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Effect of Tannic Acid on the Development and Resistance of the Gypsy Moth *Lymantria dispar* L. to Viral Infection

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It is known that crop plants' induced resistance to phyllophages, which is due to accumulation of secondary metabolites, decreases the viability of phyllophages and changes the sensitivity to entomopathogens [1]. One of the most important classes of compounds that are involved in defensive responses of deciduous plants is phenols, in particular, flavonoids and polymeric tannic compounds (tannins). Results of experiments show that correlation between the content of tannins and the viability of phyllophage depends primarily on the specific characteristics of the insect. Today, data on the effect of tannic acid on the sensitivity of insects to viral infections are almost absent, although it is known that viruses can markedly influence the populational dynamics of many species of phyllophagous insects, including the gypsy moth *Lymantria dispar* L. (Lepidoptera, Lymantriidae) [2]. The latter can regularly form foci of mass-scale reproduction nearly everywhere in Russia, except for the northern regions.

It is known that tannins, including tannic acid, can form complexes with proteins; when oxidized to quinones in the midgut of insect, they can produce semiquinone radicals and other forms of reactive oxygen species [3]. In the course of evolution, phytophages, in turn, developed a number of adaptations with respect to the secondary metabolites (allelochemicals) of plants that attenuate the deleterious effect of allelochemicals on insect's organism. These adaptations include behavioral adaptations, physiological isolation, and metabolic transformation [4]. A key role in detoxication and elimination of many xenobiotics belongs to the antioxidant system of insects, which include both enzymatic and nonenzymatic antioxidants. The second group comprises high- and low-molecular-weight thiol-containing compounds [5]. The proportion of these compounds in the midgut of insects provides information on the processes of free radical peroxidation in insect organs and is an important criterion of the so-called oxidative stress [6].

In view of this, the goal of this study was to investigate the effect of a phenolic compound, tannic acid, on the development of *L. dispar* and its sensitivity to nuclear polyhedrosis. The ratio between the concentrations of oxidized and reduced thiol-containing compounds in insect midguts—an index reflecting the antioxidant status of the insect—was used as a criterion of the effect of this allelochemical on insect's organism.

MATERIALS AND METHODS

This study was performed with the gypsy moth (*L. dispar* L., Lepidoptera: Lymantridae) caterpillars. The insects were collected at the egg phase in the natural population and then grown under laboratory conditions. The experiments were performed in spring and summer. The insects were grown either in an artificial nutrient medium in Petri dishes or on birch shoots in rearing cages at 23–25°C under at 16-h light period per day.

Forage was treated with 0.9% aqueous solution of tannic acid (Sigma, United States). This concentration corresponds to the content of tannins in birch leaves defoliated in the previous year [7]. Caterpillars of the third instar were infected perorally by finely-dispersated spraying of forage with a viral suspension at a concentration of 10^7 polyhedrons/ml.

Weight of caterpillars was determined by group weighing, and sex was determined by morphological traits of pupae—the structure of the last belly segments [2]. The mortality rate of the insects was expressed as the aggregate percentage of dead caterpillars over the entire period of larval stage. The presence of viral polyhedrons in the body of dead caterpillars was detected with a light microscope.

Biochemical analysis of the midgut was performed using the fourth-instar caterpillars on the fourth day

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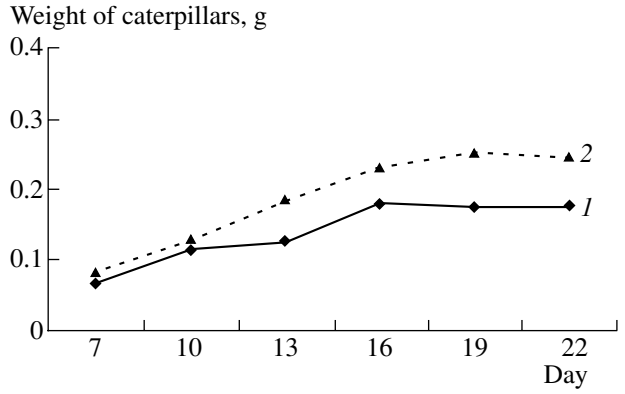

Fig. 1. Effect of tannic acid on the weight of caterpillars feeding on an artificial nutrient medium: (*1*) control, (*2*) tannic acid. Data were statistically processed using ANOVA ($p > 0.05$).

