Seed germination of woody and shrubby introduced species

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Abstract. The article presents the research results of seed germination of woody and shrubby introduced species. Depending on the depth of dormancy, the studied plant species were divided into 3 groups. The first group includes plants with the absence of dormancy or with a short dormancy; the seeds of these species do not need stratification. The second group includes plants whose seeds are in a state of a rather long intermediate physiological dormancy; they need cold stratification. The organic dormancy period of plant seeds of the second group is different – from 25 to 160 days. As a rule, these are plants with a well-formed seed bud. The physiological dormancy of the seed bud and the inhibitory effect of its covers can be an obstacle to its formation. The third group includes plants whose seeds may not germinate for several years. As a rule, the seeds of these plants are in a state of a deep physiological or morphophysiological dormancy; multi-stage stratification is recommended for their germination. The introduced species of the third group are the most difficult to study since their seeds can belong to different variants of deep dormancy, which combines the physiological immaturity of different structures of the seed bud with the incomplete morphological maturity and exogenous dormancy.

1. Introduction
Green spaces play an important role in the formation of a favorable ecological environment in urban areas. In the city of Irkutsk, there are about 6 m² of green space per person, with a norm of 11.2 m². The lack of green spaces can be replenished by cultivating woody and shrubby introduced species into the urban environment.

For greenspace expansion of the city of Irkutsk, mainly ornamental plants of the local flora are used – Betula pendula Roth, Larix sibirica Ledeb., Picea obovata Ledeb., Pinus sibirica Du Tour, Crataegus sanguinea Pall., Malus pallasiana (L.) Borkh., Sorbus sibirica Hedl., Viburnum opulus L. are less often planted. The greenspace monotonity of the city is slightly diluted with highly decorative introduced species Acer ginnala Maxim., Juglans mandshurica Maxim., Syringa josikaea Jacq. fil. ex Rchb., Populus alba L. et al. [1-3]. The introduction is considered successful if the plants do not lose their decorative properties, adapt to local conditions, go through all the stages of ontogenesis, form fruits and seeds.
Such characteristics as winter hardiness, frost resistance, qualitative signs of seeds, rhythm of seasonal development, and ability of plants to bear fruit are important for plants of Siberian regions. Evaluation of seed quality is determined by the mass of 1000 seeds, their germination, maturation of the seed bud and a number of other characteristics.

The purpose of the research was to determine the quality of seeds of woody and shrubby introduced species in the conditions of the city of Irkutsk. The research objectives were to determine the mass of 1000 seeds and to identify the germination of seeds of the studied plants species.

2. Methods and Materials
Woody and shrubby introduced species and a number of representatives of the indigenous flora was the research object. Collection of fruits and seeds was carried out from August to September. Seed quality was determined by the mass of 1000 seeds and their germination [4-10].

Seeds were germinated in the laboratory in Petri dishes. To stimulate growth, the seeds were treated with a solution of fundazol. They were also scarified by grinding with sand or using emery paper. Seed stratification was carried out in 2-4 stages: at the stage of thermal stratification, the seeds were kept at +15-20°C and at the stage of cold stratification they were kept at +5-10°C. The studies were performed in the 4-fold repetition (100 seeds of each species). The statistical processing of the experimental data was carried out according to the Dospehov method [11].

3. Results and Discussion
The climate of the city of Irkutsk is sharply continental, characterized by long dry winters and relatively warm summers with moderate precipitations. The average annual air temperature is -0.9°C. The coldest month is January (from -20-31°C to -50°C). The warmest month is July (+18-20°C to +36-37°C). The sum of active temperatures above 10°C is 1727°C. On the average, the growing season lasts 148 days [12, 13]. The average annual precipitation is about 420 mm, 77% of which comes from the warm period and 23% – from the cold one [14].

In the study area, light-gray forest soils of medium deep predominate. The humus content in the soil is 3-5%, the average content of mobile nitrogen is 20-40 mg/kg. The soils are characterized by a high degree of phosphorus availability (10-15 mg/100 g of soil) and an average degree of potassium availability (10-15 mg/100 g of soil). Their reaction is close to neutral (pH 5.6-6.0) [14].

