Chapter 6

Using Semiochemicals for Coleopterean Pests in Sustainable Plant Protection

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Additional information is available at the end of the chapter

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Abstract

Since the early twentieth century, chemical industry provides farmers large amounts of synthetic chemicals used as fertilizers and pest control products. Agriculture became intensive and the crop yields and the profit increased dramatically. Tons of toxic material for crop protection and fertilization were scattered through the gardens, fields, and orchards. But all these chemicals affect the environment, with serious negative consequences for humanity, today and tomorrow. Since 1959 until today many researchers observed and discussed the disadvantages of chemical methods of combating harmful insects and misapplied their disruptive action on cultivated ecosystems. Integrated pest management (IPM) is a process used to solve pest problems with minimum risks to people and environment. The objective of the researches presented in this chapter is to obtain and testing "*semio*chemicals"—pheromones, involved in intraspecific communication, into environmentally compatible strategies, to reduce pest populations under economic damage thresholds. Insect that are monitored and mass trapped in proposed IPM strategies are potato pest, Colorado potato beetle, *Lepidoptera decemlineata* Say (Coleoptera, Chrisomelidae); corn pests, West Corn Rootworm *Diabrotica virgifera virgifera* (Coleoptera, Chrisomelidae); and six-spined bark beetle *Pityogenes chalcographus* (Coleoptera, Scolitydae) pest in coniferous forests.

Keywords: semiochemicals, sexual, aggregation pheromones, kairomones, control, pest, Coleoptera, Colorado potato beetle (*Leptinotarsa decemlineata* SAY), western corn rootworm (*Diabrotica virgifera virgifera*), bark beetle, *Pityogenes chalcographus*

1. Introduction

The care for the next generations involves the resources protection, keeping soil, air, and waters clean. In agriculture, for a sustainable future, scientists and farmers must develop the environmentally friendly, economically viable, and socially responsible technologies [1]. Intensive agriculture uploads environment with pollutants such as pesticides, herbicides, and fertilizer. In the European Union, 38% of bird species and 45% of lepidopteran species are threatened with extinction. It is true that the high populations of insects' pests could destroy the human food sources, and therefore need to be maintained under economic damage level, but each organism has its role in the ecosystem to which it belongs. The pesticides kill not only harmful insects but also beneficial organisms, and thus the ecosystem equilibrium is modified. The most affected are the pollinators such as honeybees.

The concept of integrated protection or integrated pest management (IPM) appeared at the end of the sixth decade of twentieth century by the works of Dutch researcher, Briejer [2] and Americans: Smith and Hagen [3] and Stern and van den Bosch [4]. The disadvantages of chemical methods of combating harmful insects and misapplied their disruptive action on cultivated ecosystems were discussed recently by Gill and Garg [5].

Modern ecofriendly crop protection strategies are discussed in symposiums organized by Food and Agriculture Organization of the United Nations (FAO) and IOBC/WPRS (International Organization for Biological and Integrated Control of Noxious Animals and Plants/West Regional Paleartic Section). According to the FAO, IPM means considering all available pest control techniques and other measures that reduce the development of pest populations, with minimum risks to human health and the environment.

Development of pest management alternatives based on mediators' chemicals has been necessitated by the loss of traditional pesticides, insect pest resistance, pest resurgences, and secondary pest outbreaks often due to the effect of pesticides on all environments [5].

Semiochemicals, defined as behavior-modifying chemicals, are volatile organic compounds that transmit chemical messages, "words" in organism "language" and are used by insects for intra- and interspecies communication. The term "*semio*chemical" derived from the Greek word "semeon," which means "sign" or "signal" [6]. Insects detect volatiles semiochemicals directly from the air with olfactory receptors located in sensilla hairs on the antennae and the effect is a change in insect behavior. Semiochemicals can be classified as pheromones or allelochemicals based on how they are used and who benefits [7].

The intraspecific communication language have as "words" volatile signals, so called pheromones , emitted by an organism that produces on the receptors of the same species a behavioral change. The term "pheromone" is derived from the Greek words "pherein" (to carry) and "hormone" (to stimulate), and was introduced by Karlson and Butenandt [8]. Based on their effect, pheromones categories are as follows:

- Aggregation pheromones: signaling an important place for the life, e.g., where insects' species could find the "food" or could lay eggs.

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- Alarm pheromones: compounds that stimulate insects' escape or defense behavior.
- Sex pheromones: emitted by the female (in most of the cases) inducing male of the same species mating behavior.
- Trail pheromones: social insects as workers ants released pheromones to mark the way to a food source.
- Marking pheromones: compounds used by insects to mark the territory.

The allelochemicals are classified as allomones, kairomones, or synomones [7]. Allomones are a class of compounds that benefit the producer, but not the receiver. Allomones are often used for defense, such as toxic insect secretions. Predators also use allomones to lure prey. Kairomones are a class of compounds that are advantageous for the receiver. Kairomones are the volatiles emitted by plants that benefit many predators by guiding them to prey or potential host insects.

Synomones ("with" or "together") are compounds that are beneficial to both the receiver and the sender such as volatiles emitted by flowers that attract bees for pollination.

The practical goal of semiochemical research is to develop techniques and methods for insects' pest control. Semiochemical research is placed in Pasteur's Quadrant of the Stokes model. It is based on the research in fine synthetic organic chemistry, but the final goal is still to develop solutions for agricultural problems, insects' pest population control, through applied research in the experimental field.

Since 1880s scientists used female insects to lure males into traps. Since the 1950s up until today, more than 3000 semiochemicals connected to the chemical communication of insects have been identified. Research on semiochemicals involves continued molecular mapping, synthesis, and studies of biosynthesis. Biologist and entomologist try to understand the neurophysiological sensory functions of insects and how hormonal regulation in insects affects pheromone biosynthesis and release.

Synthetic pheromones represent a new breeding prevention method for crop pest control. In sustainable agriculture using pheromones to control pests could drastically reduce the use of pesticides. The idea is to use an artificially synthesized scent, synthetic pheromones, to "attract and kill" into a trap the pests or to disrupt mating communication between male and female pests, thus preventing them from mating and lowering the population density of the next generation of the pests. These pheromones are specific and selective, have no effect on beneficial insects, such as pests' natural enemies or on other living organisms. Synthetic pheromones mimic the natural pheromones. Fascinating and somehow ironic is that the substances involved in perpetuation of the insects species can be used to control insect pest.

"Pheromonists," chemist researchers' team from "Raluca Ripan" Institute for Research in Chemistry Cluj – Napoca, Romania, is working to develop a variety of organic synthetic insects' pheromones and with multidisciplinary teams, biologists, entomologists, agronomists as partners in projects, develop new IPM friendly environmental techniques and technologies for insects' pest control.

Synthesis of pheromones and proposed IPM environmentally compatible strategies as monitoring or mass trapping of some coleopterean species (beetles), with the aim to reduce pest populations under economic damage thresholds are presented below.

The overall objective of research presented in this chapter is to find a technique using pheromones for protect : (1) maize crop against the West Corn Rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera, Chrisomelidae); (2) potato crops against Colorado potato beetle (CPB), *Lepidoptera decemlineata* Say (Coleoptera, Chrisomelidae); (3) coniferous forests against six-spined spruce bark beetle, *Pityogenes chalcographus* (Coleoptera, Scolytidae).

