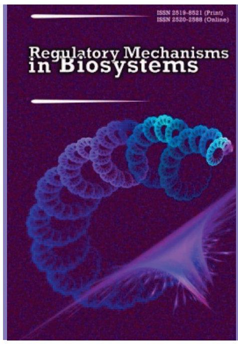

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2022, 13(1), 80–84
doi: 10.15421/022211

Effects of aromatic compounds on movement activity of *Pyrrhocoris apterus* in the conditions of a laboratory experiment

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Article info

Received 19.01.2021
Received in revised form 15.02.2022
Accepted 16.02.2022

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Nehrii, V. V., & Brygadyrenko, V. V. (2022). Effects of aromatic compounds on movement activity of Pyrrhocoris apterus in the conditions of a laboratory experiment. Regulatory Mechanisms in Biosystems, 13(1), 80–84. doi:10.15421/022211

In the conditions of an urban environment, insects, including *Pyrrhocoris apterus* (Linnaeus, 1758) (Hemiptera, Pyrrhocoridae), are subject to dozens of volatiles: solvents, food flavorings, cosmetic products. For firebugs, some attract them, some scare them off, and to some they are indifferent. In this article, in the conditions of laboratory experiment, we evaluated the influence of chemical substances on rates of movement of firebugs. Under the influence of the substances we studied, there occurred no significant increase or decrease in the activity of firebugs. The most significant effect on rates of movement of firebugs was exerted by solvent, white spirit and solvent 646 p – firebugs travelled 12 cm distance for 24.2%, 49.6% and 58.7% of the time the control group did. Movement activity of firebugs was insignificantly decreased by aromatic extracts Royal milk extract, Butcher's broom extract and chrysalis oil: firebugs travelled 12 cm distance for 132.7%, 135.2% and 146.8% of time the control group. Aromatic substances likely interact with receptors on antennae of firebugs, and therefore the insects need additional time for orienting reactions, deciding whether those substances signal presence of potential food objects in the accessibility zone.

Keywords: pheromones; attractants; repellents; kairomones; allomones; anthropogenic pollution of the environment; firebugs; litter fauna.

Introduction

Phyrrohocoris apterus (Linnaeus, 1758) is a common Palearctic species of insect, which occurs in some parts of tropical Africa and Asia (Kristenová et al., 2011). This species belongs to the Pyrrhocoridae family (Hemiptera), which consists of almost 340 species of 33 genera around the world, including only 44 species of 15 genera living in the Palearctic (Ghahari et al., 2013). The natural environment of *P. apterus* includes Europe, the Near East, Pakistan, and reaches Central Siberia in Russia; the species lives in Kazakhstan, Uzbekistan, and also Mongolia and North-West China. In Central Europe, it can be found at altitudes of up to 1,000 m (Socha et al., 2003; Endrestøl et al., 2020). *Phyrrohocoris apterus* has been introduced to North America. *Phyrrohocoris apterus* is a phytophage which prefers seeds of *Tilia* and *Malva* (Kristenová et al., 2011). *Phyrrohocoris apterus* is unable to fly, and therefore migration of certain specimens of this species does not exceed several meters – at the very most several dozens of meters a day. This species is exposed to industrial and domestic contaminants in local parks in urban conditions. Like other insects of the moderate climatic zone, *P. apterus* is subject to seasonal fluctuations in abiotic factors which form vital cycles of this species, stimulating evolution of multiple adaptive reactions at all levels of biological organization – from genetic and molecular to population (Koštál et al., 2000). Overcooling ability among some individuals of *P. apterus* strongly correlates with microclimatic environmental conditions (Tomáš et al., 2018). *Phyrrohocoris apterus* is an easily cultivated insect that can be easily kept in large groups (Frolov et al., 2017). In laboratory conditions, *P. apterus* is fed with sunflower seeds (Lopatinaelena et al., 2014).

