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Ability of honey bees to detect and recognise isomers of cresol

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Cresols are well-known volatile pollutants contaminating air, water, and soil. The ability of honey bees to learn odours of cresol isomers (*o*-, *m*- and *p*-doses 0.01 μg, 0.1 μg, 1 μg, and 10 μg of a single isomer) and to recognise the odour of the isomer learned among odours of other cresol isomers was tested. Classical olfactory conditioning of the proboscis extension reflex was used. The acquisition level was isomer- and odour intensity-dependent. Bees were able to learn the odours of *o*- and *p*-cresol at the lowest dose (0.01 μg) and that of *m*-cresol at the dose of 0.1 μg. The highest acquisition level was achieved at the dose of 1 μg of *p*-cresol and at the doses of 0.1 μg and 1 μg of *m*- and *o*-cresol. However, conditioning at the dose of 10 μg of any cresol isomer caused a response drop in honey bees. After being conditioned to one isomer of cresol, from 13.5% to 52.1% of bees responded to other isomers when tested. It was established that honey bees discriminate between the odours of *m*-cresol and *p*-cresol best: no bees were found to respond to *p*-cresol after being conditioned to *m*-cresol. The investigation showed that discrimination between the odours of *m*-cresol and *o*-cresol was the weakest: 52.1% of bees conditioned to *m*-cresol responded to the odour of *o*-cresol. The current results demonstrate the possibility and limitations of using honey bees as biosensors for detecting cresols.

Key words: cresol, pollution, *Apis mellifera*, honey bee, olfactory learning, odour recognition, olfaction, biosensor

INTRODUCTION

Environmental pollution is one of the most important problems analysed from various aspects by applied ecologists. As a result of growing human population density and rapid economic development, pollution with both inorganic (heavy metals, sulphur, nitrogen or other oxides) and organic contaminants has become a matter of great concern. Organic pollutants cresols can contaminate air, water, and soil. The major sources of cresol isomer emission into the atmosphere are car exhaust fumes, wood burning, electric power plants, coal tar, petroleum refineries, and chemical industries (Feigenbrugel et al., 2004). Higher concentrations of these pollutants are known to have a strong toxic effect on all live systems (Yokoyama et al., 1982; Keweloh et al., 1991; Vanholder et al., 1999; Lesaffer et al., 2003). Animal studies on rodents have revealed that cresols cause death of rat liver cells (Thompson et al., 1994), degeneration of cardiac, bone marrow and nerve cells in mice (Uzhdavini et al., 1972). Inhalation exposure to cresols is reported to cause respiratory diseases including the development of pneumonia, pulmonary oedema, and haemorrhage in humans (Clayton and Clayton, 1982). The U. S. Environmental Protection Agency has classified *o*-cresol, *m*-cresol, and *p*-cresol as Group C of the possible human carcinogens (U. S. EPA, 1999). Because of the increased pollution with phenols and their toxicity, more and more attention is being paid to the detection and analysis of phenolic contaminants in the environment.

To estimate pollution level, physical, chemical, and bioindication methods are used. In recent years, more attention has been directed to the living organisms as indicators of environmental health. A wide range of species and populations have been used as bioindicators (Lighthart et al., 2000; Tanabe, Subramanian, 2003; Chatenet et al., 2006 and others). It is possible to include olfactory sensory systems among the methods employed for tracing volatile chemicals in the air. Due to their especially well-developed olfaction, insects are ranked alongside the most promising pollution tracers (Houtary, Mela, 1995; Schöning et al., 2000; Park et al., 2002). Honey bees (*Apis mellifera* L.) are well known as a species extremely sensitive to many odours. Therefore, due to their ability to detect and discriminate among odours, honey bees could be explored as biosensors for detecting odoriferous substances or their mixtures. The well-developed ability of bees to learn and remember olfactory stimuli and to discriminate among a variety of odours is documented (Menzel, 1990). Studies by Vareschi (Vareschi, 1971) and the more recent ones by other authors (Laska et al., 1999, Laska, Galizia, 2001) have demonstrated that bees clearly distinguish among more than 95% of the odour pairs tested. They can be trained to detect

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not only natural odours but also those of explosives, chemical and biological warfare agents (Bromenshenk et al., 2003).

The aim of the present paper was to reveal the ability of honey bees to recognise odours of cresols, the well-known environmental pollutants. The study focussed on the two main issues: 1) to establish if bees are able to perceive cresols and to determine the most effective doses of these chemicals for learning; 2) to ascertain if they are able to discriminate among all the three isomers of cresol.

