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ORIGINAL ARTICLE

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Pathogenicity of *Phytophthora* isolates originating from several woody hosts in Bulgaria and Poland

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Abstract

Our aim was to examine the virulence of eight *Phytophthora* isolates belonging to three species (*Phytophthora cryptogea, Phytophthora plurivora* and *Phytophthora quercina*) obtained from diverse European ecosystems (in Bulgaria, Poland and Germany) towards three forest tree hosts – English oak (*Quercus robur* L.), Turkey oak (*Quercus cerris* L.) and European beech (*Fagus sylvatica* L.).

All plants grown from seeds in a greenhouse conditions were artificially inoculated under the stem bark with *Phytophthora* cultures. The tested isolates turned to be more aggressive to Turkey oaks than to English oak trees. In case of European beech, the isolates of *P. cryptogea* and *P. plurivora* exposed various virulence. The potential hazard of the introduced foreign isolates for the oak and beech forests in Poland and Bulgaria is discussed. Amongst the tested isolates, *P. quercina* P290 from German highly infected Bulgarian Turkey oaks; therefore, its negative potential impact on Bulgarian oak forests could be considered as high (if unintentionally introduced). Also, two Bulgarian isolates belonging to *P. cryptogea* and *P. plurivora* are risky for Polish beech forests, if exposed to the pathogen. The observed pathogenicity of the tested *Phytophthora* species proved their potential as important contributors to decline of valuable forest ecosystems dominated by oaks (*Q. robur* and *Q. cerris*) or beech (*F. sylvatica*), in both Poland and Bulgaria. We found that investigated *Phytophthora* pathogens could develop in the living plant stem tissues without causing any disease symptoms, which is another demonstration that phytosanitary control by simple observation of plant material is not effective.

Key words

decline, Fagus sylvatica, Quercus cerris, Quercus robur, Phytophthora, virulence

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INTRODUCTION

Alien fungal and fungal-like pathogens are considered to be one of the main causes of emerging infectious disease in forests over the past four decades (Santini et al. 2013). The oomycete genus Phytophthora comprises a large number of plant pathogens that cause significant damage to various natural and agricultural ecosystems in altered climatic zones (Callaghan and Guest 2015). Bulgaria is situated in south-eastern Europe and encompasses a wide range of environments. Since the country joined the European market, the risk of introduction and establishment of alien species is undoubtedly grown. Apart from the well-known Phytophthora pathogens occurring on the agricultural crops (Nakova 2010; Ilieva et al. 1995), there is no sufficient information available for the natural ecosystems in Bulgaria, except the report of ink disease on chestnut (Panov and Bulgariev 1982). The above records show that Phytophthora species are widespread, abundant, very diverse and poorly investigated, especially in undisturbed forest ecosystems. During the past decades, and in particular after alternating dry and wet years, an increasing number of European beech (Fagus sylvatica L.) stands across Europe have demonstrated symptoms typical for Phytophthora disease (Jung 2009). Poland is situated in central Europe, and analogically same beech and oak species could be under threat from pathogenic Phytophthora spp.

The development of DNA-based technologies has revealed much broader and more complex diversity than previously recognised and has led to the recent description of many new species (Callaghan and Guest 2015). The soil-borne species *Phytophthora quercina* T. Jung appears to be strongly involved in the European oak decline phenomenon (Jönsson et al. 2003). The species pathogenicity has been studied only on the fine roots of Central European oaks (Jung et al. 1999). Phytophthora plurivora T. Jung & T.I. Burgess has most likely been spread from Europe in the rest of the world with the nursery trade, that is, through diseased plants for plantings (Schoebel et al. 2014). The international trade has allowed pathogens to colonise new environments and/ or hosts, resulting in the growth of P. plurivora population, which is currently frequently occurring in nurseries and forest stands in Poland (Orlikowski et al. 1995, 2004; Oszako et al. 2007).

The oak forests of northern and central Europe are dominated by the deciduous forest species of Quercus robur and Quercus petraea (Matt.) Liebl. These two species are very important in Poland from both ecological and economical point of view. The most beautiful oak forests with unique wood characteristics, such as narrow annual increments, are growing in the Krotoszyn Plateau (close to Poznań in western Poland) and are very much appreciated by wood industry. In contrast, Quercus cerris is a part of flora of oak woodlands in southern and Mediterranean Europe containing evergreen oak species, including the territory of Bulgaria. The present work aims to evaluate the variation in the pathogenicity and virulence of Phytophthora isolates (Phytophthora cryptogea, P. plurivora and P. quercina) obtained from Polish and Bulgarian beech and oak stands.

MATERIAL AND METHODS

Phytophthora isolates

Eight *Phytophthora* isolates were used in this study. Six of them belonged to species *P. cryptogea* and *P. plurivora* and were obtained from the rhizosphere of damaged trees growing in various ecosystems in Bulgaria. They were identified on the basis of morphology and sequence similarity to *Phytophthora* sequences deposited in the NCBI GenBank data base (Lyubenova et al. 2016). Two *P. quercina* isolates were added in the experiments, both of them isolated from *Q. robur*, one from Poland and one from Germany (Tab. 1). Species identification of Polish isolates was performed as described by Trzewik et al. (2015).

Prior to the use of isolates in inoculation tests, they were grown on 10% vegetable juice agar media (100 g of vegetable juice 'Gemuse saft Alnatura', 3 g of CaCO₃, 10 g of plant agar (Duchefa Biochemie) and 900 ml of distilled water) in the dark at 25°C for a period of three to eight days, depending on the intensity of mycelial growth of particular species.

Plant material

Three forest tree species were tested as host plants in the pathogenicity tests: 3-month-old Turkey oaks (Q. cerris L.) of the Bulgarian origin, 5-month-old English oaks (Q. robur L.) and European beeches (F. sylvatica L.). The last two species were originated from Po-

Isolate	Host	Location
Phytophthora quercina Poland 9	Quercus robur (L.)	Piaski Forest District, Poland
Phytophthora quercina Jung ATCC [®] MYA 4084 [™] P290	Quercus robur (L.)	Germany
Phytophthora cryptogea Belasica 10/3	Castanea sativa (Mill.)	Nature Park Belasica, Bulgaria
Phytophthora cryptogea Bankya 1/1	Picea abies (Karst. L.)	Bankya, Bulgaria
Phytophthora plurivora Velingrad 1/1	Alnus incana (L.)	Velingrad, Bulgaria
Phytophthora plurivora Tulovo 4/1	Quercus robur (L.)	Protected area "Tulovska koria" Bulgaria
Phytophthora plurivora Tran 2/1	Alnus glutinosa (L.)	Along the river of Erma, Bulgaria
Phytophthora plurivora Tulovo 7/3	Quercus robur (L.)	Protected area "Tulovska koria" Bulgaria

Table 1. Phytophthoro	<i>i</i> isolates used ir	the study their hosts	and origin of the isolate
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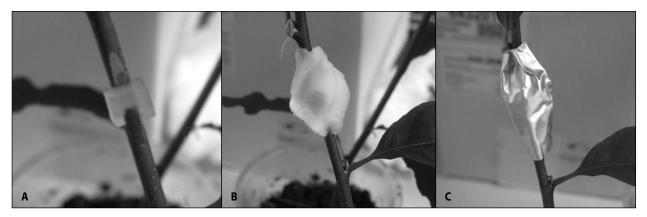


Figure 1. Inoculation procedure of the pathogenicity test with *Phytophthora* isolates. A – a piece of agar with mycelium placed on the cambium surface of the stem, after cutting the bark with a sterile knife, B – the removed bark was put back and the place was covered with the sterile moist cotton, C – the inoculation wound was sealed with Parafilm[®] and covered with aluminium foil

land (Krotoszyn Forest District, Regional Directorate in Poznań). All plants were grown from seeds (in pots containing sterilized soil) in the Forest Research Institute greenhouse under the following conditions: 24°C and photoperiod of 16 h (2000 lux) light and 8 h dark.

Inoculation procedure

The plants were inoculated with mycelium of the tested isolates placed under the bark, according to the method developed by Jung and Nechwatal (2008). The plants' bark was cut about 6 mm along the stem with a sterile knife. A piece of agar (approximately 5 cm \times 5 mm in size) with mycelium was placed on the cambium surface of each stem. Next, the removed bark was put back and the place was covered with sterile moist cotton and then sealed with Parafilm[®] and covered with aluminium foil (Fig. 1).

The aforementioned plant species were inoculated with the investigated *Phytophthora* isolates. Each isolate-host combination was replicated eight times. The same number of control plants received only the sterile agar and another eight ones were left uninjured. The inoculated plants were kept under the greenhouse conditions.

Only *Q. robur* and *Q. cerris* seedlings were inoculated with *P. quercina* isolates. Those isolates were not applied to inoculate European beeches, as *P. quercina* is a recognised pathogen infecting fine roots of oaks (Jung et al. 1999).

The evaluation of the test was performed three weeks after the inoculation. The length of the observed necrotic lesions longitudinally on the stem of each plant was measured.

Statistical methods

The resulting data was analysed using Kruskal–Wallis test. The pairwise comparisons (post-hoc test) was done using Nemenyi-Damico-Wolfe-Dunn test. All statistical analyses were performed with R (version 3.2.3.).

Re-isolation of pathogens from plant tissues

To rule out any putative contamination during plant infection experiments and to prove that the symptoms observed on plant stems are caused by organisms used for inoculation, we performed re-isolation of each pathogenic species from infected plant tissues. Several small pieces of the bark taken from the margins of necrotic lesions and healthy tissues next to inoculation wounds of each tested plant were cut, disinfected in 70% ethanol and placed on 5% vegetable juice agar media. After few days, the Petri dishes were checked under the light microscope for the occurrence of irregular hyphae, typical for *Phytophthora*.

RESULTS

Three weeks after inoculation some necrotic lesions (different length) developed around the inoculation sites on the stems on most of the plants. There were noticeable brown discolorations along the stems of *Q. cerris*. In the case of *Q. robur* plants, the observed necroses were only limited to the periphery of the inoculation wounds. It was no visible that the pathogen was developing in the plants, although it was successfully re-isolated from the stems, demonstrating their endophytic character. The *Phytophthora* isolates inoculated in *F. sylvatica* plants were also successfully re-isolated from stems, regardless of whether necrotic lesions were observed or not.

In general, *Phytophthora* isolates expressed diverse pathogenicity and aggressiveness depending on the tested host plant species, which were based on the length of necrosis developed in the stem of trees as a result of the inoculation and subsequent infection. There were no symptoms observed (e.g. leaves wilting), which could be assigned only to the specific isolate. The wounds of all inoculated control plants (with sterile agar) were healed, showing obvious release of callus and lack of necrosis. In the control, no necrotic lesions were noticed, so they are not included in the figures and are not further mentioned or discussed.

Infection of English oak (Q. robur) plants

The necrotic areas on English oak stems developed from the inoculation points when inoculated with *P. cryptogea* and *P. plurivora* isolates but not in the case of two *P. quercina* ones. Thus both *P. quercina* isolates were not able to infect stems of English oaks. Isolates of *P. cryptogea* and *P. plurivora* were pathogenic to English oak stems, but no statistically significant difference was found amongst the length of the necrotic lesions (Fig. 2). Nevertheless, all aforementioned isolates provoked necrotic lesions on the stems of English oak seedlings.

Infection of Turkey oak (Q. cerris) plants

On the contrary to English oaks, the Turkey oaks expressed the higher level of infection after the inoculation with all *Phytophthora* isolates used in the study (Fig. 3). In this case, both *P. quercina* isolates caused necrotic stem lesions, but the length of the lesions caused by the isolate *P. quercina* P290 (German origin) exceeded significantly those caused by the *P. quercina* Poland 9 isolate (Fig. 3).

According to the measurements of necrotic lesions of oak stems, there was no significant difference in susceptibility of Turkey oaks (*Q. cerris*) inoculated with two *P. cryptogea* isolates, Belasica 10/3 and Bankya 1/1.

In general, amongst the two tested oak species, the Turkey oak seems to be more susceptible to infection by *Phytophthora* isolates.

Infection of European beech (F. sylvatica) plants

Bulgarian isolates of *P. plurivora* and *P. cryptogea* expressed their pathogenicity to beech seedlings originated from Poland. Both isolates of *P. cryptogea*, Belasica 10/3 and Bankya 1/1, were pathogenic, but the first one caused more severe damage to *F. sylvatica* plants. Two from four tested Bulgarian isolates of *P. plurivora* were highly virulent to *F. sylvatica*, and the most aggressive one appears to be *P. plurivora* Tran 2/1. The isolate *P. plurivora* Tulovo 4/1 did not infect *F. sylvatica* seedlings at all (Fig. 4).

P. cryptogea isolates used in the experiments are of Bulgarian origin, obtained from plant species other than those used in the experiment on pathogenicity (Tab. 1). Both isolates of *P. cryptogea* proved to be equally aggressive to *Q. cerris* and *Q. robur*. It is rather obvious that both Bulgarian isolates are much more aggressive

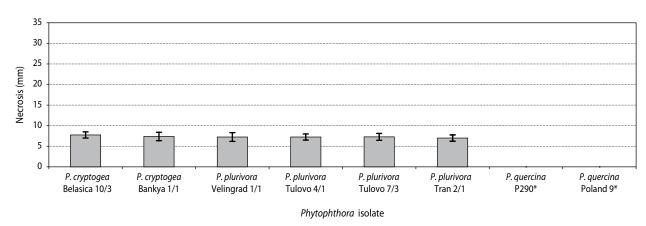


Figure 2. Mean length of necrosis caused by *Phytophthora* isolates on English oak (*Q. robur*) plants * – statistically significant at p = 0.05.

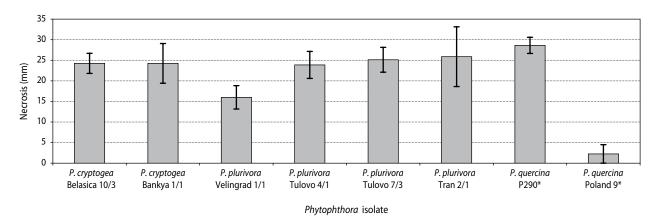


Figure 3. Mean length of necrosis caused by Phytophthora isolates on Turkey oak (Q. cerris) plants

* – statistically significant at p = 0.05.

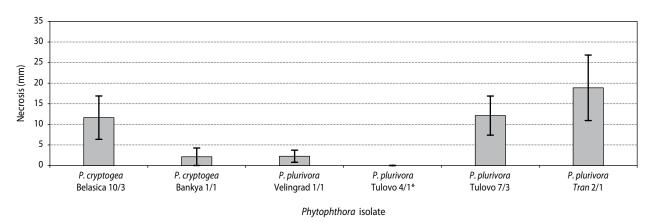


Figure 4. Mean length of necrosis caused by *Phytophthora* isolates on European beech (*F. sylvatica*) plants * - statistically significant at p = 0.05.

to Q. cerris than to Polish Q. robur seedlings. In contrary, in the case of F. sylvatica inoculation (also Polish), both the isolates differed in their virulence level. The pathogen P. cryptogea Belasica 10/3 isolated from chestnut (broadleaved species) was more aggressive to beech than the pathogen P. cryptogea Bankya 1/1 isolated from spruce (conifer species). Also, both host plant species F. sylvatica and O. robur of Polish origin were less susceptible to Bulgarian isolates of P. cryptogea in comparison to the Bulgarian host Q. cerris. It seems that aforementioned Phytophthora isolates are much better adapted to the Bulgarian oaks than to Polish host species (oak and beech), even though none were isolated from the tested plant host species. In this sense, it looks that tested Bulgarian isolates of P. cryptogea are potentially more hazardous to Bulgarian forests of Q. cerris than to Polish stands of English oak or European beech.

The reaction of English oak seedlings to the inoculation of P. plurivora is similar regardless of the isolate applied in the inoculation test. All oaks reacted by developing slight infection symptoms. Moreover, P. plurivora isolates expressed higher aggressiveness to Turkey oaks (Fig. 3). According to these findings, isolates of P. plurivora could have been expected to have smaller impacts in Poland (where English oak stands dominate) than in Bulgaria, where aforementioned pathogens could be recognised as very hazardous for Turkey oak stands. It is worth mentioning that all isolates of P. plurivora are pathogenic to both tested oak species, Q. robur from Poland and Q. cerris from Bulgaria, although two of them (Velingrad 1/1 and Tran 2/1) were isolated from alder (Tab. 1.). Four Bulgarian isolates of P. plurivora shown different level of aggressiveness to European beech (Fig. 4). Two of them are relatively highly virulent to F. sylvatica but the largest necrotic lesions are provoked by the isolate P. plurivora Tran 2/1, isolated actually from Alnus glutinosa host. Amongst all the tested P. plurivora isolates, the isolate Tran 2/1 seems to be the most hazardous one, infecting F. sylvatica and Q. cerris seedlings in a high level, and Q. robur plants in a medium level.

Both the isolates of *P. quercina* used in this study are isolated from *Q. robur* plants, one from Poland and the other from Germany. Interestingly, both aforementioned isolates did not infect Polish *Q. robur* seedlings in the performed pathogenicity tests. The isolate *P. quercina* Poland 9 originating from Poland did not infect *Q. robur* seedlings and slightly infected *Q. cerris* seedlings. On the contrary, the German isolate *P. quercina* P290 is very aggressive for *Q. cerris* but at the same time is not able to infect *Q. robur* plants.

DISCUSSION

The inoculation experiment has revealed the pathogenicity of tested *Phytophthora* isolates, confirming the results from other similar studies (Weiland et al. 2010; Jankowiak et al. 2014). In our experiment, it was demonstrated that *P. quercina* can infect not only fine roots (Jung et al. 1999; Jönsson et al. 2003) but also tissues of Turkey oak stems. Also, most of the investigated isolates show the ability to grow in the living tissues without causing any visible disease symptoms. The successful re-isolations of pathogens from healthy looking tissues demonstrated their strategy to survive. The fact that these species seem to colonise plant tissues without causing symptoms is interesting, but would require more detailed investigation.

In this study, we demonstrated the potential pathogenicity of *Phytophthora* isolates originating from two different climate zones of European regions. All tested *Phytophthora* species appeared to be pathogenic to Turkey oaks, which could have disastrous consequences for the health of this important forest tree species, especially if climatic conditions become more favourable for the development of this pathogen.

The successful infection of *F. sylvatica* by *P. plurivora* could be considered as the lack of proper defence gene induction. Probably, *P. plurivora* escapes the plant-recognition systems (Schlink 2010). In future experiments, we should check if the noticed difference in isolates' pathogenicity to beech seedlings could be related to the mentioned mechanism of the pathogen recognition by plant cells.

Detection of *P. plurivora* is important because the species is known to be a pathogen with worldwide distribution, attacking several host plants, including European beech (Werres 1995; Fleischmann et al. 2005). This plant is a dominant tree species in most of the European forests, having high environmental and economic values. The beech decline phenomenon observed in European forests during the 1980s was also associated with the interaction between climatic weather extremes

and *P. plurivora* damage caused to roots and butt collars of adult trees (Jung et al. 2000). In our study, we have shown that the Bulgarian isolates of *P. plurivora*, even if isolated from different host plants other than European beach, could be still potentially dangerous for *F. sylvatica* stands in Poland, if introduced and established in forest ecosystems.

The possible plant material for plantings or natural pathways of Polish and German *P. quercina* isolates to Bulgarian Turkey oak stands in the future may have different consequences for their health. Whilst the Polish isolate seems to have no significant impact, the German one seems to be so virulent that it can develop even in the stem tissue of oaks, which was not reported, so far. Therefore, in the case of its likely introduction to Bulgaria (sooner or later), it could have disastrous consequences for the forestry.

The oak-specific fine root plant pathogen P. quercina is a significant factor in the current phase of the European oak decline phenomenon with two subgroups were distinguished by Cooke et al. (2005), probably because of the initial introduction of isolates having different genetic backgrounds. The Polish strain of P. quercina (in contrast to the German one) did not show, under the conditions of our experiment, long lesions on Turkey oak stems, although P. quercina in Germany is considered to be the main driver of the English oak decline phenomenon, causing fatal damage to its fine roots (Jung et al. 1999). So far, according to Jung, P. quercina was only pathogenic to the roots and not to other plant tissues, but it is worth noting that in our experiment, the German isolate caused the most evident lesions (the longest necrosis, around 28 mm) in stem tissues of Turkey oaks.

Jönsson (2006) suggested the link between the root damage caused by *P. quercina* and the overall tree vitality because of different allocation of carbon within the plants. The susceptibility of English oak to this pathogen has been hypothesised that it depends on the carbon availability in roots being an essential resource for the defence system (Angay et al. 2014). The concentrations of non-structural carbohydrates (NSC) in roots depended on the alternating root/shoot growth rhythm, being high during the root flush (RF) and low during the shoot flush (SF). The infection success in the above experiment was high during RF and low during SF, resulting in a significantly positive correlation between pathogen DNA and NSC concentration in roots (Angay et al. 2014). In our experiment, we did not measure the concentration of NSC but phenological observations suggest that our inoculations were performed during the SF phase, which can explain the lower than expected success of infection. In the monitoring of oak health, it is wise to take into account the alternating growth of roots and shoots, because this phenomenon plays a crucial role for the susceptibility of lateral roots to the soilborne pathogens. The availability of NSC in oak roots has to be considered as a benchmark for susceptibility rather than resistance against *P. quercina*.

The soil properties, especially, the pH $[CaCl_2]$ value higher than 3.5 and the distinct decrease in the ground-water level enhance the virulence of *P. quercina* – the root pathogen (Thomas 2008).

The pathogenicity of south-Swedish isolates of *P. quercina* in acid forest soils under restricted water availability was studied by Jönsson et al. (2003). They found that in the acid forest soil, the percentage of fine-root dieback was high. Stress-induced susceptibility of the seedlings and/or increased aggressiveness of the pathogen in the forest soil are discussed as key factors to explain the difference in root dieback between soil types. In our experiment, oaks grew in the optimal pH (5.5–6.5) and were regularly watered, so such conditions surely did not stress the plants and did not increase the potential aggressiveness of tested *P. quercina* isolates.

The alternative explanation could be related to the research performed by Cooke et al. (2005), who distinguished two subgroups of *P. quercina* isolates, and probably, it may also be reflected in their pathogenicity. They stated relatively low genetic diversity, probably because of the predominantly inbreeding (homothallic) nature of *P. quercina*. However, the evidence on limited intra-site diversity, temporal variation and the lack of clonality within the European population suggest that some diversity is being maintained by occasional outcrossing and turnover of a reservoir of long-lived soilborne oospore (sexually derived) inoculum (Cooke et al. 2005). Probably, the above fact can explain the difference in the virulence between Polish and German isolates observed in our experiment.

