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Surface sterilization of English oak (*Quercus robur* L.) acorns using wet water steam

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Abstract

Effective and environmentally friendly methods of protection to reduce seed contamination from fungi are constantly sought. The use of thermal impulses of 100°C wet water steam to reduce fungal contamination has not been sufficiently investigated, and the potential of this physical approach has not been estimated.

The aim of the study was to investigate what impact 100°C wet water steam with different time durations had on acorn contamination with fungi, acorn germination and biometric indicators of English oak (*Quercus robur*) seedlings during the first year of growth. Different treatment durations with wet water steam were used: 2, 4, 6, 8, 10, 12 and 14 s.

Research showed that 6 seconds impact of wet steam on the surface of the oak acorn destroyed *Alternaria* spp., 8 seconds impact destroyed *Penicillium* spp. and 14 seconds impact destroyed *Mucor* spp. Using 14 seconds water steam treatment *Penicillium, Alternaria* and *Mucor* spp. fungi were eliminated.

Wet water steam treatment for 2–4 seconds not only stimulated the acorns germination by 4.0–7.6%, but also had positive influence on the root collar diameter of seedlings. Wet water steam treatment for 2–12 seconds had a positive effect on the root development of oak seedlings, however, high temperature environment had a suppressive effect on the oak seedling height.

Keywords: biological control, wet water steam, fungi, oak acorns, seedling production

Introduction

English oak (*Quercus robur* L.) is one of the most important species of native deciduous trees in Central Europe (Gniot 2007). Oaks cover 43.4 thousand ha in Lithuania and common oak takes up about 2% of all forest area (Butkus et al. 2018). Oaks play a very important role both in the forest ecosystem and in the economy because of their valuable timber (Pietras et al. 2015).

Over 1.5 million tree seedlings are grown per year in Lithuania. Approximately, 277,654 pcs. of production is grown in the nurseries, of which 7,565 pcs. are oak seedlings (Butkus et al. 2018). A phytosanitary condition of the seedling material is very important for nurseries. Most diseases known to cause problems in forestry species today are fungal ones (Lilja et al. 2010).

The future life of the forest depends on seeds. Damaged seeds cannot have a desired effect on forest regeneration even under suitable environmental conditions or cannot cause regeneration of particular species. The trees that had grown from damaged and diseased seeds grow slowly and the seeds produced by these trees will have low vitality (Ravishankar Rai et al. 2005).

Oak acorns are colonized by various fungi, which can have a negative impact on acorn viability (Washington 2003), germination (Lombardo 2009) and sprouts (Tedmund et al. 1991). By penetrating a seed, fungi are able to disrupt its cell walls, destroy starch pellets and spare proteins (Bechtel et al. 1985). Most often, acorns are infected by *Penicillium* and *Aspergillus* spp. (Tedmund et al. 1991), *Fusarium* spp. (Wit et al. 2015), *Alternaria alternata* (Fr.) Keissl., *Lecythophora* spp., *Pezicula cinnamomea* (DC.) Sacc., *Cladosporium* spp., and *Ciboria batschiana* (Zopf) N.F. Buchw. (Jankowiak 2008).

Nowadays, the importance of environmentally friendly methods (without fungicides) of destroying fungi is increasing. It is possible to improve the sanitary condition of seeds by means of thermal action only, if the pathogen is more sensitive to high temperature than the contaminated seed (Forsberg et al. 2005). Such high-temperature environments as combustion gases (Tei et al. 2003), hot water (Hanson and Ascard 2002) and wet water steam (Forsberg et al. 2002) are used to eliminate unwanted organisms.

The environment of the wet water steam is fully saturated with humidity of about 100°C temperature. Steam

condenses on the surface of a cold object. Due to the very high heat transfer coefficient during the condensation process (α = 50000–100000 W/(m^{2}K)), cold surfaces instantly reach a temperature close to the steam temperature, resulting in intensive heating of the seed surface (Sirvydas et al. 2006).

Oak acorns are colonized by a wide range of fungi (Kavosi et al. 2013). The minimum and maximum temperatures for their growth range from 5 to 65°C (Lugauskas et al. 2002). The effect of thermal sanitization treatment is based on the principle that pathogens are heat-sensitive (Forsberg et al. 2005) It has been found that the environment of 100°C wet water steam significantly reduces the amount of fungi on the seeds. The effectiveness of the treatment depends on the duration of exposure to the action of wet water steam (Sinkevičienė 2007). After the treatment of Scots pine (*Pinus sylvestris*) seeds with wet water steam for 4 seconds, the seed contamination with fungi decreased to 75.0% (Šilingienė and Vasinauskienė 2017).