Protective exudates of *P. apterus* are volatiles with low molecular weight. In larvae, as well as imagoes of *P. apterus*, molecular weight of 43 identified compounds varies 88–254 g/mol. Secretion of *P. apterus* is mostly a mixture of aldehydes. Nymphs of *P. apterus* exude (E)-2-octena,4-oxo-(E)-2-octenal chemical compounds. Those short-chained saturated and non-saturated aldehydes are very effective chemical irritants; as known, oxo-octenal was found to be the main component of the insect's defensive secretions (Farine et al., 1992). When *P. apterus* are disturbed, they are able to produce special defensive chemical substances that scare the predators off (for example, ants). Ants exposed to those substances may not only be scared away but paralyzed (Farine et al., 1992). Ants pose a serious threat for this species of insect. (E)-2-Hexenal, hexanal, hexanol, hexanoic acid, 2-butyl-2-octenal, ß-pinene, limonene, and farnesenes are pheromones produced by various *Pyrrhocoris* species for protection against ants (Jeffrey, 1988).

Flavorings and volatile biologically active compounds are used for many reasons; they may potentially have fumigant, repellent or attractive actions toward insects (Titov et al., 2021), including *P. apterus*. To combat pathogenic organisms, microbiologists and virologists study the influence of flavorings on production of food products (Boyko et al., 2016). Megapolises have green zones, where peculiar and relatively rich entomofaunas live, and where representatives of *Pyrrhocoris* (including *P. apterus*) are obviously among the commonest species of insects (Fedyay et al., 2018). For firebugs, the most significant factor is the structure of the phytocoenosis, moisture and thickness of litter, and also the mechanical composition of the soil. In urban environments, there develops a specific composition of the community of litter invertebrates, which is significantly different from the compositions of natural forest ecosystems; many invertebrates react even to insignificant changes in their environment by decrease in population (Faly et al., 2017). Many flavorings and materials they are synthesized from are approved for use in food products, are used in households, medicine and cosmetics (Boyko et al., 2019). The objective of this article was influence of various flavorings on an abundant species of insect in urban environments – *P. apterus*.

Flavorings are continuously becoming more attractive due to the increasing demand for them for medicinal, culinary and domestic purposes. There are many volatiles of plant metabolites, including well-known mixtures that became known as essential oils. Compositions of essential oils are very complex. Some components of essential oils could be useful in various spheres, such as cosmetology, veterinary and human medicines. Essential oils are used as effective additives to synthetic compounds – the obtained substances are used in the chemical industry (Hanif et al., 2019). Organic molecules used as synthetic supplements in households are non-homogenous in structure. They often contain a mixture of 50% R- and 50% L-isomers, called racemate; many volatile, synthetic compounds can contain 2, 3 and even 4 optically active atoms, and therefore, may be represented as mixtures of 4, 8 and even 16 different isomers. At the same time, the influence on olfactory receptors may be caused by only one compound of the mixture of isomer molecules. Volatiles may cause various diseases in people and animals. Man-used food additives and other chemical compounds, both synthesized and natural, enter the environment and become food for litter and soil saprophages and phytophages (Martynov et al., 2017). A large amount of those substances, production leftovers are dumped at landfills as municipals wastes. Substances used in human food enter municipal water-cleaning facilities, and therefore such substances in one way or the other come into contact with insects. In general, the influence of food supplements and other volatile chemical substances on the organisms of insects remains poorly studied (Martynov et al., 2017). There is an acute need for adequate evaluation of the influence of numerous aromatic substances used in various spheres on the environment, particularly insects. Methods of decreasing their negative influence on invertebrates are in demand and are being developed (Martynov et al., 2018).

Materials and methods

A total of 52 pure chemical aromatic compounds and their productive mixtures were used in the experiment (Table 1) in October–November, 2021. Firebugs for the experiment were collected manually in the territory of the Botanical Garden of Oles Honchar Dnipro National University, Gagarina Avenue (Dnipro), mainly near the small-leaved lime (Tilia cordata Mill.) trees. Before the experiment, firebugs were held in a 2 L plastic container with seeds and leaves of lime.