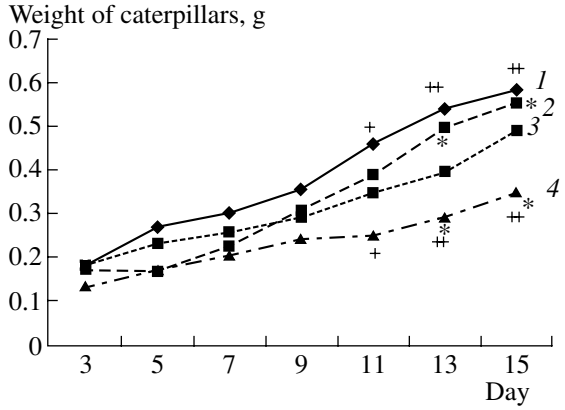

Fig. 2. Effect of tannic acid on the weight of infected caterpillars feeding on birch leaves: (*1*) control, (*2*) tannic acid, (*3*) virus, and (*4*) tannic acid + virus. Data were statistically processed using ANOVA. Asterisks and crosses indicate the points significantly differing between each other (* and +, at $p \le 0.05$; ++, at $p \le 0.01$).

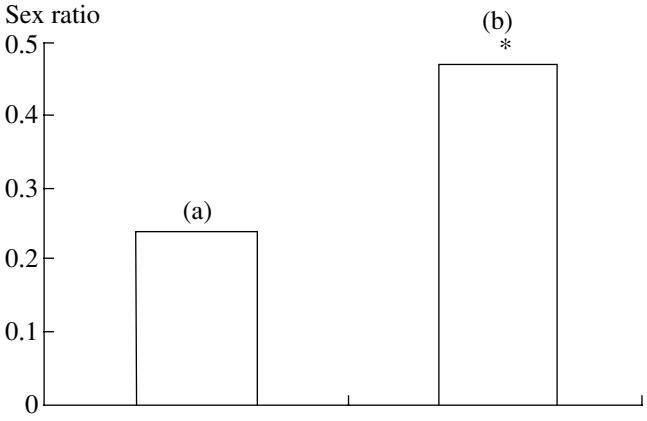

Fig. 3. Effect of tannic acid on the ratio of females to the total number of insects whose caterpillars fed on birch shoots: (a) control, (b) tannic acid. Data were statistically processed using Student's *t* test. The asterisk indicates statistically significant difference from the control at $p \le 0.05$.

after molt. The ratio of concentrations of oxidized and reduced thiol-containing components (RSSR/RSH) in gut tissues as an index of oxidative stress in the organism was determined [6]. Protein concentration in samples was determined by the method of Bradford [8] using bovine serum albumin as a standard.

The results were processed statistically using ANOVA and Student's *t* test [9].

RESULTS AND DISCUSSION

As evident from Figs. 1 and 2, neither addition of tannic acid to the nutrient medium nor the treatment of the surface of birch leaves with this compound caused changes in the weight of caterpillars throughout the larval phase ($p > 0.05$). However, the treatment with tannic acid, in combination with infection with nuclear polyhedrosis virus, resulted in a significant decrease in the weight of caterpillars grown on leaves as compared to both the control group and the group of insects treated with tannic acid (Fig. 2). Feeding the insects with the birch leaves treated with tannic acid resulted in an increase in the number of females in the experimental group (Fig. 3). The sensitivity to the virus of the caterpillars that developeding on natural forage decreased when the latter forage was treated with tannic acid (Fig. 4). When caterpillars were fed on the artificial diet, no significant changes in their sensitivity to the virus were observed, irrespective of the presence of tannic acid in the forage.

The study of the content of oxidized and reduced thiols in caterpillar guts showed that the addition of tannic acid to forage shifted the ratio of thiols towards the accumulation of oxidized forms (table). This effect was manifested irrespective of infection and the type of food (table).

