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Attempt at elucidation of the role of fatty acids in the resistance of *Pinus silvestris* L. seeds to infection by damping-off fungi in dependence on the colour of their seed shells

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Abstract

The seed shells of the pine (*Pinus silvestris* L.) contain in dependence on their colour different amounts of the particular fatty acids. Some of the latter are toxic to the fungi causing damping-off of seedlings. The difference in content of toxic fatty acids between the black and the fallow seed shells may explain the mechanism of their different resistance to infection by damping-off fungi.

INTRODUCTION

Seeds of pine and other coniferous trees may have different colours. Generally the seed shells of Scot's pine are black, brown or variegated, less frequently grey or fallow. This refers to well developed seeds, since sterile ones are usually light-coloured. In Poland in an average batch of seeds from an industrial seed extractory there is 57 per cent black seeds, 32 per cent brown ones and 11 per cent fallow ones (Grzywacz and Twaróg, 1977).

The particular trees bear seeds of the same colour (Suszka, 1967). It results, however, from investigations carried out in Sweden on plantations of grafted trees that the colour of the seeds and wings is not only genetically conditioned, but to a high extent dependent on the environment and modified by it (Simak and Gustafsson, 1954). Lighter seeds prevail in dry habitats, dark ones in moist ones (Tcherepnin, 1964: Pichelgas, 1975). The seed colour indicates the quality of seedlings and the future growth of young plantations. At an older age pines bearing black seeds also exhibit more intensive growth, trees with brown seeds are less vigorous, and those with light seeds show the poorest growth (Pichelgas, 1975).

Dark seeds with a higher specific weight are considered to be more resistant to parasitic damping-off fungi (Yakovlev after Gortchakov, 1971; Mańka, 1976). This is also true for some other plants, for instance *Linum usitatissimum* L. (Groth, Comstock and Anderson, 1970). Grzywacz and Twaróg (1977) while examining some characters of pine seeds, in reference to their resistance to damping-off of seedlings, noted that germination energy and capacity are not related to the colour of the seeds. Neither are the 1000-seed weight or the rate of water absorption in the germination process associated with colour. Black seeds have slightly heavier shells as compared with fallow or brown ones, this, however, seems of no consequence for their resistance to infection by damping-off fungi (mechanical resistance). Rosochacka and Grzywacz (1978) found wide differences in the chemical composition of seed shells according to their colour. These differences concerned the content of total nitrogen, the amino acids composition, a number of elements (metals and nonmetals) and phenol compounds. It would seem, therefore, that it is rather chemical conditions that cause the differences in the susceptibility of seeds to infection by damping-off fungi in dependence on their colour.

The natural resistance of seeds to damping-off fungi and the possibilities inherent in the selection of seeds with better properties in this respect give hopes of new methods for preventing this troublesome forest nursery disease. Pine seeds, like most small-dimension seeds, have a high fat content (they belong to the fatty-starch type). A number of fatty acids, particularly the lower ones have fungitoxic properties (Borecki at al., 1965; Torgeson, 1969). The present study was undertaken to establish the fatty acids content in the seed shells in dependence on their colour and determine the fungicidal action of these compounds.

MATERIAL AND METHODS

Seeds of Scot's pine of class I originated from the forest district Góra Śląska (Leszno District) from a pine stand of quality class III, aged 108 years and growing on a dry coniferous forest habitat (Greater Poland--Pomerania natural-forest region). The seeds were divided into 3 groups according to colours — black, brown and fallow. The seed shells were separated from the endosperm and the membrane surrounding it by means of a scalpel and a sectioning needle. They were then analysed for fatty acids content. Seed shells of extreme colours — fallow and black were examined.

Fatty acids were determined chromatographically on a PYE Unicam 104 apparatus (spiral glass column 2.7 m long, diametre 4 mm, filled with 10% PEGA on Diatomite CAW 100/120 mesh, temperature 200°C, carrying gas — argon, detector FID type, attenuation 1.0×10^{-8}—$1.2\times\times10^{-7}$ A). Quantitative interpretation was based on the peak heights product and the corresponding retention distances measured from the solvent peak. From the obtained values the contribution of methyl esters of the particular fatty acids was calculated. The acids were identified against standards.

