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The Dynamics of Phenolic Compounds and Soluble Sugars in the Leaves of the Silver Birch (*Betula pendula* Roth) after Defoliation and Their Significance in Entomological Plant Resistance

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Abstract—The dynamics of phenolic compounds, flavonols, catechines, tannins, and soluble sugars in the leaves of the silver birch *Betula pendula* Roth after strong (75%) and total (100%) artificial defoliation was studied. It was shown that the flavonol content in the leaves did not change after strong and total defoliation, while the amount of tannins did not change during the first 15 days but increased later on 1 and 2 years after 75% and 100% defoliation. The catechine content did not change during the first 15 days and increased later on 1 year after strong and total defoliation; however, it returned to the level of control plants 2 years after both types of defoliations. The amount of soluble sugars in the leaves increased 2 days after 75% defoliation; however, their content conformed to that in control plants after 10 days and it remained later 1 and 2 years after the damage. The amount of soluble sugars in the leaves also did not change 1 year after 100% defoliation.

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INTRODUCTION

Outbreaks of gypsy moth (*Lymantria dispar* L.) and strong defoliation of forests on vast areas in Northern America and Eurasia including Russia have greatly aroused interest in problems of both the phyllophage density management and plant resistance in the last decades of the past century and at the beginning of the current century (Furuta, 1982; Elkinton, 1990; Podgwaite et al., 1992; Cunningham et al., 1997; Bakhvalov et al., 2002; Koltunov, 2006; Martemyanov, Bakhvalov, 2007).

Strong and frequently total defoliation of forests including both birch and aspen forests on a huge area (hundreds of thousands of hectares) in Western Siberia induced by the gypsy moth have resulted in significant economic losses and alteration of forest ecosystems.

One important direction in studying the effect of defoliation on the stand condition is research into the phenolic compounds and soluble sugar dynamics in the leaves of damaged plants because these compounds have a significant effect on the viability and resistance to different extreme factors including herbivorous insects (Feeny, 1968; Battisti, 1988; Haukioja, 1991; Zaprometov, 1993; Shapiro et al., 1994; Schoonhoven et al., 1998; Crone, Jones, 1999). It is known that a number of defense mechanisms which can be reflected negatively in the condition of insects–phytophages at a later time are activated in response to defoliation or plant tissue damage by insects. These responses can be exhibited in the current season after damage by the insects (rapid induced resistance) and in the following vegetative season after leaf regrowth (delayed induced resistance) (Kaitaniemi et al., 1998; Bernays, Chapman, 2000; Osier, Lindroth, 2004; Haukioja, 2005). Frequently such reactions proceed with reactive oxygen species generation, for example, as a result of phenol oxidases activation with subsequent oxidation of the phenolic compounds that extensively determine the efficiency of the plant's defensive responses (Ahmad, Pardini, 1990; Zaprometov, 1993; Treutter, 2001).

It was shown that plants can enlarge their protective functions due to an increase in the allechemical production that can negatively effect the phyllophages (Ivashchenko, 1995; Dillon, Charnley, 1995; Zvereva et al., 1997; Muzatova, 2000; Ossipov et al., 2001). At the same time, there are quite a few papers reporting the absence of a negative effect of defoliation on insect viability (Watt et al., 1991; Osier, Lindroth, 2001) and even about an increase in insect viability for those inhabiting damaged plants (Watt et al., 1991; Osier, Lindroth, 2001). It was also revealed that the plant