The connection of tested *Phytophthora* species to oak decline is beyond the scope of this manuscript because an inoculation experiment alone (with three tree species) cannot be attributed to decline events. However, this assay helped us clarify to some extent the role of this group of pathogens as possible and potential contributors to the oak and beech decline phenomena in Europe. During this century, many European oak ecosystems (including the most valuable oak stands in Poland) have shown signs of stress and some have suffered periods of considerable decline and mortality (Oszako 1997; Basiewicz et al. 2007). Similar problems with oak health occurred in Bulgaria (Stefanov 1953). Many decline incidences showing typical symptoms for *Phytophthora* damage were recorded there.

However, the first recorded outbreak of oak decline took place in 1739-1748 (Thomas 2008). The suggested causes of European oak declines (apart from drought, winter cold and attacks by insects and fungi) may include root disease caused by the aggressive, exotic oomycete root pathogens belonging to Phytophthora genus (Jung et al. 1999, 2002; Jung and Nechwatal 2008), and therefore, in our experiment, we aimed to confirm it (or not) in the case of Polish and Bulgarian oaks. The significance of phytophthoras in Europeans forests is steadily rising, especially after the spectacular host jump of Phytophthora ramorum Werres, De Cock & Man in't Veld, which is the cause of the Sudden Oak Death in United States and is now killing Japanese larch trees in United Kingdom (Brasier 1999). In the past decades, pathogens from Phytophthora genus are associated with several cases of decline in European forests, causing much concern amongst scientists (Oszako 1997; Jung et al. 1999, 2002). Introduced pathogen Phytophthora cinnamomi Rands was found to be involved in Mediterranean oak decline, whilst another phytophthoras were found in the rhizosphere of damaged ash trees across Europe (Orlikowski et al. 2011). Alarmingly, since 1993, a newly emerged lethal disease is killing alders along European rivers. It is caused by the natural hybrid species Phytophthora alni Brasier & S.A. Kirk and is spreading across Europe (Brasier 1999).

Protecting plants from *Phytophthora* species is still a challenge. In the past decade, a large number of new species have been described beyond agriculture, which are known to cause enormous economic and environmental losses. The impact of exotic forest pathogens (in numbers of recognised species) on Mediterranean ecosystems becomes more severe every year (Garbelotto and Pautasso 2012; Santini et al. 2013). Under conditions of global warming, the survival and degree of root disease caused by this organisms seem likely to be enhanced and their host range might also expand (Nakova 2010; Orlikowski et al. 2011). Little is known about indigenous Phytophthora species in natural ecosystems but a diverse, trophically complex Phytophthora community is important in many forests. The number of described species has steadily increased, with a dramatic spike in recent years as new species have been split from old and new species have been discovered through exploration of new habitats (Hansen et al. 2012). The intercontinental movement and transplantation of infected plant material partially explains the appearance of new species in unexpected places. However, it is also likely that novel species arise as a result of the hybridisation and rapid evolution of introduced species under episodic selection pressures (Callaghan and Guest 2015). Hybrid progeny may possess equal or greater virulence than parent species, thereby posing an increasing risk to our natural environment and agricultural production systems. This assumption may trigger new threats posed by the introduction of plant pathogens into new environments (e.g. from Bulgaria to Poland or the other way around). The increasing trade in plants over the past decades will undoubtedly increase the risk of introducing alien species in countries where they are not yet present (Garbelotto and Pautasso 2012). Therefore, strict regulations of international trade exist particularly for harmful pathogenic organisms. The movement of plants and plant products resulting from human activity is now recognised as a major pathway for the spread of invasive alien species.

The majority of representatives from the Phytophthora genus are root rot agents, which in general are recognised as one of the key threats to European forests. The outcomes from several research projects, European COST Actions and conferences of the International Union of Forest Research Organizations (IUFRO) proved the threat posed by invasive pathogens (Santini et al. 2013). The large-scale analysis of Phytophthora infestations in Europe (Jung et al. 2015) has clearly demonstrated that the current international plant health protocols are outdated and seriously flawed. The existing phytosanitary system, despite of the European regulations posed by the European Commission (Directive 2000/29/EC), allows further introductions of potentially invasive *Phytophthora* pathogens to Europe (Jung et al. 2015). The new holistic and integrated systems

approach is urgently required. The high interest in the pathway analysis and the commodity-oriented analysis approach is very welcome.

CONCLUSION

All tested isolates belonging to *P. cryptogea*, *P. plurivora* and *P. quercina* species turn out to be pathogenic to Turkey oaks (*Quercus cerris*) of Bulgarian origin. The isolates of *P. cryptogea* and *P. plurivora*, but not those of *P. quercina*, are pathogenic to English oaks (*Q. robur*) of Polish origin.

The Bulgarian isolates of *P. cryptogea* and *P. plurivora* differ in their pathogenicity to European beeches (*F. sylvatica*) of Polish origin. From all tested isolates, especially three (*P. cryptogea* Belasica 10/3 isolated from *Cannabis sativa*, *P. plurivora* Tulovo 7/3 isolated from *Q. robur* and *P. plurivora* Tran 2/1 isolated from *A. glutinosa*) caused significant damage to European beech plants.

The observed pathogenicity of Polish and Bulgarian *Phytophthora* species have demonstrated their potential as important contributors to the declined phenomena of tested tree species (*F. sylvatica*, *Q. robur* and *Q. cerris*) in valuable ecosystems in both countries.

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ORIGINAL ARTICLE

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Preferences of people with disabilities on wheelchairs in relation to forest trails for recreational in selected European countries

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Abstract

The article presents the results of the survey on the preferences of disabled people in wheelchairs for selected features recreational trails in the woods. The study was conducted in 2015, including a sample of 130 people older than 18 years, in Poland, the Czech Republic and Slovakia (52 interviews in Poland, 21 in the Czech Republic and 57 in Slovakia). Respondents were interviewed both at the premises of the organisation as well as by email. The questions in the survey were designed to determine the preferences of the respondents in terms of recreational trails in the forests concerned: the optimal length of the route, recreational and educational points along the distribution routes of and usability of different types of forest roads. The results show that there is quite a lot of differences between the preferences of respondents from each of the analysed countries. Respondents from the Poland and Slovakia prefer shorter routes for recreation in forests, with a greater incidence of recreational and educational points along the route, whilst respondents in the Czech Republic prefer far longer routes, with a relatively larger distance between recreational points. In all the analysed countries, people with disabilities attributed highest usefulness to asphalt surfaces, concrete surfaces or surfaces made of cobblestones. The surface evaluated lowest for usability was made of wood.

Key words

disabled, development of tourism, recreation management, forest recreation management, forest roads

INTRODUCTION

Forests, in both Poland and many other countries, including the Czech Republic and Slovakia, because of the space it occupies (29.2%, 34% and 40%, respectively) as well as the spatial distribution, are seen as one of the fundamental values of the recreation. Proper management of recreational forest requires consideration of social needs and expectations whilst caring about the natural environment and landscape. In

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recent years, Poland and neighbouring countries are turning more and more attention to the adaptation of tourism infrastructure, recreation and education also for people with disabilities (Woźnicka 2007; Loučková and Fialová 2010; Jakubis and Jakubisová 2010). Currently, persons with disabilities constitute about 12% of Polish society (CSO 2014), 10% of the population of the Czech Republic (Czech Statistical Office 2014) and 8% of the population of the Slovak (Slovakia Statistical Office 2014). Tourism and recreation in forest areas play a very important role in the lives of people with disabilities. Tourism for people with disabilities should be treated according to Łobożewicz (2000) not only as entertainment and for relaxation but also as a means of therapy and education for and calming effects on the disability, allowing them to try their hand at different often difficult conditions. According to many experts on the issues of rehabilitation of persons with disabilities (Łobożewicz 2000; Wolski 1979; Weiss 1979; Jakubisová 2013) cultivation of tourism can counteract hypokinesis as well as accelerate and support the processes of renewal and regeneration of the body. Tourism also allows people with disabilities to socially integrate and adapt to normal life (Junek and Fialova 2012). The majority of tourist activities undertaken by humans takes place in the natural environment (Navratil et al. 2015). Forest gives people the opportunity to realise various forms of tourism and recreation. In all activities of tourism, recreation, leisure and education in the forests, they may participate as people with disabilities, regardless of the type of disability, age and psycho-motor possibilities. According to Łobożewicz (2000), practice shows that there is no discipline in tourism that could not be occupying for people with disabilities. The possibility of recreational use of the forest by people with disabilities is mainly conditioned by the availability of hiking trails and recreational trails including walking paths and educational paths. Linear features enable full use of the advantages of the forest environment and allow to reach interesting places in terms of natural and cultural heritage. The desired aim of this article is to determine the preferences of people with disabilities in wheelchairs, who are the inhabitants of Polish, the Czech Republic and Slovakia, in respect of the selected features of forest recreational trails, such as the optimal length of the route, recreational and educational points

along the distribution routes and usability of different types of forest roads.

MATERIAL AND METHODS

The article presents the results of surveys conducted in 2015 on a group of people with disabilities in wheelchairs. The research was conducted within the framework of the programme funded by the Visegrad Foundation simultaneously in Poland, the Czech Republic and Slovakia. The respondents were people with disabilities in wheelchairs assigned in activating associations (e.g. in Poland: Foundation for Active Rehabilitation; in the Czech Republic: League of Wheelchair Users; in Slovakia: the National Rehabilitation Centre in Kovacova, the Slovak Association of the Disabled, the Slovak Physical Disability Association, the Slovakian Paraolimpic Committee). The form of the questionnaire for respondents, the system of questions and cafeterias located in the closed questions were consulted with experienced workers and sociologists rehabilitation centres for disabled. The study was conducted jointly on trial with 130 people older than 18 years (52 interviews in Poland, 21 in the Czech Republic and 57 in Slovakia). The questionnaire was carried out either at the premises of the above organisations or by email. The questionnaire was created and published on Google pages. Information on how to complete the questionnaire directly online via the Internet was communicated on the Facebook page. The resulting research material was verified and coded. The collected material was stored in the form of a computerised database, created in Excel, on the basis of which the result tables were built.

For statistical analysis, analysis of variance was used at a significance level of 0.05, and the comparisons were performed using *post-hoc* Duncan test. Statistical analysis was performed to determine the statistically significant differences between the preferences of people with disabilities living in Poland, the Czech Republic and Slovakia. The questions in the survey were designed to determine the preferences of the respondents in terms of selected features in recreational trails in the forests concerned, such as the optimal length of the route; recreational facilities along the distribution routes, for example, holiday sites, picnic areas, educational points; and usability of different types of forest roads.

RESULTS

The results of research conducted in Poland show that according to the survey, the majority (about 71.2%) indicated that the length of trails in the forest should not exceed 4 km. The length should be 2-4 km for 32.7% of respondents and 1-2 km for 30.8% of respondent. According to 7.7% of respondents, trail length should not exceed 1 km, whilst 23% of respondents could not determine the preferred length of the trail. About 5.8% of respondents indicated that the trail length should be more than 4 km. Most of the respondents (47.6%) from the Czech Republic believed that forest trails and recreation facilities should be longer than 4 km. More than 33.3% of them preferred the route with 2–4 km in length, 14% preferred the route with 1-2 km in length and 4.8% of respondents could not specify their preferred length of the trails for recreation. On the other hand, the dominant view of people living in Slovakia (a total of 61.4% of responses), as in Poland, is that the route for recreational trails should be up to 4 km in length. The majority of respondents (38.6%) preferred route with a length of 2-4 km, 8.8% of the respondents preferred the very short route (1 km), nearly 14% of respondents were in favour of routes with a length of 1-2 km, whilst 19.3% of the respondents living in Slovakia preferred the long route (more than 4 km). Just as in studies conducted in Poland, here also we can see that about 19% of the respondents could not specify their preferred long recreational trails. The analysis of variance confirmed that the views of the respond-

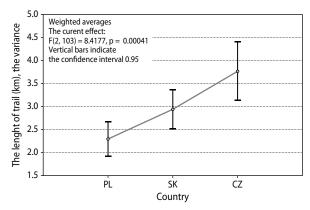


Figure 1. Comparison of the preferences of Polish respondents, Slovakia and the Czech Republic in terms of length of trails for recreation (95% trust intervals for the average)

ent from the three analysed countries differ from each other (p = 0.0004) (Fig. 1).

An in-depth analysis using multiple comparison test (Duncan's test) showed that the opinions of Polish and Slovakian respondents about the optimal length of the route do not differ from each other. It was found here that there are basically significant differences in views between respondents from Poland and the Czech Republic and Slovakia (Tab. 1).

No.	Duncan test; variables – length of trail (km) The approximate probability for <i>post-hoc</i> test Error: ASS = 1,7597, DF = 103				
	country	PL	SK	CZ	
1	PL	-	0.058	0.000	
2	SK	0.058	-	0.016	
3	CZ	0.000	0.016	-	

Table 1. Duncan test of variables - length of trail (km)

According to most of the respondent from Poland (34.6%), the distance between successive points along the recreational or educational paths should be at 200-500 m. Quite large in this case, the group of respondents (30.8%) believe that this distance should be 100-200 meters or more than half a kilometre (26.9% of respondents). Only 7.7% of Polish respondents thought that educational spots and resting places should be located from each other at a distance of no more than 100 m. The results of research conducted in Slovakia have shown, as in the case of research carried out in Poland, that the vast majority of respondents (42.1%) is convinced of the need for the deployment of recreational facilities along the routes in the distance of 200-500 m. At the same time, approximately 8.7% of the respondents were of the opinion that the holiday and education spots should be at 100-200 m. Some of respondents (24,6%) indicated smaller distance (<100 m) and longer then 500 m. On the other hand, amongst those surveyed in the Czech Republic prevailed (52.4% of respondents) the need for placing the devices along the trails for recreational holidays in the woods at a distance of not less than 500 m from each other. Quite numerous here was a group of respondents (33.3%) declaring the need to introduce recreational facilities at 200-500 m. About 14.3% of respondents were in favour of installing leisure time facilities at 100-200 m along the forest trails for recreational needs. However, there were not any respondents who prefer more frequent placement points, recreational and educational leisure routes and hiking trails that can pass through forests.

From the graphical analysis of variance it can be concluded that the respondents' views from three analyzed countries differ from each other (p = 0.023) (Fig. 2).

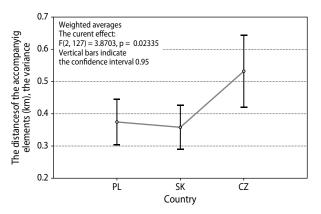


Figure 2. Comparison of the preferences of Polish respondents, Slovakian and from the Czech Republic considering (95% trust intervals for the average) the distance between successive recreational and educational points along the forest trails for recreational purposes

Analysis of *post-hoc* Duncan test showed no statistically significant differences between the views of respondents of Poland and Slovakia on above-mentioned issues. Significantly different are the preferences within the three countries on the frequency of distribution of leisure time facilities and education facilities along forest trails for recreational needs (Tab. 2).

Table 2. Duncan test of variables – the preferred distance of holiday destinations along the recreational trails

No.	Duncan test; variables – the preferred distance holiday destinations along the recreational trails The approximate probability for <i>post-hoc</i> test Error: ASS = 0,064, DF = 127				
country		PL	SK	CZ	
1	PL	-	0.789	0.008	
2	SK	0.789	-	0.005	
3	CZ	0.008	0.005	-	

Another analysed characteristics of the recreational trails was the surface. The types of surfaces such as natural, hardened gravel, asphalt, made from wood (wooden bridge), paving and pavement with concrete slabs were evaluated in terms of usability for people with disabilities in wheelchairs using a four-point scale (unnecessary, rarely needed, rather necessary, required). Research conducted in Poland showed that surfaces such as concrete slabs, asphalt and paving stones were considered to be most suitable for people in wheelchairs (high percentage of respondents (90.4%, 96.2%) and 59.6%, respectively) recognised the surface as easy and very easy to use). In Slovakia and the Czech Republic, the vast majority of respondents also pointed to the great usefulness of asphalt (94.7% and 90.9%, respectively). The views of respondents in the Czech Republic and Slovakia on the high usefulness of the surface of the concrete slabs and paving cobblestone were not as strong as those in the case of Polish respondents. The vast majority of respondents in the Czech Republic and Slovakia (36.4% and 45.6%, respectively) indicated that the surface of the concrete slab is moderately friendly for wheelchair users. In turn, the paving has been assessed by the majority of respondents from the Czech Republic (50%) as a surface unsuitable for wheelchairs. The majority of Slovaks (43.9%) considered this surface as moderately useful.

In Poland, the surface made of wood received the largest percentage of ratings (5.8%) for 'useless surface'. This surface was considered unsuitable or less suitable by 46.2% of respondents from Poland. This type of surface was negatively rated by most of the respondents (57.2%) from Slovakia, recognising it as unsuitable (14% of respondents) or low usefulness (42.1%). Similarly, a total of 50% of respondents in the Czech Republic indicated that the surface is not suitable for people in wheelchairs, including 22.7% of respondents indicated that the surface did not suit wheelchair users and 27.3% of the respondents were of the opinion that it is useful to a limited extent.

Amongst the large part of Slovak respondents (77.2%), there is a view that the least friendly surface for people in wheelchairs is the ground paved with gravel (42.1% considered this type of surface as not at all useful and 35.1% as too little useful for wheelchair users). Similarly, respondents from the Czech Republic (a total of 68.2%) recognise this type of surface to be inadequate (36.4%) or a little friendly (31.8%) for persons in wheelchairs. At the same time, only 3.5% of the surveyed Slovaks and as much as 13.6% of the Czech

respondents were of the opinion that it is a surface very friendly to people with disabilities in wheelchairs. In Poland, a total of 42.3% of the respondents recognised the hard surface gravel as unsuitable or less suitable for tourism of people with disabilities in wheelchairs.

Performed statistical analysis confirmed that only in the case of two types of surface, namely, asphalt and wood, there is no statistical difference between the preferences of people living in Poland, the Czech Republic and Slovakia. The views on usability of cobblestones are already fundamentally different from each other (p = 0.000), as shown by the analysis of variance (Fig. 3).

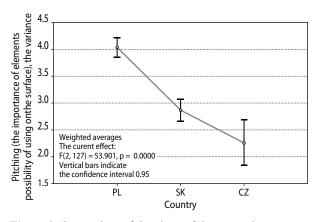


Figure 3. Comparison of the views of the respondents from the Poland, Slovakia and the Czech Republic on the suitability of the surface of the cobblestones (95% confidence intervals for the mean)

An in-depth statistical analysis using multiple comparison test showed that there are differences between the preferences of the respondents living in Slovakia and the Czech Republic, Slovakia and Poland and the Czech Republic and Poland (Tab. 3).

Table 3. Duncan test of variables – type of pavement:

 cobblestone

No.	Duncan test; variables – type of pavement: cobblestone The approximate probability for <i>post-hoc</i> test Error: ASS = 0,568, DF = 127				
	country PL		SK	CZ	
1	PL	-	0.000	0.000	
2	SK	0.000	-	0.001	
3	CZ	0.000	0.001	_	

In the case of other variants of surface (natural, paved with gravel, concrete slabs), there were statistically significant differences in the preferences of the respondents coming from the Czech Republic and Slovakia (Tab. 4).

	Duncan test; variables – type of pavement								
	country PL SK CZ								
	Error: ASS = 0.616, DF = 127								
A	typ	be of pavemen	t: concise natu	rai					
	country	PL	SK	CZ					
1	PL	-	0.008	0.000					
2	SK 0.008		_	0.236					
3	CZ	0.000	0.236	_					
в	Error: ASS = 0.720, DF = 127								
	type of pavement: paved with gravel								
1	PL	_	0.000	0.007					
2	SK 0.000		_	0.209					
3	CZ 0.007		0.209	-					
С	Error: ASS = 0.475, DF = 127								
	type of pavement: concrete slabs								
1	PL	_	0.000	0.000					
2	SK	0.000	_	0.963					
3	CZ	0.000	0.963	-					

Table 4. Duncan test of variables – type of pavement: concise natural (A), paved with gravel (B) and concrete slabs (C)

DISCUSSION

Studies were carried out on a relatively large sample that are not tested. This is due to at least two reasons, first, there is virtually no technical possibility to conduct the survey amongst people with disabilities in the woods. It is not usually difficult, because of the fact that forests are poorly adapted to the needs of people with disabilities, and besides, people with disabilities rarely visit forests. For example, the research by Woźnicka (2007) shows that 40% of the total length of roads in the forests of the city of Warsaw is available for people in wheelchairs. In large part, this is due to the poor technical condition of the surface of the road and too large vertical drops (Woźnicka 2009). Woźnicka (2006) by conducting a survey of persons with disabilities in the forests found that more than 40% of respondents rested in the woods just a few times a year. Therefore, a significant difficulty is getting to this part of the population. People with disabilities in wheelchairs do not form compact groups such as the blind or deaf. Often, for various reasons, they are also people alienated socially reluctant to seek information about their disability (Loučková and Fialová 2010).

So far in the literature in the field of tourism, development and recreation appeared different; the views on the optimal length of recreational trails are often contradictory. For example, according to Płocka (2002), walking in the woods should span a maximum length of 12 km optimally and be 8 km away. In turn, the optimal length of forest trails should be 1-2 km (Łonkiewicz and Gluch 1991). At the same time, there is a lack of information on the optimal length of routes for people with disabilities in wheelchairs, the needs and expectations of this social group. The study showed that in Polish conditions, as well as in the forests of Slovakia, recreational trail adapted for wheelchairs should not exceed 4 km. According to a research conducted in the Czech Republic, it shows that these routes may be longer. Perhaps it has to do with the fact that in the Czech Republic prevailed (50%) people moving in electric wheelchairs (0% and 14% of respondents in Poland and Slovakia, respectively), much more comfortable and easy to use. Perhaps, therefore, it was the kind of wheelchair that affects the preferences of users. This view appears to be justified in the context of the results regarding the preferred spacing/distance between the successive recreational respectively. Most of the respondent from the Czech Republic preferred the spacing of recreational facilities at 500 m, and more often, for example, 200-500 m, as was the case in respect of the respondents from Poland and Slovakia. It should also be noted that in the literature, for example, in Poland, for years functioned a notion that recreational facilities on the routes should be located at 1000-1500 m (Łonkiewicz and Głuch 1991). However, the recommended distance of positioning points along the routes was based solely on the experience of the authors of the design guidelines, rather than on the results of users' preferences of the forest recreational trails. As shown by the research presented in the article, ideas of designers do not always coincide with the opinion of tourists and vacationing in the woods.

