

POSSIBILITY OF DETECTING SPECIES FROM THE *PHYTOPHTHORA* GENUS IN THE RHISOSPHERE SOIL OF OAK TREES USING VISUAL CROWN ASSESSMENT

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Abstract

This paper explains biological background of the project. Oak decline in Polish stands in recent years has become a serious problem. Symptoms of disease like thinning crowns, yellowing of the leaves or the appearance of dark exudates on the trunks are observed. Such symptoms are characteristic for pathogenic organisms of the genus *Phytophthora*. Identification and confirmation of the presence of these pathogens is difficult. That is why we have tried to estimate the presence of pathogens in the rhizosphere soil in oak stands on the basis of visual assessment of crowns trees with symptoms typical of *Phytophthora* infection.

Keywords: *Phytophthora*, oak decline, soil, rhizosphere soil

INTRODUCTION

In European silviculture, species from the *Phytophthora* genus constitute a serious threat to forest ecosystems. According to Zentmyer and Thorn (1967) pathogenic *Phytophthora* are a destructive factor causing rotting of roots and shoot bases, leaf staining, and cancerous states, which lead to dieback of single organs or whole plants.

Pathogenic oomycetes of the *Phytophthora* genus have been found in tree nurseries, cultivations, and mature stands, which indicates high potential for infection, which may lead in turn to significant damage to different types of forest trees (ex. oak, beech, ash or alder). Over the last twenty years, the spreading of known species of pathogens of the *Phytophthora* genus, as well as the apparition of new ones, has been observed in oak stands in Europe (Jung et al., 1999, 2002; Nechwatal et al., 2001; Vettriano et al., 2002). Brasier and Jung 2003, 2006 see the cause of this in the fact that pathogens of the *Phytophthora* genus easily migrate from tree nurseries, from where they are carried to forests, stands, and riverside ecosystems, and spread from there using the river current. Many studies indicate that it is water that is the main source and vector of *Phytophthora* dissemination (Reeser et al., 2011; Orlikowski et al., 2011; Orlikowski et al., 2003, 2011b, 2012).

The dieback of oak stands is considered to be caused by a combination of abiotic and biotic factors (Balci, 2008). Participatory factors are often the direct cause of death of weakened trees. Among them are secondary insect pests, pathogenic fungi and oomycetes which cause damage to fine roots, up to 90% of their biomass (Szczerba and Robakowski, 2013; Oszako 1990, 2000). Oak crown defoliation is highly correlated with both primary and secondary abiotic and biotic factors, and in many cases there has been determined a direct relation between the presence of *Phytophthora* and the dieback and mortality of oak seedlings and mature trees (Robin et al., 1998; Tainter et al., 2000; Jonson et al., 2003; Rodriguez-Molina et al., 2005). This was also confirmed in studies involving the artificial inoculation of oak seedlings with species of *Phytophthora* (Jung et al., 1996, 1999, 2002; Maurel et al., 2001).

In California and Oregon the cause of 'Sudden Oak Death' (SOD) was *P. ramorum* Werres, de Cock & Man in't Veld (Werres et al., 2001). Successive years of intensive research on the causes of SOD led to the identification of a new species, *P. nemorosa* (Hansen et al., 2003). Dieback of oak in Spain and Portugal was linked to the presence of *P. cinammomi* (Brasier et al., 1993). In Germany, Jung and his collaborators (1996, 1999) described several species, *P. quercina*, *P. psychrophila*, *P. europea*, *P. uliginosa* and *P. pseudosyringae* (Jung et al., 1999, 2002, 2003), related to the occurring of root rot in dying oaks. *P. quercina* is considered to be the main perpetrator of oak stand dieback in Central Europe, on sand-clay soils with pH above 3.5 (Jung et al., 2000). *P. quercina* was also the species most often isolated from soils near the rhizosphere of roots of dying oak trees in Sweden (Jönsson et al., 2005). In Great Britain, studies of oaks led to the discovery of a new species, *P. kernoviae*, whose presence was found in necrotic stains on red oak (Brasier et al., 2005).

In Polish forests, these microorganisms were deemed a threat for, amongst others, stands of beech, pedunculate oak, alder, larch, and spruce (Orlikowski et al., 2011a; Oszako et al., 2007; Orlikowski et al., 2012, Stępniewska i Dłuszyński, 2010).

