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***Salix lapponum* L. vs. phytophagous insects – an assessment of the risks and the reaction of plants**

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Abstract: Since the 1950s, a significant decrease in the population numbers of *Salix lapponum* L. has been observed in Poland, particularly in the Polesie Lubelskie region. One of the most important threats comes from the biotic environment including interactions at the trophic level. An unidentified issue was the occurrence and importance of phytophagous insects inhabiting *S. lapponum* in terms of real threats to growth and development as well as specific plant reactions to herbivores. The aim of the study was to recognize the occurrence of phytophagous insects inhabiting *S. lapponum*, i.e. an endangered species, and estimation of physiological changes caused by the herbivorous species feeding.

The field study was conducted in a locality of *S. lapponum* population in the peat bog near Bikcze Lake (Polesie Lubelskie Region) in 2014–2015. In each growing season from the end of April to September, 100 stems of *S. lapponum* shoots with similar length were examined once a month. Selected parameters of chlorophyll *a* fluorescence were measured and the accumulation of H₂O₂ was visually detected in leaves collected from the control plants and from those attacked by herbivores using the 3',3'-diaminobenzidine (DAB) method. We found the presence of 8 phytophagous insect species from 4 orders (Coleoptera, Diptera, Hemiptera and Lepidoptera) on *S. lapponum*. Most of the shoots were inhabited by *Lochmea caprea*, *Aphrophora salicina* and *Aphis farinosa*. On the basis of the measurements of chlorophyll *a* fluorescence and H₂O₂ accumulation under phytophagous insects feeding, we have demonstrated that *S. lapponum* was able to maintain photosynthesis under insect attack and that the accumulation of H₂O₂ in leaf blades indicates local rather than systemic stress response to herbivore feeding.

Phytophagous insects feeding caused relatively weak disruptions to host plant physiology and *S. lapponum* is able to maintain photosynthetic efficiency under insect attack. Variation in plant traits influence host plant selection and performance of herbivorous insects. Comparative studies are needed to assess the species composition and population numbers of insects occurring on plants from other *S. lapponum* populations. Detailed knowledge of the biotic factors affecting the occurrence of *S. lapponum* would facilitate establishment of the form of its conservation.

Keywords: arthropods, chlorophyll *a* fluorescence, conservation, downy willow, H₂O₂ accumulation

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Introduction

Interactions among plants and insects create an important component of terrestrial environment. Those relationships have a crucial role in the matter recycling, energy and nutrient flows in ecosystems (Santos & Fernandes, 2010). Plants provide shelter, food resources and oviposition sites for insects. However, herbivores might be extremely harmful to plants, thus plants present a wide spectrum of different defense mechanisms including chemical and physical barriers. Moreover, both organisms are under other biotic and abiotic environmental pressures that have an important impact on interaction system (Santos & Fernandes, 2010; Charles et al., 2014). Since the 1950s, a significant decrease in the population numbers of downy willow *Salix lapponum* L. has been observed in Poland, particularly in the Polesie Lubelskie region. The populations size known from literature data have dropped by almost 80% and this trend still persists (Kaźmierczakowa & Zarzycki, 2001). Its main causes are attributed to changes in habitat conditions in natural stands of this species. These are primarily changes in water relations, which result in the process of ecological succession in peatland ecosystems (Serafin et al., 2015). The appearance of expanding species, i.e. *Phragmites australis* (Cav.) Trin. ex Steud., *Salix cinerea* L., or *Betula humilis* Schrank, adversely affects the functioning of taxa characteristic of peatland habitats, predominantly by changing the values of abiotic and biocenotic factors in such habitats (Pogorzałec et al., 2014b).

