

The influence of phosphite treatments on oak leaves and damage caused by powdery mildew *Erysiphe alphitoides*

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ABSTRACT

The aim of the project was to check the influence of ammonium phosphite preparation – Actifos on the physiology of leaves and a possible reduction of infection by the fungus a year after the application of fertilizer. Three observation plots were selected in Karczma Borowa, Krotoszyn and Piaski Forest Districts (FD). In each of these observation plots, trees were chosen randomly. In Karczma Borowa FD, the trees were treated by watering them with a 3% solution of Actifos. In Krotoszyn FD, the leaves of trees were sprayed twice (in July and September) from the plane using a 50% solution of Actifos; and in Piaski FD, only the trunks of trees were sprayed twice the same way, but in July 2012 and September 2013. In October 2013, from each tested tree, ten leaves were selected randomly from the upper, well-lit parts of their crowns. The assessment of leaf surface damaged by mycelium and chlorophyll *a* fluorescence was performed. After the application of the phosphite, no negative physiological consequences for the treated trees were noticed – neither concerning the average leaf area nor the fluorescence of chlorophyll. The manner of phosphite application (leaves, trunks or roots) did not cause any negative consequences for the vitality/health of the treated trees as compared to the control trees. A certain tendency in the reduction of oak mildew on the treated leaves with phosphite was observed, however these observations should be continued in the next years.

KEY WORDS

powdery mildew, oak, chlorophyll fluorescence, phosphite fertilizers

INTRODUCTION

Oak decline phenomenon was observed many times during the last century throughout Europe. Severe damage of oak trees is usually a combination of unfavourable climatic conditions and infection by different pathogens, especially *Phytophthora* genus (Nowakowska et al. 2007).

The powdery mildew of oak is caused by the fungus *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam (also known as *Microsphaera alphitoides* (Griffon & Maubl.) and it is a common foliar pathogen of oak throughout Europe. For example, it represented 13% of all biotic and abiotic health problems reported on deciduous oak species in France from 1989 to 2006 (Marçais and Desprez-Loustau 2014). However, this problem was not reported in Europe before 1907, when sudden severe outbreaks were observed. The causal pathogen was then reported as invasive (Desprez-Loustau et al. 2011).

In Poland, it attacks both pedunculate (*Quercus robur*) and sessile (*Q. petraea*) oaks. The disease can be very severe, particularly for seedlings growing in naturally regenerating forest, decreasing the growth of plants or even causing their death. The fungus attacks the leaves and shoots of oaks causing serious damage to them, negatively affecting photosynthesis and transpiration processes. The pathogens inflict greatest damage to young plants, especially in nurseries and plantations. Phosphites were used successfully to stop the progress of *Phytophthora* in Australia (Hardy et al. 2017). Some records of positive influence of phosphites on forest tree species in nursery, including the reduction of oak mildew infestation, were found in the past (Tkaczyk et al. 2014; Okorski et al. 2014).

The chlorophyll *a* fluorescence process can provide information on the functioning and structure of the photosynthetic apparatus (Kalaji and Łoboda 2007; Akhkha et al. 2013; Stirbet et al. 2014; Živčák et al. 2014). Moreover, the methods based on records of chlorophyll fluorescence are reliable, non-invasive, powerful and simple tools for the assessment of photosynthetic electron transport (Živčák et al. 2014) and related photosynthetic processes and allow detection of stress in plants (Baker and Rosenqvist 2004; Borawska-Jarmułowicz et al. 2014).

The aim of this project was to check the physiology of leaves and the influence of application of Actifos on reduction of fungus infection in leaves.

METHODOLOGY

Three observation plots in the Forest Districts (FD) of Karczma Borowa, Krotoszyn and Piaski were selected. In each of them, the trees were chosen randomly. In Karczma Borowa FD, 15 trees were treated only once in July 2012 by watering them with a 3% solution of the Actifos (Agropak Sp. J., Jaworzno, Poland) (100 litres under each tree), and in the control variants 8 trees were watered without the product or not watered at all (8 trees). In this case, Actifos was incorporated into the soil around the trunks of the trees. In Krotoszyn FD, leaves of the oaks (5 ha) were aerially sprayed (twice in July and September 2013) from the plane using a 50% solution of Actifos (containing ammonium phosphite). In Piaski FD, only the trunks of trees were sprayed with the same concentration of the product, two times in July 2012 and September 2013. In October 2013, from each tested tree (Krotoszyn – Actifos 8, control 6 trees; Piaski – Actifos 8, control 5 trees), ten leaves were randomly selected for analysis from the upper well-lit parts of their crowns.

