Yponomeutidae				
++	*Plutella xylostella*	DBM	10	5	-
+++	*Yponomeuta malinellus*		3	-	-
+++	*Yponomeuta padella*		3	-	-
	Thysanoptera, Thripidae				
+++	*Chloethrips oryzae*	Thrips	-	1	-
+++	*Frankliniella occidentalis*	Thrips	8	2	1
+++	*Parthinothrips dracaenae*	Thrips	-	1	-
+++	*Scirtothrips sp.*	Tea Thrips	1	-	-
+++	**Thrips tabaci**	Thrips	1	-	-

Ind. : **+++**: efficient control established; **++**: efficient control possible; **+**: control may be pos-sible after optimisation of applicationmethod; **0**: undecided, further tests necessary;efficient control impossible

Conclusions

Efficacies depend on: i). the time of application. ii). the concentration, and iii). the sensitivity of the target insect with respect to the different effects of the mode of action. Hence the optimisation of the application method of NeemAzal-T/S is decisive for the efficacy and the certainty of the success of the application in practice.

Taking into account the short half life of the active ingredient it seems useful to define operationally two groups of infestation:

1. batchwise appearance of the pest: with a good timing one application is sufficient for the control of one generation of the pest (like for: colorado potato beetle, 1. to 2. larval instar; cockshafer, ratio male : female adults ~ 1 : 1; rosy apple aphid, appearance of fundatrices),

2. persistent infestation: at the presence of young developmental stages the application should be repeated at intervals of 1 - 3 weeks (like: thrips, white fly, or green rose aphid).

The results of practical applications show that application methods can be worked out which permit an efficient control of many different pests. These efforts seem to be worth while in order to find new solutions of pest control for biological as well as for integrated farming with a toxicologically and ecotoxicologically safe product like NeemAzal-T/S.

NEEMAZAL-T/S IM KARTOFFELANBAU

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Die Alternative zur chemisch-synthetischen Insektiziden im ökologischen Kartoffelbau gegen Kartoffelkäfer *Leptinotarsa decemlineata* wurde seit langem gesucht und zuerst in den *Bacillus*-Bakterien gefunden. Die daraus formulierten umweltfreundlichen Präparate haben sehr schnell ihren Platz als Stamminsektizide eingenommen.

In 2000 wurde erstes Mal ein neues Präparat - Pflanzenextrakt aus indischem Neem-Baum - NeemAzal-T/S (1% Azadirachtin) von der Biologischen Bundesanstalt in Deutschland gegen Kartoffelkäfer in Ackerbau und gegen andere Indikationen im Zierpflanzen- und Obstbau als Pflanzenschutzmittel zugelassen.

Diese standardisierte Formulierung wurde seit 1994 im Kartoffelbau intensiv getestet. Das Präparat ist als schonend für die meisten Nützlinge und nicht bienengefährlich eingestuft; es kann ohne Wartezeit und Wasserschutzauflagen angewendet werden und ist für Vögel wie auch für Säugetiere harmlos. Toxikologische Studien zeigen, dass bei sachgerechter Anwendung von NeemAzal-T/S keine Risiken für Anwender, Verbraucher und die Umwelt zu erwarten sind; diese Ergebnisse stehen teilweise im Gegensatz zu denjenigen von Roh- bzw. nicht standardisierten einfachen Neem-Extrakten.

NeemAzal-T/S gehört zur Gruppe der entwicklungshemmenden Mitteln und zeigt deshalb keine sofortige toxische, sondern eine langsam einsetzende Wirkung. Der Wirkstoff Azadirachtin dringt in die Pflanze ein und wird von Schädlingen mit der Blattmasse aufgenommen. Schon einige Stunden nach Aufnahme ausreichender Wirkstoffmengen stellen die Kartoffelkäferlarven ihre pflanzenschädigende Tätigkeit ein. Wenige Tage später könnte einige noch auf den Blättern mit dem Hinterleib in ihren Ausscheidungen klebend zu finden sein, die sich in diesem Zustand als Beute für Entomophagen anbietet.

Im allgemein sind bei Kartoffelkäfer die folgenden Reaktionen zu beobachten: <u>Inaktivität, Fraßstop</u> - die Larven und Käfer stellen schon einige Stunden nach Aufnahme behandelter Blattmasse ihre Fraßaktivität ein und werden inaktiv; <u>Häutungshemmung</u> - bei Larven blockiert der Wirkstoff die Häutung und Entwicklung; <u>Mortalität</u> - innerhalb von 1-2 Tage verenden junge Larven, die Käfer leben länger; <u>Fertilitätsreduktion</u> - insbesondere wenn Weibchen während des Reifungsfrasses behandelte Pflanzenmasse aufnehmen, wird die Ovarienfunktion so stark beeinflußt, dass bei der Eiablage überwiegend sterile oder nicht entwicklungsfähige Eier produziert werden.

Detaillierte Versuche mit Kartoffelkäfern im Freiland zeigen einen typischen Verlauf der Wirkung von NeemAzal-T/S auf die Schädlingspopulation. Behandelt man die Pflanzen 5-7 Tage nach der Masseneiablage mit 0,5%-iger Spritzbrühe (bei Infektionsherd-Applikation) oder bringt man 2,5 L des Präparates in 400-500 L Wasser/ha aus (bei flächendeckender Applikation), bleibt die Entwicklung der Schädlinge weitgehend im ersten Larvenstadium stehen. Innerhalb von drei Wochen nach der Anwendung reduziert sich die Zahl der gefräßigen Larven auf ein Minimum, eine Verpuppung bleit aus. Trotz der nur ein bis maximal zwei Wochen andauernden Anwesenheit des Wirkstoffes ist eine Verschiebung des Verhältnisses junge zu alten Larven in der Population noch 3-4 Wochen nach der Anwendung deutlich nachweisbar. So sind auf behandelten Pflanzen überwiegend junge (LI-LII) Larven zu finden, die aus frisch abgelegten Eiern geschlüpft sind, aber sich während ihres gesamten Lebens kaum weiter entwickeln können; die Kontrollpopulation wird in diesem Zeitraum durch ältere (LIII - LIV) Larven vertreten.

Auch die versuchsweise ausgebrachte 0,5%-tiger (300 L Wasser/ha) Spritzbrühe von NeemAzal-T/S bzw. die Kombination dieser Menge mit einem *Bt*-Präparat (wie z.B. auf 50% reduzierte Dosis per ha von Novodor) sollte die vergleichbare zu empfohlener Norm der einzelnen Präparaten Wirkung liefern – dies könnte zur allgemeinen Kostenreduktion der Bekämpfung des Kartoffelkäfers beitragen. Hierbei ist jedoch auf eine genaue Terminierung zu achten und innerhalb der ersten acht Stunden nach der Anwendung sollte kein Regen fallen.

Normalerweise ist eine Behandlung ausreichend. Im Falle des massiven Zuflugs der Adulten aus Nachbarsschlägen sollte NeemAzal-T/S 10-12 Tage später neu ausgebracht.

NeemAzal-T/S hat eine effektive Wirkungszeit auf Kartoffelkäfer bis zu 10 Tage nach der Applikation - danach baut sich das Präparat biologisch ab (Halbwertzeit ca. 1 Tag), ohne Rückstände vom Wirkstoff und Formulierung in der Pflanze oder Erntegut zu hinterlassen. Analytisch läßt sich 10 Tage nach der Behandlung sogar bei 10 bis 40-fach erhöhter Aufwandmenge des Präparates kein Wirkstoff mehr auf den Blätter nachweisen und zu keinem Zeitpunkt in den Kartoffelknollen zu finden ist (Bestimmungsgrenze 10 µg/kg; Wiederfindung ca. 80%).. Es kann gefolgert werden, dass der Wirkstoff sehr schnell) in der Blattmasse abgebaut wird,

NeemAzal-T/S ist unter Raumbedingungen (kühl, trocken, dunkel) 2 Jahre nach der Herstellung ohne Verlust an der biologischen Aktivität lagerfähig; die Spritzbrühe soll am Tag der Zubereitung ausgebracht werden.

Da NeemAzal-T/S gegen Kartoffelblattläuse unter Glas gute die Wirkung zeigt, ist es wünschenswert das Präparat in Kartoffelbau als Vektor-Bekämpfung zu untersuchen.

NEUROMUSCULAR AND RESPIRATORY RESPONSES OF COLORADO POTATO BEETLE *LEPTINOTARSA DECEMLINEATA* (SAY) ADULTS ON THE TREATMENTS WITH NEEM PREPARATION

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The neem preparations were commonly used in pest control because of their antefeedant properties, and as Insect Growth Regulators possessing morphogenetic activity. Relatively high doses, considered as nonprofitables, are needed for direct toxic action. Nevertheless, the direct toxic actions of neem on the neuromuscular systems of insects are both of theoretical and practical interest.

Commonly the neuromuscular reactions of insects on toxicants were studied on their semi-isolated nerves and muscles. However, this electrophysiological invasive method was often criticized as it always causes a heavy and lasting stress state in insects.

In the present work the direct neurotoxic effects of NeemAzal T/S (neem) were studied in intact diapausing adults of Colorado potato beetle, Leptinotarsa decemlineata *(Say) by means of full uninvasive methods. We recorded the muscular activity in adult Colorado beetles by an automatic respirometer-actograph and infra-red optical actographic device. In the optical method the beetles were irradiated with infra-red light, falling on the infra-red sensitive diodes. As the indicators of the direct toxic action of neem on this beetle there were used the freaquency and amplitudes of their neuromuscular responses, which were clearly dose-dependent. Low nonlethal doses evoked irregular and externally invisible contractions of abdominal and thoracic muscles lasting from one-two days. The regular ventilating movements in neem-treated adults were lost while the irregular spontaneous muscular conteractions had a strong ventilating effect on tracheae. The neem did not cause the maximum effect − the flaccid paralysis of somatic muscles.*

The beetles usually recovered from the mild poisoning during two-three days after treatments, but they showed significantly higher standard metabolic rates (SMR) than control beetles. The increased SMR was not due to the muscular spontaneous activity, instead there was suggested the direct action on the cytochrom system of respiratory chain.

Key words: *Colorado potato beetle, neem, toxic action, muscular responses.*

Introduction

Insects treated with neem extracts die by delayed action. After the neem-treatments the insects may be living for some weeks. Sometimes the final results of neem are seen after 4-5 weeks. Eventually, new generations fail to emerge, and the population perishes. Thus the use of neem extracts does not give immediate effects like synthetic insecticides. Some patience is required after the application of neem products (Neem, 1992).

NeemAzal T/S is a important natural means for the biological control of different pest insects and its efficacy has been compared to the conventional synthetic insecticides.

However, the relatively high price of botanical insecticides has a great influence on the acceptance of neem products in many countries.

The mode of action of neem depends on its consentrations or doses. The growth inhibiting activity commonly occured when 1-10 ppm was used. Neem possesses ability to function at hormonal concentrations and produce ecdysone-type effects in susceptible insects. The strong antifeedant activity is noted in the 10-100 ppm range. The quick kill due to the direct toxicity usually requires concentrations exceeding 1000 ppm (Addor, 1995).

The direct toxicity is a notion or term not yet clearly defined. Direct toxic action commonly means the several modes of action on neuromuscular system -- knockdown effect due to axonic poisoning (action of pyrethrins and pyrethroids), or acetylcholinesterase inhibition (organophosphatases and some plants). But acute toxicosis may also be avoked by metabolic poisons (rotenoids), muscle poisons (ryanodine), and the inhibitors of chitin synthesis. It seems likely that immediate or relatively fast killing effects have often been considered an acute poisoning or toxicosis.

The clear alternative to the direct toxic effects are the antifeedant action and hormonal effects, i.e. the growth regulating or rather growth inhibiting effects involving the neuroendocrine system of insects (about the mechanisms see Addor, 1994; Mordue, 1998). It has been noted that the presence of azadirachtin alone is not as toxic as that of all Neem compounds together.

There have been published enough data indicating the direct contact action of neem on some insects. In our tests the bean aphids (*Aphis fabae*) were very susceptible to the contact action of Neem Azal T/S if used in a concentration of 50 ppm, i.e. diluted with water 200 times. On the second day no living aphids were found (Hiiesaar et al., 2000). The early instars of large white butterfly (*Pieris brassicae*) were also very susceptible to contact action of Neem Azal T/S if treated with 50 ppm (Metspalu et al., 1999). The same preparation killed by direct toxic action the first instars larvae of Colorado potato beetles if 10 ppm was used, i.e. the Neem Azal TS was deluted with water 1000 times (Hiiesaar et al., 2001). The same concentration killed on certain plants the larvae of *Aphis gossypii* and *Thrips tabaci* within one day by 10 ppm, which was suggested to be the result of direct poisoning (Hiiesaar et al., 1998).

By some studies a stronger or weaker contact toxic effect of NemAzal T/S on twospotted spider mite (*Tetranychus urticae*) was indicated (Mironova and Khorkhordin, 1997; Schauer and Schmutterer, 1980; Ballhalder and Zuber, 1997).

To morphogenetic activity and direct toxic action of neem may be enhanced by using the mixtures of neem and synergists (Lange, 1984; Lange and Schmutterer, 1982). Besides, the direct toxic action was enhanced by combining the extracts from different plants (Assabgui et al., 1997; Kuusik et al., 1999).

In contrast to present knowledge about hormonal effects in insects caused by neem products and the impact of neem treatment on insect population there is rather little information available about whether nervous cells on treated animals undergo any modifications in their activiy pattern or there occur neuronal effects (Kämper, 1997; Simmonds and Blaney, 1984).

The observation of the symptoms of poisoning is the first step in the study of the mechanism of action of an insecticide. For example, if ataxia, hyperactivity or convulsions are observed in the insects poisoned with the insecticide, one can suspect neuromuscular actions of the insecticide. On the other hand, if only paralysis occurs, the major action could either be neuromuscular blockage or metabolic inhibition (Narahashi, 1971).

The aim of the present work was to investigate the neuromuscular and respiratory responses of nonlethal doses of neem preparation in the adults of Colorado potato beetle by means of a respirometer-actograph combined with an infra-red optical actograph allowing to observe the discrete muscular contractions and SMR of intact individuals. We studied the first steps of the direct poisoning by NeemAzal T/S with topical treatments.

Insects and methods

The adults of Colorado potato beetles were collected in summer 1999 from the potato fields near Tartu. They were fed in a laboratory until they entered diapause. The diapausing beetles were held at 5…6 ^{o}C in a refrigerator.

We recorded the muscular activity patterns by means of a respirometer-actograph and by an infra-red optical method (Kuusik et al., 2001; Metspalu et al., 2001). A beetle was placed in an insect chamber of respirometer and then irradiated by infra-red emittying diodes in the ventral side whereas the signal was received by infra-red-sensitive diodes (sensors) in the dorsal side. The insect chamber was joined either to a flow-through respirometer (infra-red gas analyzer) or an electrolytic respirometer-actograph. The described respirometric actographic system allowed to record easily the real SMRs when the insect was fully restrained and motionless.

The measuring device was inserted into a selene microrefrigerator-heater (inner volume 50 cm^3) where temperatures were controlled to ±0.2 °C. All respirometric and actographic measurements were provided at 10 °C.

The beetles were treated by dipping them into water dilution of neem preparation (10 ppm) using different times expositions. The control insects were submerged into destilled water for the same time period.

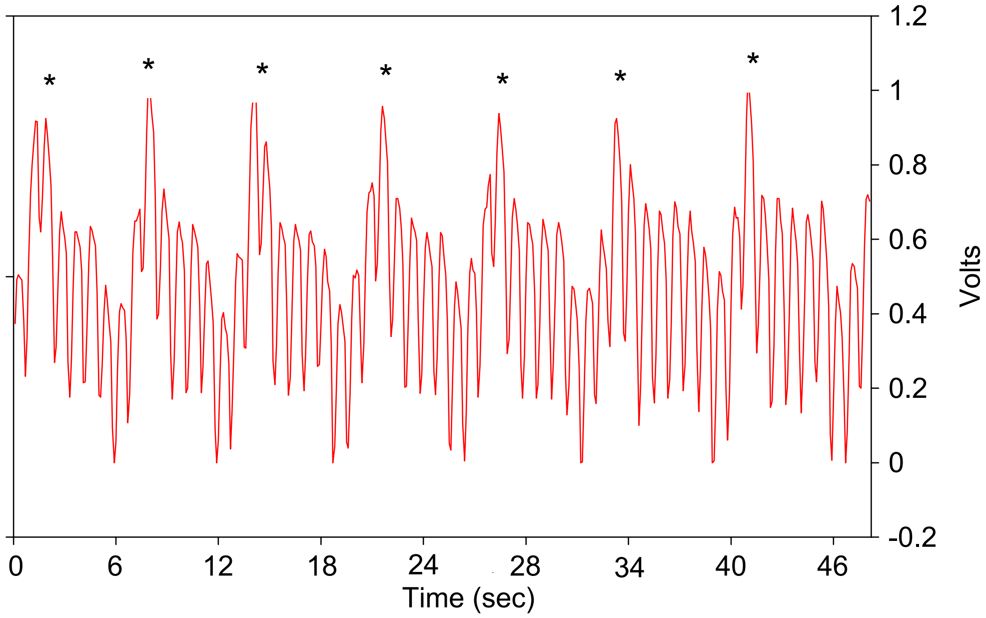

Figure 1. The clear rhythms of muscular ventilating in diapausing adult of Colorado potato beetle recorded by infra-red optical device. Note that the signals due to body ventilating movements (asterisks) are superimposed on the regular spikes caused by heartbeats

Results

The Colorado potato beetles were very active at room temperatures, and to suppress their moving activity the measurements were provided at 10 °C. This temperature was the threshold between moving activity and cold stipor of the beetles, however at the same temperature the beetles displayed clear contractions of abdominal and thoracic muscles due to tracheal ventilation or due to mild poisoning.

The beetles responded to the treatments immediately, showing spontaneous contractions of somatic muscles due to the hyperactivity of the neuromuscular system (Fig.1). It was the summary effect of handling stress and the toxicant. In the recording patterns there were not differences between stresses caused by handling and neem-treatments. The control beetles recovered from the handling stress during 15-20 minutes.

Normally the recording peaks of regular ventilating movements were superimposed on the sharper peaks due to heart pulsations measured from the third and fourth abdominal segments (Fig. 1). The freaquencies of heartbeats and ventilating movements were 0.96±0.07 Hz and 0.18±0.02 Hz respectively. Thus the recording signals from heart and tracheal ventilating were always distinguishable.

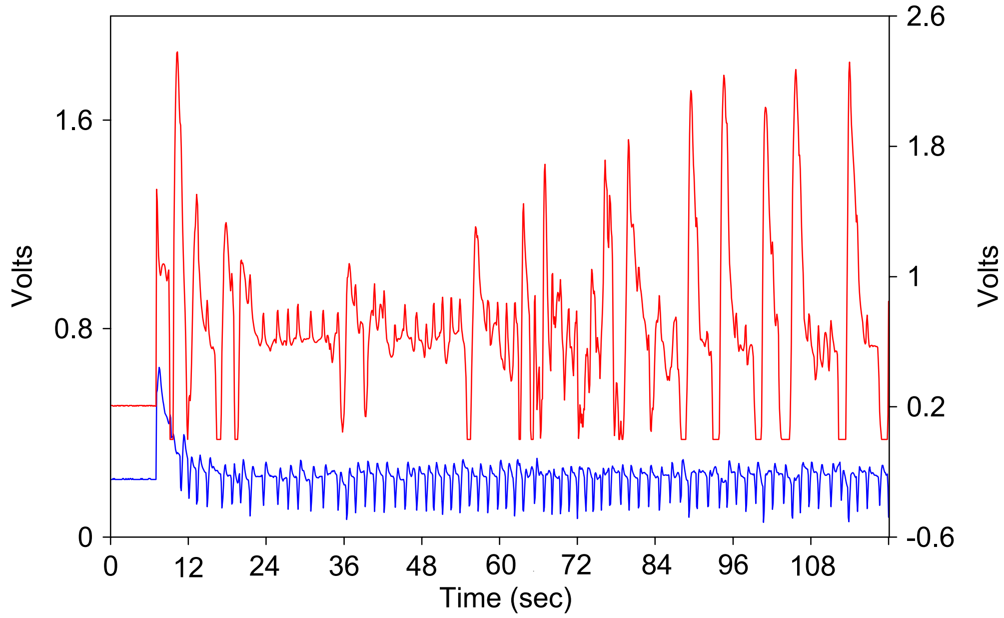

Figure 2. A typical pattern of muscular hyperactivity (upper trace) caused by treatments with neem preparation in an adult of Colorado potato beetle. Note that heart pulsations are not affected by neem (lower trace). An infra-red optical recording from abdominal 3rd segment (upper) and metathoracic segment (lower) simultaneously

The beetles submerged into neem preparation of 10 ppm (diluted with water 1000 times) for 20 seconds revealed muscular hyperactivity after treatments, and these extraordinary contractions lasted for one-two days. There were characteristic the irregular periods of muscular hyperactivity alternating with periods of stillness, which may be considered as ataxia.

Due to the muscular activity the metabolic rate increased about two times if compared with the SMR of control individuals . The muscular hyperactivity patterns were the peaks of several heights and intervals on the respirometric and infra-red optical recordings due to the discrete muscular contractions (Fig. 2). After recovering from the mild poisoning, the breathing of beetles occurred by regular muscular ventilation due to abdominal pumping. Sometimes the active ventilating movements ended and now the fully restrained beetles showed the continuous gas exchange through fully opened spiracles.

After recovering from the poisoning, when spontaneous muscular contractions were lost, the treated individuals beetles revealed significantly higher SMR than the control ones, 343±35 ml O_2 h^{-1} g^{-1} and 185±23 ml O_2 h^{-1} g^{-1} respectively. The levels of SMR did not depend on the periods of ventilating movements.

In the treated beatles the neem concentration used did not influence heartbeat rates and the regularity of heart pulses even incase of the longest exposition time lasting 30 seconds. (Fig. 2).

Discussion

There are two types of physiological effects of azadirachtin: firstly, indirect or delayed effects are modulated via the insect endocrine system, and secondly, direct effects occur where azadirachtin is acting on specific tissues.

By some earlier data the hormonally active substances commonly avoke beside the morphgenetic action, yet the direct toxic influence, even if in low doses were used.. The muscular hyperactivity and the raised metabolic rate were recognized as the symptoms of this light toxicosis of the analogues of the juvenile hormones (Kuusik and Kogerman, 1978).

The symptoms of mild poisoning by neem (10 ppm) in adult Colorado potato beetle were not externally visible. However the actographic recordings showed irregular contractions of abdominal somatic muscles of neem-treated beetles and this patterns persisted during some days. After recovering from the poisoning the beetles displayed a clear rhythm of muscular ventilating.

The neuromuscular responses of insects on toxicants have commonly been studied by a very invasive electrophysiological method, on the semi-isoleted nerves and muscle fibers (see Narahashi, 1971). In this case the heavy handling stress may be summarized to the neuromuscular stress evoked by toxicant. Kammer (1989) showed that metabolic processes and its rhythmic events must be studied in the whole intact insects and not in single semi-isolated nerves and muscular fibers. The patterns of responses on toxicants obtained from the semi-isolated nerves may be quite different from signals that are recorded from whole and intact insects. Therefore, it is necessary to study the responses in the whole organism with its interacting components. It is reasonable to employ methods suitable for registration the discrete muscular contractions in the whole insect by means of the respirometer-actographs allowing to check up the body movements together with the determination of standard metabolic rates (SMR).

In the first stage of poisoning the metabolic rate increased due to the muscular hyperactivity in neem-treated beetles. In low concentrations insecticides act on sensory nerves, inducing supernumerary impulses, which excite motor nerve via reflex arc, while motor nerves in turn induce muscular hyperactivity (see Keister and Buck, 1974; O´Brien, 1978). The toxic substances usually cause such indirect effects on insect respiration.

An interesting finding was the increase of the SMR in beetles after recovering from the poisoning, i.e. after passing the spontaneous muscular hyperactivity. By some earlier data the synthetic analogues of juvenile hormone also caused the increased metabolic rates in the pupae of large white butterfly *Pieris brassicae,* although without associating with any muscular activity, and therefore it was suggested to be a direct stimulatory effect on the respiratory enzymes (Kuusik et al., 1983). We suppose the neem acts in low concentrations also directly on the respiratory metabolism, by inducing the respiratory enzymes.

We conclude from our results that the visual observations could not be used for establishing the symptoms of nonlethal toxicosis in Colorado potato beetles, and obviously in other coleopterous species either. The symptoms of mild poisoning may be discovered and studied by special methods allowing to record even the lightest movements of tissues inside the body of an intact insect.

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RESPIRATORY FAILURES IN ADULT COLORADO POTATO BEETLES EVOKED BY NEEM PREPARATION

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The influence of neem preparation (NeemAzal TS) on the brething mode in the adults of Colorado potato beetle, Leptinotarsa decemlineata *(Say), was studied by means of a respirometer-actograph and an infra-red gas analyzer. Both respirometry were combined with an infra-red optical system allowing to record external gas exchange cycles, standard metabolic rate (SMR), and respiratory movements simultaneously. The beetles treated with nonlethal doses of neem lost their ordinary and regular discontinuous gas exchange cycles (DGCs) − the release of carbon dioxide by discrete bursts and abrupt air intakes into tracheae. The DGCs in treated beetles did not restore after the recovering of the beetles from the mild poisoning. Nevertheless, at low temperatures between 5 and 8 °C the treated beetles displayed the typical irregular microcycles of gas exchange. Authors suggest that the respiratory failures were not due to the paralyzing of the spiracles by neem, but affected were rather the neuromuscular, humoral and neuro-hormonal centers involved in the regulation of the external cyclic gas exchange.*

Key words*: neem, respiration, cyclic gas exchange, Colorado potato beetle.*

Introduction

At present time it has been well documented that neem preparations reveal several distinct modes of action: feeding deterrence, Insect Growth Regulation, oviposition deterrence, repellency, adult sterilization, the disruption of mating behaviour, the inhibition of chitin biosynthesis, and, usually mentioned in the last place, the direct toxicity or poisoning of larvae and adults due to contact immediate action on some insect species. The delayed action on insects is typical of neem.

By our knowledge the effects evoked by neem on the respiratory system of insects have not yet been studied. Insect respiration is an established index of stress imposed by insecticides. There exists an enormous literature concerning the effects of various chemicals compounds on oxygen consumption rates of insects. The respiration may be inhibited or, in other cases accelerated (Kuusik et al., 1983). Commonly the toxicants cause in insects indirect action on respiration via the exitation of the neuromuscular system resulting in enhanced respiration rates. In rare cases the toxicants induce the cytochrome system causing direct stimulatory effects on respiration.

Together with the the direct and indirect influences on oxygen consumption rated the toxic substances may also cause respiratory failures in insects abolishing the discontinuous gas exchange cycles or DGCs. The DGCs mean that insects consume oxygen and release carbon dioxide not continuously but by characterictic cycles − by abrupt intakes of oxygen followed by carbon dioxide release as discrete burst. The cyclic gas exchange has been regarded as the main water conserving mechanism in many insects (Kestler, 1980, 1985; Miller, 1974; Lighton, 1994; for reviews). The DGCs and gas exchange microcycles are the characteristic breathing mode in quiescent adults of Colorado potato beetle (Kuusik, 1976; Kuusik, Kogerman, 1983; Metspalu et al., 2001).

By some earlier data certain synthetic Insect Growth Regulators, the analogues of juvenile hormones of juvenoids, cause irreversible changes of external gas exchange: the insects treated with nontoxic and even very weak doses of juvenoids lost their normal cyclic gas exchange resulting in increased transpiration rate (Kuusik, Kogerman, 1978; Kuusik et al., 1980). In these insects no other symptoms of direct toxicosis were found. The typical DGCs were also lost in some insect species treated with extracts from certain concentrations resulted in fully abolishment of cyclic gas exchange or the so called discontinuous gas exchange cycles. During the first step of poisoning the loss of DGC was due to ventilating effect of muscular irregular contractions. Later, after the passing of the muscular hyperactivity the breathing of ther beetles occurred by regular muscular ventilating due to abdominal regular pumping movements. After some hours the active ventilating movements ended and now the fully restrained beetles showed the continuous gas exchange through the continuously opened spiracles. The normal DGCs were entirely lost and did not restore at all.

