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THE EFFECT OF THERMAL TREATMENT ON SPRING BARLEY SEED INFECTION AND GERMINATION

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Abstract

Experiments were carried out over the period 2005–2006 to study the effects of thermal treatment (hot vapour (100°C)) lasting from 1 to 10 seconds on spring barley (*Hordeum vulgare*) seed fungal infection and germination. *Fusarium* (up to 24.8% of infected seed), *Alternaria* (up to 57.8%), *Penicillium* (up to 31.8%) and *Drechslera* (up to 6.8%) genera fungi were dominant on the spring barley seed tested.

It was found that thermal treatment significantly reduced the level of fungal infection. The effect of hot vapour depended on the duration of treatment – with increasing exposure time more seed was disinfested. Pathogenic fungi *Fusarium* and *Drechslera* and saprophytic *Alternaria* were not found on barley seed after a 10 s hot vapour single treatment. However, a 10 s exposure significantly reduced seed vigour and germination. It was established that without any damage to germination, spring barley seed can be exposed to hot vapour once for up to 6 s. Under such conditions the fungi of *Drechslera* genus were completely destroyed. The seed infection by *Fusarium* and *Alternaria* markedly declined. Twice application of hot vapour especially at longer exposition gave better control of seed born fungi compared to single application; however, the two-time treatment for up to 6 s significantly reduced seed germination and vigour. Experimental findings suggest that seed can be treated by hot vapour once and the maximum seed exposure time is 6 seconds. Since moisture content of seed treated with hot vapour increases; it is necessary to continue the investigation on management of moist seed.

Key words: thermal treatment, seed born fungi, *Fusarium, Dreshslera, Alternaria, Penicillium,* seed germination.

Introduction

Barley grain can be infected by a variety of saprophytic and parasitic fungi. Fungal infection can reduce the health, grade and marketability of the grain /Clear et al., 2000; Heino, 2004/. In many cases fungi spread with cereal seed (are seed-borne) and later affect seedlings and plants. They also contaminate the soil. This is very important for grain which is used as seeding material /Chelkowski, 1991/. The summarized data of fungal infection of spring barley seed collected from different places of Lithuania showed, that fungi of *Alternaria, Penicillium, Fusarium, Drechslera,* and *Cladosporium* genera prevailed /Dabkevičius et al., 2005/.

One of the most common practices used nowadays to reduce seed fungal infection is chemical seed treatment /Carmona et al., 1999; Platz et al., 2001; Semaškienė, 2001/. However, intensive use of seed treatment fungicides contributes to environmental pollution. In organic farming there is a special demand for alternatives to chemical compounds. Different biological control agents are recommended for seed born pathogen control as environment-friendly /Knudsen et al., 2007; Hökeberg et al., 1997; Coombs et al., 2004/. Various alternative seed treatment physical methods are being tested and used: exposure to microwaves 68–75°C /Clear et al., 2002/, high-frequency waves /Cwiklinski, Hoersten, 2001/. Various thermotherapy methods: hot water, hot wet and dry air, solar energy, and wet hot vapour can be used for seed pathogen control /Agarwal, Sinclair, 1987; Clear et al., 2002; Forsberg et al., 2005/. Hot water treatment was used in practice for seed born disease control in cereals already 50–60 years ago /Strukčinskas, Žemaitienė, 1956; Doling, 1965/; however after 1960 it was replacing by efficient chemical seed treatment fungicides. Recently, research has been done to improve the properties of aerated steam treatment in Sweden /Forsberg, 2004/. The author concluded that for good efficacy of seed sanitization by aerated steam (hot humid air) treatment, a relative air humidity exceeding 85% is necessary and treatment duration can be found that gives optimum pathogen sanitization with maintained germinability. Clear et al. (2002) also pointed out, that pathogen control with no damage to seed germination is the ultimate objective of all seed treatment methods.

The objective of the present research was to study the effect of thermal treatment by hot vapour (100°C) on barley seed fungal infection and germination.