Taking into account the results of research on the functional characteristics of the surface, it can be stated

that the respondents have shown great pragmatism in selecting surface most friendly to people with disabilities in wheelchairs. Three most preferred and highest rated types of surfaces in Poland are asphalt, concrete slabs and paving stones in a little part of a forest landscape; they are certainly pavements that are so ecological and, besides, in the structure of the network of forest roads do not occur as often as ground roads. Amongst the respondent from the Czech Republic and Slovakia, paving of cobblestones or concrete slabs are seen as less suitable for wheelchair access than those from Poland. It seems that this is related to the fact that in the Czech Republic and Slovakia, surfaces made of concrete slabs and granite are quite popular in the forests, in contrast to the Polish conditions (Loučková and Fialová 2010). Granite stones is characterised by an uneven surface; therefore, it can be a big obstacle for wheelchair users. On the other hand, in the case of concrete slabs, difficulty is represented generally in the joining of the consecutive panels (Jakubisová 2013). Surprisingly, the authors of the research found that the fact that the vast majority of respondents from Poland, the Czech Republic and Slovakia have acknowledged the usefulness of the hopper surfaces made of wood is unsuitable (i.e. the wooden sidewalks). This is all the more puzzling that quite a lot of routes prepared for people with disabilities in the woods is made of wood (e.g. in Poland: 'Royal Springs' in Kozienice Forest District and 'The Royal Oak Route and Grand Dukes of Lithuania' in the Białowieża Forest District). As rightly observed (Kotásková 2010), wood surface in the rain becomes slippery and so unfriendly for people in wheelchairs. Thus, once again it proves that the views of designers and initiators of the emerging infrastructure of recreation in forests designated for people with disabilities in wheelchairs are not always consistent with their preferences, needs and expectations.

SUMMARY

The study found a number of significant differences in the views of people with disabilities surveyed in Poland, the Czech Republic and Slovakia. A proper understanding of the differences in the preferences of the respondents require further research to establish the relationship between their views on forest trails for recreation and features such as the period of use of wheelchairs, their types (stable, electric initially, the three-wheel, handcycle and others) and a group of disability (paraplegia, tertraplegia and others). Zoning traffic intensity of recreational forests and the creation of modern rules of access to and development of recreational forest must take into account the needs and expectations of disabled users of the area. This issue is still little understood. As a result, the current guidelines for recreational trails in the woods, in both Poland and neighbouring countries, hardly contribute to the formation of routes friendly to people with disabilities in wheelchairs.

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ORIGINAL ARTICLE

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Four different *Phytophthora* species that are able to infect Scots pine seedlings in laboratory conditions

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Abstract

To investigate susceptibility of young Scots pine seedlings to four *Phytophthora* species: *Phytophthora cactorum*, *Phytophthora cambivora*, *Phytophthora plurivora* and *Phytophthora pini*; seven-day-old seedlings of Scots pine (15 seedlings per experiment) were infected using agar plugs of the respective species. Control group also consisted of 15 seedlings and was inoculated with sterile agar plugs. Results unambiguously show that after 4.5 days, all seedlings show clear signs of infection and display severe symptoms of tissue damage and necrosis. Moreover, three and two seedlings in the *P. cactorum* and *P. cambivora* infected seedlings groups, respectively, collapsed. The length of largest necrosis measured 13.4±3.90 mm and was caused by *P. cactorum*. To rule out any putative contamination or infection by secondary pathogens, re-isolations of pathogens from infection sites were performed and were positive in 100% of plated pieces of infected seedlings. All re-isolations were, however, negative in the case of the control group. Detailed microscopic analyses of infected tissues of young seedlings. Therefore, our results suggest *Phytophthora* spp. and mainly *P. cactorum* and *P. cambivora* as aggressive pathogens of Scots pine seedlings and highlight a putative involvement of these species in the damping off of young Scots pine seedlings frequently observed in forest nurseries.

Key words

pathogenicity, Pinus sylvestris, Phytophthora cambivora, Phytophthora cactorum, light microscopy

INTRODUCTION

Scots pine (*Pinus sylvestris* L.) is the most common woody species in Polish forests and is present in around 58.8% of total forest stands managed by State Forests in Poland (Milewski 2015). Unfortunately, Scots pine is susceptible to several pathogenic organisms in natural stands, planted forests and nurseries, including *Heterobasidion annosum* (Fr.) Bref. (Sierota 1996; Małecka and Sierota 2003). Different needle diseases, such as red band needle blight, caused by the fungus Dothistroma septosporum (Dorog.) Morelet also play an important role in the decline of this relevant tree species (Mańka 1998; EFSA 2013).

Phytophthora species are fungal-like organisms that nest within the SAR supergroup (Lara and Belbahri, 2011; Adl et al. 2012). Till now, more than 140 species and Phytophthora taxa are known (Abad 2014). These species are able to infect different tissues (fine roots, bark, stems, leaves and shoots) on different host species in nurseries, ornamental plantings and forest stands (Erwin and Ribeiro 1996; Pérez-Sierra and Jung 2013; Jung et al. 2013). Previously, several Phytophthora spp. have been recorded as pathogens of different conifer species, including Phytophthora cactorum (Leb. and Cohn) Schröeter (Hudler 2013), Phytophthora cambivora (Petri) Buisman (Vannini and Vettraino 2011), Phytophthora lateralis Tucker and Milbrath (Hansen 2011), Phytophthora pini Leonian (Hong et al. 2011), Phytophthora pinifolia (Duran et al. 2008; Hansen 2012), Phytophthora plurivora Jung and Burgess (Jung and Burgess 2009) and Phytophthora citrophthora (R.E. Smith et E.H. Smith) Leonian (Oszako and Orlikowski 2004).

Occurrence of these pathogenic *Phytophthora* spp. in nurseries and their subsequent introduction into seminatural and natural ecosystems is the main driver of their dispersal and causes huge damage to forest ecosystems (Moralejo et al. 2009; Jung et al. 2015). In Poland, several species of *Phytophthora* were recorded in forest nurseries, including *Phytophthora citricola* Sawada on ash (Orlikowski et al. 2004), *Phytophthora cinnamomi* Rands on pedunculate oak (Oszako and Orlikowski 2005), *P. plurivora* on European beech and silver fir (Orlikowki et al. 2004; Stępniewska 2005), *P. cactorum* on European beech (Stępniewska 2003) and *Phytophthora gonapodyides* on a wide range of hosts (Oszako et al. 2007). *Phytophthora* spp. were also previously re-

n around (Orlikowski et al. 2012) was confirmed.
 e Forests Owing to widespread and importance of Scots pine in Polish forests, our main goal was to determine whether the *Phytophthora* species can threaten Scots

whether the *Phytophthora* species can threaten Scots pine plants grown in nurseries and in young stands. Pathogenicity test with four selected *Phytophthora* species were, therefore, conducted, to determine the susceptibility levels of the Scots pine seedlings with selected species. Results and implications of the study are discussed in this paper.

corded on Scots pine in forest and ornamental nurseries in Poland, and the presence of *P. cinnamomi* (Duda et

al. 2004), P. cactorum, P. citrophthora and P. plurivora

MATERIAL AND METHODS

Phytophthora species and isolates used in the experiment

Isolates of *Phytophthora* species used in the colonisation test were obtained from the Forest Research Institute-IBL *Phytophthora* culture collection. All isolates originated from different declining forest tree hosts. They were all morphologically identified, with molecular confirmation of morphological findings. Four different *Phytophthora* species were used in this experiment (Tab. 1).

Table 1. Description of isolates used in the experiment

Species	Host	Collection number	GenBank
P. cactorum	Acer pseudo- platanus	IBL325	JX276090
P. cambivora	Fagus sylvatica	IBL340	JX276088
P. pini	Poplar clone I214	IBL482	KF234656
P. plurivora	Quercus robur	IBL213	JX276023

Pathogenicity tests

In the different pathogenicity tests, non-stratified seeds of Scots pine were used (Zwoleń provenience). Seeds were incubated at 25°C under light for 24 h in glass chambers moisturised with sterile cotton wetted with sterile distilled water (Załęski et al. 1998). *Phytophthora* species used in the assay were transferred onto V8A media, prepared with 800 ml·L⁻¹ distilled water, 200 ml·L⁻¹ V8 juice (Tymbark, Poland), 18 g·L⁻¹ agar-agar (BTL, Poland) and 3 g·L⁻¹ of CaCO₃. The inoculum of *Phy*- *tophthora* spp. was recovered from the growing edges of 3-4 days old colonies incubated at $22-25^{\circ}$ C in the dark (Orlikowski et al. 2004; Milenković et al. 2012). Agar plugs with mycelium (~1 × 1 cm in size) were placed in sterilised, 90-mm glass Petri dishes containing sterile filter paper. Tips of seven-day-old Scots pine seedlings with a radicle length of 15–20 mm were placed on the top of agar plugs with mycelium in Petri dishes. The design of the experiment was completely randomised with a total of five replicates. For each replication, seedlings were used in individual Petri dishes (N = 15 seedlings per group). Control group also consisted of 15 seedlings and was placed on the sterile agar plugs.

Filter paper in the dishes was moisturised with 5 mL of sterile distilled water, and dishes were incubated under daylight at approximately 20–22°C. Dishes were monitored for every 8 h until the first seedlings collapsed, and measurements of the total lengths of seedlings and the necrosis length were performed.

To re-isolate the *Phytophthora* spp. from declining tissues, small necrotic parts of the seedlings (0.1–0.2 mm in size) were cut using sterile razor blade and plated on selective media (V8A-PARPNH), prepared according to Jung et al. (1996). Tissue fragments from control group were also plated onto V8A-PARPNH media. Observations were made under light microscope ZEISS Axioskop 2, equipped with Nikon Ds-fi1 camera, and NIS Elements AR4[®] software.

Statistical analyses

Data from the pathogenicity test were subjected to Kruskal–Wallis nonparametric test. The mean values of total length of plant, length of necrosis and percentage of necrosis were compared between the experimental groups using R software (PMCMR library).

RESULTS

Pathogenicity tests and necrosis lengths

After 4.5 days of incubation when the first seedlings collapsed, measurements of necrosis lengths as well as total length of the seedlings were performed and are presented in Table 2. Three and two seedlings, respectively, from the *P. cactorum* and *P. cambivora* groups collapsed. Photos of necrotic lesions of these seedlings are presented in Figure 1.

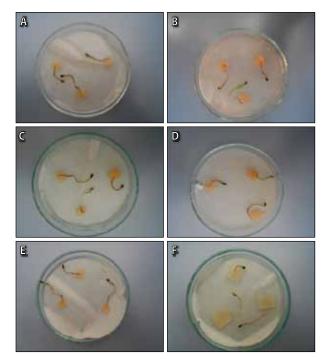


Figure 1. Necrotic lesions on seedlings caused by different *Phytophthora* species: A - P. *cambivora*; B - P. *plurivora*; C - P. *pini*; D-E - P. *cactorum*; F – control

T-11. 0 D. (1	$C_{41} = 1^{\circ}CC_{44} = DI_{44} = I_{41}$		
Table 2. Pathogenicity o	f the different Phytophthora	species inoculated on	seedlings of Scots pine

Species	Number per experi- mental group	Number of collapsed seedlings	Total length of seedlings	Necrosis length	Percentage of necrotic length
		conapsed securings	$mean \pm SD (mm)$		$\% \pm SD$
Control		0	43.06±10.51	0	0
P. cactorum		3	37.00±4.14	13.4±3.90	37.49 ±14.15
P. cambivora	15	2	36.73±9.67	11.80±3.36	32.81±7.59
P. pini		0	43.46±12.87	10.93±3.89	27.09±10.85
P. plurivora]	0	39.53±10.44	9.66±3.10	26.41±10.59

Re-isolations were successful in 100% of plated necrotic parts of the seedlings onto selective agar media, whilst the parts from control group showed no growth and were negative for the presence of any pathogenic or saprotrophic organisms.

Nonparametric test was applied to check statistical significance of seedling lengths between the tested experimental groups. No statistically significant difference between the tested experimental groups could be observed (p = 0.337). However, necrosis length between the different experimental groups was statistically significant (p = 0.000). To determine which experimental groups were statistically different, the post hoc test was applied. Results clearly suggest statistical support for the difference between each *Phytophthora*infected experimental and the control group. We could also provide statistical support for *P. cactorum* causing the largest necrosis length than all the other tested species (Fig. 2).

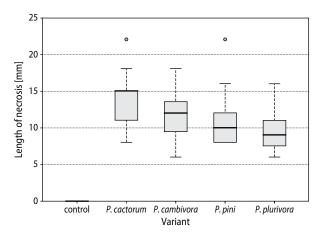


Figure 2. Necrosis lengths observed on Scots pine seedlings infected with the different *Phytophthora* spp.

Percentage of necrosis length (estimated as the percentage of observed necrosis per total plant length) was calculated for the control and experimental groups. The comparison between percentage of necrosis length amongst the different groups showed statistically significant difference between control and experimental groups infected by the respective *Phytophthora* species (p = 0.000). Between variants in which the *Phytophthora* was tested, no statistically significant difference was observed. The highest value was recorded for the *P. cactorum* experimental group (Fig. 3).

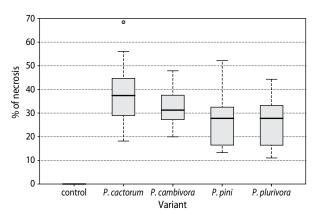


Figure 3. Percentages of necrosis length across the different control and *Phytophthora* infected experimental groups

Microscopic analyses

All tested *Phytophthora* spp. in the time course of this experiment were able to colonise the tissues of young seedlings. Observation under light microscope showed the presence of numerous *Phytophthora* structures in the tissues of all the infected seedlings. In most of the cases solely, oogonia with oospores were recorded (Fig. 4), whilst in some other cases, both oogonia and sporangia were recorded (Fig. 4C, D and H). No *Phytophthora* structures were recorded in the control group in which the tissues remained healthy.

DISCUSSION

Results obtained in the course of this study unambiguously document the ability of the four tested Phytophthora species to infect the young Scots pine seedlings. Severe seedlings symptoms and damage to infected tissues reflected by necrosis length that differ in size between the respective pathogens were observed. The most aggressive species in our experimental conditions was P. cactorum, according to all monitored parameters. This homothallic species with papillate sporangia was one of the first described species from the Phytophthora genus (Erwin and Ribeiro 1996). This pathogen is causing damages on a wide range of hosts worldwide in nurseries, forest stands, ornamental and amenity plantings (Erwin and Ribeiro 1996), including Scots pine (Hudler 2013). This species belongs to Phytophthora Clade 1 (Martin et al. 2014) and is able to operate different hybridisation events (Érsek and Man in 't Veld 2013), giving rise to more aggressive and destructive pathogens (Man in't Veld et al. 2007, 2012).

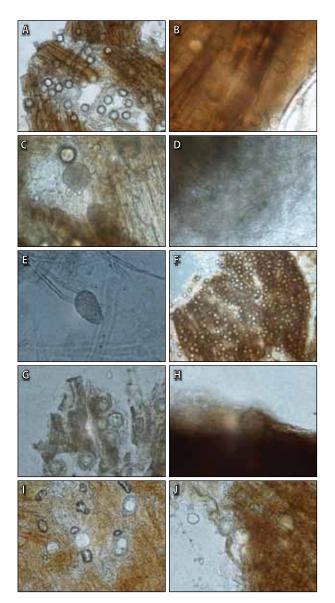


Figure 4. Microscopic analyses of the necrotic length of Scots pine tissue

P. cactorum: A – numerous oogonia in the necrotic tissue at ×200 magnification, B – oogonia in the tissue at ×400 magnification, C – oogonia and papillate sporangia in the broken necrotic tissue at ×400 magnification; *P. cambivora*: D – hypha and young sporangia at ×200 magnification, E – non-papillate, mature sporangia on the surface of infected seedlings at ×400 magnification; *P. pini*: F – numerous oogonia in the tissue at ×100 magnification, G – oogonia in the broken necrotic tissue at ×400 magnification, H – semi-papillate sporangia on the surface of necrotic tissue; *P. plurivora*: I and J – oogonia in the necrotic tissue. The second more aggressive species in our experiment was *P. cambivora*. This is heterothallic species with non-papillate sporangia, easily recognised by ornamented oogonia and two cells antheridia. It is known worldwide as the causative agent of ink disease (Erwin and Ribeiro 1996).

The third aggressive species in this experiment is *P. plurivora*, one of the most widespread species in different ecosystems worldwide. This species was previously lumped in the *P. citricola* Sawada species complex but was recognised as a new species in the study of Jung and Burgess (2009). It can parasitise a wide range of plant hosts, causing different damages and thriving in different ecological niches (Jung and Burgess 2009).

All listed species were often previously reported from different Polish nurseries (Duda et al. 2004; Orlikowski et al. 2012). However, there is no much data concerning *P. pini*. *P. pini* was described for the first time by Leonian (1925) and resurrected by Hong et al. (2011) as a part of the *P. citricola* complex. According to these authors, this species is a pathogen of at least seven genera in Europe and North America and many past damages that were assigned to *P. citricola* were actually caused by this species and *P. plurivora*. In Europe, this species was recorded in Finnish nurseries (Rytkönen 2011) and in poplar plantations in Serbia (Milenković I., unpublished data).

During the analyses of necrotic tissue using light microscopy, numerous oogonia were recorded inside and on the surface of the tissue of infected seedlings. Hyphae and sporangia of tested *Phytophthora* species were also recorded in congruence with multicyclic nature of these pathogenic organisms (Erwin and Ribeiro 1996).

Results obtained in this experiment highlight the potential risk caused by the presence of these species in Scots pine nurseries and in the first years after transferring them to the forest. On the basis of our preliminary results, a large-scale study involving sampling, isolation and species diversity determination of *Phytophthora* species in different young conifer stands and nurseries of Scots pine is warranted. Soil-infestation pathogenicity tests, as the closest experimental conditions to natural way of infection, are also required in the future to evaluate the potential of *Phytophthora* species to infect and damage mature Scots pine roots in field conditions.

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Phytophthora quercina infections in elevated CO₂ concentrations

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Abstract

In the last decades, a new wave of oak decline has been observed in Poland. The most important pathogenic organisms involved in this phenomenon are probably soil-borne pathogens *Phytophthora*genus, especially *P. quercina*. In this work, we sought to test the influence of elevated CO_2 concentration on the susceptibility of oaks (*Quercus robur* L.) to infection by *P. quercina*. In order to test the susceptibility of oak fine roots to infection, we applied phosphite-based fertiliser Actifos in 0.6% concentration. One-year-old oak seedlings were grown for one year in greenhouse with either an ambient atmosphere (400 ppm CO_2) or an elevated (800 ppm) concentration of CO_2 . Oaks grown at the elevated CO_2 concentration developed longer shoots as proved by statistically significant differences. However, there was no difference in the development of root systems. The application of Actifos had a positive significant effect on the development of shoots and the surface area of fine roots under the elevated CO_2 concentration.

Key words

Actifos, Oomycetes, Quercus robur, carbon dioxide concentration

INTRODUCTION

The *Phytophthora* species are among the most harmful plant pathogens. They belong to oomycetes, important cause of plant disease in many parts of the world. The degree to which *Phytophthora* species has been causing root damage in recent years is of great importance to the protection of Europe's forests (Brasier 1999).

In the early 1990s, the importance of these pathogens began to increase, when *P. cinnamomi* was detected as the causal agent responsible for oak damage in Iberia, and *P. alni* for the mortality among alders in Europe's riparian ecosystems (Brasier 1999). In Europe, the year 2003 brought the first record of *P. ramorum* (Lane et al. 2003) to which the phenomenon of oak decline and later Japanese larch dieback is attributed. The latter species had earlier been responsible for the damage done to trees in North America (Goheen et al. 2002). One of the most damaging species of European oaks is *P. quercina*, described as a new species by Jung et. al. (1999). Soil-

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borne species of Phytophthora were also isolated from 19 of 30 examined oak forest areas in Italy (Vettraino et al. 2002). They detected 11 Phytophthora species, including P. cambivora, P. cinnamomi, and P. cactorum in central and southern Italy and P. quercina in the northern and central part of the country. P. quercina was the only species significantly associated with declining oak trees. This situation has worsened by the lack of available anti-infection products, which could protect fine roots of trees. Studies performed in Australia, USA, and recently in Germany on the protection of natural and semi-natural ecosystems against pathogenic Phytophthora species have shown that the only way to protect forests is to use phosphite (an inorganic salt of phosphoric acid). The resulting research on the use of phosphite was not as effective as the use of phosphoric acid (MacIntire et al. 1950). Over the next 30 years of research on the use of phosphoric acid in agriculture as a fertiliser were abandoned, but the focus was on explaining its effect on plant growth. It was initially thought that the phosphite fertiliser interacts directly in a toxic manner on organisms of the genus *Phytophthora*, and therefore should be applied directly at the site of infection, and at a relatively high concentration (Fenn and Coffey 1984, 1985). Detailed studies were able to confirm that, notwithstanding a relationship between the phosphite concentration and effectiveness of its action, the use of a high concentration proved not to be entirely toxic to fungi (Smillie et al. 1989). Further studies (Grant et al. 1990) revealed a direct effect exerted by phosphite in reducing sporulation by Phytophthora fungi. The use of phosphite, thus, has an impact mediated via a modified interaction between pathogen and host as far as cell walls of hyphae are changed, and the number of suppressors masking the disease is reduced (Grant et al. 1990).

These findings prompted the rapid development of many new fertilisers based on phosphites (Lovatt 1990). In order to reduce incidences of broad-leaved tree dieback, the phosphite fertilisers were successfully applied in Germany (Jung 2008). Similarly, in Poland, the Institute of Pomology and Floriculture conducted research on the possibilities of phosphite fertiliser (Actifos) application in plant protection (Orlikowski 2004; Korzeniowski and Orlikowski 2008; Muszyńska and Orlikowski 2010; Tkaczyk et al. 2014a, 2015).

The present paper aimed to test the influence of elevated CO_2 concentration on the susceptibility of oaks (*Q. robur*) to infection caused by *P. cactorum* and *P. plurivora*. Besides CO_2 level, the effective dose of phosphite fertilisers to increase the tolerance of oaks to infection by *Phytophthora* was examined.