For each experiment, 17 firebugs were collected. In the experiment, we used a metal container with the bottom diameter of 33 cm and a 25 cm-diameter circle drawn in the center. Prior to the experiment, firebugs were put under a plastic cup (250 mL) with no bottom. To the upper part of this plastic cup, we attached a thread with a cotton ball saturated with flavoring. At the top, the container was covered by a transparent polyethylene film with a circular hole cut at the center, the diameter of which corresponded to that of the plastic cup. At the beginning of the experiment, the plastic cup was lifted from the bottom of the container at the height of 10–15 mm, giving firebugs an opportunity to run across the entire bottom of the container. Movement of the firebugs was recorded using a video camera for 120 seconds. The experiment was conducted in constant air moisture, light and temperature, because the rates of insects' movement are significantly affected by temperature and light in the place where they had been cultivated earlier for many days (Titov et al., 2021). Before the experiment and in the place where the experiment was conducted, the conditions were as follows: non-bright dispersed light, absence of gradients of temperature and moisture, absence of effects of noise and vibration of objects. It was very important to stop possible air movement above the place of the experiment, and therefore the experiment was conducted as carefully as possible. All the manipulations with the firebugs were performed carefully to avoid mechanical damage to the firebug imagoes of. The firebugs that had been already used in this experiment were not used in experiments again to prevent possible distortion of the results (Titov et al., 2021).

A total of 312 video fragments lasting 120 seconds each was recorded in the experiment (51 substances, 5 video fragments for each, 57 control video fragments without flavorings, evenly distributed on different days of the experiment). During the test of each flavoring or mixture of substances, we used 85 *P. apterus* specimens for each, 5 experiments were conducted for each of the substance with 17 firebugs. The experiments were carried out in the laboratory, with equal temperature, in the standard electronic light, avoiding exposure of the firebugs direct to sunlight (Martynov et al., 2019). Daily fluctuations of temperature did not exceed 3 °C (+18…+21 °C), relative air moisture equaled 60–70%.

Statistical analysis of the results was conducted in Statistica 8.0 (StatSoft Inc., USA). The differences between the selections were determined using single-factor dispersion analysis (ANOVA) and were considered reliable at $P < 0.05$.

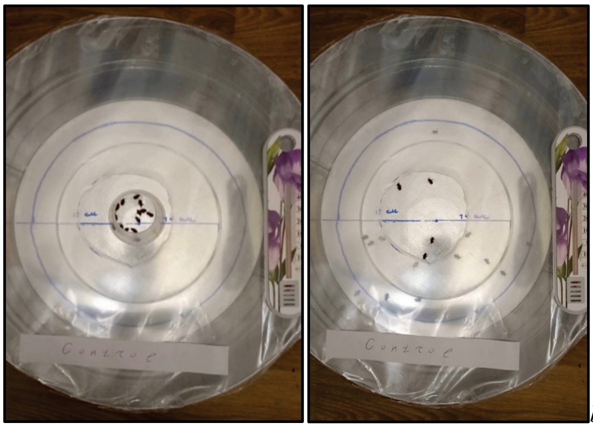
a  *b*

Fig. 1. Experiment in the container: *a* – beginning of the experiment (all firebugs in the center, within cylindrical plastic cup), *b* – middle of the experiment (firebugs running away from the center of the container)

Results

Exposure to the studied substances caused neither significant increase or decrease in moving activity of firebugs. The greatest effect on the rates of movement of firebugs was exerted by solvent, white spirit and solvent 646 p – 12.5 cm distance was travelled by the firebugs in 24.2%, 49.6% and 58.7% of the time the control group spent (Table 1). Moving activity of firebugs was insignificantly decreased by the following aromatic extracts; Royal milk extract, Butcher's broom extract and chrysalis oil – firebugs travelled 12.5 cm distance in 132.7%, 135.2% and 146.8% of the time the control group took (without flavoring).

Discussion

The highest increase in moving activity of firebugs occurred during exposure to organic solvents – solvent, white spirit, solvent 646 p, polysorbate-80, solvent 647. Volatile components of those mixtures – carbohydrate molecules – likely interact with receptors of the antennae of firebugs and increase the bugs' running speed. On the other hand, organic extracts, given in the lower part of Table 1, likely interact with receptors on the antennae of firebugs, and therefore additional time is needed for orientation, determining whether those signals are indicating a potential food object in accessible range.

Pyrrhocoris apterus is used as a model for different ecological, morphological, evolutionary, genetic and many other experimental studies (Socha et al., 2008). Chemical defense of herbivorous and omnivorous insects depends on the plants they consume (Kristenová et al., 2011). Interrelations between insects and plants have specific dynamics: changes are related both to seasonal dynamics of host plants and development cycles of phytophage insects. Such interactions are usually associated with feeding: analysis of saliva of phytophage insects helps us to better understand relations between insects and plants. For example, saliva proteins produced by aphids are similar to substances that regulate the activity of cells of consumed plants (Sharma et al., 2013).

