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Seed size and cold stratification affect *Acer negundo* and *Acer ginnala* seeds germination

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Abstract. The aim of this work is to determine how the germination of seeds of the invasive tree *Acer negundo* depends on the period of cold stratification under the snow and the duration of stratification in the air on the branches of the trees. For comparison with *A. negundo*, we used seeds of *Acer ginnala*, introduced but not invasive tree in the Middle Urals. The period of stratification in the air modeled by collecting seeds in October and December. The duration of cold stratification under the snow was 0, 1, 2, 3 and 4 months. We hypothesized that the duration of stratification in the air did not affect the germination of *A. negundo* and *A. ginnala* seeds. Cold stratification under the snow had a positive effect on seed germination of both species. The best seed germination of *A. negundo* and *A. ginnala* was after 4 months of cold stratification under the snow, the germination rate differs: in *A. negundo* 12 ± 4% (small seeds) and 79 ± 7% (large seeds), in *A. ginnala* – 1 ± 2% (small seeds) and 18 ± 4% (large seeds). In both species, large seeds germinated at 7 to 18 times more intensively than small ones. In *A. ginnala* case, even after cold stratification under snow for 4 months, no more than 22% of the seeds germinated. The germination of *A. ginnala* seeds was 4–5 times lower than that of *A. negundo* seeds.

Key words: *Acer ginnala, Acer negundo*, cold stratification, invasive plants, propagule press, seed biology.

INTRODUCTION

Acer negundo L. (boxelder maple) is a North American native tree and intentionally introduced in Europe for horticulture and landscaping purposes (Merceron et al., 2016). It is invasive in Northern Eurasia, particularly in Russia (Vinogradova et al., 2010). *A. negundo* develops mainly in disturbed and semi-natural territories (Tret'yakova, 2011; Straigytė et al., 2015; Merceron et al., 2016; Gusev et al., 2017; Kostina et al., 2017). A. *negundo* actively spread in the urbanized forests of the Middle Urals (Veselkin et al., 2018; Veselkin & Korzhinevskaya, 2018). In Europe and Asia *A. negundo* invasion leads to a replacement of economically important trees (Ryabinina & Nikitina, 2009; Bottollier-Curtet et al., 2012; Gusev, 2016). In the forest management, the search for the most efficient methods to kill and remove invasive *A. negundo* in invaded natural habitats is continuing (Merceron et al., 2016; Kostina et al., 2017).

A. negundo may form monospecific stands (Ryabinina & Nikitina, 2009; Tret'yakova, 2011; Lamarque et al., 2012; Gusev et al., 2017), reducing both native species richness and abundance (Saccone et al., 2010, Bottollier-Curtet et al., 2012). Therefore, *A. negundo* is a convenient subject for studying the penetration mechanisms of invasive alien plants into natural communities. It remains unclear how seed germination conditions related to propagation mechanisms. The outcomes of penetration depend on a number of factors, such as the generative maturity age, the spread of seeds, and their susceptibility to pathogens and others. They are important for predicting the ability to create propagule pressure in recipient communities (Colautti et al., 2006, Johnston et al., 2009; Dyderski & Jagodziński, 2018). Searching for optimal seed germination conditions needs to clarify the reproduction success of *A. negundo* over its original communities.

The seed germination of the Far Eastern maple *Acer ginnala* Maxim analyzed in comparison with seed germination of *A. negundo*. These two maple species are the most common maples in urban plantings in the Middle Ural (Mamaev & Dorofeeva, 2005). *A. ginnala* is an adventive alien decorative plant for Yekaterinburg (Tret'yakova, 2015). Single individuals planted throughout the city, but *A. ginnala* does not extend beyond plantations of trees and shrubs, despite annual fruiting. The only dense thickets of *A. ginnala* in Yekaterinburg is located in the Institute Botanic Garden (Ural Branch, Russian Academy of Sciences).

Both maple species are stable in culture, although they are slightly frostbitten during severe winters (Mamaev & Dorofeeva, 2005). Thus, *A. ginnala* is similar to *A. negundo*, but not an invasive species.

Many species of the genus *Acer* are of practical importance and are usually propagated by seeds. Therefore, some conditions for maple seed germination are known (Mamaev & Dorofeeva, 2005; Solarik et al., 2016). It was stated that the seeds of *A. negundo* do not have a dormancy (Mamaev & Dorofeeva, 2005) or have a shallow dormancy from 2–5 months (Aksenova, 1975) to 2 years (Möllerová, 2005; Kostina et al., 2017). At the same time, during the growing season, seeds of *A. negundo* may have an extended germination period (Kostina et al., 2017). Estimates of *A. negundo* seed germination vary in the ranges of 37–48% (Agishev, 2016), 36–80% (Khudonogova & Tyapaeva, 2019), 73–85% (Sofi et al, 2014). There are indications of 'high germination' (Kostina et al., 2017).

