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GENERAL BIOLOGY

Induction of Terpenoid Synthesis in Leaves of Silver Birch after Defoliation Caused by Gypsy Moth Caterpillars

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During outbreaks, forest species of phyllophagous insects are able to defoliate their host plants within huge areas. During the defoliation, affected plants lose a significant part of their photosynthetizing surface that results in some morphological, physiological, and biochemical changes [1]. Lamina destructing and the influence of elicitors (inducers), contained in the insect saliva, induce a cascade of biochemical reactions resulting in the synthesis of a wide range of secondary metabolites [2], which make the plant a less suitable food for insects. It has been shown that phenol compounds play a significant role in both constitutive and induced resistance of deciduous trees to insects, whereas terpenes and terpenoids are important in the case of conifer trees [3].

According to the recent studies, volatile compounds, such as monoterpenoids, can be of great importance in many angiospermous plants in the case of their damage caused by insects [4]; they often attract predators or parasitoids of leaf-eating phytophages and facilitate their search for prey [5]. Thus, such chemical signals, producing by a defoliated plant, may regulate the population density of herbivores via the attraction of their natural enemies; therefore, they represent one of the factors that control the changes in population density of defoliators species.

In this work, we studied the terpenoid composition of the leaves of silver birch (*Betula pendula* Roth.) growing in Western Siberia, after a significant defoliation of trees caused by gypsy moth (*Lymantria dispar* L.) caterpillars. This polyphage is a cosmopolitan species and exhibits regular outbreaks over large areas [6]. To demonstrate the development of the resistance to insects, we evaluated not only the terpenoid content of host plant leaves, but also some characteristics of the gypsy moth population (larval stage duration, survival rate, and potential fecundity). The results of our study are pioneer: earlier, the scientists who studied interrelations in the host plant–gypsy moth system paid their attention mainly to phenol compounds [7].

MATERIALS AND METHODS

The study was carried out in natural model areas occupied by birch shrubwood, from the middle of May to the end of June of 2007. The age of the trees included into our study was 9–11 years. In our experiment, we used two caterpillar *L. dispar* groups to evaluate the population characteristics of insects (the first group), and to make significant defoliation level of the trees (the second group; Fig. 1). Caterpillars developed from eggs collected in the autumn of 2006 in the natural outbreak (the density was about 10 clutches per tree) located in Novosibirsk oblast. Insects from the first group were grown under laboratory conditions up to the second instar and then transported to the field and enclosed in mesh sleeve cages (30×70 cm) on branches of trees [8]. Then, we covered the whole tree crowns with large mesh cages. In the case of ten experimental plants, we placed 250–300 caterpillars of the third instar (the second group) on each tree to provide defoliation (the trees were covered with large mesh cages). Ten control trees were also covered with cages but remained free of the insects of the second group (Fig. 1). Thus, in our experiment we used 20 trees. As a result, the first group of insects was isolated from the second (“defoliating”) group, which allowed us to observe any changes in the number of insects and in the population characteristics of this group. After the insects of the first group had eaten all leaves, we moved their small cage onto another shoot of the same tree; the shoot was preliminarily cleared from the insects of the second group. After two weeks, the experimental trees were defoliated by 70–80%, and we removed

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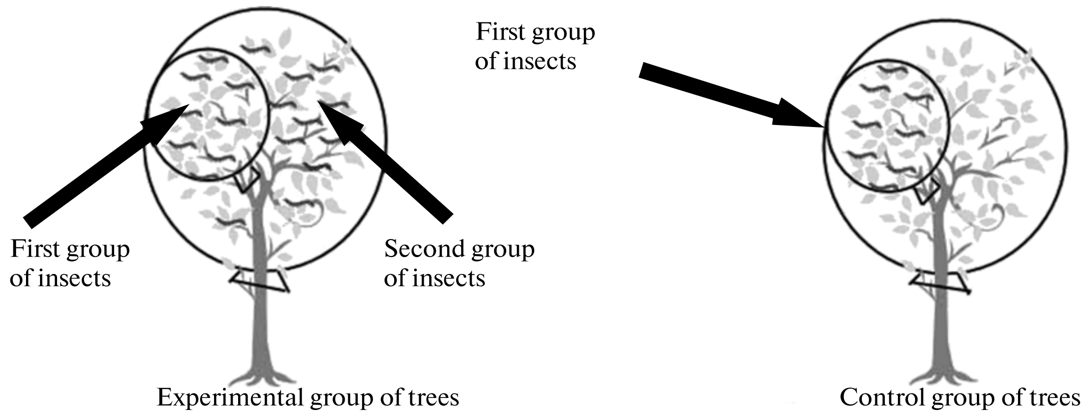

Fig. 1. Scheme of the experiment.

large mesh cages with "defoliating" group of caterpillars.