Thus, the results regarding the dynamics of the weight of caterpillars, the sex ratio, and the morality rate indicate that tannic acid has no detrimental effect on the development of *L. dispar*. It is known that polyphages may be less sensitive to the detrimental effect of tannins than the species with a narrow food specialization, owing to the structure of peritrophic matrix and the presence of high concentrations of surfactants in the midgut contents [10]. A high pH value in *L. dispar* midgut may also prevent the irreversible binding of tannic acid to proteins owing to an increase in the coefficient of dissociation of phenol–protein conjugates [11].

The increase in the RSSR/RSH ratio in the midgut of *L. dispar* caterpillars under the treatment with tannic acid is possibly due to the prooxidants properties of tannic acid, which is characteristic of the majority of phenolic compounds [12]. Similarly to many other phenols, tannic acid can undergo autooxidation to form reactive compounds, including reactive oxygen species (semiquinone radicals, superoxide anion, hydroxyl radical, peroxides, etc.). This process can be considerably

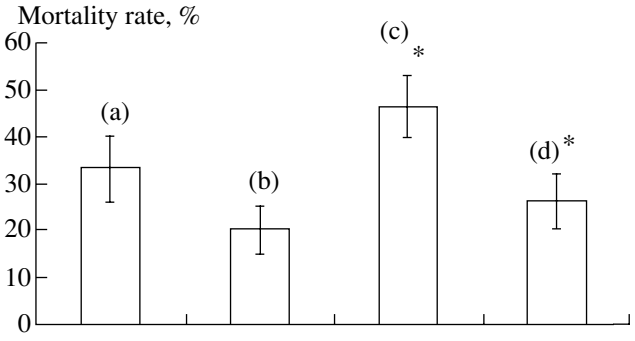

Fig. 4. Effect of tannic acid on the mortality rate of infected caterpillars fed on birch leaves: (a) control, (b) tannic acid, (c) virus, and (d) tannic acid + virus. Data were statistically processed using Student's t test. Asterisks indicate the points significantly differing between each other at $p \le 0.05$.

accelerated in the presence of phenol oxidases, which are contained in plant leaves. The process of feeding of *L. dispar* caterpillars is accompanied by the disruption of integrity of leaf-plate tissues, which leads to the release of these enzymes from cellular compartments and their subsequent activation. As a result, the processes of lipid peroxidation may be triggered in midgut epithelium, leading to the formation of peroxide radicals in the gut lumen. Reactive oxygen species generated in the midgut can damage the virions released from the protein capside in the midgut lumen during infection of the insect. On the other hand, when epithelial cell membranes are damaged, the penetration of viral particles into host cells may be disturbed. As a result, the addition of tannic acid to forage decreases the infection load exerted by the virus on the insect and reduces the number of caterpillars that die for from virosis. Earlier, a similar result was reported for the cutworm *Heliothis virescens* infected with nuclear polyhedrosis virus [13, 14]. The decrease in the weight of *L. dispar* caterpillars that fed on birch shoots treated with tannic acid was apparently determined by the aggregate damaging effect of radicals and virions retained in the midgut lumen on midgut epithelium. Possibly, this is associated with the disturbance of synthesis of digestive enzymes, which may be accompanied by deterioration of food assimilation. In addition, it cannot be ruled out that the absence of significant changes in the mortality of caterpillars that fed on the artificial tannin-containing nutrient medium after infection with the virus is apparently related to a decreased level of oxidation of tannic acid as a result of the absence of phenol oxidases in the nutrient medium. At the same time, the increase in the content of oxidized thiols in the midgut is indicative of oxidative processes (autooxidation of tannic acid) in the midgut lumen (table). However, the intensity of these processes cannot significantly influence the process of infection of caterpillars. Furthermore, tannic acid itself can interact with reactive molecules formed in the course of its oxidation, functioning as an antioxidant.

