PHENOLIC COMPOUNDS DISTRIBUTION ALONG THE LENGTH OF SCOTS PINE NEEDLES IN A POLLUTED AND CONTROL ENVIRONMENT AND ITS CONNECTION WITH NECROSES FORMATION

MARIAN J. GIERTYCH, PIOTR KAROLEWSKI

Institute of Dendrology, Polish Academy of Sciences
Parkowa 5, 62-035 Kórnik, Poland

(Received: January 17, 1999. Accepted: April 4, 2000)

ABSTRACT

Phenolic compounds distribution was studied along the length of Scots pine needles from trees in a polluted and a control site. There is no significant difference in the content of total phenols between the studied sites. There are significant differences in distribution of phenolics along the length of needles. The highest content is in the tip part of the needle and the lowest at the base. This kind of distribution suggests a role of these compounds in the processes of necroses formation. The content of phenolic compounds is positively correlated with the toxic elements – fluorine and aluminium and negatively with the macroelements – phosphorus, potassium and magnesium.

KEY WORDS: phenolic compounds, Scots pine, *Pinus sylvestris*, needle part, pollution.

INTRODUCTION

Leaf injury caused by pollution was wide examined (Malhotra and Blauel 1980; Shaw et al. 1993; Temple and Miller 1994; Wulff and Kärenlampi 1996). Pollution causes different types of the tree leaf injury and this depends on the kind of pollution and on tree species. Injuries are often of the character of necroses of whole leaves or are located only at some part. Needle tip necroses of pine are caused by fluorine compounds and sulphur dioxide (Malhotra and Blauel 1980). Jamrich (1972) and Karolewski (1990) gave a detailed description of needles with such necroses. We know a lot about chemical differences between healthy parts of needles and necroses. The content of several elements in different parts of Scots pine needles damaged by fluorine compounds were examined by Jamrich (1972). The content of free and bound proline, bound hydroxyproline, ortho-diphenols, total phenols and water in injured Scots pine needles exposed to sulphur dioxide in the control conditions was shown by Karolewski (1990). Giertych and Werner (1996) studied the content of phenolic compounds, lignin, suberine and water in different parts of injured needles from an experimental field, which was polluted by fluorine compounds and sulphur dioxide. The differences between necroses and healthy tissues result from an accumulation of toxic substances and from the senescence of damaged tissues.

In the literature there is lack of information about physiological processes in needles before visible damages appear. Needles of Scots pine are a very good material for these analyses, because it is easy to forecast where the necroses will appear.

Phenolic compounds participate in the protection and degradation processes (Swain 1977; Halverson and Stacey 1986; Richter and Wild 1992). During the process of necroses formation cell walls stiffen with lignins and other derivatives of phenolics compounds (Giertych and Werner 1996). Karolewski (1990) suggested that the high content of phenolics in the intermediate part of needles dividing the healthy part from the necrosis protects the healthy part against loss of water.

The aim of this study was to examine the distribution of phenolic compounds along the length of Scots pine needles from polluted and controls sites and determination of the relationship of phenolic compound contents with the needle part where the necrosis can appear.

MATERIAL AND METHODS

Current-year needles of Scots pine *Pinus sylvestris* were collected in January from 12-years old trees growing on two experimental areas in western Poland (Oleksyn and Białobok 1986; Oleksyn 1988). One site is in the Experimental Forest "Zwierzyniec" near Kórnik. This site was considered to be free of acute pollution (control). The second site is located at Luboń near Poznań, about 2 km from phosphate fertilizer factory. The surroundings of the factory were exposed to emissions (fluorine compounds, sulphur dioxide and nitrogen oxides) from the factory since 1917. Detailed information about emissions in the last years are given by Giertych et al. (1999).

Healthy-looking, current-year needles were taken from several branches in different parts of the crown. To minimize possible confounding effects of genetic differences, at each

TABLE 1. ANOVA summary table for needle phenolics and water content, by experiment location and needle sectors, showing degrees of freedom (df), mean squares (MS) and *p*-values (bold when *p* ≤ 0.05).