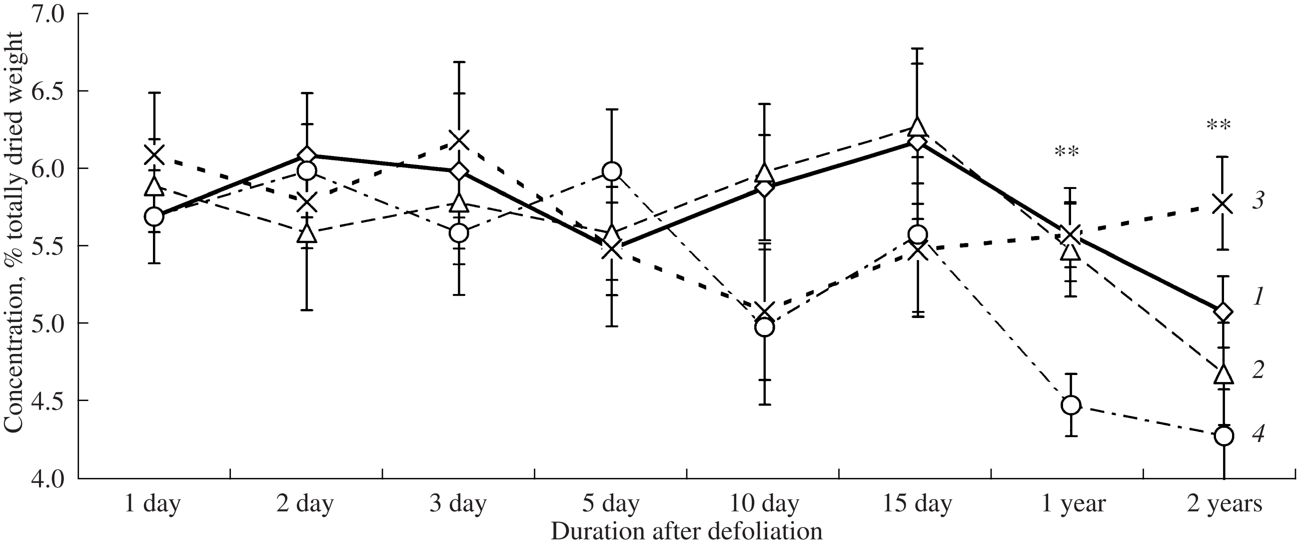

Fig. 1. Content dynamics of flavonols and tannins in the leaves of 75% defoliated and intact trees. *1*—"defoliated" flavonols; *2*—"intact" flavonols; *3*—"defoliated" tannins; *4*—"intact" tannins. $**p < 0.01$.

response to leaf loss is complex; i.e., simultaneously the level of the defensive substances in the leaves (needles) increases and the sugar level decreases; hence, the food value is reduced (Willis et al., 1999).

Earlier, we reported the effect of natural and artificial defoliation of birch forests on the dynamics of different allelochemicals in the leaves and the insect response to changes of their content (Shul'ts et al., 2004; Shul'ts et al., 2005).

In this paper we report the results of further studies on the dynamics of phenolic compounds and soluble sugars in the leaves of partly and total defoliated plants. On the basis of previous studies suggesting the importance of individual phenolic compounds for plant resistance, we studied the dynamics of flavonols, catechines, and tannins.

MATERIAL AND METHODS

Artificial defoliation was conducted in experimental plots located in several separated forest stands in the environs of Novosibirsk. Trees nine to ten years of age were used in the experiment. Control trees and trees exposed to defoliation were situated in immediate proximity to each other.

Artificial defoliation of trees was accomplished by means of mechanical removal of leaves from model trees in the last ten-day period of June when the gypsy moth, the main silver birch pest in Western Siberia, defoliates stands in natural outbreaks. Thereby, we synchronize artificial defoliation with severe defoliation in wild outbreaks in order to compare the dynamics of both phenolic compounds and carbohydrates in the artificial and natural damaged plants in future studies. The selection of the young model trees is explained by significant technical difficulties during artificial defoliation of older trees. Mechanical leaf removal in the experimental trees was accomplished in the crown uniformly by removing 3 leaves out of every 4 to induce 75% defoliation and all leaves to induce 100%. In the case of total defoliation, the sample collection was conducted at the same time but 1 and 2 years later after damaging the trees. The collected leaves were dried at 30–35°C for 3 days, and then they were used for biochemical analysis. Flavonoids were determined according to Belikov, Shraiber (1970); condensed tannins, according to Zaprometov (1974); catechines, according to Kukushkina et al. (2003); and water-soluble carbohydrates, according to Ermakov et al. (1987). Statistical analysis was carried out using the *T*-test.