Seed material germination is determined by the number of seeds germinating under certain conditions for a given period of time. Germination degree is due to the germination energy of seeds [7]. Seed material must meet the quality standard. Seed quality is estimated by the mass of 1000 seeds as well as by germination and dormancy depth [8, 9]. The Department of Dendrology of the Main Botanical Garden (Moscow, Russia) published materials on seed propagation of woody plants. The materials describe the main features that characterize the quality of seeds. Employees of Komarov Botanical Institute published a book of reference on the germination of dormant seeds. It includes information on techniques that promote seed germination of 3,000 introduced species [15].

Seed germination is an integral indicator highlighting seed quality. By the mass of 1000 seeds, introduced species differ significantly. Small-seeded plants, the mass of which in the conditions of the city of Irkutsk is hundredths of a gram, include Betula pendula (0.23), Dasiphora fruticosa (L.) Rydb. (0.25 g), Physocarpus opulifolius (L.) Maxim. (0.75 g), the genus Spiraea L. (0.06-0.09 g). Large seeds are characteristic of Euonymus europaeus L. (32.3 g), Pinus sibirica (227.12 g), Prunus virginiana L. (68.8 g), Viburnum opulus L. (45.8 g), etc. (Table. 1, 2, 3).

The results of studies on the mass of 1000 seeds of woody and shrubby species collected in Irkutsk and Moscow indicate that the mass of seeds of the same species is about the same in 48.4% of the plants: Dasiphora fruticosa (0.25 g), Spiraea media Schmidt (0.06 g), Tilia cordata Mill. (28 g), etc. Seeds of some Moscow introduced species are of a larger mass, for example, Acer negundo L. (41 g – Irkutsk, 50 g – Moscow), Cotoneaster lucidus Schlecht. (18.5 g – Irkutsk, 23 g – Moscow), Crataegus sanguinea (15.4 g – Irkutsk, 24 g – Moscow), Pinus sibirica (227 g – Irkutsk, 250 g – Moscow), Swida alba (L.) Opiz (19.8 g – Irkutsk, 26 g – Moscow), Viburnum lantana L. (29.8 g – Irkutsk, 34 g –
Moscow). 1000 seeds of Moscow introduced species have a slightly higher mass: *Berberis thunbergii* DC. (9.2 g – Irkutsk, 9.6 g – Moscow), *Euonymus europaeus* (32.3 g – Irkutsk, 32.5 g – Moscow), etc., *Lonicera tatarica* L. (3.2 g – Irkutsk, 3.3 g – Moscow), *Picea pungens* Engelm. (2.8 g – Irkutsk, 3.0 g – Moscow), *Phyllocladus amurense* Rupr. (12 g – Irkutsk, 12.5 g – Moscow), *Physocarpus opulifolius* (1.2 g – Irkutsk, 1.5 g – Moscow), *Thuja occidentalis* L. (1.2 g – Irkutsk, 1.5 g – Moscow).

The habitats of these plants are mainly European, North American or Japanese-Chinese.

Introduced species with a large seed mass in Irkutsk make up about 5%; their ranges are mainly Siberian, Far Eastern and Euro-Siberian. These species include: *Euonymus maackii* Rupr. (19.8 g – Irkutsk, 12 g – Moscow), *Malus pallasiana* (6.8 g – Irkutsk, 4.6 g – Moscow), *Picea obovata* (6.2 g – Irkutsk, 6 g – Moscow), *Prunus virginiana* (68.8 g – Irkutsk, 54 g – Moscow), *Sorbus sibirica* (2.8 g – Irkutsk, 2.2 g – Moscow), *Viburnum opulus* (45.8 g – Irkutsk, 40 g – Moscow).

The mass of 1000 seeds is a species-specific characteristic. For instance, in the genus *Spiraea*, the reaction norm by mass ranges from 0.06 to 0.09 g, in the genus *Berberis* L. – from 9.2 to 10 g, in the genus *Euonymus* L. – from 18.7 to 32.5 g.

The studied plant species, depending on the depth of dormancy of their seeds, were divided into 3 groups. The first group included plants with the absence of dormancy or with a short dormancy (Table 1). Plant seeds of this group are characterized by a morphological dormancy that lasts from several days to several months. Seed germination begins only after the final formation of the seed bud. This group also included some plants, seeds of which are characterized by a shallow physiological dormancy (from several days to 5-12 months or more). For example, seeds of *Betula pendula* and *Pinus sibirica*, which are characterized by a shallow dormancy, become photosensitive under certain temperature conditions. This feature should be taken into account for disturbance of a shallow dormancy [7].