Seed treatment with fungicides is an effective way for controlling the spread of pathogenic fungi. It remains one of the most widely used tools to reduce the damage caused by seed-borne fungal diseases. However, the intensive use of these chemicals pollutes the environment (Logrieco et al. 2003). Therefore, effective and environmentally friendly methods of protection and means to reduce seed contamination from pathogenic microflora are constantly sought (Döll et al. 2002). One of the observations is 100°C wet water steam, which can be an effective means to reduce tree seed contamination with pathogenic fungi and to limit their spread. There is lack of information in the literature on the use of 100°C wet water steam impulses to reduce the fungal contamination of acorns, and the potential of this physical technique has not been estimated.

The aim of the study was to investigate what impact 100°C wet water steam with different durations had on acorn contamination with fungi, acorn germination and biometric indicators of English oak (*Quercus robur*) seedlings during the first year of growth.

Materials and methods

The research was conducted at the Laboratories of Forest Biology and Forestry, VMU, the Biology and Plant Biotechnology Institutes and the nursery of Šakiai Forest Enterprise in 2016–2017. For testing, oak acorns were randomly collected in the oak stand in Šakiai District (55° 4’ 51.59”, 22° 58’ 52.41” (WGS)) of the first (I) selection group. The acorns were collected in autumn, on September 10, 2016, dried and stored for one month (–3°C).

The studies were carried out in two stages: under laboratory and field conditions, under eight observations. Fifty oak acorns were used during one replication.

Stage I. Laboratory tests. Treatment of acorns with wet water steam. The tests were performed according to the following scheme: observation 1: thermally untreated oak acorns, observations 2 to 8: the acorns were thermally treated with 100°C wet water steam, under different steam supply duration. The duration of each observation was 2; 4; 6; 8; 10; 12; and 14 s respectively.

In order to get even processing of the oak acorns, every sample was poured into a mesh bag (4) that was attached to the thermal insulation surface – polystyrene (EPS-70, ETNA, Lithuania), 10 cm thick) (2) so that the steam stream (3) was evenly affected by the steam diffuser (1) (Figure 1). The steam diffuser was connected to a steam boiler at a constant pressure of 0.4 bar to maintain a constant steam temperature of 99 ±1°C. Ten temperature sensors (6) recorded the steam temperature (Figure 1).

During the research, the dynamics of temperature variation in the internal tissues of oak acorns was determined by supplying the environment with wet water steam of different durations. To determine the variation of temperature, thermocouples were introduced into ten acorns up to a depth of 7.5 mm. Temperature measurements were taken using individually produced thermocouples made of Cu-Cu Ni wires having diameters of 0.07 mm. The reaction lag of these thermocouples in the case of a temperature leap of 100°C in water steam medium was established to be equal to 0.0026 s. The thermocouples were laid in accordance with the isotherm, which was 100 thermocouple diameters according to the requirements for temperature measurements (Sirvydas et al. 2006). The openings at the bottom of the drill for introducing the thermocouples into the acorns were waxed in order to prevent the wet water steam from penetrating into the deep tissues during the exposure and in order to prevent affecting the dynamics of temperature variation in the tissues.

The measurements were recorded using ALMEMO 2890-9 (Ahlborn Mess- and Regelungs Technik H, Holzkirchen, Germany) with a microprocessor data processing and gathering system, which is able to carry out up to one hundred measurements per second.

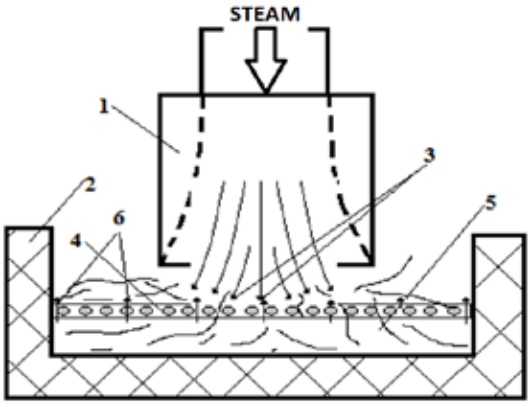

Figure 1. Technological scheme for supplying 100°C wet water steam on oak acorns: 1 – steam diffuser; 2 – thermal insulation box; 3 – steam flow; 4 – grid with oak acorns; 5 – high temperature environment; 6 – temperature sensors