Ratio between the concentrations of oxidized and reduced thiols in the midgut of infected caterpillars feeding on forage containing tannic acid

Variant	RSSR/RSH ratio	
	feeding on birch leaves	feeding on artificial nutrient medium
Tannic acid	1.738 ± 0.427*	5.04 ± 2.544***
Virus	1.536 ± 0.516	2.39 ± 1.167**
Virus + tannic acid	1.714 ± 0.354*	3.75 ± 1.347*
Control	0.999 ± 0.246	1.11 ± 0.658

Note: Data were statistically processed using Student's t test. One, two, and three asterisks indicate variants significantly differing from the control at * $p \le 0.05$, ** $p \le 0.01$, and *** $p \le 0.001$, respectively.

Thus, the results obtained in this study suggest that tannic acid does not play the key role in the development of resistance birch to *L. dispar*. We assume that one of the mechanisms by which tannic acid affects insect's organism is the effect of the products of its oxidation on midgut epithelium. Probably, evolution of *L. dispar* and its forage plant was accompanied by the adaptation of the insect antioxidant system for the action of this secondary metabolite. The results of other studies indicate that the content of tannins in birch leaves increases upon defoliation and may have a detrimental effect on the viability of insects [7], whereas the artificial increase in the content of tannins in our experiments has no adverse effect on the viability of *L. dispar*. Therefore, the response of the insect to changes in the chemical composition of the plant largely depends on the species of phytophage and, probably, on its the antioxidant status of the insect. However, additional studies are required to corroborate this assumption.

Thus, the results of this study led us to conclude that tannic acid can significantly affect the development of viral pathogenesis of *L. dispar* grown on natural forage. This fact should be taken into account in studies of relationships in three-component systems and in modeling the dynamics of abundance of forest phytophages.

ACKNOWLEDGMENTS

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REFERENCES

1. Shul'ts, E.E., Bakhvalov, S.A., Martemyanov, V.V., et al., *Dokl. Biochem. Biophys.*, 2004, vol. 394, no. 4,

pp. 42–45 [*Dokl. Akad. Nauk*, 2004, vol. 394, no. 4, pp. 551–554].

2. Bakhvalov, S.A., Il'inykh, A.V., Zhimerikin, V.N., and Martemyanov, V.V., *Evraziisk. Entomol. Zh.*, 2002, vol. 26, no. 1, pp. 101–108.
3. Ahmad, S. and Pardini, R.S., *Free Radic. Biol. Med.*, 1990, vol. 8, no. 4, pp. 401–413.
4. Baranchikov, Yu.N., *Troficheskaya spetsializatsiya cheshuekrylykh* (Trophic Specialization of Lepidoptera), Krasnoyarsk: ILiD Sib. Otd. Akad. Nauk SSSR, 1987.
5. Felton, G.W. and Summers, C.B., *Arch. Insect Biochem. Physiol.,* 1995, vol. 29, pp. 187–197.
6. Khramtsov, V.V., Yelinova, V.I., Glazachev, Yu.I., et al., *J. Biochem. Biophys. Met*., 1997, vol. 35, pp. 115–128.
7. Kaitaniemi, P., Ruohomäki, K., Ossipov, V., et al., *Oecologia*, 1998, vol. 116, pp. 182–190.
8. Bradford, M.M., *Anal. Biochem.*, 1976, vol. 72, pp. 248–254.
9. Dospekhov, B.A., *Metodika polevogo opyta s osnovami statisticheskoi obrabotki rezul'tatov issledovanii* (Methods of Field Experiments and Principles of the Statistical Treatment of the Results), Moscow: Agropromizdat, 1985.
10. Bernays, E.A. and Chapman, R.F., *J. Chem. Ecol.*, 2000, vol. 26, pp. 1773–1794.
11. Appel, H.M. and Maines, L.W., *J. Insect Physiol.*, 1995, vol. 41, pp. 241–246.
12. Zaprometov, M.N., *Fenol'nye soedineniya* (Phenolic Compounds), Moscow: Nauka, 1993.
13. Ali, M.I., Bi, J.L., Young, S.Y., and Felton, G.W., *J. Chem. Ecol.*, 1999, vol. 25, pp. 2193–2204.
14. Hoover, K., Kishida, K.T., Digiorgio, L.A., et al., *J. Chem. Ecol.*, 1998b, vol. 24, pp. 1949–2001.