MATERIALS AND METHODS

Bees

The study was carried out in May–August 2004. The colony of the honey bees *Apis mellifera* L. was housed at the Institute of Ecology, Vilnius University. The colony contained the brood in all the development stages, the mated egg-laying queen, honey and pollen. Worker bees were collected from the hive entrance and placed into plastic perforated cages (160 mm in length and 30 mm in diameter) in groups of ten individuals. The cages with bees were placed into a freezer and kept there until bees ceased moving. When anaesthetised, insects were removed from the cages and were individually restrained in a test-stand with wing-clips (Skirkevičius et al., 2000). Approximately 30 min. after fixing, the bees were used for learning experiments.

Approximately 10 min. before the start of conditioning, all the bees were tested for the motivation to respond to an unconditioned stimulus by touching their antennae with a drop of sucrose solution, i.e. without feeding. An unconditioned response (and, later, a conditioned response also) was scored if the tip of the proboscis crossed the line between the opened mandibles. If a bee failed to extend its proboscis, it was excluded from the experiment.

Stimuli and their presentation

The olfactory conditioned stimuli used were *orto*-cresol (Pure, Riedel DeHaën), *meta*-cresol (99.5%, Sigma-Aldrich) and *para*-cresol (Pure, Fluka AG). These compounds are structural isomers (Fig. 1).

Fig. 1. Molecular structures of the cresol isomers used

The conditioning trial consisted of forward-pairing of a conditioned stimulus (odour) with the unconditioned one (Bitterman et al., 1983). The conditioned stimuli were used in four different intensities. To vary stimulus intensity, only the odourant dose was changed. The highest concentration of the olfactory conditioned stimulus was produced by dissolving 1 mg of cresol in 1 ml of solvent (hexane). Serial decimal dilutions were made by adding hexane to obtain the lower concentrations needed. 0.01 ml of each solution was applied on a glass stick. Following solvent (hexane) evaporation in approximately 2 min., the stick with an olfactory stimulus was delivered to the worker bee's head at a distance of 10 mm from the antennae and was kept there for 6 sec. The rewarding stimulus used was 30% sucrose solution. Reward delivery started 4 sec. after the odourant onset by touching antennae with a drop of this solution. The stimulation elicited immediate proboscis extension, and the honey bee was allowed to feed for 2 sec. The training procedure of each bee lasted for approximately 6 sec. Stimuli were presented for, and reactions were recorded of each insect individually. Bees received training and test trials at an inter-trial interval of 7 min.

To maintain the experiment area clean, odours were removed with the help of an air exhaust system.

Dose learning

The odourants used for stimulation were single compounds. Cresol isomers (*o*-, *m*- or *p*-) at the dose of 0.01 μg, 0.1 μg, 1 μg or 10 μg were used as conditioned stimuli. The doses described refer to the amount of a substance in the solid phase on a glass stick, not to the concentration in the gaseous phase. Twelve groups, each consisting of a minimum of 35 bees, were trained in total. Each group was divided into subgroups of 8–10 bees, and those were trained on different days. All the bees received 6 training trials.

Isomer recognition

The conditioned stimuli used were three cresol isomers: *m*-, *o*- and *p*-cresol. In learning and testing phases, stimuli containing 1 μg of each cresol were presented. The dose of the stimulus was selected within the range of the doses revealed as the most effective for honey bee learning (Fig. 2). Insects received 4 training and 4 test trials. Responses to the three cresol isomers (*o*-, *m*- and *p*-cresol) and to the evaporated solvent (hexane) were tested. The testing sequence was designed as indicated in the Table. To avoid the possible olfactory pre-exposure, the honey bees that might have experienced cresols (which responded to the olfactory stimulus during the first learning-trial) were excluded from the testing procedure. Five percent of bees responding to *m*-cresol, and 3% of those responding to *p*-cresol were excluded.

Three groups, each containing a minimum of 57 bees, were conditioned in total. Each group was divided into six subgroups of 8–10 bees, which were trained on different days.

Statistical analysis

All the data were presented as the average percentage of bees that responded with proboscis extension during a given trial (mean ± one standard error). The Mann Whitney U-test was applied when statistical significance of response levels of two

Table. **Sequence of stimuli presentation while investigating the honey bees' ability to recognise cresol isomers**

*Training	Testing sequence			
	1	2	3	4
o-	*m*-	*p*-	*o*-	Evaporated solvent
m-	*o*-	*p*-	*m*-	Evaporated solvent
p-	*m*-	*o*-	*p*-	Evaporated solvent

* Bee received four forward pairing learning-trials.

o-, m- and p-, cresol isomers used as olfactory stimuli.