One of the most important threats comes from the biotic environment including interactions at the trophic level. In natural communities and in agroecosystems, insects are known to affect the growth, competitive ability, and population dynamics of their host plants (Charles et al., 2014). Direct tissue consumption by defoliators and cellular disruption by piercing-sucking insects trigger a complex of physiological responses in the host plant (War et al., 2012). These responses affect the life processes such as photosynthesis. Among the indirect effects on physiological processes, photosynthesis disruption can have the most significant influence on plant growth and performance (Nabity et al., 2009). Moreover, several studies have revealed inhibition of photosynthesis following insect oviposition, even where eggs are laid on the leaf surface in the absence of tissue damage, which suggests that down-regulation of photosynthesis is a plant-driven response to the perception of stress rather than a secondary physiological response to tissue damage (Kerchev et al., 2012). It is well established that the emission of chlorophyll *a* fluorescence provides an indicator of the primary photochemistry of photosynthesis (Murchie & Lawson, 2013). Measurements of chlorophyll *a* have

been applied to demonstrate that impairment of the photochemistry (direct or indirect) upon insect attack is a common physiological disorder (Nabity et al., 2009; Golan et al., 2015).

Photosynthesis disturbances that are often observed under insect attack are considered as the main cause of increased reactive oxygen species (ROS) accumulation and induction of oxidative stress (Kerchev et al., 2012). Therefore, the oxidative status of plants is an important strategy that enables plants to defend themselves against various biotic and abiotic stresses. Reactive oxygen species serve a variety of signaling functions and can act directly as toxins. An apoplastic burst of ROS acts as a first barrier against subsequent attack by pathogens and herbivores. Among all the ROS, highly stable and easily diffusible H_2O_2 is a crucial component of induced defense response in plants. Besides having a direct effect on phytophagous arthropods, H_2O_2 stimulates a cascade of reactions in plant cells that lead to the expression of defense genes (Torres, 2010). Moreover, oxidative changes in plants after insect attack cause oxidative damage to the insect midgut, mainly due to accumulation of H_2O_2 (Krishnan et al., 2007; Zhu-Salzman et al., 2008). Many physiological and molecular responses in plants against insect attack are triggered by H_2O_2 , and its levels remain elevated as long as the herbivore attack persists (War et al., 2012).

Plant defenses against phytophagous insects are mediated by plant characteristics that affect the herbivore's biology such as mechanical protection on the plant surface or production of toxic chemicals i.e. phenolic secondary metabolites, that either kill or retard the development of herbivores (War et al., 2012). Trichomes play an imperative role in plant defense against many insect species and exert both toxic and deterrent effects (Scott-Brown et al., 2016). Trichome density negatively affects the ovipositional behavior, feeding, and larval nutrition of herbivores (Molina-Montenegro et al., 2006). In addition, dense pubescence affects herbivory mechanically and interferes with the movement of insects and other arthropods on the plant surface, thereby reducing their access to leaf epidermis (Hanley et al., 2007). However, leaves of *Corylus avellana* L. characterized by greater thickness, density of trichomes and a higher level of defense compounds were favorable food source for larvae of monophagous beetle *Altica brevicollis coryletorum* Král (Łukowski et al., 2015). On the other hand, leaves of various *Salix* species are defended by trichomes and common secondary metabolites, mainly salicylates, which are effective against unspecialized herbivores, deterring them from feeding and increasing larval mortality (Volf et al., 2015).

An unidentified issue was the occurrence and importance of phytophagous insects inhabiting *S. lapponum* in terms of real threats to growth and

development as well as physiological plant reactions to herbivores. We attempted to verify the hypothesis that insects inhabiting *S. lapponum* could be a real threat to the proper functioning of populations and the condition of individuals of the examined species in natural habitats in eastern Poland. Field and laboratory studies were conducted to: a) determine the species composition of phytophagous insects inhabiting *S. lapponum* populations in their natural habitats (it was important to find out whether the insect species feeding on the examined plant were specific or appeared accidentally); b) indicate species that pose a real threat to the condition of plants; c) assess physiological plant response to insect feeding by determination of chlorophyll *a* fluorescence parameters and H₂O₂ accumulation in leaf tissues.