Assessing the influence of the plant defoliation on the viability of the gypsy moth population, we analyzed the following parameters: larval stage duration (the most vulnerable stage in terms of insect diseases, predators, and parasitoids), weight of female pupae (reflects the potential fecundity of an insect), weight of male pupae, and survival rate (from egg to adult). In this experiment, we placed 50 insects per cage and one cage per tree.

Averaged samples for biochemical analysis were obtained by mixing equal numbers of leaves from the top, middle, and bottom parts of the crown covered with a gauze cage. The samples were collected twice: shortly before the placement of caterpillars and two weeks after the start of defoliation (when the defoliation level reached 70–80%). Prepared leaf samples (70–100 g) were weighted and extracted with 250 ml of 96% ethanol by 72-h infusion at the room temperature. Alcohol extracts were concentrated at a room temperature using a rotational evaporator under reducing pressure to a final volume of 5–10 ml. The concentrated extracts were subjected to simultaneous hydrodistillation–solvent extraction with light petroleum (b.p. 40–70°C) as described in [9]. Solutions of volatiles fractions were dried over anhydrous sodium sulfate, filtered and evaporated at a room temperature under reducing pressure. GC-MS analysis was performed with Agilent HP 6890 equipped HP 5972A mass selective detector. Condition of analysis and identification technique were previously described [9].

The population characteristics of caterpillars were analyzed using a one-way ANOVA. Terpenoid concentrations were recalculated with repeated measures (ANOVA). If the p-level in the interaction of two factors (defoliation and time) was less or equal to ≤ 0.05, then the differences between the experimental and control groups were considered as significant.

RESULTS AND DISCUSSION

According to our analysis, the geraniol and linalool concentrations in the leaves of defoliated trees increased significantly compared to the control trees, whereas the hexadecanol content decreased (Fig. 2). In the cases of other detected terpenoids, we did not reveal any significant differences ($p \geq 0.05$).

In experiments with insects feeding on the defoliated plants, we revealed an increase in the larval stage duration of female caterpillars compared to the control group (44.9 ± 0.74 and 40.7 ± 0.92 days, respectively; $F = 10.27$, $p = 0.005$), whereas the male caterpillar phenology remained unchanged ($p \geq 0.05$). The weight of female pupae from the experimental group significantly decreased compared to that of the control group (0.55 ± 0.061 and 0.76 ± 0.036 g, respectively; $F = 9.84$, $p = 0.007$). At the same time, the weight of male pupae remained unchanged ($p \geq 0.05$). In spite of the fact that the survival rate of insects before the adult stage was very low (12.0 ± 1.51%), the survival of insects feeding on defoliated plants, significantly decreased (5.9 ± 1.55%, d.f. = 17, $df = 17$; $F = 7.87$, and $p = 0.012$). The low survival rate of the control group was mediated by the phase of population cycle in the maternal generation, which was exposed to the stress caused by a high population density and some environmental factors (a high humidity caused by abundant precipitations and the presence of small parazitoids and predators capable of penetrating through the mesh cages).

The obtained results evidence that the content of terpenoids, such as geraniol and linalool, may increase in response to considerable defoliation of silver birch caused by gypsy moth caterpillars. Earlier studies on the role of volatile substances in the chemocommunication of gypsy moth caterpillars demonstrated