MATERIAL AND METHODS

Oak seedlings were grown in the controlled conditions from seeds in autoclaved medium (to avoid infection from nursery soil). Seeds were stratified in sterile sand at 5°C for several weeks prior to the germination. Then, they were transferred to 71 pots filled with the medium (soil – 2/3, mixed with vermiculite – 1/3). They were grown for one year in the greenhouse boxes before the experiment started either with an ambient atmosphere (400 ppm CO₂) or an elevated (800 ppm) concentration of CO₂. Later, they were inoculated through the soil (in the natural way) using millet according to Vettraino's method modified by Jung (2009) and Jung et al. (1996, 2000). Several models of the experiment were proposed for ambient and elevated CO₂ conditions:

- 1. Oaks inoculated with *Phytophthora* via soil: with isolates of *P. quercina;*
- Oaks treated with Actifos (0.6%) containing phosphites (PO₃) – foliar application;
- 3. Oaks treated with Actifos (0.6%) and *Phytophthora* inoculation via soil;
- 4. Control oaks (no treatment, sterile medium put into the soil).

Four months after inoculation, the oaks were removed from soil, and fine roots were washed and scanned using WinRhizo[®] software (Regent Instruments, Canada), and EPSON Perfection V700 Photo Scanner. The diameters, lengths of shoots, and roots of each oak were assessed. The biomass of above- and below-ground parts was dried and weighed. The number of living fine roots (<2 mm) per length of mother roots (2–5 mm) was calculated, prior to the data obtained from the WinRhizo software being transferred to Excel sheets for calculation of the following representative parameters:

- 1. Fine-root length (FRL) (cm)
- 2. Fine-root length per mother root length (FRL/MRL)
- 3. Fine-root surface area (FRSA) (cm²)

A statistical analysis of these data was performed using the Kruskal-Wallis non-parametrical test (*STA-TISTICA v. 10*).

RESULTS

Oaks growing in an elevated CO₂ concentration

Actifos slightly stimulated the growth of shoots, simultaneously inhibiting the development of roots (Fig. 1A and B). However, test probability values of 0.2372 (Fig. 1A) and 0.4974 (Fig. 1B) confirm that there were no significant differences between these variants. A certain tendency of lower average length of shoots could be observed in the inoculation variant with *Phytophthora*. The inoculation variant with addition of Actifos did not change (improved) this situation, though the application of the Actifos alone was associated with elevated mean values for the lengths of shoots. This is probably mainly thanks to the content of Nitrogen above all (N = 10%). In this case, there was no detectable stimulation of the growth of roots when Actifos alone was applied.

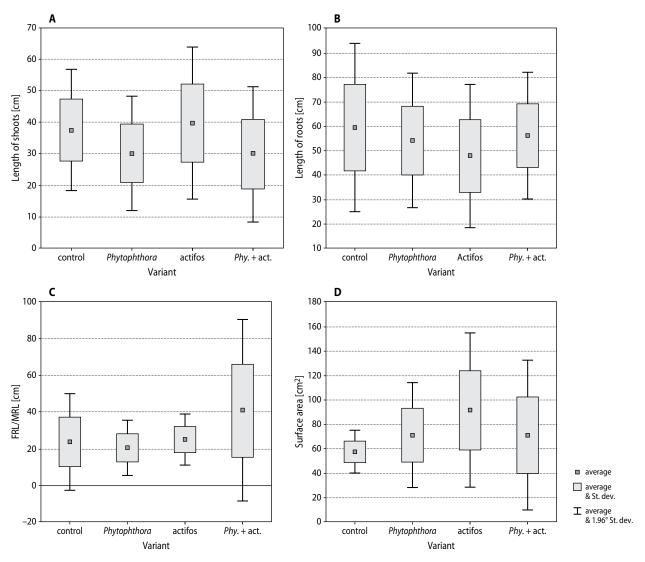


Figure 1. Comparison of the lengths of oak shoots (A), lengths of roots (B), FRL/MRL (C), and surface area (D) grown in 800 ppm CO₂

The ratio of the total length of fine roots to the length of mother roots (FRL/MRL) – yields a test prob-

ability value of 0.1454 (non-significant, Fig. 1C). Nevertheless, the lowest values were found for the variant involving inoculation of oaks with *Phytophthora*, while the highest entailed with the inoculation of plants with *Phytophthora* and simultaneous application of Actifos. This suggests positive tendency for fine roots to recover in the presence of phosphites.

The probability value of 0.1829 again failing to attest the statistically significant differences among the surface occupied by fine roots in the different experimental variants (Fig. 1D). Nevertheless, the highest values of root surface area were found where Actifos was used, while the smallest value was recorded for the control oaks.

Oak growing in an ambient atmosphere

Concerning the length of oak shoots growing in the ambient atmosphere revealed no statistically significant difference between the variants (Fig. 2A). The probability test value equalled 0.5447.

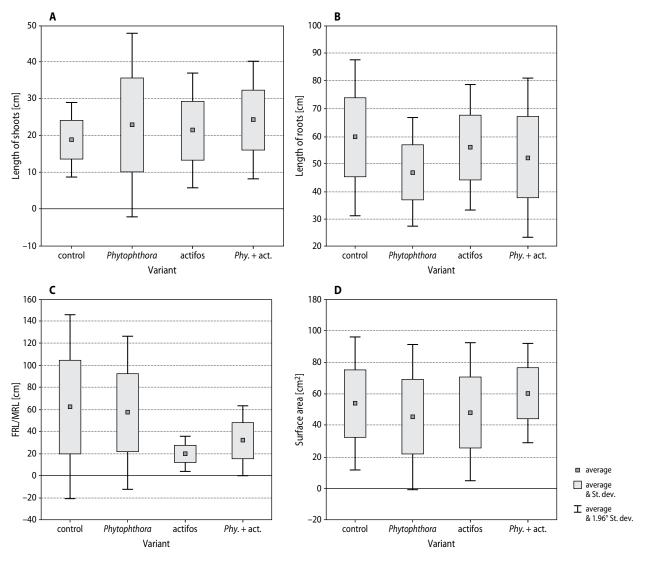


Figure 2. Comparison of the lengths of oak shoots (A), lengths of roots (B), FRL/MRL (C), and surface area (D) grown in 400 ppm CO₂

The results for root length under the control *Phytophthora*, Actifos, and *Phytophthora* + Actifos variants concerning the length of roots, no statistically significant differences among the variants could be derived with a probability test value of 0.2958 (Fig. 2B). However, a tendency for a lower average root length in the case of oaks infected by *Phytophthora* is discernible. The Actifos application offered a slight improvement at the root-level situation of investigated oaks, which is to say that some roots were saved.

The total ratio of FRL/MRL is associated with a significant difference attested by a probability test value of 0.0277 (Fig. 2C). Significant differences are detectable between control oaks and the combinations of *Phytophtora* +Actifos and Actifos only. The differences in question apply between the control and oaks inoculated with *Phytophthora*.

The surface areas occupied by fine roots, p-value of 0.5819 (Fig. 2D), indicate the lack of any statistically significant differences. The variant entailing the inoculation of oaks with *Phytophthora* and simultaneous application of Actifos proved to be the best trial, in the sense of the highest value for the root surface area. The least mean value of surface root area applies to the variant of the oak inoculation by *Phytophthora* only.

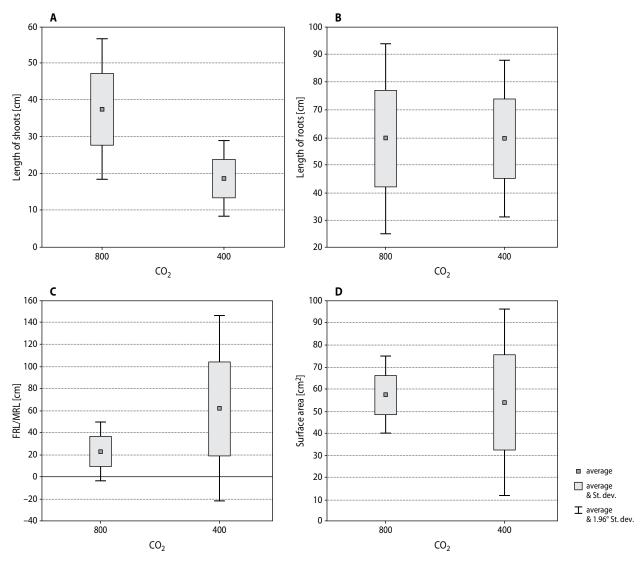


Figure 3. Comparisons of the lengths of shoots (A), and of roots (B), as well as FRL/MRL (C), and surface area (D) of oaks grown in experiment variants without inoculation

Comparison between the experimental variants

Control

The lengths of 16-month-old oak shoots, confirmed statistically significant differences (p = 0.002576). The shoots were longer at the elevated (800 ppm) concentration of CO₂ (Fig. 3A).

A comparison of the lengths of roots did not reveal significant differences (p = 0.861943; Fig. 3B).

A statistical analysis of the average root length parameter and the length of mother roots, which yielded a *p* value of 0.032278 confirmed statistically significant differences. Higher values for the FRL/MRL index were characteristic of trees growing in a normal atmosphere (CO_2 –400 ppm; Fig. 3C).

A comparison of root surface areas was associated with a non-significant test probability value of 0.862187. The root surface area was, nevertheless, greater on average at the higher (800 ppm) concentration of CO_2 (Fig. 3D).

Inoculation with Phytophthora quercina

The length of shoot, for which the differences were associated with a non-significant test probability value of 0.164161, is presented (Fig. 4A).

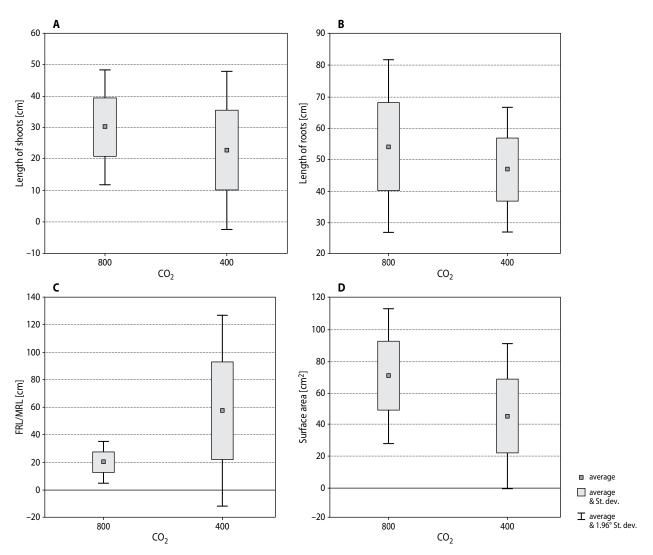


Figure 4. Comparisons of the lengths of shoots (A) and of roots (B), as well as FRL/MRL (C) and surface area (D) of oaks grown in experiment variants inoculated with *P. quercina*

Greater average values for the lengths of shoots were to be observed at the higher (800 ppm) CO_2 concentration. A similar situation applied to the root lengths, for which the non-significant test probability value was 0.270732. Again, greater average values for lengths of oak roots were to be observed at the higher CO_2 concentration. (Fig. 4B).

A comparison of values of the average root lengths with the lengths of mother roots was made. The test probability value for this is 0.0128, this attesting to the presence of statistically significant differences. Higher values for the FRL/MRL index were, in fact, characteristic for trees growing in an atmosphere containing 400 ppm CO_2 (Fig. 4C).

A test probability value of FRSA (0.072850) again denotes a lack of statistically significant differences, notwithstanding the greater average surface areas noted for fine roots in the variant at an 800 ppm concentration of CO_2 (Fig. 4D).

Application of Actifos

The test probability value associated with these differences was 0.005437, thus denoting statistically significant differences, that is, greater shoot lengths among

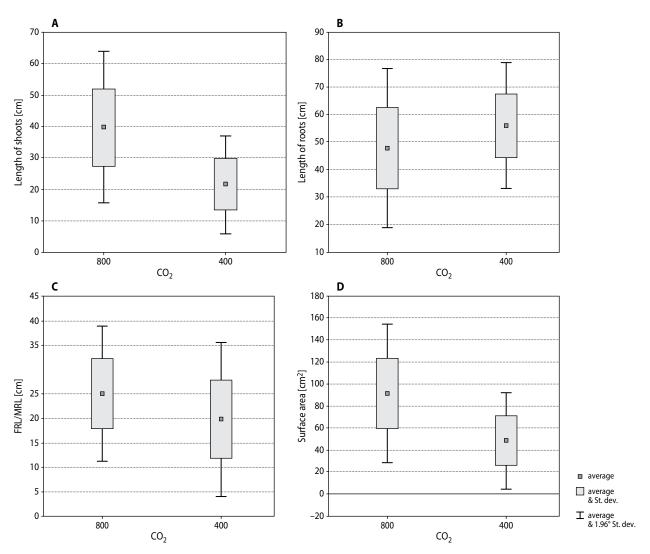


Figure 5. Comparisons of the lengths of shoots (A) and of roots (B), as well as FRL/MRL (C) and surface area (D) of oaks grown in experiment variants with Actifos

trees growing at the elevated (800 ppm) CO_2 concentration (Fig. 5A).

Where the lengths of oak roots were concerned, the test probability value of 0.164538 denoted no statistically significant differences, though roots were longer at the lower (400 ppm) CO_2 concentration (Fig. 5B).

The ratio of the FRLs was compared with the lengths of mother roots, these differences being associated with a non-significant probability value of 0.183234. A greater value for this index was nevertheless to be observed at the higher (800 ppm) CO_2 concentration (Fig. 5C).

The surface areas of fine roots, the test probability value for these differences being of 0.032278, is a value indicative of statistically significant differences. Larger average surface areas of fine roots were to be noted at the higher CO_2 concentration (Fig. 5D).

Oak inoculations with *Phytophthor aquercina* and the application of Actifos

The comparison of the lengths of oak shoots did not differ significantly (test probability value = 0.2936). Greater average values for shoot lengths were nevertheless to be observed in the variant with higher CO₂ con-

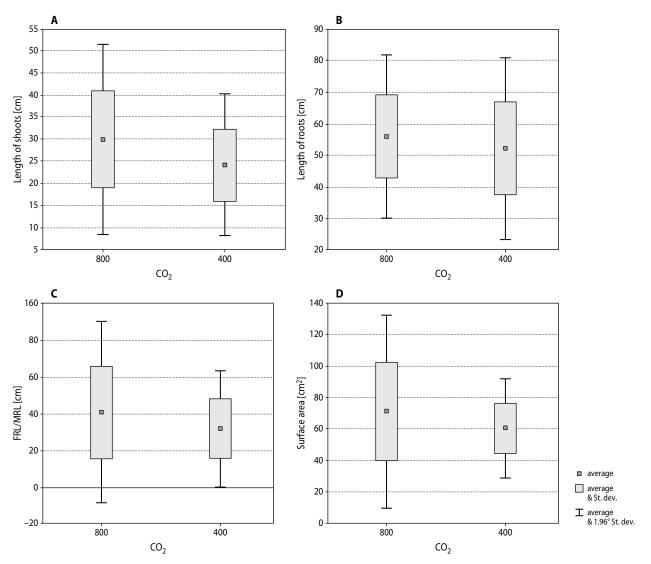


Figure 6. Comparisons of the lengths of shoots (A) and of roots (B), as well as FRL/MRL (C) and surface area (D) of oaks grown in experiment variants inoculated with *P. quercina* and treated with Actifos

centration (Fig. 6A). The comparison of the lengths of oak roots was in turn associated with a test probability value of 0.491311, again suggesting a lack of statistical significance (Fig. 6B).

The comparison for the average FRL parameter was set against the lengths of mother roots. A test probability value of 0.430898 failed to reveal any statistically significant differences, though the average values for the index were greater in the variant with a higher CO_2 concentration (Fig. 6C).

The surface areas of fine roots were compared in Figure 6D, which were not shown to differ significantly, in line with a test probability value of 0.713192.

DISCUSSION

Generally, control oaks grew better at the elevated (800 ppm) concentration of CO₂, as was evidenced by statistically significant differences in the development of shoots. However, there was no difference in the development of root systems. Probably, the span of length of greenhouse experiment (4 months) was too short to observe readable statistically significant differences. Nevertheless, some general positive observations could be drawn for forest practice. The high concentration of CO₂ better stimulated the development of oak roots than with oaks growing under low (400 ppm) concentrations of CO₂. This is evidenced by a significantly higher ratio of FRL/MRL measured at the 800 ppm concentration of CO₂. In terms of FRSA, there was no statistically significant difference between the control oaks growing at different concentrations of CO₂ (800 and 400 ppm), although slightly higher values were found in the variant with elevated CO₂, suggesting its positive influence of oak root growth, in general. Similar studies were carried out previously on the beech trees. Tkaczyk et al. (2014b) have shown that among different concentrations of CO₂, there was no statistically significant difference in the growth of beech seedlings. The influence of carbon dioxide were also tested on other organisms. Henn et al. (2000) and Stiling et al. (2003) found reduced herbivore feeding on beech and oak leaves of plants grown under elevated CO₂, while Percy et al. (2002) reported increasing herbivore damage, but no change as for rust infection by Melampsora medusae on aspen leaves under elevated CO₂.

Inoculation of oaks with *P. quercina* did not have a significant effect on the development of shoots and roots. The pathogen grew slowly (Jung et al. 1999), suggesting either that the experiment was of too short in duration, or else that the isolates we used were not especially pathogenic to the already developed woody plants. They were better developed in the atmosphere with a higher concentration of CO₂. Fast growth of oak shoots points out on efficient physiological processes (more efficient photosynthesis) ongoing in elevated CO₂ concentrations, confirmed by the reference to average values for lengths of shoots and in consequence roots in 800 pp of CO₂ variant, as well as the measured surface area of fine roots. Application of the Actifos preparation had a significant effect on the development of shoots and the surface area of fine roots under the elevated (800 ppm) CO_2 concentration. However, there were no statistically significant differences between the two concentrations of CO₂ with regard to the lengths of roots and the index calculated as the ratio of FRL/MRL. Nevertheless, the average value for the index was greater in the case of the higher concentration of CO₂, reflecting a greater number of fine roots being formed under such conditions. The lengths of shoots and roots, the ratio of FRLs to those of (thick) mother roots, and the surface area of fine roots did not show statistically significant differences in the case of oaks growing in the medium infected with Phytophthora and treated with the Actifos preparation. However, the mean values for these parameters were again consistently higher in the case of elevated concentrations of CO₂. According to some researchers, the application of Actifos can mask symptoms of disease by stimulating the growth of shoots and roots in infested oaks (Tkaczyk et al. 2016). There is, therefore, not enough justification for recommending applications of Actifos or similar products in forest nurseries (Jung, personal communication). In this light, more research should be performed on the role of phosphites as elicitors of plant resistance. Especially, if seedlings treated with phosphites (asymptomatic in nurseries) will express disease symptoms when planted in forest plantations. This is very important from seedlings selection point of view still in nurseries (distinguish healthy from diseased). In our research, the standard error and standard deviation values associated with means show high variability to measured values for the selected parameters describing oaks, including heights of shoots, lengths of roots, and so on. This denotes the non-feasibility in practice of attempts to distinguish between healthy and diseased plants (at least 1–2 years old).

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ORIGINAL ARTICLE

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Insecticidal activity of alpha-cypermethrin against small banded pine weevil *Pissodes castaneus* (Coleoptera: Curculionidae) in forest plantations and thickets

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Abstract

Scots pine (*Pinus sylvestris*) plantations and thickets damaged by biotic and abiotic factors are particularly attractive to small-banded pine weevil *Pissodes castaneus*, whose larvae excavate feeding tunnels in the stems of young trees, causing their death. There are no chemical methods that can be applied to protect forest plantations and thickets against this pest. Therefore, the studies were undertaken aimed at the assessment of the efficacy of alpha-cypermethrin used to reduce the numbers of this pest within restock areas. The scope of work included laboratory and field estimation of insecticidal activity of alpha-cypermethrin.

In laboratory, the beetles of *P. castaneus* were reared on *P. sylvestris* twigs treated with five concentrations of alpha-cypermethrin (0.0001–1%) formulated as Fastac Forest 15 SC. Insect mortality was calculated during 7-day rearing and median lethal concentration LC_{50} was calculated. The field treatments consisted of spraying of four-year old Scots pines with the insecticide in concentrations of 2% and 4%.

In laboratory conditions, the insecticide used at five different concentrations caused a 7–95% mortality of the beetles ($LC_{50} = 0.266\%$), while field sprays resulted in a 1.5–3.5-fold reduction in the colonization of trees and in higher pest mortality rates.

The results indicate the possibility of using of alpha-cypermethrin in protecting the forest against *P. castaneus* and can be the basis for the development of chemical method used in the forestry practice.

Key words

Pissodes castaneus, alpha-cypermethrin, LC50, spray treatments

INTRODUCTION

The banded pine weevil *Pissodes castaneus* (De Geer.) (Coleoptera, Curculionidae) is one of most dangerous pests in forest plantations and thickets weakened by

root fungi, deer and abiotic factors (e.g. improper planting, drought, hail) (Alauzet 1990). The beetles leave their wintering places in the first half of April and then feed on the buds and young shoots of Scots pine *Pinus sylvestris* L. trees. In the case of mass occurrence, it can lead to inhibited shoot growth and general weakening of trees. In May, the female beetles lay their eggs on the lower parts of Scots pine stems. The larvae that feed under the bark of stems, causing dieback of infested trees, are particularly harmful (Panzavolta and Tiberi 2010).

Pissodes castaneus is a species commonly found in Europe, especially in northern Italy, Austria, Germany, in the Asian part of Russia and Turkey, as well as in North Africa (CABI 2010; Panzavolta and Tiberi 2010; Santolamazza-Carbone et al. 2011). In 2001, it was introduced to South America, where it was initially described in Brazil, Argentina, Uruguay and Chile (Lede et al. 2011). In South America, it damages Pinus taeda L. and Douglas fir Pseudotsuga menziesii (Mirb.) Franco; in Europe many species of pines, primarily P. sylvestris and, in the Mediterranean region, P. pinaster Aiton and P. pinea L. In Poland P. castaneus is commonly found in P. sylvestris plantations and thickets. In the second half of the twentieth century, it did not cause considerable losses economically and has therefore not been studied in this regard. From 2000 to 2015, the area of its occurrence increased in Europe, including Poland, to over 8,000 ha per year, causing the death of young infested forests (Milewski 2015).