Isolation and morphological characterization of microscopic fungi. Different wet steam effects on acorns contamination by fungi were ascertained at the laboratory by using agarized nutrition medium method (Mathur and Kongsdal 2003). A Potato dextrose agar (PDA, Liofilchem, Italy) medium was used to isolate the fungi. Of each treatment, 200 acorns were examined for the presence of fungi. Each treated acorn with pericarp was divided into three sections, cut transversely through hypocotyl. Petri plates with a diameter of 9 cm were used. 3 cut acorns (9 pieces) were placed on the medium in a Petri plate. The acorns were placed onto the potato dextrose agar and incubated at 24 ±2°C in the dark for 7 days. The identification of fungi was determined by visual examination of colony characteristics and conidia morphology. The fungi were determined according to Leslie and Summerell (2006), Nelson et al. (1983), and Ellis (1976) descriptions.

Stage II. Field trials. Germination and root rot testing in the nursery. Acorn germination was determined according to ISTA (2009). 50 u. of acorns from each application were sown in four replicates in the plots (50 × 50 cm), in forest soil and sand mixture (1:1). The acorns were sown at 2–3 cm intervals and at a depth of 4–5 cm, with additional watering, covered with polyethylene lids, which were removed after 7 days. Germination was started to determine 7 days after sowing and continued up to 28 days. Acorns are considered germinated when the roots reach 1 cm in length. The percentage of germinated seeds was calculated (%). At the end of germination, the percentage of acorns damaged by root diseases was calculated. The germinated acorns were considered damaged by root pathogens, if their roots were blackened and the sprout stopped growing. The disease percentage was determined as follows: disease incidence (%) = number of seedlings infected/total number of seedlings × 100 (Omukhua et al. 2011).

Sowing of acorns in the nursery. The field trials were arranged according to the conventional technologies that are used in forest nurseries (Paičius 2001). The tests were carried out in sandy, loamy, shallow-grained and deep-floating soil (*Epicalcari – Endohypogleyic Cambisol*). The plot size was 0.5 × 1.0 m, which equalled to 0.5 m^2.

The oak acorns were sown in autumn, on October 20, 2016. The field study was conducted in eight observations and five replicates. The plots were arranged in an orderly manner. Batches of 250 acorns each were sown in each plot. The first plot contained thermally untreated oak acorns; the second plot contained oak acorns that were exposed to wet water steam for 2 s; the third plot with 4-second exposure; the fourth plot with 6-second exposure; the fifth plot with 8 s; the sixth plot with 10-second exposure; the seventh plot with 12-second exposure; and the eighth plot with 14-second exposure, respectively.

The acorns were sown in rows. The sowing depth was 5–7 cm. After sowing, the field was mulched with sand and fortified. One year later, after the seedling growth was over (in November) they were measured. The following basic biometric parameters were measured: height (0.1 cm accuracy), root collar diameter (0.1 mm accuracy), and main root length (0.1 cm accuracy).

The statistical analysis of the data was performed by the analysis of variance (*ANOVA*) with the aid of Statistica 12 software package (StatSoft 2013). Research data were statistically evaluated by one-way *ANOVA* of quantitative methods of evidence (Raudonius, 2017).

Results

The studies showed that *Fusarium, Penicillium, Alternaria* and *Mucor* spp. fungi were predominant on the surface of the untreated oak acorns (Table 1).

Among the identified genera, *Alternaria* spp. and *Fusarium* spp. were the most abundant. In addition to these genera, *Stemphylium* and *Cladosporium* spp. fungi were found. Their frequency did not exceed 7.0%. Among the fungi that have been identified as the most predominant on the acorns were: *Fusarium oxysporum* Schltdl., and *Alternaria alternata* (Fr.) Keissl (Table 1).

When the oak acorns were treated with 100°C wet water steam, the heat accumulated in the surface layer of the acorns and it was dependent on the retention time. Heat was transferred to the deeper layers of acorns, where the water condensation process took place (Figure 2).