A characteristic feature of infection caused by mildew is a visible white coating of mycelium on oak leaves (especially at the end of the growing season), which was used for the estimation of the infected leaf surface. For parameters like leaf surface not infected by the fungus, the 'white' area covered by mycelium was considered as damaged, and the entire surface of the leaf was measured. To achieve this goal, the WinDias 3.1 Image Analysis System (Delta-T Device, Great Britain) system was used. The measurements were made on the selected 10 leaves, as explained earlier.

The measurements of chlorophyll *a* fluorescence were performed by Handy PEA fluorimeter (Hansatech Instruments, Pentney, Norfolk, UK). After dark adaptation (20 min) the raising fluorescence transients were induced by red light (peak at 650 nm) of 3000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. The fluorescence transients were recorded for 1 s, starting from 50 μs after the onset of illumination. Measurements were performed immediately after collection.

The analysis of data involved the comparison of average values and standard deviations for a sample taken from each tree. The results were presented graphically in the form of bar graphs with marked deviations posts. In addition, an analysis of homogeneous groups using the Kruskal–Wallis test (nonparametric test) was performed at significance level $\alpha = 0.05$, in Statistica ver. 10 (StatSoft Inc., Tulsa, OK, USA).

RESULTS

Total leaf area

Based on these results, it should be noted, that the performed treatments with fertilizer containing ions of NH_4^+ had no negative effect either on the total area of

the leaves or on their physiology, regardless of the location of observation plot. This demonstrates that the product Actifos does not cause any negative changes in the physiology of trees and its application (aerial or on stems) is same from this point of view.

In Karczma Borowa FD, the average area of leaves in control trees reached 16.29 cm^2 (Fig. 1). while the average area of leaves treated with only 100 litres of water was higher and equivalent to 17.32 cm^2 (values range from 10.11 cm^2 to 26.36 cm^2). The average leaf surface in the group of trees treated with Actifos formulation ranged from 14.39 to 22.56 cm^2 (average of 17.35 cm^2). However, on the basis of statistical analysis, we cannot confirm that the conducted treatments (irrigation water or the use of the fertilizers) had a significant impact on the total area of leaves.