Solvent – hexane used as control.

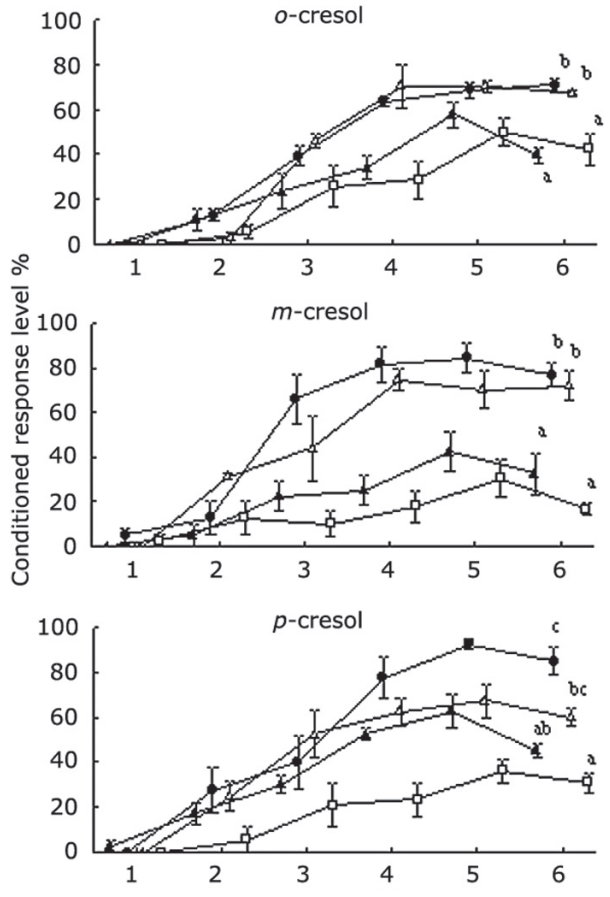

Fig. 2. The honey bee proboscis extension response to the odour in the course of six conditioning trials. The conditioned response level is the mean percentage (± SE) of the bees that responded during each learning trial prior to the presentation of the unconditioned stimulus. The bees were conditioned to one of the three cresol isomers: *o*-, *m*- and *p*-cresol. Open squares represent responses to the dose of 0.01 µg, open triangles – to the dose of 0.1 µg, solid circles – to the dose of 1 µg, and solid triangles – to the dose of 10 µg. Different letters (a, b, c) denote significant differences ($P < 0.05$, Mann Whitney U-test)

groups was evaluated. The Kruskal-Wallis test was applied when response levels of more than two groups were analysed. For the comparison of within-group responses recorded during conditioning and testing phases, the Wilcoxon signed-ranks test was used. All the analyses were carried out using Statistica software.

RESULTS

Dose learning

As a first step, we determined whether the odourant doses used could serve as effective conditioning stimuli. Twelve groups of bees were conditioned to either *o*-, *m*- or *p*-cresol at different doses.

Honey bees were able to learn the conditioned stimulus at any doses of the odourant used in the experiment (Fig. 2). The response level of 11 groups (out of 12) of bees was significantly higher in the sixth training trial than in the first one (Wilcoxon test, $P < 0.01$), except the response to the dose of 0.01 µ of *m*-cresol. In that case, the test did not show a statistically significant difference in the response level between the first and the sixth (only 16.6% of bees were responding) training trials ($P = 0.09$; Wilcoxon test). In the groups conditioned to the lowest doses either of *o*- or *p*-cresol, 42.5% and 30.8% of bees responded, respectively.

Basing on the result obtained, we conclude that the odourant doses as low as 0.01 µg of *o*- or *p*-cresol can be detected by a number of honey bees and thus the odours can function as conditioned stimuli for them.

The comparison of levels of conditioning to any of the cresols tested revealed no statistically significant differences when the presented dose of any isomer was the same (in all the cases, $P > 0.05$, the Kruskal-Wallis test).

