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Phytophthora quercina infections in elevated CO_2 concentrations

Tomasz Oszako[1], *Katarzyna Sikora*[2], *Małgorzata Borys*[3], *Katarzyna A. Kubiak*[2], *Miłosz Tkaczyk*[2] ✉

[1] Technical University in Białystok, Forest Faculty, Piłsudskiego 8, 17-200 Hajnówka, Poland

[2] Forest Research Institute, Department of Forest Protection, Sękocin Stary, Braci Leśnej 3, 05-090 Raszyn, Poland, e-mail: M.Tkaczyk@ibles.waw.pl

[3] Forest Research Institute, Laboratory of Molecular Biology, Sękocin Stary, Braci Leśnej 3, 05-090 Raszyn, Poland

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 (Maclntire 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 CO_2) or an elevated (800 ppm) concentration of CO_2. 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 CO_2 conditions:

1. Oaks inoculated with *Phytophthora* via soil: with isolates of *P. quercina;*
2. Oaks treated with Actifos (0.6%) containing phosphites (PO_3) – 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) (cm^2)

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

RESULTS

Oaks growing in an elevated CO_2 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.

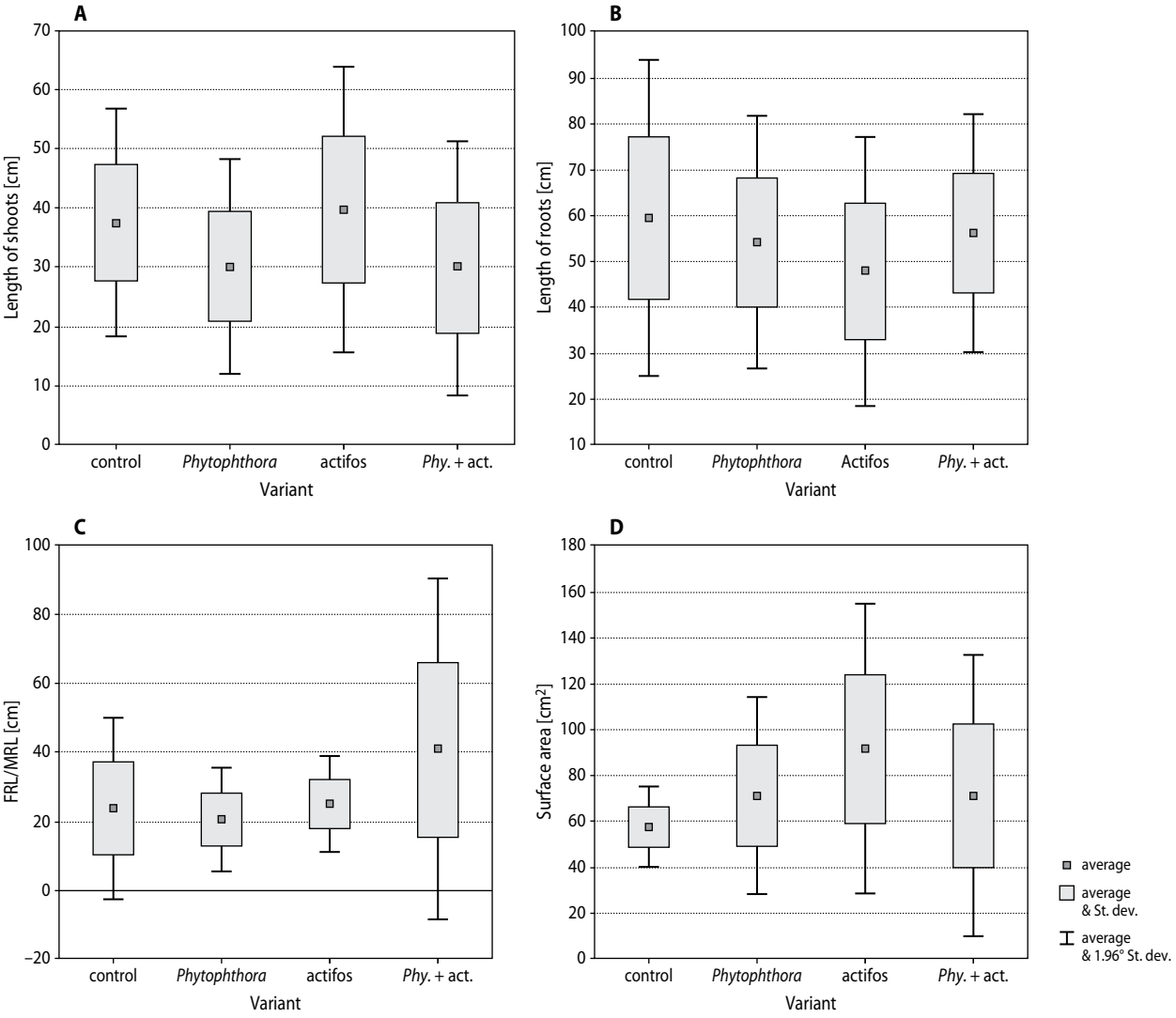

Figure 1. Comparison of the lengths of oak shoots (A), lengths of roots (B), FRL/MRL (C), and surface area (D) grown in 800 ppm CO_2

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

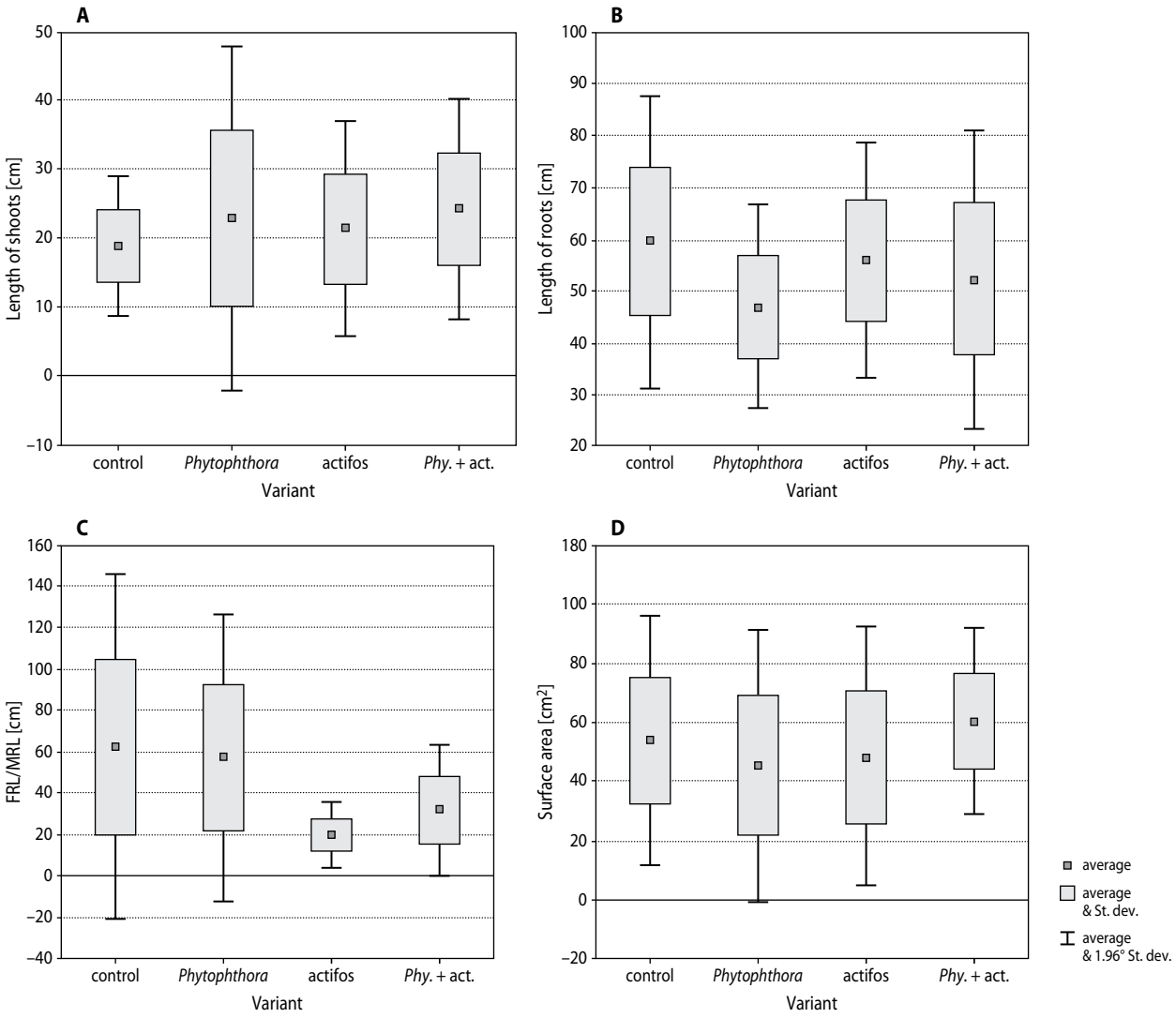

Figure 2. Comparison of the lengths of oak shoots (A), lengths of roots (B), FRL/MRL (C), and surface area (D) grown in 400 ppm CO_2

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.