The origins of fungi in the shell of the acorns and on their surface were negatively affected after 2-second im-

Table 1. Influence of wet water steam retention time on acorn contamination with fungi, %

Duration of treatments	Acorns infected with fungi, %			
	Fusarium spp.	*Penicillium* spp.	*Alternaria* spp.	*Mucor* spp.
Untreated	100 a	100 a	100 a	100 a
2 s	58.3 b	26.7 b	71.4 b	22.2 d
4 s	50.0 c	20.0 c	28.5 c	33.3 c
6 s	41.7 d	26.7 b	0 d	11.1 e
8 s	25.0 e	0 d	0 d	44.4 b
10 s	25.0 e	0 d	0 d	11.1 e
12 s	8.3 g	0 d	0 d	33.3 c
14 s	16.7 f	0 d	0 d	0 f

Note. The values are the means of four replicates. Means not sharing common letters are significantly different at $P < 0.05$.

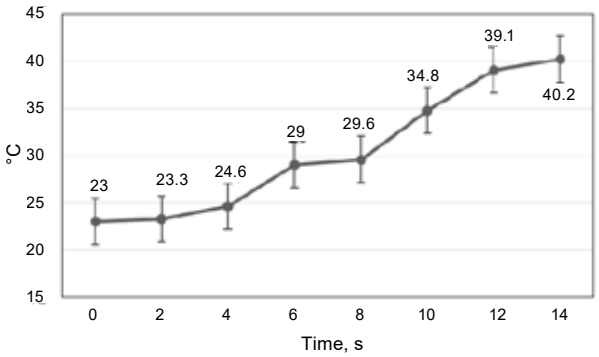

Figure 2. The dispersion of the temperature in the internal tissues of oak acorns, depending on the retention time

Note. Vertical dashed line indicates standard error in the mean.

pact by wet water steam. As a result, *Penicillium* (73.3%) and *Mucor* (77.8%) fungi decreased most significantly on the acorn surface. *Alternaria* spp. was the least affected by wet water steam and its contamination was reduced by only 28.6%.

During the 2 seconds wet water steam impact, the temperature in the internal tissues of the oak acorn rose up to 23.3°C. Fungi from *Fusarium* genus, capable of colonizing the internal tissues of the seeds, significantly decreased by 41.7% after 2 seconds time duration.

After the 4 second impact, the contamination with *Penicillium* spp. significantly decreased by 6.7%, but after the 6 second impact the contamination remained the same as after the 2-second treatment. The treatment for 4 seconds significantly reduced the amount of genus *Alternaria* by 71.5% and they were completely destroyed after the 6 s exposure. The effect of 6 second steam significantly reduced the presence of *Mucor* spp. by 11.1%. The steam impact for 4 seconds and 6 seconds had significant effect on *Fusarium* spp. contamination. Compared to the 2 s exposure to steam the amount of *Fusarium* spp. after 4 and 6 s exposure to steam significantly decreased by 8.3 and 16.6%, respectively.

The treatment with wet steam which lasted for 8 seconds completely destroyed *Alternaria* spp. and *Penicillium* spp. Under the influence of this effect, the temperature in the internal tissue of the acorns rose to 29.6°C, but the *Fusarium* spp. was not destroyed completely as the contamination remained at 25.0%. The amount of *Mucor* spp., compared to the 6 s exposure, significantly increased to 33.3% under this effect.

The treatment time of 14 seconds demonstrated the temperature rise in the tissues of oak acorns up to 40.2°C. The 14 second wet steam effect completely and significantly destroyed *Mucor* spp. However, this effect was likely to stimulate the origins of *Fusarium* fungi, and their amount was almost doubled – by 8.4%, compared with the impact of 12-second duration.

The average germination of the untreated oak acorns was approximately 51.6% (Figure 3). The treatment with short-term wet steam for 2 s and 4 s stimulated the acorns. After 2 second and 4 second treatment with wet water steam, their germination increased on average by 7.6 and 4.0%, respectively, but the difference compared to the control was not significant. After 2 and 4 s exposures to steam, the temperature of internal acorn tissues increased by 0.3 and 1.3°C, respectively, compared to the control.

As the treatments with the wet water steam durations increased, they had a negative impact on acorn germination. Compared to the control, the 6 second treatment (internal tissue temperature recorded was 29°C) reduced acorn germination to 2.8%. After the 8 s exposure to steam, the temperature of internal acorn tissues increased to 29.6°C, and germination decreased insignificantly – by 5.6%, after 10 s (at 34.8°C) – by 4.8%, 12 s (at 39.1°C) and 14 s (at 40.2°C) treatment reduced it to 12.0% (Figure 3).