Samples for chromatographic determination were prepared as follows. The pine seed shells were ground with sand and extracted with ethyl ether in columns (K u b a c k i et al., 1973). The ether was stripped with nitrogen. The fat obtained was transferred to an ampoule, it was saponified with an alcohol KOH solution. The obtained fatty acids were esterified with a solution of thionyl chloride in methanol (Ż ó r a w s k i and K u p r y s z e w s k i, 1971). The esters were separated by addition of n-heptane and a saturated NaCl solution. The n-heptane layer in which the fatty acid esters were present was taken for chromatographic analysis.

The fungitoxic value of some of the fatty acids present in pine seed shells was determined. As test organisms served species of fungi producing damping-off of seedlings such as *Alternaria tenuis* Ness., *Rhizoctonia solani* Kühn (IOLiD SGGW-AR Warszawa). *Fusarium oxysporum* (Schlecht.) Snyd. et Hans. (IOL AR Kraków, HMIPC — k, 26316/1), *Cylidrocarpon didymum* (Hartig) Wollenw. (IOL AR Poznań) Poznań.

The fungitoxic value of the following fatty acids was tested: mirystic, palmitic, oleic, linoleic, linolenic and erucic (Calbiochem, U.S.A.) spectrally pure.

Determination of the fungitoxic value was done by the medium method. Barley malt (from brewer's wort) agar medium pH 5.6 was used. Fatty acids were added to the medium in such amounts as to obtain 2, 1, 0.5, 0.1, 0.05 and 0.01 per cent concentrations. The medium consisted of agar — 20 g, brewer's wort 10° without hops, standardized, produced by the Warsaw Breweries.

As criterion for evaluation was adopted the diametre of the mycelial colony after 8 days of growth (for *C. didymum* after 14 days on account of the slower growth of this species). Each concentration was tested in 5 replications. The fungi were cultured at 25°C, relative air moisture 85 per cent. ED_{50} and ED_{100} were calculated, that is concentrations effectively inhibiting radial mycelium growth in 50 and 100 per cent in relation to its spread on medium without fungicide (in this case fatty acid).

RESULTS AND DISCUSSION

The seed shells contained (independently of their colour) largest quantities of palmitic, oleic, stearic, palmito-oleic and mirystinic acid. In whole pine seeds the main fatty acids are: oleic, linoleic and linolenic acid (Hegnauer, 1962). There are wide differences in the content of fatty acids between the seeds as a whole and their seed shells.

In black pine seed shells 12 fatty acids occurred, whereas in the fallow ones only nine; they did not contain linolenic, eicosonic and erucic acid. The black shells contained almost 9 per cent of erucic acid in reference to all fatty acids present. In the light shells this acid was completely absent.

There were wide differences between the black and fallow seed shells as regards over-all saturated and unsaturated fatty acids content (Table 1). The dark seeds contained in the shells about twice the amount in the fallow shells. In the latter the unsaturated acids had unsymmetrically arranged π bonds.

Table 1

Fatty acids content in differently-coloured seed shells of *Pinus silvestris* L. seeds

Acid	No. of carbon atoms and double bonds	Particular fatty acids content (%) in over-all amount detected	
		seed shell colour	
		fallow	black
lauric	C_{12}	2.23	2.91
mirystic	C_{14}	9.29	6.09
palmitic	C_{16}	35.24	26.32
stearic	C_{18}	16.75	9.06
arachidic	C_{20}	4.60	1.69
behenic	C_{22}	6.33	1.47
total saturated fatty acids		74.44	47.54
palmito-oleic	C_{16}^{1}	14.55	9.54
oleic	C_{18}^{1}	7.26	22.08
linoleic	C_{18}^{2}	3.75	10.39
linolenic	C_{18}^{3}	—	0.75
eicosonic	C_{20}^{1}	—	0.95
erucic	C_{22}^{1}	—	8.75
total unsaturated fatty acids		25.56	52.46