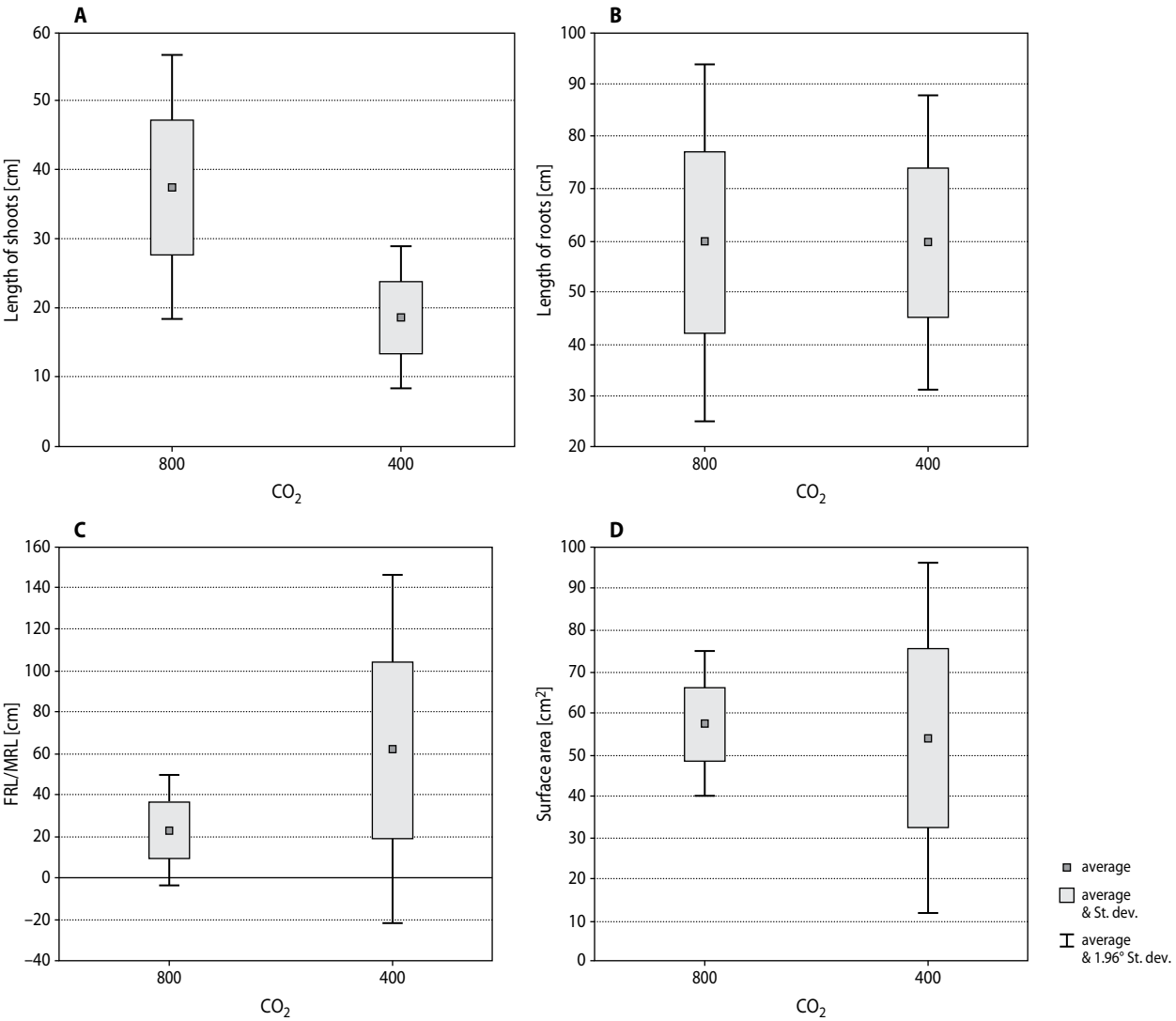

Figure 3. Comparisons of the lengths of shoots (A), and of roots (B), as well as FRL/MRL (C), and surface area (D) of oaks grown in experiment variants without inoculation

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 CO_2 (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).