According to various sources and practical recommendations, cold stratification is necessary for the germination of *Acer* species. Its duration for *A. negundo* recommended for about 40 days and for other *Acer* species for a longer time (Rastorguev, 1960). There are no information in the introduction about morphological heterogeneity of *A. negundo* and *A. ginnala* seeds and its effect on seed germination. Our study provides the first analysis of seeds size, stratification type and cold period effect on seed germination of these species.

The aim of the work was to find answers to the questions: 1. How does *A. negundo* and *A. ginnala* seed germination depend on the period of cold stratification on the soil surface under snow? 2. Does *A. negundo* seed germination depend on the time of cold stratification in the air? 3. Is *A. negundo* and *A. ginnala* seed size related to the success of their germination?

MATERIALS AND METHODS

Study area

Yekaterinburg is a large industrial city in the Middle Urals with an area of 49.8 thousand hectares with a population of about 1.4 million inhabitants. Yekaterinburg is located in the southern taiga subzone. In the surrounding territories, pine forests of natural origin prevail on soddy podzolic soil and brown forest soil. The territory of Yekaterinburg is heavily polluted (Sturman, 2008) due to a large number of industrial enterprises and the high density of the motor transport network.

The climate is temperate continental, bordered by continental, with cold winters and warm summers. The average annual temperature is + 3 °C. The average annual rainfall is 542 mm. The average snow cover depth in February is 20–25 cm, and the average snow accumulation season is 160–180 days (Borisov, 1967).

Weather conditions

The weather conditions during stratification and seed germination obtained from the meteorological observing station (experimental area in the Institute Botanic Garden, Yekaterinburg, Sverdlovsk region, Russia) and presented in Table 1.

Table 1. The weather conditions of experimental period

Month and year	Snow cover depth on the last day of the month, cm	Air temperature (°C)		
		monthly average	maximum	minimum
October 2018	11	4.3	18.5	-5.7
November 2018	10	-5.5	3.3	-19.4
December 2018	14	-11.0	-0.9	-25.3
January 2019	21	-12.6	-1.6	-22.7
February 2019	39	-11.1	1.5	- 31.9
March 2019	16	-1.1	10.3	-12.1
April 2019	9	4.3	18.8	-10.4

Seed collection

Seeds of *A. negundo* collected from dense thicket (monospecific stands) in three locations in the city Yekaterinburg. Seeds of *A. ginnala* were collected in one habitat (the only dense thicket of this maple in the city). Seeds of each species in each location were collected during two tours: October 20, 2018 (tour I) and after 52 days on December 11, 2018 (tour II).

The dry double winged fruit of maples when ripe easy breaks up into two samaras. Each of samara is one seed with wing. They were removed frome the trees by hands, then dried and stored in linen bags in a cool room. The air drying was carried out for 5 days at room temperature (20 °C). Seeds from different species, habitats (locations) were stored, sown and analyzed separately.

Determination of field germination

Vegetation experiments with stratification and seed germination were carried out from November 2018 to May 2019. The field germination experiment was the main one. Before the experiment, seeds with obvious mechanical, fungal, or bacterial damage were rejected. Then the seeds were sorted into large and small by manual separation

(Figs 1, 2). The criterion for separation was the length of the winged seeds, the seeds were considered large: more than 35 mm for *A. negundo*; seeds larger than 15 mm for *A. ginnala*. Plastic boxes of 50×30×12 cm with a standard peat substrate were used for seed germination.

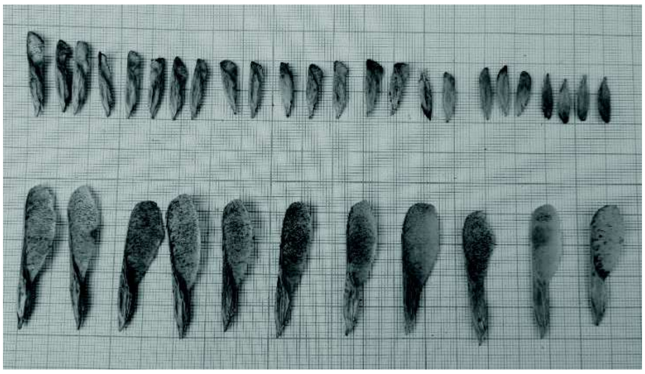

Figure 1. Seed heterogeneity of *Acer negundo*.

