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The influence of shade on phenolic compounds in Scots pine

Abstract: The influence of light reduction on the growth and phenolic compounds contents in Scots pine trees and seedlings was studied. Results of the first experiment in field conditions show that shade causes an increase of phenolic compounds and in the second experiment with seedlings under controlled shading conditions the results were opposite. It is suggested that more attention should be paid to insolation during studies on the influence of pollution on the content of phenolic compounds.

Additional key words: *Pinus sylvestris*, light reduction, bioindication, insolation, total phenols, ortho-diphenols

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Introduction

Pollution caused by industry and other human activity, also in forest regions forces us to look for new methods of monitoring the environment. One of bioindications method is the measurement of phenolic content changes (Yee-Meiler 1977, Zobel and Nighswander 1991, Richter and Wild 1994). Phenolic compounds take part in the reaction of plants to biotic stress factors (Nicholson and Hammerschmidt 1992, Bennett and Wallsgrave 1994, Oleksyn et al. 1998) but also in the case of abiotic factors phenolics can to play a role in defense reaction (Giertych and Karolewski 1993, Karolewski and Giertych 1994). Apart from typical stress factors the level of phenolic compounds is affected by other factors for example: fertilizing (Balsberg-Påhlsson 1992, Hakulinen et al. 1995, Karolewski and Giertych 1995) and high or low temperature (Forrest 1975, Pukacki and Pukacka 1987), insolation (Siegelman 1964). The influence of these factors make the use of phenolics for bioindication difficult, because they can modify the changes caused by pollution. The aim of this study was to determine the influence of shade on the content of two

group of phenolic compounds (total soluble phenols [TPh] and ortho-diphenols [o-dPh]) in needles of Scots pine trees and seedlings.

Material and methods

Plant material

The first experiment was conducted on 10-years-old trees of Scots pine (*Pinus sylvestris* L.) growing on an experimental plot in Forest Range Zwierzyniec near Kórnik. Four trees from one Polish population (Miłomłyn) were chosen to the examination. Needles were collected in September 1992 from the upper part of the crown from the insolated and shaded sides.

In the second experiment, in May 1995, one-year-old seedlings of Scots pine from a commercial nursery were planted in plastic containers. The content of nutrients and some other elements in the soil is shown in Table 1.

There were 8 containers and in each 10 pine seedlings grew. The containers were divided into four groups and placed outdoor in well insolated place.

Table 1. Content of macro nutrients (N, P, K, Ca, Mg) and S, Fe, Al and pH in the studied soil

Element	ppm
N-NH ₄	12.5
N-NO ₃	14
P	82
K	69
Ca	175.5
Mg	33.5
S-SO ₄	7.5
Fe	86.6
Mn	3.25
Al	35.1
pH	4.3

Three groups of containers were placed under special boxes limiting light and the fourth group was left as a control. The boxes were made of a plastic net, which reduced light about 50%, 70% and 85%. Light was measured using the photosynthetic light sensor (Quantum, LiCor, USA). The pattern of light changes during one day is showed in Figure 1.

The seedlings grew under these covers for four months and they were watered if it was necessary. All analyses were made at the end of growing season in Oc-

tober. Measurement of phenolics and the biometrical analyses included needles and roots of seedlings.

Analytical methods

Phenolic content was determined in 0.5g samples after double extraction for 15 and 10 minutes in boiling 95% and 80% of ethanol respectively. The analyses were performed by spectrophotometric methods distinguishing between ortho-diphenols (o-dPh) and their total pool (TPh). For the measurement o-dPh, the method described by Johnson and Schaal (1957) was used with the Arnow reagent (sodium nitrite and sodium molybdate). TPh was also determined with the method of Johnson and Schaal (1957) as modified by Singleton and Rossi (1965) with the Folin-Ciocalteu phenol reagent. The content of both groups of phenols was expressed as mmol of chlorogenic acid g⁻¹ of fresh weight (f.wt.).

Measurement of the surface and length of needles and roots was made using the AgVision system (Decagon Devices Inc., Pullman, WA, USA).

Results

The results of the first experiment show that the shade caused an increase of phenolic compounds.