Materials and methods

During 2005–2006 research on thermal treatment effect on spring barley (*Hordeum vulgare* L.) cv. ʻŪla' seed was carried out at the Plant Protection Laboratory and Heat and Biotechnology Engineering Department of the Lithuanian University of Agriculture. Based on the literature sources on cereal seed treatment with hot vapour, our research was done according to one of the latest technologies which differs from the previous ones /Forsberg et al., 2005/ by treatment temperature, relative vapour moisture and markedly shorter exposure. Forsberg (2005) treated cereal seed with 63ºC and 64ºC water vapour at 95% and 75% relative air moisture. In our research, barley seed treatment was done with hot vapour at temperature 99 ± 1°C. Special equipment was used for thermal seed treatment (Figure 1).

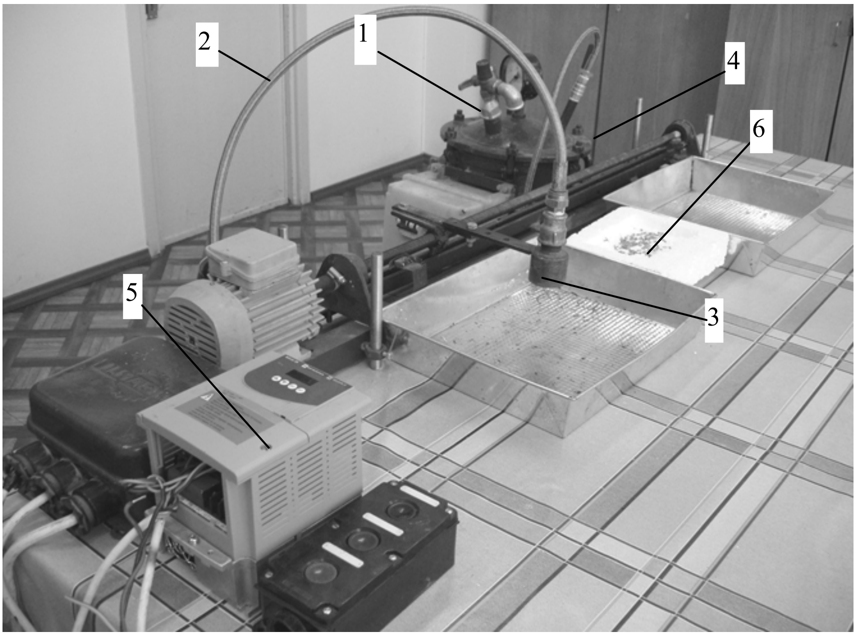

Figure 1. Thermal treatment equipment: 1 – vapour boiler, 2 – vapour transfer hose, 3 – vapour diffuser, 4 – roll, 5 – rev regulator “Altivar 28”, 6 – heat insulation panel with recess and seed
1 paveikslas. *Terminio impulso stendas: 1 – garo katilas, 2 – garo padavimo žarna, 3 – garo sklaidiklis, 4 – velenas, 5 – sūkių reguliatorius „Altivar 28“, 6 – termoizoliacinė plokštė su įduba ir sėkliniais grūdais*

Spring barley seed from the harvest years of 2004 and 2005 was used. A seed sample size for each treatment was 200 g. Barley seed treatment with hot vapour (temperature 99 ± 1°C) impulses ranging from 1 to 10 seconds at 1 s interval was made once. Twice seed treatment was made with hot vapour impulses ranging from 1 to 6 seconds. The interval between treatments was 24 hours. Exposure duration was the same for both times.

Seed moisture was measured immediately after hot vapour treatment.

Infection level of barley seed untreated and treated with hot vapour at different duration was test. Both surface non-sterilized and sterilized (internal infection) seeds were analyzed for each treatment according to Bilai (1982) and Smirnova and Kostrova (1989) methodology. From each treatment 100 barley seeds were examined for the presence of fungi. The barley seeds for internal infection were surface sterilised in a 75% ethyl alcohol for 5 minutes and thoroughly washed with sterile water. Petri plates 9 cm in diameter were used, 10 seeds per each plate. Seeds were plated onto Chapek-Dox agar and incubated in a thermostat at 26 ± 2ºC in the dark for 7 days. Identification of fungi was determined by visual examination of colony characteristics and spore morphology. The fungi were determined according to Satton et al. (2001). The results were expressed as a percentage of seed infected.

