

Susceptibility of Polish provenances and families of pedunculate oak (*Quercus robur* L.) to colonisation by *Phytophthora cambivora*

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Abstract. *Phytophthora cambivora* (Straminipila, Oomycota) causes root rot and stem canker on several deciduous tree species in Europe. However, very little is known about the variation in susceptibility to *P. cambivora* colonisation among provenances and families of pedunculate oak (*Quercus robur* L.). We studied variation in susceptibility of one French and 16 Polish provenances, representing 62 families. Samples were taken from three test plots located in the Brzesko Forest District. Oak susceptibility to *P. cambivora* was assessed by measuring lesion length following inoculation of excised shoots with two isolates of *P. cambivora*. There was significant variability in susceptibility among the 17 provenances tested. The highest susceptibility to *P. cambivora* was apparent in several provenances including Tronçais, Zaporowo, Runowo, Opole and Krotoszyn; while the most resistant provenances originated from Chojnów, Siedlce, Płock, Krotoszyn-90 and Wioska. There was also significant within-provenances variation in susceptibility to *P. cambivora*.

Key words: defense, family, *Phytophthora cambivora*, provenances, resistance, shoot, susceptibility

1. Introduction

Declining of pedunculate oak (*Quercus robur* L.) has been observed in Europe for over two hundred years. This phenomenon has a mass character with periods of higher and smaller disease intensity (Thomas et al. 2002). In Poland the symptoms of oak stands decline were repeatedly registered in Płyta Krotoszyńska (area nearby Krotoszyn) area and also in western and northern country area (Oszako et al. 2009). Intensification of trees' decline is also observed in present times (Tarasiuk, Szczepkowski 2006). A large area of this phenomenon occurrence, different habitats, different age and trees' origin indicate that the factors responsible for this disease process are both abiotic and biotic. The declining process begins often with water deficiency during the growing period, frost damages or spring frosts, and later with damages caused by insects and pathogenic fungi. The process then leads to defoliation

or frost damages, consequently leading to weakening and secretion of trees in the stand (Thomas et al. 2002; Oszako 2007).

Serious pathogens, which contribute to the damage of oaks' root system, are organisms from *Phytophthora* genus. Those fungus-like Oomycetes can be a reason for the formation of very characteristic disease symptoms accompanying oak's decline. And so, a common symptom related to trees' infection by *Phytophthora* spp. are necrosis combined with water soaked on trunks, especially in their lower parts. Over a period of time, necrosis of bark and cambium can occupy a significant trunk circuit which causes decline and leaves turning yellow in tree crown; and in the final stage of disease, even decay of whole tree (Jung et al. 2000; Vettraino et al. 2002; Jönsson et al. 2003). In Poland, the importance and incidence of organism from *Phytophthora* genus in forests are still poorly examined. In oak stands so far was reported the occurrence of *Phytophthora uliginosa*

T. Jung & E.M. Hansen (Jung et al. 2000), *P. quercina* T. Jung, *P. hedraiaandra* de Cock & E.M. Hansen (Cordier et al. 2009), *P. cactorum* (Lebert & Cohn) J. Schröt., *P. pseudosyringae* T. Jung & Delatour, *P. plurivora* T. Jung & T.I. Burgess (Olejarski et al. 2012) i *P. cambivora* (Petri) Buisman (Stępniewska et al. 2008). The last mentioned species seems to be, next to *P. plurivora* and *P. quercina* – a pathogen most frequently infecting the fine roots of pedunculate oak in Poland (Olejarski et al. 2012; Stępniewska, Jankowiak, unpublished). This species is a common deciduous tree pathogen in Europe (Brasier 2000; Jung et al. 2005), and also on pedunculate oak in France (Camy et al. 2003), Germany (Jung et al. 2000), Italy (Vettriano et al. 2002) and Sweden (Jönsson et al. 2003). Relatively high aggressiveness of *P. cambivora* towards various species of deciduous trees was confirmed in numerous infectious experiments (Brasier, Kirk 2001; Saavedra et al. 2007; Balci et al. 2008).