The results demonstrated that the acquisition levels of bees trained on high (10 µg), intermediate (1 µg and 0.1 µg) and low (0.01 µg) doses of all the three cresol isomers ($P < 0.01$ for *o*-, *m*- and *p*- cresol; Kruskal-Wallis test) differed. The conditioning levels recorded at intermediate odourant doses (0.1 µg and 1 µg) of all the cresol isomers were significantly higher than at the lowest doses (0.01 µg) (0.1 µg vs. 0.01 µg: $P < 0.05$; and 1 µg vs. 0.01 µg: $P < 0.05$; Mann Whitney U-test;) (Fig. 2). However, the conditioning levels at the highest dose (10 µg) of *m*-cresol (32.5% of bees responded) or *o*-cresol (39.7% of bees responded) were significantly lower than those at the intermediate doses (for *m*-cresol 10 µg vs. 0.1 µg: $P < 0.01$; 10 µg vs.1 µg: $P < 0.01$; and for *o*-cresol 10 µg vs. 0.1 µg: $P = 0.02$; 10 µg vs. 1 µg: $P = 0.02$; Mann Whitney U-test) and they were equal to the conditioning levels at the lowest dose of the odourants (10 µg vs. 0.01 µg, for *m*-cresol: $P = 0.24$; and for *o*-cresol: $P = 0.88$; the Mann Whitney U-test). A slightly different situation was observed when bees were conditioned to the highest dose (10 µg) of *p*-cresol (45.0% of bees responded). The conditioning level at this dose was lower compared to that at the dose of 1 µg (10 µg vs. 1 µg: $P < 0.01$; the Mann Whitney U-test), and did not differ from the conditioned response at the doses of 0.1 µg and 0.01 µg (10 µg vs. 0.1 µg: $P = 0.29$; and 10 µg vs. 0.01 µg: $P = 0.24$; the Mann Whitney U-test).

Thus, the most effective dose for conditioning slightly differed depending on a cresol isomer: for *p*-cresol it was 1 µg (85.0% of bees responded), and for *m*-cresol and *o*-cresol those were 0.1 µg and 1 µg (72.0% of bees responded to the dose of 0.1 µg and 76.7% to the dose of 1 µg of *m*-cresol, and 67.5% of bees responded to the dose of 0.1 µg and 70.8% to the dose of 1 µg of *o*-cresol). Four learning-trials were enough for bees to gain the optimal acquisition level.

Consequently, the dose of 1 µg of cresol (*o*-, *m*- and *p*-) and 4 learning-trials were used for conditioning and testing isomer recognition abilities in honey bees.

Isomer recognition

We carried out the investigation to establish whether honey bees can distinguish among the odours of *o*-, *m*- and *p*-cresol. The results indicated that during the fourth trial in the learning phase, proboscis extension responses did not differ among the three groups of the bees trained on different cresol isomers ($P = 0.47$; Kruskal-Wallis test). Over 70% of individuals responded to the odourant (Fig. 3).

When testing response to the conditioning odour, the proboscis extension response corresponded to that recorded during the last acquisition trial (in the all cases $P > 0.05$; Wilcoxon test) (Fig. 3).

In the test phase, bees from all the groups responded more often to the isomer of cresol which was used for conditioning than to the other two isomers (in all the cases $P < 0.05$; Wilcoxon test).

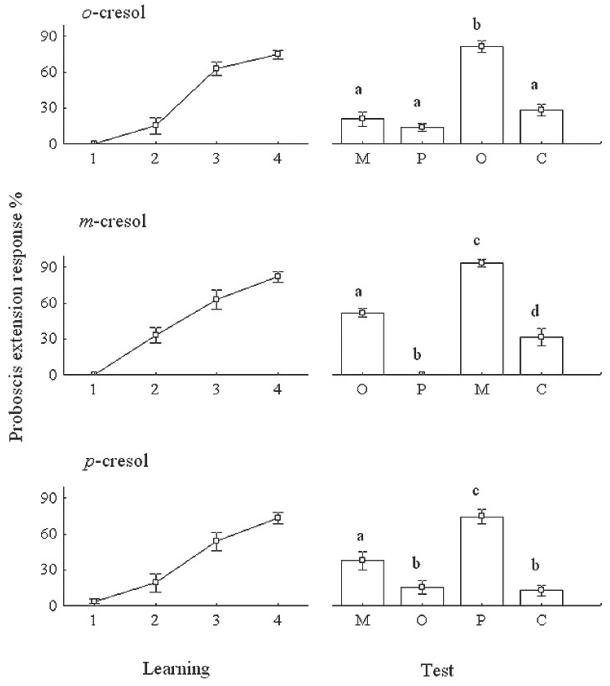

Fig. 3. The honey bee proboscis extension response to the odour in the course of conditioning and testing trials. The proboscis extension response level is the mean percentage (± SE) of the bees which responded to the odour presentation within 4 sec. of each trial. The three groups differed in the conditioned odour used: *o*-, *m*- or *p*-cresol. Responses to *o*-cresol (O), *m*-cresol (M), *p*-cresol (P), and solvent hexane as control (C) were analyzed in the test phase. Different letters (a, b, c, d) denote significant differences between columns ($P < 0.05$, Wilcoxon signed-ranks test)