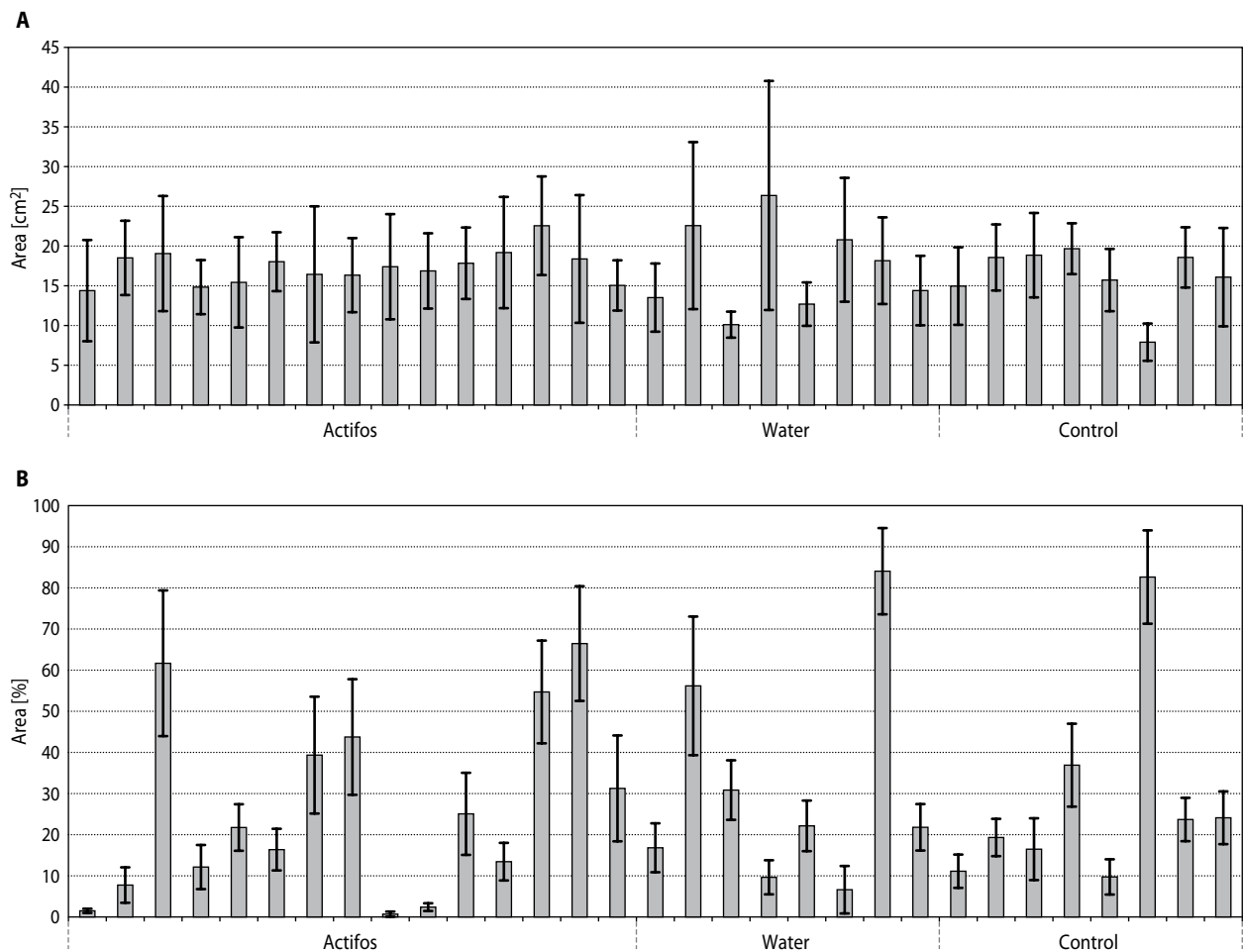


Figure 1. Total leaf area \pm S.D. (A) and leaf surface occupied by pathogen [% of total area] \pm S.D. (B) in Karczma Borowa FD

In Krotoszyn FD, the average total area of leaves in the control variant was 16.03 cm² (values ranged between 11.61 to 21.24 cm²) (Fig. 2). The total area of oak leaves treated with Actifos was 16.20 cm² and the values ranged between 10.8 to 21.79 cm². There were no statistically significant differences between the average values of total area of leaves treated and not treated with Actifos ($\alpha > 0.05$).

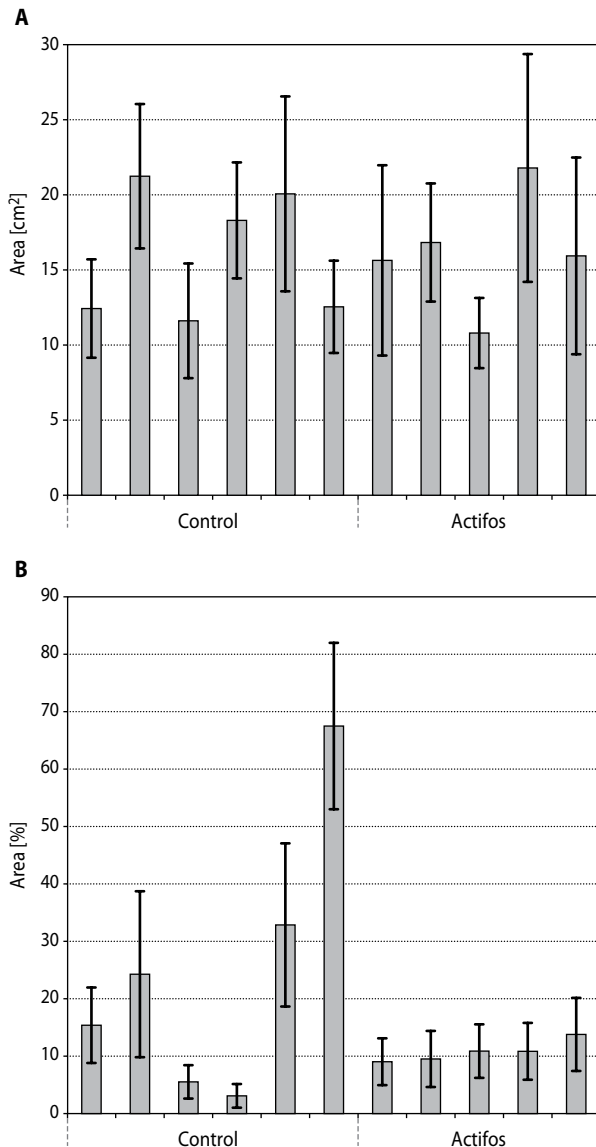


Figure 2. Total leaf area \pm S.D. (A) and leaf surface occupied by pathogen [% of total area] \pm S.D. (B) in Krotoszyn FD

In Piaski FD, the total area of leaves of control trees reached 11.95 cm² (Fig. 3). The lowest value was 8.00 cm², and the highest was 15.37 cm². The average area of leaves on the treated trees was 13.83 cm². These values varied from 10.47 cm² to 19.00 cm². In this case, the mean values of total area of leaves between control trees and treated ones were statistically significant ($\alpha \leq 0.05$).