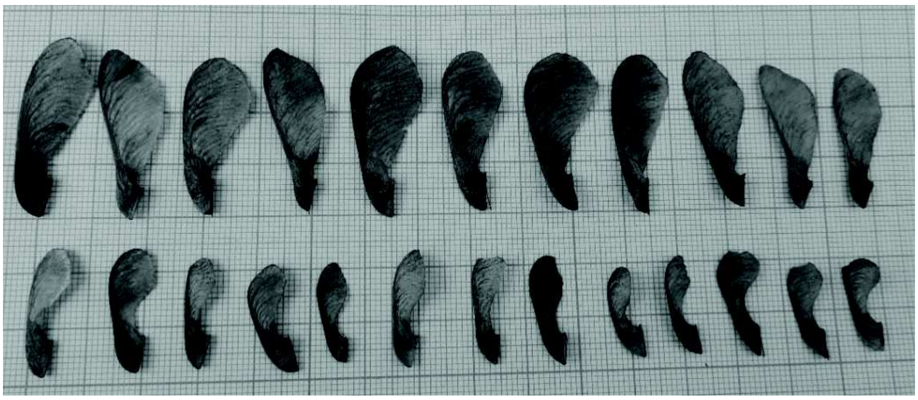

Figure 2. Seed heterogeneity of *Acer ginnala*.

On December 28, 2018, large and small seeds of *A. negundo* from each tour of collection were sown in boxes in isolation from each other. The seeds from different habitats were arranged systematically in the boxes. In each combination, 'seed size' × 'tour', a block of 5 boxes contained seeds from only one of the three habitats. In total 60 boxes, steps of 15 boxes of each variant: 15 boxes with small seeds from tour I, 15 boxes with small seeds from tour II, 15 boxes with large seeds from tour I, 15 boxes with large seeds from tour II. In each box 40 seeds were sown.

Also, large and small seeds of *A. ginnala* from each tour of collection were sown in boxes in isolation from each other (in total 20 boxes, steps of 5 boxes of each variant: 5 boxes with small seeds from tour I, 5 boxes with small seeds from tour II, 5 boxes with large seeds from tour I, 5 boxes with large seeds from tour II). In each box 40 seeds were sown.

Seeds germination was carried out in a greenhouse of Institute Botanic Garden, Ural Branch, Russian Academy of Sciences. Mean daily air temperatures during germination ranged from +18 to +23 °C. Boxes with seeds were exposed to the 10-a-clock lighting under OSRAM HPS Grow Lamps (photon flux density 150–220 µmol m^{-2} s^{-1}).

A single number of boxes with each sowing variant taken from individual habitats of *A. negundo* and *A. ginnala* was left in the greenhouse for germination without stratification under snow 12 boxes with *A. negundo* seeds, 4 boxes with *A. ginnala* seeds. Thus, the seeds in these boxes were not exposed to additional exposure to cold. The designation 'Str0' is a variant of the experiment without cold stratification under the snow.

The remaining 48 boxes with *A. negundo* seeds and 16 boxes with *A. ginnala* seeds were carried outside. Boxes were placed on the surface of the soil and covered with a layer of snow of the same height as it was in the surrounding area. Thus, the seeds in these boxes were exposed to cold - an experiment with cold stratification under the snow. From the 56 boxes, twelve boxes with *A. negundo* seeds and 4 boxes with *A. ginnala* seeds were placed in a greenhouse for seed germination on February 1 (designation 'Str1'), similarly on March 1 ('Str2'), April 1 ('Str3') and May 1 ('Str4').

Thus, the duration of cold stratification under the snow was: Str1 - 1 month, Str2 - 2 months, Str3 - 3 months, Str4 - 4 months. The number of germinated seeds was counted on the 20th day after sowing in the Str0 variant or on the 20th day after the boxes were placed in a greenhouse in the Str1 - Str4 variants.

Determination of viable seeds

On the 20th day of the germination experiment the viability of all the seeds was determined after determining germination. For this purpose, the seed peel of the unsprouted seeds was opened with a scalpel and it was determined whether the embryo was alive. The criterion of viability was the color of the embryo – cream or yellow-green without oil according to GOST 13056.7-93. Sprouted seeds were also considered viable.