Source of variation	TPh			o-dPh		H ₂ O	
	df	MS	<i>p</i>	MS	<i>p</i>	MS	<i>p</i>
Location	1	6350.207	0.2609	2663.594	0.1430	358.6292	0.0069
Tree (Location)	4	3710.429		804.603		13.6411	
Sectors	4	2553.364	0.0325	432.429	0.0100	19.5903	0.0014
L x S	4	888.381	0.3489	135.847	0.2490	3.9539	0.2510
Error	16	740.232		90.568		2.6487	

site, samples were taken from three trees (replicates) of Polish provenance (No. 7 Spala). The needles were transported to the laboratory in plastic bags. The needles were not washed. Each needle was cut with a razor blade into five equal sections proportional to the length, defined as base, tip and three middle parts. Before cutting the needles, the fascicle sheath was removed. The cutting was done over several days and during this time the needles were stored at 4°C. The sections from each tree were pooled separately.

Phenolic content was determined from 1g fresh needle samples after double extraction for 15 and 10 minutes in boiling 95% and 80% of ethanol respectively. Spectrophotometric methods distinguishing between ortho-diphenols (o-dPh) and their total pool (TPh) were used in the analyses. To identify o-dPh, the method with the Arnou reagent (sodium nitrite and sodium molybdate) described by Johnson and Schaal (1957), was used. 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 dry mass (DM).

A part of the needle samples was weighed, oven dried at 65°C and weighed again to determine (%) percent of water.

RESULTS

The distribution of phenolic compounds and water along the length of the needles was similar at both sites. There was a significant increase of phenolics (for both examined groups) from base to tip (Table 1, Figs 1-2). The differences in phenolic content between sites were not statistically significant, but needle water contents were significantly higher at the control site than at polluted site. The content of water differs significantly along the length of needles. In the control needles there was an increase from base to tip, but in the polluted needles the content of water was highest in the middle part (Fig. 3).

The content of phenolic compounds and of water were correlated (Table 2) with the content of some elements examined in earlier published parallel studies (Giertych et al. 1997). The contents of the two examined phenolics groups were significantly correlated. There were also negative correlations between the phenolic compounds and main nutrients (phosphorus, potassium and magnesium) and two microelements zinc and copper. Positive correlations were between the content of phenolics and content of the toxic elements – aluminum and fluoride. The content of water was positively correlated with the content of nitrogen, phosphorus and magnesium.

Fig. 1. Mean (±SE) content of total phenols (TPh) along needle length (five sections) and in bulk current-year needles of Scots pine trees (n=3) growing on polluted and control sites.

Fig. 2. Mean (±SE) content of ortho-diphenols (o-dPh) along needle length (five sections) and in bulk current-year needles of Scots pine trees (n=3) growing on polluted and control sites.

DISCUSSION

Our knowledge about the potential differences in morphology, physiological processes and tissue development in the particular parts of needles is very small. The differences in the content of phenolics along needle length is difficult to explain in relation to its morphology or development. We know that most needle cells are of similar age because most cells

TABLE 2. Matrix of correlation coefficients between the content of chemical elements, water and phenolics in parts of Scots pine needles (bold when $p \leq 0.05$).

Element, water phenols	N	P	K	Mg	Zn	Cu	F	Al	o-dPh
o-dPh	-0,26	-0,85	-0,71	-0,71	-0,79	-0,67	0,80	0,93	1,00
TPh	0,00	-0,72	-0,75	-0,59	-0,78	-0,56	0,77	0,87	0,95
H ₂ O	0,88	0,68	0,29	0,77	0,46	0,42	-0,35	-0,49	-0,59

Fig. 3. Mean ($\pm$ SE) content of water along needle length (five sections) and in bulk current-year needles of Scots pine trees ($n=3$) growing on polluted and control sites.

are already present in the buds and spring leaf growth occurs only by elongation.