Mean values from 3 samples

The investigations on the fungitoxic value of fatty acids revealed that only erucic acid was toxic to all the tested species of fungi. In a 2 per cent concentration it inhibited radial growth of *Cylidrocarpon* *didymum* in 100.0, *Alternaria tenuis* in 43.6, *Fusarium oxysporum* in 37,4 and *Rhizoctonia solani* in 24.8 per cent. Linoleic and linolenic acid were but slightly toxic to two species of fungi in concentrations of 1-2 per cent. With the exception of erucic acid, other fatty acids showed towards the tested fungi ED_{50} and ED_{100} in concentrations exceeding 2 per cent.

Table 2

Growth of damping-off fungi on medium containing some of the fatty acids revealed in the shells of ***Pinus silvestris*** L.

Acid	Species of damping-off fungus			
	Fusarium oxysporum	*Cylindro-carpon didymum*	*Rhizoctonia solani*	*Alternaria tenuis*
mirystic	○	+	○	○
palmitic	+	+	○	+
oleic	+	+	+	+
linoleic	○	—	—	+
linolenic	—	+	—	○
erucic	—	—	—	—

— inhibition, + stimulation, ○ influence on mycelium growth in the concentrations used not noted

Mirystic, palmitic and oleic acid either stimulated slightly growth, most distinctly in 1 and 2 per cent concentrations or they had no effect of mycelium growth in the concentrations tested. A number of fatty acids may be a source of carbon for many fungal species (P e r l m a n, 1965), hence the slight stimulation of mycelium growth after adding fatty acids (nontoxic ones) to the medium.

It is considered (T o r g e s o n, 1969) that maximum fungitoxic activity of fatty acids is exhibited by those with 11 carbon atoms in the chain. Particularly active are the acids with an odd number of carbons. Acids with branched chains show a lower activity and the introduction of unsaturated bonds increases the toxicity of the acid towards fungi and bacteria.

Better known and widely applied as fungicide is propionic acid (B o r e c k i et al., 1965; T o r g e s o n, 1969). A number of preparations for food, textiles, paper and leather, moist seeds and wood chips stored in piles (in cellulose and paper industry) conservation are produced on the basis of propionic acid.

Black seeds containing in their shells much more unsaturated fatty acids and a large amount of the particularly toxic erucic acid are at the same time more resistant to infection with damping-off fungi causing disease of seeds and seedlings.

The different content of toxic fatty acids seems to be the cause deciding of the resistance of pine seeds with different coloured shells to the parasitic damping-off of seedlings. This type of resistance is particularly important while the seeds lie in the soil after sowing and in the first phases of emergence (protection against preemergence damping-off).

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Próba wyjaśnienia roli kwasów tłuszczowych w odporności nasion Pinus silvestris L. na porażenie przez grzyby zgorzelowe w zależności od barwy ich okryw nasiennych

Streszczenie

Nasiona sosny w zależności od zabarwienia okryw wykazują różną odporność na porażenie przez grzyby powodujące zgorzel siewek (groźną i często występującą chorobę w szkółkach leśnych). Bardziej odporne są nasiona czarne, najmniej płowe. Badania składu chemicznego okryw nasiennych wykazały między innymi duże różnice w zawartości kwasów tłuszczowych w zależności od zabarwienia. W okrywach czarnych wykryto 12, a w płowych tylko 9 kwasów tłuszczowych. Okrywy czarno zabarwione zbadanych nasion posiadały znacznie więcej kwasów nienasyconych, w tym szczególnie dużo kwasu erukowego.

Badania wartości grzybobójczej 6 z wykrytych w okrywach kwasów wykazały, że kwas erukowy, a także częściowo linolowy i linolenowy są toksyczne dla testowych gatunków grzybów powodujących zgorzel siewek sosny: *Alternaria tenuis*, *Cylindrocarpon didymum*, *Fusarium oxysporum* i *Rhizoctonia solani*.

W różnej zawartości toksycznych kwasów tłuszczowych można się dopatrywać jednego z mechanizmów odporności nasion sosny na zgorzel w zależności od zabarwienia okryw nasiennych.