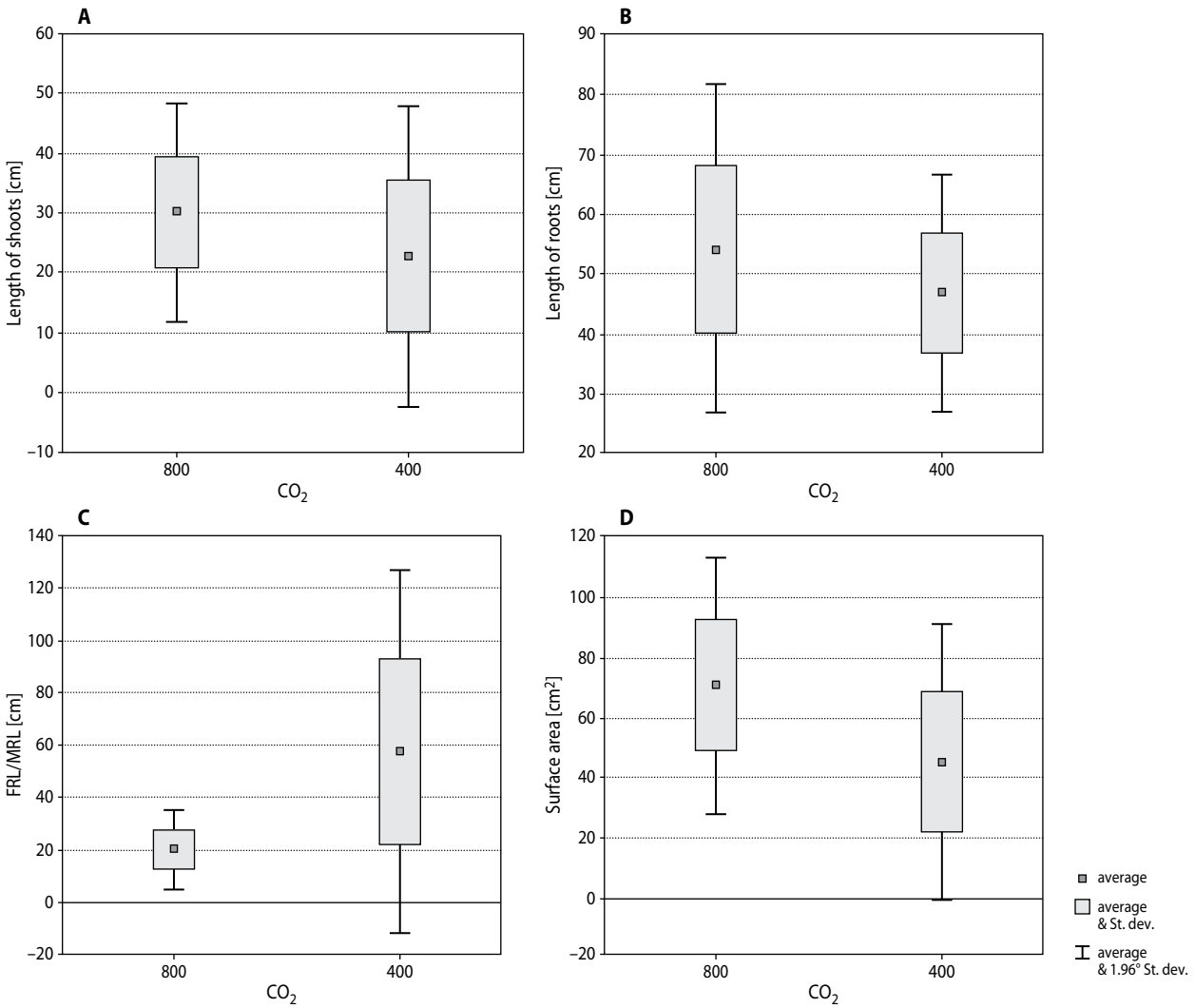

Figure 4. Comparisons of the lengths of shoots (A) and of roots (B), as well as FRL/MRL (C) and surface area (D) of oaks grown in experiment variants inoculated with *P. quercina*