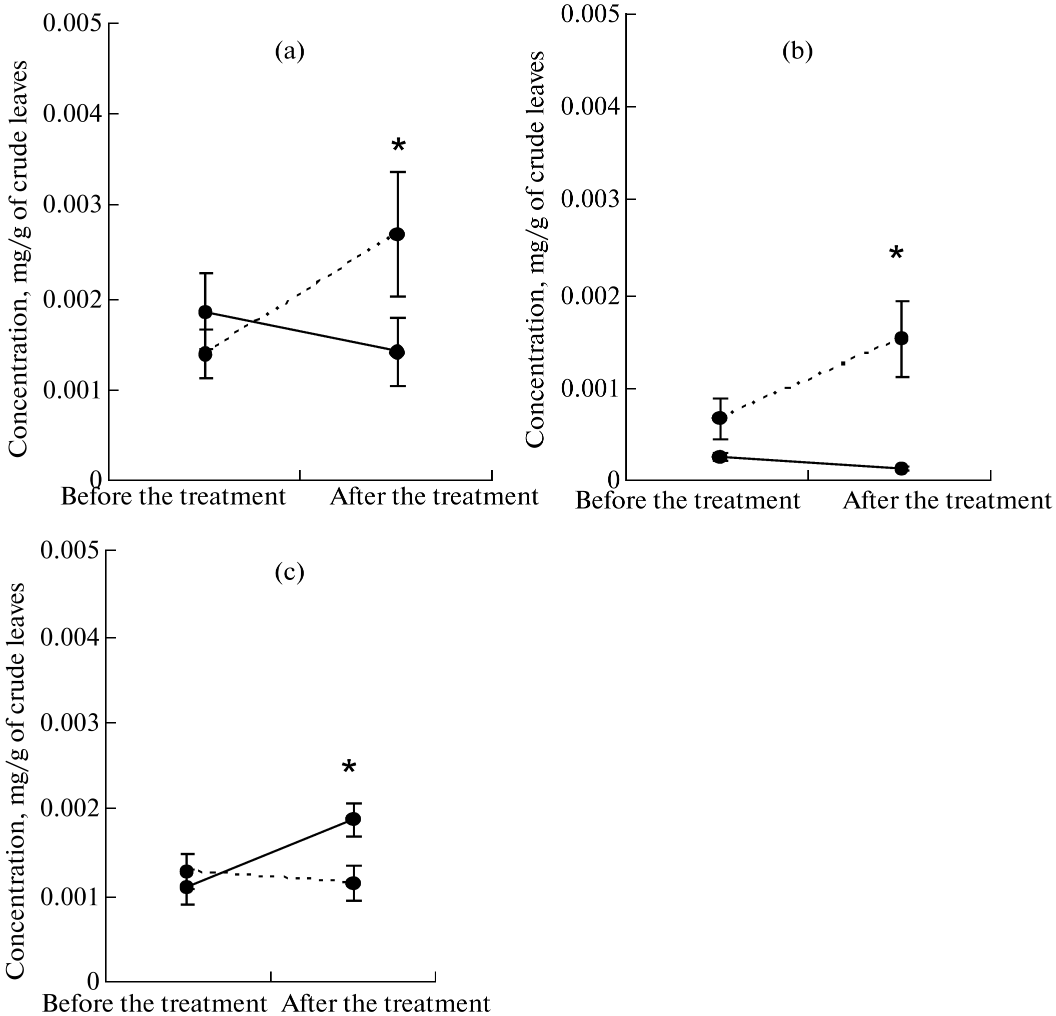

Fig. 2. The content of geraniol (a), linalool (b), and hexadecanol (c) in the leaves of *Betula pendula* after the defoliation, caused by gypsy moth caterpillars. The control and defoliated tree groups are indicated with solid and dotted lines, respectively ($p \leq 0.05$).

some repellent properties of linalool [10]. Another study demonstrated an antifeedous action of geraniol in the case of gypsy moth growth on an artificial nutrient medium [11]. Thus, the revealed increase in the content of geraniol and linalool in silver birch leaves suggests a contribution of monoterpenes to the induced resistance of birch to gypsy moth, expressed in the direct negative influence on the phytophage.

It is also known that some monoterpenoids, such as linalool, are chemical factors that play an important role in the search of a pray by parasitoids [12, 13]. Therefore, we may suppose that volatile terpenoids are involved into the induction of the birch resistance to gypsy moth in response to the defoliation; they indirectly influence on the gypsy moth population via the attraction of natural enemies of this phytophage.

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REFERENCES

1. Howe, G.A. and Jander, G., *Annu. Rev. Plant Biol.*, 2008, vol. 59, pp. 41–66.
2. Ferry, N., Edwards, M.G., Gatehouse, J.A., and Gatehouse, A.M.R., *Current Opin. Biotechnol.*, 2004, vol. 15, pp. 155–161.
3. Haukioja E., in Multigenic and Induced Systemic Resistance in Plants. L.: Kluwer Acad. Publ., 2005, pp. 279–296.
4. Arimura, G., Huber, D.P.W., and Bohlmann, J., *Plant J.*, 2004, vol. 37, pp. 603–616.

5. Par, P.W. and Tumlinson, J.H., *Plant Physiol.*, 1999, vol. 121, pp. 325–331.
6. Il'inskii, A.I. and Tropin, I.V., *Nadzor, uchet i prognoz massovykh razmnozhenii khvoe- i listogryzushchikh nasekomykh v lesakh SSSR* (Survey, Monitoring, and Prediction of Mass Reproduction of Needle- and Leaf-Grazing Insects in Forests of the Soviet Union), Moscow: Lesnaya Prom-st', 1965.
7. Hwang, S-Y. and Lindroth, R.L., *Oecologia*, 1997, vol. 111, pp. 99–108.
8. Hunter, M.D. and Schultz, J.C., *Oecologia*, 1993, vol. 94, pp. 195–203.
9. Tkachev, A.V., *Issledovanie letuchikh veshchestv rastenii* (Study of Volatile Substances of Plants), Novosibirsk: Ofset, 2008.
10. Markovic, I., Norris, D.M., Phillips, J.K., and Webster, F.X., *J. Agric. Food Chem.*, 1996, vol. 44, pp. 929–935.
11. Doskotch, R.W., Cheng, H.-Y., Odell, T.M., and Girard, L., *J Chem. Ecol.*, 1980, vol. 6, no. 4, pp. 845–851.
12. Turlings, T.C.J., Tumlinson, J.H., Heath, R.R., et al., *J. Chem. Ecol.*, 1991, vol. 17, no. 11, pp. 2235–2251.
13. Chen, L. and Fadamiro, H.Y., *Bull. Entomol. Res.*, 2007, vol. 97, no. 5, pp. 515–522.