With the aim of identifying the effect of hot vapour treatment on seed viability, germination test was done by using filter paper and sand substrate /ISTA rules, 2003/. Four hundred seeds per treatment were tested. According to filter paper methods seeds were placed directly on moist filter paper in Petri dishes which were in turn kept for incubation. Twenty seeds were planted per Petri dish. For sand test, plastic flat trays were filled with sand up to 3/4th of tray height. The sand was moistened up to 50% of its water holding capacity. The seeds were planted on the leveled layer of moist sand and covered with 20 mm of uncompressed sand. One hundred seeds were planted per tray. The Petri dishes and plastic tray with seeds were incubated in a thermostat for 7 days at 20°C temperature. First count was taken on the 4th day (vigour) and the final count on the 7th day (germination). During each count, the germinated seedlings (normal seedlings have well-developed roots and shoots) and the abnormal seedlings and non-germinated seeds were recorded.

The seed infection data expressed in per cent were transformed according to Asin (Sqr(x%)). The least significant differences of the results were computed using the software ANOVA /Tarakanovas, Raudonius, 2003/.

Results and discussion

The most common *Fusarium, Drechslera, Alternaria, Penicillium* genera were identified on spring barley seed in our research (Tables 1 and 2). The most prevalent fungi in the non-surface sterilized untreated seed were of *Alternaria* (53.8% infected untreated seed) and *Fusarium* (24.8%) genera (Table 1). The fungi of *Drechslera* and *Penicillium* genera on non-surface sterilized seed were found on 3.8 and 19.8% of seed, respectively. The fungi of other unidentified genera were found on 26.3% of seed.

Already after 1 s thermal impulse a significant reduction occurred in the fungi of *Fusarium, Alternaria, Penicillium* genera by 7.3, 11.0 and 14.3 percentage points, respectively. A more marked reduction in the fungi of *Fusarium*, *Drechslera* and *Alternaria* genera was identified having treated the seed for 4 s. After a 6 s exposure there were found no fungi of *Drechslera* genus, and *Fusarium, Alternaria, Penicillium* infection declined by 21.8; 46.5 and 13.5 percentage points. The best control of fungal infection was achieved having treated the seed for 7 and more seconds.

Alternaria, *Penicillium*, *Fusarium* and *Drechslera* genera in the surface-sterilized untreated seed was identified. Seed infection level by *Alternaria* was 57.8 %, *Penicillium* – 31.8%, *Fusarium* – 14.3% and *Drechslera* – 6.8% (Table 2). After 1 s treatment *Alternaria* infection declined by 22.5, *Fusarium* – by 8.3 and *Penicillium* – by 5.8 percentage points. A more marked reduction in *Fusarium, Drechslera, Alternaria* fungi was identified after a 5 s thermal treatment, and a 6 s treatment completely controlled an important pathogen of barley *Drechslera,* and reduced *Fusarium* and *Alternaria* infection to 0.5% and 7.3%. An 8 s thermal disinfection effectively killed all fungi, except for *Penicillium*.

Table 1. The effect of thermal treatment with hot vapour on spring barley seed-born infection (non-surface sterilized seed)

Data averaged over two experiments

1 lentelė. *Terminio apdorojimo karštais garais įtaka vasarinių miežių sėklų užsikrėtimui su sėklomis plintančiais grybais (be paviršinės grūdų dezinfekcijos)*

Dviejų bandymų vidutiniai duomenys

Treatment duration s *Apdorojimo trukmė s*	% of seed infected by / *Užkrėstų sėklų kiekis %*					
	Total *Iš viso*	*Fusarium* spp.	*Drechslera* spp.	*Alternaria* spp.	*Penicillium* spp.	Other fungi *Kiti grybai*
0[a]	100	24.8	3.8	53.8	19.8	26.3
1	100	17.5*	5.3	42.8*	5.5*	31.3
2	100	17.3*	4.5	45.0*	9.3*	32.5
3	100	12.0*	3.0	49.3*	5.0*	31.5
4	94.5	4.8*	1.0	30.3*	6.5*	58.0
5	87.0	6.0*	0.5	17.5*	7.5*	52.0
6	66.5	3.0*	0*	7.3*	6.3*	48.5
7	19.0	0*	0*	0*	1.0*	18.0*
8	8.0	0*	0*	0*	0*	8.0*
9	18.0	0*	0*	0*	0*	19.0*
10	19.0	0*	0*	0*	0*	19.0*

