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# IDENTIFICATION AND BIOLOGICAL ACTIVITY OF VOLATILE ORGANIC SUBSTANCES EMITTED BY PLANTS AND INSECTS. I. COMPONENTS OF THE NATIVE SCENTS OF *Leptinotarsa decemlineata* AND *Solanum tuberosum*

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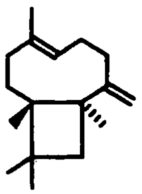
*The sesquiterpenes* trans-*caryophyllene and germacrene have been detected in the native scents of male and female individuals of the Colorado beetle* Leptinotarsa decemlineata *Say by gas-chromato-mass-spectral analysis.* trans-*Caryophyllene has also been detected in the volatiles emitted by leaves of the potato* Solanum tuberosum *L. which indicates its role as a food attractant of the Colorado beetle.*

The volatile organic substances emitted by plants and insects play an important role in the transmission of chemical information in Nature, decisively influencing intraspecies and interspecies interaction in the insect world and also interaction between insects and plants [2—4]. In this connection, there is great interest in the identification of the volatile substances emitted, on the one hand, by the imago of the Colorado beetle *Leptinotarsa decemlineata* Say and, on the other hand, by the leaves of its natural food source — the potato *Solanum tuberosum* L.

The effect of the attraction of the Colorado beetle by the scent of potato leaves has been noted by a number of researchers [5—7]. Linalool, α-terpineol, geraniol, caryophyllene and a sesquiterpene alcohol with the empirical formula $C_{15}H_{26}O$ have been identified in a steam distillate from potato leaves. There are other reports of the identification of volatile isolates from potato leaves [9, 10], but caryophyllene is not mentioned as being among them.

In an analysis of the volatile substances from a Colorado beetle imago by gas chromatography with mass-spectrometric detection (GC—MS) [11] we found [1] two compounds in an intensity ratio of 10:1, each of which had a molecular mass of 204 and corresponded to the empirical formula $C_{15}H_{24}$. Analysis of mass spectra (MS) showed that the main component was (1R,4E,9S)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene (*trans*-caryophyllene) (1). In addition to $M^{+\cdot}$ the MS of (1) contained a characteristic ion with *m/z* 189, corresponding to the splitting out of one of the methyl groups of the cyclobutane fragment, and also ions with *m/z* 133, 93, and 69, which are specific for the MS of (1) [12]. The correctness of the assignment was confirmed by the coincidence of the retention time of the compound to be identified and that of an authentic specimen of (1).



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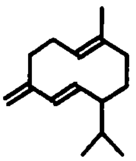

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The MS of the minor component corresponded to 3-isopropyl-6-methyl-10-methylenecyclodeca-1E,6E-diene (germacrene) (2) [12]. We must mention an ion with *m/z* 161 that is characteristic for the MS of (2), corresponding to the loss of an isopropyl group by the molecular ion, and also ions with *m/z* 119, 105, 91, and 77 characterizing the formation of alkylaromatic structures.

The identity of the compositions of the volatile products in the atmosphere from both male and female individuals was established. This shows that compounds (1) and (2) do not fulfil the function of a sex pheromone of the Colorado beetle.

Using the same method [11] and a procedure for analyzing volatile emissions of living organisms that we have developed and which is described in the Experimental part, we have identified the scent of the potato.

It was established that one of its main components is caryophyllene (1), which suggests a role of this compound as a food attractant for the Colorado beetle. In fact, olfactometric observations that we have made confirm the existence of attractive properties of (1) in relation to the Colorado beetle imago.

In order to reveal the existence of a correlation between the relative amounts of (1) in the volatile emission of the leaves of certain varieties of the potato ($Q$) and their attractiveness ($A$) for the Colorado beetle, we made an analysis of the scents of six varieties of potato. The largest amount of sesquiterpene (1) was present in the varieties Adretta and Bodalin, and the smallest amount of compound (1) was found in the varieties Romano and Kardinal (Table 1). It was also established that the values of $Q$ found correlated with the attractiveness $A$ for Colorado beetles of the potato varieties investigated. As can be seen from Table 1, varieties Adretta and Bodalin proved to be the most attractive, while the Romano and Kardinal varieties, with the lowest caryophyllene contents, were eaten considerably less willingly.