Still little is known about species of pathogens from the *Phytophthora* genus related to oak stands in Poland. *P. citricola*, *P. cactorum* and *P. gonapodeides* were isolated from soil from under pedunculated oak in tree nurseries (Oszako et al., 2007). The species *P. cinammomi* was found in tree nurseries producing pedunculated oak seedlings, as well as in 30-70 year oak stands which displayed symptoms such as cancerous states on trunks, black stains, exudations, necrosis, and crown loss (Oszako and Orlikowski 2005).

The presence of *P. quercina* was determined in the spring of 2006 in soil sampled from under the roots of a pedunculated oak showing signs of dieback of single limbs and rotting root hair roots (Oszako et al., 2007). Studies of the colonization of oak stem and leaf tissue by this pathogen have confirmed that *P. quercina* is dangerous to budding pedunculated oak seeds, and in the case of this species existing in the soil, preemergent gangrene in seedlings occurs. Jung and his colleagues described three new species in 2002, *P. europea* Hansen & Jung, *P. uliginosa* Jung & Hansen, *P. psychrophila* Jung & Hansen, where *P. uliginosa* was isolated from under declining *Q. robur* in the city of Niepołomice (Jung et al., 2002).

In 2007, in the Krzyszkowiecki Forest (Myślenice area), *P. cambivora* was isolated from rhizospheric soil from over 150 year old, declining pedunculated oaks (Stępniewska et al., 2008).

In oak stands located in the Krotoszyn Forest, symptoms were observed that could indicate the invasion of roots by pathogenic *Phytophthora*. These symptoms include thinning of tree crowns, yellowing and atrophy of leaves, damage to branches, offshoots, loss of top parts of tree crowns, outflows on trunks, necrosis of main roots, as well as atrophy and loss of smaller roots.

Considering the lack of data on the role of species from the *Phytophthora* genus in the dieback of oak stands in Poland, this study set its goals as: i) analysis of the state of crowns of selected oaks in the Krotoszyn Forest, ii) determining the presence of *Phytophthora* species in soil sampled from under declining oak trees, and iii) determining a correlation between the presence of *Phytophthora* and the state of the tree crowns.

MEANS AND METHODOLOGY

Field work

The studies were conducted in the Krotoszyn Forest area, in three stands located on land belonging to the Regional Directorate of State Forests (RDLP) Poznań. On two of these areas (Forest superintendency Karczma Borowa, intendency Nowy Świat, sections 7 and 15, and Forest superintendency Krotoszyn, intendency Jelonek, sections 181, 182, and 183) grew 120 year old oak stands, while on the third area (Forest superintendency Piaski, intendency Siedlce, section 217f) the oak stand was 60 years old. All three stands were surrounded by mixed fresh forest. 60 trees in each stand were selected and were permanently marked using ecological paint. Two soil monoliths, measuring 20x20x30cm, were extracted from the rhizosphere soil 1m away from each tree (north and south side of each tree), according to the method described by Jung et al. (1993). In addition, the state of each tree's crown was evaluated visually (defoliation and vitality) in order to estimate its state of health. The vitality test was performed using the method developed by Roloff (1989), in which the vitality is shown as tree's growth potential and its ability to regenerate the damaged crown. The basis for the assessment of the vitality is the architecture of shoots produced in the upper part of the crown. All trees were classified into one of four groups distinguished on the basis of differences in vitality, according to the criteria shown in Table 1. The assessment of crown defoliation of ash trees was carried out in early July. Both methods are based on observations from the ground. In total, soil samples were taken from under 180 trees.

Table 1. Vitality degrees (Roloff 1989)

Vitality degree	Damage level
0	Exploration phase, untouched trees, vital ones
1	Degeneration phase, weakened trees
2	Stagnation phase, damaged trees
3	Resignation phase, badly damaged trees, decaying

Isolation using the baiting method

The samples were taken to the Forestry Research Institute, where samples taken from one tree were thoroughly mixed, and a sample of 200g was set aside. This soil was placed in sterile containers, humidified, then covered with distilled water in such a way that its surface was 2 cm above the surface of the soil. 7 day oak and beech leaves were then placed on the surface of the water as traps for *Phytophthora* pathogens (Jung et al., 1999, 2000). The leaves were left in the water for 3 to 7 days, up to the moment where small dark stains were observed on them. Small samples of the leaves (8x3x3mm) were transferred to a PARPNH type medium prepared beforehand: 100 ml/l vegetable juice, 3g/l CaCO₃, 20 g/l agar, 10 mg/l natamycin, 200 mg/l

ampicillin, 10 mg/l rifampicin, 25 mg/l pentachloronitrobenzene (PCNB), 50mg/l nystatin, 50 mg/l hymexazol (Jung et al., 1996). Thus prepared Petri dishes were incubated in darkness, in a temperature of 20°C. After 24-48 hours, the observed mycelium was transferred to a V8 type medium (100 ml/l vegetable juice, 3g/l CaCO₃, 20g/l agar). The obtained isolates are stored in the Forestry Research Institute collection.