[a] – Untreated / *Neapdorota;* * – significantly differed from untreated at 0.05 probability level / *iš esmės skyrėsi nuo neapdorotų 0,05 tikimybės lygiu*

Experimental data suggest that thermal treatment with hot vapour of barley seed lasting up to 6 s can provide a relatively high control of pathogens. Under such conditions a complete control of *Drechslera* fungi was achieved, *Fusarium* seed infection level also declined markedly. Reduction of saprophytic fungi *Alternaria* infection was significant, too. The fungi of *Pencillium* genus were less sensitive to thermal treatment.

Experimental findings suggest that having treated the seed with hot vapour, depending on the treatment time, seed moisture content increased from 13–15% to 25%. One second hot vapour treatment increases seed moisture by on average 1.0% (in separate treatments from 0.4 to 1.6 percentage point), therefore moist seed should be sown immediately.

Germinability is one of the fundamental properties of a seed. The thermal seed treatment increases the temperature of seed to high levels during a short time. This induces a stress in the seed similar to the accelerated ageing occurring in a hot-air grain dryer and therefore it causes accelerated ageing of the seed /Nellist, 1981; Roberts, 1981; Forsberg, 2004/. If the thermal treatment is performed during a too long time, or at a too high temperature, the accelerated ageing will reduce the viability so much that germinability would be reduced in the seed lot /Forsberg, 2004/. In our research one and two-second hot vapour treatment did not have any effect on barley seed vigour, however, at a three-second treatment seed vigour started to decline (Figure 2). Seed vigour decreased twice having treated the seed for 7 seconds. The seed did not germinate at all for four days when increasing the exposure time to 10 seconds. The duration of the thermal impulse had a lower effect on seed germination – the negative effect was identified only after a 7-second treatment. Exposure from 1 to 5 s even slightly promoted seed germination. When the seed was treated from 7 to 10 s, its germination did not meet ISTA standards for barley, therefore such exposure cannot be applied for seed treatment.

Our experimental evidence suggests that seed treatment with hot vapour at short duration impulses for up to 3 s does not affect seed vigour, and treatment for up to 6 s does not affect seed germination. The relationship between spring barley seed vigour and duration of thermal impulse is expressed by the following equation:

$y = -1.1231x^2 + 2.4526x + 80.099$, $R^2 = 0.85$.

Curve 2 shows that thermal treatment by hot vapour at different duration impulse affects seed vigour. Maximum increase in seed vigour was determined at 1.1 s thermal treatment. A sharp decline in seed vigour occurred with increasing thermal treatment duration from 3 s, and at 6 s thermal impulse seed vigour declined to 54.3%.

The effect of thermal treatment on seed germination was less deleterious. The relationship between seed germination and duration of hot vapour treatment can be expressed by the following equation: $y = -1.0448x^2 + 6.2985x + 88.365$, $R^2 = 0.98$.

Analysis of this equation indicated that maximum increase in germination (9.5%) occurred at 3.1 s thermal treatment. Seed germination after thermal treatment for 6.0 s was 88.3%, the same as in the control treatment. As a result, thermal treatment duration up to 6.0 s did not exert any negative effect on barley seed germination. A reduction in germination starts with 7.0 s thermal treatment.

Table 2. The effect of thermal treatment with hot vapour impulse on spring barley seed-born infection (surface-sterilized seed)

Data averaged over two experiments

2 lentelė. *Terminio apdorojimo karštais garais įtaka vasarinių miežių sėklų užsikrėtimui su sėklomis plintančiais grybais (atlikus paviršinę grūdų dezinfekciją)*

