New methods for artificial scarification of *Robinia pseudoacacia* L. seeds

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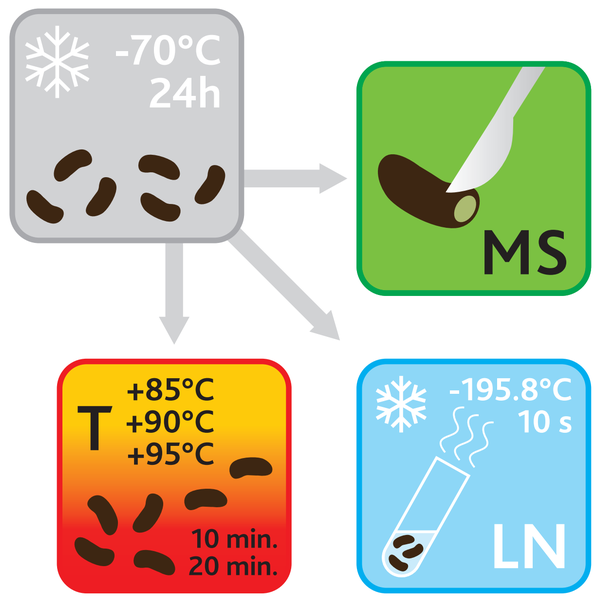
INTRODUCTION AND AIMS

Seeds of black locust (*Robinia pseudoacacia* L.) are counted to "hard" seeds. They have impermeable seed coat for water and air thus require different ways for opening. In the case of black locust seeds are usually used a mechanical or acid (concentrated H_2SO_4) scarification. Both methods are very time-consuming, especially if large numbers of scarified seeds are required, and also dangerous.
The main aim of this study was to determine the efficiency of methods for the artificial scarification of black locust seeds based on the influence of the air and liquid nitrogen temperatures.

METHODS

Seed collection and initial preparation

For the analyses were selected three populations: a managed tree stand, a selected seed stand and a seed orchard. Samples of 200 seeds (4 x 50) were randomly selected. Seeds were mechanically husked from the pods. The empty and immature seeds were separated with the pod remains to obtain pure seeds. Seeds were weighed to an accuracy of 0.01 g on an analytical balance.



Scarification methods

Effect of eight scarification methods (plus one non-scarificated as control) on germination of black locust was tested: mechanical scarification with a scalpel (MS), liquid nitrogen (LN) and air temperature (T) at 85, 90 and 95°C with two variants of time (t) of 10 and 20 min (T85_t10, T85_t20, T90_t10, T90_t20, T95_t10 and T95_t20). Before scarification, the seeds were stored at –70°C for 24 h.

Germination test

After thermal and mechanical scarification, the seeds were tested for germination at 24°C on a Jacobsen's germination apparatus for 14 days, according to ISTA rules (2013). Observations were made on days 3, 5, 7, 10 and 14 after treatment.

Statistical analysis

In order to determine whether the seed in each populations is able to germinate without scarification and to test whether there are significant differences among populations, the ANOVA (one way analysis of variance) was performed.
The final germination capacity was calculated using the following formula (1):

$$FGC = \frac{N_s}{N}\ 100\%$$

where N_s is the number of germinated seeds and N is the number of total seeds.
Germination capacity among populations and scarification variants was analysed according to a fixed general linear model as it follows (2):

$$GC_{ijk} = \mu + P_i + M_j + PM_{ij} + e_{ijk}$$

where GC_{ijk} is the percentage of germinated seeds of the i^{th} provenance, under the j^{th} scarification, μ is the overall mean, P_i is the i^{th} provenance effect, M_j is the j^{th} scarification effect, PM_{ij} is the provenance × treatment interaction effect, and e_{ijk} is the experimental error.
To determine the dynamics of germination capacity for the seed, i.e., the changes in the mean germination in time, depending on the scarification and the provenance of the seeds, we used a repeated measures analysis of variance, according to the following linear model (3):

$$GD_{ijkl} = \mu + P_i + M_j + T_k + PM_{ij} + PT_{ik} + MT_{jk} + PMT_{ijk} + e_{ijkl}$$

where GD_{ijkl} is the percentage of germinated seeds of the i^{th} provenance, under the j^{th} scarification treatment and on the k^{th} day of the trial, PT_{ik} is the provenance × day of the trial interaction effect, MT_{jk} is the treatment × day of the trial interaction effect, PMT_{ijk} is the provenance × treatment × day of the trial interaction effect and e_{ijkl} is the experimental error; the other effects are the same as in the model 2.

RESULTS

Final germination capacity

We found significant differences in germination capacity of unscarified seeds among provenances (F=6.6; p=0.017). In MTS provenance In MTS provenance it was significantly more seeds germinated (15.5%) than in the SSS (5.0%) – Fig. 1.
At the end of the test, the optimum results were obtained using the MS and LN methods (Table 1). Among the thermal-time scarification methods, the variants T85_t10 and T85_t20 were the most effective. For both time variants, 95°C had a negative effect on the germination of black locust seeds, and maintaining this high temperature for 10 min resulted in a decrease in seed germination capacity. The seeds scarified at -195.8°C (LN) were characterised by a very high germination capacity, regardless of the origin – Fig. 2.