In-house work

In total, 173 isolates of *Phytophthora* (Table 2) were obtained during laboratory work.

Table 2. List of isolates and samples from which they were derived

Superintendency Piaski		Superintendency Karczma Borowa		Superintendency Krotoszyn	
sample no.	no. of isolates	sample no.	no. of isolates	sample no.	no. of isolates
P002	1	KB003	3	K006	1
P017	6	KB003'	8	K007	4
P020	1	KB005	2	K009	1
P038	11	KB005'	10	K019	6
P044	1	KB006	2	K020	1
P047	1	KB009	1	K034	2
P049	1	KB011	5	K040	1
P055	2	KB013	4	K056	3
P061	1	KB016	9	K060	5
P064	2	KB020	4	K070	1
P068	1	KB021	4		
P072	3	Kb023	2		
P078	3	KB029	1		
P080	2	KB030	5		
P084	1	KB031	5		
P090	6	KB036	6		
P101	2	KB043	1		
P104	1	KB044	1		
P106	1	KB053	4		
P107	2	KB059	1		
P109	1	KB063	7		
P118	2	Kb071	5		
P128	3				
P154	1				
P156	2				

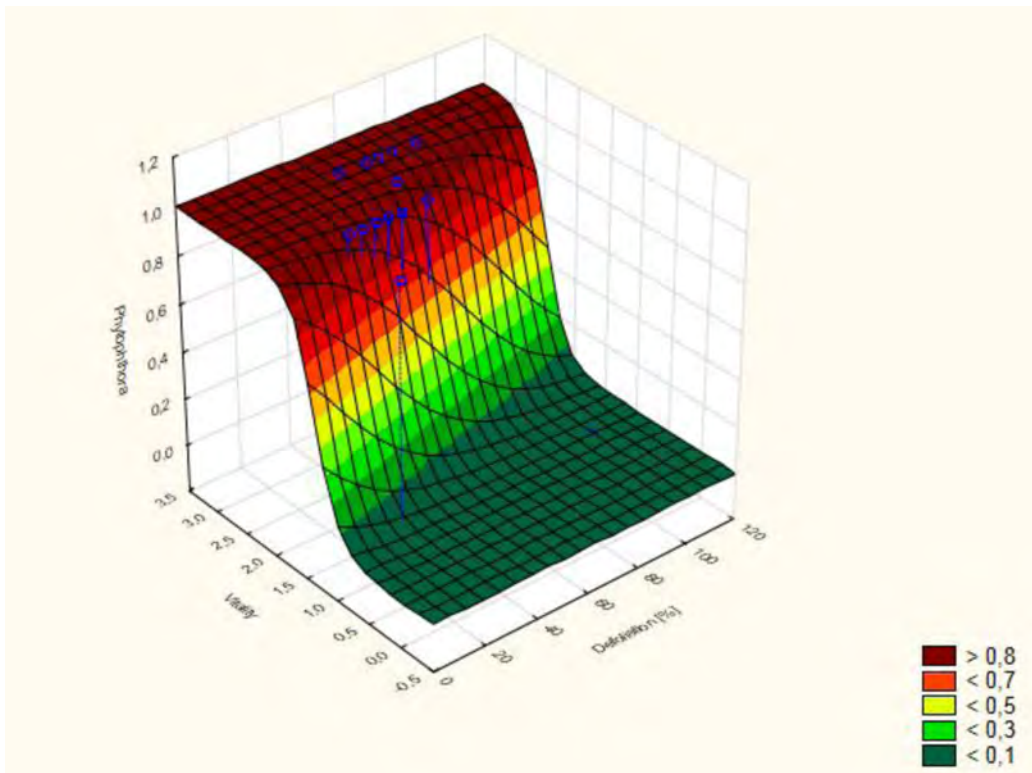
This data was then correlated, using a model of logistic regression, with the measurements of the state of health of tree crowns that were taken at the same time as the soil samples, in order to verify if the presence of species of the *Phytophthora* genus influences the visual evaluation score of oak tree crowns in the Krotoszyn Forest. All analyses were made using Statistica v.10 software.