Material and Methods

Plant species

Salix lapponum L. is a shrub reaching a height of 1 to 2 meters. It is a dioecious plant blooming in May and June. Downy willow has specific leaves with silvery grey napped underside characterized by long, thin-walled, tubular, twisted, very sinuous trichomes and a thick indumentum (Binns & Blunden, 1980; Pogorzelec, 2008a; Serafin et al., 2015). *Salix lapponum* frequently occurs in subarctic and boreal regions of northern and north-eastern Europe and western Siberia. Some isolated populations are also recorded in the mountains of Central and South Europe and in Scotland (Kaźmierczakowa & Zarzycki, 2001; Podbielkowski, 2002). It grows in sunny or partially shaded locations, and prefers oligotrophic or mesotrophic habitats rich in organic matter with a pH value of 4–6 (Pogorzelec, 2008; Serafin et al., 2015). In Poland, the *S. lapponum* species is on the verge of extinction and, as a glacial relict, it has the status of an endangered species in Poland (EN; 'Red Data Book' status) as well as a vulnerable plant (EN by IUCN) (Kaźmierczakowa & Zarzycki, 2001; Mirek et al., 2006). It occurs in the Łęczna-Włodawa Lakeland in the Polesie National Park, in the Biebrza National Park, and in Puszcza Knyszyńska (Fijałkowski & Izdebski, 2002; Urban & Gawlik, 2003; Pogorzelec et al., 2014b). There are also two populations in the Karkonosze Mountains forming an endemic *Salicetum lapponum* association W. Mat. 1965.

Study area and population

The field study was conducted in a locality of *S. lapponum* population in a peat bog near Bikcze Lake (N 51°22.724', E 023°02.563'; Polesie Lubelskie Region) in 2014–2015. This particular population was

selected due to its size (the largest in Eastern Poland – about 300 individuals), the stable sex ratio (1:1) and optimal genetic structure (lack of clonal individuals) that confirms its proper functioning (Głębocka & Pogorzelec, 2017).

Entomological analyses

In each growing season from the end of April to September, 100 random stems of *S. lapponum* with 40 cm length were examined once a month ($n = 1000$). Insect individuals and/or signs of their feeding were observed/identified in the field or collected in glass tubes sealed with a cotton wool plug and examined under a microscope in the laboratory. Intact and damaged leaves were counted on each analyzed twig. Belowground herbivores were not examined. Preimaginal forms of the insects were collected and reared in the laboratory to the adult stage. The specimen species were identified using available keys (Nowacka, 1996; Beiger, 2001; Łabanowski & Soika, 2003; Blackman & Eastop, 2017).

Physiological tests

Chlorophyll *a* fluorescence parameters are commonly used to characterize the primary photosystem II (PSII) photochemistry, which is interrelated with the photosynthetic capacity. In our study, the selected parameters of chlorophyll *a* fluorescence included the minimal (F_0) and maximal (F_m) level of fluorescence and the maximum quantum yield of PS II (F_v/F_m , where $F_v = F_m - F_0$). An increase in F_0 or a decrease in F_m and F_v/F_m reflect disturbance in photosynthesis caused e.g. by environmental stresses (Murchie & Lawson, 2013). Measurements were made using a Handy PEA fluorimeter (Hansatech Instruments Ltd., UK) on the leaves of control plants (without insects and visible damage) and on the leaves inhabited by selected insect species. The leaves of *S. lapponum* were adapted to darkness for 15 min before the measurements by attaching special light-exclusion clips. The measurements were carried out on leaves located in the middle part of the shoot, and at least 30 measurements were made for the control shrubs and for those attacked by the identified phytophagous arthropods in each growing season.

The accumulation of H₂O₂ was visually detected in the leaves using the 3',3'-diaminobenzidine (DAB) method (Deuschle et al., 2004). Healthy and damaged leaves were collected from different plants (control or inhabited by herbivores). The leaves were transported to the laboratory in plastic tubes filled with distilled water and immediately immersed in an aqueous solution of DAB (1 mg ml⁻¹ w/v; pH = 4.0) for 12 h in the dark at room temperature. The DAB solution was freshly prepared before staining in

order to avoid its auto-oxidation. The staining was terminated by immersion of the leaves in hot water and then in warm 90% ethanol until they were decolorized, except for the deep brown polymerization product produced by DAB in the presence of H_2O_2 . In our previous study using quantitative analysis and visualisation of H_2O_2 in leaves, we found that the uneven distribution of this compound within the leaf lamella may distort its quantitative analysis and, in consequence, can give quite different results even for the same leaf (Hawrylak-Nowak et al., 2015). Because the preliminary studies have shown very heterogeneous accumulation of this compound *S. lapponum* leaves attacked by insects, in this study the accumulation and location of H_2O_2 were assessed only visually and the most representative objects were photographed.