2. Colorado potato beetle aggregation pheromones in IPM techniques for potato plant protection

2.1. Chemical ecology of Colorado potato beetle

The CPB, *Leptinotarsa decemlineata* (Say), Coleoptera, Chrisomelidae, native from Mexico, identified as major pest of potato plants first time in America in 1824, arrived in Europe in 1922, via cargo ships during World War I and subsequently colonizing all of Europe except for the British Isles and Scandinavia. Then, CPB continued to expand into eastern Europe and then central Asia and western China [9].

CPB (*L. decemlineata*) attacks potatoes (*Solanum tuberosum* L.) and various other cultivated crops such as tomatoes (*Solanum lycopersicum*) and aubergines (*Solanum melongena*). It also attacks wild solanaceous plants, which occur widely and can act as a reservoir for infestation. The adults feed on the tubers of host plants in addition to the leaves, stems, and growing points [10, 11] (**Figure 1**). Both adults and larvae feed on foliage and may skeletonize the crop.

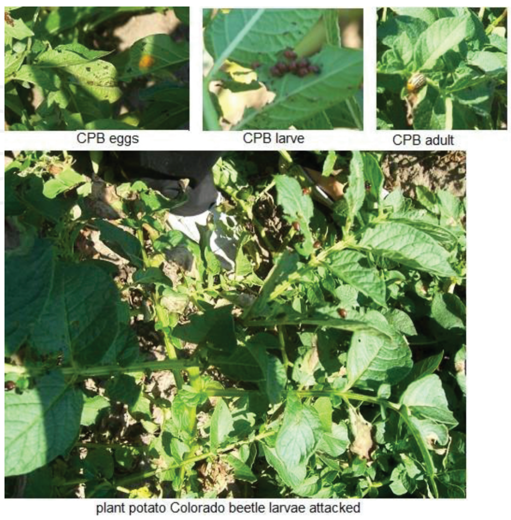

Figure 1. *Leptinotarsa decemlineata* (Say) adult on potato leaves (own photo on experimental plots).

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CPB overwinter in the soil as an adult. The beetles become active in the spring. Females lay 800 of orange colored eggs in groups of two or several dozens for a period of 4–5 weeks. Larvae hatch after 4–9 days. Larval stage lasts 2–3 weeks and then the larvae hide in the ground. During their complete larval stage (3–4 weeks), CPB larvae consume approximately 40 cm^2 of potato leaves while adults can eat up to 10 cm^2/day [12]. It is well known in Europe, where the CPB population increased dramatically during and immediately following World War II and spread eastward.

Insecticides are currently the main method of beetle control on commercial farms. However, many chemicals are often unsuccessful when used against this pest because of the beetle's ability to rapidly develop insecticide resistance. The Colorado potato beetle has developed resistance to all major insecticide classes, although not every population is resistant to every chemical. The secret of Colorado potato beetle's success as a pest is its diverse and flexible life history coupled with a remarkable adaptability.

Now because of the inevitable decline of effective insecticide treatments, research should focus even more on the development of new control methods and approaches. Some methods such as cultivating GM plants are not seen positively by consumers, and farmers have abandoned them due to lack of buyers [13]. Researches for better understanding of insect's biology and lifestyle could permit entomologists and chemists to devise new control techniques.

The use of semiochemical attractants to improve insecticide treatments should be considered as an innovative approach of CPB management. Chewing insects are indeed more sensitive to volatile organic compounds (VOCs) released by their host plants because the damage they induce in plant tissues increases the release of these compounds [14].

It is necessary but very difficult to find the cocktail of natural odors within which the quantitative proportion of each compound is as close as possible to that of the naturally emitted blend [15]. The challenge consists in finding the appropriate molecules and their ratio, instead of trying to include as many compounds in the mixture as possible [14].

Researchers from Université de Liège and from ARS-USDA Beltsville, Maryland, USA review alternative strategies to control CPB populations [16]:

- biotechnological methods using intercropping cultures for disrupt the CPB adults perception of potato VOCs;
- trapping beetles using baits with synthetic mixtures of aggregation pheromone and/or volatiles kairomones;
- antifeedant sprays on potatoes;
- the potato plant recognizes the presence of CPB through chemical signals. By genetic manipulations increase the natural capacity of the plant to trigger defense mechanisms [16].

2.2. Experimental research using synthetically CPB aggregation pheromones

Our research is related to biotechnology that uses "chemical messengers" sending or receiving information for pest control in potato crops. Such "chemical mediators," which induce a certain behavior, are aggregation pheromones—intraspecific messengers and kairomones—interspecific messengers—chemical signals emitted by the host plants.A male-produced aggregation pheromone was identified for the Colorado potato beetle, *L. decemlineata* (Say) in 2002 by Dickens et al. [17] (**Figure 2**).

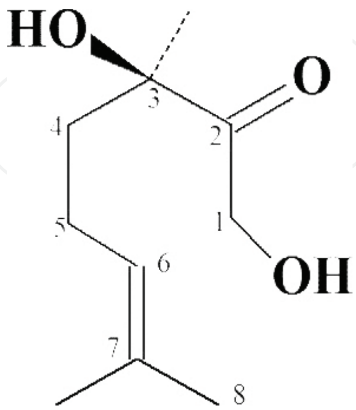

Figure 2. (*S*)-3,7-Dimethyl-2-oxo-6-octene-1,3-diol, aggregation pheromone of CPB [(*S*)-CPB].

The biological effect of (*S*)-CPB was first evaluated in a Y-olfactometer on male and female CPB, both of which were highly attracted. The (*R*)-enantiomer and the racemic mixture were not attractive [17]. CPB larvae also seem capable of perceiving the aggregation pheromone produced by adults, but further studies are needed to characterize larval behavior [18].

2.2.1. Synthesis of CPB aggregation pheromone

The synthetized (*S*)-CPB by route presented below (**Figures 3** and **4**) was analyzed and used in the field tests with extracts from potato plants (leaves) contain substances which function as kairmones.

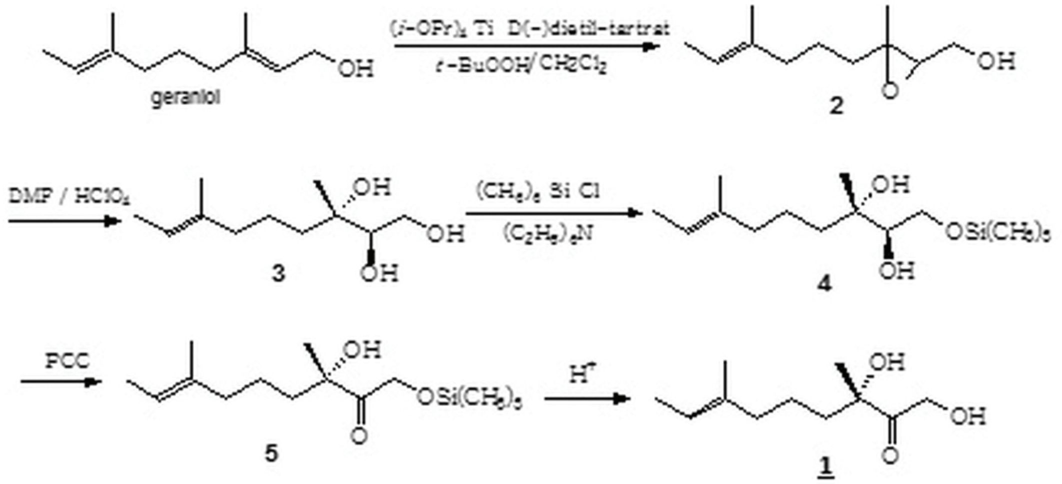

Figure 3. The way to prepare in ICCRR laboratory synthetic pheromone (*S*)-CPB.

