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The lichen genus *Pertusaria* in Poland II. Secondary chemistry of *P. flavida*

Anna Zduńczyk[1], Magdalena Dziedzic[2] & Martin Kukwa[2]

[1]Department of Botany and Genetics, Pomeranian Academy in Słupsk, Arciszewskiego 22B, PL–76–200 Słupsk, Poland.
E-mail: azdunczyk@apsl.edu.pl

[2]Department of Plant Taxonomy and Nature Protection, Gdańsk University,
Al. Legionów 9, PL–80–441 Gdańsk, Poland.
E-mails: madzia2lena@wp.pl, dokmak@ug.edu.pl

Abstract: This paper presents a study on a chemical variation of *Pertusaria flavida* in Poland. Six chemotypes were determined in the studied material, of which chemotypes IV, V and VI were never reported before. All of them produce thiophaninic acid as the diagnostic metabolite for the species and this is the only major substance in chemotype I. Chemotype II contains in addition also 2'-O-methylperlatolic acid (± confluentic acid), whereas chemotype III stictic acid complex (± minor or trace amounts of norstictic acid). Chemotype IV is characterized by the presence of 2'-O-methylperlatolic acid and stictic acid complex (± norstictic acid in minor to trace amounts). Chemotype V produce norstictic acid as a major additional substance and chemotype VI contains 2'-O-methylperlatolic acid together with norstictic acid. Chemotype II was the most common chemotype (c.75 % of specimens) in studied material. Distribution maps are presented for all chemotypes.

Kokkuvõte: Perekond lumisamblik (*Pertusaria*) Poolas II. *P. flavida* sekundaarsed samblikuained

Esitatakse uurimus samblikuliigi *Pertusaria flavida* keemilisest varieeruvust Poolas. Uuritud materjalis määrati kuus kemotüüpi, neist IV, V ja VI kemotüüpi ei olnud varem teada. Kõikides kemotüüpides esineb tiofaniinhape kui liigi diagnostiline aine, see on ka kemotüübi I ainus põhiaine. Kemotüüp II sisaldab lisaks 2'-O-metüülperlatoolhapet, ja kemotüüp III stikthappe kompleksi. Kemotüüp IV iseloomustab nii 2'-O-metüülperlatoolhappe kui stikthappe kompleksi esinemine. Kemotüüp V sisaldab norstikthapet, ning kemotüüp VI nii 2'-O-metüülperlatoolhapet kui norstikthapet. Kemotüüp II esineb kõige sagedamini (u 75% uuritud isenditest). Kõikide kemotüüpide teadaoleva esinemise kohta Poolas on koostatud levikukaardid.

INTRODUCTION

Pertusaria flavida (DC.) J. R. Laundon is a lichen species with thin or quite thick, pale or bright sulphur yellow, yellow-brown to yellow-green-grey thallus and numerous, globose to cylindrical-clavate isidia, concoloured with the thallus. Apothecia are very rarely present (not observed in Polish material), perithecia-like with 4(–8)-spored asci and large, 60–100 × 25–40 µm ascospores. The species produces thiophaninic acid as the diagnostic secondary metabolite, but also other substances can be produced as well (Hanko, 1983; Tønsberg, 1992; Chambers et al., 2009).

The species occurs in deciduous forests, open woodlands and waysides, usually on broad-leaved trees, especially on *Quercus* spp. and *Fagus sylvatica*, but also rarely on *Abies alba*, *Acer* spp., *Betula* spp. and others (Fałtynowicz, 2003; Chambers et al., 2009). In Poland *P. flavida* is frequently reported in northern part of the country, but in other regions it is rare (Fałtynowicz, 2003).

Pertusaria flavida is morphologically rather uniform, but in terms of chemistry it shows some variation; however, it was rarely a subject of detailed studies and not much has been known on the secondary metabolite composition of the species in some regions, especially in Central and Eastern Europe (see, Leuckert et al., 1969; Hanko, 1983; Tønsberg, 1992). In this paper we present the first results of such investigations in Poland. This is the second paper in the series dealing with the species of the genus *Pertusaria* DC. in Poland (see Oset & Kukwa, 2010).

MATERIALS AND METHODS

The material was studied from following herbaria: UGDA (37 specimens), KRAM (9), KTC (31), SLTC (46) and WA (2); altogether 125 specimens were examined. The chemistry was investigated with the aid of thin layer chromatography (TLC) in solvents A and C according to

Orange et al. (2001). All examined localities are mapped according to the modified ATPOL grid square system (Cieśliński & Fałtynowicz, 1993; see also Kukwa et al., 2002).