TABLE 1. Dependence of the Attractiveness ($A$) of Different Varieties of Potato on the Level of Caryophyllene in the Volatile Emission from the Leaves ($Q$)

| Potato variety | A, % | Q, % |
|---|---|---|
| Adretta | 35.5+1.10 | 9.3 |
| Bodalin | 36.5+0.52 | 8.3 |
| Detskosel'skii | 20.8+0.60 | 4.3 |
| Yunior | 26.7+0.79 | 3.3 |
| Romano | 20.3+1.04 | 2.3 |
| Kardinal | 19.5+0.44 | 1.8 |

## EXPERIMENTAL

The experiments on the identification of the volatile secretions of the Colorado beetle were carried out in the summer of 1996 in an isolated potato field, 10 × 10 m, located 100 km to the north of Ufa. After the development of potato shoots (variety Lorkh) to the budding stage, Colorado beetle larvae of the fourth stage of development were introduced into the field, and in the course of five days these withdrew into the soil. After three weeks mature specimens of the Colorado beetle appeared, which were collected in a glass jar and separated into males and females by their morphological characteristics [13]. No pairing of the individuals was observed. In the experiments to determine the dependence of the attraction of the Colorado beetle to different potato varieties on their caryophyllene content we used the potato varieties given in Table 1.

The volatile emissions were collected by the method of dynamic gas extraction followed by cryogenic focusing and GC—MS [9, 11, 14]. For this purpose, a glass sampler containing 20 males and 20 females was flushed with helium for 20 min and the volatile products were trapped in a 5 mm × 250 mm glass tube filled with the sorbent Tenax GC. Then the sorption tube was placed in a thermodesorber, and the adsorbed compounds were blown out for 20 min into a liquid-nitrogen-cooled nickel capillary with an internal diameter of 0.5 mm. The compounds to be analyzed were concentrated on a small section of the internal surface of the capillary, which enabled good chromatographic resolution to be achieved.

After the rapid heating of the nickel capillary, the organic compounds concentrated in it passed with a current of carrier gas (helium) into a chromatographic column, and the system of recording mass-spectrometric characteristics was switched on simultaneously. The GC analysis was conducted on a Finnigan 4021 instrument [quartz capillary column (0.2 mm × 50 m) with

SE-30; linear programming of the temperature: 50—250°C (6°C/min), temperature of the ion source 250°C, voltage 70 eV].

The native scents of the potatoes were investigated analogously. To take specimens, samples of the plants under investigation were covered with a filter-paper cap 500 mm in diameter with a hole in the center in which a sorption tube filled with sorbent was placed. The volatile products were trapped by the aspiration of 3 liters of air at a rate of 200 ml/min and were analyzed by the GC—MS method.

The experiments on study of the food preferendum of the Colorado beetle were conducted in Petri dishes. Disks with a diameter of 10 mm were cut out from potato leaves. To prevent the disks from drying out, filter paper moistened with distilled water was placed on the bottom of the Petri dishes and disks cut from leaves of the various potato varieties were placed on these in the form of a circle. One Colorado beetle from a group of the insects previously kept without food for two days was introduced into each dish. In a control experiment identical disks of potato leaves were kept in a Petri dish under the same conditions without insects. The time of exposure was 6 h. The experiment was conducted in 20 repetitions, and the control procedure in five. The attractiveness ($A$, %) of the leaves of the different varieties of potato for the Colorado beetle was determined in accordance with the formula

$$A=\frac{a-b}{a}\times 100,$$

where $a$ is the weight of the initial potato disk, g; and

$b$ is the weight of the residual unconsumed part of the potato disk, g.

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