It was remarkable that at low temperatures, between 5 and 8 °C, when the moving activity of beetles was suppressed, the treated beetles displayed the typical irregular microcycles of carbon dioxide release and oxygen uptakes (Fig. 2). Thus the neem application affected only the regular and larger cyclic emission of carbon dioxide, while the microbursts of this gas were preserved at low standard metabolic rates.

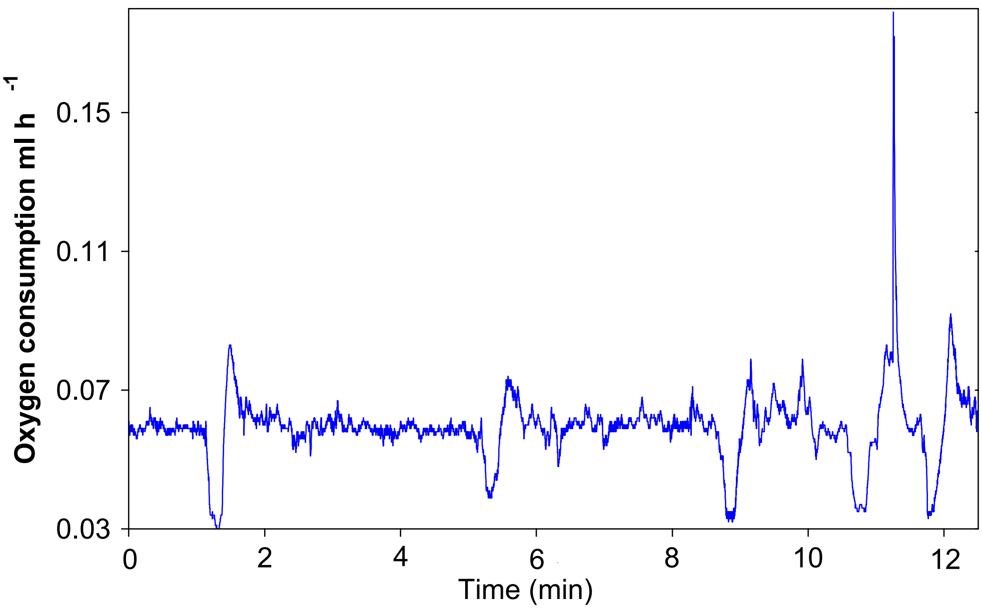

Figure 2. The gas exchange microcycles of an adult Colorado potato beetle registered by electrolytic respirometer-actograph

The DGCs in Colorado potato beetles are coupled with the opening-closing rhythms of the subelytral cavity − an airfilled space between elytra and abdominal tergites (Fig. 3). The abdominal spiracles open into the humid sublytral cavity, and thus this cavity helps lower the respiratory transpiration (our unpulished data). Normally the DGCs are synchronized with the opening-closing rhythms of subelytral cavity. In the beetles treated with neem the subelytral cavity was continuously left semi-open while no regular closing rhythm was to be seen.

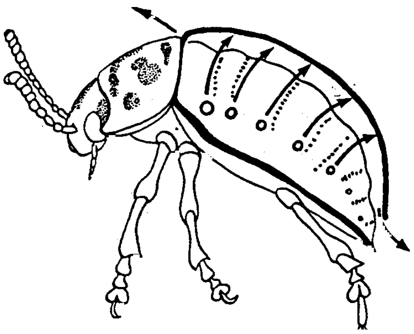

Figure 3. A scheme of the subelytral cavity of adult Colorado potato beetle

Discussion

The discontinuous gas exchange cycles are functioning in insects as a water-conserving mechanism (Kestler, 1980). This was the most probable reason why the neem-treated beetles rapidly lost their body mass. It was evident from these experiments that one of the immediate and externally invisible hide effects of the neem were the failures in the gas exchange resulting in increase transpiration and desiccation of insects. The abolishing of the cyclic gas exchange may be regarded as the earliest symptom of poisoning by toxicant in insects (see Kestler, 1991).

It may be suggested that disturbances of DGCs are due to the paralyzed opening-closing mechanisms of the spiracles. However, there also exist other factors abolishing the normal DGCs. Desiccation and very intensive handling stress lead to the same pathological carbon dioxide release pattern as the insecticide (see Kestler, 1991; Kuusik et al., 1999; Harak et al., 1998). By our results the closing-opening mechanism of the spiracles was most susceptible target to the neem in insect body. By our results the the gas exchange microcycles were preserved in neem-treated beetles indicating that the opening-closing mechanism of spiracles were not paralyzed, and thus other affecting factors were involved.

The mechanism by which insects lose their DGCs may either be autointoxication or/and release of neurohormones, or by an influence on the O_2/CO_2 thresholds for the control or on the CO_2 capacitance due to changes in the acid-base status (Kestler, 1991). Thus we conclude that the loss of the DGCs was not the result of a toxicosis evoked by neem (using here the term toxicosis in a strict sense). By our results the mechanism of spiracular regulation is the most susceptible target of the nontoxic doses of neem.. It seems most likely that the primary cause of the respiratory failures described in present work is the indirect effect of neem modulated via the insect endocrine, neurohormonal and neuromuscular centersd.

There are well documented the evidence supporting azadirachtin`s ability to to interference with neuroendocrine control of metamorphosis in susceptible insects (reviewed by Addor, 1994; Mordue, 1998). The neem classified as Insect Growth

Regulator really acts as a Insect Growth Inhibitor. Azadirachtin inhibits prothorcicotropic hormone (PTTH) in corpora cardiaca reducing or delaying ecdysone release necessary to trigger moulting.

The treatments of the last instar larvae and early pupae of Colorado potato beetles with nonlethal neem preparation doses always results in the defective metamorphosis − the emerged adults possess morphological failures on elytra. Besides, these adults display an unclear and irregular gas exchange pattern. By Pelletier (1994) the adult Colorado potato beetles with elytra deformation show high mortality rate after exposure to dry conditions during pupal stage. The beetles with deformed elytra as a rule show a high mortality rate during hibernation (our unpublishated data) evidently due to the abolishment of the water conserving mechanisms − cyclic gas exchange associated with the functions of subelytral cavity (see Cloudsley-Thompson, 1964).

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POSSIBILITIES TO CATCH OUT *LASPEYRESIA POMONELLA* AND *ARGYRESTHIS CONJUGELLA* WITH PHEROMONE TRAPS

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The shareholder company `Flora` produces rather reliable pheromone preparations for Laspeyresia pomonella *and* Argyresthis conjugella *. Taking into account, that the preparations synthesized in `Flora`are meant to be used in small gardens and the flights* Laspeyresia pomonella *and* Argyresthis conjugella *are known to peak simultaneously, an idea has emerged that pheromone dispensers for both of these species could be used in the same trap at the same time. This study was carried out to test idea in natural conditions.*

Our results showed that the preparations CP and CA-91 were most effective. In addition, we found that using two different dispensers in the same trap was also effective. However, using two different preparations simultaneously in same dispensers did not give the desired effect.

Key words: *pheromones, pheromone traps, flight dynamics,* Laspeyresia pomonella, Agryresthis conjugella.

Introduction

An essential role in the integrated pest control is played by the pheromone traps, which are the convenient tools for monitoring the pest-insect flight time and the intensity of flight. These obtained data allow determining the necessity and extent of the chemical control. The pheromones may be used in pest control by way of mass trapping and by mating disruption. In the last case such excessive pheromone concentrations are created that males become disoriented, and cannot find the scent traces lending to the female.

It was known already earlier that pheromones have an effect upon the interspecific sexual behaviour, but the serious investigations in this field have began in 1930 – 1940s (Skirkjavitsius, 1986).

In Estonia the investigations of pheromones were initiated in 1970s at the department of Organic Chemistry in the University of Tartu. Since then several specific pheromone preparations for lepidopterous pest, including the pheromones for capturing the males of codling moth *Laspeyresia pomonella* and apple fruit moth *Argyresthis conjugella.,* have been released on the market

The shareholder company ´Flora´ produces rather reliable pheromone preparations for *Laspeyresia pomonella* and *Argyresthia conjugella*. Taking into account the fact that the preparations synthesized in Flora are meant for use in small gardens and the flights of both the mentioned species are known to peak simultaneously, there has emerged an idea that pheromone dispensers for both the species could be used in the same trap at the same time. This study was carried out to test the idea in natural conditions.

Material and methods

The experiment was organized in the orchard of the Institute of Horticulture in 1998. He traps were set up to the western side of the trees on May 25. The distance between a trap and the ground was 1,5 m. The traps were checked twice a week. The following variants in our experiments were used: CP − the preparation specially synthesised for *Laspeyresia pomonella*, CA-95 − the preparation specially syntesized for *Argyrestha conjugella*, CACP − two different dispensers in the same trap mentioned, CA+CP − two pheromone preparations in one and the same dispenser (Lauk, 1998).

Results

In 1998 the flight of the codling moth began on May 29 and lasted for two months. The mass flight was observed between June 5 and July 10 (Figure 1). A good attractiveness was shown by the traps with dispenser of codling moth separately (variant CP), and by the traps including two different dispensers (variant CACP). Significantly less attractive for codling moth and apple fruit moth were the traps in which the dispenser included the mixture of pheromones of both species.

The flights of the two species studied began almost at the same time, but the flight of apple fruit moth lasted for a shorter time than that of the codling moth (Figure 2). The numerous flight of apple fruit moth were observed during the beginning of their flight, while later only a few individuals were captured with traps. Thus the attractiveness of dispensers may be estimated only by the first capture. The application of specific pheromone of apple fruit moth in a dispenser (variant CA-91) revealed the best attractiveness for that moth. With the traps including two different dispensers (variant CACP) there were captured less individuals of apple fruit moth. It was also evident that the attractiveness of two different pheromones in one and the same dispenser (variant CA+CP) was seriously affected in this combination.

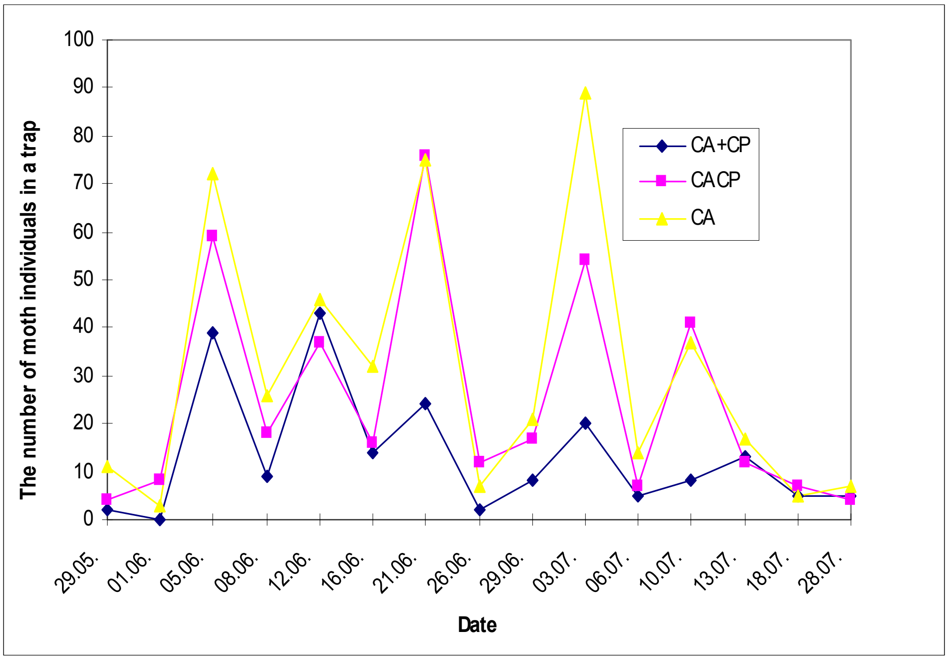

Figure 1. The capture of codling moth (mean of six replications)

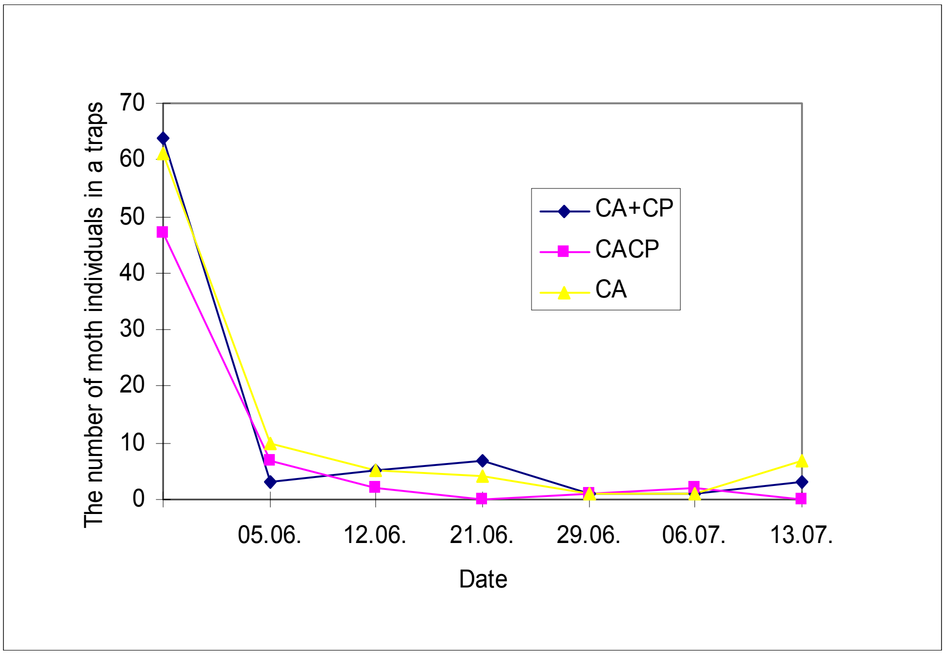

Figure 2. The capture of apple fruit moth (mean of six replications)

Conclusions

Our results showed that the preparations CP and CA-91 syntesized for one species only, were the most attractive ones. In addition we found that using two different dispensers in the one and the same trap was also attractive. However, using two different pheromone preparations in the same dispenser did not give the desired effect.

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WILDVERGRÄMUNGS- UND ABLENKUNGSEFFEKTE MIT PFLANZENEXTRAKTEN IM ÖFFENTLICHEN GRÜN IM LAND BRANDENBURG 1998 ... 2000 MIT VERSUCHSPRODUKTEN DER FIRMA *TRIFOLIO M* GMBH

M. LEHMANN

Landesamt für Ernährung und Landwirtschaft Frankfurt (Oder) - Pflanzenschutzdienst - Ringstraße 1010, D - 15203 Frankfurt (Oder)

Teilversuch 1: Verbiss-Schutz mit "Trifolio Rep 1" gegen Rehwild (*Capreolus capreolus*) an einer Schutzhecke (Kooperation mit einem Straßenbauamt)

Teilversuch 2: Wechselbeeinflussung auf öffentlichen Straßen bzw. Alleen mit Dispenser "Trifolio Rep1" bzw. "RehPell" (Kooperation mit einer Jagdgemeinschaft)

Teilversuch 3: Repellentversuch gegen Schwarzwild (*Sus scrofa*) mit „RehPell" auf einer Waldwiese (Kooperation mit einer Jagdgemeinschaft)

Nach drei Teilversuchen in den Jahren 1998...2000 zur Vermeidung von Schäden durch Wild an nicht landwirtschaftlich genutzten Pflanzenbeständen mit dem Pflanzenextrakt-Dispenser „Rep1" bzw. „RehPell" können die Wirksamkeit und die Wirkungsweise teilweise optimistisch eingeschätzt werden.

Als Repellent für Flächen, die von Wild freigehalten werden sollen, versagte dieses Präparat. Eine zum Zeitpunkt der Applikation stark verbissene vierjährige Hecke, durch welche zahlreiche Wildwechsel verliefen, konnte sowohl mit der Anordnung als Barriere außerhalb der zu schützenden Fläche als auch in direkter Belegung der Äsungspflanzen nicht wirksam vor einem sehr starken Verbiss durch Rehe (*Capreolus capreolus*) geschützt werden.

Direkt belegte, kleinvolumige Objekte (Einzelpflanzen innerhalb der Hecke, hier *Evonymus europaea* als Indikatorart) schützte es über einen längeren Zeitraum vor Verbiss. Das Ergebnis weist daher auf eine gute Verwendbarkeit bei Einzelpflanzenapplikationen, z.B. Stauden und Solitärgehölze. In diesem Falle kann jedoch die Irritation durch die relativ auffälligen, hellen Dispenser in ca. 80 cm Höhe über dem Boden nicht ausgeschlossen werden.

Eine Wirkung auf die Veränderung der Wechsel des Wildes und ein allgemeiner, großräumig abschreckender Effekt auf die Nahrungsaufnahme scheinen nicht vorhanden zu sein. Eine Barrierewirkung durch den intensiven Duft nach Knoblauch ist nicht nachweisbar. Besonders Schwarzwild (*Sus scrofa*) setzt sich nach kurzer Gewöhnungszeit über die ungewohnte Witterung hinweg, obwohl sie vom Menschen noch mehrere Wochen als belastend empfunden wird. Bereits wenige Tage nach Applikation der Dispenser als Barriere um eine Waldwiese traten auf der zu schützenden Grasfläche die unerwünschten Schäden durch das Aufwerfen der Pflanzendecke wieder auf.

Bei Wildunfällen im Straßenverkehr im Land Brandenburg werden nicht nur die beiden Unfallpartner sondern auch häufig die am Straßenrand stehenden Alleebäume beschädigt. Mit hoher Geschwindigkeit befahrene Bundes-, Landes- und

Kreisstraßen in der Nähe von Ortschaften fallen vielfach durch hohe Unfallfrequenzen und hohen materiellen Schaden auf. Im vorliegenden Fall erreichte die Unfallziffer einen offizeillen Wert von bis zu 10 Tieren je laufenden Kilometer im Jahr.

Deshalb wurden in einem weiteren Teilversuch auf einigen aufeinander folgenden Abschnitten einer 1,2 km langen Strecke mit Alleebäumen (*Quercus rubra*, ca. 90 Jahre alt) RehPell-Dispenser im Abstand von ca.10 Metern zueinander auf beiden Straßenseiten in 80 cm Höhe an den Bäumen befestigt, um einen Einfluss auf die sehr zahlreichen und zunehmenden Unfälle durch Kollision wechselnder Rehe mit Kraftfahrzeugen zu verringern. Hierzu wurde die Teststrecke in fünf Abschnitte unterteilt, in den Jahren 1998 bis 1999 die drei ersten, im Jahre 2000 die drei letzten Abschnitte belegt und die Position sowie die Anzahl gefallener Rehe registriert. Die Dispenserapplikation geschah in jeweils zwei bekannten „Hauptunfallperioden“ im Jahr. Sowohl bei einer ersten Anordnung (Teilstrecke 1, 2, 3) als auch bei veränderter Position (Teilstrecke 3, 4, 5) der Dispenser wurde in der Periode der zu erwartenden Wirksamkeit des Duftstoffes eine Verlagerung der Unfallhäufigkeit hin zur ungeschützten Teilstrecke nachgewiesen. In dieser Zeit waren keine Veränderungen in der Dichte und Konzentration des Rehwildes, in der Nahrungsaufnahme, in der Nutzung und Gestaltung des Landschaftsteiles und in der Verkehrsdichte feststellbar, die diese Verlagerung und Veränderung bewirken konnten. Ein Wechsel der Rehe über die Straße und eine Nahrungsaufnahme (Raps, Gras, Getreide, Eicheln) entlang der Straße fanden weiterhin statt.

Zur Reduzierung von Verkehrsunfällen in Wald-Agrar-Landschaften mit Wildbeteiligung sind Effekte der verwendeten Dispenser mit einem Wirkstoff aus Pflanzenextrakt erkennbar, die jedoch nur eine Vermeidung oder Verringerung der sorglosen Straßenüberquerung bei Rehen ausweisen. Auch hier ist eine totale Blockierung der Wechsel nicht möglich und auch nicht erwünscht. Die "RehPell"-Dispenser lösen offensichtlich durch den intensiven und weitreichenden Duftreiz bei Rehwild eine Sensibilisierung aus, die jedoch nicht bis zu einer Abschreckung geht. Sobald ein weiterer Reiz die leichte Irritation durch die Dispenser überdeckt, werden die Reaktionen der Rehe entweder verstärkt oder abgeschwächt. Die folgenden möglichen Reaktionen sind dann, auf die jeweilige Situation bezogen, entweder - bei nahenden Kraftfahrzeugen - „zurück von der Straße und abwarten" oder - zum Beispiel bei Einfluss durch wildernde Hunde - „ungewohnte Witterung ignorieren und trotzdem weiter laufen". Die Wirksamkeit hält ca. vier Monate an. Der Versuch wird fortgesetzt.

PRELIMINARY RESULTS ON THE USE OF ANTI -MARTEN DISPENSERS "MARDER-STUNK" ON MOTOR CARS

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Martens (*Martes foina*) create defects on motor cars in country settlements in Germany. They allmost cause damages on insulation matters and rubber-coated ignition cables, hydraulic and heating systems. A lot of sytems to prevent them from doing this have been developed with more or less effects.

The tested Renault flotilla includes three and after summer 1998 four cars standing in an open carport under equal conditions. First serious marten damages happened in 1996 an 1997 on bonnet insulation and ignition cables. Bone leftovers were found above the engine.

This attacks motived to apply "Marder stunk", a garlic-based pheromone dispenser against martens, since autumn 1998 in one car in comparison to 2 resp. 3 further cars to the ignition cables inside the test object. Only one pheromone stick was placed each. New dispensers got fixed in the months April/Mai and October.

The treated car Renault *Laguna* realised no further "unasked visits" and damages. Only two Renault *Clio* had defects in winter 1999, in early summer 2000 (ignition cable) and late summer 2000 (washer sleeves). A *Renault 19* and one of the *Clio* bare "fragrant trees" inside the cars with more or less success. The "Marder stunk" dispensers seem to produce a repellent effect ton the martens and prevent them from damaging ignition cables.

The smell irritations by the anti-marten dispensers to humans are relatively small.

About 60 sets of "Marder stunk" dispensers in 1999 and 2000 were sold by a Renault dealer. Only three car drivers noticed no action of the dispensers: Two of them forgot to take off the cap of the dispensers and one left the dispensers in his cars boots.

ON APPLIED RESEARCH OF THE INSECT PHEROMONES AND PLANT EXRACTS IN ESTONIA

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An overview is given about the history of the research of insect pheromones and plant extracts in Estonia.

Key words: *pheromones, plant extracts, Estonia.*

Leida Leivategija, an assistant professor of the Estonian Agricultural University (former Estonian Agricultural Academy), started the research on insect pheromones. In 1973 she made the first field experiments with codling moth pheromones synthesised in the Institute of Biological Methods in Plant Protection in Moldova. During the 1970-s she experimented with codling moth preparations of different origin (USA, Hungary) (Leivategija, 1978, 1979). In 1979 at the University of Tartu the Laboratory of Fine Organic Synthesis was organised and the first Estonian pheromone preparations were synthesised. In collaboration with the Institute of Forestry the aggregation preparations of bark beetles were tested (Mihkelson, Õunap, 1983; Õunap,1883). On the initiative of the Laboratory of Fine Organic Synthesis the chemical company Tartu Flora started the production of pheromones of different moth species from genera *Archips, Argyrestia, Cydia, Grapholita, Mamestra, Synanthedon.* In collaboration with Tartu-Flora the researchers and lecturers together with students of the Estonian Agricultural University were helping in field testing of preparations (Leivategija,1982; Lauk, Mirma,1985; Lauk, Lilleste,1986; Lauk,1987, Leivategija, 1987; 1989; Hiiesaar et al., 1994a; Lauk et al., 1998). The Institute of Chemistry and the Institute Zoology and Botany of the Estonian Academy of Sciences were active in the research of pheromones of click beetles (Merivee, Erm, 1993). In the 1980-s Estonian researchers had good contacts and co-operation with different scientific institutions in the former USSR: the Institute of Biological Methods in Plant Protection in Krasnodar, the Institute of Ecology in Vilnius, the Institute of Ecology and Ethology of Animals in Moscow, the Institute of Plant Protection in St. Petersburg, the Research Institute of Plant Protection Chemicals in Moscow. The sex pheromones of different moth species with special traps from Tartu were tested in all these institutions and recommended for practical use in different places of the former USSR. Regularly scientific meetings took place in Tartu, Vilnius, Moscow, etc. Tartu was one of the centres of pheromone research in the former USSR, where almost 1000 scientists were involved in it (Mõttus, Granat, 1983; Rjabchinskaja, Kolesova, Mõttus, 1986). In Estonia after the collapse of the USSR at the beginning of the 1990-s the pheromone research was concentrated to the Laboratory of Ecochemistry in the Estonian Agricultural University (Mõttus et al.,1996.1998).. The chemical company Tartu Flora lost its pheromone preparations market in republics of the former USSR where sex attractant dispensers of cotton-, vineyard- and orchards pests were very widely used in theSouthern republics. Still Tartu - Flora has certain chemical compounds and is able to produce some preparations on special order (Table 1).

Table 1. Some pheromone dispensers that can be produced in Tartu Flora nowadays

Insect name	Dispensers
Orchard pests	
Adoxophyes orana	Feroflor AO
Archips podana	Feroflor AP
Archips rosana	Feroflor AR
Argyresthia conjugella	Feroflor AC
Cydia pomonella	Feroflor CP
Enarmonia formosana	Feroflor EP
Grapholita funebrana	Feroflor GF
Grapholita molesta	Feroflor GM
Hedya nubiferana	Feroflor XH
Lithocolletis blancardiella	Feroflor MM
Pandemis heparana	Feroflor PH
Spilonota oscellana	Feroflor SO
Synanthedon tipuliformis	Feroflor ST
Polyphagous and vegetable	
pests	Feroflor CE
Agrotis exclamationis	Feroflor CO
Agrotis segetum	Feroflor HM
Cydia nigricana	Feroflor MB
Mamestra brassicae	Feroflor PM
Plutella xylostella	

The interest in plant compounds as botanical insecticides first arose in the Laboratory of Experimental Entomology of the Institute of Zoology and Botany at the end of the 1970-s (Kuusik, 1977). More profound and directed research on modes of action of extracts of different local plants started in the 1990-s when researchers of the Laboratory of Experimental Entomology were incorporated into the Institute of Plant Protection of the Estonian Agricultural University. The physiological changes in muscular, respiratory and transpiration activity in yellow *mealworm (Tenebrio molitor*), greater wax moth (*Galleria mellonella*) and large white butterfly (*Pieris brassicae*) caused by some plant extracts were studied (Kuusik et al 1993; Harak, 1994; Kuusik et al., 1995).

In the years 1994 –1997 two projects on the research of plant extracts financed by the Estonian Science Foundation were under way in the Institute of Plant Protection.

In the project – Using some plant extracts as insecticides and insecto-acaricides of diverse mode of action against greenhouse pests (project leader L. Metspalu) around 100 local plant species were tested against twospotted spider mite (*Tetranychus urticae*), greenhouse whitefly (*Trialeurodes vaporariorum*), aphids (*Aphididae*) and thrips (*Thripidae*). The plants with more toxic, repellent and deterrent properties were established (Metspalu, Hiiesaar,1993;1994a;b;c; Hiiesaar et al.,1994b, Metspalu et al, 1997. The further investigation has shown that there is possible to enhance the insecticidal activities of plant extracts by synergetic effects evoked in certain mixtures of these extracts. For example, the effectiveness of absinthe water extract on *Aphis gossypiella, Thrips tabaci, Trialeurodes vaporariorum* and *Tetranychus urticae* was significantly increased from nearly 50% to 80-90% by combining the extract with pine shoot extract at a mixing ratio of 1:1. The behavioural reaction of the pests due to the deterrent/or repellent effects was also synergised by mixing absinthe and pine shoots extracts (Kuusik et al.,1999; 2000). It is important to take into account the fact that plant extract influence can be dependent on the host-plant. The extract of *Allium sativum* evoked strong deterrent actions on whitefly only on the tomato variety Holland and did not on the Mato. That can be caused by difference in allelochemicals of the tomato varieties (Hiiesaar et al., 2000a).

