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Influence of cutting off distal ends of *Quercus robur* acorns on seedling growth and their infection by the fungus *Erysiphe alphitoides* in different light conditions

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Abstract: *Quercus robur* seedlings are very often infested by oak powdery mildew caused by *Erysiphe alphitoides*. This disease attacks primary leaves with high insolation. In our experiment seedling growth in different light conditions was observed. Seedlings were derived from intact seeds or from seeds where the distal ends of acorns were cut off. Acorns were sown in pots at four light conditions (2%, 8% and 30% light transmittance and in full sunlight). For half of the seedlings we reduced the influence of powdery mildew by using a fungicide. Height of seedlings, shoot dry mass and the degree of damage caused by *Erysiphe alphitoides* were measured. The highest damages were in full light conditions and the lowest in the 30% light treatment. The influence of acorn reduction on powdery mildew infection was not significant (p=0.0763), however, in the full light conditions the seedlings from cut acorns were ca. 30% less damaged. The cutting of acorns also alters the height of seedlings and shoot mass. The tallest seedlings were from cut seeds growing in the 30% light treatment. The influence of the fungicide was lowest in the 30% light treatment where the decrease in damage was only ca. 10%. For nursery practice we can say that oak seedling production from cut off acorns under 30% light transmittance should give the best results of seedling growth even without the fungicide application.

Additional key words: pedunculate oak, container nurseries, oak powdery mildew, phenology

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Introduction

Pedunculate oak (*Q. robur*) is one of the most economically and ecologically important forest tree species, especially in Europe (Stein et al. 2003). Forest decline, particularly in this species, was observed in Europe in the 1970s and 1980s (Liese and Siwecki 1991; Oleksyn and Przybył 1987). Oak powdery mildew caused by *Erysiphe alphitoides* is a common disease of pedunculate (*Quercus robur)* and sessile (*Quercus petraea*) oaks (Mougou et al. 2008). Disease attacks especially the young trees and seedlings, also in nurseries, and it causes a decrease of net CO_2 assimilation, stomatal conductance and leaf nitrogen content (Hajji et al. 2009). European oaks – pedunculate (*Quercus robur* L.) and sessile oak (*Q. petraea* (Matt.) Liebl.) germinate unevenly under natural conditions. The difference between time of first and last germinating acorns can be up to a few weeks (Suszka et al. 2000) and this may be the species phenological strategy allowing some seedlings to miss certain pathogens or insects. Production of seedlings in containers requires simultaneous germination and emergence because if there are large differences in the time of germination, the earlier germinated plants quickly develop leaves which overshadow neighboring seedlings and restrict access to water (Suszka 2006). A technique that helps cause faster and more uniform germination in germination tests, is cutting off the acorn at the scar and removing the pericarp from the distal end of the seed (ISTA 1999). A modified version of this method – to cut off about 1/3 of the distal ends of the acorns – is sometimes used in container nurseries (Suszka 2006). Cutting off a small part of the acorn did not have negative consequences on seedling growth (Giertych and Suszka 2011), but it is possible that by faster emergence this procedure will alter also the level of *Erysiphe alphitoides* damage.

The infection intensity of plant leaves by pathogens depends on light conditions. Oak powdery mildew attacks seedlings mainly in full light conditions (Kelly 2002). The limitation of light in the container nurseries can also decrease the level of infection. The availability of light influences the amount of assimilated carbon and the direction of its allocation (Dudt and Shure 1994; Osier and Lindroth 2006) and it can also limit the growth of seedlings.

The aim of this study was to examine the influence of cutting off the distal end of acorns, light conditions and fungicide on damages caused by *Erysiphe alphitoides* and on growth of *Quercus robur* seedlings. We hypothesized that seedlings from cut acorns will be less damaged and that the reduction of cotyledon reserves will be compensated by speeding up emergence and prolonging the first growing season so that they achieve a similar size as seedlings grown from whole acorns. We further hypothesized that limitation of light will decrease damage caused by mildew but will also restrict growth of the seedlings.

Material and Methods

Cultivation of seedlings

Acorns were obtained from the forest storehouse in Jarocin, Poland. Mature acorns were collected in Autumn 2008, they were subjected to a standard procedure of thermotherapy and fungicide – Dithane M-45, 1.5g/kg (Suszka et al. 2000). After seven months storage (at –3°C) they were randomly divided into two groups (May 12th 2009). Acorns in the first group ca. 1/5 of the distal end cut off and the second group were whole acorns (control). All acorns were placed vertically in plastic boxes with peat-perlite 1:1 mixture (ca. 80 acorns in one box) and kept at 20°C. The apical end of acorns was above ground to allow observation of germination. Acorns with cut distal ends germinated almost immediately and on May 18th and they were sown horizontally at 2–3cm depth individually in 2 liter pots filled with a 1:1 ratio (v/v) mixture of forest soil and peat with the addition of Osmocote® fertilizer, with a controlled release over a period of 5–6 months. In total we sowed 160 cut acorns. The pots were placed into six shade houses covered with polypropylene shade cloth that produced treatments with 2%, 8% and 30% light transmittance and full sunlight conditions. Each light variant was represented by two blocks with 20 pots. In each shade house the pots were divided into two groups: sprayed with fungicide and control. We used the fungicide Nimrod 250 EC (dose proposed by producer with 0.1% concentration) and sprayed the seedlings three times at one week intervals (first spray June 28th). Uncut acorns were sown in these same conditions, successively over time depending to germination, from May 25th to one month later on June 24th. Germinating acorns were randomly assigned to light and spray treatments.