So far, there are no methods to reduce populations of P. castaneus, except the practiced method of uprooting and burning of trees inhabited by the pest. Until recently, Polish regulations on the use of pesticides allowed for the testing and application of many products registered for the protection of forests against the weevils. Most of this work focused on the efficacy assessment of new formulations registered against the large pine weevil Hylobius abietis L. (Coleoptera, Curculionidae), which is considered as the most dangerous pest of one- to three-year-old coniferous plantations (Örlander 1997, Skrzecz 1998, Långstrom and Day 2004). The lack of chemical methods, which can be applied in the crop protection against P. castaneus and the fact that populations can last for many years, justified the undertaking of studies for developing a chemical method to reduce the number of pest populations in forest plantations and thickets. Alpha-cypermethrin, formulated as Fastac 100 EC (10 g of active ingredient l⁻¹, producer BASF), has been used for many years in forest protection against weevils, mainly H. abietis (Głowacka et al. 1991). In 2013, a new formulation of alpha-cypermethrin, Fastac Forest 15 SC (15 of active

ingredient 1⁻¹), was registered in Europe. This product is used as a water emulsion in a concentration of 4% for dipping the seedlings before planting or spraying after planting to guarantee protection against H. abietis beetles. Preparations based on cypermethrin and its derivatives are currently the only insecticides permitted to protect forest crops against weevils in Poland. They are contact insecticides and paralyse the nervous system, causing, in the case of H. abietis beetles, death within two to three days (Garbaliński 1995). Thus this study was conducted to evaluate the insecticidal activity of alpha-cypermethrin against the beetles of P. castaneus and the assessment of its efficacy in the protection of Scots pine against this pest. The scope of this work carried out in 2012-2013 included testing of insecticide activity under laboratory conditions and field spraying of trees.

MATERIAL AND METHODS

The *P. castaneus* beetles used in laboratory experiments were collected in April and May 2013 during their feeding on shoots of five-year-old Scots pines growing in central Poland (52°03'34.9" N; 21°23'11.5" E). Alphacypermethrin (formulated as Fastac Forest 15 SC) was tested in five water concentrations prepared by serial dilutions: 1.0, 0.1, 0.01, 0.001, and 0.0001%, which corresponded to a content of the active ingredient from 1.5⁻² to 1.5⁻⁶ g in 1 ml. The twigs collected from Scots pine trees on which the beetles fed were then divided into sections (5 cm long and 1 cm in diameter) without needles, dipped for five seconds in the above-mentioned concentrations of alpha-cypermethrin and placed on aluminum foil to air-dry. Control-untreated twigs were dipped in water. The prepared shoots were placed individually in Petri dishes (height 1.5 cm, 9 cm in diameter) with one beetle. After three days, the twigs were replaced with untreated ones. In each variant (five concentrations and one untreated) were tested 45 beetles, a total of 270 beetles. The experiment was conducted during seven days at 20°C, 70% RH and a photoperiod of 16:8 (L:D) h. Insect mortality was estimated each day and based on the results, LC₅₀ (concentration required to cause 50% mortality) was calculated using probit regression analysis of alpha-cypermethrin concentrations that have been logarithmically transformed according to the Finney method (1962) using the BioStat Pro 5.9.8. program (AnalystSoft Inc.).

Field experiments were performed according to the procedure EPPO PP 1/127 (2) prepared for *H. abietis* and modified for *P. castaneus*.

Field treatments were performed in 2014 in central Poland (Forest District Celestynów: 52°04'50.6" N; 21°20'54.0" E) in four-year-old Scots pine plantations in which the P. castaneus were found feeding on trees in April of the same year. Alpha-cypermethrin was tested in the form of a water emulsion of Fastac Forest 15 SC in the concentrations 2 and 4%. Spraying was performed in the second half of May, i.e. during the period of egg laying by P. castaneus females. The treatments consisted of spraying the parts of the stems from the root collars to a height of about 20 cm, where the females lay eggs and the larvae hatch. The liquid was applied with the use of the knapsack sprayer SOLO 425, at 0.4 MP and a dose of approximately 100 mL of preparation per tree. The comparative control consisted of trees sprayed with the same dose of water. In each variant of the experiment (two concentrations of insecticide and untreated), 100 trees were sprayed in the spatial distribution of 20 trees in five rows (repetitions). The efficacy of treatments was estimated after six months (the second half of September) based on the numbers of trees with symptoms of colonization by P. castaneus: yellow needles, upper shoots hanging down and resin leaks on the stems. These trees were taken out and stripped in the laboratory, and the larvae located on the stems from the root collar to a height of 20 cm were counted. The numbers of seedlings colonized by the pest as well as the numbers of its larvae in all variants of experiment were analysed using Kruskal-Wallis test with an adopted significance level of $P \leq 0.05$, followed by post-hoc test (multiple comparisons of mean ranks for all groups) to find treatment effects that are significantly different from each other. The Statistic v.10 (StatSoft®) package was used in the analysis.

RESULTS AND DISCUSSION

The laboratory experiment demonstrated a systematic increase in mortality of *P. castaneus* beetles in all variants of insects reared on twigs dipped in alpha-cypermethrin (Fig. 1).

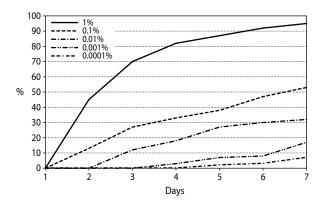


Figure 1. Cumulative mortality of *Pissodes castaneus* larvae feeding on *Pinus sylvestris* twigs treated with different concentrations of alpha-cypermethrin

The mortality of insects increased proportionally with increasing insecticide concentrations and was highest in the group of beetles that fed on the twigs treated with 1% of alpha-cypermethrin. After 24 hours of insect feeding on twigs dipped in 1.0 and 0.1%, beetle mortality increased respectively to 45 and 13% and survived insects showed signs of paralysis. At that time, in other variants (0.01-0.0001%), there were no signs of insect paralysis and the presence of excrements testified the insect feeding. After two days of feeding, the beetles were found dying on twigs treated with a concentration of 0.01%. On the last day of the experiment, the insect mortality had exceeded 50% only in variants in which the beetles fed on twigs treated with the highest concentrations of alpha-cypermethrin (1.0, 0.1%). In other variants mortality reached 7% (concentration 0.0001%) to 32% of insects (0.01%). There were no dead individuals in the control experiment. The final LC₅₀ was 0.266% with a standard error of 0.043%. In the available literature, there is no information on the impact of pyrethroides (including alpha-cypermethrin) on P. castaneus. A study of this scope were primarily focused on the white pine weevil Pissodes strobi (Peck), a pest of white pine (Pinus strobus L.) and 10 species and hybrids of spruce (Picea spp.) (Russel et al. 1990, Alfaro et al. 1995), as well as the large pine weevil Hylobius abietis, which damages young coniferous trees. DeGroot and Helson (1993) exposed P. strobus weevils to white pine twigs sprayed with permethrin and observed the knockdown of insects with LC_{50} of 0.053 and 0.095 $\mu g/cm^2$ of twig bark after, respectively, 1 and 14 days. Similar to presented results, Malinowski (1995) obtained similar

results, who stated up to 90–100% mortality of *H. abietis* beetles reared during 10 days on Scots pine twigs treated with alpha-cypermethrin with concentrations: 0.0125, 0.025, 0.05%.

Assessment of colonization of trees treated with alpha-cypermethrin formulated as Fastac Forest 15 SC was carried out six months after the treatment and showed statistical differences between the mortality of Scots pines in individual variants of experiment (Kruskal–Wallis test H = 12.321, p < 0.0021) (Tab. 1). The lowest number of trees killed by P. castaneus was found in the plot sprayed in the concentration of 4%. More than twice as many dead trees were on plots protected with the insecticide in the concentration of 2%. Most trees killed by the insect were found in the untreated control variant. Only larvae of P. castaneus were found in the stems of observed trees. Usefulness of alpha-cypermethrin formulated as Fastac 100 EC in concentration of 1% was confirmed by Korczyński (2001a, b) who observed 2.5-fold reduction of numbers of treated trees that were damaged by H. abietis. He also compared the extent of bark damages on treated and untreated trees and found more than 10-fold reduction of area eaten by the large pine weevils.

Table 1. Colonization of *Pinus sylvestris* trees treated with 2and 4% of alpha-cypermethrin by *Pissodes castaneus*

Treatment	Mean (± SD) number of dead trees	of <i>P. castan</i> found on the	D) number neus larvae stem section length)
		alive	dead
2%	9.4±1.6 ^{a*}	2.6±1.1ª	2.8±1.6 ^a
4%	4.4±1.1 ^b	1.6±0.7 ^a	5.4±1.2 ^b
untreated	16.0±5.4°	8.0±2.3 ^b	1.25±0.4°

* Different letters show statistical differences at p = 0.05.

Treatments resulted in reducing the intensity of colonization of trees by the pest. In the trees sprayed with alpha-cypermethrin, there were three- to four-fold less living larvae than under the bark of unsprayed trees. Statistical analyses showed no differences be-tween the numbers of the living larvae in trees treated with both concentrations of alpha-cypermethrin and confirmed the differences compared to untreated trees (H = 36.129; p < 0.0001). Also dead *P. castaneus* larvae were found in all groups of experimental trees. The

mortality of larvae statistically differed depending on treatment (H = 38.223; p < 0.0001). Most dead larvae were found in the feeding tunnels in the trees treated with an insecticide concentration of 4%. Twice less numbers of dead larvae were found after removing the bark from the trees sprayed with a 2% concentration of the insecticide. The death of larvae in treated trees was most likely caused by their contact with the insecticide, because there was no sign of infestation of larvae by parasitoids. The mortality of pest larvae developing in untreated trees was the lowest and caused by parasitoids from the Braconidae family, which are the main factor causing a reduction of *P. castaneus* populations (Kenis et al. 1996, 2004).

CONCLUSIONS

- Alpha-cypermethrin used as Fastac Forest 15 SC caused higher mortality (up to 95%) of *P. castaneus* beetles rearing on *P. sylvestris* twigs treated with insecticide.
- Spraying of four-year old *P. sylvestris* trees with alpha-cypermethrin resulted in the reduction of numbers of trees colonized by *P. castaneus* and led to the higher mortality of pest larvae developing in the infested pines.
- The results indicate the possibility of using alphacypermethrin in the protection of forests against *P. castaneus* and can be the basis for the development of chemical methods used in forest management.

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Symptoms of the naturalisation of the Turkey oak (*Quercus cerris* L.) in Polish forests

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Abstract

The Turkey oak (*Quercus cerris* L.), the natural range of which embraces southern Europe and Asia Minor, belongs to trees rarely introduced into Polish forests. Tree stands where it appears, established before the Second World War, can be found in some 20 localities, mostly in the western part of the country. Because this species is capable of a natural renewal in a woodland environment, a research was made to find in what conditions and how far it undergoes spontaneous naturalisation. Three study sites were chosen in the forests of central Wielkopolska. An inventory was made of mature stands of the Turkey oak and its generative renewal. Plant communities in which the young generation of Q. cerris usually appears were characterised. It was found that self-sown seedlings of this species grew at a distance of up to 2,500 m from parent trees. The highest number and the greatest density of specimens of the secondary generation of the Turkey oak were found at 'Racot', which is a 100-hectare, mid-field woodland island where mesotrophic habitats predominate and where about 50% of the area is occupied by communities with manmade pine tree stands. At all sites, Q. cerris penetrates primarily this type of deformed phytocoenoses, developing mostly on former farmland. It has become a permanent component of the underbrush and undergrowth in them, and in some places, it also makes up the tree layer. It was observed that in the study area, it penetrated the woodland environment much more effectively than Quercus rubra, considered an invasive species. The expansion of the Turkey oak in several of the examined localities can be regarded as a basic manifestation of its naturalisation in places where there are phytocoenoses with pine stands in broad-leaf forest habitats in the neighbourhood of parent trees.

KEY WORDS

alien tree species, introduction, establishment, regeneration dynamics, dispersal

INTRODUCTION

The Turkey oak (*Quercus cerris* L.) is an important forest tree in the countries of southern Europe and Asia Minor, where it occurs in a natural state: from southern France, through Italy, Switzerland, Austria, the Balkan Peninsula, to Lebanon (Meusel et al. 1965; Browicz 1982; Menitski 1984; Bozano and Turok 2003). Outside its natural range, it is mostly cultivated as a fast-growing ornamental tree with modest soil requirements and a strong resistance to drought. In a few regions, especially in north-western France and southern Great Britain, it has been found in spontaneously developed secondary plant communities (Jalas and Suominen 1976; Preston et

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al. 2002). Attempts at an introduction of the Turkey oak to Polish forests for productive purposes have failed. The introduction was probably only intended to test the acclimatisation properties of the species, rather than its use at an economic scale because no research was made on the growth dynamics and productivity of the trees. Possibly, even preliminary observations of the frost damage of its tree trunks caused the range of the experimental cultivation of the Turkey oak in forests to be restricted (Danielewicz et al. 2014). Only fragments of tree stands containing this species have survived until today, mostly those established before the Second World War and usually on the land formerly belonging to the Prussian sector of divided Poland. Even so, it merits interest as one of the plants permanently established in Poland, and at a local scale, it may prove to be an invasive species requiring the control of its population dynamics in the wild (Danielewicz and Maliński 2003; Tokarska-Guzik et al. 2012). A summary of basic information about the 22 localities of the Turkey oak in Polish forests can be found in Danielewicz et al. (2014). Those authors observed symptoms of its spontaneous regeneration from seeds in almost all places where this species was represented in a mature tree stand by a dozen or more trees. However, it was not everywhere that further development of selfsown seedlings of Q. cerris and its penetration into forest communities was recorded.

The research reported in this paper was intended to find in what conditions the naturalisation of the Turkey oak took place in the woodland environment of selected sites in Poland, and how advanced is this process. The aim of the research was to document symptoms of this process, determined on the basic of properties in the local, secondary population of *Q. cerris*, such as the spatial range of spontaneous proliferation, abundance, density, and diversity of the size of individuals, which are formed by natural, generative regeneration, as well as a part of this tree in the structure and floristic composition of plant communities in which it occurs most frequently.

MATERIAL AND METHODS

Study sites

Out of the 22 localities of the Turkey oak found in Polish forests (Danielewicz et al. 2014), 3 sites were chosen for study, in which this species was observed to display the greatest ability for spontaneous dispersal away from places where seed-bearing trees grow. They all lie in central Wielkopolska (Fig. 1), from 20 to 47 km in a straight line from the region's capital, the city of Poznań. They were designated by the names of the forest districts where they are situated.

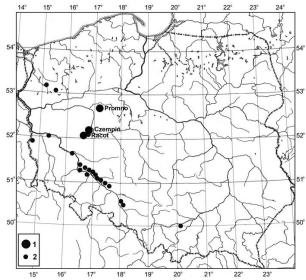


Figure 1. Location of study plots (1) relating to other *Quercus cerris* localities in forests of western Poland (2)

Site 1: 'Racot' in the Kościan Forest Inspectorate. The meso-region of the Leszno Upland. A small woodland area of 105 ha, surrounded by agricultural land. Divisions 197-201. The highest proportion of the woodland area is occupied by fertile and moderately fertile habitats: a fresh deciduous forest (Lśw), 50%, and a mixed fresh forest (LMśw), 36% (according to the classification of forest habitats (forest site type), used by the Polish foresters (Kliczkowska et al. 2004)). Half of the area is occupied by anthropogenic communities with Pinus sylvestris, the rest being mostly broad-leaf forests (of the oak-hornbeam forest type) with Quercus robur, less frequently with Acer pseudoplatanus, as well as phytocoenoses with Q. rubra, Pseudotsuga menziesii, Picea abies, and Larix decidua. The area with old-growth stands of Q. cerris, 123 years old (division 199 g) is 0.82 ha.

Site 2: 'Czempiń' in the Konstantynowo Forest Inspectorate. The meso-region of the Leszno Upland. A small woodland area of 41 ha, surrounded by agricultural land. Divisions 261 and 262. Predominant habitats of fertile broadleaf forests: a fresh deciduous forest, 50%, a moist forest, 39%, while the proportion of mesotrophic habitats of a fresh mixed forest is a mere 9%. Tree stands with predominantly broad-leaf species (*Quercus robur, Fraxinus excelsior, Fagus sylvatica*, and *Alnus incana*) occupy 78% of the woodland area, and those with the pine, 19%. The greatest cluster of 25 Turkey oaks, over 120 years old, occurs in a multispecies broad-leaf tree stand in division 261b.

Site 3: 'Promno' in the area of the Czerniejewo Forest Inspectorate. The mesoregion of Wielkopolska Lakelands. Its north-western fragment, called the Czerniejewo Forests, is a large woodland island occupying 423 ha, surrounded by agricultural land, and in the north-east neighbouring on the village of Pobiedziska-Letnisko. Divisions 215-231. The highest proportion of the woodland area, 48%, is occupied by a fresh mixed deciduous forest, and 28% by a fresh deciduous forest. The habitats of a fresh mixed coniferous forest account for 7% of the woodland area, and a fresh coniferous forest, 2%. Stands of Pinus sylvestris occupy 47% of the area, Quercus sp. 35%, Alnus sp. 12%, and other species as Fagus sylvatica, Carpinus betulus, Fraxinus excelsior, or Populus tremula - 6%. The area with 61 oldgrowth stands of *Q.cerris*, 122 years old (division 229 a) is 0.40 ha.

Structure of the thickness of the oldest stands of the Turkey oak

Measurements of tree thickness were taken in 2015 in the oldest stands of *Q. cerris* in the case of which there was no doubt that they had been planted. It was only at 'Promno' that such measurements were also performed many years earlier, in 1967 (Juwa 1968), hence only in this case was it possible to present the structure of the stand in the period preceding the substantial decline in the number of the trees. Because of the scattering of the oldest planted Turkey oaks over a few places at 'Czempiń', the structure of the tree stand with this species at this site is not presented in the article.

Distribution and structure of the natural regeneration of the Turkey oak

This part of the study was conducted at 'Racot' and 'Promno'. No such work was carried out at 'Czempiń' because of the concentration of the undoubtedly spontaneous regeneration of the Turkey oak in only one place at a distance from the parent tree stand. Besides, the origin of trees of this species in a few sapling stands in this area is not clear. They may have been planted together with native oaks, although it is possible that they have appeared there spontaneously.

The search for the natural regeneration of *Quercus* cerris embraced all areas with old-growth stands of this species as well as forests situated in their neighbourhood. No study was made of the regeneration of the Turkey oak outside forests, for example, at roadsides and on railway embankments. The position of each specimen coming from a self-sown seedling was determined in terms of geographical coordinates using Trimble Juno 3B GPS equipment. At 'Racot', where there is a mature stand of *Q. rubra*, young specimens of this species were also recorded. Because of changes that have recently occurred as a result of forest husbandry in division 198f (tree cutting and artificial forest regeneration) at the 'Racot' site, use was made of materials collected in 2006.

Measurements were made of the height of the oaks forming a natural regeneration (with the exception of one-year-old seedlings) and their diameters at an altitude of 1.3 m, if greater than 5 cm.

Plant communities with the Turkey oak

At all sites accordance with the methods used in phytosociology, relevés were made in places with mature tree stands containing the Turkey oak (15 relevés) and in communities where this species had appeared spontaneously and now covers at least 5–25% of the area (24 relevés). Also, in this case, relevés in division 198f come from the year 2006 for the reason explained earlier.

RESULTS

Thickness structure of the oldest stands with the Turkey oak

It can be assumed that the only source of seeds through which the Turkey oak could disperse over the study area was tree stands containing this species established at the end of the 19th and the beginning of the 20th centuries that attained generative maturity some 50–60 years ago. At each site, there is one such stand outside of which not even single trees planted at that time were

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found. The probability that they grew in other places, but were overlooked, or that they had been cut down before the research began, is very low.

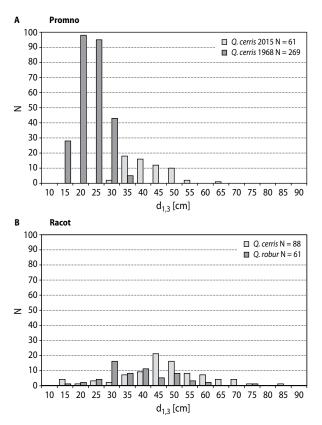


Figure 2. Thickness structure of the oldest stands of *Quercus cerris* and *Q. rubra* – other alien tree from the genus *Quercus*, which spreads in the same area

In 1967, there were 269 introduced Turkey oaks at 'Promno', which means that more than 200 trees had disappeared over the nearly 50 years since then. Because there are no detailed data on how this number kept declining, one can only suppose that the reduction was mostly due to tending cuts, which is reflected in the distribution of breast-high diameters characteristic of managed tree stands (Fig. 2A). In the mixed stand of *Quercus cerris* and *Q. robur*at 'Racot', the range of tree thickness is greater and the distribution closer to normal (Fig. 2B), which may be connected with tending cuts being less intensive here. At both sites, however, the Turkey oak stands established over 100 years ago have gone through all development stages and attained generative maturity at least a few decades ago.

Distribution and structure of the natural regeneration of the Turkey oak

The most exuberant natural regeneration of the Turkey oak was recorded at 'Racot', with 1,516 specimens from self-sown seedlings growing at a distance from

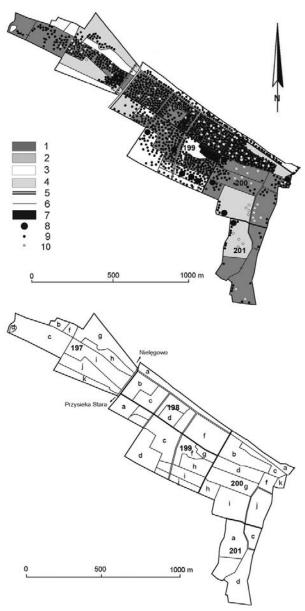


Figure 3. Distribution of localities of the Turkey oak at 'Racot' forest: 1 – *Pinus sylvestris* stands, 2 – *Quercus robur* or *Q. petraea* stands, 3 – other broad-leaf stands, 4 – non-forest environment, 5 – roads, 6 – forest subsection boundaries, 7 – oldest *Q. cerris* stands, 8 – young plantation of *Q. cerris*, 9 – natural regeneration of *Q. cerris*, 10 – natural regeneration of *Q. rubra*

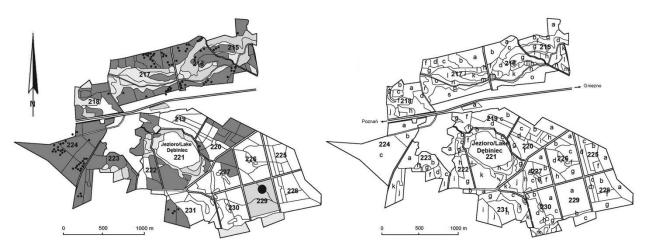


Figure 4. Distribution of localities of the Turkey oak at 'Promno' (for description see Fig. 3)

the parent tree stand. They can mostly be found in the north-eastern parts of divisions 198 and 200, fragments of division 199 near the old *Q. cerris* trees, and the part of division 197 farthest from them (Fig. 3). In the young natural regeneration and undergrowth, a much rarer species is *Q. rubra* (108 specimens), the distribution of which is presented in Figure 2, which indicates that it plays a decidedly lesser role than *Q. cerris* in the penetration of the woodland environment at this site. It occurs most frequently in the places of exuberant regeneration of the Turkey oak (divisions: 198f, 200b).