Studies over within-species variability of pedunculate oak were initiated by Kienitz in Germany (Kleinschmit 1993), however not earlier than the last 20 years significant research development in this range could be observed (Jensen 2000; Baliuckas, Pliura 2003; Bogdan et al. 2004; Barzdajn 2009; Banach 2011). In the mentioned studies, a significant variation was noted in the examined oak populations with reference to survivability, trunk form, phenology or the content of organic compounds in the leaves. The object of interest of researchers was also susceptibility of different oak origins to insect pests (Crawley, Akhteruzzman 1988; Skrzypczyńska 2001; Banach, Lenowiecki 2011). So far, there have been no investigations evaluating the resistance of pedunculate oak provenances to infections by pathogenic fungi, although already in the 60s Leibundgut (1969) had analysed the susceptibility of different oaks provenances to *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. In Poland, preliminary studies in this range were performed also by Zwaduch (2005), who indicated different degree of affected pedunculate oak's leaves by this pathogen within the examined oak populations. Lately Szczepkowski (2010) also examined resistance of pedunculate oak's wood, collected from trees of various health conditions, derived from 7 polish populations, on decomposition caused by *Coniophora puteana* (Schumach.) P. Karst., *Laetiporus sulphureus* (Bull.) Murrill and *Trametes versicolor* (L.) Lloyd. For other forest trees, considerable amount of research in this range was performed on elms in reference to *Ophiostoma novo-ulmi* Brasier (Santini et al. 2005) and on pines and spruces towards *Gremmeniella*

abietina (Lagerb.) M. Morelet (Roll-Hansen 1971; Hansson 1998), and also inter alia to *Cenangium ferruginosum* Fr. (Kuzmina, Kuzmin 2008). Similar research works were performed for *Mycosphaerella pini* Rostr. ex Munk (Eldridge, Dowden 1980), *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton (Smith et al. 2002), *Fusarium circinatum* Nirenberg & O'Donnell (Hodge, Dvorak 2007) and *Mycosphaerella* spp. (Carnegie et al. 2004). Relatively many studies were dedicated to genetic variability of different populations of *Castanea sativa* Mill. to infections by *P. cambivora* (Miranda-Fontania et al. 2005; Robin et al. 2006). In Poland so far resistance of polish larch origins was examined to infections of *Lachnellula willkommii* (Hartig) Dennis (Kulej 2006) and health condition of spruces was estimated, which belong to 1100 provenances representing different regions of Europe on experimental area in Krynica (Zółciak et al. 2009). In quoted studies, in many cases, a large susceptibility variation was found of different trees populations to infections by dangerous plant pathogens.

Presently various methods are used allowing evaluation of trees' susceptibility to infection by root pathogens. It may be a shoot's and root's inoculation of living plants or a method of so-called "excised shoots" (Miranda-Fontania et al. 2005). In both methods, trees' susceptibility is estimated by the length of necrosis produced after artificial inoculation of plants with isolate of pathogenic organism. The method of "excised shoots" is a most frequently used method for estimating trees' susceptibility to infection by pathogens; however, results obtained by this method may not reflect fully the situation occurring on living trees. Lately however, Robin et al. (2006) showed that inoculation of shoots collected from trees with necrosis measurement is an appropriate method for estimating trees' susceptibility to infection caused by root pathogens.

The aim of this research was the estimation of susceptibility of different provenances and families of pedunculate oak to colonisation by oomycete *Phytophthora cambivora*. The authors with their studies wanted to answer the following questions:

- Is there an inter- and inner-population variability of pedunculate oak in reference to infections by *P. cambivora*?
- Is it possible to select resistant and susceptible provenances and families of pedunculate oak occurring in Poland?
- Is there a difference in resistance of different provenances of pedunculate oak depending on *P. cambivora* isolate properties?

2. Material and methods

The samples of shoots were collected in October 2010 from randomly chosen oaks growing on tree provenance-family experimental plots (Chrostowa I and II and Jodłówka), established in years 1996-2000 on the area of Brzesko Forest District (Banach 2010). Within each family, shoots were collected from two trees without any visible disease symptoms. From each randomly selected tree were cut 20 two-year old shoots around 50 cm long with an average thickness of 0,78 cm (from 0,40 cm to 1,22 cm). Next, the shoots were put into a paper bag, which included description of the origin and family number. The whole material was moved into plastic bags and transported to laboratory. Subsequently the samples of shoots were put into freezer for 24 hours. In total, 2480 shoots were collected from 16 Polish and 1 French pedunculate oak's provenance. The collected shoots came from oaks which belonged to 62 families (from 3-5 families per provenance) (Fig. 1, Table 1).