Dviejų bandymų vidutiniai duomenys

Treatment duration s *Apdorojimo trukmė s*	% of seed infected by / *Užkrėstų sėklų kiekis %*					
	Total *Iš viso*	*Fusarium* spp.	*Drechslera* spp.	*Alternaria* spp.	*Penicillium* spp.	Other fungi *Kiti grybai*
0[a]	100	14.3	6.8	57.8	31.8	31.3
1	100	6.0*	11.0	35.3*	26.0	52.8
2	100	7.3*	5.0	47.5*	18.0*	59.5
3	98.8	5.3*	2.0*	36.3*	20.5*	49.5
4	98.1	11.5*	7.0	17.3*	38.3	29.8
5	84.5	3.0*	1.0*	7.0*	29.5	65.0
6	71.0*	0.5*	0*	7.3*	23.3	46.5
7	24.0*	4.0*	0*	2.0*	7.0*	15.0*
8	27.0*	0*	0*	0*	14.0*	17.0*
9	16.0*	0*	0*	0*	10.0*	6.0*
10	15.0*	0*	0*	0*	8.0*	7.0*

[a] – Untreated / *Neapdorota;* * – significantly differed from untreated at 0.05 probability level / *iš esmės skyrėsi nuo neapdorotų 0,05 tikimybės lygiu*

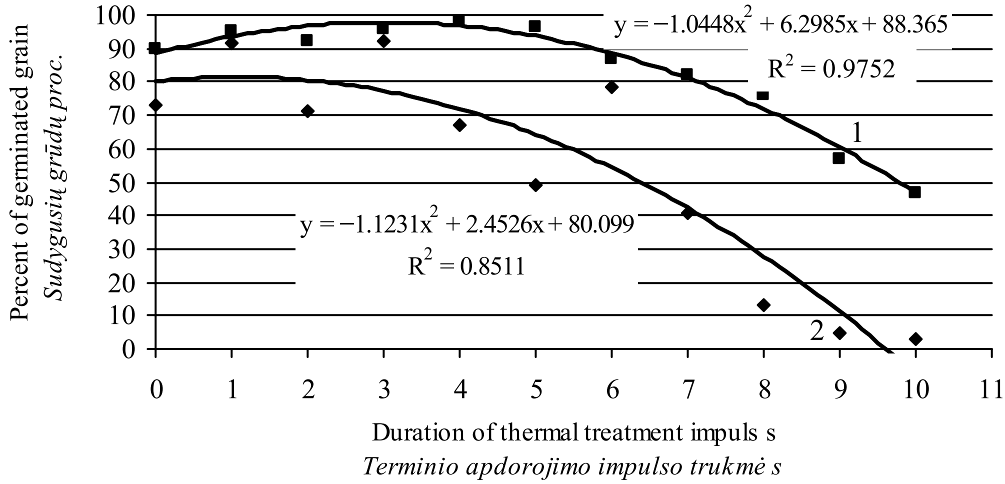

Figure 2. Relationships of spring barley germination (curve 1) and vigour (curve 2) and duration of thermal treatment impulse
2 paveikslas. *Vasarinių miežių daigumo (1 kreivė) ir dygimo energijos (2 kreivė) priklausomumas nuo terminio apdorojimo karštais garais impulso trukmės*

Having identified the duration of thermal impulse at which no damage to seed germination is done and which gives relatively good control of fungi, and seeking to achieve higher efficacy in reducing the seed infection level, research was conducted on twice application of thermal treatment. The hypothesis of this study is that single thermal treatment affects but does not completely control fungi, therefore they will be more sensitive to repeated treatment. The effect of single and twice application of thermal treatment on barley seed infection was studied.

Single application at different duration of thermal impulse significantly reduced infection level by *Fusarium, Dreshlera, Alternaria* and *Penicilium* genera compared with untreated (Table 3). The percentage of seed infected with the fungi, like in the first experiment, consistently declined with increasing exposure time. Twice application of hot vapour at different duration in many cases declined spring barley seed infection by *Fusarium, Dreshlera, Alternaria* and *Penicilium* genera compared with single application. Longer exposition time (4, 5 and 6 seconds) of twice application with hot vapour resulted in higher reduction of seed infection, especially for *Fusarium* and *Alternaria* spp., than the exposition for 1, 2 or 3 seconds.

In these experiments, like in previously conducted ones, it was found that having treated the seed with hot vapour once for up to 6 s, its germination and vigour slightly declined; however, germination remained within the range allowed by the standard – not lower than 85% (Figure 3). Having twice treated the same seed, its vigour and germination were markedly lower – in the case of two applications for 6 s seed vigour declined by 20.3% and germination by 18.5%. The seed treated twice for 4, 5 and 6 seconds was of lower germination than the standard allows, therefore such seed treatment is deleterious to seed.

