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Regular research paper

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DIFFERENCES IN DEFENCE STRATEGIES AGAINST HERBIVORES BETWEEN TWO PIONEER TREE SPECIES: *ALNUS GLUTINOSA* (L.) GAERTN. AND *BETULA PENDULA* ROTH.

ABSTRACT: The different defence strategies of trees against herbivores are very often connected with succession status, leaf life span and the level of secondary metabolites. We examined the effect of simulated leaf grazing on the differences in the leaf life span and defence chemistry of two pioneer tree species that belongs to the same family (*Betulaceae*), black alder (*Alnus glutinosa* (L.) Gaertn.) and European white birch (*Betula pendula* Roth.). At the beginning of the growing season, mature leaves were perforated using a paper punch. The holes removed about 10% of the leaf surface. Each species was represented by six trees – one branch was chosen for perforation and one branch as a control. All leaves were counted every week until their abscission. Additional damages caused by grazing insects were also noted.

Undamaged birch leaves were held much longer than those of alder. The average difference in half leaf life span between control and perforated leaves was 28 days in birch and 6 days in alder. The control unperforated alder leaves were significantly ($P < 0.05$) more often grazed by insects than those that were perforated. Leaf perforation in alder increase phenolic concentrations in the new, young leaves. In birch we did not observe these changes.

The comparison of alder and birch indicate that the species with similar successional status can have different strategies of leaf defence. The birch leaves were characterized by a longer leaf life span, constitutive defence, a lack of induced

defence accumulation of phenolics and earlier shedding of damaged leaves in comparison to the control. The black alder foliage had a shorter leaf life span, induced defence reaction (produced more phenolics after perforation), and only slightly earlier shedding of damaged leaves than the control.

KEY WORDS: insect herbivore, phenolic compounds, artificial damage, black alder, European white birch, leaf life span

1. INTRODUCTION

Almost all plants growing in the natural environment have herbivore enemies. At the same time it is evident that they are able to use different defence strategies. It is known that the degree of plant resistance to grazing reflects a compromise between the benefits of reduced herbivory and the costs of defence (Simms 1992). There are four basic strategies by which plants can reduce damage by herbivores: association with other species, escape in space or time, tolerance of reduction of leaf area, and physical or chemical defence (Kennedy and Barbour 1992). The most expensive kind of defence is chemical defence because the carbon in the organic C compounds cannot be used in building pro-

cesses. Chemical defence is divided into constitutive and induced defence. Koricheva *et al.* (2004) considers that plants are jacks-of-all-trades and may successfully produce several types of defence without considerable trade-off. However, it is known that pioneer plants (early succession status) have low amounts of defence metabolites (Coley *et al.* 1985) because they need the carbon to assure fast growth, but they tolerate reduction of leaf area better than late succession status plants.

Among the tree species examined in this paper, *Alnus glutinosa* is common in sun to partially shade sites and in the range of soil types including wet, dry and infertile soil. *Betula pendula* requires sunny and moist sites with well drained soil, but will tolerate dry soil (Table 1). In a previous paper (Oleksyn *et al.* 1998), we showed some differences in the defence strategies between these species. The gas exchange and leaf chemistry measurements suggested that in contrast to *Betula*, *Alnus incana* (L.) Moench and *Alnus glutinosa* possess leaf-level physiological adaptations and defence mechanisms, which can attenuate negative effects of herbivory. Since *Betula* does not defend leaves by producing secondary metabolites, we want to advance the hypothesis that the defence strategies against insect attack are different among species in the same family (*Betulaceae*) which represent the same successional status (pioneer trees). The aim of this study was to answer the following question: Do trees shed damaged leaves earlier than undamaged ones? Based on previous work (Oleksyn *et al.* 1998), we hypothesized

that damaged leaves of *B. pendula*, which lacks a constitutive defence strategy, will be shed earlier in comparison to control than preventively defended leaves of *A. glutinosa*. We also explored whether perforated leaves contain higher levels of phenolic compounds in comparison with the control. In addition we tested whether perforated leaves will be avoided or preferred by insects.

2. MATERIAL AND METHODS

All experiments were conducted on 7-year-old trees of black alder (*Alnus glutinosa* (L.) Gaertn.) and European white birch (*Betula pendula* Roth.) growing at the Arboretum of the Institute of Dendrology, Kórnik, Poland (52°15'N and 17°04'E). Each species was represented by six trees. On each tree, two branches (with 18 to 39 leaves) were perforated using a paper punch (10% of leaf surface was removed) and on each tree the leaves from two unperforated branches were used as a control. The leaves were damaged after they fully unfolded on May 4 and 18, 1999 for alder and birch, respectively. After one week, perforated and control leaves from one branch for each tree were sampled for chemical analyses. In addition we also sampled new leaves from the same branches that developed after perforation. The leaves from the second branch were counted every week. Additional damage caused by grazing insects was also visually assessed. The results of additional insect grazing were evaluated only after two and three weeks after perforation due to differences between species in leaf life span.