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

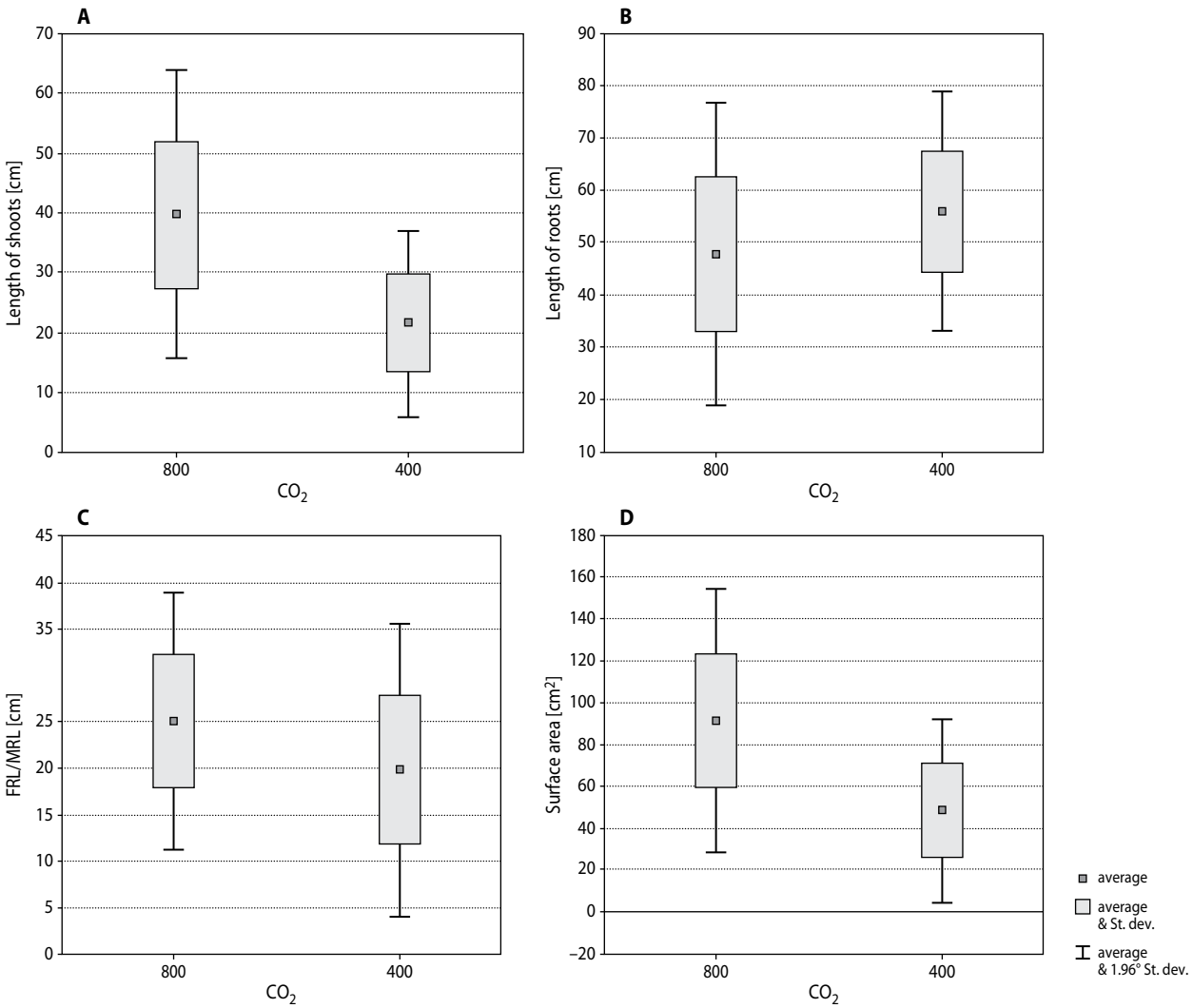

Figure 5. Comparisons of the lengths of shoots (A) and of roots (B), as well as FRL/MRL (C) and surface area (D) of oaks grown in experiment variants with Actifos