When *m*-cresol was used for conditioning, not a single bee responded to the odour of *p*-cresol used in the test. Meanwhile, 52.1% of bees responded to *o*-cresol, but that response was significantly lower compared to the response to the odour of *m*-cresol (93.5%) (*m*-cresol vs. *o*-cresol: $P < 0.01$; Wilcoxon test).

The group conditioned to *o*-cresol also responded to *m*-cresol and *p*-cresol when tested. However, the response to *o*-cresol was significantly higher (81.3%) compared to that to *m*-cresol (20.6%) or *p*-cresol (13.5%) (*o*-cresol vs. *m*-cresol: $P < 0.01$; *o*-cresol vs. *p*-cresol: $P < 0.01$; the Wilcoxon test). There was no significant difference between the responses to *m*-cresol and *p*-cresol (*m*-cresol vs. *p*-cresol: $P = 0.09$; the Wilcoxon test).

The bees conditioned to *p*-cresol also responded to the odours of *o*-cresol (15.3%) and *m*-cresol (37.6%) during testing, however, these responses were significantly lower compared to the response to *p*-cresol (74.7%) (*p*-cresol vs. *o*-cresol: $P < 0.01$; *p*-cresol vs. *m*-cresol: $P < 0.01$; the Wilcoxon test). In that case, honey bees responded more often to the odour of *m*-cresol than to that of *o*-cresol (*m*-cresol vs. *o*-cresol: $P < 0.01$; the Wilcoxon test).

Thus, the results allow us to conclude that honey bees are able to recognise and distinguish the odour of *m*-cresol from that of *p*-cresol very well. However, it was more difficult for them to discriminate between the *o*-cresol odour and that of *m*-cresol.

DISCUSSION

Dose learning

Our results revealed that honey bees were able to learn and recognise the odours of *orto*-, *meta*- and *para*-cresols. The effective doses for conditioning to cresols as well as the number of learning-trials required to obtain optimal acquisition results were estimated. The acquisition level was dependent on the dose and cresol isomer. Approximately 30% of individuals learned the lowest dose used (0.01 μg) of *o*- and *p*-cresol. The lowest learned dose of *m*-cresol was 0.1 μg. The most effective doses for bee conditioning were 1 μg and 0.1 μg of *o*- and *m*-cresols, and 1 μg of *p*-cresol. An increase in the dose of any of cresols caused a decrease in the acquisition level. This effect, in our opinion, may be explained by the repellence of cresols. An indication of this was the changed behaviour of bees: contrary to the typical behaviour of bees, observed when they were trained on the lower doses, bees would not direct their antennae to the stimulus or extend proboscis and would behave as if they were avoiding stimulation. Some cresols are known as repellents of honey bees. There are some field data indicating that the foraging activity of honey bees in plots of blooming dandelions sprayed with *o*-cresol (100 ml/l, 26 gallons per acre) decreases in an hour after the application of this chemical (Mayer, Lunden, 1999). It is also known that bees do not forage on flowers of some *Araceae* and *Apocynaceae* plants containing *p*-cresol (Kite, 1995; Andreas et al., 2006).

The learning curves demonstrate that honey bees learn odours of cresols during the 4th or the 5th learning-trial. Half of the obtained curves show a slight decrease at trial 6, which suggests an effect of satiation with sucrose reward (Menzel et al., 2001). So, the isomer recognition experiment involved only 4 training-trials.

Isomer recognition

The results of our experiments involving different cresol isomers for conditioning and testing demonstrated that bees distinguish the odour of the learned isomer from that of other isomers. The excellent performance of *Apis mellifera* observed here is in agreement with the earlier studies revealing high ability of this species to discriminate among different kinds of odours (Vareschi, 1971; Laska et al., 1999; Laska, Galizia, 2001). Honey bees in our study distinguished among all the isomers of cresol. However, there were some bees, which were unable to recognise isomers of cresol and made mistakes when tested. The percentage of such bees was low. The best discrimination by honey bees was observed after their conditioning to *m*-cresol and testing with the *p*-cresol odour. There were no bees responding to *p*-cresol. However, the ability to discriminate between the odours of *m*-cresol and *o*-cresol proved to be the lowest (52.1% of bees conditioned to *m*-cresol responded to *o*-cresol). In other cases, when bees were conditioned to the odour of *o*- *or p*-cresol, up to 30% of bees responded to the odour of the tested isomer. Thus, bees responded more often to the isomer used for learning than to the tested one.