In the experiment two isolates of oomycete *P. cambivora* were used: 528.08 and 303.07, which were

isolated from soil collected from oak stand by Stepniewska and Jankowiak. Those isolates were identified on the base of morphologic features and comparison of ITS rDNA sequences (ITS1–5.8 S–ITS2) with reference sequences obtained from NCBI base (Jankowiak et Stepniewska, unpublished data). First isolate was obtained in 2008 from Babice (Rudy Raciborskie Forest District), and the second one in 2007 from Krzyszkowice Forest (around Wieliczka). Cultures were obtained from fungal culture collection of the Laboratory of Department of Forest Pathology, Agricultural University of Cracow, Poland.

Isolates grew for 14 days on medium composed of: juice V8 (vegetable juice) – 200 ml, CaCO_3 – 3 g, agar – 15 g, distilled water – 800 ml. Preliminary pathogenicity test of mentioned isolates revealed that isolate 528.08 showed a much higher aggressiveness degree to pedunculate oak than isolate 303.07. In an experiment conducted on 2-year old pedunculate oak seedlings, only isolate 528.08 caused declining of 2-year old seedling. This isolate also generated much greater necrosis on seedling stems than isolate 303.07 (Jankowiak, Stepniewska, unpublished).

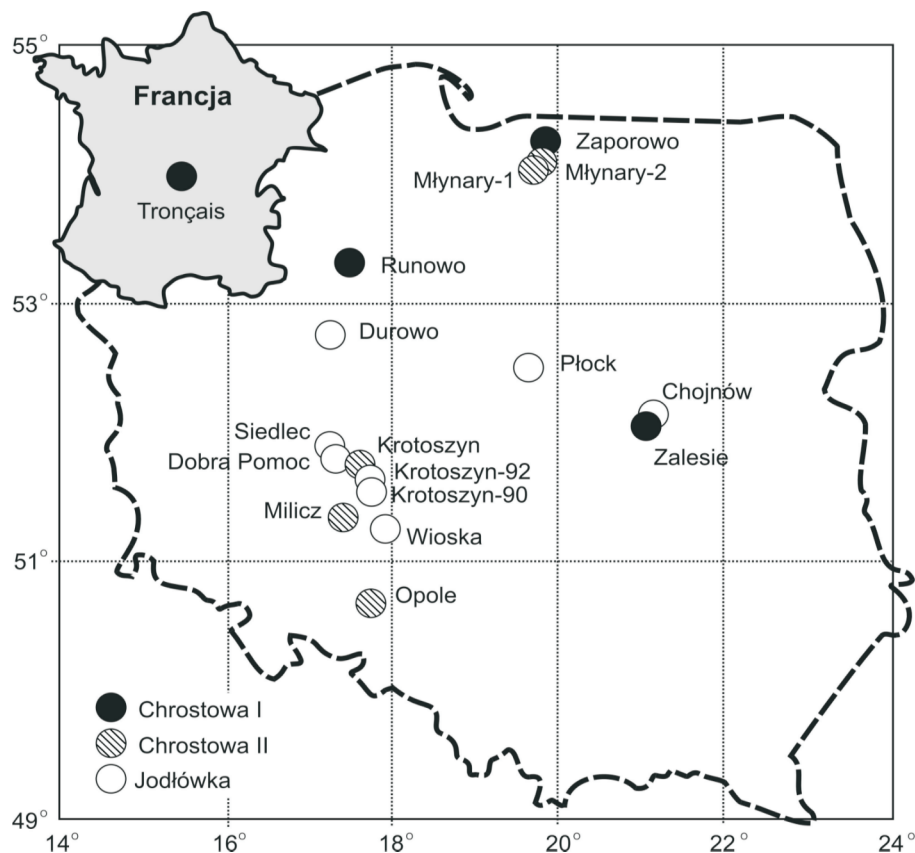


Figure 1. Location of pedunculate oak provenances used in the test

Table 1. Provenances and families of pedunculate oak used in the investigations

Experimental plot	Provenance (Forest district)	Forest range	Georg. coordinates		Family No
			latitude	longitude	
Chrostowa I	Zalesie (Chojnów)	Zalesie Dolne	52° 01'	21° 01'	203, 206, 210, 212, 222
	Zaporowo	Kurowo	54° 14'	19° 45'	236, 237, 238, 247
	Runowo	Dąbie	53° 19'	17° 27'	257, 259, 280
	Tronçais	–	46° 00'	02° 00'	283, 290, 295, 296, 299
Chrostowa II	Młynary-1	Kisielew	54° 01'	19° 40'	36, 38, 40, 41, 66
	Młynary-2	Słobity	54° 06'	19° 43'	28, 43, 48, 50, 86
	Opole	Narok	50° 44'	17° 47'	9, 13, 14
	Milicz	Kaszowo	51° 30'	17° 20'	54, 55, 56, 59
	Krotoszyn	Smoszew	51° 40'	17° 30'	88, 91, 92, 95
Jodłówka	Chojnów	Chojnów	52° 03'	21° 03'	123, 153, 176
	Dobra Pomoc	Dobra Pomoc	51° 49'	17° 08'	65, 162, 144
	Durowo	Dębina	52° 48'	17° 08'	19, 48, 151
	Krotoszyn-90	Borowina	51° 39'	17° 35'	86, 90, 143
	Krotoszyn-92	Jelonek	51° 46'	17° 35'	18, 63, 141
	Płock	Brwilno	52° 36'	19° 37'	35, 83, 177
	Siedlec	Siedlec	51° 50'	17° 07'	80, 81, 109
	Wioska	Wioska	51° 21'	17° 42'	21, 87, 127