In the project – Plant extracts in the control of some vegetable- and forest cultures pests - the influence of water extracts of more than 30 plant species was tested in cabbage butterfly and great pine weevil (Luik, 1997). All the tested extracts had a polyfunctional influence on cabbage butterfly, they were acting as oviposition and feeding deterrents and also intoxicated caterpillars via food, as well as in a contact way. Against cabbage butterfly the most effective extracts were *Artemisia absinthum, Matricaria inodora, Tanacetum vulgare, Rheum rhaponticum* and *Lycopersicum esculentum.* Practically with the treatment of cabbage plants with extracts it is possible to influence oviposition of butterflies as well as the survival of developing caterpillars. All tested plant extracts also modified the maturation feeding intensity of the pine weevil. Extracts of *Asarum europaeum* and *Narcissus poeticus* acted as feeding stimulants on pine weevils. Under the influence of the extract of *Padus. avium* the total activity of weevils was inhibited. The extracts of *Allium sativum, Taxus baccata, Primula veris* and *Heracleum sosnowsky* acted as feeding deterrents for a short term in laboratory conditions. In nature these extracts lost their influence quite quickly. Some stabilisation of extracts is needed.

At the end of the 1990-s thanks to the new contacts with the German firm Trifolio an opportunity opened for the Institute of Plant Protection to test commercialised plant – neem - preparations for comparisons to Estonian plant extracts. The results of tests with NeemAzal-T (5% azadirachtin content) and NeemAzal-T/S (1% azdirachtin) showed that the plant compound – azadirachtin – affected the maturation feeding behaviour of the pine weevil both in laboratory and forest conditions. In forest conditions the repellent influence was long lasting if a higher concentration treatment was used (Luik, 2000; Luik et al., 2001). In laboratory conditions NeemAzal T/S caused the high mortality of mite *Tetranychus urticae* and some insects-*Aphis gossypii* and *Thrips tabaci* (Hiiesaar et al., 2000b). NeemAzal T/S and plant extracts tests are still continuing in Colorado potato beetle.

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ON DIFFERENT MODES OF ACTION OF NEEMAZAL T/S IN CABBAGE BUTTERFLY

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The influence of NeemAzal T/S 0.05% and 0.1% water emulsions in III instar cabbage butterfly caterpillars was studied. NeeamAzal T/S had a deterring effect for feeding which was stronger when a higher concentration was used. The poisoning of caterpillars via food was also quicker in case of 0.1% concentration and 100% caterpillars were killed during 4 days. NeemAzal T/S caused high mortality of caterpillars also in a contact .

Key words: *NeemAzal T/S, cabbage butterfly , deterrence, mortality.*

During the last ten years new interest has arisen in natural botanical insecticides. They are environmentally less harmful than syntethical pesticides and acting in many insects in different ways (Schmutterer, 1990,1992; Bergen,1994; Metspalu, Hiiesaar, 1994; Luik,1997; Metspalu et al.,1997). Among natural pesticides the compounds from neem (*Azadirachta indica* A. Juss) have a number of properties useful for insect pest management. These include repellence, feeding and oviposition deterrence, insect growth regulator activity, low mammalian toxicity and low persistence in the environment (Schmutterer, 1990; Koul, 1992; Hiiesaar et al.,2000). Neem is also less toxic to nonphytophagous insect species than many conventional insecticides, including pest natural enemies and insects-pollinators (Hoelmer et al., 1990;McCloskey et al.1993; Nauman et al., 1994a,b). Some experiments are made with neem for forest pest management. The neem seed extracts had a systemic influence on bark beetle *Dendroctonus ponderosae*. The lodgepole pines treated with neem were less attacked by *D. ponderosae* and the mortality of larvae was increased on applied trees (Naumann et al., 1994a). In weevils *Hylobius pales* the application of pine logs with neem extract significantly inhibited feeding during 24 h (Salom et al., 1994).

The German firm Trifolio is producing a commercialised neem preparation – NeemAzal, containing active compound triterpenoid azadirachtin – the secondary metabolite of the neem tree. The influence of the commercial preparation on insect is not only depending on the main acting compound but also on additives, which can modify the mode of action. Therefore it is important to test the preparation in different pests while different species in different developmental stages can respond differently. The results of tests with NeemAzal-T (5% azadirachtin content) and NeemAzal-T/S (1% azdirachtin) showed that the plant compound – azadirachtin – inhibited the maturation feeding of the pine weevil *Hylobius abietis* both in laboratory and forest conditions. In forest conditions the inhibition was long lasting if higher concentrations treatments of conifer seedlings were used (Luik,2000; Luik et al., 2001). In laboratory conditions NeemAzal T/S caused the high mortality of mite *Tetranychus urticae* and some insects- *Aphis gossypii* and *Thrips tabaci* (Hiiesaar et al.,2000). The aim of present study was to explain the influence of NeemAzal T/S to the feeding behaviour and toxicity via food and in contact in cabbage butterfly *Pieris brassicae* III instar caterpillars.

Material and methods

The eggs of cabbage butterfly were collected from the field and incubated in petri dishes at laboratory conditions. Newly hatched caterpillars were fed with fresh cabbage leaves. If caterpillars reached III instar the experiments with them were started. The NeemAzal T/S water emulsions in two concentrations 0.05% and 0.1% were tested.

Choice feeding tests and the following development observation in caterpillars. .Pieces of cabbage leaves with the area of 400 mm² were used in choice feeding tests. For treatment with the Neem/Azal T/S emulsion the pieces were dipped into 0.05% or 0.1% emulsion for 2 seconds. Later leaf pieces were dried for 15 minutes on filter paper and put together with the untreated control piece to petri dish. A treated (0.05% or 0.1%) and an untreated pieces of cabbage leaf were placed in moistened paper sleeves (to prevent drying of leaf pieces) within a petri dish. One III instar caterpillar, starved for 8 h was placed in each dish (100x15 mm). The petri dishes were exposed in a laboratory at natural light conditions in temperature + 20±1 °C. All the dishes were numbered and during three days after every 24h the eaten leaf area was measured in dishes. In both variant (0.05% and 0.1%) of the choice feeding test 35-40 caterpillars were used. On these data the average leaf area eaten by caterpillars was calculated. For the estimation residual influence of NeemAzal after 3 days choice feeding test all the caterpillars of the experiment were fed with fresh untreated cabbage leaves and the mortality of caterpillars during the following development was fixed. For the establishment of the rate of natural mortality the 30 caterpillars were continuously reared in the same conditions at the laboratory and their mortality was registered. They were also used as control variant for other tests.

Estimation of NeemAzal T/S influence on caterpillars via food. The III instar caterpillars were fed with the cabbage leaves treated with NeemAzal T/S 0.05% or 0.1%. The caterpillars were reared in 1-l glass jars, where food was changed and excrements removed every day. The leaves were dipped into the corresponding emulsion and dried on filter paper and after that placed into a glass jar. In both concentration variants 40 caterpillars were reared in the laboratory conditions and their mortality was fixed during their development.

Estimation of NeemAzal T/S contact influence on caterpillars . Thirty III instar caterpillars were dipped for 2 seconds into 0.05% or 0.1% NeemAzal emulsion. After drying they were fed continuously with fresh cabbage leaves in laboratory conditions in glass jars and their mortality was fixed. In both variants 40 caterpillars were used.

Results

During the first 24 h in the choice feeding test the caterpillars did not touch the leaves treated with NeemAzal T/S in both concentrations (Tabel 1). They were eating only untreated control leaves. On the second day caterpillars obviously got used to the odours and slightly started to feed also on treated leaves. On the third day when ¾ part of control leaves were eaten the caterpillars really started to eat leaves treated with 0.05% NeemAzal emulsion. In the leaf treated with a higher concentration of NeemAzal it still deterred feeding of the caterpillars quite strongly.

Table. 1. Feeding area of the III instars caterpillars of the cabbage butterfly on untreated control and treated cabbage leaves with NeemAzal T/S 0.05% or 0.1% emulsions.

Days	**0.05%**		**0.1%**	
	Control leaf	Treated leaf	Control leaf	Treated leaf
I	132.8± 8.8	0	144. 8±15	0
II	312.6±.31.3	1.0± 0.3	317.2±32.3	0.1±0.02
III	323.1± 30.5	73.2±10.1	318.8±30.0	0.5±0.2

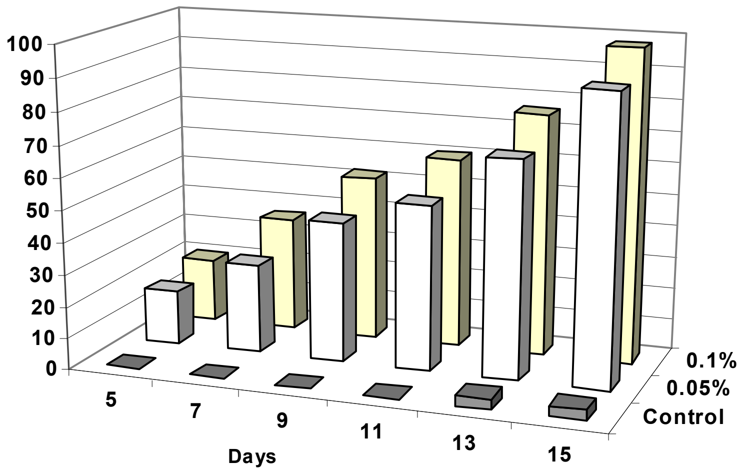

Figure 1. Mortality (%) of cabbage butterfly in different developmental stages after 3 days lasting choice feeding tests (with untreated and treated with Neem-Azal T/S (0.05% or 0.1%) leaves).

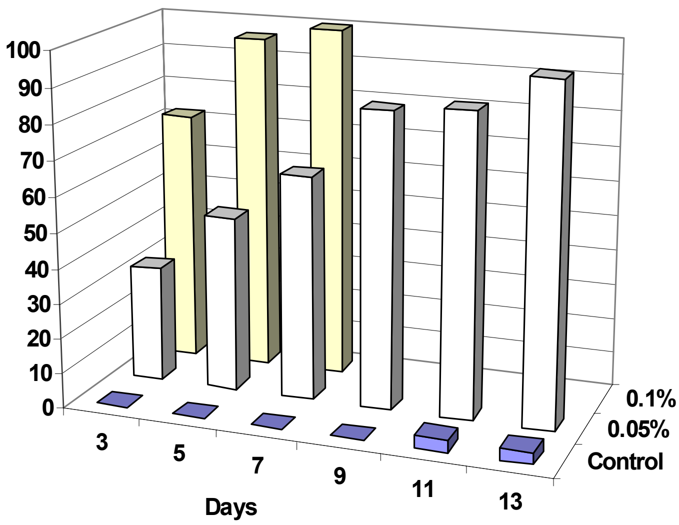

Figure 2. Mortality (%) of cabbage butterfly caterpillars in feeding on untreated and treated with Neem AzalT/S 0.05% or 0.1% emulsions cabbage leaves.

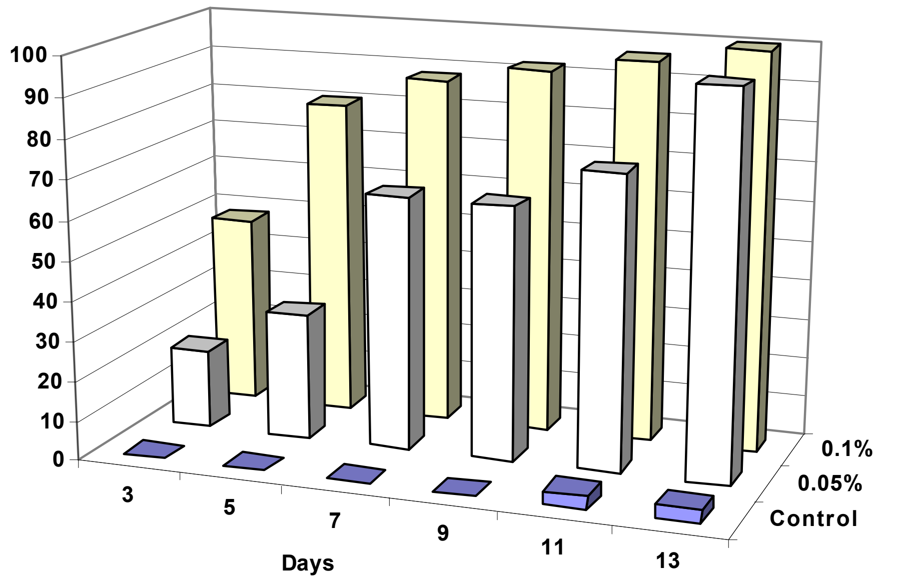

Figure 3. Mortality (%) of cabbage butterfly caterpillars in following development dipped in III instar into Neem Azal T/S 0.05% or 0.05% emulsions.

It is possible to conclude that the deterring influence of feeding of the NeemAzal on cabbage butterfly is depending on concentration.

After the choice feeding test during which the caterpillars were three days exposed in NeemAzal odours the mortality of caterpillars increased in the following development (Figure 1). Mortality of caterpillars was higher if caterpillars were exposed in stronger odours of NeemAzal.

Consequently, the residual effect of NeemAzal odours expressed in further development in specimens and they were killed if not earlier then in the pupal stage (13- 15 days of development).

When caterpillars were fed with poisoned by NeemAzal then in the case of a higher concentration all the caterpillars were perished during 7 days (Figure 2). In using 0.05% emulsion half the test insects were killed at the same time, others were killed, if not earlier, then in pupal stage after 13 days development.

The contact influence of NeemAzal appeared in high mortality on the seventh day after dipping caterpillars into emulsions (Figure 3). When the concentration was lower, the mortality rate was higher than 50%. When the concentration was higher the mortality rate was higher than 80%. On the 13-th day after dipping all specimens treated with 0.1% emulsion were perished . In the case of a lower concentration they totally perished a bit later in the pupal stage.

There is possible to conclude that tested NeemAzal water emulsion in concentrations 0.05% and 0.1% had a deterring influence on feeding in the III instar cabbage butterfly caterpillars and the inhibition effect was stronger in the case of a higher concentration. The residual effect of NeemAzal appeared in the following rearing of caterpillars of the choice feeding test. The main part of this test population perished during 13 days. That is explicable with the influence of NeemAzal odours. NeemAzal

T/S 0.05% and 0.1% caused high mortality in caterpillars both via food as in contact . Higher concentration influenced the caterpillars more quickly.

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HOW TO INCREASE HONEYBEE POLLINATION EFFICIENCY IN ENCLOSURE

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All fieldwork was carried out in a 2-year old seed field of alfalfa in Põlva county (South-Eastern Estonia). We tested honeybees and bumblebees in the field conditions and in enclosure. The influence of honeybee behaviour on alfalfa pollination efficiency was examined using bud beehives placed in net cage (enclosure experiments). The bud colony with all instars of brood frames covered with bees was separated from the dominant beehive. After some time the foragers left the experimental hive, which was removed from the mother hive for some hours. So the foragers from the bud hive could return to the mother hive and the result was an experimental hive with young bees without foragers.

*The honeybee (*Apis mellifera *L.) is the most numerous visitors of alfalfa flowers in the field conditions. They make up more than 90% of the total number of visitors in the alfalfa fields of Estonia (Fig. 1). Their abundance amounted to 30–40 individuals per 10 m^2, and their working speed (18.52±1.14 flowers per minute) was comparable to that of solitary bees (17.50±1.25) and bumblebees (16.71±0.91). On collecting nectar, 1.100±0.003% (n=1182) of flowers were tripped occasionally, while only 0.4% of the flowers were tripped by alighting on the flower directly from the front and releasing the tripping mechanism* (Fig. 2)*. This ensured effective cross-pollination.*

The number of pollen collectors in the cages arises in the first two days until 19% of all visitors. The mean of 47.1±1.9% of visited flowers was pollinated successfully by honeybees. Thus the tripping efficiency of the honeybees increases rapidly in enclosure (Fig.2). In the field conditions the effectiveness of pollen collection of control beehives persisted as before the experiment.

The removal of the beebread filled combs from the experimental hive and addition of brood frames full of young larvae in the bud hive caused the protein deficiency in the hive and forced honeybees to collect more effectively the pollen. Bee colonies need pollen as a protein food for feeding larvae. If in the hive there is not sufficiently bee bread, the foragers have to collect pollen even in extreme conditions and from the plants not usually preferred.

In Estonian conditions, the honeybee can be pollinate more effectively only in isolators by creating moderate beebread deficiency in the colony, but this would be feasible only in alfalfa variety breeding and in the multiplication of high quality seeds.

***Key words:** honeybee,* Apis mellifera, *bumblebee,* Bombus lucorum, Bombus veteranus, *pollination, pollination in enclosure, pollination in greenhouses.*

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Introduction

The honeybee (*Apis mellifera*) is the most numerous and effective pollinator of different crops. The honeybee living in compact colonies can be easily and at low cost transported from one field to another to raise seed harvests. They are used also in the pollination of greenhouse crops. Honeybees often visit numerously also the flowers of alfalfa. The bees foraging on the flowers of alfalfa can be divided into three groups according to their behavioural patterns: 1) nectar robbers, who do not trip the flower; 2) nectar collectors, who trip and pollinate the flowers; and 3) pollen collectors (Vansel, Todd, 1946; Bolton, 1962; Reinhardt, 1952). In Estonian conditions the honeybee belongs to the first group and they mainly steal nectar from the side of the flower without pollinating the flower. They occasionally sometimes trip visited flowers and also pollinate them (Mand *et al.*, 1994; Martin *et al.*, 1998). In Estonia this behaviour has led to contrasting opinions as to the usefulness of the honeybee as an alfalfa pollinator: some researchers deny the bee any importance as an lucerne pollinator, others are of the opposite opinion (Laur, 1962; Niglas, 1980).

The aim of our study was to investigate the efficiency of alfalfa pollination by bumblebees and honeybees contrastively in field and in enclosure conditions. Secondly we investigated also the possibility of increasing the effectiveness of honeybee in the pollination of alfalfa in greenhouse conditions and using them in hybridisation experiments.

Materials and methods

All fieldwork was carried out in a 2-year old seed field of alfalfa in Põlva county (South-Eastern Estonia). Flower visitors were assessed in two ways. We tested honeybees and bumblebees in the open i.e. field condition, and in enclosure, namely in cage surrounded by a fine net.

The influence of honeybee behaviour on alfalfa pollination efficiency was examined in more detail using bud beehives placed in net cage (enclosure experiments). The bud colony with all instars of brood frames covered with bees was separated from the dominant beehive. After some time the foragers left the experimental hive, which was removed from the mother hive for some hours in the distance of 40–50 m. So the foragers from the bud hive could return to the mother hive and the result was an experimental hive with young bees without foragers.

Six 2x5 m^2 observation plots were selected and marked in the open field. Three 2x5 m^2 plots of alfalfa were isolated from outside insects with netting cages in the same field for investigation of: 1) honeybees, 2) medium-tongued *Bombus veteranus*, uncommon pollinator in open conditions, 3) control, without bees.

During a plot count of insects, the observer walked slowly around the plot and all bees were counted and their behaviour has recorded. We counted also tripped and untripped flowers in every evening and estimated the pollination efficiency. We also estimated the speed of flower visits of honeybees and bumblebees and separately counted nectar and pollen collectors. Counts were made three times per day from 12.00 h to 15.00 h in the period of flowering peak in July on all plots. At the end of the flowering season we estimated the pod and seed set of testing plots in the open field and in enclosure conditions by counting empty and fertilized seedpods (Martin *et al.* 1998). The pod and seed set was estimated also by artificially and self-tripped flowers.

Alternatively, the mobile observer followed the visit sequences of particular pollinators (picked at random by following the next bee sighted, as the previous one was lost from view). The observance time and behaviour of the bee were recorded.

Results

The honeybee (*Apis mellifera* L.) is the most numerous visitors of alfalfa flowers. They make up more than 90% of the total number of visitors in the alfalfa fields of Estonia (Fig. 1). At times, their abundance amounted to 30–40 individuals per 10 m^2, and their working speed (18.52±1.14 flowers per minute) was comparable to that of solitary bees (17.50±1.25) an bumblebees (*B. lucorum*) (16,71±0,91). On collecting nectar, 1.100±0.003% (n=1182) of flowers were tripped occasionally, while only 0.4% of the flowers were tripped by alighting on the flower directly from the front and releasing the tripping mechanism (Fig. 2). This ensured effective cross-pollination because the bees were, as a rule, covered with pollen. The bees found in the field were presumably newly formed foragers who had no experience how to collect nectar from the side. After some time they learned to steal and collect nectar from the side without tripping and pollinating the flower.

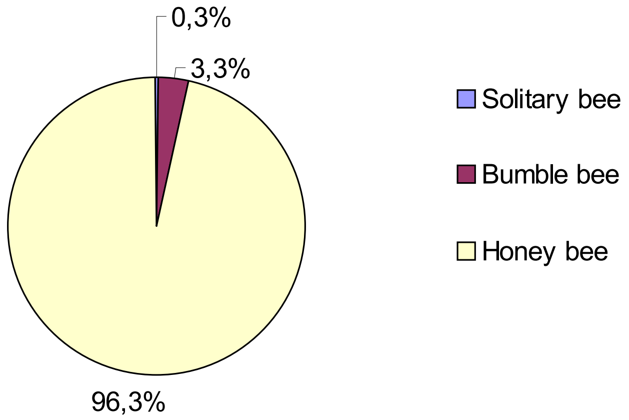

Figure 1. Relations between alfalfa visitors in the field conditions of Estonia

The number of pollen collectors in the cages arises in the first two days until 19% of all visitors. At first the pollen collecting behaviour of honeybees was nervous. They were obviously newly formed foragers. Afterwards they calmed down and the behaviour of the honeybees became similar to the behaviour of solitary bees (*Melitta leporina* and *Megachile* sp). The mean of 47.1±1.9 % of visited flowers was pollinated successfully by honeybees. Thus the tripping efficiency of the honeybees increases rapidly in enclosure **(Fig.2).** In the field conditions the effectiveness of pollen collection of control beehives persisted as before the experiment **(Fig. 2).**

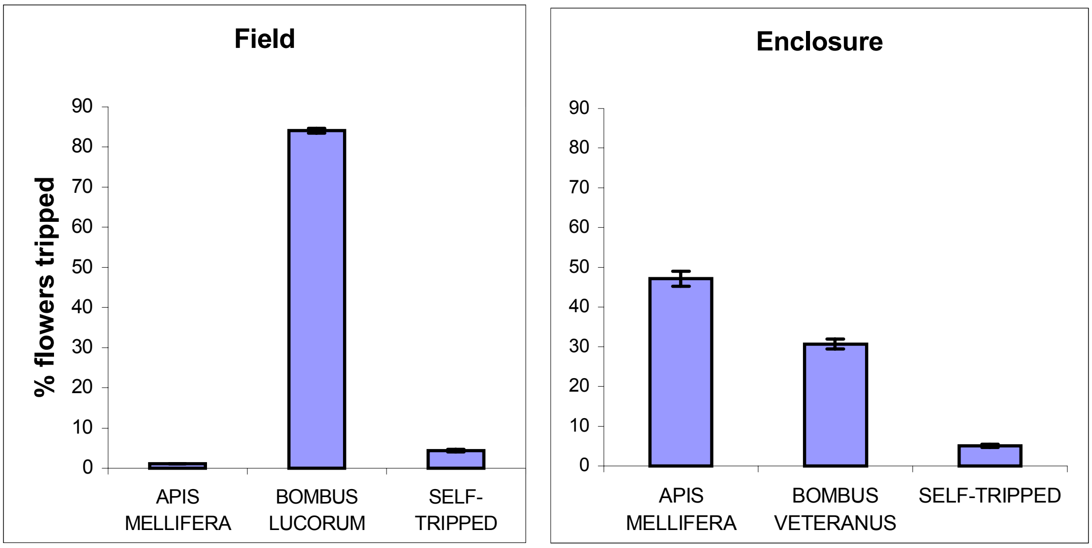

Figure 2. Pollination efficiency of alfalfa by *A. mellifera*, *B. veteranus* in the field and enclosure conditions

The honeybees density had a significant positive correlation with the total number of flowers in the experimental plots (r=0.53; n=27; P<0,05), but there was no significant correlation with the percentage of tripped flowers (Fig. 2). At the same time the percentage of tripped flowers increased with the increasing of bumblebee density in the field, reaching a maximum of 80–90% tripped flowers (maximum 92.3%) (Fig. 3) when 0.6 bumblebee occurred per 10 000 flowers in the field (Mänd *et al.* 1996). When the nectar production was limited due to draughts, only a few honeybees visited the flowers.

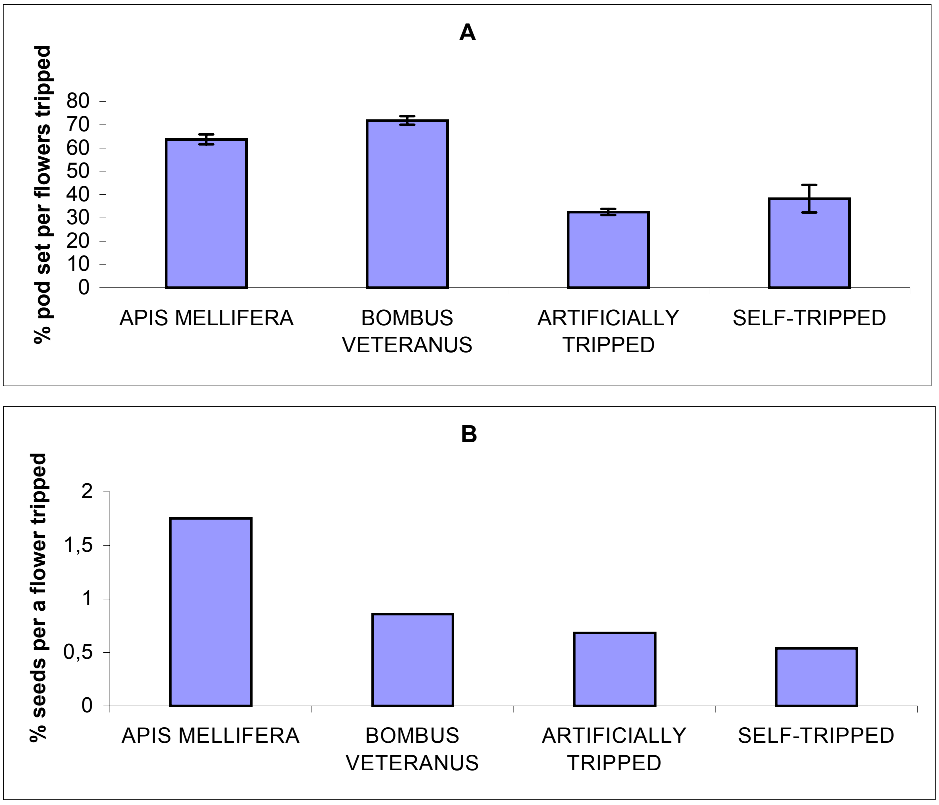

Figure 3. Pollination efficiency, pod (A) and seed set (B) of alfalfa by self tripped, artificially tripped and by different species of bees.

Discussion

In arid regions such as in Arizona and California, high yields of alfalfa seed have been set by honeybee pollination. In semi-deserts, where alfalfa is grown under irrigation and competing flowers are absent during the flowering period of alfalfa, the main pollinator is honeybee (Bolton, 1962).

In Estonian conditions, where around the alfalfa fields there are numbers of natural and seminatural habitats with host of competing wild flowers within the flight range, honeybees very seldom visit alfalfa for pollen collection (Martin *et al*., 1998; Mand *et al*. 1997a,b). Different entomophilous field crops, as clovers *(Trifolium sp.),* rape, mustards *(Brassica sp.),* fruit trees and berries, various weedy plants and wild flowers are visited in preference to alfalfa. Even the increase of beehives in the field does not help the pollination efficiency in Estonia due to the high percentage of natural and seminatural habitats within the feeding territory (optimal 1.5 km) of the honeybee. Where mowing down can minimize these pollen sources, the potential value of honeybees for setting alfalfa seed can be realized better in some extent.

A similar situation has been described in Sweden where honeybee pollination effectiveness rises to 0.83% of visited flowers (Lešins *et al*., 1954). Whereas we can easily manipulate with beehives and increase the number of honeybees in the fields, it is also possible to rise the seed set to some extent. Using honeybees in northern regions is probably ineffective in increasing the alfalfa seed production in the field conditions and thus uneconomic for seed growing farms.