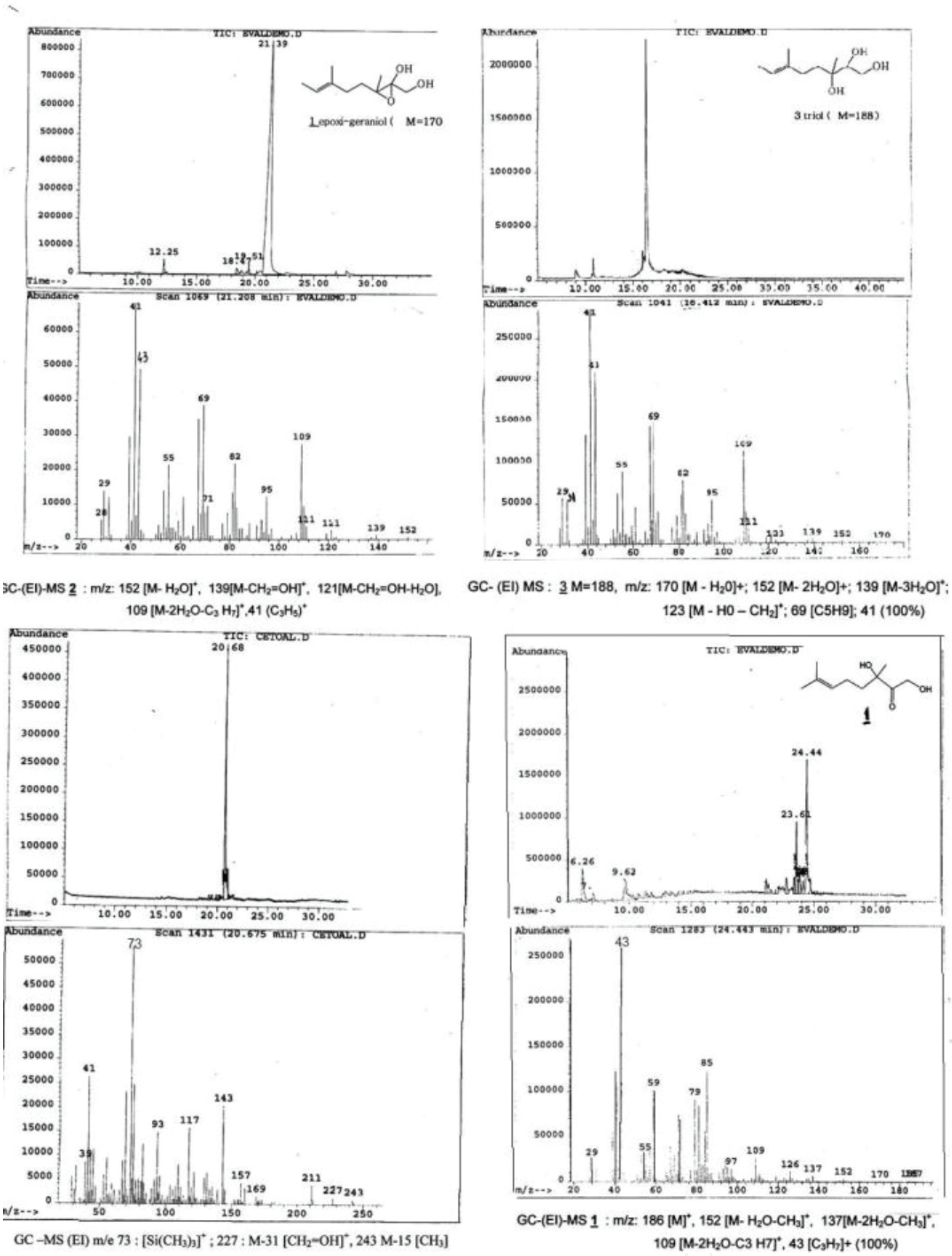

Figure 4. (EI) GC-MS analysis (Hewlett-Packard 5972 GC-MSD, capillary column HP-5MS (30 m × 0.25 mm × 0.25 μm) synthons and *S*-(CPB) aggregation pheromone.

2.2.2. Testing S-CPB attractivity for L. decemlineata: field experiments

Field experiments were conducted in three different locations from Transylvania area, Romania: Research-Development for Potato Station Targu-Secuiesc (**Figure 5**), University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, USAMV Research Station situated in Jucu-Cluj county, and Agricole Research-Development Station Turda (**Figure 6**).

Figure 5. Experimental plots from RDPS Targu-Secuiesc (own photo on experimental plots).

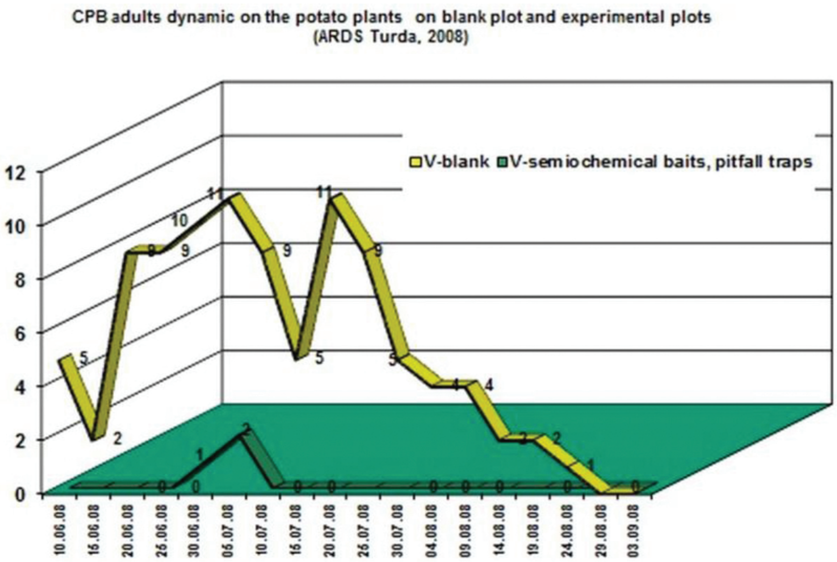

Figure 6. Adult CPB migration dynamic on potato plants in two plots (blank and experimental) ARDS Turda, Cluj, România.