RESULTS

So far the three chemotypes of *Pertusaria flavida* were known:
(I) with thiophaninic acid and a trace of O-methylmonochloronorlichexanthone;
(II) with thiophaninic acid, a trace of O-methylmonochloronorlichexanthone, 2'-O-methylperlatolic and ± confluentic acids;
(III) thiophaninic acid, a trace of O-methylmonochloronorlichexanthone, and stictic acid complex with stictic acid as major substance with trace to moderate amounts of constictic, cryptostictic, menegazziaic and norstictic acids (Hanko, 1983; Tønsberg, 1992).

All those chemotypes were found in Polish material, but in our study we could not determine O-methylmonochloronorlichexanthone reported by Tønsberg (1992). We found, however, one to several unidentified xanthones, of which one may represent the mentioned substance.

As result of our studies, three new chemotypes were additionally found. They are characterized by the following substances:
(IV) thiophaninic acid, unknown xanthone(s) related to thiophaninic acid, 2'-O-methylperlatolic acid, stictic acid complex with stictic acid as major substance with trace to moderate amounts of constictic, cryptostictic, menegazziaic and norstictic acids;
(V) thiophaninic acid, unknown xanthone(s) related to thiophaninic acid, norstictic acid with trace or minor amounts of connorstictic acid;
(VI) thiophaninic acid, unknown xanthone(s) related to thiophaninic acid, 2'-O-methylperlatolic and norstictic acids with trace or minor amounts of connorstictic acid.

In chemotype IV and VI we did not found confluentic acid, which was recorded together with 2'-O-methylperlatolic acid in chemotype II. This substance could be found in those two chemotypes in the future when more material will be available. The summary of chemical content of all chemotypes is presented in Table 1.

Tønsberg (1992) reported that chemotype II was the commonest in Norway (73% of specimens). This is also the case for Poland; this chemical race was found in 97 specimens (c. 75% of samples). Chemotype I was the second one (21 specimens, 16.8% of specimens). Others were extremely rare, and chemotypes III and V were represented by one specimen each, IV by three specimens and VI by two samples. Due to the scarce material of chemotypes III–VI it was impossible to compare the morphological variation between them and the common ones, but no morphological distinctness was observed between chemotypes I and II; they show more or less the same variability in the size, shape and development of isidia. That corroborates, in our opinion, that they all belong to one chemically and morphologically variable taxon.

The distribution maps of all chemotypes in Poland were compiled (Fig. 1). No special distributional pattern was observed for any of them. As the chemotypes are unequally frequent, it was impossible to compare their substrate preferences, but in general *Quercus* spp. and *Fagus sylvatica* were the most preferred trees (except for chemotypes V and VI, which were found exclusively on *Fraxinus excelsior* and *Carpinus betulus*).

Table 1. Chemical composition of chemotypes of *Pertusaria flavida* in Poland (traces of xanthones not included); + – substance always present; ± – substance sometimes absent; trace – substance found in trace amount only

Substances	I	II	III	IV	V	VI
thiophaninic acid	+	+	+	+	+	+
2'-O-methylperlatolic acid		+		+		+
confluentic acid		±				
stictic acid complex			+	+		
norstictic acid			trace	trace	+	+