RESULTS AND DISCUSSION

The dynamics of both flavonols and tannins in the leaves of the 75% defoliated and intact trees are shown in Fig. 1. The amount of flavonols in the leaves of the damaged trees during the first 15 days and later 1 and 2 years after leaf removal was not different from that in the leaves of the control plants. The tannin content in the leaves of the damaged trees in current vegetation season did not differ from that in the leaves of intact trees. However, the tannin content in the leaves of damaged plants was significantly higher in comparison with leaves of the control plant in the next two vegetation seasons, i.e., 1 and 2 years after defoliation. The amount of catechines in the leaves of damaged trees was higher then that in the leaves of intact trees only later, 1 year after the damage was found. Their amount

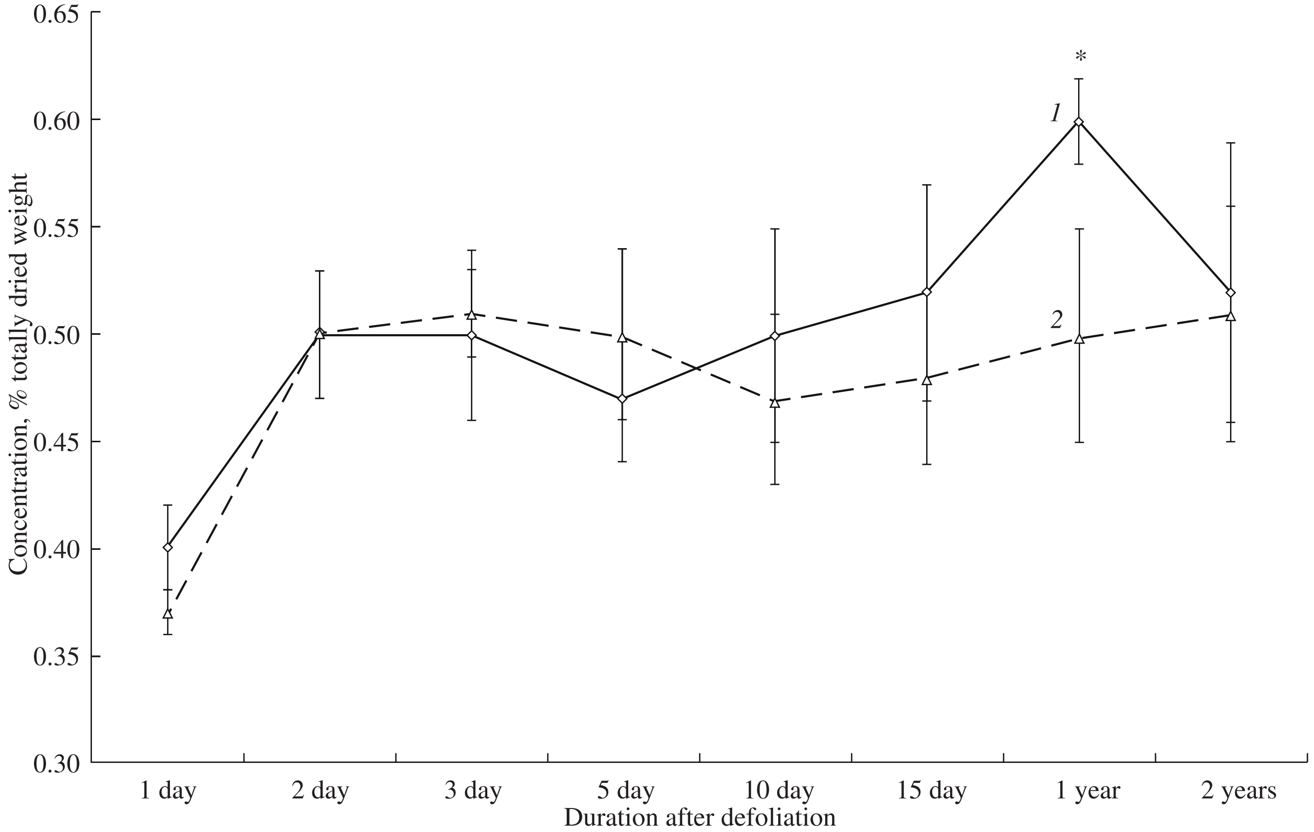

Fig. 2. Content dynamics of catechines in the leaves of 75% defoliated trees (*1*) and intact trees (*2*). $*p < 0.05$ (for Figs. 2–4);

did not differ from that in the leaves of the control trees during the first 15 days and later on 2 years after defoliation (Fig. 2).

The water soluble carbohydrate content increased 2 days after defoliation in the leaves of damaged plants; however, their content was sharply reduced on the third and fifth days. The amount of carbohydrates did not differ from the control later 10 days after damage (Fig. 3). Significantly differences in the sugar concentration were not observed between the test and control plants in the following two years after strong defoliation.