The maximum distance between the parent stand of Q. cerris and specimens of its natural regeneration at 'Racot' is 1,468 m, close to the border of the woodland area. At 'Promno', where the regeneration is much less exuberant (102 specimens) and located much farther from the old trees, the distance extends to 2,460 m (Fig. 4). A common feature in the distribution of the secondary localities of Q. cerris that can be observed on the maps of those two sites (Fig. 3 and 4) is that most of them are located in forests with pine tree stands ('Racot') or exclusively in such forests ('Promno').

The highest density of the natural regeneration of the Turkey oak at 'Racot' is almost 100 specimens/ha in division 200b (fresh mixed coniferous forest [BMśw], a pine tree stand aged 87), located in the close vicinity of an old-growth stand of this species (Tab. 1). There were 50 (3%) *Q. cerris* specimens lower than 1 m at this site and 757 (50%) specimens with a breast-high diameter of under 5 cm (Fig. 5 and 6). The lowest Turkey oaks coming from natural regeneration grow farthest away from the parent stand (division 197), and in division 201, where their number is the smallest. In divisions 198 and 200, where the number and density of the secondary generation of the oak are the highest, 70% and 79% trees, respectively, were part of the undergrowth, in some cases of the young natural regeneration, and the rest, 30% and 21%, reached the lower tree layer. With a lower natural regeneration rate in division 199, the proportion of specimens in the tree layer is much higher, at 74%. At 'Promno', the predominant height of *Q. cerris* is 1–5 m (86%), with only 9 trees (8%) higher than 5 m.

Table 1. Number of Turkey oak specimens from naturalregeneration per ha at "Racot"

Forest	Numbe	er of indiv	viduas	in fores	t subsec	tions
sections and		N			N/ha	
subsections (area in ha)	min.	max.	total	min.	max.	aver- age
197 f-k (27,07)	22 (i, k)	185 (j)	305	6 (k)	41 (j)	11
198 a-f (18,18)	19 (d)	161 (f)	401	13 (d)	25 (f)	21
199 a-f, h-j (21,37)	11 (a, j)	88 (h)	293	5 (a)	51 (h)	14
200 a-b, d-k (24,01)	5 (i)	370 (b)	499	1 (i)	97 (b)	21
201 a, c-f (11,44)	2 (f)	8 (a)	18	1 (d)	3 (f)	2

The spatial differences in the size of Turkey oaks from natural regeneration at 'Racot' are also reflected

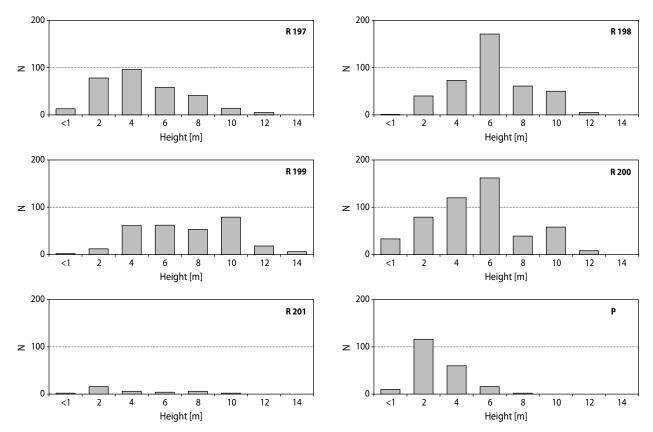


Figure 5. Differences in the height of the Turkey oak from natural regeneration at 'Racot' (R) and 'Promno' (P), number of forest sub-compartment

in the distribution of their thickness (Fig. 6). In four divisions: 197, 198, 200, and 201, the highest proportion is that of oaks with a breast-high diameter of under 5 cm (including those that have not attained the height of 1.3 m): 52%, 68%, 50%, and 56%, respectively. Their proportion in division 199 is the smallest, at 23%. Apart from division 201, where there are only 18 young specimens, the most numerous are trees 5–9 cm thick, but in division 199, their proportion in the number of all trees with a breast-high diameter of 5 cm and more amounts to 45%, markedly lower than in the other divisions (197 - 73%, 198 - 63%, and 200 - 60%). At 'Promno' as a whole, Turkey oaks with the smallest thickness values predominate.

Communities with the Turkey oak

At all the study sites, phytocoenoses with the old growth of the Turkey oak occurred in areas where the potential association in fresh deciduous forest habitats is the Central-European oak-hornbeam forest *Galio*

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sylvatici-Carpinetum, but the actual vegetation is dominated by communities deformed by forest husbandry. Also, the phytocoenoses under study differ considerably from the natural oak-hornbeam communities in terms of structure and floristic composition (Tab. 2). Tree stands are usually oak monocultures developed in accordance with the principles of forest cultivation. It is only at 'Promno' that Carpinus betulus and Fagus sylvatica are an admixture. The proportion of forest ground-layer plants typical of fertile and moderately fertile broad-leaf forests is small. What distinguished the 'Promno' and 'Czempiń' phytocoenoses is only a slightly greater proportion of species characteristic of the class Querco-Fagetea. A symptom of the advanced degeneration of the examined communities with the Turkey oak is a substantial representation of the class Artemisietea, and at 'Racot', a markedly higher contribution of species of the class Epilobietea. In many places of this site, Rubus sp. forms lush clusters; hence, a high proportion of forest phytocoenoses show

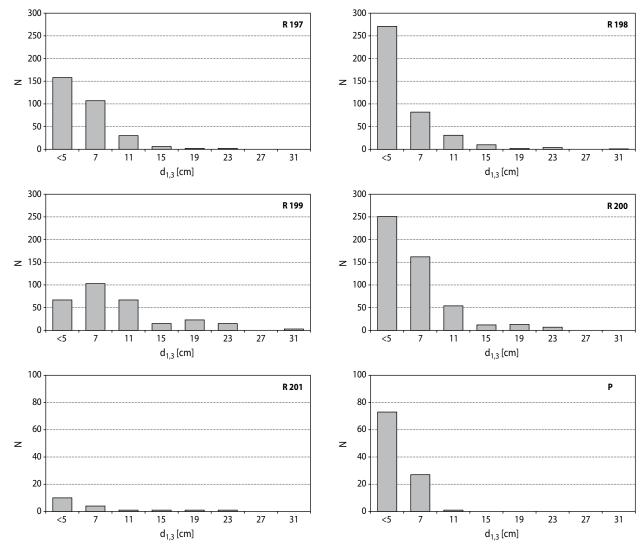


Figure 6. Differences in the thickness of the Turkey oak from natural regeneration at 'Racot' (R) and 'Promno' (P)

signs of so-called rubietisation, one of the six forms of forest communities degeneration distinguished by Olaczek (1972,1974), involving mass development of those plants. What is characteristic here is a more frequent occurrence of Q. *cerris* in the undergrowth and in the herbal layer.

Succes	sive Nº	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Date	Day Month Year	07 06 15	07 06 15	07 06 15	01 06 15	01 06 15	15 06 15									
Density of a,	a1*	60*	60	60	70	80	80	70	70	90	70	60	70	50	60	70
Density of a2	2	30														
Density of b		20	20	40	5	5	40	40	30	10	30	50	40	60	50	10

Table 2. Communities with old-growth Turkey oak

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
80	90	60	90	70	70	80	70	80	60	40	70	80	90	80
< 5	< 5	< 5	5	5	5	< 5	10	< 5	-	-	-	-	< 5	-
400	400	200	400	400	200	200	200	200	200	200	200	200	200	200
Р	Р	Р	С	С	R	R	R	R	R	R	R	R	R	R
2	2	2	2	2	1	1	1	1	1	1	1	1	1	1
			-	-	-	-	-	-	-	-	-		-	9
-	-	-	-	-	-	-	-	-	-	-	-	-	-	9 9
			-	-	-	-	-			-	1			g 107
														20
20	54	23	57	51	27	25	27	20	17	10	23	21	20	20
1 2*	13	11	2.2	22	11	3.2	2.2	1 1	22	2.2	2.2	2.2	2.2	4.3
4.5		4.4	2.2	2.2	-				2.2					2.2
•		•							•					2.2
							2.2							
	•	•			-	-	•					•		1.1
	•				•		•		•		•		•	1.1
	•	•	•	•			•	•	•	•		•		+
	•		•	•	-	3.3		•			2.2			•
					1.1	•	•	•	•	2.2		4.4	3.3	
			•		•	•			•	•	+			
	•		•		•	•		•	•	•	•	•		•
						•	2.2	+	+					
					•	•					•			•
-			•		•			•	•	•	•	•		•
	2.1													
		1.1												
2.2			•		•									
2.2	1.1				-									
									1.1					
							1.1		2.2					
			•		1.1			1.1		2.2	2.2			
			+	+		+	+	1.1		+	1.1	+		+
+	+		+		1.1		+				1.1	+		1.1
				+	.	+							+	
			+		2.2	1.1				1.1	1.1		1.1	
			1.2	+						+			+	
	1.2						1.1	2.2	2.2					
								1.1	2.2					
			1.1											
				+			1.1	+						
										+				
									+	+				
					+		+	•				•	•	
	•	•	•	•	+	•	+	•	1.2		1.2	1.2	•	
	< 5 400 P 2 2 9 a 122 26 - - - - - - - - - - - - -	80 90 < 5	80 90 60 <5 <5 <5 400 400 200 P P P 2 2 2 2 2 2 9 9 9 a a a 122 122 122 26 34 23 $4.3*$ 4.3 4.4 . $+$ $.$ $4.3*$ 4.3 4.4 . $+$ $.$ $4.3*$ 4.3 4.4 . $+$ $.$	80 90 60 90 < 5 < 5 < 5 5 400 400 200 400 P P P C 2 4.3 4.4 2.3 37 4.3 4.4 2.2 1.2 1.2 $.$	80 90 60 90 70 <5 <5 <5 5 5 400 400 200 400 400 P P P C C 2 4.3 * 4.3 2.3 3.7 31 4.3 * 4.3 2.4 2.2 2.2 $.$ $.$ $.$ $.$ $.$ $.$ $.$ $.$ $.$ $.$ $.$ $.$ $.$ $.$ $.$ $.$ 4.3 * 4.3	80 90 60 90 70 70 <5 <5 <5 5 5 5 400 400 200 400 400 200 P P P C C R 2 2 2 2 2 2 1 2 2 2 2 2 2 1 9 9 9 9 1 1 9 a a b g 122 122 122 123 123 107 26 34 23 37 31 27 $4.3*$ 4.3 4.4 2.2 2.2 4.4 $.$ 1.2 122 123 107 3.2 $4.3*$ 4.3 4.4 2.2 2.2 4.4 $.$ 1.1 1.1 1.2 1.2 4.4 $.$ 1.1 1.1 1.2	80 90 60 90 70 70 80 < 5 < 5 < 5 5 5 < 5 < 5 400 400 200 200 400 400 200 200 P P P C C C R R 2 2 2 2 1 1 9 9 9 9 1 1 9 9 a a a b b g g g g 122 122 122 123 123 107 107 26 34 23 37 31 27 23 4.3* 4.4 2.2 2.2 4.4 3.2 3.3 1.2 2.2 	80 90 60 90 70 70 80 70 < 5 < 5 < 5 5 5 < 5 < 5 < 10 400 400 200 400 400 200 200 200 P P P C C R R R 1	80 90 60 90 70 70 80 70 80 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 5 < 10 < 200 200 P P P C C R R R R R 2	80 90 60 90 70 70 80 70 80 60 < 5	80 90 60 90 70 80 70 80 60 40 <5 <5 <5 5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 <5 $<$	80 90 60 90 70 70 80 70 80 60 40 70 <5	80 90 60 90 70 70 80 70 80 60 40 70 80 <5	80 90 60 90 70 70 80 70 80 60 40 70 80 90 < 5

Successive Nº	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Crataegus monogyna c				+		•			•	+.2	•	•			•
Frangula alnus b	•	•	•		•	+	•	•	•	•	•	•			•
Frangula alnus c	•	•	•	+	•	•	•	•	•	•	•	•	•	•	•
Rhamnus cathartica b	•		•	1.1	•						•		•		
Rhamnus cathartica c				+		•						•			
Pinus sylvestris c													+	+	•
<i>Betula pendula</i> c	•		•	•	•	•	•	•	•	•	•	•	+	+	
Ch. Querco-Fagetea															
Poa nemoralis	1.2	1.2	1.2	3.3	1.2	2.3	2.2	2.3	1.2	2.2	2.3	2.3	2.2		3.3
Atrichum undulatum d	1.2	1.2	1.2	1.2	1.2	1.2	1.2	2.2	1.2				•	1.2	
Dryopteris filix-mas	2.2	2.2	1.2	1.2			2.2					1.2	2.2	1.2	
Anemone nemorosa	2.2	1.2	2.2										•		
Milium effusum	1.2	1.2	1.2												
Brachypodium sylvaticum		1.2		1.2	1.2										
Scrophularia nodosa		1.2		2.2	+										
Viola reichenbachiana	+	+		1.2											
Circaea lutetiana	+	+	+												
Ajuga reptans		2.2	1.2												
Dactylis polygama	1.2				1.2										
Carex sylvatica	1.2	1.2													
Stachys sylvatica	1.1	+													
Ch. Quercetaea robori-pet	raeae														
Carex pilulifera						1.2		1.2				1.2	1.2		2.2.
Pteridium aquilinum			1.2						1.2	1.2					
Veronica officinalis				2.2	1.2										
Holcus mollis				1.2				1.2							
Dicranella heteromalla d													+.2	1.2	
Polytrichum formosum d						+		1.2							
Ch. Epilobietea				1		I				1		1		1	<u>. </u>
Rubus apricus						2.3	2.2	3.3	3.3.	1.2	1.2	3.3	3.3	4.5	2.3
Rubus seebergensis						2.2	2.2	1.2	2.2	1.2	1.2	1.2	2.2	2.2	2.3
Rubus gracilis		1.2				2.3	2.2	2.2	2.2	1.2		1.2	2.2	2.2	2.2
Calamagrostis epigeios					2.2			2.3	2.3	1.2	2.3		1.2	2.3	
Rubus macrophyllus										1.2		1.2		+.2	
Rubus idaeus	+		1.2	2.2											
Rubus lamprocaulos						1.2						1.2			
Fragaria vesca		+			+										
Ch. Atremisietea	I				I	I			I	I	I	I		I	<u> </u>
Moehringia trinervia	1.1	1.2	+	1.2	2.2	1.2	1.2	1.2	1.2		+	1.2	1.2	+	+.2
Impatiens parviflora	3.4	+	3.3			2.3	3.3		2.2	3.3	2.3	2.2	3.3	2.3	2.3
Fallopia convonvulus	+	1.2	1.2		1.2	1.2.	+.2	1.2	1.2			1.2	1.2	+.2	+
Urtica dioica	2.2	2.2	2.2	1.2	1.2		+.2				·	2.2	1.2		2.2
Galeopsis pubescens	2.1	1.2	2.2	1.2	1.1		+	. 1.2		1.2					+.2
Galeopsis pubescens	2.1	1.2	2.2	1.2	1.1	•	+	1.2	•	1.2	•	•	•	•	+.2

Successive Nº	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Galium aparine				1.2	1.2		+.2	1.2		+.2					+
Rubus caesius				1.2	1.2	2.2.	+.2	+						+.2	
Geranium robertianum	1.2	+	1.2	1.2		1.2									
Mycelis muralis	+	1.1	1.1	1.1	1.2										
Alliaria petiolata	+	2.2	1.2	+											
Ch. Calluno-Ulicetea															
Agrostis capillaris								+.2			+.2				+.2
Others															
Dryopteris carthusiana	+	+				1.2	+.2		1.2		+		+.2	+.2	
Juncus conglomerates						+	+.2	1.2				+	1.2		+.2
Carex ovalis						1.2	2.2				+.2	1.1			+.2
Carex pallescens						1.2			+.2			1.2			
Veronica chamaedrys		+		2.2	1.1										
Festuca rubra				1.2	2.2										
Poa pratensis				2.2	1.2										
Oxalis acetosella	1.2		1.2												
Anthoxanthum odoratum				1.2	1.2										
Deschampsia caespitosa		1.2	1.2												
Carex hirta							+.2					+.2			
Hypericum perforatum		+													+
Hypericum montanum				+	+										

Sporadic:

Trees and shrubs: Crataegus laevigata b 2 (+); Prunus avium c 6 (1.1); Ribes uva-crispa b 5 (1.2); Sambucus nigra c 3 (+); Ulmus glabra b 9 (1.1) Ch. Quercetea robori-petraeae: Festuca ovina 4 (1.2); Hieracium lachenalii 4 (+); Solidago virgaurea 4 (+)

Ch. Epilobietea: Rubus grabowskii 15 (2.2); Rubus radula 5 (+);

Ch. Artemisietea: Geum urbanum 2 (+); Glechoma hederacea 5 (+.2); Humulus lupulus b, c 5 (1.2); Rubus pseudidaeus 6 (+);

Others: Acinos arvensis 5 (1.2); Brachytheeium rutabulum d 4 (1.2); Dryopteris dilatata 12 (+.2); Euhrynchium hians d 2 (1.2); Euphoprbia cyparisias 4 (+); Galium verum 4 (1.2); Luzula multiflora 12 (+.2); Myosoton aquaticum 3 (+); Plagiomnium affine d 2 (1.2); Rubus nessensis 8 (1.2); Vicia cassubica 11 (+)

Explanations: P - "Promno", C - "Czempiń", R - "Racot"

The Turkey oak coming from natural regeneration, covering no less than 2% of the area in the shrub layer, was usually found in substitute communities with pine tree stands in habitats of the mixed fresh forest type corresponding in the potential vegetation of central Wielkopolska to poor forms of oak-hornbeam forests or acidophilous oak forests (Tab. 3). Only in one place at 'Racot', the tree layer consisted of Betula pendula, and in two, the habitat was a fresh mixed coniferous forest. The age of tree stands at the time the relevés were made ranged from 59 to 87 years, which means that they reached the stage of a maturing or mature tree stand. They usually had a small proportion of additional species and a single layer. The most stable components of the shrub layer, apart from the Turkey oak, were Sorbus aucuparia, Quercus robur (at 'Promno'

also Q. petraea) and Frangula alnus, and sometimes also Prunus serotina. The most popular species in the ground layer was *Pleurozium schreberi* accompanied, but only at 'Promno', by two other species characteristic of the class Vaccinio-Piceetea. An insignificant role was played by species diagnostic for other forest communities from the classes Quercetea roboripetraeae and Querco-Fagetea. Popular components of those phytocoenoses were plants representing the class of clearing communities, Epilobietea angustifolii, like Calamagrostis epigejos and various species of the genus Rubus, as well as Agrostis capillaris from the class Calluno-Ulicetea and Rumex acetosella from the class Koelerio-Ulicetea. In the group of accompanying species, the most important were the ferns Dryopteris carthusiana and D. dilatata.

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Table 3. Communities with Turkey oaks from self-sown seedlings

Successive N ^o	-	2	3	4	5	9	7	8	6	10	11	12	13	14 1	15 1	16 1	17 18	8 19	20	21	22	23	24
Acer platanoides b	•	•							1.1								<u> </u>	•	•	•	•	•	•
Sorbus aucuparia b	2.1	1.1		2.2	2.2	•	1.1	1.1		2.2	2.1		+	2.1 2.1		2.1 1	1.1 1.1	1 3.2	2.2	2.2		::	
Sorbus aucuparia c					+			+		+	+	+					•	2.2	2.2	2.2	•	+	
Frangula alnus b	+		1.1	1.2		1.2			+	1.1	+	+	+	+ 1.1			. 1.1	1 1.1	1.1	•	1.1	+	
Frangula alnus c				+		+		+						+			•	•		+		+	
Prunus serotina b	1.1		1.1	3.3	3.3	2.2				+		3.3 3	3.3				2.2	2 1.1	•	2.2			
Prunus serotina c	+		+	+	+	+						1.2	+			2	1.2 2.2		+	·	•		
Prunus padus b	•			•	1.2	·	•	 .	 .		+	2.2 2.	2	+	-	+	1.1 2.	5				1:1	
Prunus padus c						•											·	•	•	•	•	+	
Acer pseudoplatanus b							1.1	+			2.1			+++	- 1.1		1.1		•	•			
Acer pseudoplatanus c						+			+					+	+		•	•	•	•			
Quercus petraea b	3.3	2.2	2.2	•	+												•	•	•	•			
Quercus petraea c				•			+										•	•	•	•			
Quercus rubra b				•													1.1	1		•	1.1		
Quercus rubra c	•	•		•								+					•	+	+	•	•	•	•
Prunus avium b																	•	1.1	•	•	•		
Prunus avium c	+	+		•		•	•										· 	+	•	•	•	•	
Carpinus betulus b					+	1.1											•		•	•			
Fagus sylvatica b	•		1.1	•			•										·	•	•	•	•	•	
Fagus sylvatica c			+			•											· 		•	•	•	•	
Fraxinus excelsior b	•	•		•													•	•	•	•	•		+
Fraxinus excelsior c	•			•		+								·			•	•	•	•	•	•	
Sambucus racemosa b																. 1.1						1.1	
Sambucus racemosa c	•					•									т —	+	•	•	•	•	•	•	
Robinia pseudoacacia b	•			•													•	•	•		1.1		•
Robinia pseudoacacia c														·	-		•	•	+	•	+		
Sambucus nigra b				•												-	•	•	•	•	•		+
Sambucus nigra c				+										•			•						
Crataegus monogyna b		1.1												•		-	•						
Crataegus monogyna c	•			•		+									·		•	•		•	•	•	•