Considering aggregation pheromone as attractant we try to get out the pest from potato field attract CPB a pitfall trap, a container with potato leaves alcoholic extract. Baits were placed on potato plants, close to the pitfall trap, "attract and kill" technique. The pheromonal baits are 0, 1 g *(S)-3,7-dimethyl-2-oxo-6-octen-1,3-ol* and *2-phenyl-ethan-1-ol* [(*S*)-CPB] impregnated on rubber stopper (**Figure 7**).

Experiments and observations from Research Development for Potato Station (RDPS) Targu-Secuiesc are presented below.

The experimental plots were artificially colonized with CPB. Each plot have 28 rows spacing 0.75 m and a distance between plants 0.30 m. Thirty CPB adults, collected from elsewhere, were placed on potato plants row nos. 13 and 14. The pitfall with kairomone (potato lives

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extract) and baits with pheromones are located on row no. 4 from the edge of the plot. In days 1, 3, 5, 7, and 10, CPB migration from row no. 14 ("start point") to row no. 4 "finish point" was observed. After day 10, 13 CPB adults were found on row no. 4, area with pheromonal baits.

Figure 7. Pheromonal baits and pitfall traps with kairomones.

The experiment conclusions are as follows: aggregation pheromone bait attracts beetle, but CPB did not reach into the trap, pitfall with kairomone is not efficient, probably the trap design is inadequate, to capture CPB must be used another type of trap such as a small wing trap (**Figure 20**).

2.2.3. Field experiments in USAMV Research Station situated in Jucu-Cluj county

In the field, the Colorado beetles were observed on potato plants. The experimental plots were located at 200 m distance from the blank plot. Pitfall traps and pheromone baits were placed in 40 m^2 area each, the experimental plot was at 20 m distance. A significantly number of CPB adults were identified by counting, crowded around traps, relative to the place where no pheromone traps were placed. The adults were aggregate, during egg lying, around traps in an area of about 18 m^2, with a circle radius of 2.5 m. This result shows that the behavior induced by this pheromone attract the beetles into the area, but these beetles do not try to touch the pheromone source, as occurs if the attractants are the sexual pheromone. Noteworthy, there was a higher concentration of adults in plots' edges and especially an affinity for plants infected with viruses.

2.2.4. Experimental research in the field of Agricola Research-Development Station Turda

At this location, the traps were placed on 0.8 ha potato plants' experimental plot, located at 20 m distance between them. Data placement of traps were made from June 5, following the evolution of both generations of the pest. Observations were made from June 10, and continued until September. Dynamic observation was performed each 5 days. Besides the abundance of adults, an observation on attack frequency (%) in each variant (experimental and blank) was performed. In 2008, the abundance of adults was lower compared to previous years, around 80 adults in the period between June 10 and late July, untreated version. In the experimental lot, the abundance was 19 adults in the mentioned period in the six traps.

Because the abundance of adults was lower this year, the frequency of attacks was insignificant. Thus, in the untreated lot, the attack rate was of about 40% and in the experimental lot it was 10%.

Pitfall with (*S*)-CPB as bait-attractant compositions was placed in the potato plant field. The effect of aggregation pheromone has been a migration, colonization beetles of both sexes, for "frontier" where they were installed traps and dispensers. In all the experiments, in the pitfall were found other insects of the same order as CPB, and many CPB adults were found around, but not in trap. The above results show a good aggregation capacity of the pheromone, but the traps still have to be perfection, because not all pests attracted by the pheromone are also captured.

By capturing their pest population, the results show a fall below economic threshold without affecting the potato crop or ecosystem.

It is necessary to continue to explore alternative control methods using semiochemicals and studying to better understand behaviors generated by these semiochemicals. The chemical ecology of CPB is not yet completely understood and this incomplete knowledge makes semiochemical-based approaches inefficient when compared to traditional insecticide treatments. The management strategies for CPB control must be flexible and adaptable to ever-changing circumstances [16].

3. Pheromones for maize crop protection

3.1. Chemical ecology of western corn rootworm (*D. virgifera virgifera* LeConte)

Pest description: Class: Insect; Order: Coleoptera; Family: Chrisomelidae; Genus: Diabrotica.

Western corn rootworm (WCR) *D. virgifera virgifera* LeConte infests corn crops in North America and since 1992 has been reported in Europe [19]. In Romania, WCR was first reported in 1996 at Nadlac (Arad County), near the Hungarian border and this quarantine pest migrates eastward.

The WCR beetles are about 5–7 mm long. Adults have a dark head, a yellow pronotum, and a yellow abdomen. The legs covered with short hairs are dark brown in males and brown in

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females. Male's body color is greenish-yellow and female's body has a yellow color [20] (**Figure 8**).

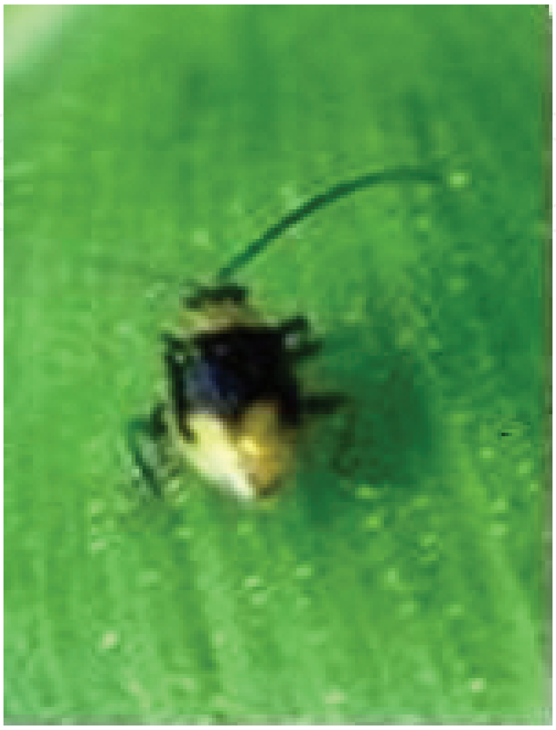

Figure 8. WCR (Diabrotica virgifera virgifera LeConte) adult (own photo on experimental plots).

The main damage is caused by WCV larva which lives in the soil and feed the roots and the adults feeding damage on corn silk and the maize in the milk stage, sometime on maize leaves or other species of plants from the spontaneous flora, but the multiplication of this species is assured by the maize crop [21] WCR (cucurbits, bean). Adults lay eggs in the soil and WCR larvae become active in May, attacking the roots of corn plants in development by drilling the cortical parenchyma, create tunnels in the central vascular tissue, which lead to the fall of the plant in windy day.

Factors that influence the pest propagation are as follows:

Soil: With good physical, chemical, and biological properties, loose and rich in humus, slightly acidic or alkaline, moist on top, favoring the breeding. Sandy soil is unfavorable for larvae, especially for the young during drought.

Climate: Gentle winter, with snow, free of strong winds; moistly spring; high air temperature for adult activity (until 30°C), favors the breeding.

Host plant: Larvae can feed on 22 species of plants, but they prefer maize and soybean. *Human intervention*—early seeding, the high density of the maize plants, excess nitrogen fertilization, irrigation, all these technologies contribute to the pest breeding.