Growth parameters and damage analyses

At the end of growing season (October 13th) 64 seedlings (4 for each experimental treatment) was chosen for analyses. We measured height of seedlings, shoot dry mass and the degree of damage caused by *Erysiphe alphitoides*. Damages were measured using an image analysis system and the WinFolia Pro Software (Regent Instruments, Inc., Quebec, Canada). All leaves from each seedling were scanned. Differences in color of infected parts of leaves allow measurement of the percent of leaf surface damage.

Statistical analyses

Analysis of variance (ANOVA) was used to asses the influence of preparation treatment, light conditions and fungicide on height, shoot mass and damages. The results expressed in percent were arcsin transformed for ANOVA analyses. All analyses were conducted with JMP software (version 7.0.2; SAS Institute, Cary, NC, USA).

Results

Light conditions and fungicide treatment had a significant influence on seedling damage caused by *Erysiphe alphitoides* (Table 1). The highest damages were in full light conditions and the lowest in 30% light (Fig. 1). Fungicide decreased the percent of damage from 10% to 70% depending on light conditions, with the lowest damage levels at the 30% light treatment. The influence of acorn cutting on leaf damage was

Table 1. Summary of ANOVA results for percentage of oak leaves damage caused by *Erysiphe alphitoides*, shoot mass, seedlings height and number of leaves at the end of growing season with preparation treatment, fungicide treatment, light conditions and interactions (*P* – values bold then $P \leq 0.05$)

	Source variance	df	Mean Square	F	*P*
Damages	Treatment (T)	1	778.22	3.29	0.0763
	Fungicide (F)	1	1646.52	6.96	**0.0114**
	Light conditions (L)	3	1208.63	5.11	**0.0040**
	T*F	1	31.85	0.13	0.7153
	T*L	3	188.58	0.80	0.5017
	F*L	3	166.69	0.71	0.5540
	T*F*L	3	73.59	0.31	0.8171
	Error	45	236.41		
Shoot mass	Treatment (T)	1	4.73	13.23	**0.0007**
	Fungicide (F)	1	0.93	2.62	0.1126
	Light conditions (L)	3	4.32	12.10	**0.0000**
	T*F	1	0.06	0.17	0.6795
	T*L	3	1.78	4.97	**0.0045**
	F*L	3	0.36	1.00	0.4008
	T*F*L	3	0.12	0.32	0.8074
	Error	46	0.3570		
Seedling height	Treatment (T)	1	449.63	8.03	**0.0068**
	Fungicide (F)	1	8.07	0.14	0.7059
	Light conditions (L)	3	206.74	3.69	**0.0183**
	T*F	1	15.75	0.28	0.5985
	T*L	3	158.18	2.82	**0.0490**
	F*L	3	81.07	1.45	0.2412
	T*F*L	3	30.65	0.55	0.6524
	Error	46	55.99		
Number of leaf at the end of growing season	Treatment (T)	1	385.94	12.57	**0.0009**
	Fungicide (F)	1	197.88	6.45	**0.0147**
	Light conditions (L)	3	745.40	24.28	**0.0000**
	T*F	1	31.12	1.01	0.3194
	T*L	3	52.42	1.71	0.1789
	F*L	3	200.67	6.54	**0.0009**
	T*F*L	3	9.46	0.31	0.8194
	Error	46	30.07		

small. The leaves of seedlings from cut acorns were less damaged but the differences were not significant (Table 1). Acorn cutting also altered the height of seedlings and shoot mass (Table 1). The tallest seedlings were from cut acorns growing in the 30% light treatment. In the case of other light treatments the influence of acorn reduction was not significant (Fig. 1). Similar results were obtained in the case of shoot mass (Fig. 1). Decrease of shoot mass caused by low light conditions was appreciable and the influence of other experimental treatments was not significant. For both growing parameters we observed a significant interaction between preparation treatment and light conditions (Table 1). Reduction of acorns had a significant influence under the 30% light conditions. Fungicide treatment did not influence growth parameters. All experimental treatments examined altered the number of leaves at the end of growing season (Table 1). Cutting of acorns and fungicide increased the number of leaves whereas shade decreased it.