Successive N ^o	-	2	3	4	5	9	7	8	6	10	11	12 1	13 1	14 15	5 16	5 17	7 18	19	20	21	22	23	24
Ch. Vaccinio-Piceetea																							
Pleurozium schreberi d	5.5	4.4	3.3	3.3	3.3	3.3	•	3.3	3.2	4.4 3	3.3 1	1.2 2	.2 3.	.3 3.3	3 3.3	3.	3 3.3	3 2.3	2.3	3.3	3.3	3.3	
Pseudoscleropodium purum d			2.2	3.3	2.3	+	1.2	·			 .				<u> </u>		· ·	·					
Vaccinium myrtillus			1.2	1.2				 .															
Ch. Quercetea robori-petraeae																							
Polytrichastrum formosum d		2.2	1.2	1.2	1.2	2.3	•		•		+ 2	2.2 3.	.2			•	1.2	2.3	2.3	•	2.3		
Carex pilulifera				•		•	•		1.2	1.2		. 1	.2 1	-2 +		·	•	•	•		+	1.2	
Hypnum cupressiforme d		2.2	1.2	•	+	•	1.2	•	•		+			· 	· ·	1	.2	•	•	•			
Holcus mollis		•	•	•										•		•	•	1.2	•		+		•
Festuca ovina	1.2	1.2		•			·				 .			· ·		•	·	•					
Calamagrostis arundinacea				1.2	1.2									· ·	<u> </u>	•	·	·					
Ch. Querco-Fagetea																							
Dryopteris filix-mas			+	+	1.2	1.2		+							<u> </u>	·	•	·	•	•	•		
Poa nemoralis				•			1.2	•						· ·		•	•	•	•				1.2
Atrichum undulatum d		•	•	•			1.2	•	•	•	•	•		•		•	•	•	•	•	•		1.2
Ch. <i>Epilobietea</i>																							
Calamagrostis epigeios	2.2	2.2	2.2	1.2	1.2		1.2	1.2		1.2 1	2	1.2		2.2 3.3	ц Ч	2 2.2	2 1.2	1.2	1.2	1.2	1.2	2.2	1.2
Rubus gracilis	1.2	1.2	1.2	•			2.2		2.2	2.2 1	2	+	+	2.2	2.2	2	2 2.2	•		1.2		2.2	2.2
Rubus apricus				•				3.3		2.2 3	3.3	+	. 2	2 2.	2 2.2	2	2.2	2.2	2.2	+	2.2	2.2	
Rubus seebergensis				•			2.2	2.2			1.2	+			2.	.2	1.2		1.2	1.2	2.2	2.2	
Rubus idaeus	1.1	+	+	1.2	1.2	2.2	·	1.2	•	•		•		•	•	•	+	•	•	•	•	•	5.4
Rubus koehleri				•		•	•	2.2	3.3 2	2.2					1.	2	·	•	1.2		•		
Rubus lamprocaulos				•			3.3	2.2	2.2		2.2			· 		•	•						
Rubus plicatus	+			1.2						• •	2.2			· 	•	•	•	•	•				
Rubus radula					2.2		•							· 	· ·	•	•	•					2.2
Rubus grabowskii			1.2				•		2.2				_	· 	·	•	•						
Ch. Calluno-Ulicetea																							
Agrostis capillaris	3.3	4.4	3.3	3.3	3.2	3.3	•		2.2	2.2	+	+	1	.2 1.2	.2 1.	2 1.2	2 1.2				+	+	
Ch. Koelerio-Ulicetea																							
Rumex acetosella			+	+		+	•		•	1.2 1	2		<u>'</u>	+	2		+	•		+		1.2	
Others																							
Dryopteris carthusiana	1.2		1.2	+	+	1.2		1.2	2.2	2.2 2	2		-	2 1.5	2	2	<i>с</i> і +	1.2	1.2	1.2	2.2	1.2	
Dryopteris dilatata			1.2		+		•		2.2						2 2	2 1.5	5 +		1.2		1.2		
Anthoxanthum odoratum		1.2	1.2	1.2	1.2	•	•							•	·	•	•				+		1.2

Successive N ^o	-	7	ю	4	5	9	7	×	6	10	Ξ	12	13	14	15	16	17	18	19	20	21	22	23	24
Deschampsia flexuosa	2.2			1.2				2.2		1.2													1.2	•
Carex nigra	•													1.2		1.2						+		•
Molinia caerulea														2.2	+									
Nardus stricta									1.2	1.2														•
Moehringia trinervia			+																				1.2	•
Humulus lupulus					+																			1.1
Geranium robertianum				•	+	+																		
Geum urbanum						+																		+
Sporadic:																								

Prunus spinosa b 7 (1.2); Rosa canina b 2 (1.1)

Ch. Vaccinio-Piceetea: Dicranum scoparium d 11 (1.2)

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Ch.

Querco-Fagetea: Brachypodium sylvaticum 24 (1.2); Dactylis polygama 3 (1.2) Ch.

Artemisietea: Fallopia convonvulus 7 (+); Galium aparine 24 (+); Glechoma hederacea 6 (+) ; Impatiens parviflora 11 (2.2); Mycelis muralis 4 (1.2); Rubus caesius 24 (1.2); Urtica dioica 24 (1.2)

arix ×marschlinsii a 7 (2.1); Poa pratensis 24 (1.2); P. trivialis 24 (1.2); Rubus hirtus 8 (2.2); Veronica chamaedrys 6 (+); V. officinalis 1 (+); Viola sp. 24 (+) <u>Others</u>. Carex hirta 1 (1.2); Dactylis glomerata 4 (1.2); Fragaria vesca 1 (+); Galium verum 2 (+); Juncus conglomeratus 7 (+);

Explanations: P – "Promno", R – "Racot", C – "Czempiń"

2005; Straigyté and Žalkauskas 2012; Chmura 2013, 2014; Gazda 2013; Major et al. 2013; Woziwoda et al. 2013, 2014; Myczko et al. 2014; Dyderski 2015). This is due to the fact that in many regions, this has been the broad-leaf tree of alien origin cultivated most frequently, and its capacity for spontaneous reproduction and expansion in forests has long been known. Although the dynamic tendencies of Q. rubra have been an object of study in a variety of environmental conditions and over large areas, to this day, its capacity for a spontaneous penetration of plant communities has not been satisfactorily explained (Woziwoda et al. 2013, 2014; Danielewicz and Wiatrowska 2014). Because of its little significance for forestry and the

In the literature on the naturalisation of alien oaks in central Europe, most attention has so far been paid to the establishment and dispersal of Quercus rubra (Vor

small introduction range, the Turkey oak has not been such an attractive research object. This paper presents several pieces of evidence showing Q. cerris to have a greater invasive potential, at the scale of a few sites, which suggests that the penetration of the woodland environment by this species could be wider if its cultivation was more widespread.

As has been demonstrated, the most probable sources of seeds for the dispersal of the Turkey oak are individual, small tree stands established more than 100 years ago in habitats suitable for the cultivation of native oak species. At 'Racot', the distance separating the parent trees from the farthest localities of their young generation is almost 1.5 km. This is the distance over which acorns of *Ouercus robur* and *O. petraea* are carried by jays (Ouden et al. 2005). But, an intriguing question is whether the young Turkey oaks at 'Promno' found that some 2.5 km from old trees of this species can be proof of a greater distance over which its acorns are dispersed via zoochory. Among interesting issues connected with this is the chronology of Q. cerris entering new localities. Is it a gradual and steady process, or rather one intensive in only some periods when acorns develop most abundantly? And, how does it affect the fruiting of the other oak species growing in the same area, but producing fruits at a different time? Answers to those and similar questions can be supplied by detailed studies of the age structure of the natural regeneration and biology

DISCUSSION

of the Turkey oak in secondary localities and the role of zoochory in its dispersal.

An analysis of the size characteristics of the Turkey oak specimens from self-sown seedlings in the study area makes it possible to state that this species is not only established, but locally meets all the criteria by which it should be classified as belonging to the group of neophytes or species attaining the stage of naturalisation (cf. Faliński 1968, 1969; Richardson et al. 2000; Pyšek et al. 2004; Tokarska-Guzik 2012). Such plants are capable of regular (rather than sporadic) reproduction and of producing permanent secondary generations the existence of which does not depend on direct human activity.

CONCLUSIONS

Quercus cerrisis capable of a natural renewal in a woodland environment in Poland. In the light of the various definitions concerning the synanthropisation of the plant cover, it can be problematic to regard the Turkey oak as an invasive plant in forests. While it penetrates forest communities spontaneously and has a stable position in the undergrowth or even lower tree layers in them, those phytocoenoses greatly depart from the permanent natural communities in terms of structure and floristic composition. The dispersal of Q. cerris in them is similar to that of other oaks species, often renewing under the canopy of pine tree stands (Mosandl and Kleinert 1998; Pigan and Pigan 1999; Gómez 2003; Sokołowski and Paluch 2003; Gniot 2007). In many cases, this reflects the regeneration of forest communities in which native plants can be accompanied by alien species. If we assume, following Sukopp's (1962) conception, that a neophyte appearing in such 'unsaturated' phytocoenoses does not push out native species, and the proportion of the Turkey oak to the already present community components, according to Faliński's (1968) criteria, can be called compensatory, at least for the time being, then classifying it as an invasive plant is disputable in terms of definitions emphasising the threat to biological diversity posed by such plants. However, if we were to use the criterion of the rate of dispersal of alien species - over a distance longer than 100 m in a period shorter than 50 years (Richardson et al. 2000), then Q. cerris would have the status of an invasive plant.

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Prospects for agricultural lands afforestation in Poland until 2020

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Abstract

The aim of the paper is to present the potential for afforestation of agricultural lands under the 'National Programme for Expanding of Forest Cover' (KPZL) in Poland until 2020. The analysis is based on the ongoing social and economic changes in rural areas in the past decade as well as factors limiting implementation of the Programme. The data used have been derived from annual reports of the Agricultural Property Agency (ANR), the Central Statistical Office and also other official documents and legal acts related to the issue.

Assuming that the area of agricultural lands transferred by the ANR to the State Forests Holding – an institution responsible for implementing the KPZL on state-owned lands – remains approximately 350 ha per year, the overall extent of afforestation on state-owned lands will not exceed 4,500–4,600 ha until 2020. In case of private lands, a further decline in annual afforestation area will be observed. The lack of a stable financing system is a major reason for the decrease in the area of agricultural lands transferred for afforestation. The projections show that the average annual afforestation area on private lands will not exceed 2,500 ha until 2020. Altogether, it can be expected that during the period 2015–2020, approximately 20,000 ha of agricultural lands will be afforested.

The study shows that the current rate of afforestation is insufficient for reaching the target defined in the KPZL (afforestation of 680,000 ha of lands during the period 2001–2020). Low supply of private lands for afforestation and insufficient financial support for farmers are the most important factors limiting the implementation of the KPZL. The situation could be improved by free transfer of public lands for afforestation from the ANR to the State Forests Holding and by implementing financial instruments for afforestation of private lands from the Forest Fund resources.

Key words

agricultural policy, forest cover expansion, forest policy, rural development

INTRODUCTION

Expansion of forest cover is one of the main aims of the Polish forest policy. According to the 'National Policy on Forests', adopted by the Council of Ministers in 1997, the forest cover should reach 30% of the total area of the country by 2020 and 33% by 2050 (National Policy on Forests 1997). According to the Central Statistical Office, forests currently cover 29.4% of the land area of Poland (Forestry 2014).

Afforestation in Poland is performed under the 'National Programme for Expanding of Forest Cover'

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(KPZL). The Programme was adopted by the Council of Ministers in 1995 (National Programme 1995), but it never became a so-called 'government programme', because no financial means for its implementation in long term had been guaranteed. The aim of KPZL is to ensure that conditions are favourable for expansion of forest cover to up to 30% of the country's land area, secure optimal distribution of afforestation, set up ecological and economic priorities as well as programme implementation instruments. The Programme has been implemented since 1995 and will be completed in 2020. From 1995 to 2000, a preparatory phase of the Programme was implemented (National Programme 1995).

According to KPZL, from 2001 to 2020, the expected afforested area would be spread over 680,000 ha, which would include 130,000 ha of lands owned by the State Treasury (state-owned lands) and 550,000 ha of private lands. In detail, it was assumed that, for individual periods, area of new forests cover on agricultural lands would be as follows:

- 2001–2005 24,000 ha annually, including 14,000 ha of private lands,
- 2006–2010 32,000 ha annually, including 24,000 ha of private lands,
- 2011–2020 40, 000 ha annually, including 36,000 ha of private lands (National Programme 2003).

Between 2001 and 2014, approximately 163,000 ha of agricultural lands were afforested throughout the country, 101,600 ha of which accounted for private lands and 61,400 ha for lands owned by the State Treasury. The rate of implementation of the Programme for this period amounted to 37%.

The rate of implementation of KPZL was particularly unfavourable during the past decade (2005–2014): only 32% of lands owned by the State Treasury and 21% of private lands were afforested (the overall implementation rate amounted to 23%). Since 2010, the annual rate of implementation of KPZL has decreased from 18% to 9% (Kaliszewski et al. 2014).

The most important obstacles in the way of implementation of the 'National Programme for Expanding of Forest Cover', which actually caused its collapse, arose mostly from socioeconomic changes in the rural areas following Poland's accession to the European Union (EU) in May 2004. Research results indicate that the most important factors limiting the performance of KPZL during the past decade involve:

- high competitiveness of transferring agricultural lands for non-agricultural and non-forest purposes,
- high competitiveness of direct payments for agricultural production compared to afforestation premium,
- lack of financial support for afforestation of lands within Natura 2000 areas, which are yet to be covered by protection plans,
- exclusion of permanent grasslands from afforestation,
- enlarging minimal plot areas supported by afforestation premium from 0.1 to 0.3 ha in 2004 and up to 0.5 ha in 2007,
- weak promotion of financial support for afforestation among farmers and their training courses,
- complicated procedures for granting financial support.

Most of the aforementioned factors appear to be permanent and long term (Kaliszewski 2012; Kaliszewski et al. 2014).

The Rural Development Programme (RDP) of 2014–2020, which is the most important instrument for supporting afforestation of private lands, introduces several significant changes in comparison to the previous RDP of 2007-2013 (RDP 2007) in terms of rules and procedures for granting financial aid for afforestation. They include, inter alia, verified afforestation payments, annual premium per hectare to cover the costs of agricultural income foregone and maintenance, covering agricultural lands afforested with EU financial support in previous years with area payments (from 2015 onward) and decreasing to 0.1 ha - in certain cases - a minimal afforested area eligible for granting financial aid (RDP 2014). The Programme, however, does not remove any other significant barriers that arose after 2004. For the analysis of prospects for afforestation of agricultural lands until 2020, it was assumed that in case of lands owned by the State Treasury (state-owned lands), the implementation of KPZL would be mostly limited by a deficit of lands suitable for establishing new forests, while in the private sector, afforestation would be mostly limited and hampered by a shortage and insufficiency of financial means, discouraging farmers from planting new forests on agricultural lands and permanently excluding them from agricultural production (Kaliszewski 2012; Łazowy 2015).

The aim of this paper is to present the potential for afforestation of agricultural lands under the KPZL in

Poland until 2020. The analysis is based on ongoing social and economic changes in rural areas in the past decade as well as factors limiting the implementation of the Programme, presented by Kaliszewski et al. (2014).

Methods

The potential supply of state-owned lands suitable for afforestation in the period of 2015–2020 was estimated based on the information about availability of land for afforestation in the resources of the Agricultural Property Agency (ANR) and the State Forests National Forest Holding. The data were derived from annual reports of the ANR and from the Central Statistical Office (GUS).

The projection for afforestation area of private lands until 2020 was made based on the observed 10year trend of the area afforested annually and also taking into account the rules for financing afforestation provided in the RDP 2014–2020 (RDP 2014), as well as the results of the survey on the factors limiting afforestation in non-state lands (Kaliszewski et al. 2014).

Legal regulations and their amendments have been characterised based on the legislation listed in the Legal Acts Database (ISAP), managed by the administration services of the Sejm (the lower chamber of the Polish Parliament) (Online Legislation System 2016).

RESULTS

Afforestation of lands owned by the State Treasury

State-owned lands for afforestation are transferred to the State Forests (which performs establishment of new forests in the field) by the ANR. The ANR is a government institution authorised by the State Treasury to perform process of restructuring and privatisation of agricultural property of the State Treasury. The property taken over by the ANR constitutes the Agricultural Property Stock of the State Treasury.

The transfer of land is made according to the provisions of the Forest Act (Act of 28 September 1991) and the Act on Management of Agricultural Property of the State Treasury (Act of 19 October 1991). The current wording of the Article 24 par. 4 of the latter act states that, at the request of the ANR, a local district authority shall transfer free of charge separate plots intended for afforestation in an area development plan or a zoning permit to the State Forests Holding. Until 2010, the ANR could also transfer to the State Forests free of charge forested areas as well as lands suitable for afforestation, other than those specified in Article 24 par. 4 quoted previously. It was possible to make such a transfer by means of a contractual agreement between the Head of the ANR and the General Director of the State Forests (par. 4a). In 2010, this regulation was overruled by the Parliament (Act of 26 November 2010). Furthermore, in 2011, the Agency was obliged, first of all, to sell the state-owned property instead of transferring it free of charge, even in case of lands intended for afforestation (Act of 16 September 2011). Thus, by this means, all possibilities of free distribution of state-owned lands belonging to the Agricultural Property Stock of the State Treasury was ultimately strictly limited.

From 1992 to 2014, the ANR transferred free of charge to the State Forests altogether 153,700 ha of lands for afforestation. They included mainly lands of VI and VIz classes (i.e. very weak arable lands), lands located in watershed and groundwater reservoir areas, and enclaves surrounded by forests managed by the State Forests and adjacent to these forests (Report 2015).

Between 2004 and 2014, the ANR transferred to the State Forests more than 21,400 ha of lands for afforestation. During this period, new forests were established on more than 21,200 ha of land (Fig. 1). Until 2011, an average area transferred by the Agency amounted to 2800 ha annually. However, from 2011 onwards, it has substantially decreased to about 380 ha per year, reaching its minimal value in 2012 (232 ha). The decline resulted from the previously mentioned legislative changes. Nevertheless, at the end of 2014, the ANR was in possession of 29,800 ha of land formally destined for afforestation (Report 2015).

According to the Local Data Bank of the GUS, at the end of 2014, the area of non-forest lands designated for afforestation by the State Forests amounted to 3200 ha (Local Data Bank 2015). Assuming that until 2020 80% of those land would be afforested (because of the delay between the lands transfer and their actual afforestation) and the area of lands transferred by the ANR would amount to approximately 350 ha annually, in the period 2015–2020, approximately 4500–4600 ha of state-owned lands may be afforested (Fig. 2).

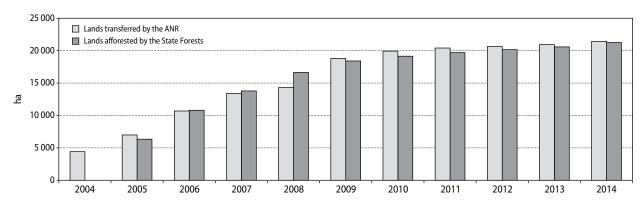
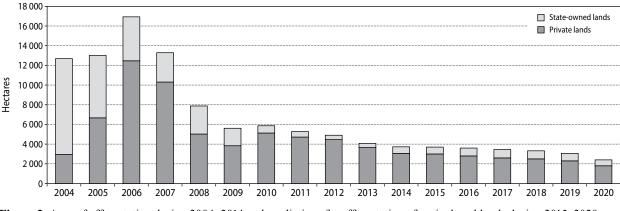
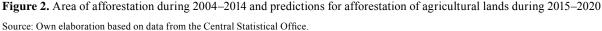


Figure 1. Cumulated area of lands for afforestation transferred to the State Forests by the Agricultural Property Agency (ANR) and cumulated afforested area during 2005–2013. For 2004, only the area of transferred lands has been presented

Source: Own elaboration based on data from the ANR and the Central Statistical Office.





As regards regional distribution of lands for afforestation possessed by the ANR, most areas of lands are managed by its branch offices in Szczecin (11,300 ha), Wrocław (10,000 ha), Olsztyn (3600 ha) and Gdańsk (1300 ha) (Report 2015). Thus, it is very likely that afforestation will be carried out mainly in the following provinces: Zachodnio-Pomorskie (north-western Poland), Dolnośląskie (south-west), Wamińsko-Mazurskie (north-east) and Pomorskie (northern Poland). Afforestation in the Małopolskie, Podkarpackie and Świętokrzyskie Provinces (southern and central Poland) can only be performed on the lands already (up to 2015) transferred to the State Forests, as the ANR does not possess anymore lands formally destined for afforestation in those regions.

Afforestation of private lands

Between the years 2014 and 2020, afforestation of private (non-state) agricultural lands will be predominantly carried out under the RDP 2014–2020, which is the most important support instrument for development of agriculture and rural areas. Its 'Afforestation and creation of woodland' is the only activity under the 'Investments in forest area development and improvement of the viability of forests' in the Polish RDP 2014–2020 (RDP 2014).

The total aid (i.e. along with national co-financing) provided under the 'Afforestation and creation of woodland' in the RDP 2014–2020 amounts to EUR 301 million for the entire duration of the programme. The total financial follow-up commitments for the 'Afforestation of agricultural land and afforestation of non-agricultural land' of the RDP 2007–2013 and the 'Afforestation of

The limited financial resources allocated for the ac-

tivity of 'Afforestation and creation of woodland' under

the RDP 2014-2020 urge to search for other sources of

agricultural land' of the RDP 2004–2006 have been estimated at about EUR 270 million. These commitments will be financed under the 'Afforestation and creation of woodland' of the RDP 2014–2020 and the amount covers new applications submitted by farmers in 2014 (RDP 2014).

The average total amount of aid granted for afforestation (i.e. new applications) in the period 2009–2013 amounted to PLN 43.5 million per year (Reports 2010–2014). For new afforestation to be implemented in the period 2015–2020, about EUR 31 million have been allotted (RDP 2014). Based on the average exchange rate as of September 2015 (EUR 1 = PLN 4.19; National Bank 2015), it makes some PLN 129.9 million, which is below PLN 22 million annually.

Payments for afforestation under RDP 2007–2013 (altogether 27,400 ha) made by the Agency for Restructuring and Modernization of Agriculture, the accredited paying agency, amounted to PLN 538.8 million. Only in 2014, they totalled PLN 90.6 million (Report 2014). Given these values, the sum of approximately PLN 129.9 million allocated for this purpose for the period 2015–2020 seems to be dramatically insufficient not only to increase the annual extension of afforestation but even to maintain it at the current level.

Between 2007 and 2013, the area of private lands afforested annually decreased from 10,300 to 3,600 ha. In 2014, less than 3,100 ha were afforested. With no stable system of financing of afforestation, further decline in the annual area of afforestation may become a fact. Based on the above assumptions, it can be presumed that from 2015 to 2020, the extent of new afforestation on the private lands will not exceed 15,000 ha, that is, on average, 2,500 ha annually (Fig. 2).

It is difficult to determine the regional distribution of the oncoming afforestation, because they are not limited by potential agricultural land availability. Based on the analysis of current trends in regional distribution of afforestation, it is most likely that new private forests may be located in Warmińsko-Mazurskie, Mazowieckie and Lubelskie Provinces, that is, in north-eastern, central and eastern regions of Poland. The areas with least afforestation will probably be in Opolskie, Śląskie and Małopolskie Provinces, that is, in the southern part of the country (Kaliszewski et al. 2014).