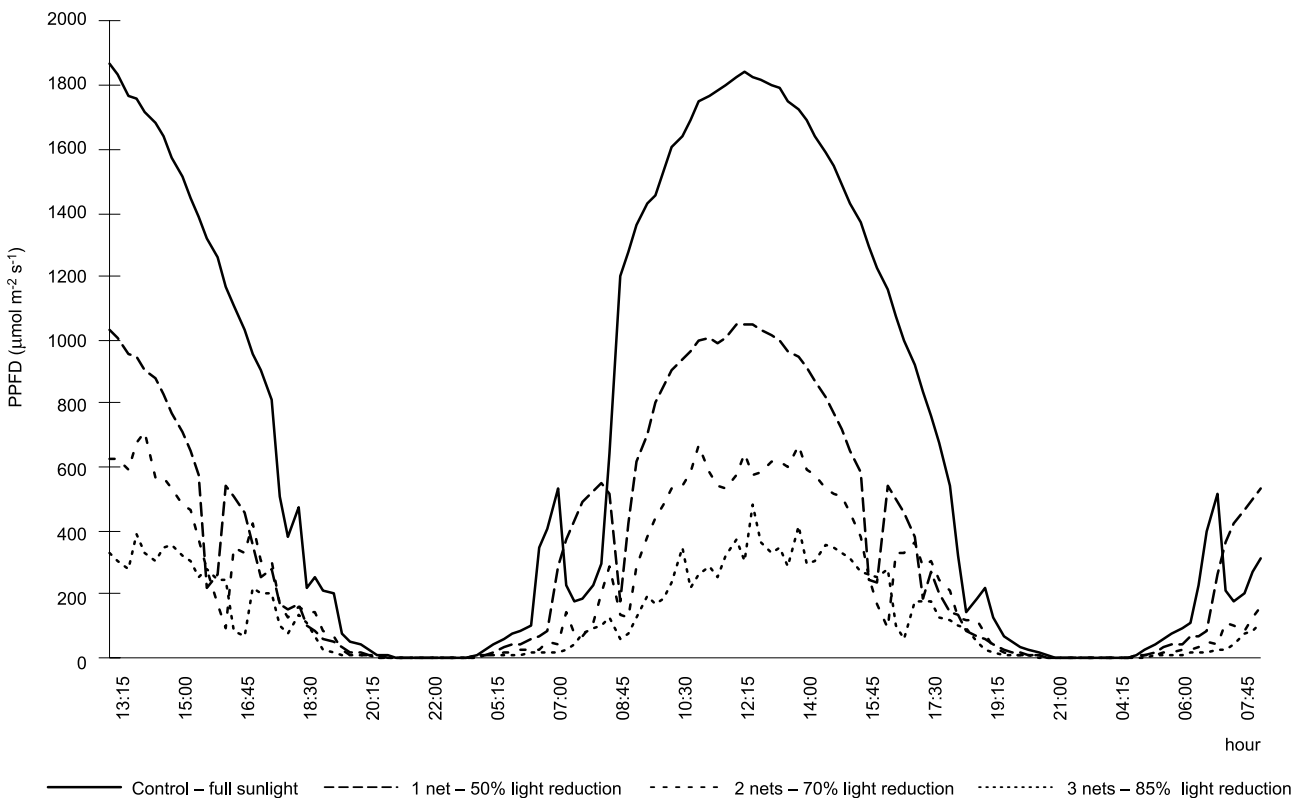


Fig. 1. Changes of light intensity depending on light reduction and hour

Needles from the shaded side of the crown had more of both TPh and o-dPh (Fig 2). The differences were significant in the case of o-dPh ($p < 0.0015$).

In the second experiment the content of both examined groups of phenolic compounds (o-dPh and TPh) in the needles and roots of Scots pine decreased with an increase of shading (Fig. 3). This decrease was not statistically significant (Tab. 2).

The reduction of light caused some changes in Scots pine seedling growth. An increase of shade caused a decrease of seedlings biomass (Fig. 3). The decrease of biomass was mainly caused by a decrease of root mass. Linear reduction of roots length, area and weight with increase of shading was confirmed (Fig. 3). The influence of the light reduction on the changes in length, surface and weight of needles were not significant.