Quercus robur “excised shoots” inoculation procedure with *P. cambivora* isolates looked as follows. Collected branches after removing them from freezer were disinfected using cotton wool saturated with 96% ethyl alcohol. After drying, shoots were cut for sections 30 cm long. With the use of sterile scalpel from the middle part of shoot, a bark was taken on 0,5 cm section, revealing cambium. Into this wound with the use of sterile inoculating loops, the inoculum disc (4 mm diameter) was placed. The inoculum was taken from the 14-day-old *P. cambivora* culture. Ten shoots were inoculated with isolates representing each family. After placing the inoculum in wound, each artificially infected fragment of shoot was covered up tightly with a parafilm strip and placed into a 300 ml flask with distilled water. Similar procedure was conducted with control with one difference, that inoculum was replaced with clean V8 medium. Flasks with shoots were placed such that they could be exposed to day light activity. Altogether 1860 shoots of pedunculate oak were inoculated.

After 5 days from shoots' inoculation, a size of necrosis was measured. From shoots a parafilm was unwind and then a periderm was removed gently with sterile scalpel. The length of necrosis was measured parallel to the shoot's axis, and next, from each shoot a re-isolation of *P. cambivora* was made. For this purpose from each inoculation point one fragment of discoloured cortical parenchyma was collected with sterile scalpel

(4 × 4 mm) and transferred to Petri dishes with V8 medium enriched with pimaricin (5 mg·l⁻¹), ampicillin (250 mg·l⁻¹), rifampicin (10 mg·l⁻¹), pentachloronitrobenzene – PCNB (100 mg·l⁻¹) and hymexazol (50 mg·l⁻¹).

On the bases of received necrosis measurements, statistical analysis was made using considered as very weakly pathogens of NIR test – based on marking the smallest significant differences. Analysis was made in program ‘Statistica® 9.0’ (polish version, StatSoft, Tulsa, USA).

To define the influence of origin and family on diversity of necrosis' length, a hierarchical variance analysis was made on the basis of the following formula (Żuk 1989):

$$y_{kmn} = \mu + P_k + F_{m(k)} + E_{n(km)}$$

where:

y_{kmn} – phenotypic value of n -th individual in m -th family and k -th provenance,

μ – general average,

P_k – effect of k -th provenance,

$F_{m(k)}$ – effect of m -th family in k -th provenance,

$E_{n(km)}$ – effect of n -th individual in m -th family in k -th provenance (error).

Also a Pearson's correlation coefficient was calculated on the origin and family level between

the average necrosis length caused by both used *P.cambivora* isolates.

Due to lack of normal distribution and homogeneity of variance before performing statistical analysis, measurement data have been subjected to logarithm.

3. Results

After 5 days of the experiment, the artificially infected oak's shoots were well visible – dark discolorations localised in cortical parenchyma and phloem. Average necrosis length caused by isolate 528.08 was 1,58 cm, and 0,50 cm by isolate 307.07. On control shoots a poor response was noticed for introduced inoculum and a lack of necrosis, except for Chojnów origin (0,01cm), Wioska (0,04 cm) and Krotoszyn-92 (0,04 cm) (Table 2).