**Table 1.** Woody and shrubby introduced species with the absence of dormancy or with a short dormancy; the seeds do not need stratification.

| Species, variety | Mass of 1000 seeds, g | Germination, % | Scarification a, b |
|------------------|-----------------------|----------------|-------------------|
| *Acer ginnala* Maxim. | 25.0 | 51.0±0.28 | + |
| *Betula pendula* Roth | 23.50±0.74 | 80.06±0.30 | - |
| *Dasiphora fruticosa* (L.) Rydb. | 0.25 | 42.02±0.30 | + |
| *Euonymus nanus* M. Bieb. | 0.23±0.03 | 54.18±0.40 | + |
| *Picea obovata* Ledeb. | 0.25±0.02 | 69.15±0.41 | - |
| *Picea pungens* Engelm. | 16.80±0.58 | 80.11±0.73 | - |
| *Pinus sylvestris* L. | 6.20±0.55 | 98.00±0.25 | - |
| *Populus alba* L. | 6.20±0.55 | 85.49±0.32 | - |
| *Spiraea bumalda* Burv. | 0.76±0.20 | 98.01±0.72 | - |
| *Spiraea japonica* L. | 0.07±0.008 | 98.02±0.65 | - |
| *Spiraea margaritae* Zab. | 0.07±0.003 | 98.25±0.68 | - |
| *Spiraea media* Schmidt | 0.09±0.004 | 95.16±0.44 | - |
| *Spiraea vanhouttei* (Briot) Zab. | 0.07±0.002 | 94.19±0.39 | - |
| *Thuja occidentalis* L. | 1.20±0.22 | 42.25±0.55 | + |
| *Ulmus laevis* L. | 5.60±0.46 | 23.50±0.37 | + |

a + – seeds that require scarification; b – seeds that do not require scarification

All the introduced species of the first group do not require stratification, or the disturbance of a shallow dormancy occurs under the influence of short-term cooling (within a few hours, days, or weeks). The seeds of some of them, for example, *Acer ginnala*, *Dasiphora fruticosa*, *Euonymus nanus* M. Bieb., *Thuja occidentalis*, *Ulmus laevis* L., have a waterproof, thick cover and germinate faster after scarification.
Seed germination of plants of this group ranges from 23.5% (Ulmus laevis) to 80.06-98.25% (Betula pendula, Picea pungens Engelm., Pinus sibirica, S. bumalda Burv., Spiraea margaritae Zab., Spiraea media, Spiraea japonica L., Spiraea vanhouttei (Briot) Zab.).

Populus alba is characterized by the complete absence of dormancy. It takes only 10-12 days from the moment of seed ripening in Populus alba (when capsule glumes open) and until the complete germination. Freshly harvested Populus alba seeds germinate in 2-4 days. Seed germination of this species is 85.49%.

The second group includes plants whose seeds are in a state of a rather long intermediate physiological dormancy (Table 2).

Table 2. Woody and shrubby introduced species with intermediate physiological dormancy of seeds; cold stratification is required for seed germination.

| Species, variety | Mass of 1000 seeds, g | Germination, % | Stratification, days |
|------------------|-----------------------|----------------|---------------------|
|                  | Moscow | Irkutsk | With stratification | Without stratification | Thermal, 15-20°C | Cold, 5-10°C |
| Berberis amurensis Rupr. | 10  | 9.4±0.8 | 73.40 ± 0.30 | 2.06 ± 0.31 | - | 125 |
| Berberis sibrica Pall. | 10 | 9.4±0.6 | 72.01 ± 0.42 | 69.32 ± 0.27 | - | 85 |
| Berberis thunbergii DC. | 9.6 | 9.2±0.3 | 65.11 ± 0.58 | 40.08 ± 0.25 | - | 60 |
| Lonicera tatarica (L.) Borkh. | 3.3 | 3.2±0.5 | 49.33 ± 0.29 | 0 | 15 | 30 |
| Berberis thunbergii DC. | 4.6 | 6.8±0.8 | 48.42 ± 0.15 | 0 | 15-20 | 30-50 |
| Physocarpus opulifolius (L.) Maxim. | 12.13 | 12.0±0.1 | 86.23 ± 0.49 | 2.01 ± 0.40 | - | 25-30 |
| Prunus virginiana L. | 1.1 | 0.75±0.05 | 74.05 ± 0.33 | 72.50 ± 0.21 | - | 25 |
| Physocarpus opulifolius Diabolo | 1.5 | 1.2±0.02 | 69.41 ± 0.38 | - | - | 25 |
| Swida alba (L.) Opiz | 54 | 68.8±0.5 | 54.12 ± 0.25 | 2.10 ± 0.50 | 30 | 50-60 |
| Tilia cordata Mill. | 26 | 19.8±0.2 | 87.52 ± 0.56 | 62.30 ± 0.40 | - | 50 |
| Viburnum lantana L. | 28 | 28.0±0.4 | 52.60 ± 0.33 | 0 | - | 160 |