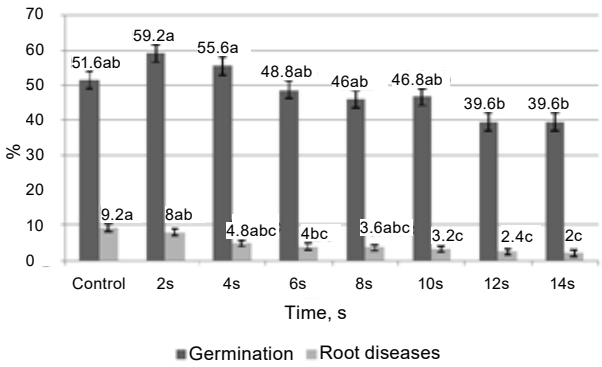

Figure 3. Influence of wet water steam treatment duration on oak acorn germination and root diseases prevalence on 1-month-old germinated acorn

Note. The values are the means of four replicates. Means not sharing common letters are significantly different at $P < 0.05$.

The share of 1-month-old germinated acorns damaged by root diseases in the nursery fields, which had been untreated, was 9.2% (Figure 3). The effect of wet water steam reduced the presence of pathogens of root diseases on the acorns, and the number of sick seedlings decreased accordingly. After 2, 4 and 6 s treatments, the number of seedlings with root disease decreased by 1.2; 4.4 and 3.6%, respectively, but the differences were insignificant in comparison to the control. The treatment with wet steam for 10 s, 12 s and 14 s significantly reduced the root diseases of oak seedlings from 6.0 to 7.2% and these differences were statistically significant to the control and the 2 second observations. After the treatment, which lasted from 4 to 14 s, the percentage of seedlings infected by the root disease decreased non-significantly from 4.8 to 2.0%.

One of the main biometric features is the height of the seedlings. During the research, the negative effect of the high temperature environment on the height of oak seedlings emerged. The treatment of 6 s had the least impact on the average seedling height, it decreased by 1.05 cm (Table 2).

The most significant difference was found between the control ones and the treatments of 10 s and 14 s. The seedlings were on average 3.7 and 3.9 cm shorter than the untreated seedlings, respectively. A significant differ-

Table 2. Influence of wet water steam treatment duration on the biometric parameters of oak seedlings

Duration of treatments	Biometric parameters of seedlings, cm		
	Stem high, cm Mean ± SE	The length of the main root, cm Mean ± SE	Diameter in the root collar, mm Mean ± SE
Untreated	21.40±1.29 a	21.00±1.26 g	0.76±0.04 ab
2 s	18.88±0.86 c	21.50±0.96 f	0.85±0.04 a
4 s	19.35±1.07 c	26.97±1.64 c	0.84±0.04 a
6 s	20.35±1.13 b	27.48±0.96 b	0.75±0.04 ab
8 s	19.25±0.92 c	28.30±1.87 a	0.75±0.05 ab
10 s	17.75±1.16 d	23.05±1.24 e	0.69±0.04 b
12 s	19.10±1.28 c	23.65±2.18 d	0.69±0.04 b
14 s	17.50±0.98 d	19.70±0.94 h	0.76±0.04 ab

Note. The values are the means of five replicates. Means not sharing common letters are significantly different at $P < 0.05$. SE stands for Standard error.

ence in height was found between the seedlings treated with wet water steam for 12 s and 14 s. The seeds treated for 2 s, 4 s and 8 s were lower by 2.52 cm, 2.05 cm and 2.15 cm, respectively, and these differences were insignificant compared to the control (Table 2).

The study has shown the positive effect of wet water steam on the development of seedling roots. It was found that the seedlings grown from the wet water steam-treated acorns formed a longer main root than that in the control (Table 2). The average length of the seedling root in the control was 21.0 cm. As the wet water steam retention time increased from 2 s to 6 s, the main roots of the seedlings were substantially longer than 0.50 to 6.48 cm, compared to the root of seedlings grown from the untreated acorns. The effect of the 8 s treatment with wet water steam on the root length has been found. They were 7.3 cm longer than in the control, and the difference was substantial.

However, after the acorns were treated with wet water steam for 14 seconds, the average root length dropped substantially by 1.30 cm compared to the control.

The treatment with wet water steam had little effect on the diameter of the root collar in the seedlings grown from the treated acorns. A positive effect of treatments with durations of 2 s and 4 s was observed during the study, but the difference was not significant compared to the control.