We can use honeybees for alfalfa pollination only in enclosure conditions, where the source of pollen is absents for brood. Bee colonies need pollen as a protein food for feeding larvae. If in the hive there is not sufficiently bee bread, the foragers have to collect pollen even in extreme conditions and from the plants not usually preferred. We have seen honeybees collecting pollen from wind pollinated plants. Sometimes they collect pollen fallen down on leaves or from the ground surface.

To encourage honeybees to pollinate alfalfa more effectively we placed beehives without old foragers in the enclosure where the alfalfa test plots were cultivated. The removal of the beebread filled combs from the experimental hive and addition of brood frames full of young larvae in the bud hive caused the protein deficiency in the hive and forced honeybees to collect more effectively the pollen. After few days newly formed foragers started to work in the enclosure without falling into stress. When we used a previously active bee hive in enclosure, which had had its own outside feeding territory, the old foragers fell into stress and they died in a few hours in their greenhouses and netting cages, because they could not find the exit to their own feeding territory. We can avoid the dying of the old foragers putting artificial swarm without outside workers into greenhouses or into netting cages. To enhance pollen collection from alfalfa and other plants not preferred by honeybees it is necessary to maintain sufficiently brood combs especially with eggs and larvae and remove the combs with bee bread.

For optimal pollination of 1000 m² of alfalfa the enclosure needed a beehive with 5–7 brood combs. The described method suits presumably also for pollination of cucumber and other greenhouse entomophilous crops.

In our conditions, the honeybee can be made to pollinate more effectively only in isolators by creating moderate beebread deficiency in the colony, but this would be feasible only in alfalfa variety breeding and in the multiplication of high quality seeds.

Acknowledgements

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THE REDUCTION OF DAMAGES CAUSED BY PEA MOTHS (*LASPEYRESIA SPP.*) BY SPRAYING THE PEAS WITH NEEMAZAL T/S

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We investigated the efficiency of NeemAzal T/S on the pea moth's Laspeyresia dorsana *and* L. nigricana *in the field. The formula was diluted with water and tested by concentrations of 0.5%. The pea was sprayed six times with weekly intervals. The spraying with NeemAzal T/S reduced the damage caused by caterpillars of the pea moths 2-3 times. In the control variant the occurrence-curve of the caterpillars of pea moths and the flying-curve of moths coincided, there is an about one-month shift in time, i.e. the maximum of flight happened in mid July whereas the number of damaged pods was greatest in August. In the year of trial, the level of damage caused by pea moths remained considerably low, the most damaged test (50%) proved to be the one from the end of summer. In the variant treated with NeemAzal T/S the amount of damaged pods stayed on one and the same level during the whole trial period, not exceeding 18 percent.*

Key words: *pea moth's, NeemAzal T/S, damage of pods.*

Introduction

The pea moths (*Laspeyresia dorsana* and *L. nigricana*) are widely distributed in Estonia. In vegetable gardens, there caterpillars cause every year substantial damage, to nearly 80 percent of pods. Emergence and moth flight towards pea crops begins at the end of June and continues until the end of July. Each caterpillar damages up to 6 seeds, but only 1 or 2 are severely gnawed. Embryonic development lasts from 1 to 3 weeks on the stipples or the leaflets. The caterpillar goes through a very short wandering stage (1 day) before penetrating a young pod. Larval development lasts 18 to 30 days, after which the larva leaves the pod and migrates to the ground where it spins a cocoon containing particles of soil, and then hibernates. In spring, the caterpillar leaves its winter-cocoon, and pupates in a slight cocoon just below the surface of the ground. In Estonia pea moths have one generation per year.

By means of appropriate sowing seasons it is tried to reduce the damage caused by pea moths. Since pea is one of our most frost-resistant vegetable cultures, and its early sowing helps to keep the damage by pea moths on a lower level, it is usually sown at the end of April. In addition, there are plenty of toxic chemicals available. The pollution of environment and the acquired resistance of insects to pesticides bring about the necessity to seek for new ways of restricting the number of insects.

Many plants contain chemicals that are toxic to insects. Because these naturally occurring insecticides are derived from plants, they are called botanical insecticides or botanicals. Throughout history, plant products have been successfully exploited as insecticides, insect repellents, and insect antifeedants (Dethier et al., 1960; Dethier, 1970; Schoonhoven, 1982; Addor, 1995; Mordue (Luntz), 1998). Jacobson (1989) suggested that the most promising botanicals were to be found in the families

Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae and Canellaceae. The neem tree (*Azadirachta indica* A Juss, Meliaceae) is widely used for its insecticidal properties. Many biologically active compounds can be extracted from neem: triterpenoids, phenolic compounds, carotenoids, steroids and ketons. This mixture of compounds significantly reduces the possibility that tolerance or resistance might develop in any of the affected organisms. The tetranortriterpenoid azadirachtin has mostly received attention as a pesticide because it has shown biological activity on a wide range of insects. Besides azadirachtin, salinnin, nimbin and other components in small concentrations are considered the component parts of neem. Natural defences of plants against herbivores almost always rather consist of the mixtures of closely related compounds than of single toxicants alone. This phenomenon is well exemplified among botanical insecticides (Isman, 1997).

The extracts of neem seeds have been tested on several orders of insects, mites and nematodes in different regions of world. The neem (*Azadirachta indica*) products work by intervening at several stages of an insect's life. Various neem extracts are known to act on insects in the following ways: disrupting or inhibiting the development of eggs, larvae or pupae; blocking the moulting of larvae or nymphs; disrupting mating and sexual communication; repelling larvae and adults; deterring females from laying eggs, sterilising adults; deterring feeding; blocking the ability to swallow; sending metamorphosis away at various stages; inhibiting the formation of chitin. The action of neem depends on the pest species, the part of plant extracted and the concentration used. (Schmutterer et al., 1980, 1984; Schoonhoven, 1980; Jacobson, 1987; Parmar, 1987; Koul et al., 1990; Addor, 1995; Mordue (Luntz), 1998; Hiiesaar et al., 2000).

At present time, the Trifolio-M Company in Germany manufactures and markets the formulation of Neem-Azal T/S (NAZ), where 1% azadirachtins have been included. The aim of the present work was to elucidate the action and efficiency of that botanical insecticide on the pea moths in the field.

Material and methods

For small-field trials the pea was sowed on May 24. All the tests were carried out in three replicates, each representing three rows of 6-meter length. The placing of the test series was systematic, i.e. the test series and replicates were distributed evenly by Matthews (1997). We diluted the formula with water and tested by concentrations of 0.5%. The spray volume was increased as the plant size increased. As the insecticidal activity of Neem-Azal persists on plants from one week to ten days (our observations; Kleeberg, Hummel, 2001), the pea was sprayed six times with weekly intervals. We sprayed the control with pure water. Six times a certain amount of test pods (50-60 pods) was taken from each replicate to determine the damage. Each time the pods of more or less the same stage of ripeness were collected. The halves of the pods were green and juicy and the seeds inside had diameters of 7-8 mm. In such pods the larvae of pea moths had reached there the third instar, being easily noticeable.

Results and discussion

An important condition in using neem is that a dry period of 4-10 hours should follow the spraying. During this time the preparation is absorbed into the leaves (translaminar action), and there is no rain to wash it off the plants. NAZ stays active for up to ten days, after which it decomposes without leaving any side-effects to the plants.

In the control variant the occurrence-curve of caterpillars of pea moths and the flying-curve of moths coincided, there existing an about one-month shift in time, i.e. the maximum of flight happened in mid July whereas the number of damaged pods was greatest in August (Figure 1). In the year of trial, the level of damage caused by pea moths remained considerably low, most damaged test (50%) proved to be the one of August. In the variant treated with NAZ the amount of damaged pods stayed on one and the same level during the whole trial period, not exceeding 18 percent.

As each day of our tests was accompanied by rain, washing away part of the sprayed neem, the effectiveness of our activity was decreased. But even in such weather conditions the spraying with NAZ reduced the damage caused by pea moths 2-3 times (Figure 2).

The decrease of damage may have several reasons. In selecting a plant suitable for oviposition insects use their senses of taste, smell, touch and sight. Female adults are provided with taste sensillae on the tarsi, mouth appendages and an ovipositor. Tactile hair is spread all over the body. Treating an insects host plant with an extract of a non-host plant can make finding the plant and laying eggs there impossible for the insect (Bernays and Chapman, 1994).

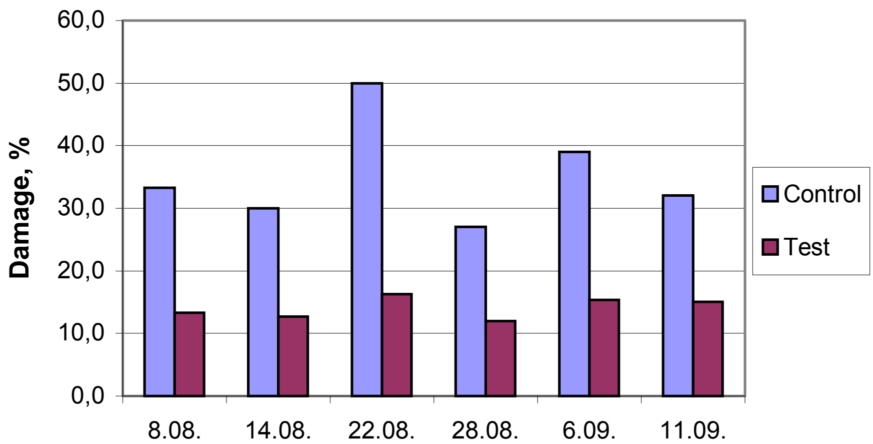

Figure 1. The dynamics of the damage caused by pea moths (*Laspeyresia spp.*) in the control variant and in the test (P <0.05)

Since ancient times, neem has been used as an insect repellent for stored grains and in houses against crawling bugs and bed bugs. At present, neem repellent properties are known to affect more than 200 insect pest species (Miller, 2000).

Data from literature reveal that neem is useful as an ovipositional repellent for the protection of crops like tobacco, groundnut, cotton and sweet potato from the damages caused by tobacco caterpillar or tobacco cutworm, a serious polyphagous pest of several crops (Larew, 1990; Liu and Stansly, 1995). Neem extracts have been found to be repellent to *Aedes pipiens* (the northern house mosquito), *Anopheles* mosquito, biting flies, sand fleas and ticks (Ascher, 1993). In our tests NAZ proved to be repellent to the adults of large cabbage butterfly (*Pieris brassicae*) and cabbage army moth (*Mamestra brassicae*) (Metspalu et al., 2001). Meadow et al. (2001) founded the repellent effect of neem extracts against oviposition of turnip root fly (*Delia floralis*) and the cabbage moth (*Mamestra brassicae*). Saxena et al. (1993) reported that neem extract disrupted mating signals in *Nilaparvata lugens* (Homoptera, Delphacidae). Thus the adults of pea moths may have been faced with difficulties in locating the plants sprayed with NAZ, and those few plants located showed little suitability for egglaying.

Secondly, NAZ possibly affected the eggs of pea moths laid on plants. It is known that in case of bigger doses the preparation may have a direct cytotoxic effect, comparable to that of a pesticide. In such cases the eggshells wrinkle and sunken, the embryonic development fails to take place, and, consequently, the eggs perish. In treating the egg clutches of *Leptinotarsa decemlineata* we obtained the same results. Weaker doses do not prevent embryonic development, although in some cases young larvae are unable to hatch (Hiiesaar et al., 1999; Kuusik et al., 2000). According to the data by Mordue (Luntz) (1998) the azadirachtin containing in the preparation induced the weakness of muscles, and thus led to the inability of the larvae to hatch. The hatched larvae did not eat eggshells polluted with the preparation and which are antifeedant to larvae. After that, it is possible that the caterpillars hatched and the extract acted as contact or stomach poison, when the larvae ate the contaminated remains of their eggs (Jenkihau, et al., 2001).

The tests with pea moths will be repeated to obtain more detailed information on the activity of neem kernel seed extracts. The initial tests showed that NAZ is capable of reducing the damage caused by pea moths in gardens.

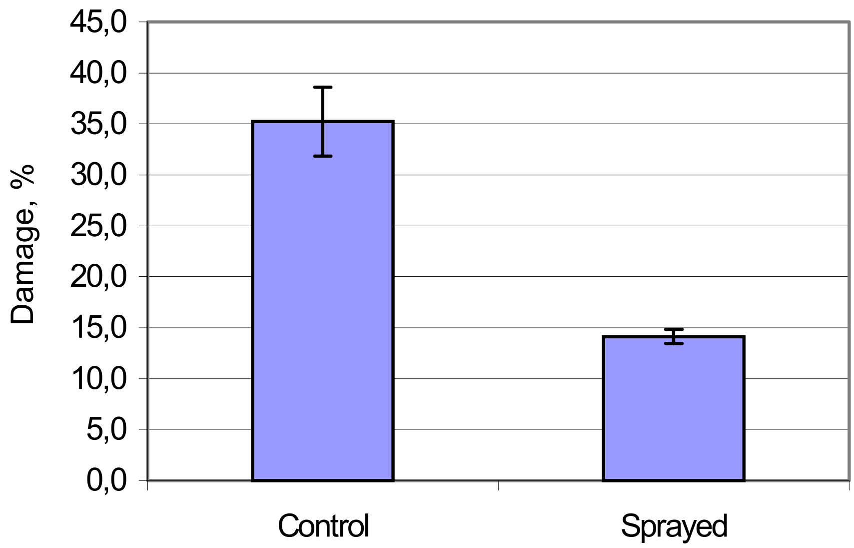

Figure 2. The percentage of pods damaged by pea moths (*Laspeyresia spp.*) in the variant sprayed with NAZ and in the control variant (P<0.05)

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THE EFFECTS OF CERTAIN TOXIC PLANT EXTRACTS ON THE LARVAE OF COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY)

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The influence of the extracts of Heracleum sosnowskyi, Artemisia absinthium, Artemisia dracunculus, Rheum rhaponticum, Tanacetum vulgare, Levisticum officinale and Achillea millifolium on the larvae of Colorado potato beetle (CPB) (Leptinotarsa decemlineata Say) was estimated. The biggest death rate (75-80%) was caused by giant hogweed (Heracleum sosnowskyi) extract. Wormwood (Artemisia absinthium) was also toxic, killing off 70% of the first-instar larvae, but its effect diminished in each following instar, being different from control only in case of the first and second larval instars. Common yarrow (Achillea millifolium) proved to have low toxicity, only the number of dead first-instar larvae was bigger than in the control, but the difference was not statistically reliable. Tarragon (Artemisia dracunculus) was moderately toxic, 47% of the first-instar and 43% of the second-instar larvae died. The death rate of the third-instar larvae was at the same level as in the control. The larvae of the first instars did not perish during the first days, but the death rate increased in the next days of development, which indicates a growing toxication in the organism leading to death due to deepening physiological failures. When the larvae of CPB were eating the treated leaves of common tansy (Tanacetum vulgare), 24% of them perished. Hot-water extract was made from lovage (Levisticum officinale), and it became the most effective of all the hot-water extracts. In this variant 33% of the larvae died, which was significantly greater than the control variant. The extract from rhubarb (Rheum rhaponticum) was not toxic for the larvae of CPB.

Key words: *Heracleum sosnowskyi, Artemisia absinthium, Artemisia dracunculus, Rheum rhaponticum, Tanacetum vulgare, Levisticum officinale, Achillea millifolium, larvae of Leptinotarsa decemlineata, ethanolic extracts, boiling water extracts.*

Introduction

It can be affirmed that after the extensive invasion of Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say), both by air and by being thrown up on the sea beach, in 1973, it has locally become a permanent pest in Estonia. The sum of active temperatures (above 10 °C) in the South, and especially the Southeast of Estonia, is sufficient for the beetle to go through its developmental cycle even in case of a moderately warm summer.

Almost all the synthetic insecticides in the world have been tested, and many of them have been put to good use in the control of CPB. Every new preparation was effective for 3 - 4 years, after which its effect started to decrease, especially when it was used only one-sidedly, because CPB develop an inherited resistance. The CPB has developed resistance to all registered synthetic insecticides by all three resistance mechanisms; decreased cuticular penetration, enhanced metabolic detoxification, and altered target site sensitivity (Weisz, et al., 1994; Kuusik, et al., 1995, 1997).

The quick development of poison-resistance and the high price of pesticides have forced many laboratories in the world to search for new ways of controlling CPB and other pests as well. The botanicals (botanical insecticides – toxins derivated from plants) have been used in agriculture long before synthetic chemicals were made available. Plant extracts are not yet widely used in conventional commercial agriculture, but small-scale organic growers and home gardeners are using them more extensively. In many temperate-zone plants there has been discovered a deterrent or a repellent (repelling by means of odour) action on CPB. The actions of our, Estonian plants, on CPB are yet to be studied. The aim of the present research was to investigate the influence of the extracts of *Heracleum sosnowskyi*, *Artemisia absinthium, Artemisia dracunculus, Rheum rhaponticum, Tanacetum vulgare, Levisticum officinale* and *Achillea millifolium* on the larvae of CPB.

Methods

Plant extracts can be obtained from raw materials or powders of dried plants by using either water or solvents. Since different solvents and means of procedure extract different substances from the plants, both extraction by means of ethanol and hot-water extracts were used in the present research. It was endeavoured to collect as standard plant material as possible from the gardens of Tartu, because the amount and composition of active substance agents in plant extracts depends not only on the means of procedure but also on the environment, weather and the age of the plant. Raw plant material was cut into as small pieces as possible.

In the first variant 85% ethanol was added to the plant pieces. The mixture was shaken for 20 minutes and left to stay covered for 24 hours. After that the mixture was filtered and vaporised in a vacuum-thermostat at 90 °C. Ethanol extracts diluted in water were prepared once and kept in the refrigerator at 5 °C. The residue was diluted in water into a 10% w/v concentration, into which potato leaves were immersed for 10 seconds. Then the leaves were dried to remove the residue of the solution, and fed to the first-, second- and third-instar caterpillars of CPB respectively.

In the second variant boiling water was poured to the ground raw material - 100 g plant material used per 1 litre water. Next day the extract was filtered, and potato leaves were immersed into it for 10 seconds. For example I. Tammaru (1981) and A. Ploomi (1998) have prepared such extracts. The leaves were dried and fed to the larvae of CPB during the whole larval period. As the experiments have shown, in a refrigerator the qualities of the extracts remain active for up to 3 days, every third day new hot-water extracts were prepared. In one test the larvae were fed with treated leaves, in the other with leaves immersed into water. In the first variant (ethanol extracts) the death rate of the larvae was estimated, and in the second (boiling water) the death rate of the larvae and the length of larval period. Each series was carried out in three replications.

Results

In the first trial variant, where ethanol extracts from plants were used, the biggest death rate was caused by giant hogweed (*Heracleum sosnowskyi*) extract (75-80%), difference from the control being: $t=15.1$ and $p<0.0001$. The afore mentioned extract had more or less the same effect on all the instars (Figure 1, E). Hogweeds contain a variety of alkaloids, furanocoumarins, and phenols, whereas it is known that furanocoumarins affect insects both as feeding deterrents (Klocke, et al., 1989**;** Berenbaum 1992) and toxicants (Hiiesaar, et al., 1994).

In the first trial variant the wormwood (*Artemisia absinthium*) was also relatively toxic, killing off 70% of the first-instar larvae, but its effect diminished in each following instar, being different from the control ($p<0.0001$) only in case of the first and second larval instars (Figure 1). For centuries wormwood has been used as a moth repellent, general pesticide, and as a spray to repel slugs and snails. Wormwood is rich in essential oils, including thujone, thujyl, absinthum, absinthol, sesquiterpene lactones (absinthin, etc) flavonoids, azulenes, and glycosides. Sesquiterpenes are a group of compounds that have shown antifeedant effects on many insect species. Sesquiterpenes isolated from the family Asteraceae are an important source of CPB antifeedants (Hough-Goldstein 1990)**.** Thujone containing in wormwood affects insects as a neuorotoxic substance, but the effect may be synergized under the joint influence of thujone, absinthum and absinthol (Luik, 1997). The hot-water extract of wormwood had a considerably smaller effect on the larvae of CPB (Figure 2). During the trial period slightly

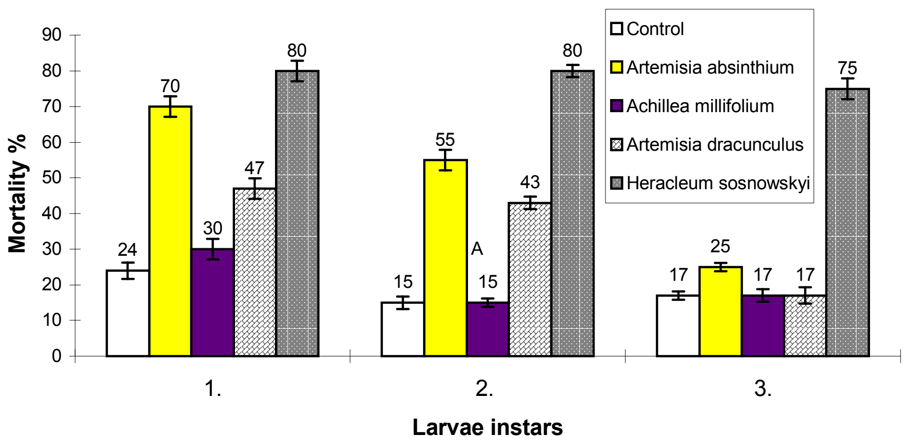

Figure 1. The toxic effect the ethanol extracts from plants. The larvae of different instars were fed with potato leaves treated with plant extracts (10% concentration) and their death rate was observed. Three replications, each with 20 larvae.

over 20% of the larvae died there. Thus it may be concluded that the toxic components of wormwood dissolved in water to a lesser extent than in ethanol.

Common yarrow (*Achillea millifolium*) was extracted with ethanol. The extract proved to have low toxicity, and only the number of dead first-instar larvae was bigger than in the control, but the difference was not statistically reliable (Figure 1, 24%, and 30%). The death rate of second- and third-instar larvae remained at the same level as the control, which indicates that caterpillars of different instars are not similar in their physiological state. Common yarrow proved to reveal little toxicity for the caterpillars of large white butterfly as well (Luik,1997). There was no hot-water extract made from common yarrow. Over hundred biologically active compounds have been identified from common yarrow. It yields a volatile oil containing azulene, also gum, tannin, resin, chlorides of calcium and potassium, and various salts: such as nitrates, malates, and phosphorus, cineol and proaculene, achilleine, and vitamin C.

Tarragon (*Artemisia dracunculus*), which contains pinene, camphene, myrcene, iodine, terpinene, linalol, was extracted with ethanol. There 47% of the first-instar and

43% of the second-instar larvae died. The death rate of the third-instar larvae was at the same level as in the control. The larvae of the first instars did not perish during the first days, but the death rate increased in the next days of development, which refers to a growing toxication in the organism leading to death due to deepening physiological failures.

Common tansy (*Tanacetum vulgare*) is generally considered to be effective in restricting the numbers of pests on cruciferous crops (Luik, 1994, 1997; Lauk, Kaseorg, 1994). We used hot-water extract against the larvae of CPB. When eating the treated leaves, 24% of the larvae of CPB perished. It shows that other procedures and usage for common tansy should be searched for. Common tansy is a strong-smelling garden favourite that has been studied most extensively. Tansy has been used as a general-purpose insect repellent (Hough-Goldstein, et al., 1990). Tansy oil produces avoidance behaviour in CPBs (Panasiuk, 1984). CPB populations have decreased from 60-100%, when the host plant has been interplanted with tansy (Panasiuk, 1984). Tansy contains approximately 100 different compounds: essential oil (borneol, thujone, camphol), sesquiterpene lactones, pyrethrins, flavonoids (quercitin, jaceidin), vitamin C, citric acid, butyric acid, oxalic acid, malic acid, resin, tannins (Schearer, 1984; Hough-Goldstein, et al., 1990).

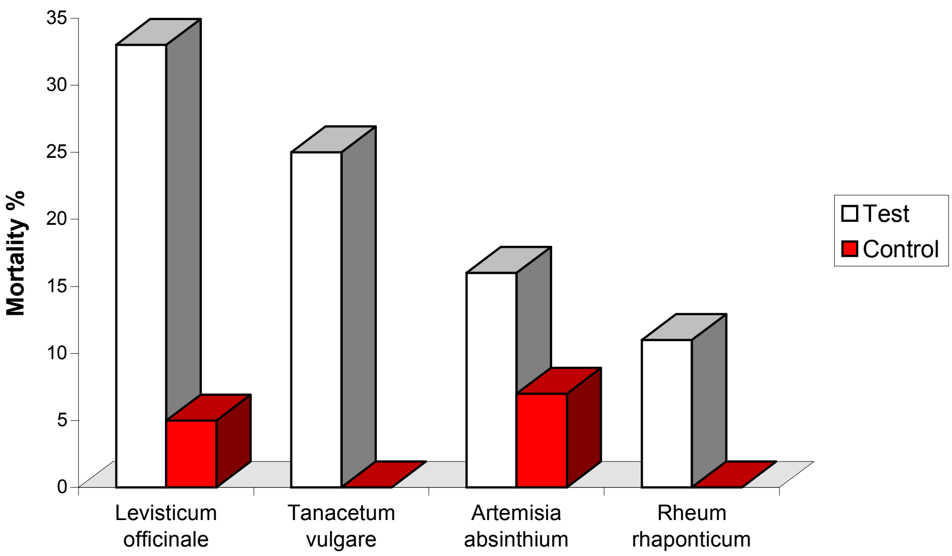

Figure 2. The death rate of the larvae fed on potato leaves treated with hot-water extract ($P<0.05$

Hot-water extract was made from lovage (*Levisticum officinale*), and it became the most effective of all the hot-water extracts. In this variant 33% of the larvae died, which was significantly bigger than in the control variant ($p<0.01$). Lovage contains essential oils (phthalides, terpenes, pinene, carvacrol, pellandrene), coumarins (bergapten, psoralen), and furocoumarins beta-sitosterol, resins, angelic acid (Duke, 1992).

The extract from rhubarb (*Rheum rhaponticum*) was not toxic for the larvae of CPB. There are data in literature saying that water extracts from rhubarb have been employed in repelling several bugs, but there are also warnings that the oxalic acid crystallises out, so the product should be washed thoroughly (Bonhage-Hale, 1999).

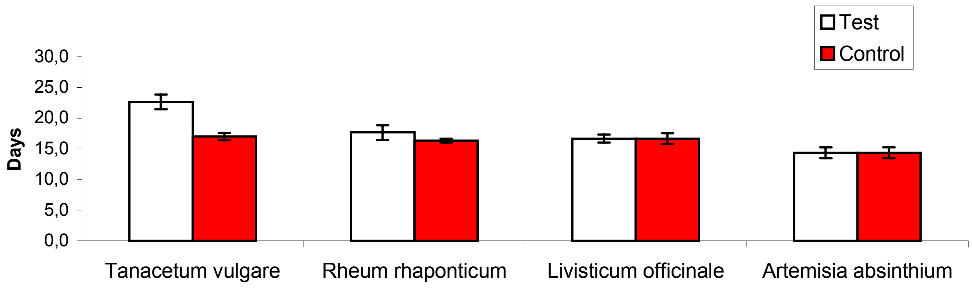

Figure 3. The duration of the larval instar of CPB depending on the water extracts of plants used for treating the food given to the larvae

In the post-ingestive phase plant compounds can influence digestion, nutrient assimilation, growth and reproduction. In the given tests the antifeedant action of the extracts was reflected in the course of larval stage, but the larvae were continually fed with potato leaves dipped into plant extracts (Figure 3). Common tansy slowed down the growth of the larvae (difference from the control: t=03.18 and p=0.024) while rhubarb and absinthe did not, although they revealed a slight toxic effect.

Discussion

Plant stimuli (visual, mechanical, and chemical) can influence animal behaviour positively by attracting them from a distance or by stimulating oviposition and feeding on contact. Negative reactions are triggered by repellents acting at some distance or elicited by antifeedans on contact (Städler, 2000). A means of saving plants from pests would thus be disguising their natural odour with an extraction from a repellent plant, i.e. the odour of another plant having repellent effect on insects. From ecological aspect it would be the most perspective method: by changing the behaviour of the insect it is scared off the plant. The pest is not destroyed, it is only directed into another part of biotope. The present trial was performed in laboratory conditions, where the insects had no choice. They were raised in test boxes and forced to feed on a plant treated with the extract. In such conditions it is impossible to evaluate the repellent action of the extracts.