Data analysis

One-way analysis of variance (ANOVA) followed by Tukey's test at the 0.05 probability level was used to investigate the influence of feeding of the most numerous insects (*Aphrophora salicina*, *Aphis farinosa* and *Lochmaea caprea*) on chlorophyll *a* fluorescence parameters of *S. lapponum*. The images of histochemical detection of H_2O_2 were collected from at least fifteen leaf blades using at least four different individuals for both the control shrubs and those attacked by the herbivore insects. The leaves were harvested twice during each growing season, but between seasons and between the harvest dates, we did not observe any visual differences in the accumulation and localization of H_2O_2 in the control and damaged leaves. Therefore we have presented the most representative images for given objects.

Results

We detected the presence of 8 phytophagous insect species from 4 orders on the *S. lapponum* shoots (Table 1). Most species belonged to the order Coleoptera. Both larvae and imagines had chewing mouthparts. *Crepidodera aurata* Marsh. and *Lochmaea caprea* (L.) fed on willow leaves, while *Meligethes aeneus* (Fabr.) fed on pollen. Two species, *Caloptilia stigmatella* (Fabr.) and *Pandemis heparana* (Den. & Schiff.), were classified to the order Lepidoptera. Only larvae were observed on the plants. They had chewing mouthparts and fed on willow leaves. The guild of sap-sucking specialists (Order Hemiptera) was formed by *Aphrophora salicina* (Goeze) and *Aphis farinosa* (Gmel.). Aphids fed on the top parts of the shoots and young leaves, while planthoppers fed only on the shoots. The order Diptera was represented by one species, *Rhabdophaga rosaria* (Loew). Its larvae were observed in characteristic galls.

Most of the shoots were inhabited by larvae of *L. caprea* and *A. salicina*, i.e. 65.2% and 43.2% of the analyzed shoots, respectively (Table 1). Colonies of *A. farinosa* were noted on 16% of the *S. lapponum* shoots. Other species inhabited from 1.4 to 4.2% of the analysed shoots. The larvae of *L. caprea* scraped the leaves, which reduced their biologically active surface. They fed in groups and damaged more than 47% of leaves on the attacked plants. The larvae of *A. salicina* fed on the shoots inside the typical foam nest. We observed more than four nests per shoot on average. *Aphis farinosa* colonies consisting of up to 30 specimens inhabited the bottom side of young leaves and the tops of the shoots. In the case of both sap-sucking species, no visible damage was noted on the shoots. *Caloptilia stigmatella* inhabited 4% of

Table 1. Phytophagous insects infesting *Salix lapponum* L. growing in a peat bog in 2014–2015

Insect species/systematics	Developmental stage	Mouthparts	% of infested shoots	Mean number of damaged leaves on infested shoots [in %]
<i>Aphrophora salicina</i> (Goeze)	larvae	sap-sucking	43.2	4.3*
Hemiptera: Auchenorrhyncha: Cercopidae	imagines		8.1	not found
<i>Aphis farinosa</i> (Gmel.)	larvae and imagines	sap-sucking	16.4	2.3*
Hemiptera: Sternorrhyncha: Aphididae				
<i>Lochmaea caprea</i> (L.)	imagines	chewing	5.3	not found
Coleoptera: Chrysomelidae	larvae		65.7	47.1
<i>Crepidodera aurata</i> Marsh.	imagines	chewing	2.1	not found
Coleoptera: Chrysomelidae				
<i>Meligethes aeneus</i> Fabr.	imagines	chewing	1.4	not found
Coleoptera: Nitidulidae				
<i>Caloptilia stigmatella</i> (Fabr.)	larvae	chewing	4.2	1.6
Lepidoptera: Gracillariidae				
<i>Pandemis heparana</i> (Den. & Schiff.)	larvae	chewing	3.6	2.7
Lepidoptera: Tortricidae				
<i>Rhabdophaga rosaria</i> (Loew)	larvae	minute styliform mandibles	2.5	1*
Diptera: Cecidomyiidae				

*mean number of colonies/galls on an infested shoot.