The comparison of the test results obtained when conditioning was performed to one isomer of cresol and testing to another, reveals some asymmetry. Thus, when conditioned to *m*-cresol or to *p*-cresol, honey bees demonstrated different responses to the *o*-cresol odour. Honey bees responded more often to *o*-cresol after being conditioned to *m*-cresol than after being conditioned to *p*-cresol ($P < 0.01$; Mann Whitney U-test). When bees were conditioned to *m*-cresol and *o*-cresol, the response levels to *p*-

cresol were low and not significantly different (P = 0.27; Mann Whitney U-test). Similarly, when bees were conditioned either to *o*-cresol or *p*-cresol, their responses to *m*-cresol did not differ significantly (P = 0.30; Mann Whitney U-test).

When analysing the testing data, we observed that the test stimulus, first presented after learning, elicited higher response compared to that of the second test stimulus (the difference in responses to the first and second stimulation was statistically significant, except the test after conditioning to *o*-cresol) (Fig. 3). There may be some reasons behind this fact. After the learning phase, during which the conditioned stimulus was presented with reward periodically, bees were already sensitised and anticipated the following stimulus with reward. Therefore, they responded to the test stimulus with proboscis extension. The unknown stimulus reduced this response and maybe inhibited the following one when another unknown stimulus was provided. Moreover, bees conditioned to individual compounds or to mixtures generalize their responses to a wide range of other olfactory stimuli, and sometimes this generalization is asymmetric (Pelz et al., 1997; Sandoz et al., 2001). That is the case when bees respond more to the odour B after learning the odour A than in the reverse situation. We hypothesize that this phenomenon might be observed in two cases: 1) lower responses of bees to *m*-cresol after being trained on *o*-cresol than to *o*-cresol after being trained on *m*-cresol 2) or higher responses of bees to *m*-cresol after being trained on *p*-cresol than to *p*-cresol after being trained on *m*-cresol. Also, when not-reinforced tested stimuli are very similar (in our case, isomers of cresol), the habituation (or even extinction) of proboscis extension response might occur. It might be assumed that it was the effect of the habituation process that was observed in test-trial 2, when response to the second test-stimulus was considerably lower than response to the first one during test-trial 1 (in case *p*- and *m*-cresol) (Fig. 3). However, when honey bees received the odour of the conditioned stimulus in test-trial 3, response greatly increased (74.7% of bees responded to *p*-cresol, 81.3% of bees to *o*-cresol and 93.5% of bees responded to *m*-cresol). In our opinion, this result provides evidence on the ability of honey bees to recognize isomers of cresol. The question whether the obtained asymmetry in response to test stimuli is the result of periodicity in the presentation of stimuli and, consequently, habituation, or the result of asymmetric generalization, has still to be investigated in the future.

Olfactory biosensors may be applied for the detection of both cresols and other pollutants or specific volatile compounds in the environment (explosive, narcotic or toxic substances). Electrophysiological methods recording responses of receptors of insect antennae, *i.e.* the peripheral olfactory system, are used for these purposes most often (Houtary, Mela, 1995; 1996; Schöning et al., 2000; Park et al., 2002). As our study has demonstrated, the behavioural responses (proboscis extension) of honey bees using olfactory conditioning must be made use of together with the above mentioned biosensors to record the presence of volatile compounds in the environment. One can suppose that the usage of the whole insect as a biosensor, rather than the peripheral olfactory system alone, will only enhance the sensitivity and discriminating ability.