Cold stratification is necessary for plant seeds of this group. The organic dormancy period for plant seeds of this group varies from 25 to 160 days (Table 2). As a rule, these are plants with a well-formed seed bud. The physiological dormancy of the seed bud and the inhibitory effect of its covers can sometimes be an obstacle to its formation. As a result of cold stratification, seeds of some plants grow faster: Physocarpus opulifolius (L.) Maxim (25 days), Phyllodendron amurense (25-30 days), Viburnum lantana (30 days). A longer dormancy period was found in Berberis amurensis Rupr. (125 days) and Tilia cordata Mill. (160 days). The combination of thermal and cold stratification was
studied using the example of Lonicera tatarica, Malus pallasiana and Prunus virginiana, the duration of seed germination of which range from 45 to 85 days.

Seed germination of plants of the second group is different — from 0-72.5% in unstratified to 30–87.5% in stratified seeds. The seeds of Lonicera tatarica, Malus pallasiana, Tilia cordata, and Viburnum lantana do not germinate without the use of stratification techniques. As a result of cold stratification, seed germination is significantly increased in Berberis amurensis (from 2 to 73.4%), Phyllodendron amurense (from 2 to 86.2%), and Prunus virginiana (from 2 to 54%).

According to M.G. Nikolaeva [7], the seeds of Physocarpus opulifolius are recommended to be stratified at a temperature slightly below +10°C for 2 months. According to our data, seed germination of Physocarpus opulifolius is 69.41-72.5% in the case of stratification of the seeds for 25 days. They can germinate at room temperature, but in this case the sprouting process is very long.

The third group includes plants whose seeds may not germinate for a long time — 1-4 years. As a rule, their seeds are in a state of deep physiological or morphophysiological dormancy. Introduced species of the third group are the most difficult to study since their seeds can belong to different variants of very deep dormancy when the physiological immaturity of different structures of the seed bud is combined with the incomplete morphological maturity and exogenous dormancy [7]. These plants include Cotoneaster lucidus Schlecht., Crataegus sanguinea, Euonymous europaeus, Euonymous maackii, Euonymus verrucosus Scop., Pinus sibirica, Sorbus sibirica, Viburnum opulus.

For the plant seeds of the third group, multi-stage stratification was studied. The research results showed that in some cases, the techniques of the four-stage stratification were not effective. For example, the combination of scarification and stratification did not affect seeds germination of Crataegus sanguinea, Pinus sibirica, and Viburnum opulus; their seeds did not germinate. The seed buds of Viburnum opulus were in a torpedo stage, while all the elements of the future seed bud were in a morphologically unripe state; the plant root increased to 1 mm in the first stage of thermal stratification and no visible changes occurred in the first stage of cold stratification. The second thermal stratification led to the growth of the hypocotyl to 2 mm; the root also increased and was near the micropyle, but it did not go beyond the seed. To clarify the reasons that prevent seed germination, further detailed study of the embryogenesis of the studied species is necessary.

Seed germination of Euonymus verrucosus and Euonymus maackii, subjected to the four-stage stratification was 39.12 and 53.60%, respectively (within 250-270 days). For seed germination of Euonymus europaeus (48.05%) and Cotoneaster lucidus (44.26%), stratification for 300-310 days was sufficient. The seeds of Sorbus sibirica germinated after the two-stage stratification (within 210-240 days); their germination rate was 58.02% (Table 3).

Long-term germination of seeds for several years and their deep dormancy play an important role and contribute to the preservation of the species, but this creates certain difficulties for breeders and landscapers and encourages them to find ways to solve these difficulties. In most cases, agrotechnical methods of scarification and stratification of seeds contribute to more simultaneous and early seed germination. However, as the studies have shown, they are effective not in all cases. There is a lot of contradictory information in the literature about the duration of stratification and the mode of its implementation. This information should be thought over by breeders and introducers.