Table 2. Selected parameters of chlorophyll *a* fluorescence in *Salix lapponum* L. leaves under biotic stress caused by insect feeding

Insect species	F_0	F_m	F_v/F_m
<i>Aphis farinosa</i>	442.29 ± 73.63 a	1562.71 ± 168.17 ab	0.705 ± 0.06 n.s.
<i>Aphrophora salicina</i>	354.50 ± 45.01 b	1394.00 ± 268.36 b	0.742 ± 0.03
<i>Lochmaea caprea</i>	372.00 ± 92.64 ab	1568.75 ± 282.59 ab	0.759 ± 0.06
control	432.27 ± 44.83 a	1662.86 ± 106.60 a	0.740 ± 0.03

Mean values (±SD) sharing the same letter in the column are not significantly different at $p < 0.05$ ($n = 60$); n.s. = not significant.

the *S. lapponum* twigs. Its larvae fed in characteristic mines in the apical surface of the leaves. *Pandemis hep-arana* was noted on 3% of the shoots. Its larva created a nest by rolling the leaves together with silk. It fed inside, damaging almost 3 leaves on the shoot on average. Rosette galls of *R. rosaria* were noted on 2% of the *S. lapponum* shoots.

The analysis of the chlorophyll *a* fluorescence parameters show that only the presence of *A. salicina* exerted a significant effect on F_0 and F_m values, contributing to their decrease in relation to the control plants. On the other hand, the maximum quantum yield of PS II photochemistry (F_v/F_m) was not affected by the phytophagous insect feeding, regardless of the insect species (Table 2).

Histochemical detection of H_2O_2 in leaf blade tissues using DAB staining revealed that the accumulation of H_2O_2 occurred near wound sites caused by insect feeding. However, H_2O_2 did not spread throughout the veins and tissues and was mainly localized in the damaged zone (Fig. 1).

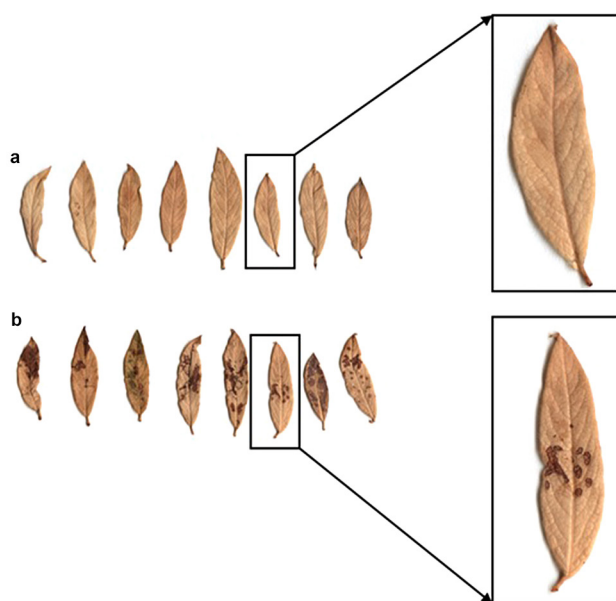


Fig. 1. Histochemical detection of H_2O_2 using DAB staining in the control (a) and herbivore-infested (b) leaves of downy willow. The intense deep brown color indicates the presence and accumulation of H_2O_2 .

Discussion

In this study, we found 8 species of insects belonging to 4 orders feeding on *S. lapponum*. Photosynthetic efficiency did not seem affected by the presence of the insects, and the accumulation of H_2O_2 in leaves after damage was localized rather than systemic response to herbivores feeding.