Table 3. The effect of single and twice thermal treatment with hot vapour on spring barley seed-born infection (non-surface sterilized seed)
3 lentelė. *Vienkartinio ir dukartinio terminio apdorojimo karštais garais įtaka vasarinių miežių sėklų užsikrėtimui su sėklomis plintančiais grybais (be paviršinės grūdų dezinfekcijos)*

Treatment duration s *Apdorojimo trukmė s*	% of seed infected by / *Užkrėstų sėklų kiekis %*											
	Total *Iš viso*		*Fusarium* spp.		*Drechslera* spp.		*Alternaria* spp.		*Penicillium* spp.		Other fungi *Kiti grybai*	
	1	2	1	2	1	2	1	2	1	2	1	2
0[a]	100	100	13.5	9.5	4.0	1.5	44.0	35.5	28.5	24.0	55.0	52.0
1	100	100	7.0*	6.0	3.0	0.5	38.0	22.0*	19.5*	16.0*	69.5	61.5
2	100	100	7.5*	5.5	2.5	1.5	28.0*	23.5*	14.5*	11.0*	57.5	62.5
3	100	100	5.5*	5.0*	1.0	0.5	18.5*	21.5*	18.0*	12.0*	60.0	69.5
4	100	99.4	5.0*	1.0*	1.0	1.5	21.5*	13.5*	5.0*	3.5*	67.0	81.0
5	96.0	93.4	3.0*	1.5*	0.5	0.5	17.5*	2.5*	8.5*	7.5*	86.0	87.5
6	83.0	50.0	1.5*	0.5*	0.5	0	19.5*	0.5*	8.0*	5.0*	54.0	47.0

[a] – Untreated / *Neapdorota*
1 – After the first treatment / *Po pirmojo apdorojimo*
2 – After the repeated treatment / *Po pakartotinio apdorojimo*
* – significantly differed from untreated at 0.05 probability level / *iš esmės skyrėsi nuo neapdorotų 0,05 tikimybės lygiu*

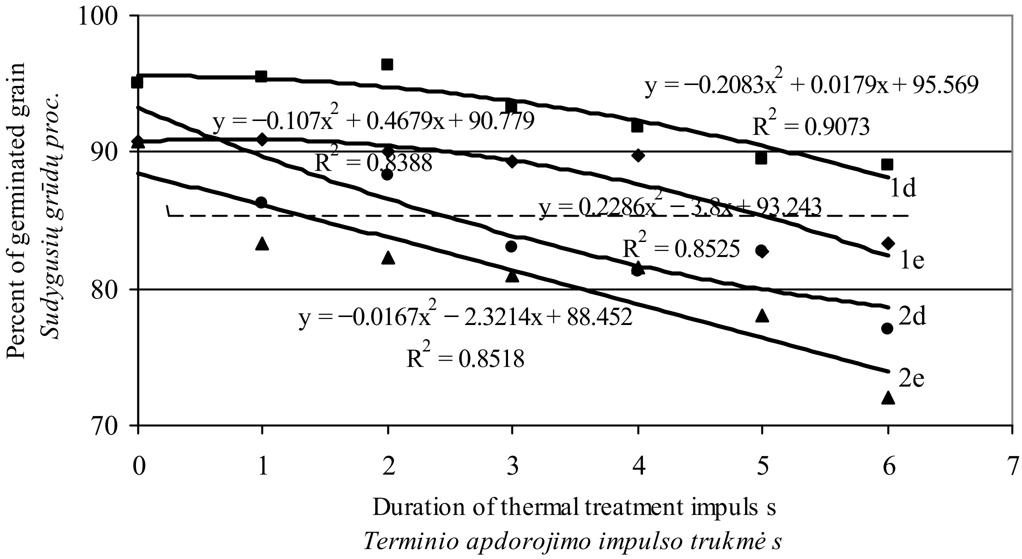

Figure 3. The effect of single (curve 1) and twice (curve 2) thermal treatment with hot vapour on spring barley seed grain vigour (e) and germination (d). The dotted line marks the lowest allowable limit (85%) of the germination standard
3 paveikslas. *Vienkartinio (1 kreivė) ir dukartinio (2 kreivė) terminio apdorojimo karštais garais įtaka vasarinių miežių sėklų dygimo energijai (e) ir daigumui (d). Punktyrinė linija žymi žemiausią daigumo standarto leidžiamą ribą (85 %)*