Hierarchical variance analysis for origins and families in origin showed, that with significance level $p \leq 0,01$ a size of necrosis created by two isolates of *P. cambivora* differed on origin and family level (Table 3).

Correlation between average necrosis length caused by both isolates used in studies was high and amounted to 0,73 for origins and 0,75 for families which indicates a large comparability of their negative influence on analysed oaks, regardless of isolate origin.

3.1. Provenance variation

Large susceptibility differences were proved of various pedunculate oak's provenance to *P. cambivora* colonisation. For isolate 528.08, relatively small necrosis occurred on oak shoots which belong to the following populations: Siedlec, Durowo, Dobra Pomoc, Chojnów, Młynary-1, Młynary-2, Milicz, Krotoszyn-90, Krotoszyn-92 and Płock. The largest average necrosis was caused by isolate 528.08 on shoots from provenance Tronçais, Runowo, Opole, Krotoszyn and Zalesie (Fig. 2).

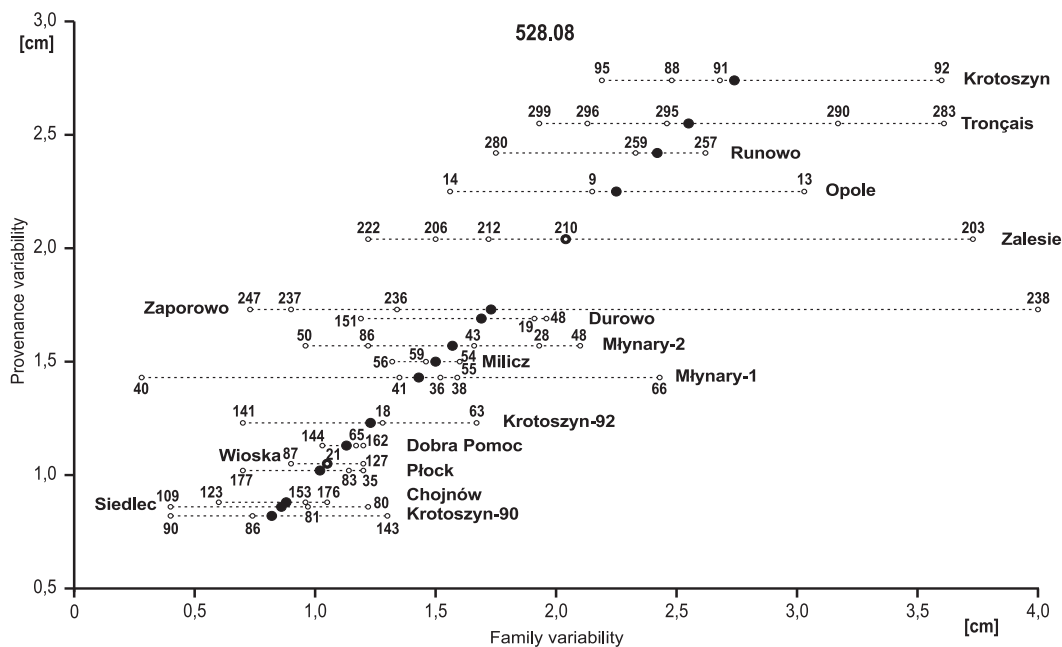
In contrast to isolate 528.08, isolate 303.07 caused much smaller necrosis on shoots. The biggest average necrosis was observed in case of populations from Tronçais, Runowo, Zaporowo; smallest one's in case of oaks from Krotoszyn-90, Milicz and Chojnów. Additionally, much smaller differences between oak populations were observed than in case of isolate 528.08, because as many as 12 origins had necrosis of similar size. They were: Krotoszyn-90, Siedlec, Chojnów, Płock, Wioska, Dobra Pomoc, Krotoszyn-92, Młynary-1, Milicz, Młynary-2 and Durowo (Fig. 3).