Table 1. Comparison of ecological traits for *Alnus glutinosa* and *Betula pendula*. Amax – area-based net photosynthesis under light-saturated conditions.

Trait	<i>Alnus glutinosa</i>	<i>Betula pendula</i>
Successional status	pioneer	pioneer
Light requirements ¹	half-light	full light with partial shade
Soil moisture ¹	high	middle
Soil pH ¹	5.5–6.5	4.5–6.5
Soil richness ¹	rich	moderately rich
Soil mechanical composition ¹	heavy clay and loam	sand and clay
Leaf life span (weeks) ²	10	18
Leaf nitrogen (%) ³	2.8	2.6
Amax ($\mu\text{mol m}^{-2}\text{s}^{-1}$) ⁴	6	12
N-fixer	yes	no

¹Zarzycki *et al.* 2002; ²This study; ³Oleksyn *et al.* 2000; Reich and Oleksyn 2004; ⁴Oleksyn *et al.* 1998.

The content of total phenols was determined in a 0.1 g sample of leaf dry mass after a boiling extraction for 15 min. in 95% ethanol and 10 min. in boiling 80% ethanol. The content of phenolic compounds was measured colorimetrically using Folin and Ciocalteu's Phenol Reagent (SIGMA F-9252), following Johnson and Schaal's (1957) method as modified by Singelton and Rossi (1965). The leaves were sampled one week after perforation. The content of total phenols was expressed in μmol of chlorogenic acid g^{-1} dry mass.

Analysis of variance (ANOVA) was used to assess the influence of perforation on the total phenolic compound content in the leaves and the influence of leaf perforation on the amount of damage caused by insects. Survival analysis (Log-Rank test) was used to analyse the time of leaf shedding. A Paired t test (one-way) was used to analyse the increase of phenolic compounds in old and young leaves after perforation. All analyses were conducted with statistical analysis software (JMP 4.0.2; SAS Institute, Cary, NC, USA).

3. RESULTS

The studied tree species differed in their leaf life span. Birch held its leaves much longer than alder. Birch shed 50% of control (not damaged) leaves after about 126 days and 90% of its leaves after 168 days while the 50% of alder control leaves were shed after about

70 days and 90% after 98 days (Fig 1AB). The perforation of birch and alder leaves resulted in faster abscission. The survival analysis showed statistically significant differences (Log-Rank test $P < 0.001$ for birch, $P < 0.01$ for alder) in the leaf life span between perforated and control leaves (Fig 1AB). The difference in the half life span between control and perforated leaves was approximately 28 days for birch and 6 days for alder. Total life span of the foliage was shortened by 22% and 8% for birch and alder, respectively.

We found that after 3 weeks the control alder leaves were significantly ($P < 0.05$) more often grazed (61% of leaves had damage caused by herbivore insects) than those that were manually perforated (39%). In the case of birch, control leaves were also more often grazed but the differences for extra damage (grazing) were not significant (Fig 2).

Control leaves of birch have significantly higher concentration of phenolics in comparison to alder leaves (Fig 3). Although the changes in the phenolic concentrations after artificial perforations were not significant, they were greater for alder (Table 2). A marginally significant increase ($P=0.07$) of phenolic concentrations in the new, young leaves that developed after foliage perforation was observed in the alder. In the case of birch, these changes were very small and not significant statistically ($P=0.36$). These differences between studied species indicate possible existence of induced defence reactions in the alder but not in birch leaves.

Table 2. Mean (SD) content of total phenolic compounds in the old and young leaves from control and perforated branch of *Alnus glutinosa* and *Betula pendula* one week after manual perforation of leaves. Statistical analyses include Paired t test and ANOVA.

Speciesa	Age of leaves	Control branch	Perforated branch	Paired t test P	Statistical analysis			
					ANOVA			
					Treatment	d.f.	F	P
<i>Betula</i>	old	185(33)	199(45)	0.24	Perforation	1	0.38	NS
					Age	1	2.67	NS
	young	215(28)	221(45)	0.36	Perforation x Age	1	0.06	NS
					Error	20	1.03	
<i>Alnus</i>	old	84(11)	92(18)	0.13	Perforation	1	2.94	NS
					Age	1	42.09	0.0001
	young	176(42)	228(71)	0.07	Perforation x Age	1	1.53	NS
					Error	20	15.52	

NS – not significant ($P > 0.1$)