Since plants contain very different compounds simultaneously, they exert not only repellent but also antifeedant, morphogenetic and toxic effects on insects (Dethier, et al., 1960; Dethier, 1970; Schoonhoven, 1982; Schmutterer, 1992; Luik, 1994; 1997; Kuusik, et al., 1995). The consequences of these effects can be determined in laboratory conditions. The larvae of CPB have contact receptors, (sensillae) which are used for analysing the edibility of a plant. It must be noted that the extracts affected the larvae of CPB both through food and touch while they were moving on a leaf.

Three requirements exist for a substance to be classified as an antifeedant. The insect must prefer the untreated plant to the treated plant. If an insect dies from starvation rather than eats the treated plant, the substance is an absolute antifeedant. The second requirement is that a starving insect should choose any food, treated or untreated. A substance inhibiting feeding only for a defined time is called a relative antifeedant. The third requirement for an antifeedant is to have a toxic action if the insect ingests it (Danielson, 1996). Three modes of action exist for antifeedants (the two main modes of action, by which secondary plant compounds act, are deterrency and toxicity. Deterrents prevent the insect from further feeding by affecting the peripheral nervous system. Toxicants work once digested by the insect by disrupting cellular, biochemical, and physiological processes (Mendel, et al., 1991[a], 1991[b]). Most other insecticidal compounds act by affecting the insects central nervous system (Ortega, et al., 1995; Gonzales-Coloma, et al., 1995). Most frequently available allelochemical compounds to cause antifeedancy are alkaloids flavonoids, terpene, lactones and phenoles (Smith, 1989).

Several plant extracts used in the trials caused a slight toxication, and their consequences were revealed only in later larval instars and/or during the next instars, acting as complementary agents of stress in the populations with especially high numbers of insects.

Acknowledgement

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CALCULATION AND USING OF PHEROMONE COMMUNICATION CHANNEL PARAMETERS FOR OPTIMIZATION OF PHEROMONE DISPENSERS

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1. Introduction

Environmental management, technological and social progress are evidently aspects of sustainable development. Natural and agroecosystems are in continuos changes, influenced both natural and antropogenic factors. Environment monitoring is a process by which we collect information about the current state and resulting trends. Great social and economical changes in post-soviet countries, Estonia included, are known. But remarkable changes in environment technology in Northern Europe have taken place as well. The usage of pesticides has generally decreased and many former agricultural lands are abandoned where the wasteland plant communities are developing. Biodynamic agriculture is in advance. At the same time some climatic tendencies probably connected to the global warming occur, for example higher temperatures and thinner cover of snow in winter. The northern region in Europe and Scandinavia needs effective monitoring facilities.

All the living organisms, including insects, are part of ecosystems. The monitoring of insect populations can yield us information valuable both in basic ecological research and in some applied research as plant protection. We can use different biological characteristics as indicators providing information about ecosystem and the quality of environment. Purposes of monitoring of moth populations can be defined as follows:

1. to evaluate relative diversity of moth fauna in different areas (cultivated lands, orchards, morasses, bushes);
2. to compare species density and spreading in different habitats and/or in different places on some extent;
3. to determine moth species flight dynamics and number of generations;
4. to investigate local and long- distance migration of moths.

Long term-studies are needed to associate the changes in populations with some natural process or human impact.

The pheromone trapping systems can be a valuable method to organise evaluations of the changes in moth populations in natural conditions in a wide scope. By now sex pheromones of about 1500 moth species are identified. Modern methods in integrated plant protection and forest pest management employ synthetic insect pheromones in various ways. Beside of prognosis of pest population activity and timing of pest control methods this includes mass trapping and mating disruption of males to control pests population. Despite of 35-years experience, today the use of pheromone traps for both plant protection and environment monitoring is limited and the need for refined pheromone materials is evident.

However, besides the usage of pheromones in plant protection, some successful trials using pheromones for environment monitoring are described earlier by different researchers. For instance, Kozlov and Haukioja (1993) used the data on population density of *A. podana* in Lipetsk to determine the environment situation in industrial zone and found the positive correlation of *A. podana* population density and air pollution. This suggests, that *A. podana* may have potential as an indicator of air pollution, similar to widespread use of lichens (lichenoindication).

The aim of this paper is to conclude the quantitative studies in moth sex pheromone communication and in dispenser modelling.

2. Pheromone traps and pheromone monitoring

The principle of environment monitoring is to detect the species of interest (indicator species) in certain area and to evaluate some quantitative parameters (relative population density, spreading etc.) of it by means of trap catches. So the result of the pheromone monitoring leads to numerical value: the mean trap catch. The informative value of the method depends on the knowledge about factors that determine the trap catch. All kind of pheromone blends, used for monitoring, should act as attractants. Attractant dispensers used in monitoring systems must provide representative trap catches in natural conditions. Their quality must be constant to get comparable data from place to place and year to year.

Consequently, there are two aspects in creating of pheromone monitoring methods:

1. producing of pheromone materials of higher reproducibility;
2. methods of usage of pheromone traps to get the reproducible results over the long period of monitoring (year to year).
3. The purpose of studying moth pheromone dispensers for monitoring is to get quantitative data about the influence of several factors on the trap catch:
4. the dose of the pheromone blend;
5. the ratio of components in evaporated blend (effluvia);
6. effect of decomposition products of attractants (inhibition, attractivity for other species etc.);
7. the dispenser design - its shape, surface structure etc.
8. The development of quantitative models for pheromone materials enables to prepare attractants and dispensers of certain predicted and controlled attractivity.

3. Concept of pheromone communication channel

It is believed that, during the course of evolution, many animal signals have become very distinctive to avoid ambiguity, and they tend to occupy an information band of a certain width which does not overlap too much with that used by other animals. From this arises the concept of channels of communication (Howse et al, 1998).The channel of communication - in this case chemical - is always of a defined width. That is to say, only certain chemical compounds in certain ratios to which the receiver is selectively sensitive, can carry the message. However, the channel of communication is as much a function of the emitter as it is of receiver.

Experiments with *Pectinophora gossypiella* and *Argyrothaenia velutinana* confirm the assumtion, that the maximally attractive ratio between pheromone components for males is the one that most closely approximates the natural average ratio emitted by females (Löfstedt,1990). The narrow variation in female-produced component ratios contrasts with relatively wider response windows found for male moths by means of trap catch.The lack of species-specifity appears mainly in case of synthetic sex attractants. This can happen, if the component ratio appears to be in the overlapping area for two species. Incoplete blends, where some species isolating compounds are missing, can also attract several species. The complete overlapping of natural pheromone communication channels and the importance of spatial or temporal isolation in prevention of cross-attraction for some *Yponomeut*a spp. is reviewed (Löfstedt,1990).

4. Calculation of pheromone communication channel parameters

The concept of pheromone communication channel can be used to find correlation between the pheromone component ratio and blend attractivity. Having experimental trap catch data for dispensers with different ratios of components (x_i), we applied the Gaussian curve

$$f(x)=\frac{A}{\sigma\sqrt{2\pi}}\exp\left(-\frac{(xi-\mu_i)^2}{2\sigma^2}\right)+y_0 \qquad (1)$$

where μ is central moment and σ is standard deviation, to calculate the optimal content of one compound x_{max} in two-component attractant blend. For our purpose the equation (1) may be written as

$$f(x)=\frac{A}{w_c\sqrt{\frac{\pi}{2}}}\exp\left(-\frac{(x_{\max}-x_i)^2}{w_c^2}\right)+y_0 \qquad (2)$$

where w_c is the width of pheromone communication channel (approximately equal to two standard deviations) and x_{max} is used instead of the central moment of population μ .

The standard deviation may be calculated as

$$w_c = 2\sigma = \sqrt{\frac{\Sigma(x_{\max}-x_i)^2}{\Sigma(y_i)-1}} \qquad (3)$$

where y_i is trap catch if the content of component in effluvia is x_i. Subsequently, the width of pheromone communication channel w_c characterizes the influence of component ratio on trap catches. As seen from equation (1), if the content of a substance is equal to x_{max} ± 0.5 w_c, the trap catch equals $y = e^{-0,5}$ = 0.61 or 61% of maximum trap catch. In case the change of the component ratio in effluvia equals to x_{max} ± 0.2 w_c, the mean trap catches are 10% lower than y_{max}. Evidently, w_c may be used as a measure of pheromone communication channel width (Mõttus et al., 2001b).

5. Variability in pheromone communication within species

We used the calculation method described above to determine the optimal pheromone component ratio and the communication channel width for *Archips podana* Scop. The species is highly variable in fore and hind wing colouration and venation in imagoes (Galetenko, 1964; Razowski, 1977). T. Ivanova showed geographical variability in *A. podana* male genitalia (Ivanova & Mõttus, 1986). Later Kozlov and Motorkin (1987, 1988) and (Safonkin, 1987a, 1987b) described the different number and position of apical (A) and lateral (L) spikes on the aedeagus (A, L, 2L, AL, A2L, 0 as main phenotypes). On the basis of detailed research Kozlov & Esartia (1991) separated Caucasian subspecies of *A. podana* (male's phenotype L), named *A. podana meridiana* Kozl. et Esart. Later Brun et al. (1991) described the population of *A. podana* with males type L as "Corsican variety". Variations or dialects in pheromone communication of *A. podana* were assumed (Ivanova & Mõttus, 1986, Kozlov et al., 1991).

To make clear the need for special attractant blends for different phenotypes, we used trap catch data from Estonia (Uhti and Otepää), north-western Russia (Velikie Luki) and North Caucasus (Krasnodar). A phenotype males dominate in Estonia and north-western Russia, whereas North Caucasian population consists mainly of L phenotype males, see Table 1 (Liblikas et al., 2001).

Table 1. Intraspecific variability of *Archips podana* in 2000 based on the position of spikes on aedeagus. Males trapped by Z11-14:Ac and E11- 14:Ac in ratio 60:40.

Collection locality	Phenotypes, nr. and % ( ) of males				Total
	A	AL	L	0	A+AL+L+0
Krasnodar, Russia, (orchard)	0	1(0.2)	423(89)	52(11)	476
Velikie Luki, Russia, (orchard)	565(69)	87(11)	89(11)	72(9)	813
Velikie Luki, Russia, (forest)	546(73)	66(9)	52(7)	84(11)	748
Pushkin, Russia, (orchard)	8(89)	1(11)	0	0	9
Uhti, Estonia, (orchard)	282(84)	16(5)	16(5)	20(6)	334
Otepää, Estonia, (forest)	337(84)	13(3)	34(8)	20(5)	404

The optimum ratio of 11-tetradecenyl acetate isomers has value 61,3±1,3% of Z-11-14:Ac (Table 2). The results demonstrate the high stability of named parameters in in areas with different dominating aedeagus types and in diverse biotopes. There was no significant correlation between the attractant component ratio and the percentage of A, L, AL and 0 phenotypes in trap catch at $p < 0,05$ and $p < 0,1$ as well (Liblikas et al., 2001).

Table 2. Optimum ratio of 11-tetradecenyl acetate isomers and width of pheromone communication channel of *A. podana* in different regions.

Region	Time	Generation	Biotope	Optimum ratio	Channel width
Velikie luki	1990	I	Orchard	**62.2**	9.3
Krasnodar	1990	I	Orchard	**60.2**	11.2
Krasnodar	1990	II	Orchard	**62.9**	14.1
Estonia, Uhti	1999	I	Orchard	**61.2**	14.3
Estonia, Otepää	1999	I	Forest	**60.0**	9.8
Mean				61.3	11.7

Calculated activity curves for Estonian orchard and forest populations (mainly phenotype A) are given in Figure 1 and for the I and II generation in Krasnodar (mainly phenotype L) in Figure 2. Because of the lack of differences in pheromone communication of *A. podana* in all tested areas, the optimized pheromone dispenser may be used for plant protection (Kolessova, 1993) and environ-ment survey (Kozlov and Haukioja, 1993).

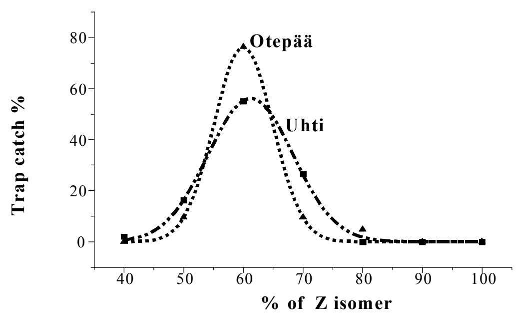

Figure 1. Gaussian fits of trap catch results for experiment at Otepää (forest) and Uhti (orchard) in 1999.

6. Species specifity of attractant blend

The optimum ratio of Z11-14:Ac and E11-14:Ac for three tortricid species, *A. podana*, *Aphelia paleana* Hübner. and *Pandemis chondrillana* was compared (Mõttus et al., 2001b). For *A. paleana* x_{max} =89 % of Z11-14:Ac, w_c =9,6, for and for *P. chondrillana* x_{max} =60 % of Z11-14:Ac, w_c =22,3. The response windows for *A .paleana* and *A. podana* overlap in the region where the content of Z11-14:Ac is 70 - 90%, but the difference between attractivity maxima is 28%. It confirms, that the reproductive isolation of these species may be based on different blend component ratio. The optimised two-component dispensers exhibited no cross-attraction in field tests.

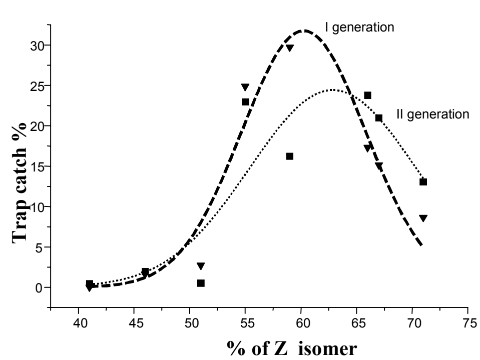

Figure 2. Gaussian fits of trap catch results for experiments at Krasnodar in 1990.

For *A. podana* and *P. chondrillana*, the attractivity maxima are similar, the dispenser having 60% Z11-14:Ac is not species-specific in regions where both species are present (Kolessova et al., 1993). The pheromone of *P. chondrillana* is not identified, the specifity of communication channel may be probably achieved by some additional pheromone components. The dispensers, capturing both *A. podana* and *P. chondrillana*, can be labour-saving in monitoring of wide range. However, if there is only one species of interest or if high sensitivity sampling is needed, the species-specific dispenser is more applicable.

7. Influence of component ratio on trap catch

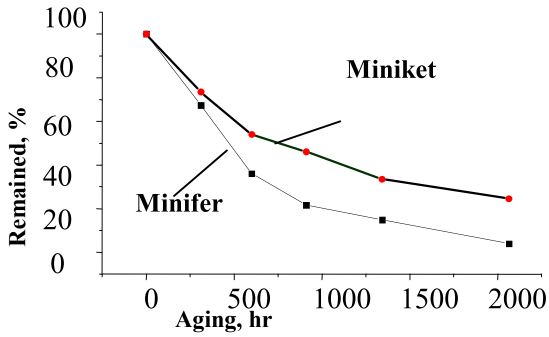

Figure 4. Evaporation dynamics of (I) from Miniket and Minifer dispensers in orchard. Tartu, 1998. Rubber Minifer dispensers ave the first order evaporation.

The of pheromone dispenser has the maximal attractivity, if the ratio of attractant compounds in evaporated blend (effluvia) coincides with the species specific ratio x_{max}. According to equation (1) , if the content of a substance equals to $x_{max} \pm 0.5\ w_c$, the trap catch equals to y = 0.61 or 61% of the maximum trap catch (Figure 3). If the content of a substance equals to $x_{max} \pm 0.2\ w_c$, the mean trap catch diminishes 10%, which is statistically insignificant attractivity loss of dispenser in field tests. The deviation of ± 9 % from optimal ratio can cause significantly lower trap catch, as shown for *A. podana* (Ojarand et al., 2000).

The calculation of communi-cation channel parametres provides to set the limits for pheromone component ratio in effluvia. For example, reasonable attractivity and species specificity for *A. podana* and *A. paleana* can be achieved, in case the monitoring dispenser emits effluvia with accuracy of ± 5 % of Z11-14:Ac (Mõttus et al., 2001b). Most moth species have pheromone compounds with different evaporation rates and the ratio of components in effluvia will change in time.

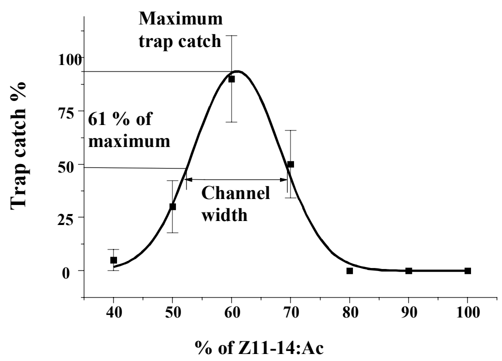

Figure 3. Influence of component ratio on trap catch of *A. podana*

8. Calculation of the emission from dispenser

Emission from the dispenser is affected both by diffusion and evaporation processes and in case the evaporation speed from the surface of dispenser is slower than diffusion to the evaporating surface, thus being the limiting process, the first order process equation

$$c_0 = c_t e^{-kt} \qquad (4)$$

where c_0 is the amount of compound in the dispenser at the beginning of evaporation, *ct* is the amount of compound at time *t* and *k* is evaporation rate constant, may be used to calculate the pheromone is evaporation rate constant, may be used to calculate the pheromone loss (McDonough & Butler, 1987, Mõttus et al., 2000).

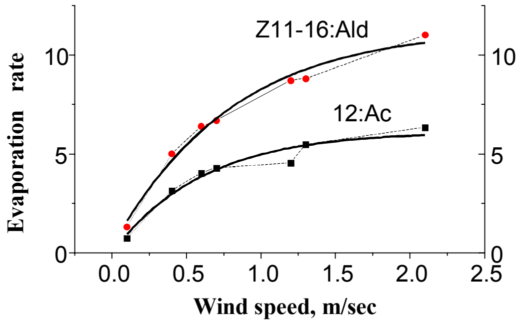

Figure 5. Influence of wind speed on rate constants of 12:Ac and Z11-16:Ald. Feroflor dispenser, at 25 °C

Constant *k* characterises the evaporating compound and it is dependent from wind speed, temperature, surface structure of dispenser and mostly of dispenser design (evaporation area to volume ratio, maximal diffusion way length etc.). In case of the first order process a half of the amount of the substance will be evaporated in time $\tau_{1/2}$, called half-life. Half-life time may be calculated using eq. 4

In case *ct* equals to 0.5 c_0, the equation (5) may be written as

$$\tau_{1/2} = \frac{1}{k}\ln\frac{c_0}{c\tau} = \frac{1}{k}\ln 2 = \frac{0.693}{k} \qquad (5)$$

Equation (5) indicates that the evaporation rate diminishes two times after a half of pheromone is evaporated. It is clear that in effluvia the ratio of substances will change significantly in case the half-life times of component are different more than two times. Evaporation rate constants of Feroflor dispensers have been measured for most alkenols and alkenyl acetates by Mõttus et al*.* (1993).

Equation (4) is valid in case the evaporation from dispensers is not limited by diffusion. It is so for the most dispensers, such as Thomas dispensers, Feroflor dispensers from AS Tartu-Flora or Minifer type dispensers from our laboratory (Mõttus et al*.* 1993, Mõttus et al., 2001a). Miniket dispensers are diffusion limited. This causes lover than the first order evaporation and lesser changes in evaporation rate in time, see Figure 4. This effect is essential, it leads to higher trap catches and equal activity of dispensers for longer ageing period. For instance, more than two-months active period was achieved in case of many-component pheromone blend for *Cydia pomonella* (Mõttus et al, 2001a).

9. Influence of wind speed on evaporation rate

At laboratory of Ecochemistry EAU the influence of wind speed on evaporation rate was measured in special tube. The rise of wind speed from 0.1 m/sec to 0.6 m/sec increased about 6 times the rate constant. Further elevating of wind speed up to 2.5 m/sec caused two times increase in rate.

The wind dependence of rate constant may be assumed to be due of porosity of dispenser's surface. If so, the major changes in rate constant value may be expected at low (<0.5 m/sec) wind speeds. Data in Figure 5 (Mõttus et al., 1993) confirm this assumption.

The wind effect was higher in case of dispensers having smaller volume and large evaporation surface, such as Miniket (weight of 0.15 g) to compare with the larger Feroflor dispensers (weight of 0.15 g). Evaporation constant dependence from wind speed in case of Feroflor dispensers is given in Figure 5. In traps the wind speed exceeds 0.5 – 0.8 m/sec rarely and wind effect does not seems of significant. Dispenser used in open air should have special protection from wind speed influence.

10. Influence of temperature on evaporation rate

Both evaporation and diffusion will accelerate at higher temperature. For diffusion processes the influence of temperature may be written as

$$D = D_0 e^{-\frac{E_0}{RT}} \quad (6)$$

where E_0 is activation energy. Similarly to diffusion the evaporation is accelerated in accordance with Clausius-Clapeyroni equation

$$\frac{d \ln p^0}{dT} = \frac{\Delta H_{vap}(T)}{RT^2} \quad (7)$$

where p^0 is vapour pressure.

Using equation (7) McDonough (1989) yielded the equations to calculate half-life of evaporating substance:

$$\ln(1/k) = \frac{\Delta H_{vap}}{RT} + y_0 \quad (8)$$

where y_0 is constant, ΔH_{vap} vaporisation heat and T temperature. Dependence of vaporisation heat on temperature is complicated. The vaporisation heat for some pheromone components has been measured on the basis of evaporation rates (McDonough, 1989, Olsson et al., 1983, Hiraooka & Suvanai, 1978). Results of different measurements vary greatly and evidently the published data cannot be used to calculate rate constants for ambient temperature in practise. Moreover, assumption, that evaporation rate constant is determined by vaporisation heat only is evidently not correct and changes of diffusion constant D should be regarded. Empirical resolution of the calculations seems more rational.

We assumed that in case of relatively limited temperature changes (Δt <30 °C) the linear dependence the linear dependence of k value on temperature can be expected. Writing equation (8) for two temperatures T_1 and T_2 we obtain

$$\ln(1/k_1) - \ln(1/k_2) = \frac{\Lambda H_{vap}}{RT_1} - \frac{\Lambda H_{vap}}{RT_2} \quad (9)$$

and $k_2 = k_1 c^{\Lambda t \eta}$ (10)

where Δt is difference in temperature and constant η characterizes substance and dispenser.

McDonough et al. (1989) calculated half-lives for many pheromone components. Using experimentally measured initial evaporation rates the first half-life was determined by means of extrapolation. Consequently these results do not regard the diffusion. Calculated by us from results by McDonough et al. (1989) evaporation rate constants k are listed in Table 3.

Table 3. Evaporation rate constants of some alkyl acetates from rubber dispensers at wind speed of 0.2 m/sec. Rate constants are calculated from results by McDonough et al. (1989)

Substance	Rate constant, hr^{-1}		
	15°C	25°C	35°C
10:Ac	0.00362	0.00941	0.0229
12:Ac	0.00045	0.000134	0.0037
14:Ac	0.000056	0.000191	0.000603

Listed rate constants are used to calculate η for 15 - 35 °C (Table 4). Assuming linear temperature dependence of *k* a simple equation may be written

$$k_2 = k_1 \delta^{\Delta t} \qquad (11)$$

where δ = changes of evaporation rate per temperature degree.
Table 12 lists the calculated constants δ and η, and calculated rate constant for *t* = 25 °C on the basis of rate constants for temperature 35 °C.

Table 4. Predicted evaporation rate constants for ambient temperature on the basis of listed in table 11 rate constants for 35 °C.

Substanc e	δ	η	Rate constant (hr-1), 25 °C	
			table 11	eq. 11
10:Ac	1.10	0.09533	0.00941	0.0091
12:Ac	1.11	0.10534	0.00134	0.00136
14:Ac	1.13	0.11874	0.00019	0.000184

Results in Table 4 acknowledge that temperature dependence coefficient δ is relatively permanent for narrow temperature range and can be used for predictive calculations. Table 5 lists the results of our laboratory experiments on temperature dependence of some alkyl acetates for Feroflor dispenser. Calculated coefficient δ is essentially the same as calculated from data by McDonough et al. (1989).Calculated temperature dependence of some alkyl acetates from Feroflor dispenser are given in Figure 6.

Table 5. Temperature dependence of rate constants for some alkyl acetates, Feroflor dispenser. δ is calculated by equation (11)

Substance	Wind speed m/sec	Δ t	ln Δt/ t	δ
12:OH	0.6	14	0.0932	
12:OH	1.3	13	0.0997	
12:OH	2.1	11	0.0965	
Mean			0.0958	1.10
12:Ac	0.1	6	0.102	
12:Ac	0.4	11	0.106	
12:Ac	0.6	14	0.103	
Mean			0.104	1.11
14:Ac	0.1	7	0.109	
14:Ac	0.6	14	0.116	
14:Ac	1.3	12	0.119	
Mean			0.114	1.12

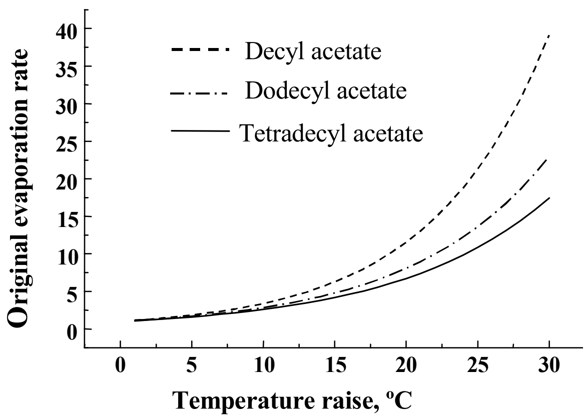

Figure 6 Dependence of evaporation rates of some alkanols from temperature.

11. Conclusions

Calculation of communication channel parameters based on normal distribution is an effective tool in pheromone research and in dispenser modeling. Several aspects in pheromone communication, such as the optimal ratio of components, thecorrelation between the ratio of components and blend attractivity, the species specificity and the geographical variability may be estimated.

Predictive calculation of eva-poration rates of attractant blend components is significant as it allows to determine ratio of attractant components in effluvia. Aging time of attractant dispensers is limited mainly by changing in time ratio of components less than by stock of active ingredients in dispenser. First order evaporation causes two-time changes in component ratio during period approximately equal to the half-life of more volatile compound. Due to diffusion limitation evaporation process is less than the first order. This leads to prolongation of active period of dispensers.

Diffusion limitation leads to the lesser concentration of substances on evaporating surface. This will have significance in case of compounds, which tends to oxidize, polymerize or isomerize, having contact with air or air pollutants. Inside the dispenser using of antioxydants and other stabilizing compounds, the attractant blend components may protected by mean of nonvolatile reactants.

Rise of temperature leads to the loss of substances at day-time; at evening and early morning, the period of elevated activity of many pest moths, amount of substances on evaporating surface is exhausted which leads to diminished concentration of attractant blend and inadvisable ratio of components in effluvia. Decomposition products of pheromone components have often lesser volatility. Consequently, the diminished evaporation of active blend ingredients may lead to higher concentration of inhibiting products in effluvia.

These factors may be cleared, calculated and used for optimization of substrate dispensers. Using described in these paper formulae we have worked out the Miniket dispensers in purpose to achieve higher attractivity and species specificity. As we hope, the optimized dispensers fit better the needs of insect population monitoring for plant protection and environment monitoring.

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USAGE OF PHEROMONE DISPENSERS FOR MONITORING OF MOTHS IN NORTH-EUROPEAN AREA

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Pheromone traps can offer a reliable technique for monitoring and controlling different pest moths in agriculture, forest management and environment monitoring. Traps are used in two different ways:

- to detect and monitor pest population: this allows other methods to be organized more effectively;
- large numbers of pheromone traps can be used to attract and remove as many of the reproductively active males as possible.

In traps a pheromone bait or dispenser is used. The behavior of insects, as they approach, enter, and stop flying close to the bait is affected by the quality of pheromone materials used. Not all attracted insects enter the trap, Zvereva et al. (1991) estimated the trapped amount of *A. podana* as 30 % of those attracted. Beside of maximally high trapping efficiency, the attractant dispenser should have stable attractivity over the flight period of insect. Amount of trapped males, species specificity, and aging of dispensers depend on the load amount of substances and their ratio, amount of degradation products, type of substrate, and other characteristics of dispenser. Due to different evaporation rates, the ratio of attractant components in effluvia will change in time. Consequently, the attractiveness of a dispenser is not constant and the component ratio of the emitted blend differs from the optimal. It is evident that in many cases the dispensers should be designed for specific climate conditions.