The rate of implementation of KPZL on private lands (according to the objectives set out in the Pro-

gramme's revision of 2003) is likely to reach about 22%. The Programme will be most successful in the following provinces: Warmińsko-Mazurskie (87%), Kujawsko-Pomorskie (59%) and Lubuskie (40%), while the least success rate will be attained in Śląskie (8%), Wielkopolskie (9%), Małopolskie (11%), Świętokrzyskie (13%) and Łódzkie (14%).

DISCUSSION

The estimates presented for the period of 2015–2020 show that altogether afforestation of approximately 20,000 ha of agricultural lands, mostly private ones (75%), may be expected. Thus, throughout the period of the implementation of 'National Programme for Expanding of Forest Cover' (2001–2020), approximately 183,000 ha would be afforested, which means 27% of the originally planned extent. The main factors affecting the breakdown of the KPZL implementation are long-term and it does not seem possible to displace them in the coming years.

According to Łazowy, from farmers' point of view, under neither the RDP 2007-2013 nor the RDP 2014–2020, were the full cost of changing agricultural lands to forests financially compensated. After agricultural lands are afforested, the landowners permanently lose the possibility of converting their afforested lands back to agricultural lands. The current support system under the RDP does not take it into account. The landowners are granted only costs of afforestation, tending young stands and a possible income loss from agricultural production as a result of establishment of a new forest (for a period of 12 years). Thus, it seems reasonable that landowners are also compensated for the permanent loss of their lands and thus not being able to engage in more profitable agricultural production. This would also require granting them a land value equivalent (Łazowy 2015). The average price of poor arable land (land of V and VI classes) in 2013 amounted to PLN 20,200 per 1 ha (Statistical Yearbook 2014) and based on such a sum, the aid for farmers should be increased (Łazowy 2015).

sources, namely, those allocated to the Forest Fund, which is the internal compensatory fund of the State Forests Holding. The legal basis for domestic support is delivered by the Commission Regulation (EU) No 702/2014, declaring certain categories of aid in the agricultural and forestry sectors and in rural areas compatible with the internal market in application of Articles 107 and 108 of the Treaty on the Functioning of the European Union (Regulation 702/2014). According to the Art. 32 of the Regulation, additional national aid may be granted for afforestation or the creation of woodland on agricultural and non-agricultural land and may include, inter alia, costs of the plantation material, plantation costs and costs directly linked to the plantation, costs of necessary prevention and protection material as well as costs of replanting necessary during the first year of afforestation. However, because of the Forest Fund legal structure, such aid would have to exclude direct profits for landowners and thus include only lands other than agricultural ones, that is, only those forested (partially) through natural succession or lands protected because of their watershed and soil protective functions, where as a rule no afforestation premium for forest owners is granted.

Commission Regulation (EU) No 702/2014 specifies that, in order to ensure compliance with the Regulation of the European Parliament and of the Council (EU) No 1305/2013 of 17 December 2013 for support of rural development by the European Agricultural Fund for Rural Development (EAFRD) and to simplify the rules for approving state aid for co-financed part by the EAFRD and additional funding of RDPs, the aid should be granted only under the RDP and in accordance with that programme (par. (62) and Art. 32 par. 2) (Regulation 702/2014). As the responsibility of the paying agency in the Polish RDP 2014–2020 is borne by the Agency for Restructuring and Modernization of Agriculture, it would be reasonable if it would support administratively the aid from the Forest Fund.

The increase in the extent of afforestation rate in state-owned lands would be feasible after the possibility to transfer lands allocated for afforestation from the ANR to the State Forests is restored. At the end of 2014, there were approximately 30,000 ha of such lands (Report 2015). The main obstacle is caused by the obligation for the ANR to sell the property it manages in the first place, which considerably limits free land distribution to other public agencies and institutions.

CONCLUSIONS

The results of the study and data analysis allow to draw the following conclusions:

- According to the observed dynamics of afforestation, it may be assumed that it will not be possible to reach a goal formulated in the KPZL to afforest 680,000 ha of agricultural lands in the period of 2001–2020.
- Currently, the most important limiting factors are shortage of lands suitable for afforestation (stateowned property) as well as insufficient financial resources available for afforestation and related compensations (private lands).
- It is recommended to restore the opportunity to transfer lands designated for afforestation free of charge from the ANR to the State Forests.
- In view of the limited resources allocated to the measure of 'Afforestation and creation of woodland' under the RDP 2014–2020, it seems reasonable to consider the possibility of launching domestic aid for this purpose from the Forest Fund.

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SHORT COMMUNICATION

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European oak decline phenomenon in relation to climatic changes

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Abstract

The complex phenomenon of decline in European oak is currently triggered by changing climatic conditions and their consequences like heavy rains, local floods and pest development. Especially, pathogens from *Phytophthora* genus profit from soil saturation with water. They are alien invasive species, which attack and severely damage fine roots. In drought conditions occurring in the subsequent year, many oaks die as they encounter problem with water uptake. Additionally, insect defoliators followed by oak mildew infections accelerate the level of tree mortality. Secondary insects, butt and root pathogens are usually the final cause of death of many oaks. More research is needed in the direction to determine (i) measurable factors (e.g. chlorophyll florescence) that can indicate that the process of tree decline has already started, (ii) the correlation between the root decay and the crown symptoms (scanners, software), (iii) which combination of stressors stimulate the best development of pathogens that lead to the high plant mortality and (iv) the difference between the mortality caused by the native and the invasive *Phytophthora* species.

Key words

Quercus spp., Phytophthora, Armillaria spp., climate change, pathogen, pests

INTRODUCTION

The impact of recent global climate change has been well documented through changes in plant phenology, morphology and abundance (Richardson et al. 2013). Damages to forests caused by the extreme climatic events have also been well documented (Linder et al. 2010). The climate change scenarios for the 21st century had predicted changes in the ecosystems, which will cause many difficulties for oaks to adopt and mitigate these new environmental conditions (Corcobado et al. 2014; Linder et al. 2010; Borja et al. 2008). During the 21st century, the temperature will continue to raise (according to different scenarios) between 1.1 and 6.4°C (IPCC, 2007), causing many problems to plants and even more complex ecosystems such as forests.

Plants are continuously affected by below- and above-ground abiotic and biotic stressors (Milanović et al. 2015). Because of the complexity of the forest ecosystems, it is becomes very complicated to hypothesise the effect of climatic changes, especially in an interaction between plants and their dependent communities of organisms (including pathogenic ones). The impact of climate change on plants and their interaction with herbivores and pathogens are usually based on models, because of lack of data or insufficient data about these changes (Poutasso et al. 2012; Milanović et al. 2015). The plant diseases driven by pathogens are expected to be exacerbated by the negative effect of weather extremes on forest ecosystems (Santini et al. 2013). The pathogen ecology is strongly dependent on environmental factors, such as temperature, moisture and changes in winter temperatures as a result of global warming. This can influence their distribution and development or even the pathogenicity. The negative effects have also been reported for herbivore and root-damaging insects (Masters et al. 1993).

This review focuses on the importance of climatic changes and how they directly (changing weather conditions) and indirectly (increased pest and diseases) affect the European oak tree populations. Different causes of the oak decline phenomena are mentioned and discussed in this review.

DROUGHT AND WATER LOGGING

The whole of Europe will be, according to climate model simulation, under pronounced periods of droughts and/or water logging (Linder et al. 2010, Potausso et al. 2012, Portz et al. 2011). These two factors cause stress and increase the vulnerability of oak trees, which are among the most valuable broadleaved trees in Europe. The different stages of oak stands affected by the phenomenon of decline were observed in the last decade throughout Europe. The complexity of oak decline process and spectra of involved biotic agents were reviewed by Oszako and Delatour in "Recent advances in oak health in Europe" (Oszako and Delatour 2000). The main biotic factors involved in oak decline are root pathogens (*Armillaria* and *Phytophthora* species) and insect defoliators.

FACTORS AFFECTING OAK DECLINE

Phytophthora

Plant pathogens of the genus *Phytophthora* have a global distribution and play a crucial role in damaging both agricultural crops and forest ecosystems (Nilsson and Diunker P. 1997; Oβwald et al. 2014; Milenković et al. 2012). Several *Phytophthora* species (*P. quercina*, *P. cambivora*, *P. plurivora*, etc.) were linked with the decline phenomenon of oak trees. The level of fine root damage often reaches 90% or more (Fig. 1).

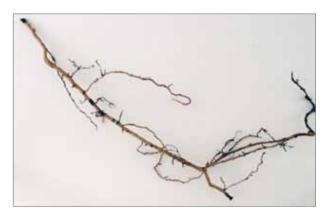


Figure 1. Fine roots of pedunculate oak (over 100-year-old), damaged in 90%

Those species are able to infect both juvenile and mature pedunculate (Quercus robur L.) or sessile oak (Q. petraea Liebl.) trees and affect their vitality by decreasing their photosynthesis, the growth of roots and their functionality (Jönsson et al. 2003, Barajas-Morales et al. 1997). An invasive pathogen such as P. cinnamomi, which can potentially deteriorate the health status of Mediterranean oak trees (Oszako and Orlikowski 2005), has been recently discovered at a high incidence in the study of three native pedunculate oak forests in Poland (unpublished 2015). However, P. cinnamomi was recorded on pedunculate oaks in Poland already in 2005 (Oszako and Orlikowski 2005). As this pathogen is highly dependent on soil water content and mild temperatures, a combination of observed climate warming and frequent weather extremes could enhance the decline process of the abovementioned populations of oaks, which is of much concern for local foresters.

ARMILLARIA

The Armillaria species are opportunistic pathogens that cause damage to a wide range of woody hosts (Shaw and Kile 1991; Fox et al. 2000). Usually, they attack the already stressed oaks and cause decay in the main roots and the sapwood. These fungal species demonstrate different levels of their pathogenicity, which is highly correlated with the production of rhizomorphs (Morrison 2004). The shift in the pathogenicity of opportunistic pathogens, *Armillaria gallica* (Fig. 2) and *A. cepistipes*, was observed in Europe during the oak decline incidents in the past (Keča et al. 2006; 2009; Lushaj et al. 2010; Keča and Solheim 2011; Cleary eta I. 2012, Cleary and Holmes 2011). They also often follow damage that *Phytophthora* causes to fine roots infecting the vicinity of cankers occurring on mother roots (Fig. 3).

Insect defoliators

The insect defoliators regularly cause damage to oaks and other broadleaf forest tree species, particularly in the Northern Hemisphere. A recent study showed that the leaves of *Quercus rubra* L., naturally infected by *Phytophthora plurivora* are more susceptible to an attack by *Lymantria dispar* L., than leaves of healthy trees (Milanović et al. 2015).

OAK DECLINE PHENOMENA AND RESEARCH GAPS

There are considerable knowledge gaps about the oak decline phenomenon; especially processes leading to the deterioration of tree health and possible death are barely investigated. The visible symptoms of disorders are often too general and therefore it becomes difficult to find practical solutions to mitigate its consequences. In particular, there is little understanding of how the extreme weather conditions influence the subtle interaction between the local population of tree-hosts, pathogens and insect pests. How do host-pathogen interactions change if abiotic stress disrupts physiological processes in oaks? It is of major importance to recognise the decline process in the initial phase, because once the decline syndrome starts, it is hard to stop its further development. Also the involvement of contributing factors such as secondary insect pests (Cerambyx spp., Agrillus spp., Scolytus intricatus (Ratz.) (Coleoptera: Scolyti-



Figure 2. Mycelial fans of *Armillaria gallica* developing under the bark od declining oak trees



Figure 3. Lateral roots completely damaged by pathogens of *Phytophthora* genus. The canker (in the middle) is being healed by callus tissues (from both sides) but rhizomorphs of *Armillaria* sp., which are attached to the root surface, (above) started secondary infection

nae), etc. or stem-decaying fungi (Fomes fomentarius (L.) J.J. Kickx, Kretzschmaria deusta (Hoffm.) P.M.D. Martin (Fig. 4), Stereum spp., Trametes spp., Phellinus igniarius (L.) Quél. and P. robustus (P. Karst.) Bourdot & Galzin, etc) is usually hard to prevent.



Figure 4. Mycelium of *Kretzschmaria deusta* destroying bark and wood of stressed oak trees

Oak decline phenomena are complex, multi-factor disease, resulting in a progressive weakening of trees and leading to the dieback of their crowns (Manion and Lachance 1992). In their study, they observed the loss of vitality of oaks and the reduction in their growth rate, as well as an increase in their susceptibility to abiotic and biotic stressors. The decline syndromes generally affect trees of all age classes and ultimately cause their death, unless the stress is removed (Woo 2009). Though many researchers had studied forest decline from different points of view, the main cause of this widespread phenomenon is still not well understood. In the light of effects of climatic factors (single or combined), such as summer drought or heavy rains, the decline of trees, in particular oaks, could be even more readable in the course of the 21st century. Knowledge of physiological processes accompanying different disorders, ultimately leading to the mortality of oaks, is crucial to understand their susceptibility to predisposing (soil properties, drought, stand structure, etc.), inciting (primary insects, oak mildew, winter frost, etc.) and contributing (root rot diseases, stem decay fungi, secondary insect pests, etc.) factors (Figs. 3 and 4) (Ceisla and Donauber 1994). To understand this complex process, the factors contributing to the decline process and their interactions under controlled conditions need to be separately tested. The susceptibility of pedunculate oak (Q. robur) to climate extremes like drought/ flood, followed by the root infection with the native pathogens like Armillaria gallica, P. plurivora or the invasive one as P. cinnamomi, is still unclear. The decline process is strongly correlated with the destruction or decay of the root system and crown defoliation (Figs. 1 and 2). However, in the case of mature trees, it is very hard to find any correlation between the amount of decayed roots and symptoms observed in their crowns.

Armillaria gallica is the native pathogen to oak stands representing an opportunistic species that attacks stressed trees and coarse roots, while P. plurivora is a biotroph that colonises mainly fine roots. It is still unknown if the loss of the fine roots will have a stronger influence on the decline of stressed trees, e.g. by insect defoliators, than the loss of the coarse roots. Also, there is evidence that the synergistic activity of both pathogens may occur during the decline of oaks (Jung et al. 2016; Oßwald et al. 2014; Keča et al. 2015). The invasive P. cinnamomi could cause much more serious problems if it is introduced in a new ecosystem, similar to as it was observed in oak stands (Quercus coccifera L., O. ilex L. and O. suber L.) in the Mediterranean region (Brasier 1996; Brasier and Scott 1994; Corcobado et al. 2014; Linaldeddu et al. 2014).

PHYSIOLOGICAL ASPECTS OF TREE REACTION TO DROUGHT AND PEST ATTACK

The high carbon availability during the root growth should increase an oak's defence capacity against the root pathogens (Jönsson 2006). Another study showed that the ability of trees to mobilise non-structural carbohydrates against root-rot pathogens enhances its susceptibility and speeds up the decline process (Angay et al. 2014). So, there is no consensus reached on the influence of photosynthetic assimilates (simple sugars) on the activity of harmful organisms (insect defoliators, fungi, Oomycetes).

The root pathogens strongly influence the water relations in the woody plants, which particularly implies the transpiration and photosynthesis of leaves. The measurements of gas exchange and the fluorescence of leaves may be used to calculate the electron transport rate (Laisk and Loreto 1996). The differences between the electron transport rate calculated from the fluorescence ratio and that calculated from the gas-exchange measurements have been used to estimate the mesophyll diffusion resistance and chloroplast CO_2 concentration and non-photosynthetic carboxylation and decarboxylation rates. These parameters could be used for an assessment of the process of photosynthesis and the health condition of oaks.

Furthermore, plant hormones, like abscisic acid (ABA), play an important role in many aspects of plant growth. The adaptation to drought and low temperatures is regulated by the combinatorial activity of interconnected ABA-dependent and ABA-independent signalling pathways (Mauch-Mani and Mauch 2005). By contrast, the plant hormones like salicylic acid (SA), the jasmonate acid (JA) and the ethylene (ET) play a major role in the trees' resistance to pathogens and insects (Flors et al. 2008). The combination of these hormones could be crucial in the assessment of the stress in trees as well as in the resistance/susceptibility of trees to later pest attacks (Thaler and Bostock 2004).

GENETIC VARIATION VS HEALTH STATE OF OAK STANDS

Thirteen oak (*Q. robur*) populations from the Krotoszyn and Elbląg Forest Districts, located in central and northern Poland (51°68' N, 17°44' E and 54°20' N, 19°73' E, respectively), were analysed in order to determine genetic variation and similarity in relation to health and resistance to stress factors (Nowakowska et al. 2007). Chloroplast DNA was investigated using PCR-RFLP markers. Different frequencies of six haplotypes variants ('1', '4', '5', '7', '10' and '12') of 'DT' and 'CD' loci primers were determined. All oak populations studied were characterised by high genetic variation ($G_{ST} = 0.818$), which indicate higher genetic variation observed within the stand than among them (Nowakowska et al. 2007). Going into details, higher genetic variation was observed in trees from central Poland (Krotoszyn; $H_T = 0.809$) in comparison to the northern part (Elblag; $H_T = 0.785$). Oaks stands from Krotoszyn mostly originated from the Balkan and Iberian oak refugees, as demonstrated by the presence of prevalent haplotype pattern composed of variants '5', '10' and '12', respectively. Within this region, noticeably Jarocin stand was composed by trees with one Balkanian haplotype '7' to the extent of 87%. Conversely, most stands of the Elblag Forest Region presented the haplotype pattern typical of the Apennines post-glacial oak refuge (with predominating variants '10' and '12'). One population from this region, Młynary, exhibited slightly different pattern with a high proportion of Balkanian haplotype '4' (40%) and Iberian haplotype '12' (38%).

Within these oak populations, Jarocin (Krotoszyn FD) and Młynary (Elblag FD), stand health was monitored from 1999. Different levels of injury symptoms were observed between stands but mortality was higher in Jarocin, less genetically variable (with predominant frequency of one Balkanian haplotype '7'). Severe damage affected 60% of the trees in Jarocin stand after 2003, which was probably due to a combination of unfavourable climatic conditions and infection by pathogens, e.g. Phytophthora and Pythium. It has been shown that in Poland, oak trees have been colonised mainly by P. quercina, P. cactorum, P. plurivora, P. cambivora and P. cinnamomi (Oszako and Orlikowski 2005; Jankowiak et al. 2014; Nowakowska et al. 2016). Comparing chloroplast DNA variation data obtained for Młynary from central Poland and Jarocin from northern Poland, the population with higher cpDNA differentiation (Młynary) appeared better adapted to the changing environmental conditions (drought, high temperature) than Jarocin with lower genetic variation (Nowakowska et al. 2007).

RECOMMENDATIONS FOR FURTHER RESEARCH

Further research has to be done in order to determine (i) measurable factors that can indicate that the process of tree decline has already started, (ii) the correlation between the root decay and the crown symptoms, (iii) which combination of stressors stimulate the best development of pathogens leading to high plant mortality, (iv) the difference between the mortality caused by the native and the invasive pest species.

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SHORT COMMUNICATION

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Open access to research data in the data warehouse of the Forest Research Institute

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The concept of open science has a long history. Its origin dates back to the 17th century and is connected with the release of the first scientific journal in Europe: *Journal des Savants* and *Philosophical Transactions of the Royal Society.* Today worldwide, there are more than fifty four thousand scientific journals, of which more than a million scientific articles are published annually. The contemporary concept of open science includes not only an open access to the research results, that is an open access to scientific articles, but also more and more open models for other areas of scientific work like access to research data or conducting research with 'open notebook'. The dissemination of open science concept is a global trend, which is connected with the development of information and communication technologies.

In Poland, the policy guidelines on open access to publications and research results are consistent with the Commission Recommendations of 17 July 2012 on the access to and the preservation of scientific information (2012/417/EU). Among the scientific institutions conducting studies on forest ecosystems in Poland, the Forest Research Institute was the first to use advanced information technology for open access to scientific data. The project 'Forest Information Centre - information platform of environmental monitoring in Poland' (The Innovative Economy Programme 2010-2014) was developed as a model of the scientific data warehouse. The data warehouse designed based on the concept of Business Intelligence uses the following tools: SQL Server Integration Services, SQL Server Analysis Services, SQL Server Reporting Services and SQL Server Report Builder. The adopted technological solutions enable integration and standardization of data from distributed sources, loading multidimensional data structures (OLAP cubes), and perform multidimensional data analysis (Fig. 1). The process of integrating the data warehouse may include archival data of the Institute, data from operational bases in current projects, as well as data from external sources like the Information System of State Forests (SILP). The structures of the multidimensional warehouse (OLAP cubes) are designed regardless of the source databases. There are two basic categories of data in the warehouse, namely facts and dimensions. Facts are the analysed data. For example, in case of the Institute, facts relate to the growth of trees, the occur-

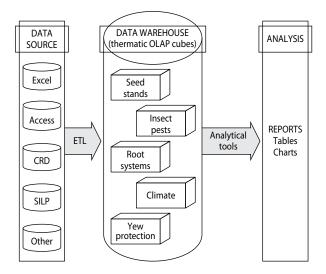


Figure 1. Scheme of data warehouse architecture of the Forest Research Institute

rence of insect pests, the development of root system of forest trees, etc. Facts are described by measures such as the height of trees, the number of insects per 1 ha of stands, root biomass, etc. Dimensions specify the condition of analysis; in case of the Institute, dimensions relate to the time (year, month) and location (geographical coordinates or forest address consisting of the codes, such as Regional Directorate of State Forests, forest districts, precincts forest, forestry, division, sub-division, etc.). Scientific data selected for integration is subjected to a process ETL, which is responsible for the selection and downloading of data from the source databases, data transformation, cleaning, conversion, sorting, standardization, and then loading of data to the structures warehouse. The integrated scientific data through the use of modern computer tools create new sources of valuable scientific information, such as, on the environmental changes in an era of global climate change. The results of the analysis can be presented in the report as graphs or tables or they can also be exported to CSV files, PDF, Excel, Word or XML. The results of the analysis are stored in thematic catalogues (e.g., seed stands, root systems, insect pests, climate, yew protection, etc.) and they are available in the Repository Reports of Data Warehouse (Fig. 2). In the last decade, a number of scientific institutions in Poland have undergone modernization, and have expanded their information systems based on modern technologies. Modern database systems guarantee data security, fast search of information and provide authorized access to data. Data warehouse enables the storage of historical data and operational data at one place and at the same time, allows various kinds of analyses like identifying trends, directions of change, development forecasts, etc. The possibility of data mining changes the natural environment in Poland and gives significant support for the development of forest research and scientific cooperation.

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Figure 2. Distribution of thematic data warehouse of the Forest Research Institute

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