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References

1. Andreas J., Dötterl S., Meve U. 2006. The chemical nature of fetid floral odours in stapeliads (Apocynaceae-Asclepiadoideae-Ceropegieae). *New Phytologist*. Vol. 172. P. 452–468.
2. Bitterman M. E., Menzel R., Fietz A., Schäfer S. 1983. Classical conditioning of the proboscis extensions reflex in honeybees (*Apis mellifera*). *J. Comp. Psychol.* Vol. 97. P. 107–119.
3. Bromenshenk J. J., Henderson C. B., Smith G. C. 2003. Biological systems (Paper II). In: MacDonald et al. (ed.) *Alternatives for Landmine detection*. RAND's Science and Technology Policy Institute, U. S. P. 273–283.
4. Chatenet P., Froissard D., Cook-Moreau J., Hourdin P., Ghestem A., Botineau M., Haury J. 2006. Populations of *Myriophyllum alterniflorum* L. as bioindicators of pollution in acidic to neutral rivers in the Limousin region. *Hydrobiologia* Vol. 370. P. 61–65.
5. Clayton G. D., Clayton F. E. 1982. *Patty's Industrial Hygiene and Toxicology*. Third, revised ed. New York: John Wiley Sons. P. 213.
6. Feigenbrugel V., Le Calvé S., Mirabel P., Louis F. 2004. Henry's law constant measurements for phenol, o-, m-, and p-cresol as a function of temperature. *Atmospheric Environment*. Vol. 38. P. 5577–5588.
7. Huotari M., Mela M. 1995. The blowfly olfactory biosensor: its sensitivity and specificity. *Transducers '95. Eurosensors IX*. P. 462–465.
8. Huotari M., Mela M. 1996. Insect olfactory biosensors for amines and human sweat odours. *18th Annual International Conference of the IEEE Engineering in Medicine and Biology Society. Biosensors II*. Amsterdam, 1996. P. 95–96.
9. Keweloh H., Diefenbach R., Rehm H. J. 1991. Increase of phenol tolerance of *Escherichia coli* by alterations of the fatty acid composition of the membrane lipids. *Arch. Microbiol.* Vol. 157. P. 49–53.
10. Kite G. C. 1995. The floral odour of *Arum maculatum*. *Biochemical Systematics and Ecology*. Vol. 23. P. 343–354.
11. Laska M., Galizia C. G. 2001. Enantioselectivity of odor perception in honeybees (*Apis mellifera carnica*). *Behav Neurosci*. Vol. 115. P. 632–639.

12. Laska M., Galizia C. G., Giurfa M., Menzel R. 1999. Olfactory discrimination ability and odor structure-activity relationships in honeybees. *Chemical Senses*. Vol. 24. P. 429–438.
13. Lesaffer G., De Smet R., Belpaire F. M., Van Vlem B., Van Hulle M., Cornelis R., Lameire N. Vanholder R. 2003. Urinary excretion of the uraemic toxin *p*-cresol in the rat: contribution of glucuronidation to its metabolization *Nephrol. Dial. Transplant*. Vol. 18. P. 1299– 1306.
14. Lighthart B., Prier K., Loper G. M., Bromenshenk J. 2000. Bees scavenge airborne bacteria. *Microb. Ecol*. Vol. 39. P. 314–321.
15. Mayer D. F., Lunden J. D. 1999. Effects of different chemicals on honey Bee foraging of dandelion. *Proceedings of the 73rd Annual Western Orchard Pest & Disease Management Conference*. Portland, USA, 6–8 January 1999. Washington: Pullman, Washington State Univ. P. 83.
16. Menzel R. 1990. Learning, memory and cognition in honey bees. In Kesner R. P., Olten D. S. (eds.). *Neurobiology of Comparative Cognition*. Lawrence Erlbaum, Hillside, NJ, USA. P. 237–292.
17. Menzel R., Manz G., Menzel R., Greggers U. 2001. Massed and spaced learning in honeybees: the role of CS, US, the intertrial interval, and the test interval. *Learning and Memory*. Vol. 8. P. 198–208.
18. Park K. C., Ochieng S. A., Zhu J., Baker T. C. 2002. Odor discrimination using insect electroantennogram responses from an insect antennal array. *Chem. Senses*. Vol. 27. P. 343–352.
19. Pelz C., Gerber B., Menzel R. 1997. Odorant intensity as a determinant for olfactory conditioning in honeybees roles in discrimination, overshadowing and memory consolidation. *The Journal of Experimental Biology*. Vol. 200. P. 837–847.
20. Sandoz J., Pham-Delègue M., Renou M., Wadhams L. 2001. Asymmetrical generalisation between pheromonal and floral odours in appetitive olfactory conditioning of the honey bee (*Apis mellifera* L.). *J. Comp. Physiol*. Vol. 187. P. 559–568.
21. Schöning M., Schroth P., Schütz S. 2000. The use of insect chemoreceptors for the assembly of biosensors based on semiconductor field-effect transistors. *Electroanalysis*. Vol. 12. P. 645–652.
22. Skirkevičius A., Blažytė L., Skirkevičienė Z. 2000. Influence of keeping bees *(Apis mellifera carnica* Pollm.) in colonies or caged on the formation of conditioned reflex on the queen bee pheromones. *Pszczelnicze zeszyty naukowe*. Rok XLIV. Nr. 2. P. 43–53.
23. Tanabe S., Subramanian A. 2003. *Bioindicators suitable for monitoring POPs in developing countries*. Center for Marine Environmental Studies (CMES), Ehime University, Japan. P. 130.
24. Thompson D. C., Perera K., Fisher R., Brendel K. 1994. Cresol isomers: comparison of toxic potency in rat liver slices. *Toxicol. Appl. Pharmacol*. Vol. 125. P. 51–58.
25. U. S. Environmental Protection Agency. 1999. *Integrated Risk Information System (IRIS) on Tricresol*. National Center for Environmental Assessment, Office of Research and Development, Washington, DC.
26. Uzhdavini E. R., Astafyeva K., Mamayeva A. A., Bakhtizina G. Z. 1972. Inhalation toxicity of o-cresol. *Trudy Ufimskogo Nauchno-Isseldovatel'skogo Instituto Gigiyeny Profzabolevaniya*. Vol. 7. P. 115–119.
27. Vareschi E. 1971. Duftunterscheidung bei der honigbiene - Einzelzell-ableitungen und verhaltensreaktionen. *Z. Vergleich. Physiol*. Vol. 75. P. 143–173.
28. Vanholder R., De Smet R., Lesaffer G. 1999. *p*-Cresol: a toxin revealing many neglected but relevant aspects of uraemic toxicity. *Nephrol. Dial. Transplant*. Vol. 14. P. 2813–2815.
29. Yokoyama M. T., Tabori C., Miller E. R., Hogberg M. G. 1982. The effects of antibiotics in the weanling pig diet on growth and the excretion of volatile phenolic and aromatic bacterial metabolites. *Am. J. Clin. Nutr*. Vol. 35. 1417–1424.