4. Conclusion
1. The mass of 1000 seeds and the germination is determined in 36 species and varieties of plants. The mass of 1000 seeds of the studied plants ranges from hundredths of a gram (the genus Spiraea L. (0.06-0.09 g)) to 227.12 g (Pinus sibirica). Some coniferous tree seeds are characterized by high germinability (Pinus sylvestris – 98%, Picea pungens – 80.11%). The same situation is observed in some deciduous plants (Spiraea bumalda – 98.01%, Spiraea japonica – 98.02%, Spiraea margaritae – 98.25%, Populus alba – 85.49%, Berberis amurensis – 73.4%, Physocarpus opulifolius – 74.05%, Swida alba – 87.52%, etc.). Low germinability was found in Ulmus laevis (23.5%).
Table 3. Woody and shrubby introduced species with a deep physiological or morphophysiological period of seed dormancy; 2-4 stage stratification is required for seed germination.

| Species, variety | Mass of 1000 seeds, g | Germination, % | Stratification, days |
|------------------|------------------------|----------------|---------------------|
|                  | Moscow | Irkutsk | t, 5-10°C | t, 15-20°C | t, 5-10°C | t, 15-20°C |
| **Acer negundo**  | 50 | 41.04±0.77 | 68.30±0.51 | 50-60 | 20 | 30 | 10 |
| L. Cotoneaster lucidus Schlecht. | 23 | 18.50±0.32 | 44.26±0.25 | 150 | 30 | 120 | - |
| Crataegus sanguinea Pall. | 24 | 15.45±0.15 | - | 365 | 60-90 | 90 | - |
| Euonymous europaeus L. | 32.5 | 32.33±0.86 | 48.05±0.82 | 160 | 30 | 120 | - |
| Euonymous maackii Rupr. | 12 | 19.81±0.52 | 53.60±0.30 | 90 | 30 | 130 | 20 |
| Euonymous verrucosus Scop. | 18-26 | 18.70±0.20 | 39.12±0.64 | 150 | 30 | 30 | 40 |
| Pinus sibirica Du Tour | 250 | 227.12±0.51 | - | 150 | 15 | 30 | - |
| Sorbus sibirica Hedl. | 2.2 | 2.82±0.59 | 58.02±0.20 | - | 60-90 | 150 | - |
| Viburnum opulus L. | 40 | 45.84±0.60 | - | - | 30 | 120 | 40 |

2. The seeds of Acer ginnala, Betula pendula, Dasiphora fruticosa, Euonymus nanus, Picea obovata, Picea pungens, Pinus sylvestris, Populus alba, Spiraea bumalda, Spiraea japonica, Spiraea margaritae, Spiraea media, Spiraea vanhoueit, Thuja occidentalis, and Ulmus laevis do not need stratification. The dormancy period of seeds of these species is from several days to 5-12 months. The seeds of Acer ginnala, Dasiphora fruticosa, Euonymus nanus, Thuja occidentalis, and Ulmus laevis have a waterproof, thick cover and germinate faster after scarification. Seed germination of plants of this group is different – from the complete absence of dormancy (in Populus alba) to 80.06-98.25% (in Betula pendula, Picea pungens, Pinus sibirica, Spiraea bumalda, Spiraea margaritae, Spiraea media, Spiraea japonica, Spiraea vanhoueit).

Cold stratification is necessary for the seeds of Berberis amurensis, Berberis sibirica, Berberis thunbergii, Lonicera tatarica, Malus pallasiana, Physocarpus opulifolius, Prunus virginiana, Swida alba, Tilia cordata, Viburnum lantana. The organic dormancy period of the seeds of these plants varies from 25 to 160 days. As a result of cold stratification, seed germination significantly increases in Berberis amurensis (from 2 to 73.4%), Physocarpus opulifolius (from 2 to 86.2%), and Prunus virginiana (from 2 to 54%).

For the seeds of Acer negundo, Cotoneaster lucidus, Crataegus sanguinea, Euonymus europaeus, Euonymous maackii, Euonymous verrucosus, Pinus sibirica, Sorbus sibirica, Viburnum opulus with the multi-year dormancy period, the multi-stage stratification is recommended (seed germination was 39.12 – 68.30 %). In some cases, according to the research results, the techniques of the four-stage stratification are not effective. For instance, the seeds of Crataegus sanguinea, Pinus sibirica and Viburnum opulus did not germinate.

3. Seeds of 33 introduced species studied can be used for greenspace expansion of miniparks and parks of Irkutsk.

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