RESULTS

After conducting statistical analyses it was determined that on all three areas of study the presence of species from the *Phytophthora* genus was significantly correlated with the vitality and defoliation of tree crowns. In the case of the experimental area of Karczma Borowa, the value of test probability was 0.000; in the Krotoszyn area 0.001; while in the Piaski area this value was 0.0006.

An equation of the logistic regression model for each area was created, which made it possible to evaluate how the probability of observing organisms of the *Phytophthora* genus changes when the parameters of corona health (vitality and defoliation) change. In the Karczma Borowa area, in a 130 year old oak stand, it was possible to determine using the logistic regression model that a decrease of the defoliation parameter along with an increase of the vitality parameter increases the probability of finding pathogens of the *Phytophthora* genus in the rhizosphere soil (pic. 1). This is represented by the negative value taken in the equation shown below by variable x representing defoliation and the positive value taken by variable y representing vitality.

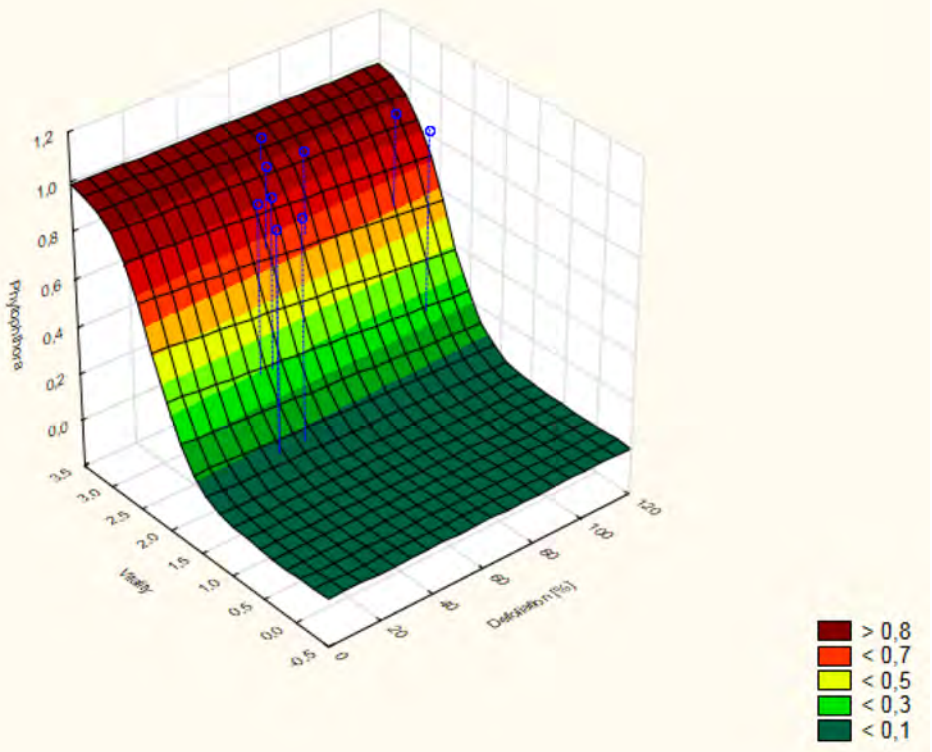
$$P(Y : 1) = \frac{e^{-7,806-0,059x+6,051y}}{1 + e^{-7,806-0,059x+6,051y}}$$



Pic. 1. Logistic regression model for the Karczma Borowa area

Similar results were obtained in the Krotoszyn area, where the trees studies were also 130 year old oaks. Once again, the logistic regression model takes a negative value for parameter x (defoliation) and a positive value for parameter y (vitality). This means that, in a manner analogical to the Karczma Borowa area, a lower value of the defoliation parameter and a higher value of the vitality parameter increase the probability of finding pathogens of the *Phytophthora* genus in the rhizosphere soil (pic. 2).

$$P(Y : 1) = \frac{e^{-7,416-0,004x+3,338y}}{1 + e^{-7,416-0,004x+3,338y}}$$



Pic. 2. Logistic regression model for the Krotoszyn area

The results obtained in the Piaski area, where the stand is half as old (60 years) as in the two aforementioned areas, are significantly different. The model of logistic regression designed for this area shows that both an increase in the value taken by the parameter of defoliation and that of vitality cause an increase in the probability of finding pathogens of the *Phytophthora* genus in the rhizosphere soil (pic. 3). This is shown by the positive value of indicators x and y (defoliation and vitality respectively) in the equation of logistic regression shown below.