Discussion

In the presented study the influence of light reduction on phenolic compound content and growth of Scots pine trees and seedlings was examined. The results of the first experiment show that the insolation can decrease the level of phenolic compounds. There is lack of published information that light reduction can cause an increase of phenolic compounds in needles. However, there are many papers

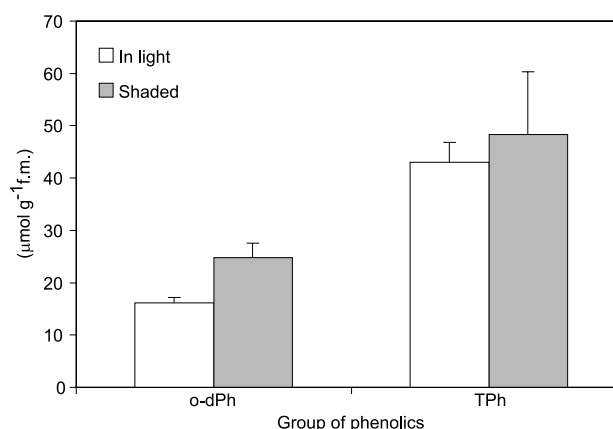


Fig. 2. Content of phenolic compounds in the insolated and shaded needles of Scots pine

confirming an increase of phenolic compounds under the influence of light (Siegelman 1964, Kubackova et al. 1994, Lewis et al. 1998). An increase of phenolic compounds in needles can be an artifact related to changes in water or carbohydrates levels (Żytkowiak et al. 1999). In the second experiment the decrease of phenolic compounds content with reduction of light confirms earlier information. Higher content of phenolic compounds in insolated needles can be linked with the role of phenolics in protecting

Table 2. Summary of ANOVA for biometric parameters and content of phenolic compounds in needles and roots of Scots pine seedlings, by shading (DF – degree of freedom, MS – mean squares, and p -values (bold then $p \leq 0.05$))

Source of variation	Estimated parameter									
	Seedling fresh mass			Needle fresh mass		Needle dry mass		Needle surface		
	DF	MS	p	MS	p	MS	p	MS	p	
Shading	3	48.7661	0.1057	2.02315	0.3957	0.15967	0.3364	557.59	0.2670	
Block	4	12.0917	0.2506	1.58438	0.3638	0.10431	0.5200	289.505	0.4757	
Error	16	8.0908		1.36266		0.128036		314.226		
	Total needles length			Shoot dry mass		Root fresh mass		Root dry mass		
	DF	MS	p	MS	p	MS	p	MS	p	
Shading	3	158906	0.1815	0.06638	0.5099	9.8179	0.0000	0.43869	0.0054	
Block	4	59064	0.6374	0.07265	0.1826	0.00847	0.9997	0.01879	0.7824	
Error	16	91370		0.04085		0.78114		0.04334		
	Roots surface			Roots length		Mean needle thickness		Needle surface to length ratio		
	DF	MS	p	MS	p	MS	p	MS	p	
Shading	3	8631.1	0.0081	1076285	0.0130	29.5320	0.3866	155.052	0.1277	
Block	4	460.2	0.7856	74422	0.8105	22.5035	0.4386	44.035	0.2491	
Error	16	1072.9		189267		22.6146		29.359		
	TPh in needles			TPh in roots		o-dPh in needles		o-dPh in roots		
	DF	MS	p	MS	p	MS	p	MS	p	
Shading	3	227.292	0.6765	28.4046	0.8698	19.9024	0.8296	31.5833	0.7580	
Block	4	415.958	0.0805	122.218	0.1508	67.9538	0.1476	77.9486	0.0354	
Error	16	163.802		62.6245		34.4641		23.1988		