Thus, 75% defoliation of silver birch causes an increased amount of catechine in the plant leaves only later on, 1 year after damages; however, their amount decreased to the level of the control plants after one more year. At the same time, the increase in the amount of tannin 1 year after defoliation remains until the next season, i.e., 2 years after infliction of damage. It is possible to assume that the delayed induced entomological resistance of trees is provided with catechines and tannins. However, the action of tannin is longer than that of catechine. Earlier it was reported by other researchers that catechines and tannins can negatively affect the period of larval stage and weight of the female pupae when caterpillars of *Epirrita autumnata* fed on damaged trees of silver mounting (Ruuhola et al., 2007).

The artificial 75% defoliation also causes a short-term increase in the amount soluble sugars in the leaves of damaged trees with the subsequent decrease in their amount over several days. However, the amount of sugar in the leaves of damaged trees does not significantly differ from the control a year after defoliation and even has a tendency to increase. Probably, the primary increase in the sugar content is connected with dissociation of polysaccharides on mono- and disaccharides; however, afterwards the sharp reduction of the photosynthetic surface is not able to compensate for the loss of soluble sugars and polysaccharides. It is possible to assume that the reduction of the amount of soluble sugars in the leaves 3 days after damage ddecreases the food quality and lead to low accumulation of energy by folivore and this can result in a decrease in their fecundity. However, taking into account the moderate reduction in the level of soluble sugars in a quantitative sense, it is difficult to suppose that this adversely affects insect feeding. Most probably, insects easily compensate for insignificant sugar loss by an increase in leaf consumption. Moreover, earlier we showed that artificial 75% defoliation of silver birch did not result in reliable changes in the insects performance