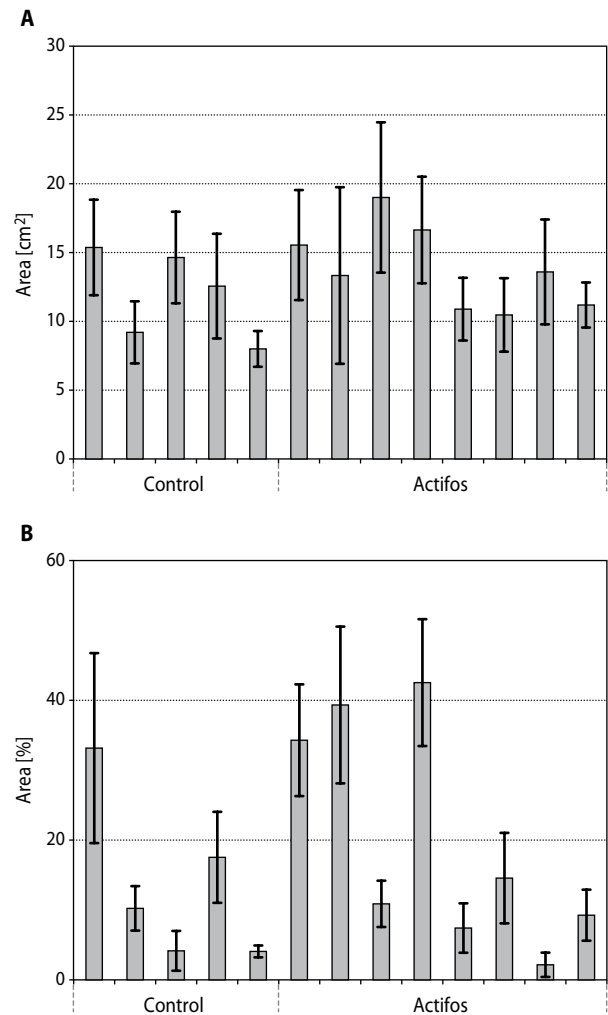


Figure 3. Total leaf area \pm S.D. (A) and leaf surface occupied by pathogen [% of total area] \pm S.D. (B) in Piaski FD

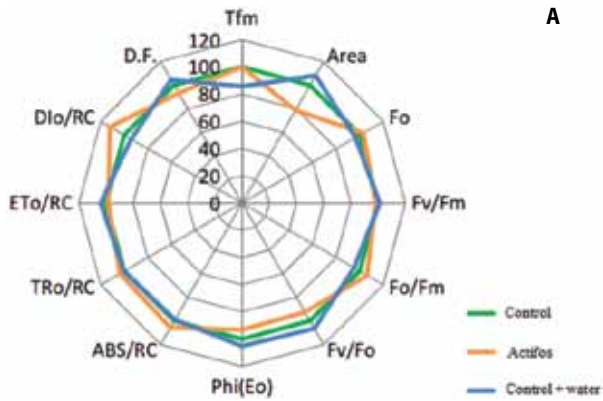
Leaf area infected by oak mildew

In Karczma Borowa FD, the average value of leaf area occupied by the pathogen in the variant trees amounted to 28% of the entire leaf area (Fig. 1). The average leaf

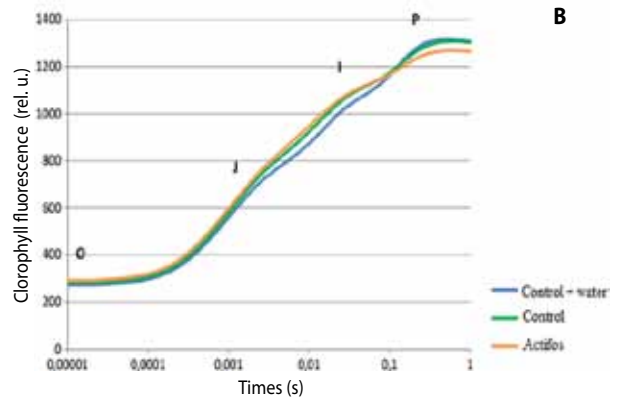
surface occupied by the pathogen in trees treated with water reached 31%. On the research plots treated with 'Actifos' the average leaf area occupied by the patho-

gen was the smallest – 26.55%. The values of leaf area occupied by the pathogen ranged from 0.7% to 66.4%. Considering the reduction of development of powdery