In Baltic countries and Russia Feroflor type substrate dispensers from AS Tartu-Flora have been successfully used in last twenty years in different agrotechnologies (Bykhovets et al., 1986, Zolotov et al., 1993; Kolessova et al., 1993, Yemelyanov et al., 1996). The production list of pheromones is summarised in Table 3. To meet needs of new environment technology and biodynamic agriculture, the small-size Minifer and Miniket dispensers were designed and tested for many pest insect pheromone blends. To avoid competition between traps, the used loading of the attractant was minimal. The substrate of Miniket dispensers is a special polymer composition (Mõttus et al., 2000a). In this paper we summarise our results on usage of Feroflor and Miniket dispensers and creating and introducing new pheromone preparations for practical plant protection. Beside of different laboratory tests, all the pheromone dispensers are surveyed in field bioassay experiments.

Table 1. Some sex attractants evaluated by field screening method in Estonia, Byelorussia and in Voronezh and Krasndar regions of Russia

Species	Blend omponents	Ratio	References	Host plants
Eupoeccilia sanguisorbana H-S*	Z11-14:Ac E11-14:Ac 12:Ac	0.7 0.3 1.5	Ryabtcinskaya et al, 1986	*Sanguisorba officinalis* L.
Aleima loeflingiana L.*	Z11-14:Ac E11-14:Ac	0.2-0.3 0.8-0.7	Ivanova et al., 1986	*Quercus* L.
Arhips lafauriana Bag*	Z11-14:OH	1.0	Ivanova et al., 1986	polyphagus
Choristoneura diversana Hbn *	Z11-14:Ac 12:Ac	1 1-2	Ryabtcinskaya et al, 1986; Ivanova et al., 1986	Rosacea, *Corylus* L
Thiodia caradjana Kenn	Z11-14:Ac E11-14:Ac Z9-12:Ac	0.9 0.1 0.2	Ryabtcinskaya et al, 1986	Compositae
Eucosma cumulana Gn	Z8-12:Ac (E,E)-8,10-12:Ac	0.6 0.4	Ryabtcinskaya et al, 1986	*Inula salicina* L.
Gypsonoma nitidulana L.*	Z11-14:OH Z11-14:Ac E11-14:Ac	0.1 0.9 0.1	Ryabtcinskaya et al, 1986	*Populus* L.
G. oppressana Tr.	Z11-14:Ac E11-14:Ac	0.2 0.8	Ryabtcinskaya et al, 1986	*Populus* L.
G. dealbana Fröl.	Z8-12:Ac (E,E) 8,10-12:Ac E11-14:Ac	0.4 0.6 0.2	Ryabtcinskaya et al, 1986	*Betula* L. *Corylus* L
Cydia medica-ginis Kuzn*	(E,E) 8,10-12:Ac	1.0	Tchmyr & Mõttus, 1982	*Medicago* L.
Cydia (Grapholita) compositella F*	(E,E-)6,8-10:Ald Z8-10:Ac	0.9 0.1	Tchmyr et al., 1981	*Medicago* L. *Trifolium* L.
Grapholita delineana Walk.*	(E,E-)6,8-10:Ald	1.0	Mõttus et al., 1985	*Cannabis* L.
Pammen	Z8-12:Ac	1.0	Ryabtcinskaya et al,	*Acer* L.

e germana Hbn			1986	*Crataegus* L.
Dicrorampa petiverella L.*	Z11-14:Ac E11-14:Ac	0.2 0.8	Bykhovets et al, 1986	*Achillea* L. *Tanacetum* L.

- The tested blend had attractivity similar to pheromone preparations and was used for monitoring population density. In most cases, the blend compositions are optimized.

The field screening has been a successful tool to find pheromone type attractants as well. For some moths, attractive blends are optimised using this method. In 1980 – 1990, a co-ordinated field-screening project was accomplished in the European part of the former Soviet Union. Most of the results were published in different local journals in Russian and were therefore not reviewed by abstract journals and by Pherolist. Table 1 lists some attractive blends ascertained by research scientists of our laboratory, participating in this project.

Leaf rollers (Tortricidae) are well known pests of orchard ecosystems, occurring annually, frequently giving mass outbreaks. Different species vary in voltinism, trophic relations and larval habitats, being harmful during the whole period of apple vegetation. *Hedya nubiferana* Hawk., *Spilonota ocellana* F., *Ptycholoma lecheana* L. and *Choristoneura diversana* Hbn. damage burst buds, leaf and flower trusses. Maximal population density of their larvae is found on apple trees of autumn varieties in phenophase of blossoms bursting. They may have economical significance in named region sporadically, in case of outbreaks. In regions close to Estonia, for instance Lithuania, Byelorussia, and Pskov district of Russia, significantly higher population densities of these moths are noted (Bykhovets et al., 1986). In Estonia they may be surveyed as an indicator factor of biodiversirty as it may be expected that population density of these moths depends on climate and human activity, which was the reason to test the pheromone dispensers (Nikolaeva, 1998; Yemelyanov and Nikolaeva, 1997, Yemeyanov et al., 1996).

H. nubiferana and ***E. foenella*** are often trapped by the same blend. Data in Table 2 demonstrate that in case of optimized dispensers the selectivity of blends will be higher, and as we hope, in case of controlled release rate of blend components, practically full species selectivity of dispensers may be achieved.

Table 2. Tested Miniket type dispensers for *Hedya nubiferana* and *Epiblema foenella.* Orchard at Uhti, 1998

Dispenser code	Blend components (mg)			Insects per trap	
	12:OH	E8,E19-12:Ac	E8-12:Ac	*Epiblema foenella*	*Hedya nubiferana*
EPF	0,5	0,15	0,35	22.4	0.2
HNM2	0,5	0,25	0,25	7.8	5.6
HNM	0,5	0,35	0,15	5.2	8.4

Pheromone preparations for ***C. diversana, S. ocellana*** and ***P. lecheana*** are tested for pest monitoring in orchards and included for regular use, see in Table 3.

The ***Codling moth, Cydia pomonella*** L. is the most important pest of the period of fruit growing and development, inhabiting up to 19-20 % of apple fruits of earlier and middle varieties (Yemelyanov, 1995). Species diversity, acyclic development and unexpected mass outbreaks create difficulties in forecasting their density and timing of treatments. Development of pheromone methods enabled to get additional information on biology and population density of orchard moths and to use the collected information for ecological pest management. Application of codling moth and leaf rollers sex attractants begun in 1978 in and a number of publications have concluded the research (Yemelyanov et al., 1996; Mõttus et al., 1997; Yemelyanov et al., 1998; Yemelyanov and Bulyginskaya, Yemelyanov et al., 2000, Kolessova et al., 1993).

In areas where the *C. pomonella*, has one generation, the control of pest insects can sometimes be achieved by mass trapping methods. To control the *C. pomonella* population, pheromone traps were used in an isolated apple orchard (Madsen et al., 1976). In Estonia, Leivategija (1982) introduced this method to many small private orchards after a successful demonstration of the technique. The pheromone kits, consisting of Feroflor dispensers, available from AS Tartu-Flora, Estonia were used. The mass trapping method was introduced in Velikie Luki, in the region with climate closer to that of Estonia (Yemelyanov et al., 2000). Eleven compounds were identified in the pheromone blend of *C. pomonella* (Causse, 1988). Despite repeated identification by several investigators, the mood of activity of pheromone blend components is not clear and McDonough et al. (1993) considered the only active compound to be (E,E)-8,10-dodecadienol (I). Arn et al. (1985) have published experimental data on navigation activity of dodecan-1-ol (12:OH). In Minifer dispenser, a mixture of (I) and 12:OH was used in two different ratios. We discovered that the Miniket UMD dispenser, loaded with the two-component attractant blend, had elevated attractivity to *C. pomonella*, compared with Feroflor dispensers containing the two components or only (I).

The ***Pea moth, Cydia nigricana*** F., uses (E,E)-8,10-dodecadien-1-yl (II) acetate as the main component of female sex pheromone (Greenway, 1984; Witzgall et al., 1993). Male attraction to synthetic E8-E10-12Ac declines after a few days in the field due to isomerisation, 5 % of isomeric impurities caused significant inhibition (Witzgall et al., 1993). The isomerisation of conjugated dienes in dispensers is influenced by the substrate used. There is no evidence of any substrate suppressing isomerisation sufficiently in field conditions, but a propheromone compound, [(8,9,10,11-n)- E8-E10-12Ac] iron, releases isomerically pure (II) (Streinz et al.,1993, Witzgall et al., 1996).

A pheromone mimic, (E)10-dodecenyl acetate, was recommended to monitor *C . nigricana* (Macaulay et al., 1977). This compound is less attractive than E8-E10-12Ac, but ensures constant attraction over the whole flight period. According to Tuovinen (1982), it can be used for the timing of a spray in Finland, but the correlation between total catch and damaged peas was not strong enough for prognosis. High-dosed Feroflor HM dispensers, loaded with 3 mg of (II) have been found to be suitable for indication of economic threshold and timing of sprays (Tschmyr, 1981; 1985) in Voronezh region, Russia.

The blend of (II) and the false isomers of (II) (30 g/ha) was an effective mating disruptant in Sweden. Males were observed to fly out of the treated field and their attraction to calling females in the cages was almost entirely suppressed. Larval infestation decreased from 36 % to 2 % , if the immigration of mated females from other fields was prevented (Bengtsson et al., 1994; Witzgall et al., 1996).

Different sex attractant preparations containing Z-11-hexadecenyl acetate (Z11-16:Ac) and Z-13-octadecenyl acetate (Z13-18:Ac) in variable ratios have been used to determine the population density of the **Apple fruit moth*, Argyrestia conjugella*** Zell. Female pheromone has not been identified, attractant was ascertained by field screening (Booij et al, 1984). On the basis of many-year experiments in Finland, Tuovinen (1991) concluded that the attractant preparation, which contains Z11-16:Ac and Z13-18:Ac, is not specific enough for growers' use, but it may be used by trained personnel to determine the need of sprays. AS Flora produces Feroflor type dispensers of different dose and composition for *A. conjugella*. Feroflor CA-71, having load ratio of Z13-18:Ac and Z11-16:Ac of 3:7, was used widely in Estonia (Mõttus et al., 1996). In field tests with the small-size Miniket type dispensers, 1:1 blend had the highest attractivity (Mõttus et al., 1999a). At the same time, the need of identification of *A. conjugella* pheromone blend is evident, none of the tested mixtures had attractivity similar to a female pheromone.

In regions with two generations the **Large fruit-tree tortrix*, Archips podana*** Sc., is considered to be an important pest (Persoons et al., 1974), in regions of one generation, it may cause damage sporadically (Paternotte, 1999). Lately it was demonstrated that pheromone traps offer a worthwhile method of investigating *A. podana* population densities and other biologically interesting correlations resulting from the effect of human activity or changes in climate (Kozlov and Haukioja, 1993). Sex pheromone of *A. podana* was identified by Persoons et al. (1974) as 1:1 mixture of Z11-14Ac and E11-14:Ac. The optimum ratio of Z11-14Ac and E11-14:Ac in pheromone blend in Byelorussia (Bykhovets et al., 1986), Central Russia (Ryabtchinskaya et al., 1986), and in Carpathian region (Safonkin, 1990) has been described as 6:4 of Z/E. The Gaussian normal distribution curve was used for calculation of the optimal ratio of compounds in the latest experiments in Estonia. Calculated values of optimal content of Z11-14:Ac for *A. podana* in two different habitats were close, being respectively 58.6 % and 60.0 % (Ojarand et al., 2000). Our field experiments are aimed of optimizing the dispenser and of using pheromone methods for plant protection and monitoring climate changes.

The **Currant shoot borer**, ***Lampronia*** (***Incurvaria) capitella*** Cl., *is* a significant pest of red and white currant, causing damage to its pods. On the basis of our experiments, we estimated the amount of trapped male insects to be about about 50 000 per hectare. Severe damage may be caused and in Estonia some red currant plantations were cut off due to too high population of *L. capitella*. It is considered a harmful pest in neighboring areas as well (Samersov, 1998, Tuovinen, 1997).

The **Antler moth, *Cerapteryx graminis*** L., (Noctuidae, Hadeninae), is a Holoarctic species with the southern range limit of about 45° N (Balachowsky, 1972). The species has one generation per year and its larvae feed on almost all species of grasses. Infestation of extensive meadows and pastureland, leads to a decline in grassland productivity (Zolk, 1931). In Estonia, outbreaks of *C. graminis* are described in 1936 and in 1980 – 1990.

There are two known imaginal forms and some variable color morphs of *C. graminis* (Forster and Wohlfahrt, 1971, Pyöronilä et al., 1979). Maercks (1943) and Schenker (1950, 1956) described the life history of *C. graminis*, but there is little scientific understanding what factors cause antler moth outbreaks.

One reason for higher population, as we consume, may be higher rainfalls during the period of feeding and pupating of *C. graminis*. The monitoring of *C. graminis* may have some importance because the influence of environmental changes on its population, as it occurred in 1980s in Sudeten Mountains, Poland (Klukowski, 1993), and in Denmark (Berris and Graveland, 1988). Danell and Ericson (1990) have concluded that in northern latitudes, the larval density was negatively correlated with the mean temperature in June. The sex pheromone of *C. graminis* has not been identified, field tests have shown the attractivity of Z11-16:Ac (Mõttus et al, 1981) and 1:1 mixture of Z11-16:Ald and Z11-16:Ac (Priesner,1986). The attractant blend was optimised in experiments in Estonia and Poland as 7: 3 – 4:6 of Z11-16:Ac and Z11-16:Ald (Mõttus et al.,1998).

The **Timothy tortrix*, Aphelia paleana*** Hbn., is cued to many *Pocaceae* (*Gramineae*) and its economical significance is not known. Mixture of Z11-14:Ac and E11-14:Ac (9:1) is attractive to *A. paleana* (Booij and Voerman, 1984). This mixture had the highest attractivity for *A. paleana* in Krasnodar Territory (Ivanova et al., 1986). In a detailed study, it was demonstrated that the pheromone communication channel for *A. paleana* was narrower than that for *A. podana* and the attractant dispensers should have component ratio accuracy better than ±3% (Mõttus et al., 2001).

There are not many **cruciferous pest insects** having long distance attractant pheromones. Consisting Z11-16:Ac dispenser Pheroflor MB2 for the **Cabbage moth, Mamestra brassicae** L. has been successfully tested together with other blends for Noctuidae (Zolotov and Mõttus, 1985).

Economically more impotant **Plutella xylostella** L., the **Diamondback moth**, has, in recent years, caused economic damage to cabbage and some other cruciferous plants, such as oilseed rape, in Estonia and measures to control the pest are needed (Mõttus et al, 1997). It has one generation per year. How it overwinters successfully in temperatures below 0 ^{0}C for 5 - 6 months (November - April) is not clear. In Estonia, pheromone traps usually start catching male P. xylostella in early June, when cabbage plants are transplanted to fields

The pheromone blend of *P. xylostella* has been identified by Tamaki et al. (1977) as mixture of (Z)-11-hexadecen-yl acetate (Z11-16:Ac) and (Z)-11-hexadecenal (Z11-16:Ald). In our practice small dispensers were more attractive to compare with Feroflor type dispensers (Mõttus 1998).

Control of the **Currant-clearwing, *Synanthedon tipuliformis*** Cl., in red, white, and black currants is difficult due to the larvae hidden in the wood and the short flight period (2–3 weeks) of adults. A mixture of 2E,13Z -18:Ac and 3E,13Z-18:Ac in a ratio of 100:3 at doses of 0.010-1.0 mg is recommended for species-specific field monitoring of *S. tipuliformis* in Hungary and New Zealand (Szocs et al., 1998).

Mass trapping using of 25 traps/ha decreased larval damage by 50 % and increased black currant yield by 1.56 t/ha in Byelorussia (Samersov et al., 1998). In Velikie Luki *S. tipuliformis* was considered an economically significant pest and Feroflor type dispensers have been successfully used for population survey (Yemelyanov and

Nikolaeva, 1996). In Lithuania, investigation of 16 blackcurrant plantations in 1989–91 showed that *S. tipuliformis* damaged up to 1% of branches in young bushes not yet producing berries and 1– 63% (average 13%) of those producing berries (separate branches were regarded as those commencing no higher than 30 cm above the ground). Normally, there was 1 larva per branch, while 2 larvae occurred less often and 3 only when over 20% of branches were damaged; 3 being considered a suitable criterion of heavy infestation. Study of seasonal dynamics using pheromone traps showed that the time of commencement of flight varied by 2 weeks according to weather conditions and most adults flew in the last 10 days of June (Buda, 1993).

As a result of 30 years research the pheromone methods have been introduced into practice of agricultural technology in Russia and Baltic States. Pheromone dispensers of more than 30 different insects have been tested and recommended for monitoring and control of insect population (see Table 3) during this period.

Further coordinated research has aim to work out dispensers of higher quality and to pay more attention on Noctuidae and Geometridae.

Table 3. List of insects, having significance for plant protection and environment monitoring. The listed dispensers are produced by AS Tartu-Flora (Feroflor type dispensers) or available from Laboratory of Ecochemistry (Miniferr type dispensers)

Insecta	Dispenser type	
	Minifer	Feroflor
Adoxophyes orana	*ADO*	AO
Agrotis segetum	----	CO
Agrotis exclamationis	in progress	CE
Aphelia paleana	*PAL3*	----
Archips lafauriana	LAF	----
Archips podana	**POD**	AP
Argyresthia cojugella	ARC	CA
Archips rosana	**ARO**	AR
Autographa gamma	----	AG
Cerapteryx graminis	CER	----
Choristoneura diversana	DIV	----
Clepsis spectrana	CLS	----
Croesia holmiana	CRH	----
Cydia nigricana	in progress	HM
Cydia pomonella	UMD	CP
Dichrorampa petiverella	DIP	----
Enarmonia formosana	ENF	EP
Epiblema foenella	EPF	----
Eulia ministrana	**MIN**	----
Grapholitha funebrana	GRF	GF
Hedya dimidiana	HDI	----
Hedya nubiferana	HNM	XH
Heliothis armigera	in progress	XC
Lampronia capitella	LNC	----
Lobesia botrana	----	KM
Mamestra brassicae	MBR	MB
Mamestra thalassina	MTH	----

Table 3 *(cont.)*

Insecta	Dispenser type	
	Minifer	Feroflor
Pandemis heparana	**HEP**	PH
Phalonidia manniana	MAN	----
Plutella xylostella	PLX	PM
Ptycholoma lecheana	LEC	----
Rhopobota naevana	RON	----
Spilonota ocellana	----	SO
Synanthedon tipuliformis	----	ST
Synanthedon myopaeformis	----	SM
Syndemis musculana	SYM	----
Tortrix viridana	TV1	TB

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WIRKSAMKEIT VON NEEM AZAL TS GEGEN DIE KIRSCHBLÜTENMOTTE (*ARGYRESTHIA PRUNIELLA* L.) IM RAHMEN DER BEKÄMPFUNG DES FROSTSPANNERS (*OPEROPTHERA BRUMATA* L.) IN SAUERKIRSCHEN

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Die Kirschblütenmotte (*Argyresthia pruniella L.*) konnte in den letzten Jahren regional in sächsischen Sauerkirschanlagen als ein bedeutender Schädling festgestellt werden. Ohne entsprechende Bekämpfungsmaßnahmen sind Ertragsausfälle bis über 80 % möglich. Die Fraß-schäden sind bei flüchtiger Betrachtung relativ unscheinbar und können dadurch leicht übersehen werden. So kam es sicher auch schon in früheren Jahren vereinzelt zu einem stärkeren Befall. Der Schaden wurde dann jedoch häufig anderen Ursachen zugeschrieben (z. B. Blütenfrostschäden).

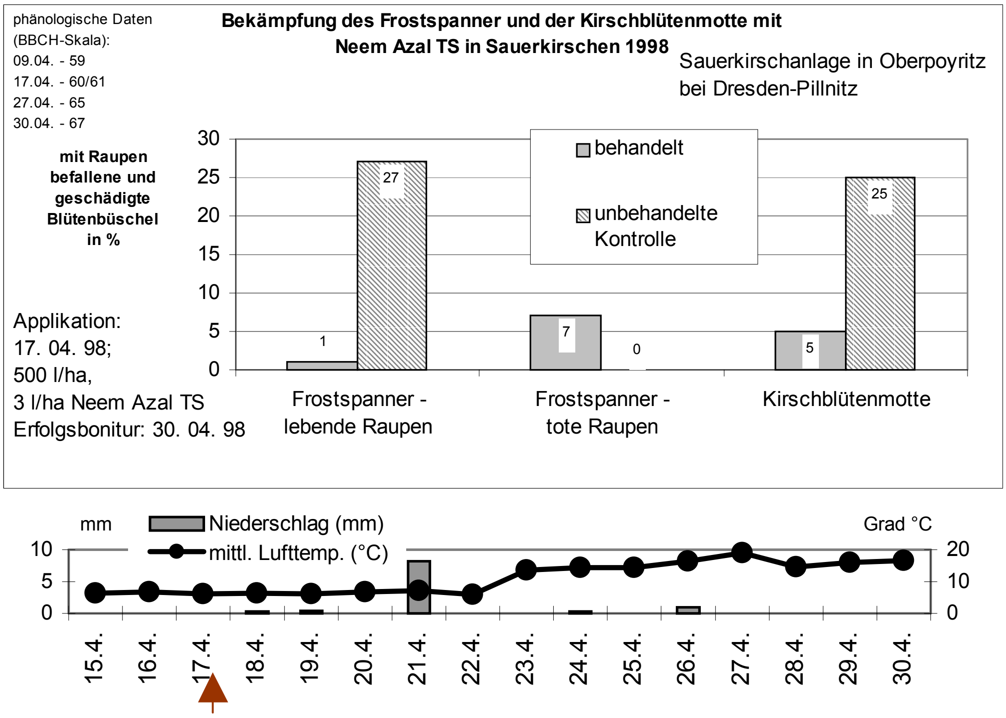
In der Regel wird die Kirschblütenmotte bei Bekämpfungsmaßnamen gegen den Frostspanner gut miterfaßt. In einigen Fällen konnte sie aber auch schon als alleiniger Hauptschädiger festgestellt werden.

Die gezielte Überwachung ist relativ schwierig. Die Eiablage erfolgt sehr versteckt in Rindenritzen und unter aufgeplatzter Rinde. Vorbeugende Austriebsspritzungen mit Mineralölpräparaten bleiben dadurch weitgehend wirkungslos. Der Raupenfraß an den Blüten- und Blattknospen beginnt sehr unscheinbar zur Zeit des Knospenschwellens. Der Falterflung kann im Sommer mit Pheromonfallen überwacht werden.

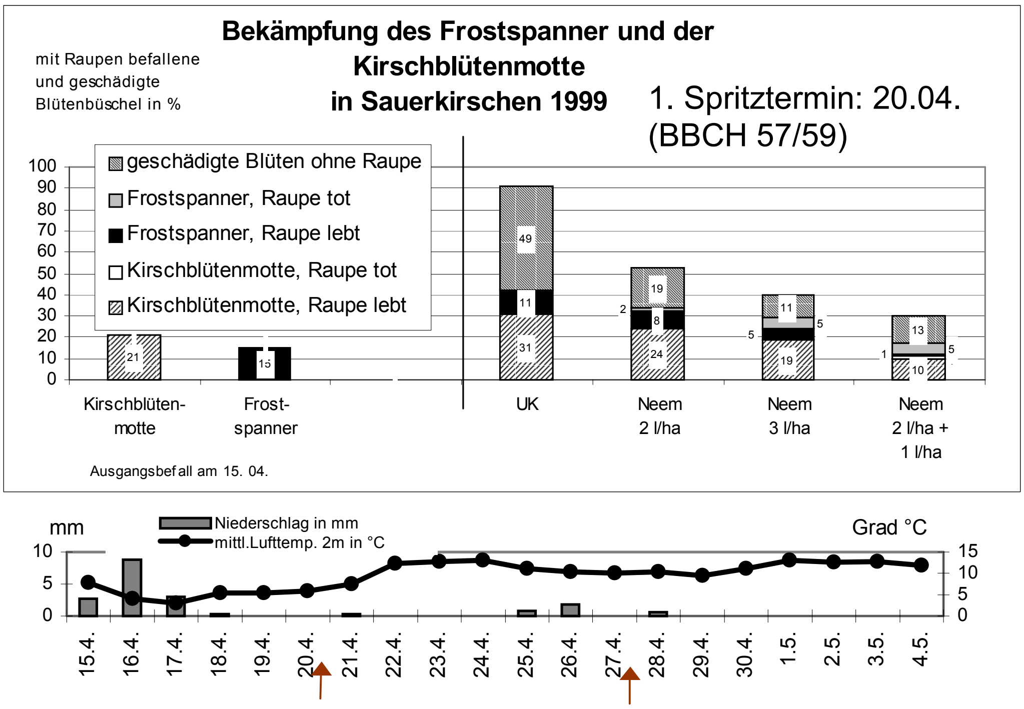
In Sachsen fiel nach den bisherigen Beobachtungen die Raupenaktivität annähernd mit der des Frostspanners zusammen. So schlüpften die ersten Räupchen 1999 in Dresden-Pillnitz in der Zeit vom 25. bis 29.03., knapp eine Woche vor den ersten Frostspannerraupen.

Neem Azal TS wurde 1998 und 1999 versuchsmäßig in einer Sauerkirschanlage in Oberpoyritz bei Dresden erfolgreich gegen den Frostspanner erprobt. Dabei konnte über beide Jahre eine gute bis sehr gute Nebenwirkung gegen die Raupen der Kirschblütenmotte festgestellt werden. Der Spritztermin richtete sich in beiden Jahren nach der Bonitur auf den Hauptschlupf der Frostspannerraupen (1998 BBCH 57/59 - ‚Rote Knospe' bis ‚Ballonstadium', 1999 BBCH 60/61 - ‚Erste Blüten offen' bis ‚Beginn der Blüte'). 1998 konnte mit einer einmaligen Applikation mit 3l/ha Neem Azal TS beim Frostspanner ein Wirkungsgrad von 96% und bei der Kirschblütenmotte von 80% erzielt werden (Grafik 1). 1999 wurden 3 Varianten erprobt (siehe Grafik 2). Die Variante mit 2 Applikationsterminen (2 l/ha + 1 l/ha) zeigte dabei sowohl beim Frostspanner (ca. 90% WG) als auch bei der Kirschblütenmotte (ca. 68% WG) die besten Ergebnisse. Offensichtlich wurde die sich in diesem Jahr über einen etwas längeren Zeitraum hinziehende Schlupfperiode bei beiden Schädlingen durch die Splittingvariante optimaler abgedeckt.

Entsprechend der spezifischen Wirkungsweise von Neem Azal TS ist bei starkem bis sehr starkem Befall (wie in der Versuchsanlage) der Blütenschaden nicht ganz zu verhindern. Gemessen an der Befallsentwicklung in der unbehandelten Kontrolle konnte er jedoch in beiden Jahren spürbar reduziert werden. Bei großflächiger Behandlung ist damit eine sehr wirksame Verminderung der Population für das Folgejahr möglich.