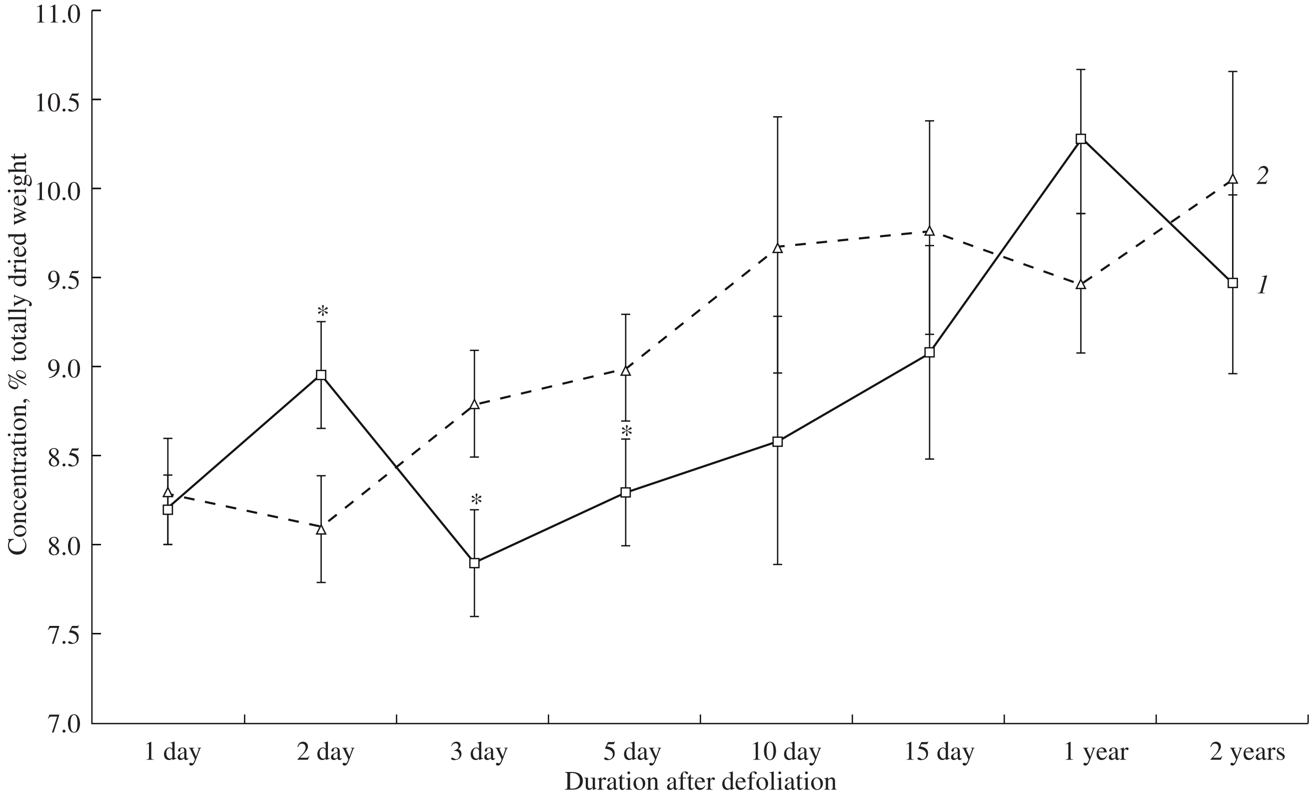

Fig. 3. Content dynamics of soluble sugars in the leaves of 75% defoliated trees (*1*) and intact trees (*2*).

(Bakhvalov et al., 2006). Consequently, this result also confirms the statement that the reduction of the nutritive value is not the main defensive reaction of the plant against insects.

The flavonol content 1 and 2 years later and the sugar content a year after damage does not significantly differ from the control when the tree was totally (100%) defoliated (Fig. 4). The total loss of leaves as in the case of 75% defoliation results in an increase in the amount of both catechines and tannins during the next 2 years; however, the increase in the amount of catechine 2 years after defoliation does not reach a significant value in comparison with the control (Fig. 4). It is to be noted that about 18% of the completely defoliated trees (4 individuals out of 22) died, which indicated the strong stress induced by the full loss of the photosynthetic apparatus. Nevertheless, even so the induced stress did not significantly increase the amount of catechines and tannins or the reduction of the amount of sugars in the leaves of totally damaged plants as compared with leaves of the 75% damaged silver birch.

A sharp decrease of density of herbivorous insects including the gypsy moth in the natural outbreaks after strong or totally damage to the leaves was repeatedly observed (Bachvalov et al., 2002; Belyaev et al., 2005; Martemyanov and Bakhvalov 2007). Basing on current results it is possible to note that phenolic compounds and sugars were unlikely the main cause of the drop in phyllophages. Most likely, the negative effect of both cathechines and tannins on insects is shown when they act jointly with other allelochemicals. Furthermore, it is not ruled out that protection of the allelochemicals against insects is shown more strongly after natural defoliation by caterpillars. It is known that in this case elisitors contained in the insect saliva that can induce additional signal pathways in a plant, which results in a response differing from the induction after mechanical defoliation, have an important role (Baldwin et al., 1997; Korth, Dixon, 1997; Kahl et al., 2000).