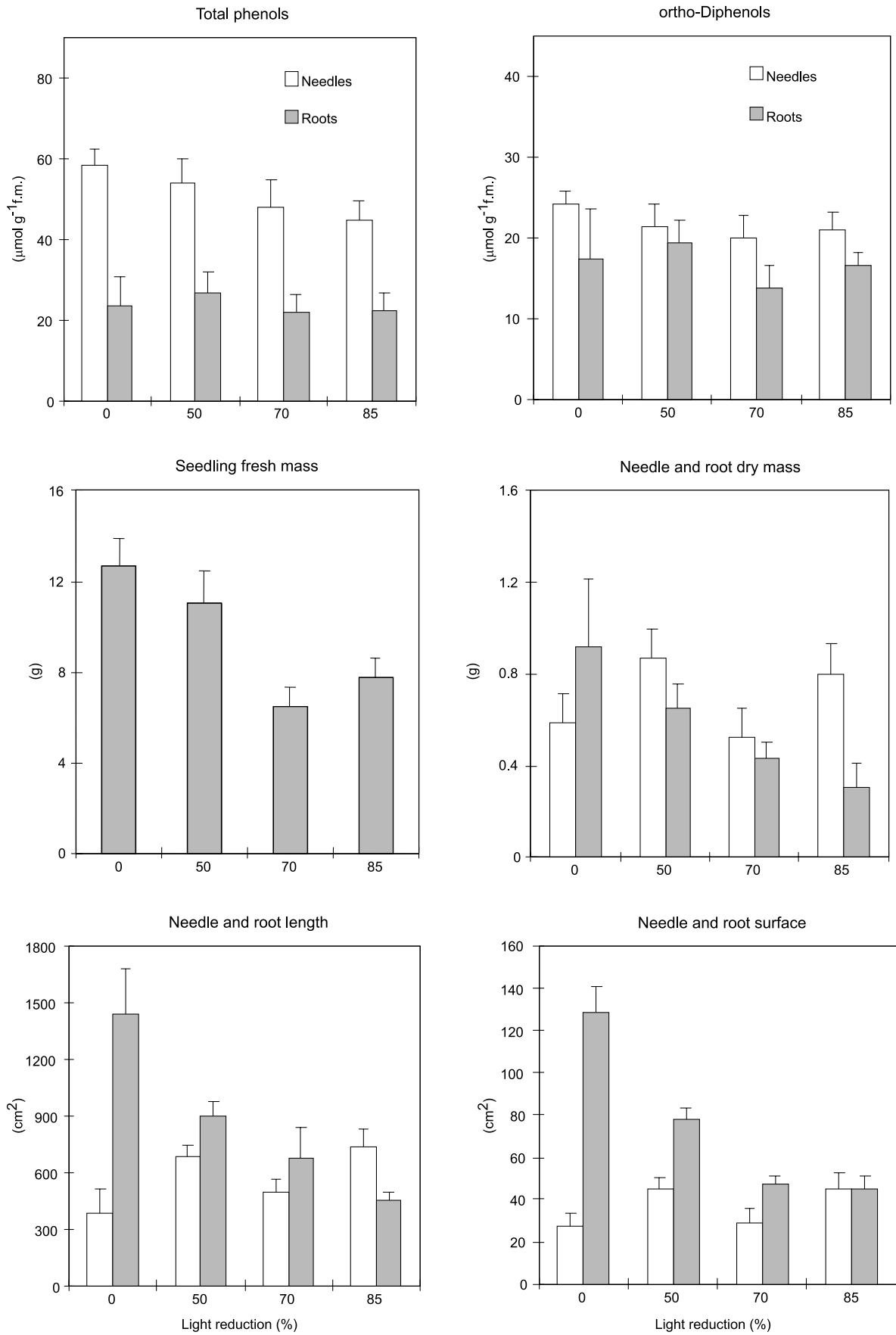


Fig. 3. Changes of phenolic compounds, seedling fresh mass, needle and roots dry mass length and surface as dependent on light reduction

against UV-B light (Cooper-Driver and Bhattacharya 1998). Another explanation can be the higher lignification of insolated needles, which were thicker and stiffer in comparison with needles from shaded seedlings. It is interesting that there were no changes in the content of roots phenolics, in spite of the fact that there were significant changes in roots mass, surface and length. The influence of shade on roots system was shown by Noland et al. (1997) and Reich et al. (1998). Shade is often given as a factor limiting the needles length (Kovalev and Antipova 1983, Lemke and Woźniak 1992). In our experiment shade did not cause significant changes in the length, surface or mass of needles.

The level of phenolic compounds increases in stress conditions (Yee-Meiler 1977, Grill et al. 1975, Giertych and Karolewski 1993, Karolewski and Giertych 1994). The phenolics as compounds associated with the reaction of plants to stress factors are considered good metabolite bioindicators of pollution (Zobel 1996). However using phenolic compounds as bioindicators of pollution we must respect the influence of light. Good insolation or shading can affect changes of phenolic content caused by pollution. There is still a lack of studies showing the influence of shade on the changes of phenolic compounds in forest trees. The results presented in this paper are not clear. We suggest that when taking plant material for bioindication we must to draw the attention to the light condition and always take material equally insolated.

Acknowledgements

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