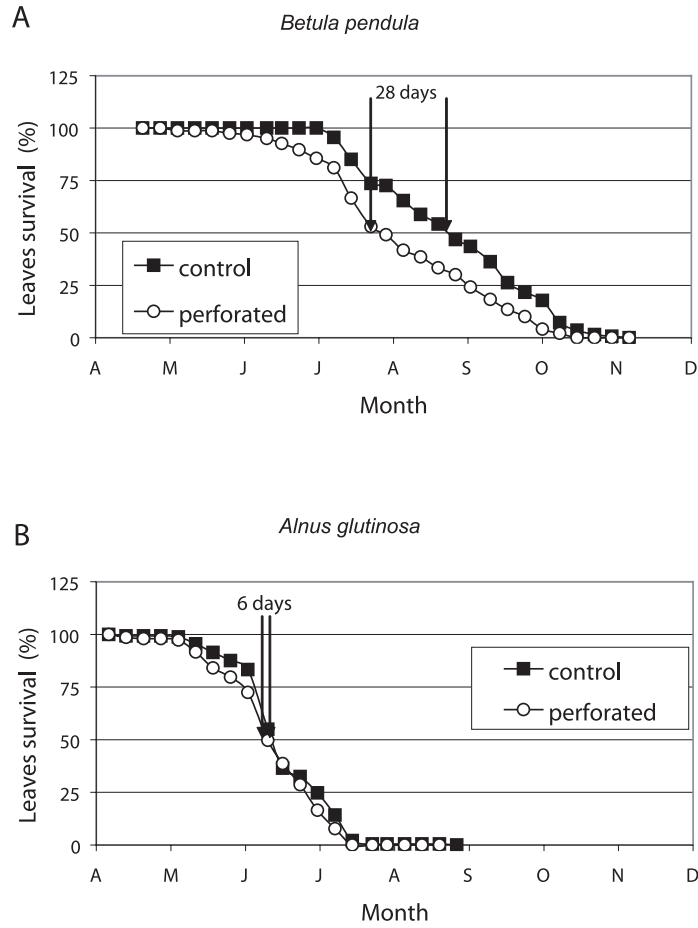


Fig. 1. The percent (mean for six trees) of perforated (○) and control (■) leaves of birch (A) and alder (B) noted in next successive weeks after perforation. Arrows indicate differences between damaged and control leaves when 50% of leaves had fallen.

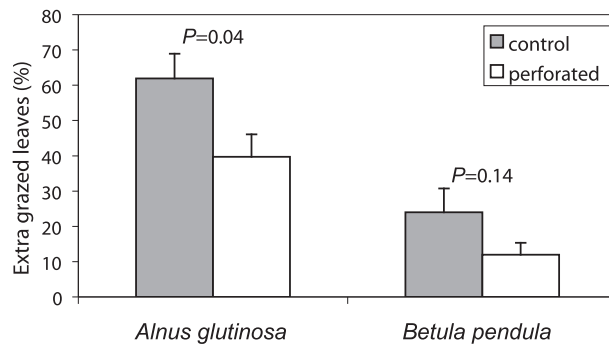


Fig. 2. The percent (mean for six trees) of extra grazing on perforated (□) and control (■) leaves of alder and birch three weeks after perforation.

4. DISCUSSION

We have shown that simulated insect grazing significantly shortened the leaf life span of both *Alnus glutinosa* and *Betula pendula* trees (Fig. 1). However, leaf life span reduction was much smaller for *A. glutinosa*, (8%) than those for *B. pendula* (22%). Both species also differ by 4 weeks in the leaf life span of control leaves (Table 1, Fig. 1). These differences are relatively large among early successional deciduous tree species (Wright *et al.* 2004).

Leaf abscission of grazed leaves may be considered as a defence strategy. It was previously documented that removal of one half of the canopy leaves from *Betula pubescens* ssp. *tortuosa* plants resulted in a 35% enhancement in area-based net photosynthesis of the remaining leaves (Hoogesteger and Karlsson 1992). The increased photosynthetic rate was correlated with an increased leaf N concentration in the remaining leaves.

Our data support the idea that the defence strategies against herbivores may be related to the leaf life span (Coley *et al.* 1985). Species with longer leaf life span usually had higher concentrations of immobile defences such as tannins and lignins (Coley 1987), while those with short leaf life span species have higher photosynthetic rates (Reich *et al.* 1992, Wright *et al.* 2004, Wright *et al.*

2005) and during herbivore attack can rapidly produce mobile defences or better tolerate the reduction of leaf surface area. We have found that *B. pendula* trees, with a leaf life span of 18 weeks, had ca. 60% higher total phenolics concentration (Fig. 3) of control unperforated leaves than *A. glutinosa* with a leaf life span of only 10 weeks.