Monitoring: Pheromone traps and yellow sticky traps were used.

Control: From nonpollutant methods it can be mentioned: pheromone traps, color traps, autochthonous natural enemies, and biological product Spinosad 240 SC (based on filamentous bacteria *Saccharopolyspora spinosa*) [22].

In the establishing of the pest's control strategy, an important part it has the prognosis of its appearance, which is based on the number of adults/plant (sticky traps with sexual pheromones baits), the number of larva and eggs/sample, the intensity of the caused damage to the silk of the corn cobs.

The reduction of the adult population is an important part in the reduction of the larva population of the next year and in the reduction of the damage to the cobs, which influences the production of beans and the quality of the seeds. It is recommended the control of adults, because their act by of destruction on the silk and implicitly compromise the pollination process when it is registered a density of more than 10 adults/plant at the commercial hybrids and five adults/plant at corn for seed [23].

3.2. Experimental research using synthetically sexual pheromone for WCR

Sexual pheromone of the *D. virgifera virgifera* Le Conte was identified by Guss et al., from virgin females of the WCR as 8-methyl-2-decanol propanoate (1,7-dimethyl-nonan-1-yl propanoate) [24].

The way of synthesis proposed and carried out in "Raluca Ripan" Institute for Research in Chemistry Laboratory is described in **Figure 9** and have five stages [25]. On this way, the racemic mixture of the four enantiomers was obtained [26].

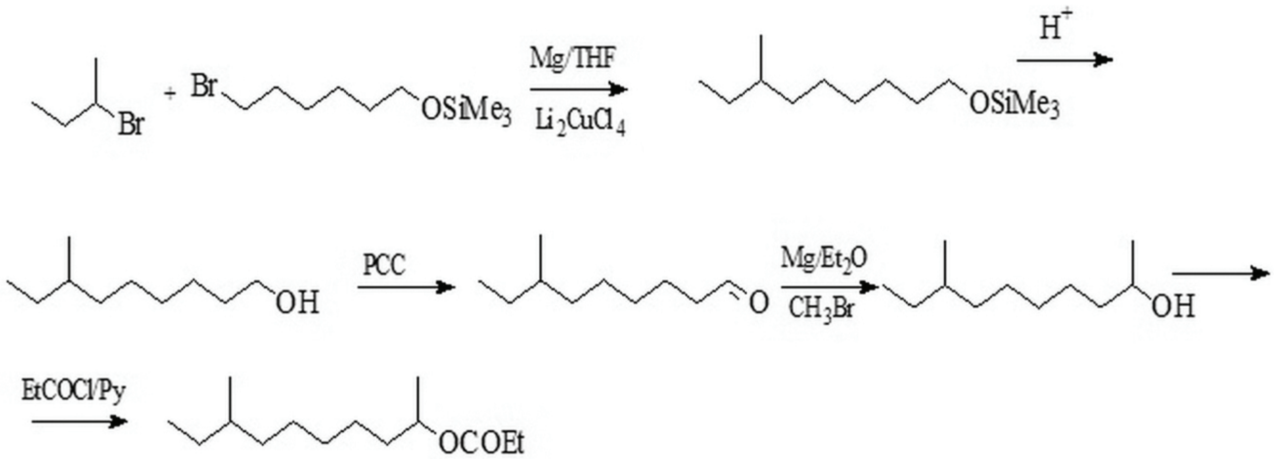

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Figure 9. 1,7-Dimethyl-nonan-1-yl propanoate, WCR sexual pheromone synthesis.

The reaction yields for each stage was >74% and the intermediary and reaction products were identified through GC-MS, IR, and NMR [26].

In the case of the 1,7-dimethyl-nonan-1-yl propanoate there are four optically active forms or four optical isomers afferent to the two asymmetric carbon atoms C_1 and C_7. The four isomers, respectively: 1*R*, 7*R*; 1*S*, 7*S*; 1*R*, 7*S*; 1*S*, 7*R*; form two pairs of enantiomers (the **A** pair: 1*R*, 7*R*; 1*S*, 7*S*, the B pair: 1*R*, 7*S*; 1*S*, 7*R*) The GC-MS analysis carried out this time with a chiral column separates two pairs of diastereoisomers without separating each isomer (**Figure 10**, **Figure 11**).

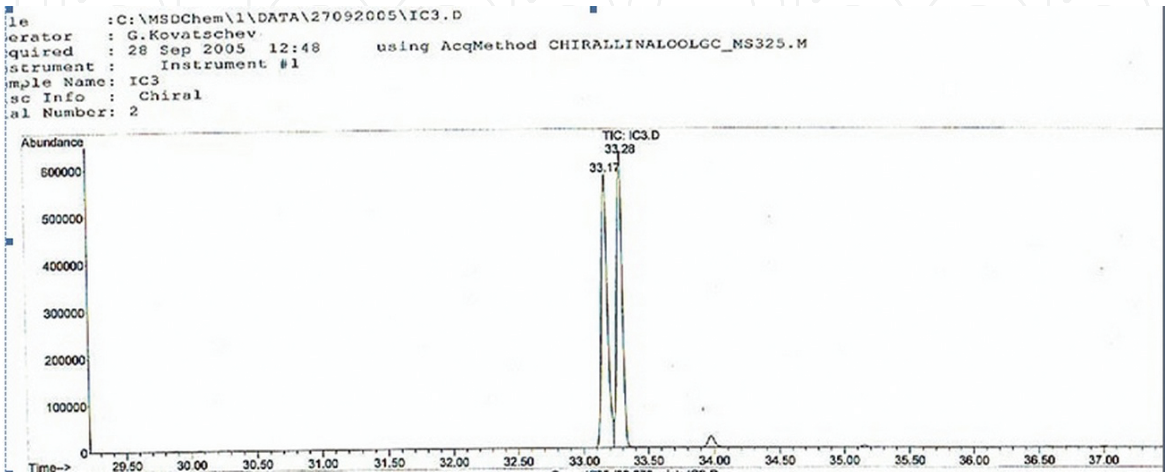

Figure 10. GC analysis of the 1,7-dimethyl-nonan-1-yl propanoate (GC Hewlett-Packard 5972 GC-MSD, capillary column HP-5MS (30 m × 0.25 mm × 0.25 μm).

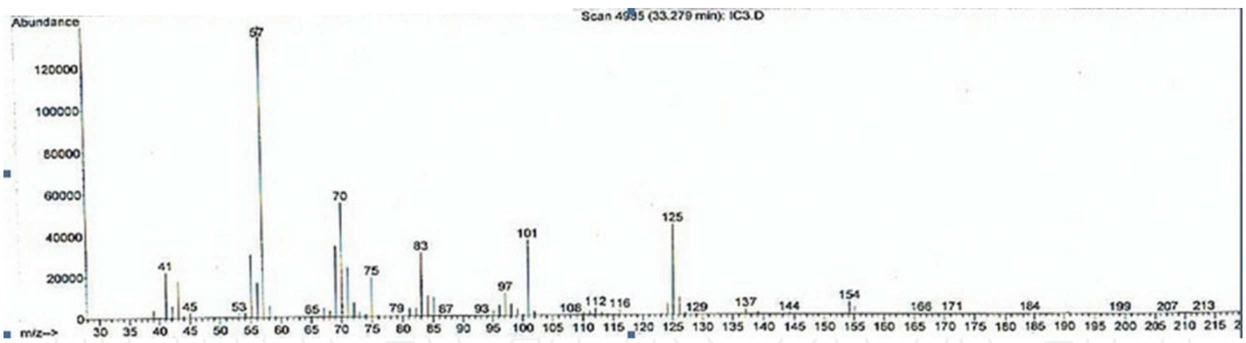

Figure 11. (EI)-MS spectrum of the 1,7-dimethyl-nonan-1-yl propanoate: GC-MS: tr = 13.91 min; *m*/*z*: 171 (M^+–COEt), 154 (M^+–OCOEt), 136, 125, 112, 101, 97, 83, 74, 70, 57 (100%), 43.