Germination dynamics

Depending on the provenance of seeds and scarification treatment, the germination capacity was significantly affected by time duration of the thermal treatment (interactions P×T and M×T, in both cases p<0.001). However, there were no significant differences in dynamics of germination of seeds from the different provenances, even when they were scarified using different methods (interaction P×M×T, p = 0.83).
On the third day of the incubation, significant differences were observed between the analysed populations. The highest percentage germination was found in MTS population (47.1%). The lowest percentage germinated seeds were found in SO (31.6%). This trend was continued until the end of the incubation period. After 14 days of incubation, there was no significant differences observed between MTS and SSS populations, whose seeds germinated significantly better than the seeds from SO – Fig. 3.

CONCLUSIONS

- Seeds not treated with scarification are characterised by much lower germination capacity.
- Average germination capacity can depend on the management of forest stands or their type of reproduction.
- Uniform germination of seeds was obtained by using liquid nitrogen for scarification.
- Scarification of black locust seeds at 95°C for 10 or 20 min reduced the germination capacity.
- Thermal scarification of the seeds of black locust was most effective in the temperature range 85–90°C, with exposure times of 20 and 10 min, respectively.
- Thermal methods and the use liquid nitrogen may provide alternatives to the labour intensive cutting of seeds and methods that require the use of specific equipment (e.g., soaking in sulphuric acid).

ACKNOWLEDGMENTS

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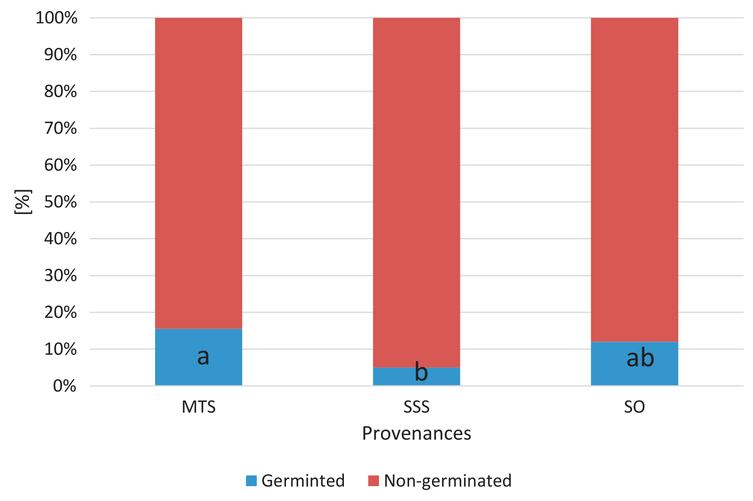

Fig. 1. Percentage of germinated and non-germinated seeds of black locust collected from three sites. MTS managed tree stand, SSS selected seed stand, SO seed orchard. Means (germinated seeds) with the same lowercase letter are not significantly different at p ≤ 0.05.

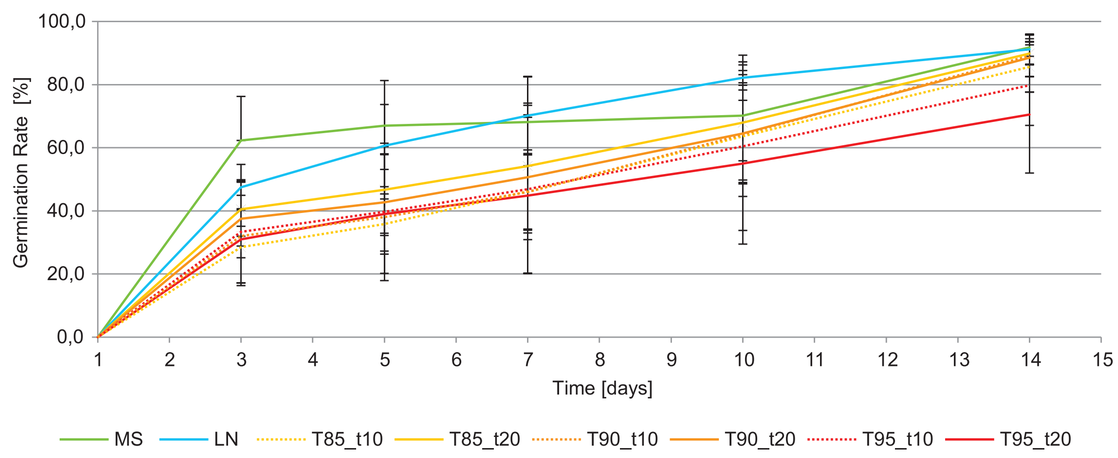

Fig. 2. Germination dynamics of seeds treated with different scarification methods. MS mechanical scarification, LN liquid nitrogen. T85_t10 – 85°C (air temperature) by 10 min, T85_t20 – 85°C by 20 min, T90_t10 – 90°C by 10 min, T90_t20 – 90°C by 20 min, T95_t10 – 95°C by 10 min, T95_t20 – 95°C by 20 min. Bars are standard deviations (SD).