The control alder leaves were significantly more often grazed by feeding insects in comparison to artificially perforated leaves (Fig. 2). It is likely that herbivore-induced plant feedbacks are responsible for the preferences and performance of herbivores (Rodríguez-Saona *et al.* 2005). In our prior studies we found that in *Alnus* leaves grazed by the alder beetle (*Agelastica alni* L.), total phenolics concentration increased exponentially with increasing perforation (Oleksyn *et al.* 1998). At the same time, total phenols concentration in perforated leaves of *Betula pendula* trees declined compared with those not perforated. In general, the comparison of feeding preferences of insect herbivores on undamaged and perforated leaves of *A. glutinosa* and *B. pendula* (Fig. 2) indicates induced defence reactions in the alder leaves and the lack of such a mechanism in birch leaves. A similar reaction was also observed by Cornelissen and Fernandes (2001) in the leaves of the tropical tree *Bauhinia brevipes* (Vog.). They found that higher concentrations of tannins in damaged leaves signifi-

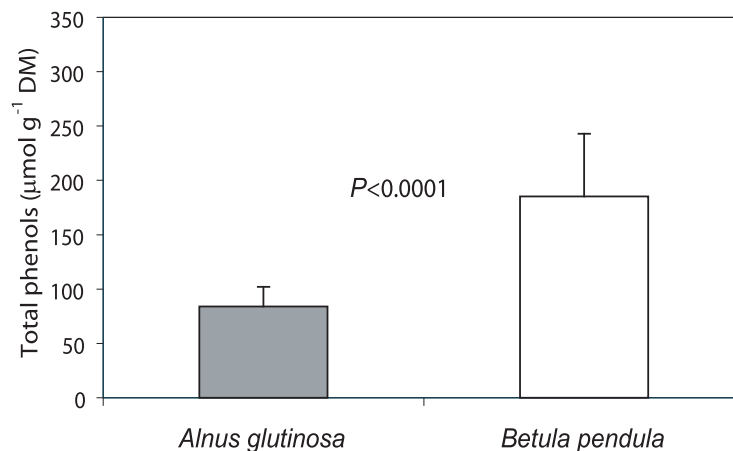


Fig. 3. Total phenolic content in control leaves of *Alnus glutinosa* (■) and *Betula pendula* (□).

cantly lowered the rate of herbivore grazing compared to control leaves.

Nitrogen is considered to be a limiting nutrient for herbivorous insects, and an increase in nitrogen content in their diet has been frequently found to result in improved insect herbivore performance (Mattson 1980, Ikonen 2001, Giertych *et al.* 2005a). As early successional trees with high foliar N concentration (Table 1), both *B. pendula* and *A. glutinosa* have a high probability of insect attack. However, despite their taxonomical relationship and many shared ecological traits (Table 1), these species used different defence strategies. Evidence presented in this paper and accumulated in the literature suggests that *B. pendula* relies largely on constitutive and *A. glutinosa* on inducible defence strategies (Baur *et al.* 1991, Oleksyn *et al.* 1998, Tscharntke *et al.* 2001). Apparently, the primary benefit of inducible defences is that they incur the cost as a function of actual attack, not probability of attack (Zangerl and Bazzaz 1992).

One of the reasons for the constitutive defence strategy used by *B. pendula* may be related to the photosynthetic rate of this species (50% higher than that of *A. glutinosa*, Table 1) and high foliar N concentration (Oleksyn *et al.* 1998, Table 1). Leaves that operate at high photosynthetic rate, and rich in nitrogen and water are most nutritionally rewarding for herbivores (Moreau *et al.* 2003, Giertych *et al.* 2005b). Plants that have a high probability of attack should not rely on induced defence, but rather on a more potent constitutive defence (Zangerl and Bazzaz 1992).

In summary, the leaf life span and leaf chemistry measurements suggest that *Alnus glutinosa* and *Betula pendula* possess different leaf-level adaptations and defence mechanisms which can attenuate negative effects of leaf grazing. Leaf grazing caused both rapid and delayed induced resistance in alders (Fig. 2, see also Baur *et al.* 1991, Seldal *et al.* 1994, Oleksyn *et al.* 1998, Tscharntke *et al.* 2001). These inducible defences can reduce the effects of feeding damage by changing in raw fiber, decreasing water content and increasing trichome density of the leaves (Baur *et al.* 1991, Baur and Rank 1996). Our prior experiments have also shown sub-

stantial stimulation of photosynthetic rates following leaf perforation in *Alnus* but not in *Betula* trees. The compensatory photosynthetic increase in alders was related to a decline in leaf starch and increase of stomatal conductance in grazed leaves. In contrast, *Betula pendula* trees rely on constitutive defence (high level of phenolic compounds in the control leaves, Fig. 3) and lack inducible defences except early shedding of injured leaves. Early leaf abscission of plants in response to insect herbivory might represent a phenological defence (Faeth *et al.* 1981).

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