Grafik 1: Bekämpfungsversuch 1998



Grafik 2: Bekämpfungsversuch 1999

POSSIBILITIES TO INFLUENCE MATURATION FEEDING OF THE LARGE PINE WEEVIL, *HYLOBIUS ABIETIS* L., WITH PLANT EXTRACTS AND NEEM PREPARATIONS

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The effect of water extracts of 20 plant species and neem preparations NeemAzal-T and NeemAzal-T/S (Trifolio-M GmbH, Germany) on the maturation feeding of Hylobius abietis *L. was studied in laboratory and field experiments. In laboratory tests, water extracts of* Allium sativum, Taxus baccata, Heracleum sosnowskyi, Primula veris, Urtica dioica, Achillea millefolium, Colchicum autumnale, Phaseolus vulgaris *and* Tussilago farfara *decreased the feeding activity of the large pine weevil significantly. In field conditions, only extracts of* Taxus baccata *and* Primula veris *displayed a short-term repellent effect. The repellent influence of neem preparations lasted during the whole vegetation period, however, at higher concentrations (20 %) the preparations had some phytotoxic effect on conifer seedlings.*

Key words: Hylobius abietis*, plant extracts, neem preparations, maturation feeding, Estonia.*

Introduction

The large pine weevil, *Hylobius abietis* (L.) (Coleoptera, Curculionidae) is the most destructive and economically important pest inhibiting regeneration of coniferous forest in Europe. Over time, various methods and measures from ultratoxic chlorine and phosphorus organic insecticides to expensive labour-consuming mechanical protectors as well as various kinds of traps have been applied to control pine weevils, or to reduce the damage caused by them (Eckstein, 1915; Lindström et al., 1985; Nordlander, 1987; Eidmann, von Sydow, 1989; Glowacka et al., 1991; Voolma, 1994; Hagner, Jonsson, 1995; Zumr, Stary, 1995; Eidmann et al., 1996; Örlander, Nilsson, 1999; Watson, 1999, etc.). To minimize use of pesticides and to avoid pollution of the environment, natural antifeedants and deterrent and repellent substances have been searched for pest control during recent decades (Schmutterer, 1992; Salom et al., 1994; Lindgren et al., 1996; Luik, 1997; Klepzig, Schlyter, 1999; Govindachari et al., 2000). By treating conifer seedlings with such substances, it is possible to disguise fragrances attracting pine weevils, or to dispel the pests from cultivated plants.

Within the last five years the effect of altogether 20 water extracts of native Estonian or introduced plants as well as the neem preparations NeemAzal-T (5% Azadirachtin) and NeemAzal-T/S (1% Azadirachtin) (Trifolio-M GmbH, Germany) on the maturation feeding behaviour of the large pine weevil has been studied both in laboratory and natural conditions at the Estonian Agricultural University (Luik et al., 1995, 1998, 2000). The aim of this paper was to present the results of these experiments.

Material and methods

For laboratory experiments, adults of *H. abietis* were collected from trapping pitfalls in a fresh clearcutting area in the forest district of Räpina, Southern Estonia. Scots pine twigs 10 cm long and 5 mm in diameter were halved; one half was treated with a plant extract or neem emulsion, and the other one remained untreated as control for comparison. One treated and one untreated twig were placed each on a separate moistened paper sheet (to prevent contact between twigs) in a petri dish. One weevil, starved for 12 h, was placed in each dish (100x15 mm). The petri dishes were exposed to natural light conditions at a temperature of +20±1 °C. In each variant 35–40 weevils were used, while each specimen was tested only one time. The area of feeding traces left by weevils on the bark surface of twigs was measured, using transparent scale-paper, after 24 h, 48 h and 72 h. All treatments were performed in duplicate. The sex of the beetles used in the experiments was established for the estimation of feeding differences between the sexes.

For plant extracts, raw leaves (in case of garlic *Allium sativum*, cloves) of cowslip *Primula veris*, garden rhubarb *Rheum rhapunticum*, red elder *Sambucus racemosa*, marsh ledum *Ledum palustre*, corn mayweed *Matricaria perforata*, Sosnowskyi cow parsnip *Heracleum sosnowskyi*, yarrow *Achillea millefolium*, yew *Taxus baccata*, stinging nettle *Urtica dioica*, haricot bean *Phaseolus vulgaris*, coltsfoot *Tussilago farfara*, common tansy *Tanacetum vulgare*, bird cherry *Prunus padus*, greater celandine *Chelidonium majus*, lily of the valley *Convallaria majalis*, horse chestnut *Aesculus hippocastanum*, meadow saffron *Colchicum autumnale*, asarabacca *Asarum europaeum,* and poet's narcissus *Narcissus poeticus* were ground and exposed in water for 24 h. The content of plant material formed 20% of the extracts. After filtration, the extracts were used for dipping of pine twigs for 5 seconds. After dipping each pine twig was dried for 5 seconds on filter paper and was then placed, together with an untreated control twig, in a petri dish.

Feeding deterring extracts, prepared in the laboratory, were tested in field conditions by treating Norway spruce seedlings with them. Differently treated three-year-old seedlings were planted in randomised blocks (each of the 40 blocks contained differently treated seedlings and an untreated control) in a fresh clear-cut area. The number of seedlings damaged by weevils was established with weekly intervals during the vegetation period.

Results and discussion

In laboratory conditions, all tested plant extracts and neem emulsions modified to a certain extent the maturation feeding intensity of the pine weevil, while males were more susceptible to extracts than females.

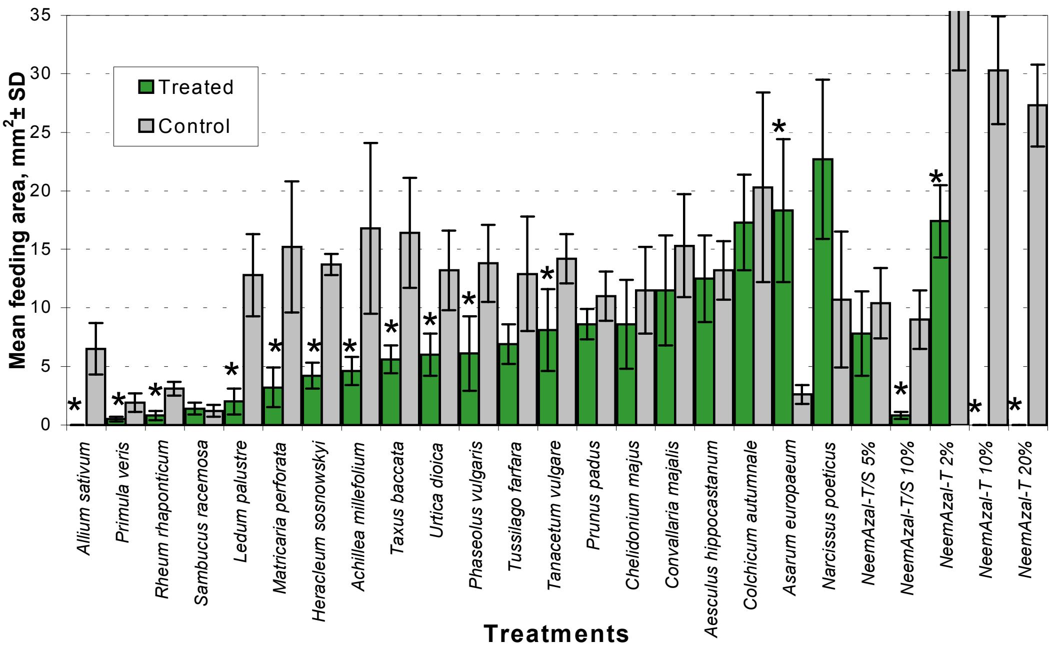
In laboratory conditions, 20% of the water extracts of *Allium sativum, Taxus baccata, Heracleum sosnowskyi, Primula veris, Urtica dioica, Achillea millefolium, Colchicum autumnale, Phaseolus vulgaris, Tussilago farfara* reduced significantly the feeding activity of the large pine weevil. Treatments with the extracts of *Matricaria perforata, Sambucus racemosa, Rheum rhaponticum* had a minor effect and *Aesculus hippocastanum* did not have any effect on feeding activity.

The extracts of *Allium sativum, Taxus baccata, Ledum palustre, Primula veris, Heracleum sosnowskyi, Phaseolus vulgaris* and *Tussilago farfara* acted as antifeedants for the short term (24–72 h) in laboratory conditions, while the feeding activity of males was more inhibited than that of females.

General feeding level in laboratory conditions was low with the use of *Prunus padus* extract. The extracts of *Chelidonium majus, Asarum europaeum, Narcissus poeticus, Convallaria majalis, Phaseolus vulgaris* and *Tussilago farfara* acted as feeding stimulants.

NeemAzal-T and NeemAzal-T/S exerted an antifeedant and a repellent effect on the pine weevil in laboratory conditions in all treatment variants, respectively (Figs. 1–3). The general smell environment of NeemAzal-T increased pine weevils' feeding intensity, while direct exposure to this substance inhibited it.

A



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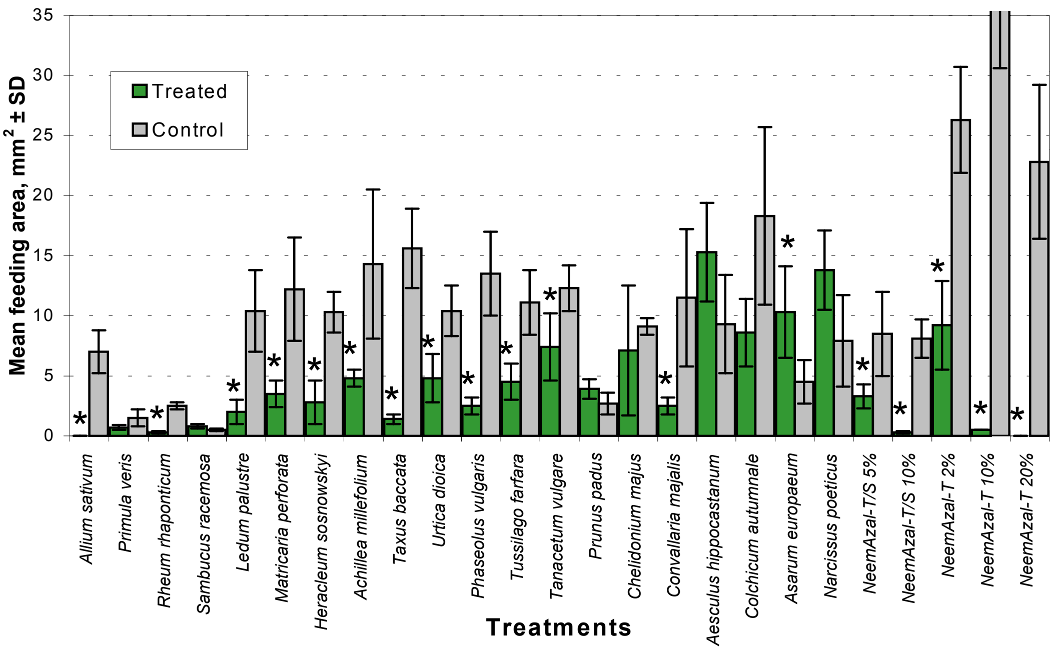


Fig. 1. Mean (± SD) feeding area (mm^2) of females (A) and males (B) of *Hylobius abietis* during 24 hours in pine twigs treated with different plant extracts and neem preparations.

* Significant difference between treated and untreated control twigs, Student t-test, $p<0.05$

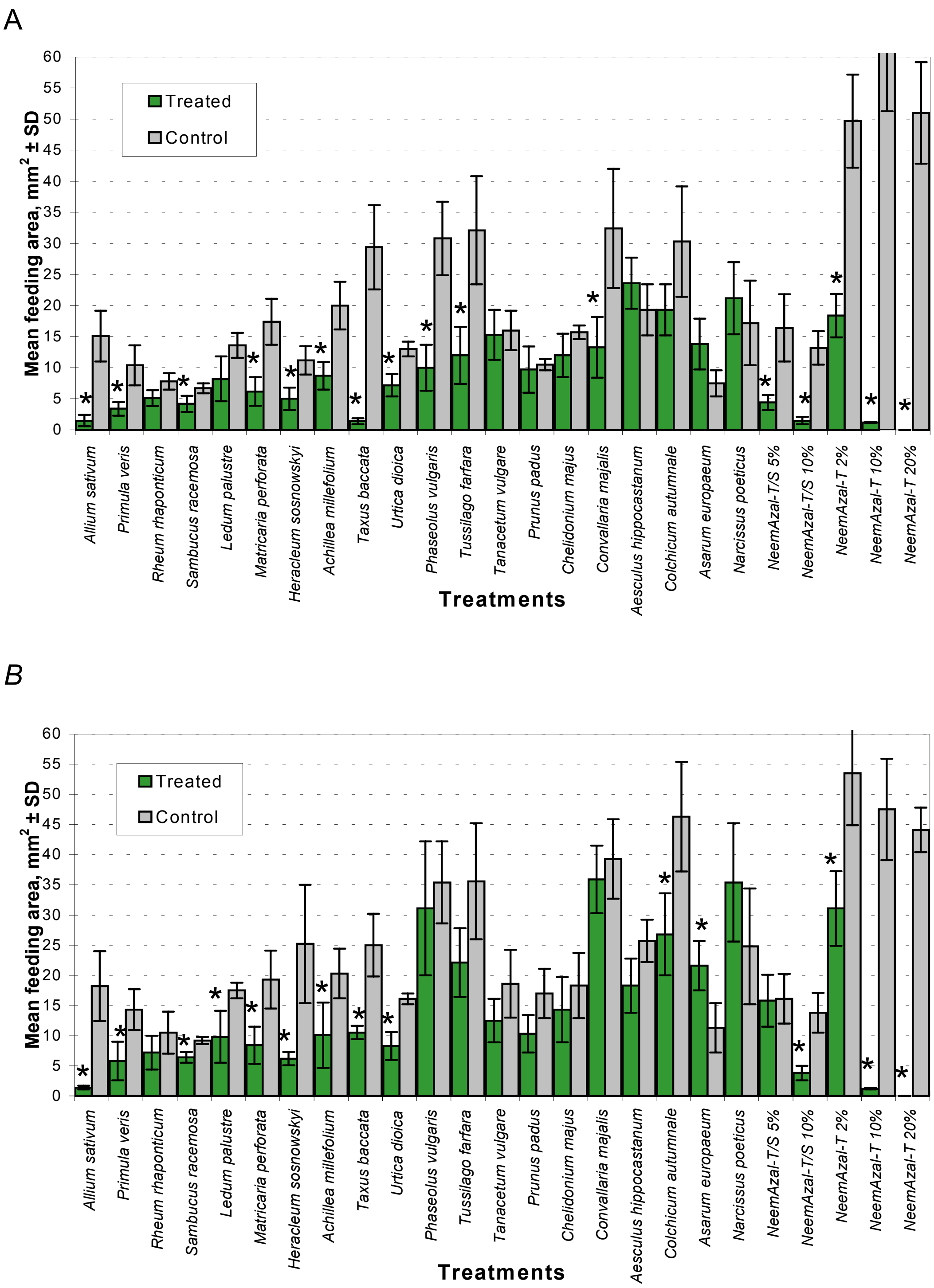
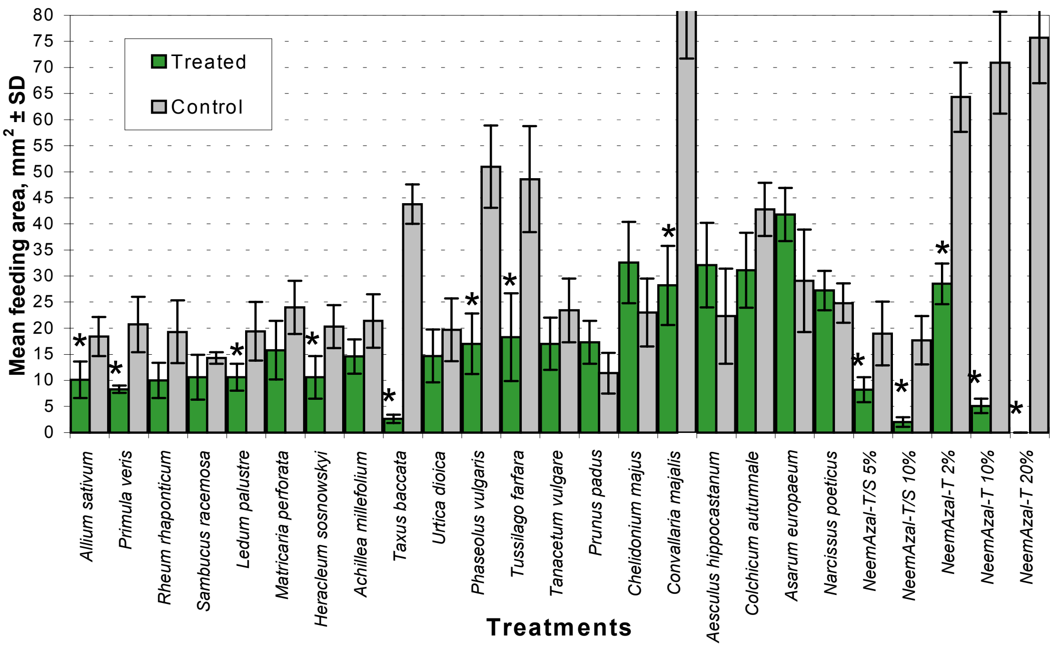


Fig. 2. Mean (± SD) feeding area (mm^2) of females (A) and males (B) of *Hylobius abietis* during 48 hours in pine twigs treated with different plant extracts and neem preparations.

* Significant difference between treated and untreated control twigs, Student t-test, $p<0.05$

A



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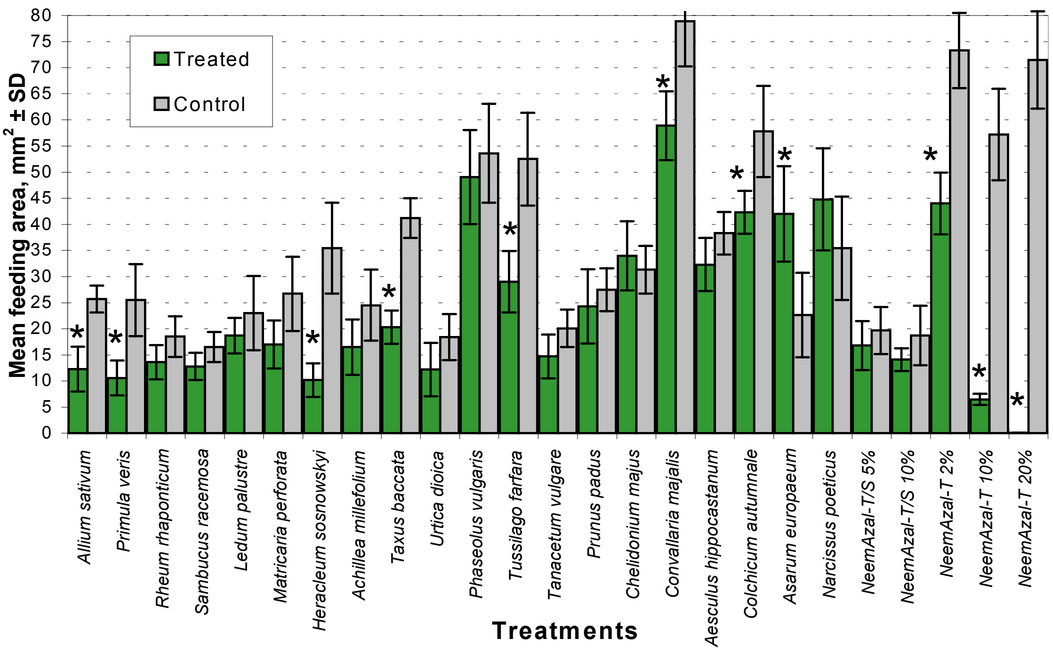

Fig. 3. Mean (± SD) feeding area (mm^2) of females (A) and males (B) of *Hylobius abietis* during 72 hours in pine twigs treated with different plant extracts and neem preparations.

- Significant difference between treated and untreated control twigs, Student t-test, $p<0.05$

In field conditions, the treatment of 3-year-old Norway spruce seedlings with plant extracts did not have a long-term effect – the treated seedlings proved to be later even more damaged (Fig. 4.). The treatment with the extracts of *Taxus baccata* and *Primula veris* had a short-term repellent effect on the large pine weevil; however, *Tanacetum vulgare, Allium sativum, Heracleum sosnowskyi* appeared to be attractive to the large pine weevil. It is possible that compounds of the extracts decompose into attractive ingredients. On the other hand, the treatment of seedlings can cause additional stress which can result in a release of attractive volatiles from trees.

The effect of NeemAzal-T on pine weevils in the field conditions was more prolonged compared with the effect of the water extracts of native plants (Fig. 5). The repellent influence of the neem preparation lasted during the whole vegetation period and was more stable in treatments with higher its concentrations. At the same time, the neem emulsion at higher concentrations (20%) had some phytotoxic effect on conifer seedlings.

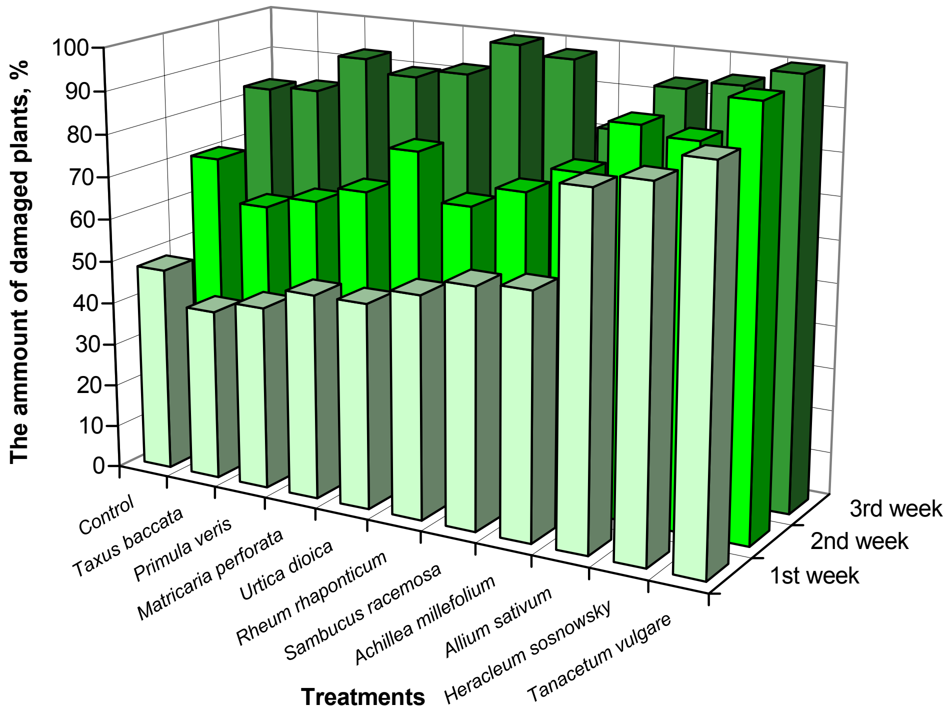

Fig. 4. Proportions of damaged by *Hylobius abietis* 3-years-old spruce seedlings treated with different plant extracts in a fresh clear-cutting in 1-3 weeks after planting

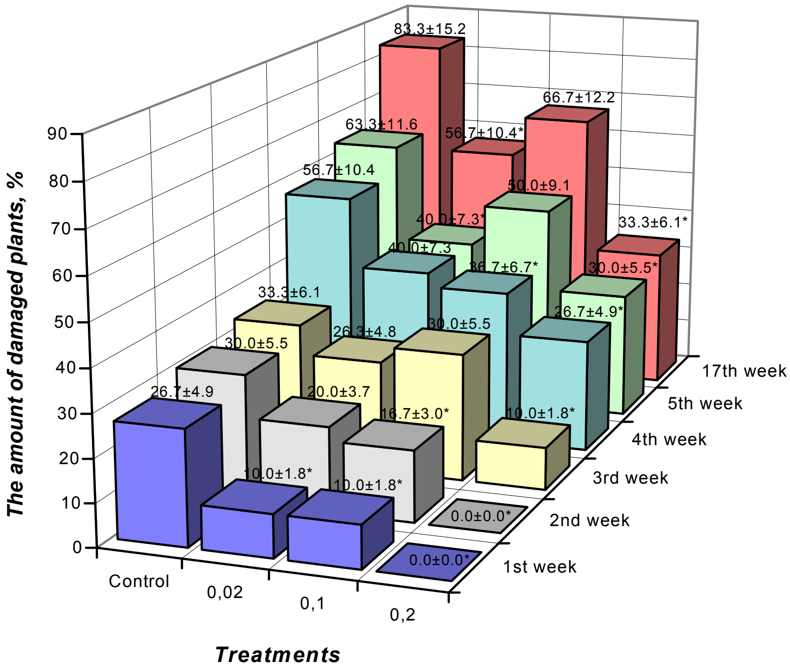

Fig. 5. Proportions of damaged by *Hylobius abietis* 3-years-old spruce seedlings treated with water emulsion of NeemAzal-T in the concentrations of 2%, 10% and 20% and untreated seedlings in a fresh clear-cutting in 1-5 and 17 weeks after planting.

* Significant differences between treated and untreated seedlings, Student t-test, $p<0.05$

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THE PRELIMINARY RESULTS OF TRIALS CONDUCTED WITH NEEM AND COMBINATIONS OF NEEM AND *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* IN GYPSY MOTH (*LYMANTRIA DISPAR* L.) CONTROL IN SLOVAKIA

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The gypsy moth, Lymantria dispar *L. (Lep. Lymantriidae), is the most important defoliator of oak stands in Slovakia. If necessary, control is done by aerial applications of* Bacillus thuringiensis *var.* kurstaki *(*Btk*) and viruses preparations during outbreaks till now. However, when population density is very high (over 5 egg masses / tree) the larvae cause heavy defoliation in spite the fact, that treatment by (*Btk*) or viruses was done. From this reason alternative ecological possibility for control it is necessary to find. The effects of 2.0 (NAI2.0) and 4.0 (NAI4.0) l NeemAzal – T/S combined with 2.0 l Istroecol (rape oil formulation) per ha on larvae of gypsy moth in aerial application is shown. Larvae treated by 2.0 l showed 50.1 % mortality, larvae treated by 4.0 l showed 78.6% mortality. Average defoliation reached 3.85% in (NAI2.0) and only 3.15% in (NAI4.0) and was significantly lower as in control – 24.75 and 27.5 % (ANOVA, Scheffe test). Surface trials were carried out on another experiment. The effects of combination of NeemAzal and* Btk *products (A:* Btk *–0.02 l; B: NeemAzal –T/S –0.005 l +* Btk *-0.005 l; C: NeemAzal –T/S –0.01 l +* Btk *-0.01 l; and D: NeemAzal –T/S –0.02 l +* Btk *-0.01 l - each combination with 0.01 l of Istroecol diluted in 5 l of water) on gypsy moth larvae is shown. All used variants (A, B, C and D) reached 100.0% mortality (natural mortality was high as well –70%). Average defoliation reached 78.6% in A, 41.4% in B, 34.3% in C and 6.2% in D and was significantly lower that in control – 91.2 and 92.9% (ANOVA, Tukey HSD test). Defoliation in A was significantly higher as defoliation in B, C and D.*

Key words: *NeemAzal products, Bacillus thuringiensis var.kurstaki, Lymantria dispar control*

Introduction

The gypsy moth, *Lymantria dispar* L. (Lep. Lymantriidae), is the most important defoliator of oak stands in Slovakia. The control is done by aerial applications of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) and viruses preparations during outbreaks (every 6-10 years). When population density is very high (over 5 egg masses / tree) the larvae cause heavy defoliation in spite the fact, that treatment by (*Btk*) and viruses was done. Trials to control gypsy moth by application of NeemAzal products and combination of NeemAzal and *Btk* products in oak stands were conducted in spring 1998 to confirm, if NeemAzal preparations in combination with *Btk* could decrease or minimise defoliation (due to antifeedant effect).

The antifeedant activity of Neem is well known (Singh, 1993). However, different insect species showed varying degree of sensitivity to various extracts and pure compounds. For example cabbage leaves sprayed with 0.4% neem seed kernel suspension significantly reduced the damage by *Pieris brassicae* larvae. On the other hand, at 1 to 2% concentration, the Neem oil failed to give any significant protection to castor leaves against the hairy caterpillar, *Amsacta albistriga* (3 to 4% concentration protected castor leaves to the extend of 70.7 and 91.4% respectively).

Nicol and Schmutterer (1996) confirmed high mortality of gypsy moth larvae after treatment by NeemAzal-T (5% azadirachtin). In the treated stand the majority of the larvae died 3-4 weeks after application and hardly reached the second or the early third larval instar. The high reduction of production of faeces and gain in mass of untreated and treated larvae of gypsy moth was observed as well. Only 17 % of larvae had not died, however, the resulting adults were fertile. The feeding activity of gypsy moth larvae in the treated stands decreased rapidly (second antifeedant effect) (Nicol and Schmutterer, 1996).