For example, limonene – the main component of essential oil from *Citrus aurantium* L. – is an efficient insecticide against imagoes of weevils (Jacobson, 1982). Unlike other substances, 24 h action of D-limonene, even in 10 g/L concentration, caused death to 50% of nematode larvae of *Strongiloides ransomi* (Boyko et al., 2017). From a phytochemical perspective, plants are producers of chemical substances, while insects are receivers of chemical aromatic signals (Stanley et al., 2020). In our experiment, D-limonene only caused slight (up to 128.7% compared with the control group) statistically insignificant increase in time it took for the insects to travel the distance. That is, this substance displayed neither fumigant (lethal) nor repellent activities toward firebugs, since it did not increase, but even slowed the migration of insects to the source of smell.

Table 1
Changes in moving activity of firebugs *P. apterus* under the influence of various chemical substances and their mixtures (x ± SD, n = 85)

Variants of the experiment	x	SD	Compared with the control, %
Control	38.5	34.0	100.0
Sovent	9.3	6.0	24.2
White spirit	19.1	22.1	49.6
Solvent 646 p	22.6	28.0	58.7
Polysorbate-80	23.4	28.5	60.7
Solvent 647	24.0	31.7	62.3
Extract of *Calendula officinalis*	26.4	29.2	68.6
Extact of *Quillaja saponaria*	28.3	27.9	73.4
Rerosene	28.6	30.7	74.3
Butyl acetate	30.4	30.5	79.1
Cyolohexane	31.4	30.4	81.4
Flower extract of *Malva sylvestris*	31.5	31.6	81.9
Ceramide complex	31.7	32.2	82.3
Xylene	32.1	33.5	83.3
Pomegranate extract	32.5	33.4	84.3
Cocamidopropilbetaine	33.7	34.5	87.6
Chitosan	33.8	34.8	87.7
Horse chestnut extract	33.9	31.9	88.1
Iactic acid	34.7	30.7	90.1
Mousse babassu	35.1	34.0	91.0
Sapindus makorossi	36.2	32.4	93.9
Avobenzone	36.3	31.2	94.3
Methoxypropane-2-ol	36.8	33.6	95.5
Parsley extract	38.3	35.0	99.4
Butoxyethanol	39.9	35.6	103.6
Anisaldehyde	40.2	36.3	104.3
Honeyquat	41.5	36.3	107.7
Formic acid	42.4	36.9	110.2
Farnesol	43.4	37.1	112.6
Ethyl acetate	43.8	38.9	113.6
Huile de ricin sulfatec	44.1	31.3	114.4
Gamm-Undecandctone-4-Olde	44.6	36.9	115.9
Citral natnral	45.4	37.6	117.8
Comelina oil	45.7	30.1	118.8
Acrylate W2000	46.3	33.9	120.2
Lenciolal	46.7	36.1	121.2
Sage extract	47.4	36.4	123.1
Biogold	47.5	37.1	123.2
Fruit acids	48.0	34.4	124.8
Dimengi sulroxide	48.2	33.2	125.1
Benzal asetate	48.6	35.7	126.2
Red pepper extract	49.2	36.1	127.7
Isoamyl acetate	49.3	37.0	128.0
D-limonene	49.5	38.6	128.7
Benzaldehyde	49.8	36.1	129.4
Cucumber extract	50.1	34.2	130.0
Complex benzoate and sorbate	50.2	34.0	130.3
Silk protein hudrolyzate	50.4	35.7	130.9
Gasoline	50.6	38.2	131.3
Royal milk extract	51.1	35.5	132.7
Butcher's broom extract	52.0	37.1	135.2
Chrysalis oil	56.5	36.7	146.8

Note: x – mean time (seconds) firebug needs to leave the 25 cm circle, SD – standard deviation.