As a group of trees and shrubs widespread in Europe, *Salix* genus provide habitats of rich biodiversity in their native environments. More than 200 arthropod species inhabit willows in Central Europe. As an important food source, they are associated with a wide range of phytophagous insects (Georgiev et al., 2004; Canty et al., 2016). However, only a relatively small number of herbivorous species regularly cause considerable damage to *Salix* trees, which reduces their economic or environmental value (Charles et al., 2014). Insect herbivores have been divided into generalists tolerating a wide array of defenses present in most plants, and specialists using a specific range of host plants releasing defense compounds (Fürstenberg-Hägg et al., 2013). Most of the phytophagous insect species observed in 2014-2015 on *S. lapponum* were presumably specific to the Salicaceae family. It seems that, these herbivores were not directly related to *S. lapponum*. Their presence in the biocoenosis of the peat bog near Lake Birkze was probably caused by the species composition of the phytocoenosis, with the numerous occurrence of other willow species (*S. cinerea*, *Salix rosmarinifolia* L., *Salix pentandra* L., *Salix aurita* L.) (Pogorzelec, 2008a; Pogorzelec & Wojciechowska, 2011; Pogorzelec et al., 2014).

Lochmea capreae occurs in Europe, Lapland, the British Isles, the Balkans, and Lake Baikal. Adult beetles make holes in willow and birch leaves and attack the buds and bark of annual shoots (Rokytová et al., 2004). This species was indicated in the Polesie National Park and recognized as eudominant among chrysomelid species in transitional and high peat bogs (Ścibior, 2010). Contrary to literature (Rokytová et al., 2004), in our study, eggs of this species were noted on the *S. lapponum* leaves. The beetles of another chrysomelid, *C. aurata*, produce small holes or pits in the leaf by eating through the epidermis and underlying tissues. This species is very common

throughout Poland as well as abundant and having a wide range in Europe (Canty et al., 2016). However, research conducted by Ścibior (2010) did not reveal *C. aurata* in the peat bogs of the Polesie National Park. We found only few individuals and no visible damage caused on infested leaves. *Crepidodera aurata* and *L. capreae* occur abundantly in *Salix* plantations in south-eastern Poland (Czerniakowski, 2000; 2006). *Aphrophora salicina* was found to attack about 42% of the *S. lapponum* twigs. This species commonly inhabits various species of willow, and is also mentioned as a pest in willow plantations (Nijak, 2009; Czerniakowski, 2010; Ropek & Frączek, 2016). *Aphis farinosa* was the only aphid species found on *S. lapponum* in our study. Small colonies inhabited the bottom side of young leaves and the tops of shoots. Of 120 aphid species inhabiting willows worldwide, only 6 have been indicated on *S. lapponum* (Blackman & Eastop, 2017). In spring and early summer, *A. farinosa* formed dense colonies, attended by ants, on young shoots of the willows. It is a monoecious species with a sexual generation occurring already in summer (Blackman & Eastop, 2017). Both hemipterous species namely, *A. farinosa* and *A. salicina* are serious pests of ornamental willows in Polish nurseries (Łabanowski & Soika, 1998). Less common gall inducing insects were represented by *R. rosaria*. Adult females oviposit into expanding willow shoots. The activity of the gall former inhibits further lengthwise growth of the shoot, with concomitant reduction of leaf growth and increased production of trichomes. A characteristic 'leaf rose' is formed at the tip of a galled shoot (Nyman et al., 2011). This species is mentioned as a local pest of *Salix* species (Charles et al., 2014). In our study, caterpillars of *C. stigmatella* and *P. heparana* inhabited 4% and 3% of the *S. lapponum* shoots, respectively. The larvae of *C. stigmatella* were found to feed on different species of willows and poplars. Young larvae mine the leaves of their host plant, while older instars continue living freely under a leaf fold (Łabanowski & Soika, 2003). Wrzesińska and Wawrzyniak (2012) found this species in high densities (as a eudominant) on *Salix viminalis* L. plantations in the Kujawy and Pomorze Province. *Pandemis heparana* has two generations per year, and its larvae are considered a pest of trees and shrubs. They live in rolled leaves and are polyphagous, feeding on various deciduous trees and shrubs, including *Quercus* sp., *Salix* sp., *Betula* sp., *Lonicera* sp., *Sorbus* sp., *Malus* sp., and *Pyrus* sp. (Łabanowski & Soika, 2003).