During my experiments, I have tried to test the mixture of Neem product (low defoliation, but high expenses for treatment) and *Btk* product (low price, but sometimes high defoliation). My idea was to confirm if reduced amount of Neem preparation could be replaced partially by *Btk* and efficiency stay at the same (similar) level.

Material and methods

Trials focused on control of gypsy moth by application of NeemAzal products and combination of NeemAzal and *Btk* products in oak stands were conducted in May 1998. I wanted to confirm, if combinations of NeemAzal and *Btk* preparations could decrease or minimise defoliation. The effects of 2.0 (NAI2.0) and 4.0 (NAI4.0) l NeemAzal – T/S combined with 2.0 l Istroecol (rape oil formulation) on larvae of gypsy moth was studied in the first study. Applications were carried out by aeroplane with the ingredients diluted in 100 l water / ha. Artificially reared gypsy moth larvae were exposed into cloth bags after treatment on treated and control plots (10 branches each variant, 2 repetitions). They were taken into lab after 3 weeks together with sample branches. Surface trials were carried out on another experiment at the beginning of June 1998. The effects of combination of NeemAzal and *Btk* products (A: *Btk* –0.02 l; B: NeemAzal –T/S –0.005 l + *Btk* -0.005 l; C: NeemAzal –T/S –0.01 l + *Btk* -0.01 l; and D: NeemAzal –T/S –0.02 l + *Btk* -0.01 l - each combination with 0.01 l of Istroecol diluted in 5 l of water) on gypsy moth larvae was studied. Again gypsy moth larvae were exposed on sprayed trees into cloth bags on treated and control plots and taken into lab after 3 weeks together with branches. Mortality on treated and control plots was evaluated by Abbott formula. Defoliation was evaluated on the basis of 20 leaves, which were taken from each sample branch (10 branches each variant, 2 repetitions). Analysis of variance was used for comparison of differences in average defoliation (Tukey HSD Test and Scheffe Test).

Results and discussion

Aerial applications

Larvae treated by NAI2.0 showed 50.1 % mortality, larvae treated by NAI4.0 showed 78.6% mortality 3 weeks after treatment. Average defoliation reached 3.85% in (NAI2.0) and only 3.15% in (NAI4.0) and was significantly lower as in control – 24.75 and 27.5 % (ANOVA, Scheffe, test) (Table 1). Differences between defoliation caused in NAI2.0 treatment and NAI4.0 treatment were low and not statistically significant. Low defoliation in control it is caused by high natural mortality of reared larvae (egg masses for caterpillars rearing were collected in outbreak conditions).

Table 1 Differences in defoliation - aerial application [Scheffe test; (P <0.01), n=20, MAIN EFFECT: Treatment]

Treatment	Neem 1	Neem 2	Control 2	Control 1
Average def.	3.850	3.150	27.500	24.750
Neem 1	●	0.998010	0.000000**	0.000003**
Neem2		●	0.000000**	0.000001**
Control2			●	0.898061

Surface treatments

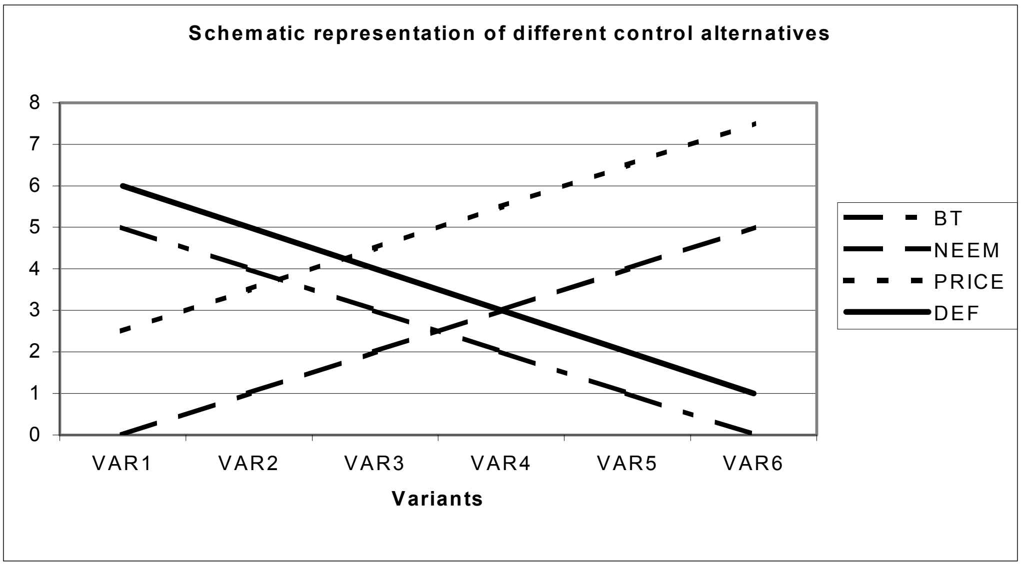
All used variants (A, B, C and D) reached 100.0% mortality after 3 weeks. Natural mortality was extremely high as well –70%. There were 2 reasons – 1st (egg masses for caterpillars rearing were collected in outbreak conditions) and 2nd experiments were leaded at the beginning of June, when leaves are not optimal for young gypsy moth larvae feeding. Average defoliation reached 78.6% in A, 41.4% in B, 34.3% in C and 6.2% in D. In all variants, it was significantly lower that in control – 91.2 and 92.9% (ANOVA, Tukey HSD test). Differences in defoliation among all variants were statistically significant (Table 2).

Table 2. Differences in defoliation - surface application [Tukey HSD test; (P <0.01), n=20, MAIN EFFECT: Treatment]

Treatment	(A)	(B)	(C)	(D)	CONTROL1	CONTROL 2
Average def.	78.571 %	41.389 %	34.286 %	6.238%	91.191 %	92.857 %
A	●	0.0012**	0.00012 **	0.00012 **	0.00669 **	0.00137 **
B		●	0.33555 **	0.00012 **	0.00012 **	0.00012 **
C			●	0.00012 **	0.00012 **	0.00012 **
D				●	0.00012 **	0.00012 **
CONTROL1					●	0.99708

The results of preliminary experiments with combinations of *Btk* and Neem products showed some possibilities for using this control strategy. Combinations of *Btk* and Neem it could be possible to use in aerial treatment mainly, where application of chemical preparations is forbidden, but protection of forest stands against defoliation it is necessary. Aerial treatments against gypsy moth are frequently done in forest stands influenced by oak decline by using *Btk*, or viruses. However, when population density is very high (over 5 egg masses / tree) the larvae can cause heavy defoliation in spite the fact, that treatment was done. In forest stands influenced by oak decline even medium defoliation (over 40%) can cause deterioration of tree health. Successfully testing the *Btk* and Neem combination could be involved to strategy “Control in Advance” NOVOTNÝ & TURČÁNI (1997) in the most endangered stands (in that strategy, the treatment is done in time, when abundance is not extremely high).

Application of pure Neem products it is impossible to do in conditions of Slovak forests, because high prive of application. However, some situations are known when application of Neem could be suitable to use. As it is possible to see from Figure 1, defoliation during high abundance of caterpillars can reach 60 (sometimes more) % in spite the treatment by using *Btk* only. The relative price of this application reachs coefficient Kp = 2,5. After totally replacement *Btk* by Neem products it is possible to expect defoliation only 10 (or less) %, but the price of this application is 3 times higher (Kp=7,5). On the basis of data from literature (Novotný and Surovec, 1992) it is known, that defoliation up to 30 (40)% does not cause to defoliated trees serious deterioration of health. Decreasing the defoliation together with still high efficiency of treatment could be probably reached by using optimised structure of applicated preparations (combinations of *Btk* and Neem). In that case it will be necessary to use sublethal dose of Neem preparations together with sublethal dose of *Btk*. To prepare the optimised combination of *Btk* and Neem it is necessary to lead another experiments. During making these experiments it is necessary to study the mechanisms of activity of both preparations (*Btk* and Neem) on target organisms. Not everything is clear on that area. For example, how is it possible, that total effect of Neem and *Btk* on target insect is stronger, when it is possible to predict, that relation between them will be negative? It is mainly antifeedant effect of Neem, which can negatively influences affect of *Btk* (because caterpillars have to consume some area of foliage, to be infested by spores of *Btk)*.



Conclusions

1) Unfortunately, the experiments were leaded in 1998 only and were not repeated on different climate conditions. From this reason they could be considered as preliminary only.

2) Defoliation in oak stands treated by 2.0 l and 4.0 (NAI4.0) l NeemAzal – T/S combined with 2.0 l Istroecol was statistically different as in control and reached only 3 to 4 %. The efficiency of Neem on gypsy moth larvae seems to be high.

3) Among different variant as the most suitable variant was found C (NeemAzal –T/S –0.01 l + *Btk* -0.01 l) defoliation 34% and D (NeemAzal –T/S –0.02 l + *Btk* -0.01 l) - defoliation 6%.

4) It is necessary to continue experiments to optimise treatment variants for different health conditions of oak stands (healthy stands, stands influenced by oak decline...).

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CURRENT STATUS OF BIOPESTICIDE USE IN LATVIA

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In Latvia, the Law of Plant Protection regulates all plant protection actions and registration of plant protection products according to EEC/94/414.

Totally, 135 plant protection products, including 101 active ingredients and 17 biological products are registered in Latvia. There are six biological products on fungal basis, four insects – predators and seven parasitoids. There are no pheromones and plant extracts registered for commercial use in Latvia by this time. In spite of that, pheromones are used for the research and forecasting and warning purposes. In most cases, the company Flora *from Estonia is a cradle of the applied pheromones in Latvia. Pheromones are used on agricultural fields and forests.*

During the vegetation period, decision support about necessity of plant protection measures is facilitated providing information to farmers through forecasting and diagnostic units. These units carried out monitoring of pests` activity using different methods and measures, including pheromones.

The obtained information about actual situation on the fields is recorded in the automatic responder and everyone can phone and get information free of charge.

Introduction

All over the world there are known and synthesised over 5000 animal pheromones. Perspective for wide range use, perspective could be approximately 300 - 400 insect pheromones. In the plant protection practice wider used are those insect pheromones that are considered as significant pests of forest or agricultural crops. They are mainly moth and beetles pheromones, seldom sucking insects` or *Hymenoptera* pheromones. The last ones are coming in the market only just now.

Purpose of pheromone application is to motivate decision support for integrated plant protection systems and to fix appearing of specific species of insects, to determine number of generations, in the season, to estimate the population density and damage threshold of certain species or to cause the vacuum of insect males in the definite region. The last activity is applicable if it economically pay off.

Materials and methods

At the existing forecasting and diagnostic service units and State Forest Service there are used mainly insect sex pheromones and a few aggregation pheromones to provide farmers with critical information on the need for additional control measures. In Latvia, insect pheromones are not used for creating of phenological models.

Placing and testing of pheromones occurred according to the following scheme.

- determination of purpose of pheromone use – identification of species, estimation of damage threshold, or mass trapping (direct population reduction);
- choice of placement – in orchards within the framework of a definite orchard; for field crops within the framework of crop and intermediate; in the forest by necessity putting 2-4 sets per hectare or according to recommendations of the company;
- the choice of time – before known earlier flight of certain species in the definite region;
- traps and other sets have to be tested 3 times a week;
- registration of data and dissemination of information (Arn,1990)

Results and discussion

During the years 1995 – 2001, totally 135 plant protection products, including 101 active ingredients and 17 biological products are registered in Latvia. There are six biological products registered on the fungal basis, four insects – predators and seven parasitoids. There are no pheromones and plant extracts registered for commercial use in Latvia by this time. In spite of that, pheromones are used for monitoring and forecasting and warning purposes in Latvia Seven products of the above mentioned are offered on the basis of fungus *Trichoderma harzianum, Trichoderma viridae, Streptomyces griseoviridis, Rhizobium meliloti, Azotobacter chroococcum, Pseudomonas putida, Verticillium lecanii* or a mixture of them. Others are insect parasitoids: *Trichogramma embryophagum*, *Dacnusa sibirica, Dacnusa isaea, Encarsia formosa, Aphidoletes aphidimyza, Aphidius colemani* and predators of insects or mites: *Amblyseius cucumeris, Macrolophus caliginosus, Phytoseiulus persimilis, Hypoaspis aculeifer.*

There are no available standardised plant extracts for control of pests in Latvia. Due to non- - aligned efficiency comparatively widely advertised spruce extract Fitoekol-IF is not included in the official list of the registered plant protection. In the biological farming nettle, camomile, horse-tail, milfoil non – standardised extracts are used for the control of several harmful organisms.

In Latvia, insect pheromones were widely used during, 70^{ies} and 80 ^{is} of the 20th century in orchards and vegetable fields, later monitoring and identification of species were carried out in forest. According to Cinitis, 2001 (unpublished data), totally 18 insect feroflors and countless modifications of them have been tested

From different types of pheromones, mainly insect sex pheromones are used (Biopesticides, 1998, Table 1.). Time tested that that was that not advisable to use pheromones in small gardens, wherewith decreasing area of orchards decrease demand of pheromones too.

Table 1. Types of pheromones used in Latvia

Type of pheromone	Target pest	Using practice in Latvia
Alarm	Aphids, spider mites etc	No
Foot mark	Ants	No
Aggregation and host kairomones	*Coleoptera* (storage and forest pests)	2 (forest - bark beetles)
Sex pheromones	Mainly *Lepidoptera* **Coleoptera**	Mainly *Lepidoptera*

At the present there were used 9 sex pheromones by State Plant Protection Service, Forecasting and Warning units and 2 aggregation pheromones by State Forest Service

(Ozols,1985).

Table 2. Pheromones used for forecasting and warning purposes

Crop	Target pest	Type of pheromone
Peas	pea moth *Laspeyresia nigricana (= Cydia nigricana)*	sex
Orchards	mountain ash moth *Argyresthia conjugella*	sex
	codling moth *Carpocapsa pomonella* (=*Cydia pomonella)*	sex
	Yponomeuta padellus (= *Hyponomeuta padella)*	sex
	fruit moth *Grapholitha funebrana*	sex
	currant clearwing moth *Synanthedon tipuliformis*	sex
	gypsy moth *Lymantria dispar*	sex
Cabbage	cabbage moth *Barathra brassicae* (= *Mamestra brassicae*)	sex
	Plutella maculipennis	sex
Forest	spruce bark beetle *Ips typographus*	aggregation
	ambrosia beetle *Trypodendron lineatum*	aggregation and host kairomones
	pine beauty moth *Panolis flammea*	sex
	nun moth *Lymantria monacha*	sex
	gypsy moth *Lymantria dispar*	sex

On the agricultural crops, pheromones are placed according to the above mentioned scheme and monitoring of target pests is the principal action.

In the forest, density of trap use depends on the homogeneity of the forest stand and purpose of use: detection of species, threshold catches or direct population reduction (mass trapping), large amount of traps should be placed, approximately 10 times more in comparison with the monitoring needs.

Conclusions

In the nearest future it would be necessary to enlarge the list of the used pheromones for monitoring and control of *Elateridae, Agrotis*, different storage pests and forest pests for instance, *Neodiprion sertifer, Bupalus piniarius, Hylobius* spp. (Ozols, 1985).

For the biological farming, it is highly necessary to find out opportunities to control insects with standardised plant extracts like neem-tree extracts and local plant insecticides.

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THE INFLUENCE OF PYRETHRINS ON LARGE WHITE BUTTERFLY (*PIERIS BRASSICAE* L.) LARVAE

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The effect of local pink pyrether (Tanacetum parthenium) and commercial insecticide Spruzit were comparatively studied on larvae of Large white butterfly (Pieris brassicae) in Estonian cruciferous fields. T. parthenium ethanol extract of 4 % and 2 % concentration was compared to Spruzit 0,1 % and 0,01 % solution. Pure tap water was used as control. Larvae were grown in Petri dishes in the start, later in 1 l glass jars. Larvae were fed with cabbage leaves treated with T. parthenium extract or Spruzit or with leaves immersed into water as control. The highest death rate was observed on larvae fed with leaves treated with Spruzit 0,1 due septicaemia. In other treatments the rate of mortality did not differ from control results. In all treatments the antifeedant and repellent effect of pyrethrum extract as well as Spruzit to larvae was clearly evidenced. In all treatments the antifeedant and repellent effect of pyrethrum extract as well as Spruzit to larvae was clearly evidenced. Although the antifeedant effect of pyrether was smaller than that of 0,1 % Spruzit the significant differences from control were observed. Larvae treated with 0,1 % Spruzit had longer moulting which caused uneven and later pupation than in control. The III stage larvae treated with 4% pyrether and 0,01% Spruzit underwent the pupation at the same time with control but the prepupation period was somewhat shorter.

Key words: *Large white butterfly,* Pieris brassica*, pyrethrins, mortality of larvae, weight of larvae.*

Introduction

The history of use plant extracts as biological insecticides extend to age-old civilizations. Pyrethrum was identified in antiquity in China. It spread West to Percia probably via the Silk Roads during the Middle Ages. Dried powdered flower heads were known as “Persian Insect Powder”. Records of use date from the early 19th century when it was introduced to Dalmatian, France, United States and Japan.

The term pyrethrins is used collectively for the six insecticidal constituents present in extracts of the flowers *Tanacetum cinerariaefolium* and other species. The extract in refined using methanol or supercritical carbon dioxide. Current production comes from East Africa, Ecuador and Papua New Guinea and Australia.

Pyrethrum extract is defined as mixture of three naturally occurring, closely related insecticidal esters of chrysanthemic acid, Pyrethrins I and the three corresponding esters of pyrethrin acid, Pyrethrins II. The three components of Pyrethrins I are pyrethrin I, jasmolin I and cinerin I; the components of Pyrethrins II are pyrethrin II, jasmolin II and cinerin II (Elliott, Janes, 1969).

The level of toxicity of phyrethrins is low because, unlike organophosphorous insecticides, it does not inhibit enzyme cholinesterase. Phyrethrins are non-systemic insecticide with contact action. Active ingredient binds to sodium channels, prolonging their opening and thereby causing knockdown and subsequent death.

They are effective against a broad range of insects. The major drawback to the use of pyrethrum in that it is rapidly degraded by the ultraviolet component of sunlight, greatly limiting its efficacy out-of-doors. (Isman, 1994).

Insects' organism shows resistance against all insecticides, including pyrethrins. Pyrethrins may be oxidased by oxidases to the less toxic compounds (Kuusik, 1977). Pyrethrins stability decreases in light and air, oxidation causes losses in insecticidal activity. Therefore pyrethrins are combined with stabilizing compounds called synergist, e.g pipenorylbutoxide that prevent detoxification. Synergists inhibit the oxidation of pyrethrins in insects and promote the penetration of pyrether through chitin (Lange, 1984; Henrick, 1995; Isman, 1997). Pyrethrins disable the movement of Na- and K- ions in nerve fibres subsequently causing total paralysis. The slower the paralysis occurs the lowers are the chances to recover and the more likely the organism dies (Hayashi et al., 1968). On a slighter attack insect may recover from paralysis, on a more serious poisoning it dies (Camougis, 1973; Kuusik, 1977). Pyrether can act as a repellent when applied in a small quantities (Kuusik, 1977).

There are many products composed on the basis of naturally occurring pyrether, the most known being Spruzit, produced by company Neudorff, Germany. In this product the dried flowers of *Chrysanthemum cinarariefoium*, grown in Africa, are extracted and piperonylbutoxid is added as synergist. This contact insecticide has to drop onto insects' sceletion. The product is recommended for control of chewing and sucking insects (e.g. aphids, bugs, beetles), and spider mites on fruit, vegetables, field crops, ornamentals, glasshouse crops, and houseplants. It is not harmful for bees (Gesamtkatalog, 1993; "Bio Fibel", 14/92; Schmidt and Grothmann, 2000).

The aim of a present research was to investigate comparatively the effect of local pink pyrether (*Tanacetum parthenium*) and commercial insecticide Spruzit on larvae of Large white butterfly (*Pieris brassicae*) in Estonian cruciferous fields.

Material and methods

The ethanol extract of *T. parthenium* was chosen for one of the studied products because of the special properties of its active ingredient. Compared to water extract ethanol extract remains stabile at a relatively low temperature (+ 4…6 °C) over a long time period. Plant extract consisted of dried flowers of *T. parthenium* and 85 % ethanol. Mixture was shaken during 20 minutes and left to stay covered for 24 hours. After that mixture was filtered and vaporized in vacuum-thermostat at 90 °C. Solution was diluted in water to 4 % and 2 % concentration. These were compared to Spruzit 0,1 % (according to common recommendations) and 0,01 % solution. Pure tap water was used as control. There were 5 treatments in total at the experiments.

Large white butterfly eggs were collected on cabbage fields around Tartu area. Larvae were grown in Petri dishes in the start, later in 1 l glass jars. Larvae were fed with cabbage leaves treated with *T. parthenium* extract or Spruzit or with leaves immersed into water as control. The rate of mortality and gaining of larvae as well as the length of larval period was estimated.

Results

In development of larvae the most essential factors are the quantity and the composition of food, main elements being P, K, N and Fe. The elongation of larvae and the weight of pupae decrease when food is insufficient or of a low feeding value. Feltwell (1973) found that cabbage white larval period was longer and death rate was higher when they were fed with inner leaves of cabbage containing less vitamin A and carotine. Allen (1954) found that deficiency of F and N causes slow development of larvae, elongation of pupation period and increases occurrence of death.

In consequence of lethal toxicity insect will perish either direct toxicity or septicaemia. After sublethal toxicity insects recover but latently perturbation in organisms can persist disturbing insects' growth and development.

The highest death rate was observed on larvae fed with leaves treated with Spruzit 0,1 %. Day after eating 30 % of larvae were dead, in 2-3 following days 22 % more larvae were dead due septicaemia. In other treatments the rate of mortality was relatively low during larvae period and did not differ from control results (figure 1.)

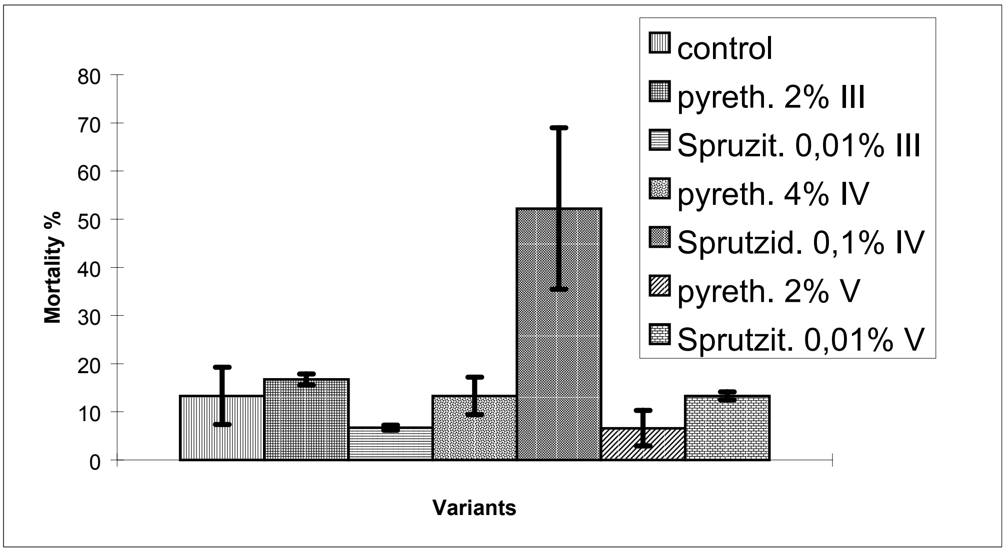

Figure 1. Mortality of Large white butterfly (*Pieris brassicae*) larvae in different treatments

In all treatments the antifeedant and repellent effect of pyrethrum extract as well as Spruzit to larvae was clearly evidenced. In many cases larvae eat less or did not eat at all cabbage leaves treated with insecticides compared to control. Treated food was available during two days but the poisoning due eating was not very likely because larvae preferred starving to death to eating treated leaves.

Mortality occurred to some extent also in control variants mainly because of the daily measurements and scaling procedures that constantly disturbed larvae. In some cases the larvae became infected by microsporidios (latently existing in Large white butterfly populations naturally) and bacterial diseases. This may have been caused by a little bit higher temperature that it is said to be optimal for larvae growth in insectarium. Due to the technical facilities it was impossible quarantee optimal temperature in insectarium as well as in ambient atmosphere. However, all treatments were maintained at the same conditions and therefore the data are comparable.

Optimum temperature for Large white butterfly development is 20° - 26 °C (Maercks, 1934), above that the speed of development but also the mortality increase. Large white butterfly larvae are able to grow and undergo metamorphosis at the temperatures between 10° and 32 °C (Klein, 1932). At the temperature +15 °C the larvae stage lasts 37 days, at the temperature +28,8 °C only 11 days. Larvae can also stand temporary low temperatures (near 0 °C) at which time they do not feed. At a constant temperature the last instar is the longest one. At this stage larvae are the hungriest eating 11 times more than is their weight (Chlodny, 1967).

At larvae stage the growth is accompanied with the increase in weight. There are several factors preventing feeding or digestion of food (antifeedants) that may cause the loss of weight due to the starving (Kuusik *et al.*, 1995). The reason is the loss of water that usually happens via excretion. In case of poisoning the water is lost orally as well as anally. The stronger is poisoning the faster the larvae lose water (Greenwood *et al.*, 1985).

Our trial showed that incrementation was slower and more unequal in treated larvae compared to control (figure 2). The greatest difference from control was observed in IV stage larvae treated with 0,1 % Spruzit. The 0,02 % pyrether and 0,01 % Spruzit, applied in III and V stage, gave equal results. Although the antifeedant effect of pyrether was smaller than that of 0,1 % Spruzit the significant differences from control were observed.

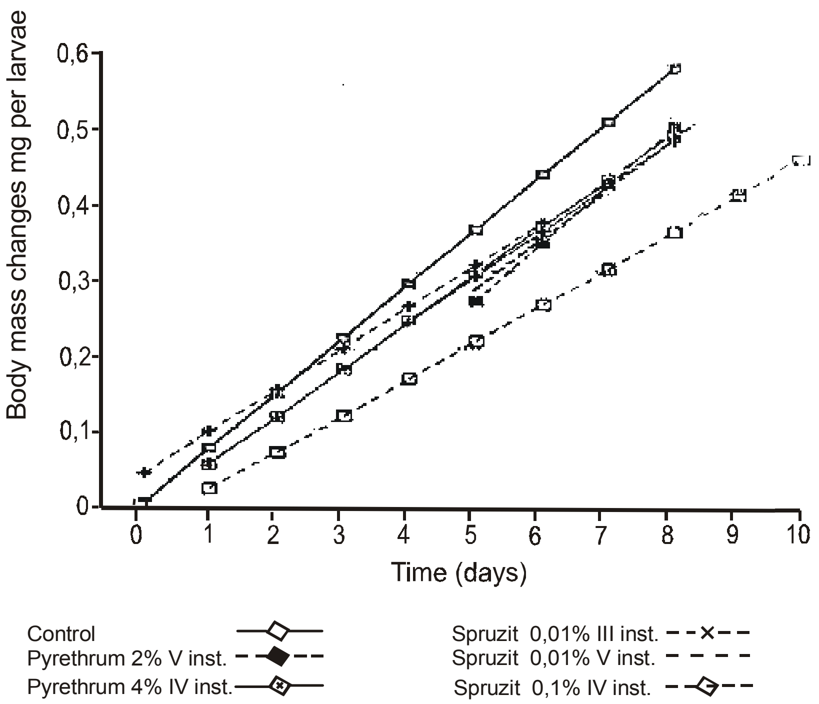

Figure 2. The body mass increasing of the Large white butterfly (*Pieris brassicae*) larvae

We also estimated the time larvae needed for exchanging instars. Larvae treated with 0,1 % Spruzit had longer moulting which caused uneven and later pupation than in control. The III stage larvae treated with 4% pyrether and 0,01% Spruzit underwent the pupation at the same time with control but the prepupation period was somewhat shorter.

It is known that moulting and pupation cannot happen before larvae have stored a certain amount of nutrients. In a lack of nutrients larvae cannot reach to the weight and size necessary for pupation and that leads to the insufficient preparation for winter period. Our trials indicated that commercially produced Spruzit has the best effect on the control of cabbage white. Having strong antifeedant influence the locally prepared pink pyrether is on many cases satisfactory in reducing the abundance of Large white butterfly in Estonian cruciferous fields.

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