Discussion and conclusions

The study showed that the oak acorns, which had not been treated with 100°C wet water steam, were contaminated with *Fusarium, Penicillium, Alternaria, Mucor, Stemphylium*, and *Cladosporium* spp. fungi (Table 1). The dominance of these genera of fungi among forest trees is confirmed by similar studies (Lazarev et al. 2005, Sutherland et al. 2002, Mittal and Wang 1993).

The research has shown that the abundance of different fungal genera in oak acorns can be effectively reduced by thermotherapy. This was confirmed by Hauptman et al. (2013) on ash seedlings (*Fraxinus excelsior*) affected by ash dieback (*Hymenoscyphus fraxineus*). The efficient use of hot-water treatments may reduce biosecurity risks associated with the transfer of ash material between diseased and non-diseased areas (Hauptman et. al. 2013). Thermal treatment of cereal seed with aerated steam is a widely applied method for the control of seed-borne diseases (Forsberg et al. 2002, Tinivella et al. 2009).

Having established the mycological contamination of oak acorns, *Alternaria* spp. was the most common genus. According to Lugauskas et al. (2004), *Alternaria* spp. was one of the most common genera of fungi which are usual in Lithuanian climatic conditions. If the fungus is seed-borne, it may attack seedlings, causing damping-off, stem lesions, or collar rot (Thomma 2003, Pegg et al. 2014). A short-term exposure of 2 seconds to 100°C wet water steam had a negative impact on the abundance of *Alternaria* spp. fungi, while 6 s impacts destroyed *Alternaria* spp. fungi completely. It can be assumed that most of the fungi of this genus were spread on the surface of the acorns and were completely destroyed by the effect of 2 to 6 s steam at 100°C. After 2 s and 6 s of thermal effects, the temperature of internal tissues of the acorns increased from 23.3 to 29.0°C. *Alternaria* spp. can grow at a temperature range in between 15°C to 25°C, above and below the optimal range of temperature fungi shows poor growth and sometimes mortality may occur. Fungi growth slows down at 35°C and stops completely at 45°C (Kaur and Kumar 2019). Sensitivity to saturated steam (at 50°C, for 15–20 min.) was determined by treating *Lobelia erinus* L. seeds; it resulted in a commercial use of saturated steam against *Alternaria* infection (Hall and Taylor 1983).

The effect of wet steam of different durations was effective for reducing the amount of *Penicillium* spp. fungi. Already the 2 s 100°C steam treatment was very effective (73.3% less infections). The treatment of 8 s exposure (in internal seed tissues at 29.6°C) they were completely destroyed. The thermal disinfection against *Penicillium* spp. would be very important for the acorns which are prepared for storing as the fungi of this genus can also damage the seeds that are not injured. *Penicillium* spp. develop best when the ambient temperature is 20–25°C (Lugauskas 2004). When seeds are stored, *Penicillium* spp. can penetrate the wheat grain aleurone layer and endosperm cells, damage the germ, reduce vitality, contaminate with toxins, and change the colour of the seed (Chelkowski and Cierniewski 1983). Therefore, it would be most effective to treat the acorns before *Penicillium* penetration into deeper tissues.

Mucor spp. are cosmopolitan soil fungi, which are often found on plant seeds (Lugauskas 2004). In our study these fungi reacted to the effects of wet water steam depending on the duration of exposure: fungal spores which survived had been stimulated by a short time stress. After treatment with wet water steam for 4, 8 and 12 s, the number of isolated *Mucor* spp. increased by 11.1%; 33.3% and 22.2%, respectively. Only the long-lasting 14 seconds steam exposure completely destroyed *Mucor* spp.

Wet water steam at 100°C did not effectively destroy one of the major agents of seedling root diseases, the fungi of the *Fusarium* genus (Ocamb et al. 2002). In the nursery, *Fusarium* spp. are the most common cause of damping-off (Sutherland and Davis 1991). They often surround the root of the plant and cause their rot and wilt (Lugauskas 2004). *Fusarium oxysporum* was one of the most frequently identified fungi in the studies (more than 10 isolates). *F. oxysporum* was able to kill 2-month-old seedlings and it could be responsible for the decay of roots. It could also be involved together with other factors (pathogenic fungi, droughts, frost) in the dying process of European ash trees (Przybył 2002). *Fusarium* spp. has the ability to germinate and grow under different environmental conditions (Marin et al. 1996). This was also confirmed by our studies, which

showed that 14 second treatments with wet water steam at 100°C were too short to destroy the *Fusarium* infection – it only reduced acorn contamination with the fungi of this genus, but did not destroy them completely.