The observed increase of phenolic compounds from needle base to tip can be connected with the natural process of development and ageing. The similarity in content of phenolic compounds along the length of Scots pine needles between needles from polluted and control environments suggests this kind of explanation.

On the other hand the highest content of phenolics in the tip part of the needle, especially in needles from the polluted environment, can result from pollution. The pollutants – sulphur dioxide, fluorine compounds – caused an increase of phenolics in Scots pine needles (Yee-Meiler 1977; Karolewski and Giertych 1995). This increase is connected with the role of phenolic compounds in defense reactions against pollution (Karolewski 1990; Pflanz et al. 1994; Kainulainen et al. 1995; Booker et al. 1996). Phenolic compounds, through the lignification and suberization of cell walls, take part in the creation of the intermediate band separating the healthy part of needle from necroses (Karolewski 1990; Giertych and Werner 1996). On the other hand phenolic substances play a role in necroses formation (Richter and Wild 1992).

Tip-necroses of needles are characteristic for the pine trees polluted by sulphur dioxide and fluorine compounds (Malhorta and Blauel 1980; Karolewski 1990). Our present research was made on the visually health needles, i.e. before visible damages appear. The formation of necroses must be preceded by changes in the metabolism. One of these changes is an increase of phenolic compounds. The high level of phenolics in the tip part of needles without damages is probably connected with the beginning of necroses formation. In the tip part of the needle Giertych et al. (1997) found in a parallel research high contents of toxic elements (aluminum and fluorine),

which are accumulated by the plant in the part it expects to lose first, and low levels of macroelements (nitrogen, phosphorus, potassium) which can be usefully retranslocated to other tissues. This confirms that in the tip part of needles necroses will be occurring.

Differentiation in the content of phenolic compounds and some elements along needle length suggests that also that other metabolites may have different contents in particular needle parts. This subject requires further investigation, but just now we can say that it is necessary to consider it during bioindication using pine needles.

LITERATURE CITED

- BOOKER F.L., ANTONEN S., HEAGLE A.S. 1996. Catechin, proanthocyanidin and lignin contents of loblolly pine (*Pinus taeda*) needles after chronic exposure to ozone. *New Phytol.* 132: 483-492.
- GIERTYCH M.J., DE TEMMERMAN L.O., RACHWAŁ L. 1997. Distribution of chemical elements along the length of Scots pine needles in a heavily polluted and a control environment. *Tree Physiology* 17: 697-703.
- GIERTYCH M.J., KAROLEWSKI P., DE TEMMERMAN L.O. 1999. Foliage age and pollution alter content of phenolic compounds and chemical elements in *Pinus nigra* needles. *Water, Air, and Soil Pollution* 110: 363-377.
- GIERTYCH M.J., WERNER A. 1996. Phenolic compounds in needles of Scots pine (*Pinus sylvestris* L.) damaged by industrial pollution. *Arbor. Kórnickie* 41: 165-172.
- HALVERSON L.J., STACEY G. 1986. Signal exchange in plant-microbe interaction. *Microbiol. Rev.* 50: 193-225.
- JAMRICH V. 1972. The localization, accumulation and binding of fluorine from industrial fumes in the tissues of the organs of *Pinus sylvestris*. [In Czech with English summary.] *Zbornik Vedeckých Prac VSLD vo Zvolene* 14: 89-99.
- JOHNSON G., SCHAA L.A. 1957. Accumulation of phenolic substances and ascorbic acids in potato tuber tissue upon injury and their possible role in disease and resistance. *Amer. Potato J.* 34: 200-202.
- KAINULAINEN P., HOLOPAINEN J. K., OKSANEN J. 1995. Effects of gaseous air pollutants on secondary chemistry of Scots pine and Norway spruce seedlings. *Water, Air, and Soil Pollution* 85: 1393-1398.
- KAROLEWSKI P. 1990. Visible and invisible injury to Scots pine (*Pinus sylvestris* L.) needles caused by sulphur dioxide. *Arbor. Kórnickie* 35: 127-136.
- KAROLEWSKI P., GIERTYCH M.J. 1995. Changes in the level of phenols during needle development in Scots pine populations in a control and polluted environment. *Eur. J. For. Path.* 25: 297-306.
- MALHOTRA S.S., BLAUER R.A. 1980. Diagnosis of air pollutant and natural stress symptoms on forest vegetation in western Canada. *Environ. Can., Can. For. Serv., North. For. Res. Cent. Edmonton, Alberta. Inf. Rep. NOR-X-228.*
- OLEKSYN J. 1988. Report on the IUFRO-1982 provenance experiment on Scots pine (*Pinus sylvestris* L.). *Arbor. Kórnickie* 33: 211-229.
- OLEKSYN J., BIAŁOBOK S. 1986. Net photosynthesis, dark respiration and susceptibility to air pollution of 20 European provenances of Scots pine *Pinus sylvestris* L. *Environ. Pollut. Ser. A* 40:287-302.