Table 2. Average length of the necrosis (cm) for individual provenances of *Q. robur* (a–i – homogeneous groups, NIR test, $p \leq 0,05$)

Provenance	Experimental plot	Isolate				Reference test
		528.08		303.07		
		mean	rank	mean	rank	
Krotoszyn-90	Jodłówka	0,82 ^a	1	0,13 ^a	1	0,00
Siedlec		0,86 ^{ab}	2	0,22 ^a	4	0,00
Chojnów		0,88 ^{ab}	3	0,19 ^a	2	0,01 ^a
Płock		1,02 ^{ab}	4	0,22 ^a	4	0,00
Wioska		1,05 ^{ab}	5	0,32 ^a	9	0,04 ^b
Dobra Pomoc		1,13 ^{abc}	6	0,34 ^a	12	0,00
Krotoszyn-92		1,23 ^{bcd}	7	0,27 ^a	7	0,04 ^b
Młynary-1	Chrostowa II	1,43 ^{cde}	8	0,32 ^a	11	0,00
Milicz		1,50 ^{de}	9	0,19 ^a	2	0,00
Młynary-2		1,57 ^e	10	0,31 ^a	9	0,00
Durowo	Jodłówka	1,69 ^e	11	0,29 ^a	8	0,00
Zaporowo	Chrostowa I	1,73 ^{ef}	12	1,11 ^{cd}	15	0,00
Zalesie		2,04 ^{fg}	13	0,92 ^{bc}	14	0,00
Opole	Chrostowa II	2,25 ^{gh}	14	0,23 ^a	6	0,00
Runowo	Chrostowa I	2,42 ^{hi}	15	1,18 ^d	16	0,00
Tronçais		2,55 ^{hi}	16	1,43 ^e	17	0,00
Krotoszyn	Chrostowa II	2,74 ⁱ	17	0,75 ^b	13	0,00
Mean length of necrosis		1,58	–	0,50	–	0,01

Table 3. Influence of provenance and family on length of necrosis caused by *P. cambivora*

Source of variance	Degree of freedom	Isolate				Reference test	
		303.07		528.08		<i>F</i> -test	significance (<i>p</i>)
		<i>F</i> -test	significance (<i>p</i>)	<i>F</i> -test	significance (<i>p</i>)		
Provenance	16	3,272	<0,001	2,985	<0,002	2,177	<0,021
Family within provenance	45	8,411	<0,001	8,181	<0,001	1,934	<0,001

**Figure 2.** Variation among provenances and families of pedunculate oak with respect to mean length of necrosis caused by *P. cambivora* (isolate no 528.08); ● – average for provenance, ○ – average for family, 9–299 – numbers of families

3.2. Family variation

The resistance of oak shoots to *P. cambivora* colonisation differed significantly depending on family ancestry in one origin. Of all examined origins, the longest necrosis on oak shoots was found- regardless of isolate – in case of Zaporowo/238. Average necrosis' length for both isolates was 2,39 cm (Fig. 2–3). Relatively large necrosis occurred also on oak shoots from Tronçais/283 family (1,82 cm for isolate 303.07, and 3,61 cm for isolate 528.08), Zalesie/203 (1,69 cm for isolate 303.07, and 3,73 cm for isolate 528.08) and Krotoszyn/92 (1,33 cm for isolate 303.07 and 3,60 cm for isolate 528.08). Average length of necrosis on shoots from those families for both isolates was respectively: 1,81, 1,81 and 1,64 cm. To families on which the smallest

necrosis sizes were recorded (for both isolates) were: Siedlec/109, Młynary-1/40 and Krotoszyn–90. The average necrosis length measured on shoots from these families were 0,14, 0,14 and 0,18 cm respectively (Fig. 2–3).

3.3. Re-isolation of *P. cambivora* from infected shoots

From almost 90% of families, isolate 528.08 was re-isolated. In the case of those families, this isolate was recovered in 80-100% inoculated shoots (isolate was successfully detected in all inoculated shoots from 50% of families), and it was only from 8 families (Zalesie/203/222/206, Tronçais/295, Zaporowo/237/244, Milicz/59 i Runowo/257) that the share of successful re-isolations was 40–70%.