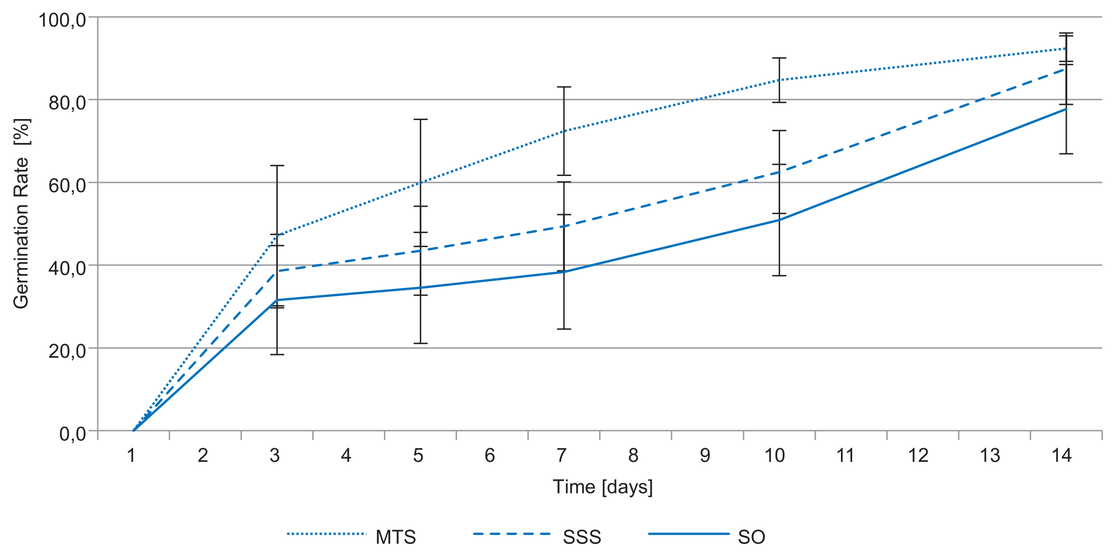

Fig. 3. Germination dynamics of seeds with different provenances. MTS – managed tree stand, SSS – selected seed stand, SO – seed orchard. Bars are standard deviations.

Scarification methods	FGC (%) Mean ± SD			Total for scarification methods
	MTS	SSS	SO	
MS	94.0 ± 0.00ab	94.5 ± 4.12a	87.0 ± 8.41b	91.8 ± 4.19A
LN	95.0 ± 3.46a	92.5 ± 3.00ab	86.0 ± 1.63b	91.2 ± 4.65A
T85_t10	93.0 ± 2.00a	93.0 ± 1.15a	81.5 ± 5.26b	89.2 ± 6.64A
T85_t20	94.0 ± 3.27a	88.0 ± 2.83a	87.5 ± 5.97a	89.8 ± 3.62A
T90_t10	86.0 ± 5.89ab	93.5 ± 4.73a	77.5 ± 10.38b	85.7 ± 8.01AB
T90_t20	94.5 ± 1.91a	88.5 ± 4.43ab	82.5 ± 3.42b	88.5 ± 6.00A
T95_t10	92.5 ± 1.91a	80.0 ± 4.32b	67.0 ± 6.83b	79.8 ± 12.75B
T95_t20	90.0 ± 6.00a	69.5 ± 3.00b	52.5 ± 4.12c	70.7 ± 18.78C
Total for provenances	92.4 ± 3.01a	87.4 ± 8.63b	77.7 ± 12.18c	

Table 1. Final germination capacity (FGC) of black locust seeds for different scarification methods. Means with the same lowercase letter within a row or means with the same uppercase letter within a column are not significantly different at p ≤ 0.05; SD – standard deviation; MTS – managed tree stand, SSS – selected seed stand, SO – seed orchard; MS – mechanical scarification, LN – liquid nitrogen, T85_t10 – 85°C (air temperature) by 10 minutes, T85_t20 – 85°C by 20 minutes, T90_t10 – 90°C by 10 minutes, T90_t20 – 90°C by 20 minutes, T95_t10 – 95°C by 10 minutes, T95_t20 – 95°C by 20 minutes.

Graphic design: Urszula Wojciechowska

SEE ALSO

Jastrzębowski S., Ukalska, J, Kantorowicz W., Klisz M., Wojda T., Sułkowska M., 2017. Effects of thermal-time artificial scarification on the germination dynamics of black locust (*Robinia pseudoacacia* L.) seeds, European Journal of Forest Research, Vol. 136, Issue 3, pp 471–479.