Karczma Borowa FD

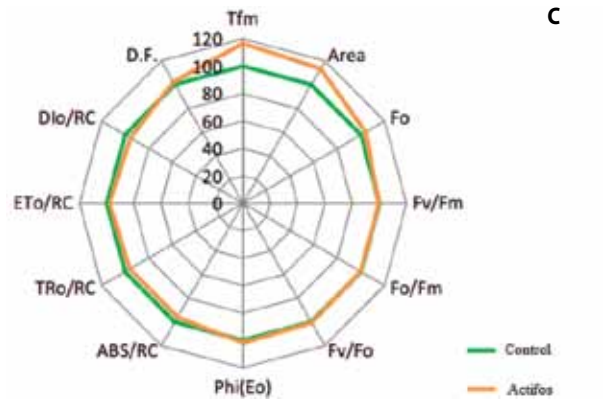


A

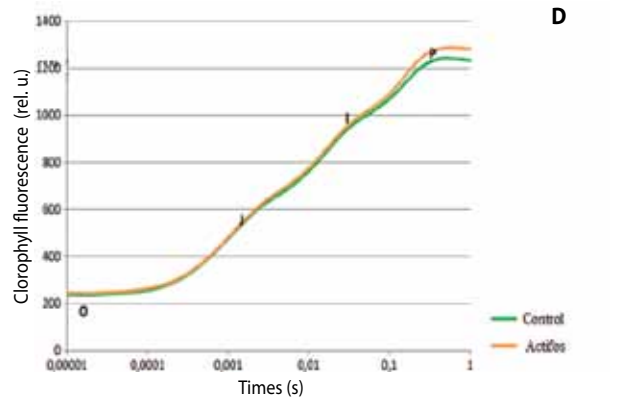


B

Krotoszyn FD

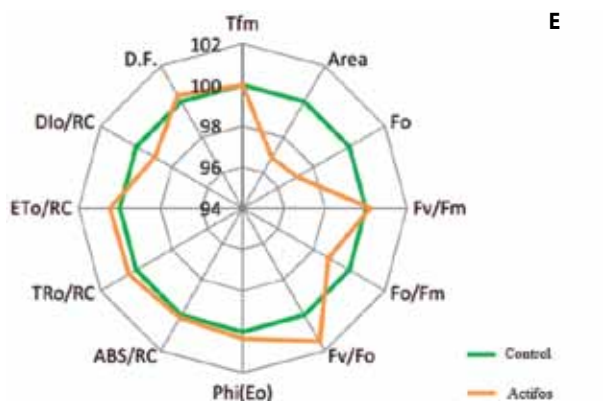


C

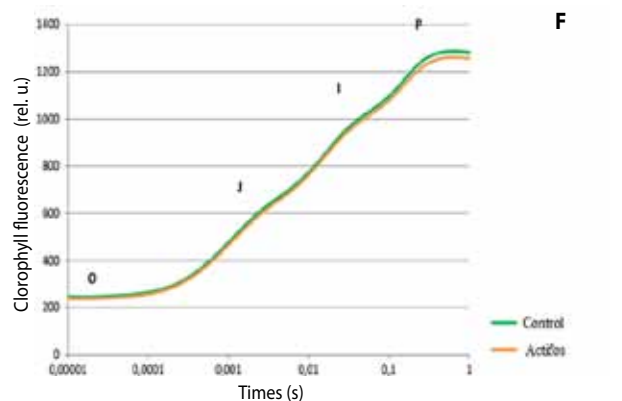


D

Piaski FD



E



F

Figure 4. Chlorophyll fluorescence in the Karczma Borowa FD (A, B), Krotoszyn FD (C, D) and Piaski FD (E, F)

mildew in the Karczma Borowa FD, the lowest values of the area occupied by the pathogen was demonstrated in the case of the areas of the 3 trees.