Couture and Sutton (1980) found that *Fusarium* spp. could be completely destroyed by long-term dry heating. Gilbert et al. (2005) exterminated *F. graminearum* in wheat grains, keeping them at 70ºC in dry heat for 5 d. Clear et al. 2002, found that *F. graminearum* could be effectively killed only after prolonged 15 days exposure at 60°C, 5 days at 70°C or 2 days at 80°C.

Our study of 2 s and 4 s duration exposures has shown positive effects of wet water steam on the germination of oak acorns. The treatment with wet water steam for 2 s and 4 s, when the temperature inside inner tissues of the acorn increased from 23 to 24.6°C, acted as a stimulant and a positive effect on germination growth was determined. According to Morozov et al. (2013), heat treatment of seeds with an increase in seed temperature of 1–3°C increases seed pre-sowing quality. But longer treatments of 12 and 14 seconds (when the temperature inside the seed rises to 39.1 and 40.2°C, respectively) reduce the germination of acorns. The negative effect of a long-term impact of hot water on the germination of acorns has also been identified in the studies conducted by Knudsen et al. (2004). During the studies the germination of the acorns kept for 2.5 h in 41°C water decreased from 85 to 60%. After the wet water steam treatment for 5 hours at 44°C, 96% of the embryos were still alive 21 days later. In fact, 76% of the embryos were actively greening, growing and turning into seedlings, which suggested that this treatment also broke dormancy.

As the effect of wet water steam increased, the morbidity of acorns with root diseases decreased. Nursery seeds or seedlings are usually destroyed by seed-borne *Fusarium* spp. and soil-borne *Rhizoctonia* spp., *Pythium* spp. and *Phytophthora* spp. pathogens (Kraft et al. 2000). The roots of the damaged seedlings and the lower part of the seedlings blackens and the seedling dies (Landis 2013). During the studies, the effects of water steam of 2 and 4 s exposures both stimulated the germination of the acorns and prevented the pathogens during the germination period.

It is known, that before the germ of roots and stem are developed at the expense of nutrients of the seed: set of amino acids and starch. The more of these substances and how quickly they are absorbed by cells of embryo seed, so the more it develops powerful root system and quickly goes through all stages of growth and development of plants (Morozov et al. 2013). According to Morozov et al. (2003), active biochemical processes in physically stimulated seeds contributed to a more rapid absorption of nutrients. In our study, the exposure to wet water steam positively influenced the development of the seedling root system. The acorns stimulated with water steam for 4–8 s produced the seedlings with the longest roots, and the exposures of 2 s and 4 s resulted in the longest root collar. However, there was no long-term effect on the further growth of the plant growth, particularly stem length.

The research has shown the advantages and disadvantages of this thermal treatment method for seeds. The treatment of acorns by wet water steam for 2–4 s reduces their contamination by *Alternaria, Penicillium, Mucor, Fusarium* spp. and manifestation of root diseases, as well as positively affects the length and diameter of the main root of the seedling without affecting the germination of acorns. However, the effect of the 2–4 s thermal impulse is too short to destroy fungal contamination because the efficiency of this technique depends on the fungal localization in the acorns. By increasing the steam exposure time at the surface of the acorns, the use of longer wet steam exposure times of 6 s to 8 s eliminates *Alternaria* spp. and *Penicilium* spp., which is particularly important for the acorns in storage; however, the acorn germination and biometric characteristics of the seedlings are negatively affected: the root diameter and stem length are reduced. After 10 to 14 s exposure to steam, *Penicillium, Alternaria* and *Mucor* spp. were completely destroyed and the number of seedlings affected by root-borne diseases decreased, however, the acorns remained contaminated with *Fusarium* spp. and fungi. The effects of these exposures reduced the germination of the acorns, height of the seedlings and diameter of the main root.

Our study showed that of various durations of exposure to wet water steam, the optimal one was the treatment of acorn seed material with 100°C wet water steam for 2–4 s, which not only reduced the contamination of the seeds with fungi and had no negative effect on its germination, but also stimulated it and thus the biometric indicators of the seedlings improved. Wet steam treatment of acorns would be a good alternative to pesticides in forestry. It is a cheap and environmentally friendly method of treating seed material. However, further and detailed studies are still required to investigate the effectiveness of this method.

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