In the WCR, adults monitoring tests were carried out in the fields of Agricole Research and Developments Station (ARDS) Turda-Cluj county, Romania, the baits with racemic mixture using sticky traps showed a good attractivity.

The data from the graphic confirm both the existence of the attack of *D. virgifera virgifera* in the corn culture at the Turda Station, and the efficiency of the Romanian pheromone in comparison with others type of traps (**Figure 12**).

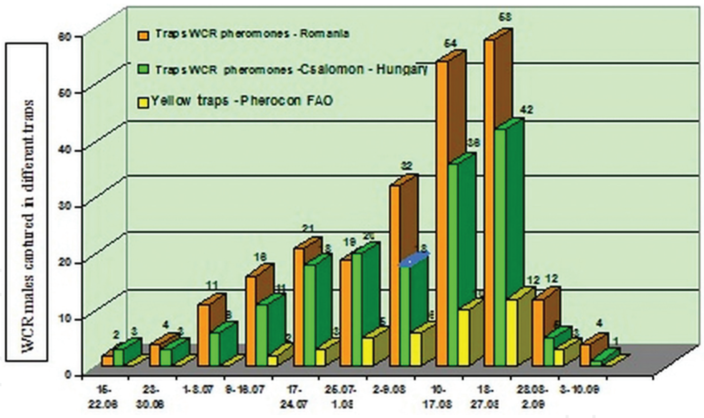

Figure 12. The efficiency of the Romanian pheromone in comparison with the imported one.

In 2005, at ARDS Turda, WCR was monitoring in field conditions, in two crop rotations: bean-wheat-corn and soy-wheat-corn, using sticky traps with bait sexual pheromones prepared in "Raluca Ripan" Institute for Research in Chemistry Cluj-Napoca.

The observations were carried out between July 12 and September 7, the number of WCR adults in this period being quite high: 921 WCR adults in the soy-wheat-corn crop rotation and 680 adults in the bean-wheat-corn crop rotation.

This pest can also develop in the soy culture, as shown in WCR adults numbers in the crop rotation with soy, as compared to the other one. The massive appearance of adults took place starting from the end of July and until the end of August, with a large number of adults in the second decade of August, when, in the traps with sexual pheromones were registered 100–248 adults/week.

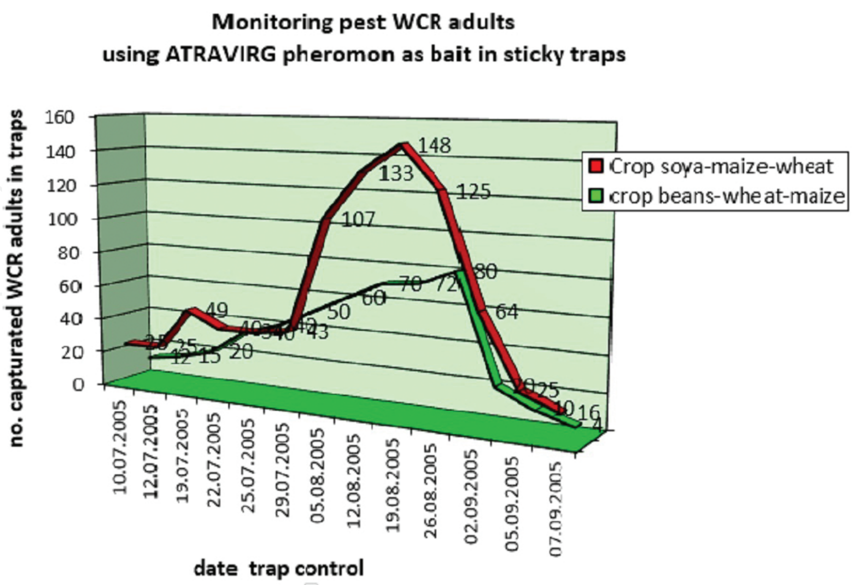

Figure 13. WCR males captured in two different crops.

The total number of adults of *D. virgifera virgifera* captured in the traps with pheromones, in the mentioned period, was of 1601 (**Figure 13**).

In Romania, start with July 1996, western corn rootworm (*D. virgifera virgifera*) was quarantine pest. In Transylvania, adults were monitored in the corn fields, e.g., 2002–2005 in ARDS Turda (**Figure 14**).

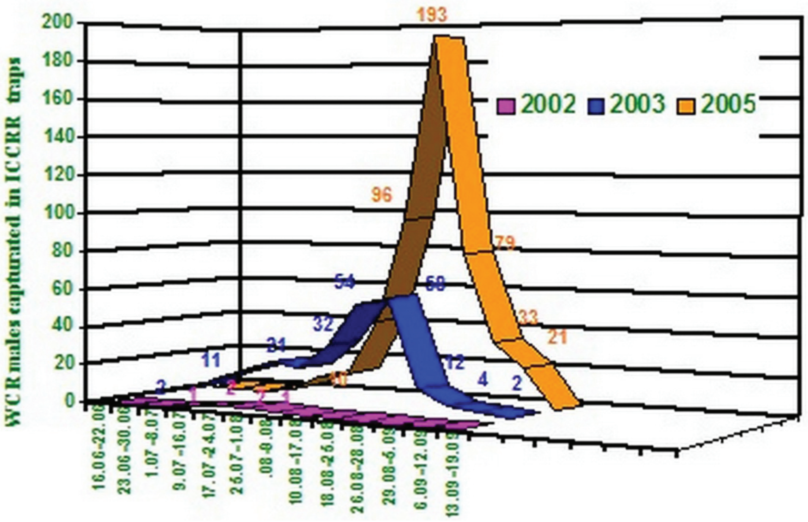

Figure 14. WCR pest "fly" dynamic in ARDS Turda fields (2002–2005).

As bait in sticky traps are 1,7-dimethyl-nonyl propanoate (WCR sexual pheromones) synthetized in "Raluca Ripan" Institute for Research in Chemistry (RRIRC) (**Figures 15** and **16**).

Figure 15. One single RRIRC sticky trap bait WCR sexual pheromones after 2 weeks exposed in corn field, ARDS Turda, Romania, 2005 (own photo on experimental plots).

Figure 16. RRIRC sticky traps (own photo on experimental plots).