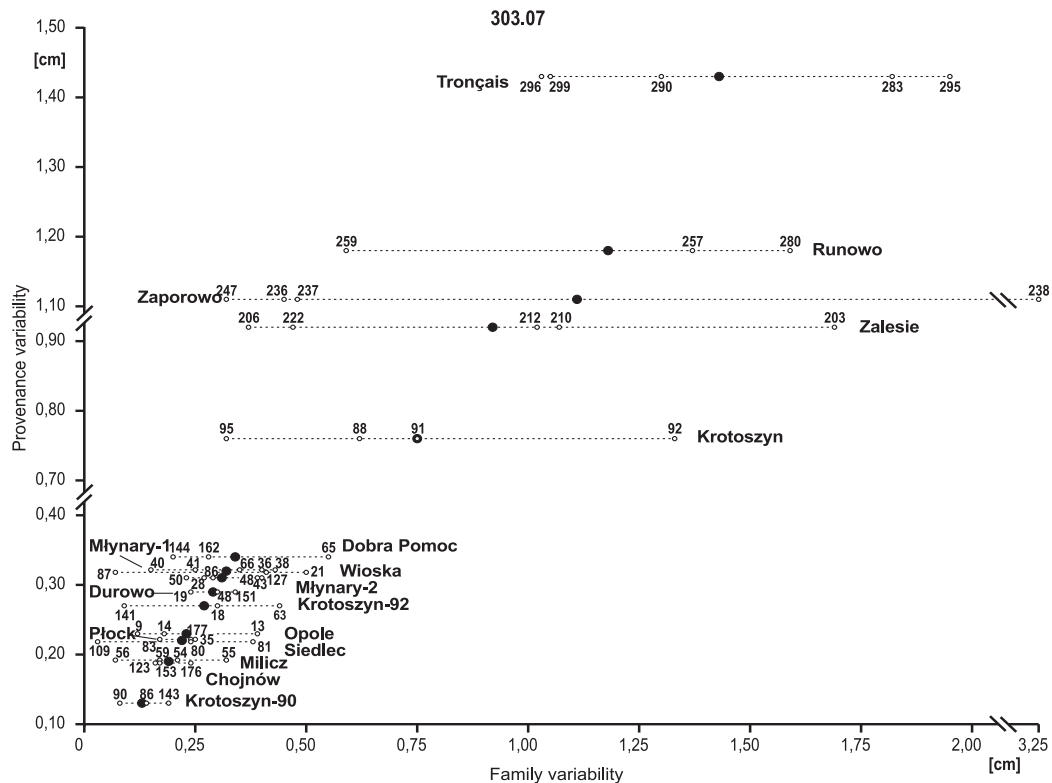


Figure 3. Variation among provenances and families of pedunculate oak with respect to mean length of necrosis caused by *P. cambivora* (isolate no 303.07); ● – average for provenance, ○ – average for family, 9–299 – numbers of families

The re-isolation of 303.07 *P. cambivora* isolate was much less effective. Only at 36% of families the isolate could be detected in all artificially infected shoots, and from 4 families (Milicz 59/56 i Opole 9/14) isolate was re-isolated from less than a half from inoculated shoots.

4. Discussion

This study showed a relevant differentiation of pedunculate oak's shoot colonisation speed by *P. cambivora*, both on origin and families level. Similar variation was observed in studies of different European populations of *C. sativa* artificially infected by *P. cambivora* organism (Miranda-Fontania et al. 2005; Robin et al. 2006). In the following studies, necrosis' character on oak shoots was similar to those on chestnut.

In many other studies conducted in Europe and North America a large susceptibility variability of different trees' population to different fungal pathogens was also reported. Eldridge and Dowden (1980) have documented the occurrence of large differences in colonisation of

various *Pinus ponderosa* Dougl. ex Lawson origins by *M. pini*, while Hansson (1998) observed variable susceptibility of different provenances of *Pinus sylvestris* L., *Pinus contorta* Dougl. ex Loud and *Picea abies* (L.) H. Karst. on artificial infection with *G. abietina*.

The results obtained in this study are consistent also with those of Banach (2002), who found a high variability of some breeding features (inter alia height and spring flushing of pedunculate oak tested on experimental area Chrostowa I). It seems, that polish pedunculate oak populations are strongly differentiated not only in reference to those features, but also because of susceptibility to pathogenic fungi. It can also be partly confirmed by Szczepkowski (2010), who in laboratory conditions showed the occurrence of differences between 7 polish oak populations in terms of oak's wood weight loss caused by three species of rot fungi.