- PFLANZ H., OPPMANN B., WOLF P., LOMSKY B., GEIBEL M. 1994. Detoxification of air pollutants in presence of apoplastic phenols. *Acta Horticulturae* 381: 360-366.
- RICHTER CH.R., WILD A. 1992. Phenolic compounds in needles of Norway spruce trees in relation to Novel Forest Decline. *Biochem. Physiol. Pflanzen* 188: 305-320.
- SHAW P.J.A., HOLLAND M.R., DARRALL N.M., McLEOD A.R. 1993. The occurrence of SO₂-related foliar symptoms on Scots pine (*Pinus sylvestris* L.) in an open-air fumigation experiment. *New Phytol.* 123: 143-152.
- SINGELTON V.I., ROSSI J.A. 1965. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagent. *Am. J. Enol. Viti.* 16: 144-158.
- SWAIN T. 1977. Secondary compounds as protective agents. *Ann. Rev. Plant Physiol.* 28: 479-501.
- TEMPLE P.J., MILLER P.R. 1994. Foliar ozone injury and radial growth of ponderosa pine. *Can. J. For. Res.* 24: 1877-1882.
- WULFF A., KÄRENLAMPI L. 1996. Effects of long-term open-air exposure to fluoride, nitrogen compounds and SO₂ on visible symptoms, pollutant accumulation and ultrastructure of Scots pine and Norway spruce seedlings. *Trees* 10: 157-171.
- YEE-MEILER D. 1977. Phenole als Indikatoren metabolischer Störungen bei fluorexponierten Waldbäumen. *Mitt. Eidg. Anst. Forstl. Versuchswes.*, Ber 53: 203-229.

ZAWARTOŚĆ ZWIĄZKÓW FENOLOWYCH WZDŁUŻ IGIEŁ SOSNY ZWYCZAJNEJ Z TERENU SKAŻONEGO I KONTROLNEGO ORAZ ICH ROLA W PROCESIE TWORZENIA NEKROZ

STRESZCZENIE

Badano rozmieszczenie zawartości związków fenolowych wzdłuż igieł sosny zwyczajnej z terenu skażonego i kontrolnego. W igłach z obu powierzchni stwierdzono istotne różnice w rozmieszczeniu fenoli. Najwyższą zawartość stwierdzono w szczytowej części igły, a najniższą w nasadowej. Ten rodzaj rozmieszczenia fenoli sugeruje rolę tych związków w procesie tworzenia nekroz. Poziom związków fenolowych jest dodatnio skorelowany z zawartością toksycznych pierwiastków (fluorem i glinem) a ujemnie skorelowany z zawartością makroelementów (fosforem, potasem i magnezem).

SŁOWA KLUCZOWE: związki fenolowe, sosna zwyczajna, *Pinus sylvestris*, części igły, zanieczyszczenie.