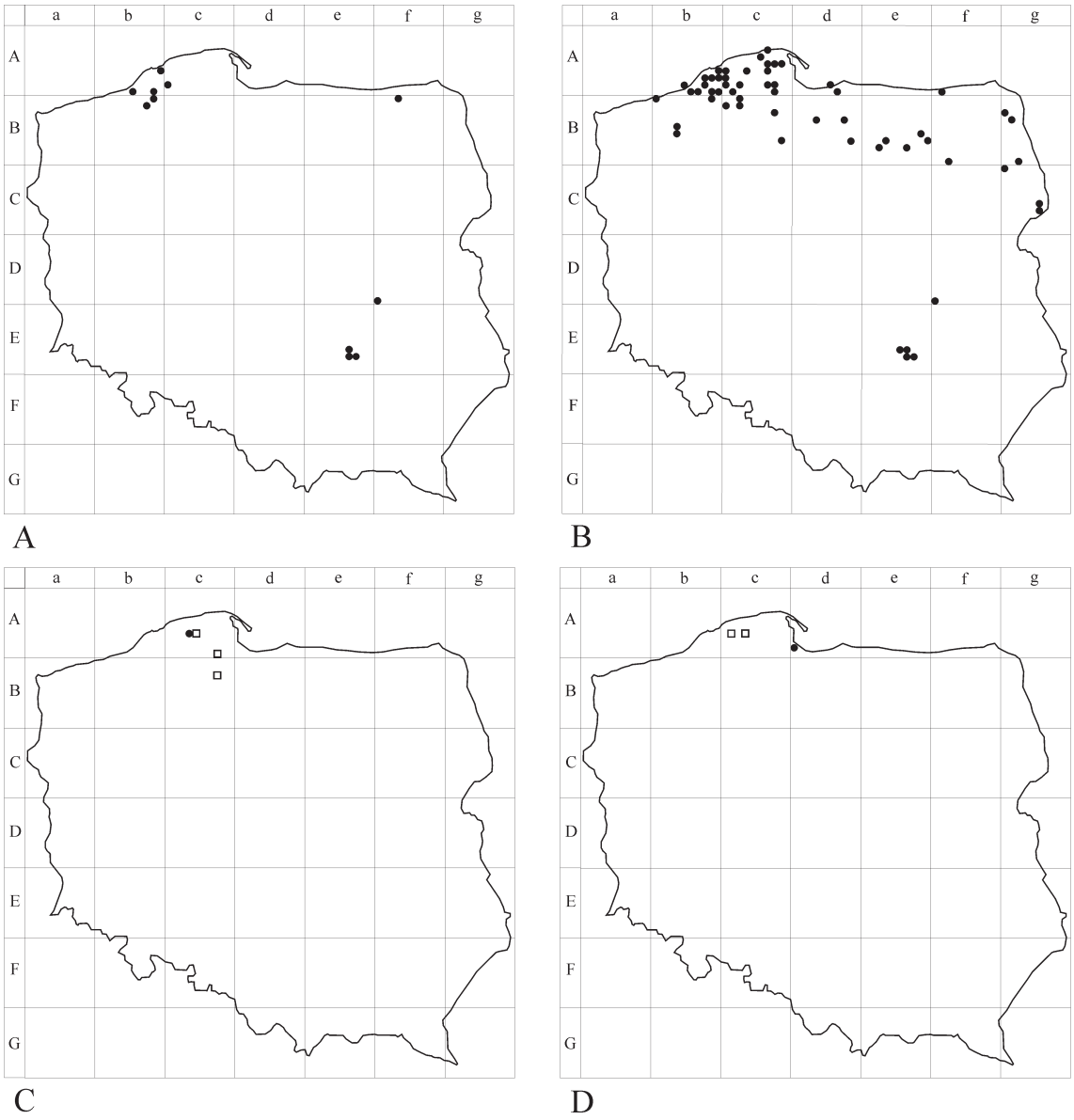

Fig. 1. Distribution of chemotypes of *Pertusaria flavida* in Poland: A – chemotype I; B – chemotype II; C – chemotypes III (●) and IV (□); D – chemotypes V (●) and VI (□).

Specimens examined.

Chemotype I (selected). [Ab–69] – Równina Sławieńska, forest section No. 291, NW Charnowo Słupskie railway station, 54°33'01"N, 16°55'20"E, on *Quercus* sp., 14.06.1976, leg. I. Izydorek (SLTC); **[Ac–66]** – Pojezierze Kaszubskie, vicinity of Paraszyno village, forest section No. 150, on *Fagus sylvatica*, 17.11.1966, leg. T. Sulma (UGDA-L–18084); **[Ac–80]** – Równina Sławieńska, Leśny Dwór forest inspectorate, forest section No. 597a, 54°22'N, 17°09'E, on *Quercus robur*, 18.11.2002, leg. A. Krupska, I. Izydorek (SLTC); **[Ac–97]** – Pojezierze Kaszubskie, Zamkowa Góra hill, vicinity of Kartuzy