The main event in production of cedar oils was the establishment of industries in China and Texas. Oil that is being extracted in Texas is likely obtained from *Juniperus chinensis* L. (Pinales, Cupressaceae). In 1986, 420 T of cedar oil, worth 847,000 US dollars, was imported to the USA. At the same time, production of cedar oil allowed the US to export 1 M kg of it, worth $5.6 M (Swan, 1989). Small doses of essential oils gradually decrease the survivability potential of insect pest, notably reducing its appetite and oviposition. Many essential oils prepared from herbs exert insecticide activity (Abdel-Sattarab et al., 2010). Farmers have developed various methods of using bamboo, wooden planks, dirt, bricks, cow manure, various parts of plants or plant extracts as natural insecticides during storage of industrial grain (Kabrambam et al., 2021). Essential oils transmit chemical signals, which allow plants to control and regulate the environment: deter phytophages, attract insects for pollination, inhibit germination of seeds of other species of plants, and compete with various plant species for water and light (Hanif et al., 2019).

Three elements are required for relationship between organisms: an organism that produces a biological signal, the environment through which it is transmitted, and another organism which should somehow receive signal and react to it. Pheromones are chemical substances used for the communication between animals of one species. Despite multiple exceptions, pheromones are a more important way of interaction between insects of one species than eyesight and hearing. Pheromones that are a stimulus for a receiving individual cause immediate response and are called "releaser pheromones" (Shorey, 1973). Exocrine glands produce biological compounds onto the surface of an insect's body, which serve for defensive purposes, as repellents, or attractants. An example of attractant is pheromones that are a complex mixture of chemical substances (Cerkowniak et al., 2015). The main physiological roles of odorant glands in *P. apterus* are storing and emitting volatile substances with a strong odour as a defense against predators (Brian, 1979).

In an experiment, the destructive effect of four alcohols was compared, produced separately or as various combinations; synthetic pheromone (B)-trans-pityol was used in all experiments, because the previous work (Pierce et al., 1995) had revealed that it is as effective at luring insects into traps as insect-produced (c)-trans-pityol (Groot et al.,1999). Understanding dynamics of interaction between insects and plants promotes understanding about various aspects of relationships between plant-consuming insects and their host plants (Sharma et al., 2013).

Working with volatiles from green leaves demonstrated that they disrupt aggregation of several species of bark beetles. Using synthetic pesticides against insect pests has led to the emergence of pesticide-resistant insects, severe pollution of the environment, negative impact on natural enemies of phytophages (parasites, predators), leading to ecological imbalance in ratio of predators to their prey, and poses a threat to human health. Therefore, ecologically pure alternative strategies of combating insect pests are a priority of further innovative activity in the sphere of protecting plants in agriculture and forestry (Gopalakrishnan et al., 2014).

Conclusion

The problem of the effects exerted by aromatic compounds on insects has recently become more relevant, which is first of all related to the increasing amounts of production of chemical food additives, broad use of carbohydrates for domestic purposes, use of essential oils in cosmetics. The problem of utilization of domestic wastes also plays an important role in environmental pollution of urban agglomerations. All of this causes uncontrolled environmental pollution by aromatic compounds of various chemical composition. Their effects on various insect groups, particularly on *P. apterus*, remain completely unstudied. We do not fully understand the possible consequences of the broad spread of aromatic compounds for fauna of urban invertebrates.

In our laboratory experiment, we evaluated influence of 51 aromatic compounds and their mixtures on *P. apterus*. Exposed to the studied substances, *P. apterus* was seen to have no significant increase or decrease in moving activity. The most significant increase in the rates of movement of firebugs was exerted by solvent, white spirit and solvent 646 p – to travel 12.5 cm-distance, the firebugs spent 24.2%, 49.6% and 58.7% of the time the control group had spent. Movement activity of firebugs was insignificantly decreased by the aromatic extracts Royal milk extract, Butcher's broom extract and chrysalis oil – to travel 12.5 cm distance, it took firebugs 132.7%, 135.2% and 146.8% of the time taken by the control group. Such results confirmed our hypothesis that aromatic compounds have a weak effect on *P. apterus*. This species of insect dominates in the conditions of industrial pollution, near human homes. This is likely related to the poor reaction of *P. apterus* to aromatic compounds and their mixtures, used by people.

The research "Ecological evaluations of flavorings as attractants and repellents for invertebrates important in agriculture and forestry" was supported by the Ministry of Education and Science of Ukraine No. 0122U001225.

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