3.3. Recommended biotech corn crop protection using pheromone traps

Together with other measures to reduce *D. virgifera virgifera* larval population (crop rotation, the seeding, and seed treatments), using synthetic sex pheromone traps is a recommended option for the pest management.

Traps used for western corn rootworm *Diabrotica virgifera virgifera* are placed on a stick in the ground or in more vigorous corn plant at the height of 1 m, usually 1–2 weeks before presumptive appearance of the pest (in June).

Adults begin to emerge, usually in late June, when the corn plant already has silk (the beetle's favorite food). If used for monitoring, 9 traps/ha have to be installed at the edge of the maize lot. The traps are inspected twice a week when count the captured beetles and clean the trap by removing butterflies, insects or leaves that accidentally entered on the sticky surface. Sticky plate is replaced twice per month, pheromone bait once per month, and observations are made in 5–7 days. Depending on the number of adults captured, the chemical treatment is indicated or not. So, monitoring and treatments recommended are as follows:

- If the number of catches is 5–8 WCR adults/trap in next year. If corn is sown on the same plots, the roots will be attacked by larvae. It is necessary either seed treated with insecticide or treatment ground for larvae. Treatment with granular soil insecticide is done either when seeding or at first diggings. If there are larvae (i.e., eggs deposited in the previous year), only ground treatment is insufficient.

- If the number of capture is 10 adults/trap—corn for consumption or five adults/trap—corn for sowing, treatment is required for adults.

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The field tests show how important are traps with pheromonal baits for monitoring the appearance of WCR adults in crops and for decreased adult populations during mating season so that generations of larvae in the next year are reduced.

4. Aggregation pheromones used in pine forest protection

4.1. Chemical ecology of six-spined spruce bark beetles *P. chalcographus* L.

Pest description: Class: insect; Order: Coleoptera; Family: Scolytidae; Genus: Pityogenes.

P. chalcographus infests Norway spruce [*Picea abies*], especially the younger trees or, in competition with another bark beetles *Ips typographus*, the upper regions of older trees. Bark beetles (Coleoptera, Scolytidae) must compete for food and space in which to reproduce within the relatively thin phloem layer of their host tree [27]. *P. chalcographus* is rather small for bark beetles, being only 2 mm long and weighing 1.2 g, the color is dark brown almost black [28].

Biology: In the Nordic countries, *P. chalcographus* has a single generation in a year. Romania had two flights per year. The first flight was in April-June (76–94% from the flight on all growing season), the second flight was in July-August (6–24% from the flight on all growing season) [29].

Damage: Both sexes are aggregated through male pheromone released. The attracted males want to join the attack and secure an area for his and several female's young. The female deposits their eggs in galleries excavated in the vascular cambium and secondary phloem. The phloem layer is only about 2–4 mm thick and rich in nutrients; successful breeding is dependent on the death of these tissues. Larval galleries have a length of 2–4 cm, are dense, well printed on bark and wood weak [30]. Aggressive bark beetle species like *P. chalcographus* are associated with pathogenic blue stain fungi, which help them to overcome the defense reaction of the host tree. Some of the fungi in this group are pathogenic and may play an important role in the death of the tree by blocking water conduction [31] or indirectly by overstimulating tree defense mechanisms that may exhaust the host [32, 33].

Host: Norway spruce is the preferred but not the only host. Bark beetles belong to the family of ypids. Usually, these insects are attracted to and breed on trees felled or broken by wind, on trees affected by fire or sunstroke, on trees severely debilitated by drought or pollution, and the trees having lost defense capability. Most of the time, these beetles live under the bark of trees, feeding inner part of the bark, and leave this place just to seek new sources of food.

Monitoring: At the temperature of 16.8–17°C, beetles *P. chalcographus* become active and could be monitored [34].

Control: Pest control is carried out by pheromone traps. The use of pheromones by "mass trapping" technique is one of the few ways to protect the forest ecosystem.

4.2. Experimental research using synthetically aggregation pheromone for bark beetle *P. chalcographus*

4.2.1. Chemical synthesis of the main component

The bark beetle *P. chalcographus* aggregation pheromone is a "cocktail" with four components: 2-ethyl-1,6-dioxaspiro-[4,4]-nonane (Chalcogran)—the main component [35] and secondary components: methyl-*E*2,*Z*4-decadienoate; α-pinene and ipsdienol.

The proposed synthesis *for 2-ethyl-1,6-dioxaspiro*-[4,4]-*nonanne* is represented in (**Figure 17**, **Figure 18**), it has four steps with 1,6-hexanediol as starting substance. It is an original reaction path except for the last stage, where the reaction conditions used by Cekovic and Bosnjak [36] at the cyclization of 1,7-nonanediol were modified [37, 38].

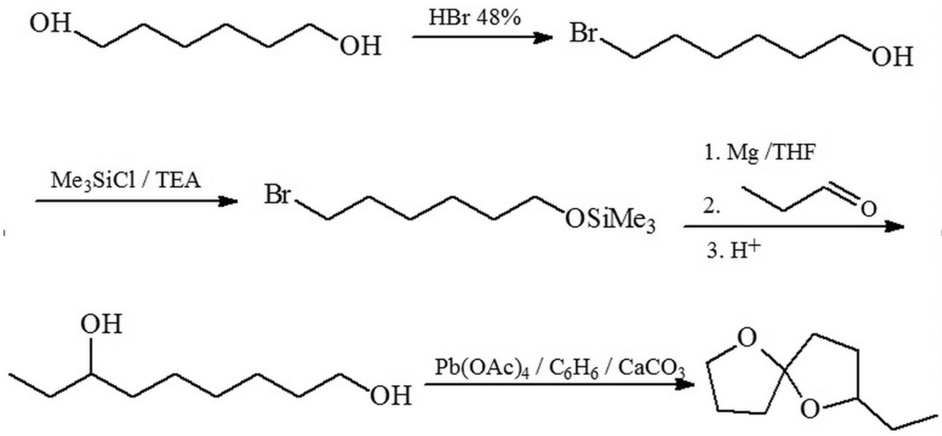

Figure 17. The reaction path which was proposed and carried out in laboratory.

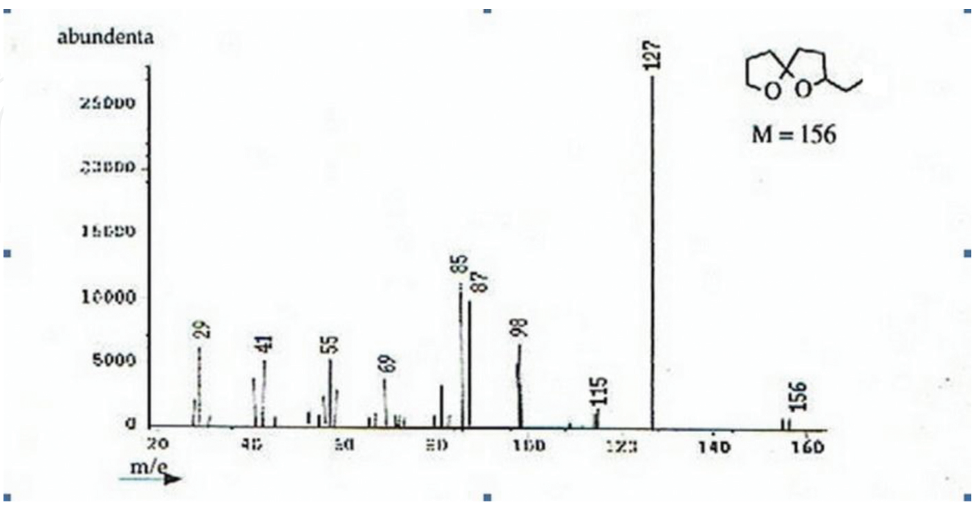

Figure 18. The mass spectra of the main product of reaction: 2-ethyl-1,6-dioxaspiro-[4,4]-nonane (**1**).