Thus, the obtained results suggest that strong and total defoliation of the silver birch does not cause changes in the flavonol content but results in an increase in the contents of both catechines and tannins after 1 and 2 years, which most likely determines the delayed induced resistance against gypsy moth. At the same time, the amount of soluble sugars was reduced in the first days after damage and later on did not differ from the control. Presumably, the content of soluble sugars in the silver birch leaves does not determine the plant resistance to gypsy moth.

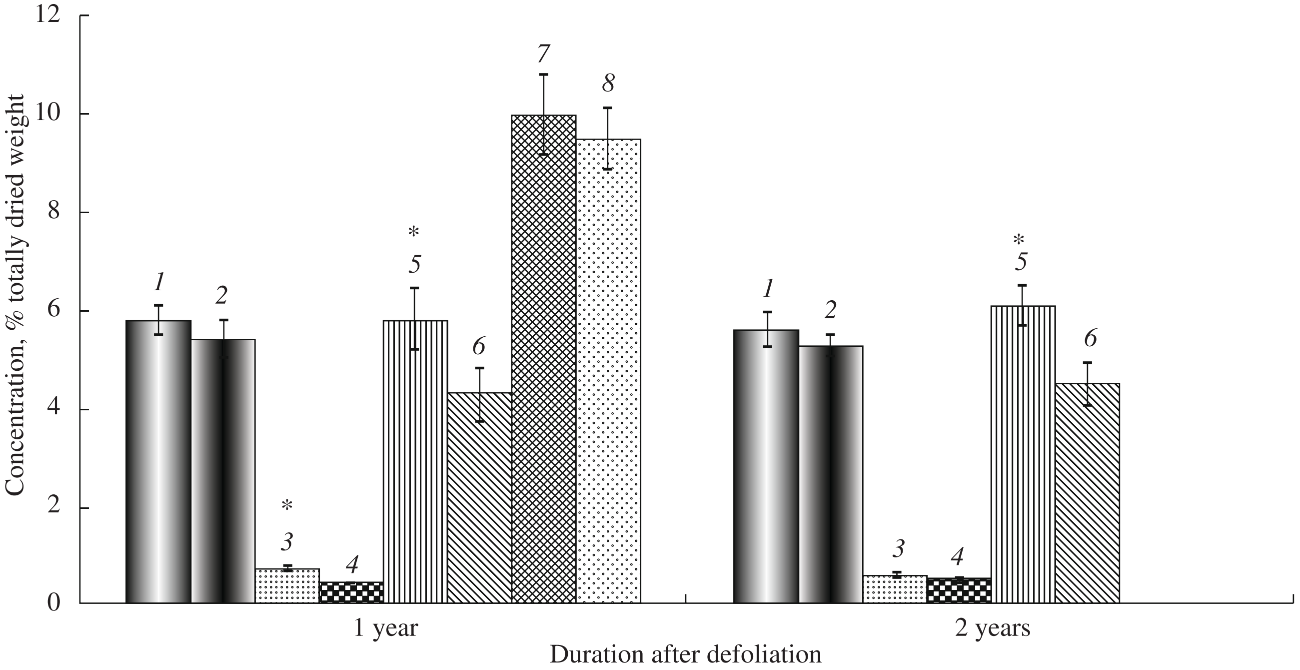

Fig. 4. Content dynamics of allelochemicals and sugars in the leaves of 100% defoliated and intact trees. *1*—defoliated flavonols; *2*—intact flavonols; *3*—defoliated catechines; *4*—intact catechines; *5*—defoliated tannins; *6*—intact tannins; *7*—defoliated sugars; *8*—intact sugars.

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