In Krotoszyn FD, the average area occupied by the foliar pathogen, for which the samples were collected from the control plot, amounted to nearly 11% (Fig. 3). In contrast, the average leaf area covered by the pathogen on treated leaves was more than twice higher – 24.7%. In the Krotoszyn, on average, the area of one leaf was occupied by the pathogen within the group of oaks treated with Actifos and the control ones did not differ significantly. However, in two control oaks, the surface of leaves colonized by the powdery mildew was significantly higher than in the case of the treatment.

In Piaski FD, the treatments performed with Actifos on the trunk of the trees did not have any significant effect on the leaf surface occupied by the pathogen (Fig. 3).

Chlorophyll *a* fluorescence (ChlF)

In all the plots, there were no significant differences between the control trees and trees treated by Actifos in terms of the efficiency of processes occurring in photosystem II (PSII) (Fig. 4). In Karczma Borowa FD, the best results for different parameters of chlorophyll *a* fluorescence were obtained for the trees that were watered (Fig. 4A). The trees that have been studied in Krotoszyn FD didn't show any differences in ChlF parameters. In Piaski FD, the differences were observed for Area, F_0 , F_v/F_0 and DIo/RC (Fig. 4E), suggesting that the trees in that plot were under some kind of stress, probably high competition among trees for water (Kalaji and Łoboda 2010).

CONCLUSIONS

Our findings show that phosphite application causes no negative physiological consequences on the foliage of the treated oaks, both for the average leaf area and for the fluorescence of chlorophyll. The manner of phosphite application (leaves, trunks or roots) did not negatively influence the physiology of the treated oaks as compared to the control. The tendency in the reduction of oak mildew on leaves of oaks treated with phosphite observed is encouraging, but these observations should be continued in the coming years.

REFERENCES

- Akhkha A., Boutraa T., Kalaji H., Ahmad P., Dąbrowski P. 2013. Chlorophyll fluorescence: a potential selection criterion for drought tolerance in selected durum wheat (*Triticum durum* Desf.) cultivars. *Signpost Open Access Journal of NanoPhotoBio-Sciences*, 1, 147–156.
- Baker N.R., Rosenqvist E. 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *Journal of Experimental Botany*, 55, 1607–1621.
- Borawska-Jarmujłowicz B., Mastalerczuk G., Pietkiewicz S., Kalaji M.H. 2014. Low temperature and hardening effects on photosynthetic apparatus efficiency and survival of forage grass varieties. *Plant, Soil and Environment*, 60, 177–183.
- Desprez-Loustau M.-L., Feau N., Mougou-Hamdane A., Dutech C. 2011. Interspecific and intraspecific diversity in oak powdery mildews in Europe: coevolution history and adaptation to their hosts. *Mycoscience*, 52, 165–173.
- Hardy G.E.St.J., Barrett S., Shearer B.L. 2001. The future of phosphite as a fungicide to control the soil borne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. *Australasian Plant Pathology*, 30, 133–139.
- Kalaji H.M., Łoboda T., 2010. Fluorescencja chlorofilu w badaniach stanu fizjologicznego roślin. Wydawnictwo SGGW, Warszawa.
- Marçais B., Desprez-Loustau M.-L. 2014. European oak powdery mildew: impact on trees, effects of environmental factors, and potential effects of climate change. *Annals of Forest Science*, 7, 633–642.
- Nowakowska J.A., Oszako T., Bieniek J., Rakowski K. 2007. Genetic characterization in relation to the health state of oak populations in the Elbląski and Krotoszyński regions of Poland. *Leśne Prace Badawcze*, 3, 33–51.
- Okorski A., Pszczółkowska A., Oszako T., Nowakowska J. 2015. Current possibilities and prospects of using fungicides in forestry. *Leśne Prace Badawcze*, 76 (2), 191–206.
- Stirbet A., Riznichenko A., Yu G., Rubin A.B., Govindjee. 2014. Modeling chlorophyll *a* fluorescence

- transient: relation to photosynthesis. *Biochemistry* (Moscow), 79 (4), 291–323.
- Tkaczyk M., Nowakowska J.A., Oszako T. 2014. Nawozy fosforynowe jako stymulatory wzrostu roślin w szkółkach leśnych. *Sylwan*, 158 (1), 3–9.
- Živčák M., Olšovská K., Slamka P., Galambošová J., Rataj V., Shao H.-B., Kalaji M.H., Brestič M. 2014. Measurements of chlorophyll fluorescence in different leaf positions may detect nitrogen deficiency in wheat. *Zemdirbyste-Agriculture*, 101, 437–444.