The 2-ethyl-1,6-dioxaspiro-[4,4]-nonane (**1**), having two asymmetric centers, has two pairs of optical diastereoisomers: **A** (2*R*,5*S*-**1**; 2*R*,5R-**1**) and **B** (2*S*,5*S*-**1**; 2*S*,5*R*-**1**) (**Figure 19**). The natural pheromone contains the pair of diastereoisomers (2*S*,5*R*)-**1**—biologically active and (2*S*,5*S*)-**1** —biologically inactive [39, 40].

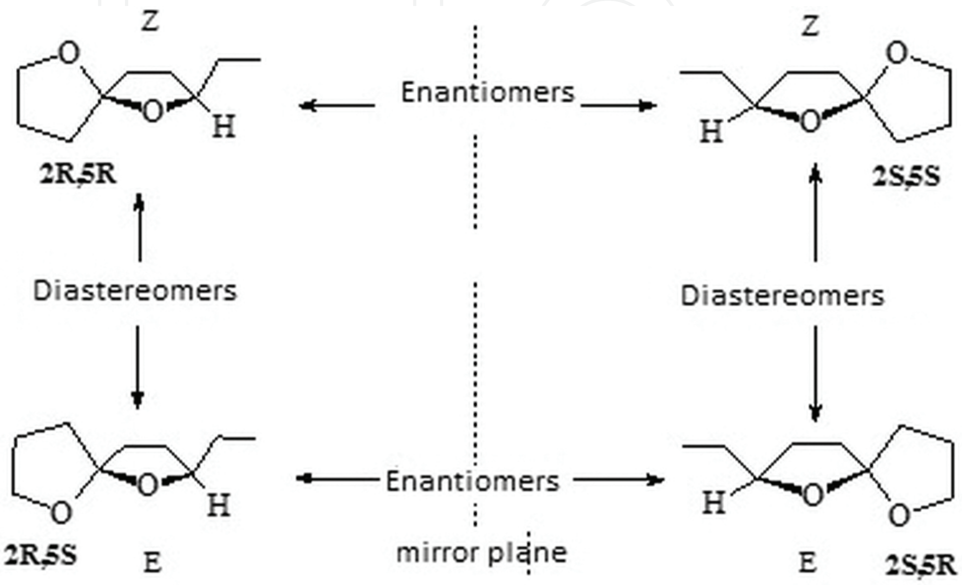

Figure 19. Enantiomers and diastereomers for 2-ethyl-1,6-dioxaspiro-[4,4]-nonane.

All the four stereoisomers were obtained by synthesis and this racemic was tested.

4.2.2. Field experiments

The biological activity, respectively the efficiency of the Romanian pheromone baits is tested in comparison with other imported compound. All the tests were obtained in Brasov area (Romania) between 2001 and 2004 (**Tables 1** and **2**).

Year	Location of the experiments Romania, Brasov	The type of trap	The type of bait	No. of traps	The period and duration of observation (days)	The number of captures	The intensity of the attraction
2001	Gârcin (OS Săcele)	Wing	Atrachalc	3	16.05–12.07 (58 days)	1.405	8.07
			Baits import	3		70	0.4
2004	Tamina (OS Braşov)	Theysohn	Atrachalc	3	31.05–20.07 (51 days)	1.292	8.4
			Baits import	2		503	4.9

Table 1. Experimental results in Romanian forests, 2001–2004.

Year	Location of the experiments	The type of Trap	The type of bait	No. of traps	The period and duration of observation (days)	The number of captures	The intensity of the attraction
2002	Gârcin (OS Săcele)	Wing	Atrachalc	5	21.05–28.07 (68 days)	35.531	104.5
2003	Gârcin (OS Săcele)	Wing	Atrachalc	3	19.05–1.07 (43 days)	35.679	276.5

Table 2. Experimental results , Romanian forests, 2002–2003.

Figure 20. *Pityogenes chalcographus* wing trap installed in forest (own photo on experimental plots).

4.2.3. Conclusions

(1) In 2001 and 2004, tests show the increased attractiveness of baits with the Romanian pheromone—Atrachalc, compared to another baits (import), irrespective of the type of trap used.

(2) In 2002 and 2003, the tests carried out with the Atrachalc baits and wing traps show in the same location different no captured beetles according to the period of time when the traps were placed and the observations were made. Besides the time factor, weather conditions or other elements from the ecosystem could influence catches

(3) 2009 comparative tests in Brasov area with different lures obtained higher level of the captured beetles using Atrachalc—Romanian baits. Wing traps with Atrachalc lure are recommended by experts for Romanian forests [41].

4.3. Recommended biotechnique: *P. chalcographus* "mass-trapping"

For monitoring and control bark beetles, pheromone lures are placed in wing-type trap (**Figure 20**). The traps are placed at the forest edge at about 5 m in the case of old forests and about 15 m in the case of young forests. Between the traps, distance is 30–50 m. To a high infestation, 2–3 traps/ha are used and for low infested forest one trap/ha is used. Traps are installed in late April to late August usual on a tree already attacked by bark beetles. Pheromone baits are replaced at no more than 6 weeks.

5. Conclusions

A changing climate with higher growing season temperatures and altered rainfall patterns make control of native and invasive insects an increasingly urgent challenge. Treatments with increasing amounts of insecticides are not a solution; it is time to intensify interdisciplinary research on semiochemicals based on a scientifically sound understanding of pest biology to provide the urgently needed and cost-effective technical solutions for sustainable insect management worldwide [42].

Using pheromones in order to protect the above-mentioned crops is first of all an ecofriendly method, avoiding these ways the overloading environment with insecticides. The ecosystem remains unaffected due to the high selectivity and specificity of the semiochemicals. Sex pheromone baits in sticky traps attract insects very selective, only the species that emitted for mating the natural sex pheromons.

This method does not affect another components of the ecosystem such as soil, air, water, or animals. The insect pest population falls below the economic damage threshold. In the case of the bark beetle is the most efficient combating method, because this pest lives (acts) underneath the bark, a place where insecticides cannot be applied.

The same happen in the case of WCR because it is very difficult to use pesticides in the maize crop. Because Colorado potato beetles develop rapidly resistance to insecticides using aggregation pheromone as bait in a proper trap could be a solution to control this pest.

All studies and experimental reviews above-mentioned have enhanced knowledge of chemical communication in and highlight the potential of semiochemicals as a component of future integrated management strategies.

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