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MEDUNEŠIŲ BIČIŲ GEBĖJIMAS APTIKTI IR ATPAŽINTI KREZOLIO IZOMERUS

S a n t r a u k a

Krezoliai yra gerai žinomi aplinkos taršalai, aptinkami tiek ore, tiek vandenyje, tiek grunte. Tirtas bičių gebėjimas išmokti atpažinti krezolio izomerų dozes (0,01 μg, 0,1 μg, 1 μg, 10 μg) ir skirti izomerus (*orto*-, *meta*- ir *para*-). Mokymas atliktas taikant klasikinį sąlyginio reflekso sudarymo metodą, paremtą bitės atsaku refleksiškai iškišti liežuvėlį sacharozės tirpalu dirginant skonio receptorius. Tyrimų rezultatai parodė, kad bičių mokymosi geba priklausė nuo naudoto izomero ir jo dozės. Bitės išmoko atpažinti mažiausias naudotas *o*- ir *p*-krezolių dozes – 0,01 μg. Mažiausia *m*-krezolio dozė, kurią gebėjo išmokti bitės, buvo 0,1 μg. Didžiausias išmokusių atpažinti stimulą bičių skaičius buvo pasiektas panaudojus 1 μg *p*-krezolio, o *m*- ir *o*-krezolių – 0,1 μg ir 1 μg. Mokant didesnėmis krezolių dozėmis (10 μg) reaguojančių bičių itin sumažėjo. Bičių, maišiusių sąlyginiu stimulu naudoto krezolio izomero kvapą su kitų dviejų izomerų kvapais, skaičius kito nuo 13,5 iki 52,1%. Geriausiai bitės skyrė *m*-krezolį nuo *p*-krezolio. Po mokymo *m*-krezolio kvapu nebuvo reaguojančių į *p*-krezolio kvapą. Blogiausiai bitės skyrė *m*-krezolio kvapą nuo *o*-krezolio kvapo. Tarp mokytų atpažinti *m*-krezolio kvapą buvo 52,1% bičių, reaguojančių į *o*-krezolio kvapą. Pateikti rezultatai rodo bičių darbininkių kaip biosensorių aptikti krezoliams panaudojimo galimybes bei kai kurias diskutuotinas ribas.

Raktažodžiai: krezolis, tarša, *Apis mellifera*, olfaktorinis mokymas, kvapų atpažinimas, uoslė, biosensoriai