Determination of laboratory germination

Laboratory experiments were carried out in May 2019. We performed laboratory measurements according to standard (GOST 13056.6-97) to compare with other authors results. Samples were germinated on filter paper with wicks dipped in water (daylight 300 lux, temperature from +18 to +23 °C). We used only the seeds from second lot of collection (tour II). We took the combined average sample of the seeds from three habitats of *A. negundo* and average sample of the seeds of *A. ginnala* from one habitat. Then the seeds were sorted into large and small by manual separation (Figs 1, 2). The seeds were not additionally treated by cold stratification under the snow. Large seeds were sown apart from small to compare laboratory and field germination. Each average sample had 200 seeds (100 large and 100 small). There were 4 sowing variants in total: for *A. negundo* large and small seeds; for *A. ginnala* large and small seeds. The number of seedlings was counted on the 25th day of germination.

Data analysis

The influence of all tested factors on field germination was evaluated by 4-way ANOVA. Factors in ANOVA in different combinations were: 1) the species of tree – *A. negundo* or *A. ginnala*; 2) seed collection tour – I or II; 3) the duration of cold stratification – Str0, Str1, Str2, Str3 or Str4; 4) seed size – large or small. Another analyzed source of variability was habitat (factor 'location'), i.e. the place where the seeds were collected. In ANOVA, viable and sprouted seed fractions were analyzed after arcsine transformation.

The figures and text show the untransformed values of viable and sprouted seeds. Designations in the text and in the table: *SD* – standard deviation; *dF* – the number of degrees of freedom; *F* – the value of the Fisher criterion; *P* – the significance level. All differences were considered statistically significant if $P < 0.05$.

RESULTS AND DISCUSSION

Germination

The species of the seeds, the duration of stratification on the soil surface and the size of the seeds significantly influenced the germination of the seeds individually and in some interactions (Table 2). The duration of stratification in the air did not affect seed germination either independently or in interactions with other factors (Table 2). Consequently, the presence of seeds on the tree branches in October – December (additional 52 days) did not affect their germination. In further analysis, the time of air stratification was not considered.

Table 2. The effect of species membership ('species'), duration of stratification in the air ('tour'), duration of cold stratification under snow ('stratification') and seed size ('seed size') on the germination and viability of seeds (*dF* – the number of degrees of freedom; *F* – the value of the Fisher criterion; *P* – the significance level). All differences were considered statistically significant if $P < 0.05$

Factors and interactions	*dF*	Germination		Viability	
		F	*P*	*F*	*P*
Species [1]	1	32.89	< 0.0001	3.87	0.0560
Tour [2]	1	1.86	0.1800	2.76	0.1046
Stratification [3]	4	51.40	< 0.0001	1.07	0.3829
Seed size [4]	1	53.53	< 0.0001	726.15	< 0.0001
[1] × [2]	1	0.05	0.8281	0.00	1.0000
[1] × [3]	4	10.48	< 0.0001	0.47	0.7573
[2] × [3]	4	0.62	0.6538	1.43	0.2409
[1] × [4]	1	11.59	0.0015	4.10	0.0497
[2] × [4]	1	0.33	0.5713	3.14	0.0841
[3] × [4]	4	15.44	< 0.0001	0.82	0.5207
[1] × [2] × [3]	4	0.42	0.7902	0.45	0.7720
[1] × [2] × [4]	1	0.61	0.4394	0.20	0.6582
[1] × [3] × [4]	4	1.98	0.1166	0.96	0.4426
[2] × [3] × [4]	4	0.47	0.7602	0.27	0.8932
[1] × [2] × [3] × [4]	4	0.07	0.9912	0.14	0.9659

Seed germination of two *Acer* species almost exponentially depended on the duration of their stratification under the snow (Fig. 3). In the absence of cold under snow stratification and with under snow cold stratification of 1 month, the seeds did not germinate. After 4 months of cold stratification under the snow, the germination rate differs: in *A. negundo* 12 ± 4% (small seeds) and 79 ± 7% (large seeds), in *A. ginnala* –

1 ± 2% (small seeds) and 18 ± 4% (large seeds). In both species, large seeds germinated at 7 to 18 times more intensively than small ones.

Habitat conditions did not affect seed germination (Fig. 3). In the 3-way ANOVA (factors: 'stratification', 'seed size', and 'location'), the differences associated with the factor 'location' were insignificant (F (2; 30) = 2.24; P = 0.1234). After 4 months of cold stratification under the snow, the range of germination average values in three habitats was small: at 73 to 83% for large seeds and at 10 to 14% for small seeds of *A. negundo*.