$$P(Y : 1) = \frac{e^{-3,346+0,0107x+1,553y}}{1 + e^{-3,346+0,0107x+1,553y}}$$

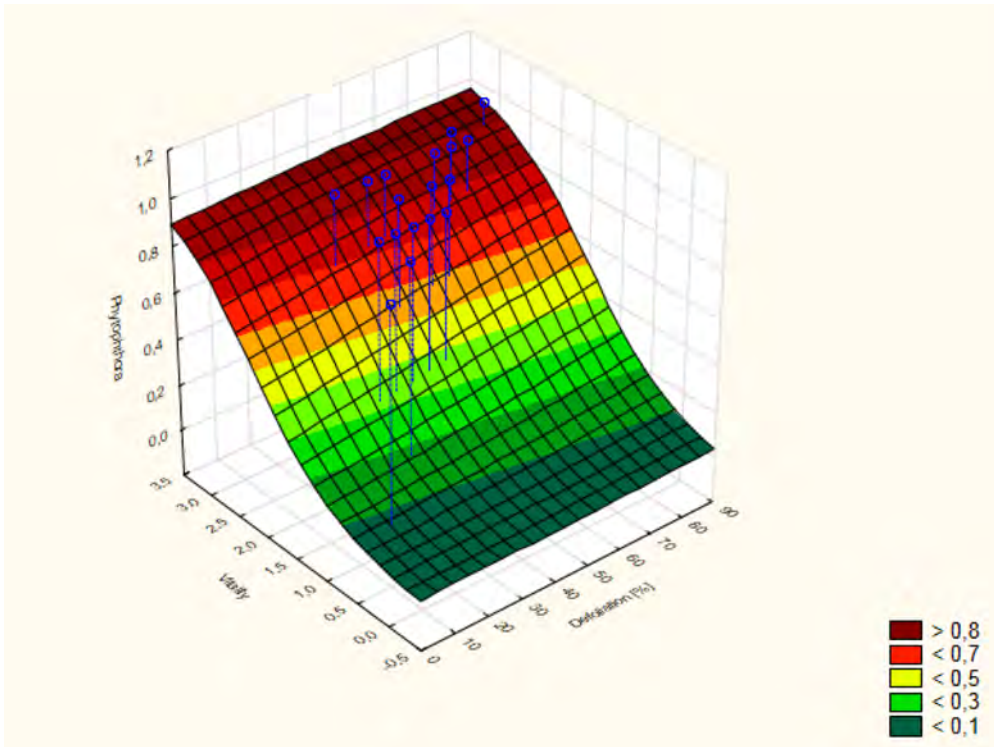


Fig. 3. Logistic regression model for the Piaski area

DISCUSSION

The conducted research clearly shows that changes observed in the crowns of oak stands are closely related to the presence of pathogens from the *Phytophthora* genus and its harmful influence on the host plants. Research done earlier allows us to reach similar conclusions. Jung et al. (2000) found a similar dependence between the presence of *P. quercina* and the vitality and defoliation of trees in oak stands in Germany. This can mean that the changes in the corona are the effect of damage done to the roots by species of the *Phytophthora* genus. Additionally, the proposed equations of logistic regression allow us to determine with greater probability, using parameters that are relatively easy to assess (vitality and defoliation), the presence in rhinosphere soil of oomycetes from the *Phytophthora* genus. The infection of roots and the ensuing dieback leads to disturbances in mineral and water management in the plant, and finally to its weakening and decay (Erwin and Ribeiro). This is the reason why it is so important to detect the damage and detrimental action of these organisms as soon as possible.

Despite the statistically significant relationship between the crown healthiness and the presence of the pathogen in the soil, results of analysis cannot be interpreted literally. It should be noted that the trees are biological part of the forestry ecosystem and all the time are influenced by many biotic and abiotic factors. A deficiency of minerals in the soil, the annual outbreak of pest insect may lead to a reduction in tree health, which can be seen in the crown defoliation. In addition, the use of traditional, subjective assessment methods of defoliation and vitality may increase the risks of misclassification of trees, as infected. Therefore the proposed models in this paper are a tool which may suggest that *Phytophthora* pathogens are present in the roots of the tree, but this is not an indicator that can be used conclusively.

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