This study indicates that the oaks most susceptible to infections by *P. cambivora* are the trees from Tronçais, Krotoszyn, Runów, Opole and Zalesie. To origins that are resistant can be the oaks from Chojnów, Siedlce,

Płock, Krotoszyn-90 and Wioska. Interestingly, it was observed, that some of the most susceptible populations belong to the fastest developing oaks (Runowo, Zalesie, Tronçais) (Banach 2002). Majority of oaks susceptible to *P. cambivora* colonisation came from polish western areas (Krotoszyn, Runowo, Opole). High susceptibility of oaks from Krotoszyn region can be correlated with their mass dying in this region in the 1980s (Przybył 1995). Perhaps too frequently dieback of oaks from Krotoszyn could have contributed to the mass occurrence of pathogens from *Phytophthora* genus, as happened in Germany (Jung et al. 2000). It seems however, that fungus-like organisms from *Phytophthora* genus can be only one of the elements in decreasing oaks vitality. According to Thomas and others (2002) the oaks decline is a complex disease, in which fundamental pathogenic role is played by abiotic factors such as drought in growing season and cold winters.

In this study a significant difference in shoots' colonisation rate by *P. cambivora* on family level was revealed. It seems that it plays even a bigger role than differentiating between provenances. In majority of populations single families more susceptible or resistant to colonisation by *P. cambivora* were detected. For example among 5 families belonging to origin Młynary-1, family 40 should be acknowledged as strongly resistant, while family 66 as strongly susceptible to colonisation by *P. cambivora*. Also provenances from Krotoszyn are characterised with similar variability, wherein here the majority of families showed higher susceptibility. Similarly high differentiation of other feature, for example spring leaf development, oaks' growth and their susceptibility to insect pest on family level, was reported also by other researchers (Banach 2002; Baliuckas, Pliura 2003; Bogdan et al. 2004; Barzdajn 2008; Banach, Lenowiecki 2011).

Results obtained in the following experiment have to be interpreted carefully. Inoculation method of "excised shoots", which is commonly used for resistance research of various trees' provenances with organisms from *Phytophthora* genus, also has some limitations. Firstly, results obtained from "excised shoots" inoculation, therefore declining shoots, do not always have to reflect the situation occurring on living shoots or trees' roots. Apart from that, experiment conducted on 2-year old shoots may not reflect fully the mechanisms of resistance of older trees. On the other hand, detailed research conducted on oak in France (Robin, Desprez-Loustau 1998) showed that inoculation results of cut fragment of shoots and roots of living seedling are tightly correlated. Similar conclusions emerge from the latest work of

Robin and others (2006) in which it was proved that the method of "excised shoots" is the most appropriate for examining chestnut to *P. cambivora* infection.

In the conducted experiment, isolate 528.08 generated much bigger necrosis on shoots than isolate 303.07. Those differences may be linked with various levels of aggressiveness characterising both isolates. It is due to high aggressiveness that isolate 528.08 could colonise oak shoots much faster than less aggressive isolate 303.07. In this study relatively large differences in colonisation of shoots and families of pedunculate oak between two isolates of *P. cambivora* were also noted. However it is difficult to explain the cause of these differences. It seems that the most significant role could have been played by chemical substances in oak shoots, which may have differentiated the development of *P. cambivora* in shoots' tissue. Perhaps some individuals (isolates) are better adapted to live in trees' tissues characterised by high content of chemical substances with fungistatic properties. The most important chemical substances which inhibit fungi and other organisms' development in plants' tissues are secondary metabolites such as phenols, carbolic acids, flavonoids and tannins (Witzell, Martín 2008). Therefore the next research stage should be learning the content of some chemical compounds in oaks' tissue in reference to susceptibility to infections by various pathogenic organisms.

5. Conclusions

1) Within polish pedunculate oak provenances there was a high level of inter-population variability of shoots' susceptibility to *P. cambivora* colonisation. Provenances particularly susceptible were: Zaporowo, Runowo, Opole and Krotoszyn, whereas origins from Chojnów, Siedlec, Płock, Krotoszyn-90 and Wioska were relatively resistant.

2) It was proved, that within majority of analysed pedunculate oak populations exist families of different susceptibility to *P. cambivora* colonisation.

3) There were some differences in susceptibility of different provenances and families of pedunculate oak to colonisation by examined *P. cambivora* isolates. Therefore it seems that in research on pedunculate oak susceptibility with the use of "excised shoots" method should be used on more isolates of this organism.

4) The results of the conducted experiment indicate a possibility of selection in the future provenance or families of pedunculate oak characterised with high resistance to infection by *P. cambivora*.

Acknowledgments

The research studies were carried out in the framework of the project no. DS-3414/ KFL, financed by grant for education awarded by MNiSW; and project BLP-364, financed by the General Management of State Forest.

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