Discussion

As we expected, seedlings from cut acorns (which emerged earlier) were less damaged by oak powdery mildew. The differences were not significant when we analyzed all light treatments. However, if we take into consideration only the most damaged treatment (full light), leaves of seedlings from reduced acorns were significantly less damaged, especially in the non fungicide treatment (Fig. 1). This is probably connected with the earlier emergence of cut acorns, which had earlier leaf flush occurring before the maximal potential dispersal of fungal conidiospores. Biotrophic fungal pathogens are adapted to their host plants for phenological synchrony, to optimize the possibility of contacts leading to infections (Desprez-Loustau et al. 2010). *Erysiphe alphitoides* causes heavy infestation and substantial loss of foliar assimilation capacity only if the presence of young leaves coincides with a high inoculum potential of fungal conidiospores (Thomas et al. 2002). In Central Europe the initial infection occurs in late spring (Przybył 2006). The secondary infections occur during the summer and concern mainly the young foliage regenerating after insect defoliation (Thomas et al. 2002). In young oak seedlings a secondary leaf flush is rare, so powdery mildew infects mainly the first leaves. So the acceleration of leaf development by cutting off acorns is advantageous for seedlings because it caused a mismatch with the timing of pathogen development.

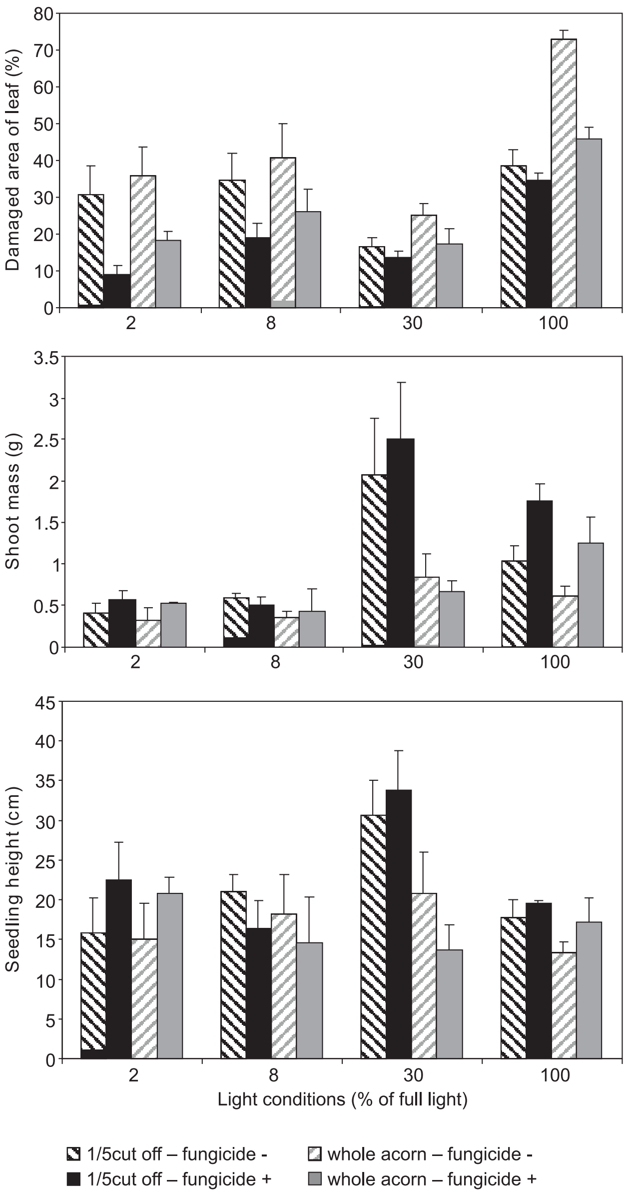

Fig. 1. Influence of experimental treatments (light conditions, cutting off the distal end and fungicide) on mean pedunculate oak damaged area of leaf, shoot mass and seedling height (cm) at the end of the growing season. The vertical lines represent standard error of the mean

Reduction of light also decreased the level of mildew damage, confirming an earlier study (Kelly 2002). The higher level of infection in the deep shade treatments (2 and 8%) than in 30% of sunlight is probably connected with differences in leaf structure. The leaves from shade conditions (higher leaf SLA) are softer and thinner (Reich et al. 1999), moreover they have lower levels of phenolics (Cronin and Lodge 2003; Chacón and Armesto 2006) and therefore poorer chemical defenses. According to our expectations, fungicide decreases mildew damage especially in seedlings from uncut acorns.

The increase seedling height and shoot mass after acorn reduction differs from our earlier experiment where we did not observe any significant influence of cutting off distal ends of cotyledons on growing parameters (Giertych and Suszka 2011). Significantly higher and heavier seedlings from reduced acorns were especially found in the 30% of sunlight treatment. The seedlings from whole acorns emerged 2–5 weeks later and in the lower light conditions (30% of sunlight) could not make up for the reduction of the growing season. In full light and in deep shade the differences in growing parameters between the acorn reduction treatments are not so visible.

In conclusion we can say that cutting off distal ends of oak acorns as a pre-sowing procedure, in addition to the advantages of more simultaneously emergence (Giertych and Suszka 2011), also caused a lower occurrence of powdery mildew infection. This is especially visible when the seedlings are growing in full light conditions where the mildew infection was the highest. The production of seedlings from cut acorns under some reduction of light (30%) can yield taller and less damaged seedlings, even without fungicide treatments,

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