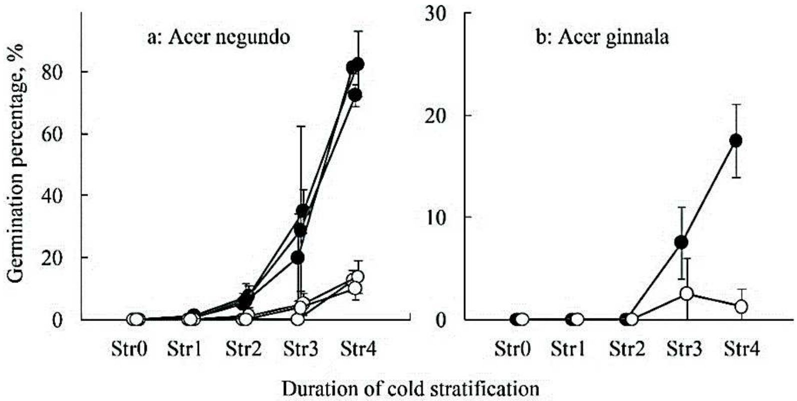

Figure 3. Germination (± SD) of large (●) and small (○) seeds of *Acer negundo* (a; data for three different habitats) and *Acer ginnala* (b) depending on the duration of cold stratification under the snow (Str0 - without stratifications; Str1 – Str4 – stratification 1–4 months, respectively). Attention should be paid to the difference in scale along the ordinate axis in Figures a and b. SD – standard deviation.

Viability

Viability was significantly related to the size of the seeds (Table 2) and was not related to other factors ('species', 'tour', 'stratification', 'seed size'). Seed viability also did not differ significantly between the two species of maples. Large seeds were more viable (78–93%), small seeds were less viable (5–30%) (Fig. 4). Viability depended neither on the time of seed collection (October or December) nor on the duration of cold stratification under the snow (Table 2).

The results confirmed that the duration of cold stratification under the snow is crucial for stimulating seeds germination of both *Acer* species. In the absence of cold stratification under snow or if its duration was at 1 to 2 months, the germination of *A. negundo* and *A. ginnala* seeds is limited. In such cases, no more than 10% of germinable seeds sprouted on the soil (Fig. 3).

Laboratory germination, which is determined without stratification, is also low in both types of maples. The average laboratory germination of *A. negundo* large seeds was 13%, small – 0%. The average laboratory germination of *A. ginnala* large seeds was 6%, small – 0%.

The average field germination of *A. negundo* seeds was about 50–70%. This value depends on the ratio of large/small seeds and it was 2/1 based on our assessment. This is close to other similar estimates, under which *A. negundo* seed germination ranges between 37–48% (Agishev, 2016), 36–80% (Khudonogova & Tyapaeva, 2019) and 73–85% (Sofi et al., 2014). The average germination of *A. negundo* and the ratio of large/small seeds in the climatic and environmental conditions of the Middle Ural and other regions need to clarify.

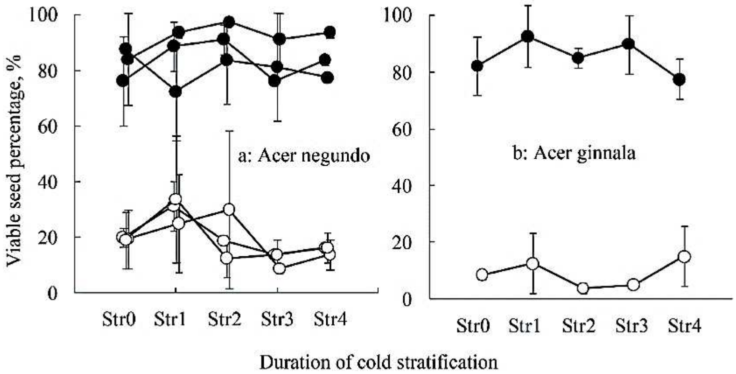

Figure 4. Viability (± SD) of large (●) and small (○) seeds of *Acer negundo* (a; data for three different habitats) and *Acer ginnala* (b) depending on the duration of cold stratification under snow (Str0 – without stratifications; Str1 – Str4 – stratification 1–4 months, respectively). SD – standard deviation.

The duration of cold stratification under the snow of about 4 months raises the level of the actual *A. negundo* seeds germination toward the optimal germination (Fig. 3). After such stratification, the proportion of germinated *A. negundo* seeds from seeds that are capable to germinate was 93% among large seeds and 80% among small ones.