The magnitude of the insect herbivory effect on photosynthetic capacity is multifaceted, depending mostly on the type of feeding habits and the specificity of plant defense responses (Nabity et al., 2009). It has been found that plant genotypes that are capable to maintain photosynthesis under insect attack often exhibit greater resistance, which supports the

hypothesis that insect-induced changes in photosynthesis are genetically programmed (Kerchev et al., 2012). In general, healthy plants have photosynthetic efficiency (F_v/F_m) of 0.8–0.9, while plants suffering from stress have ratios of ca. 0.3–0.7 (Öquist & Wass, 1988). The F_v/F_m obtained in our study ranged between 0.705 and 0.759 and were a slightly below the optimal values given by Öquist & Wass (1988) and Murchie & Lawson (2013). We believe that under natural conditions, due to large fluctuations in environmental factors affecting photosynthesis, the F_v/F_m recorded by us for *S. lapponum* can be considered as optimal. Moreover, we did not find significant impairment of photosynthesis (e.g. F_v/F_m as a proxy for maximum quantum yield of PSII) in the plants inhabited by phytophagous insects, compared to the control plants. Only *A. salicina* feeding reduced the F_0 and F_m values, but the decrease in F_0 is not a sign of the negative impact of stress factors on chlorophyll *a* fluorescence (Murchie & Lawson, 2013). Therefore, we assume that the photosynthetic capacity of *S. lapponum* can be maintained in conditions of plant infestation by the examined insect species. There is very little information available on the measurement of chlorophyll *a* fluorescence in *S. lapponum*. Odasz-Albrigtsen et al. (2000) reported that *S. lapponum* growing in the birch forests of Norway had an F_v/F_m value ranging between 0.594 and 0.695.

The involvement of ROS in defensive responses of plants towards insect herbivores is well established. Infection or the action of pathogen-derived elicitors cause an oxidative burst characterized by overproduction of H_2O_2 (Gatehouse, 2002). In our study, we found that the feeding of herbivores on *S. lapponum* leaves induced accumulation of H_2O_2 very close to the area of damage. Similarly, other studies have provided direct evidence for H_2O_2 accumulation following plant infestation by either chewing or piercing-sucking insects (Kerchev et al., 2012, and references therein). For example, a study concerning infestation of lima bean by the chewing caterpillar *Spodoptera littoralis* Boisd. revealed accumulation of H_2O_2 close to the zone of wounding within 3h following either mechanical injury or caterpillar feeding (Maffei et al., 2006). The high levels of H_2O_2 have been implicated in the induction of cell death in the hypersensitive response to pathogens. The accumulation of H_2O_2 has previously been observed at a distance away from the injured leaf area, but only after insect feeding, suggesting a potential function of H_2O_2 as a systemic diffusible signaling molecule (Gatehouse, 2002; Kerchev et al., 2012). However, in the present study, we did not detect H_2O_2 in vascular bundles, which implies local rather than systemic stress response to herbivore feeding.

Czerniakowski (2006) and Nijak (2011) reported that different insect species showed a clear

preference for cultivars and clones of *S. viminalis*. This is associated with the morphological and anatomical structures of plants and with biochemical interactions between the insect and the host plant. To avoid insect attack, plants produce specialized morphological structures or secondary metabolites and proteins (War et al., 2012). Binns and Blunden (1980) revealed the thickest indumentum on both adaxial and abaxial epidermis of *S. lapponum* leaves in comparison to 19 species and 12 hybrids of *Salix*. Trichomes play a crucial role in plant defense against many herbivores. Pubescence negatively affects the ovipositional and feeding behavior, hinder the movement of arthropods on the plant surface, and reduce their access to leaf epidermis (War et al., 2012 and references therein).

During the current study, we found 8 phytophagous insect species inhabiting *S. lapponum*. The small number of herbivores can be associated with the thick indumentum of the plants. Only 3 species, namely *L. caprea*, *A. salicina*, and *A. faronosa*, inhabited the analyzed shoots in higher densities. However, the pest feeding caused relatively weak disruptions to host plant physiology and the *S. lapponum* specimens were able to maintain photosynthetic efficiency under insect attack. Comparative studies are needed to assess the species composition and population numbers of insects occurring on plants from other *S. lapponum* populations. Comprehensive knowledge of the biotic factors affecting the occurrence of downy willow would facilitate establishment of the form of *S. lapponum* conservation.

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