As was mentioned above, twice application of hot vapour especially at longer exposition gave better control of seed born fungi compared to single application; however, the two-time treatment for up to 6 s significantly reduced seed germination and vigour. Experimental findings suggest that seed can be treated by hot vapour once and the maximum seed exposure time is 6 seconds. Our research showed that hot vapour treatment can be a promising way to reduce cereal seed infection with *Fusarium*, *Drechslera*, *Alternaria* and *Penicillium* fungi. This seed treatment method is environment-friendly and can be effectively used on organic farms.

Summarised experimental data suggest that thermal treatment with hot vapour of barley seed lasting up to 6 s can provide a relatively high control of pathogens with no damage to seed. It is noteworthy that the moisture content of such seed is by about 6% higher compared with that before the exposure. As a result, it is necessary to continue the study on moist seed management.

Conclusions

1. Thermal seed treatment with hot vapour is a promising method for limiting the spread of seed-borne fungi of *Fusarium, Dreshlera, Alternaria* and other genera.
2. Thermal treatment duration with no damage to spring barley seed germination can be no longer than 6 s.
3. Since moisture content of seed treated with hot vapour increases, it is necessary to continue research on moist seed management.

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TERMINIO SĖKLŲ APDOROJIMO ĮTAKA JŲ LIGOTUMUI IR DAIGUMUI

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Santrauka

2005–2006 m. tirta terminio apdorojimo (karštais garais (+100 °C)), kurio trukmė nuo 1 iki 10 s, įtaka vasarinių miežių (*Hordeum vulgare*) sėklų užsikrėtimui su sėklomis plintančiais grybais ir jų daigumui. Tyrimui naudotose sėklose dominavo *Fusarium* (iki 24,8 užsikrėtusių sėklų), *Drechslera* (6,8 proc.), *Alternaria* (57,8 proc.) ir *Penicillium* (31,8 proc.) genčių grybai.

Nustatyta, kad terminis sėklų apdorojimas iš esmės mažino jų ligotumą. Apdorojimo karštais garais veiksmingumas priklausė nuo apdorojimo trukmės – ilgėjant ekspozicijos laikui, daugiau sėklų buvo apvalyta nuo su sėklomis plintančių grybų. Po 10 s ekspozicijos karštuose garuose miežių sėklose visiškai nerasta *Fusarium*, *Drechslera*, *Alternaria* grybų. Tačiau 10 s trukmės terminis sėklų apdorojimas iš esmės sumažino jų dygimo energiją ir daigumą. Buvo nustatyta, kad sėklos karštais garais (nepažeidžiant jų daigumo) gali būti apdorojamos vieną kartą iki 6 s. Šiomis sąlygomis visiškai sunaikinami su sėklomis plintantys *Drechslera* genties grybai, gerokai sumažėja *Fusarium* ir *Alternaria* genčių grybais užsikrėtusių grūdų. Dukartinis sėklos apdorojimas karštais garais, ir ypač esant ilgesnei ekspozicijai, buvo veiksmingesnis nuo su sėklomis plintančių grybų nei vienkartinis, tačiau, dukart termiškai apdorojus sėklas iki 6 s, jų dygimo energija ir daigumas sumažėjo iš esmės. Tyrimas parodė, kad miežių sėklos gali būti apdorojamos karštais garais vieną kartą ir ne ilgiau kaip 6 s. Pažymėtina, kad drėgmės kiekis apdorotose karštais garais sėklose padidėja, todėl ateityje būtini sėklų džiovinimo tyrimai jas apdorojus be rizikos sėkloms iš naujo užsikrėsti.

Reikšminiai žodžiai: terminis sėklų apdorojimas, su sėklomis plintantys grybai, *Fusarium, Dreshslera, Alternaria, Penicillium,* sėklų daigumas.