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 CO_2 con-

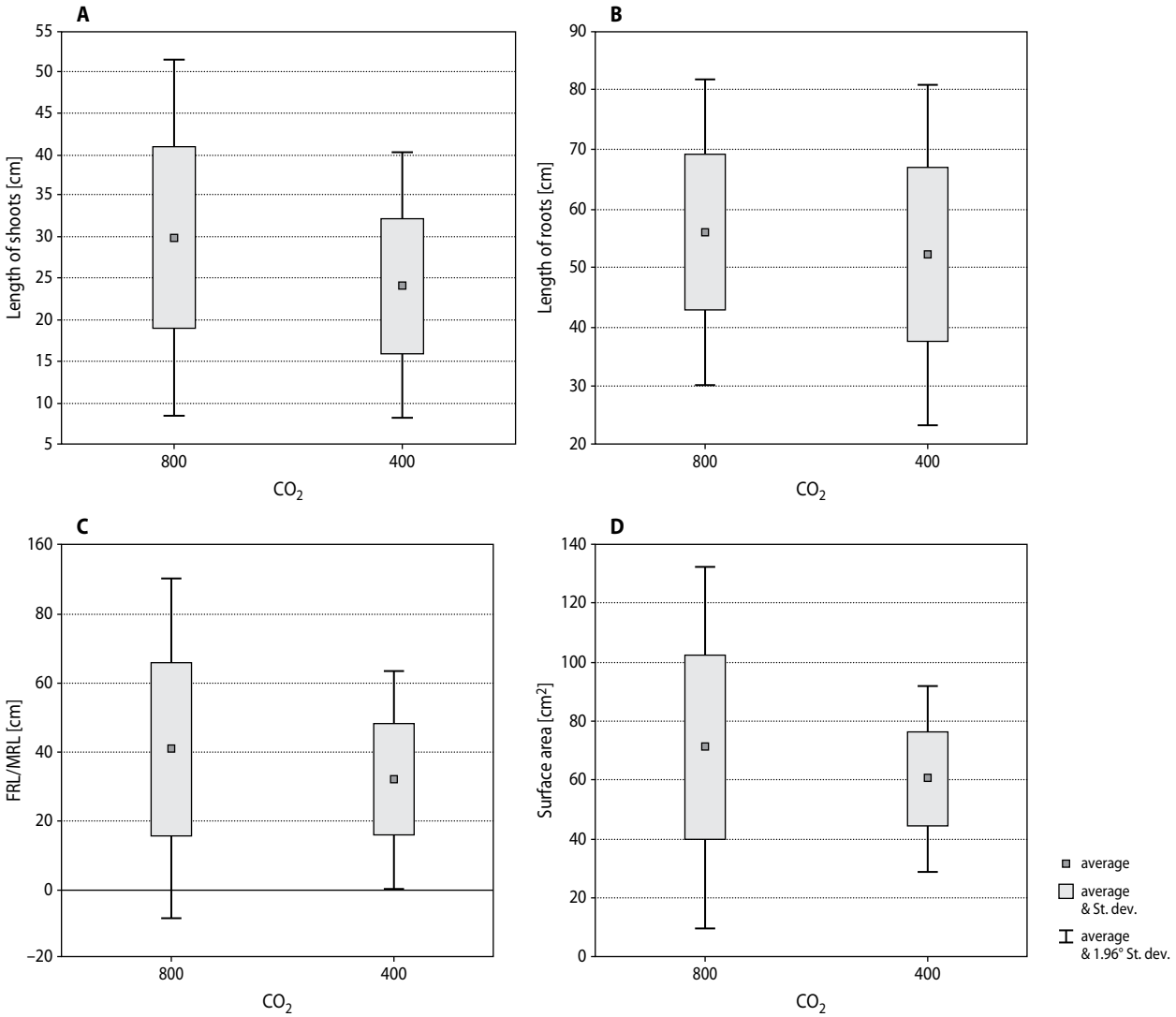

Figure 6. Comparisons of the lengths of shoots (A) and of roots (B), as well as FRL/MRL (C) and surface area (D) of oaks grown in experiment variants inoculated with *P. quercina* and treated with Actifos

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 CO_2, 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 CO_2 better stimulated the development of oak roots than with oaks growing under low (400 ppm) concentrations of CO_2. This is evidenced by a significantly higher ratio of FRL/MRL measured at the 800 ppm concentration of CO_2. In terms of FRSA, there was no statistically significant difference between the control oaks growing at different concentrations of CO_2 (800 and 400 ppm), although slightly higher values were found in the variant with elevated CO_2, 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 CO_2, 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 CO_2, while Percy et al. (2002) reported increasing herbivore damage, but no change as for rust infection by *Melampsora medusae* on aspen leaves under elevated CO_2.

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 CO_2. Fast growth of oak shoots points out on efficient physiological processes (more efficient photosynthesis) ongoing in elevated CO_2 concentrations, confirmed by the reference to average values for lengths of shoots and in consequence roots in 800 pp of CO_2 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 CO_2 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 CO_2, 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 CO_2. 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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