The proportion of viable seeds in *A. ginnala* is the same as in *A. negundo*. But in *A. ginnala* case, even after cold stratification under snow for 4 months, no more than 22% of the seeds germinated (Fig. 3). Thus, *A. ginnala* seeds apparently require some other conditions for optimal germination.

Our estimates are generally consistent with existing knowledge on *A. negundo* and *A. ginnala* seed germination. It is known that the viability of *A. negundo* and *A. ginnala* seeds are close (Aksenova, 1975), as in our estimate. It is also known that the seeds of *A. ginnala* germinate much less vigorously (Khudonogova & Tyapaeva, 2019), as we found. Thus, our data confirm that the *A. negundo* ability to create propagule-pressure is higher than that of *A. ginnala*. This is consistent with its invasive status in the region. In particular, this is in agreement with the existence of a strong positive relationship between the adult plant number and young plant number of *A. negundo* in urban forest parks (Veselkin & Korzhinevskaya, 2018).

Our estimates point to the need for a long, up to 4 months, cold stratification under the snow for *A. negundo* seeds, while in the literature were recommended 30–40 days as enough time of stratification (Rastorguev, 1960; Aksenova, 1975). Seed germination of *A. negundo* and *A. ginnala* did not change after 52 days of additional stratification in the air during the cold season. This is consistent with the fact that the seeds of *A. negundo* that have fallen to the soil surface either sprout quickly or lose their germination rate quickly (Aksenova, 1975), but seeds held on branches retain germination ability to 2 years (Kostina et al., 2017). The opinion that the seeds of *A. negundo* do not have dormancy (Mamaev & Dorofeeva, 2005) has not been confirmed.

Our results showed that the seed size of *A. negundo* and *A. ginnala* is uniquely associated with germination. In other words, size is a convenient criterion for selecting quality seeds. Perhaps seed size affected germination due to the different stages of fruit maturity.

Seed size may reflect the fitness of the parent plants (Fenner, 1985; Silvertown, 1989). Large seeds of trees and herbs have usually higher germination in comparison with small ones (Gross, 1984; Shipley & Parent, 1991; Bursem & Miller, 2001; Deb & Sundriyal, 2017). One possible explanation of such success is the large seeds have more resources that provide greater survival (Harper, 1977; Fenner, 1985; Moles & Westoby, 2004). Seed size is the principal factor that affects the germination and seedling growth along with many internal and external factors (Moles & Westoby, 2004; Sahi et al., 2015).

The seed heterogeneity of *A. negundo* may be manifested for example, due to abiotic conditions of the habitat of mother trees (Erfmeier et al., 2011). But we did not study this phenomenon. We collected seeds in different habitats on the territory of a big city. These were both relatively vast areas of green space, and areas near residential buildings and highways. However, the seed germination of *A. negundo* did not differ significantly among seeds collected in the different habitats.

Our data allow us to raise some questions. Is the achieving of maximum germination after 4 months of cold stratification related to the synchronization of the *A. negundo* seeds ripening with the regional climate? How geographic variation in the optimal stratification period for *A. negundo* seeds differ in other regions?

The results of this study allow us to discuss the reasons for *A. negundo* distribution in Northern Eurasia. We assume that the absence of prolonged cold stratification under the snow can limit the distribution of harmful *A. negundo* in regions where the climate is hot. Probably global climate warming should reduce the *A. negundo* propagation activity and the ability to spread.

Our results can be used in the control of *A. negundo* invasion. Cold stratification under the snow affects the success of germination. Therefore, all ways to disturb this optimal condition should be effective to diminish seed germination.

CONCLUSIONS

We elucidated that *A. negundo* seeds germinate significantly greater than the *A. ginnala* seeds to improve understanding of invasive plants biological characteristics. This may be one of the explanations of the different status of these species in the region: *A. negundo* is invasive, actively spreading species; *A. ginnala* is alien, but not spreading plant. In other respects, our research is mainly monitoring. The results are consistent with previously known.

These are two conclusions: 1) the duration of cold stratification under the snow is crucial for the germination of seeds from *A. negundo* and *A. ginnala*; 2) the duration of cold stratification of seeds from *A. negundo* and *A. ginnala* in the air does not affect their germination. In practical terms, our data allow us to recommend the use seed size of *A. negundo* and *A. ginnala* as an effective predictor of their ability to germinate and it requires prolonged cold stratification under the snow to ensure their optimal germination.

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