town, on *Tilia cordata*, 02.09.1961, leg. T. Sulma (UGDA-L–18082); **[Bb–17]** – Wysoczyzna Polanowska, Kręg forest district, forest section No. 87, 54°07'N, 16°41'E, on *Fagus sylvatica*, 26.08.1977, leg. I. Izydorek (SLTC); **[Bf–03]** – Pojezierze Ełckie, Puszcza Borecka, Diabla Góra nature reserve, 54°08'00"N, 22°09'00"E, forest section No. 4, on *Quercus* sp., 01.08.1988, leg. J. Zielińska (WA); **[Df–90]** – Równina Kozienicka, Zagożdżon nature reserve, on *Quercus* sp., 23.11.1969, leg. S. Cieśliński (KTC); **[Ee–66]** – Góry Świętokrzyskie, Świętokrzyski National Park, S slopes of Góra Miejska Mt., c. 50°55'00"N, 20°56'00"E, forest section No. 15, on *Quercus* sp., 09.06.1982, leg. S. Cieśliński (KTC); **[Ee–76]** – Góry Świętokrzyskie, Świętokrzyski National Park, Agata boulder scree, 50°52'39"N, 20°59'01"E, on *Fagus sylvatica*, 27.11.1982, leg. S. Cieśliński (KTC). **Chemotype II (selected). [Ab–84]** – Pobrzeże Koszalińskie, Łazy (54°18'28"N, 16°11'47"E), on *Fraxinus excelsior*, 13.07.1974, leg. J. Nowak (KRAM-L); **[Ab–98]** – Równina Sławieńska, Janiewice village, 54°16'21"N, 16°46'06"E, on *Fagus sylvatica*, 26.08.1987, leg. W Fałtynowicz (KRAM-L, UGDA-L–3611); **[Ac–57]** – Pojezierze Kaszubskie, Zamostne village, on the way to Góra village, 54°38'N, 18°07'E, on *Fagus sylvatica*, 04.10.1967, leg. T. Sulma (UGDA-L–18088); **[Ac–70]** – Wysoczyzna Damnicka, E of Krzywań village, 54°24'45"N, 17°08'53"E, on *Quercus rubra*, 17.06.1991, leg. E. Szeflińska, I. Izydorek (SLTC); **[Af–91]** – Kraina Węgorapy, Dąbrówka Nowa village, 54°17'00"N, 21°50'00"E, on *Quercus* sp., 02.09.1988, leg. S. Cieśliński (KTC); **[Bc–02]** – Wysoczyzna Polanowska, Chomice forest district, N of Kępice village, 54°09'N, 17°26'E, on *Fagus sylvatica*, 06.08.1976, leg. I. Izydorek (SLTC); **[Bc–27]** – Pojezierze Kaszubskie, Orle nad Jeziorem Dużym nature reserve, c. 54°02'35"N, 18°13'09"E, on *Quercus* sp., 21.08.1962, leg. T. Sulma (UGDA-L–18033); **[Bc–68]** – Bory Tucholskie, Stara Rzeka village, 53°39'00"N, 18°17'46"E, on *Quercus* sp., 13.06.2004, leg. M. Kukwa 3297 (UGDA-L–11775); **[Cg–65]** – Równina Bielska, Białowieża Primeval Forest, forest section no. 425, Żebra Żubra trail, 52°42'17"N, 23°48'03", on *Carpinus betulus*, 03.05.2004, leg. M. Kukwa 3260 (UGDA-L–12721); **[Ee–77]** – Góry Świętokrzyskie, Świętokrzyski National Park, Łysa Góra Mt., 50°51'42"N, 21°02'20"E), on *Quercus* sp., 21.07.1957, leg. B. Halicz, S. Kuziel (KTC). **Chemotype III. [Ac–63]** – Wysoczyzna Damnicka, S o Podole Wielkie village, forest section No. 114, 54°34'N, 17°29'E, on *Quercus* sp., 12.11.1991, leg. D. Gnacińska (SLTC); **Chemotype IV. [Ac–64]** – Pradolina Łeby i Redy, Lębork town, 54°32'10"N, 17°44'51"E, on *Fagus sylvatica*, 12.06.1964, leg. T. Sulma (UGDA-L–13984); **[Ac–97]** – Pojezierze Kaszubskie, Zamkowa Góra hill, vicinity of Kartuzy town, forest, on *Fagus sylvatica*, 02.10.1964, leg. T. Sulma (UGDA-L–18055); **[Bc–27]** – Pojezierze Kaszubskie, Orle nad Jeziorem Dużym nature reserve, c. 54°02'35"N, 18°13'09"E, on *Quercus* sp., 21.08.1962, leg. T. Sulma (UGDA-L–18085); **Chemotype V. [Ad–80]** – Pobrzeże Kaszubskie, Gdańsk Oliwa town, c. 54°21'11"N, 18°37'52"E, dolina Ewy valley, forest section No. 92, on *Fagus sylvatica*, 06.07.1984, leg. W. Fałtynowicz (UGDA-L–1506). **Chemotype VI. [Ac–61]** – Wysoczyzna Damnicka, Damnica forest inspectorate, forest section No. 159, by Łupawa river, 54°30'N, 17°16'E, on *Fraxinus excelsior*, 22.10.1992, leg. A. Kozłowska, I. Izydorek (SLTC); **[Ac–63]** – Wysoczyzna Damnicka, S o Podole Wielkie village, forest section No. 115, 54°34'N, 17°29'E, on *Carpinus betulus*, 12.11.1991, leg. D. Gnacińska (SLTC).

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