EFFECTS OF EARLY CONE COLLECTION AND CONE STORAGE ON GERMINATION OF PINUS SYLVESTRIS L. SEEDS

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Abstract

This experiment was conducted to determine the effects of the date of cone collection and of cone storage on germination characteristics of Scots pine seeds. Cones were collected on six occasions between September and November, 2000, from two altitudes in Bolu, Turkey, and stored at +5 °C for 1 month-1 year to test possible effects of after-ripening on seed germination. The low level of cone moisture content (21-23 %) was reached in late November. Seed moisture content ranged from 21 to 29 % during the month of September and October, and fell slowly to 14-15 % in December. Germination parameters were affected by the date of cone collection, and the cone and seed maturity improved up to 2nd November. Germination percent and rate were also greatly affected by germination condition. The seeds collected in September and October germinated poorly in darkness at 10 and 20 °C. If collection occurred as early as late September and stored at +5 °C for at least one month before extraction, cones gave seed with the same good properties as the seed from cones which had ripened under natural conditions on the trees. Following appropriate handling, germinability of autumn-harvested seed was not weakened, even after 1-year storage.

Key words: Pinus sylvestris L., germination, cone storage, light, temperature

INTRODUCTION

Pinus sylvestris L. is widely distributed in Turkey and being in mixed and pure stands. It makes up 5.5 % of total forest area and occupies about 738 000 ha in Turkey (OGM, 1980). Its altitudinal range is from sea level on the coastal belt of the Black Sea to about 2700 m in the northeastern Turkey and most Scots pine forms more or less close stands between 1250-2000 m a. s. l. (Kayacik, 1963; Coode, Cullen, 1965; Elicin, 1972).

A seed’s ability to germinate is determined largely by its degree of maturity. Anatomically or physiologically immature seeds germinate slowly even in favorable conditions. Thus, the effective forest tree procurement requires cone collections at the time of seed maturation.
Anatomically immature Scots pine seeds are morphologically dormant. Also anatomically mature Scots pine seeds collected in the autumn, prior to natural seed fall, sometimes exhibits physiological immaturity or dormancy with a low rate of germination process (Remrod, Alfjorden, 1973). One way of improving the technical quality of seeds from seed stands is to artificial ripening, i.e., the cones are collected when the seeds are still immature and are then allowed to ripen under more favourable conditions than in nature. Several investigations on artificial ripening of Scots pine seeds have been made in Sweden and Finland (Asplund et al., 1973; Kardell, 1973; Remrod, Alfjorden, 1973; Sahlen, Bergström, 1993). Generally, storage of cones at low temperatures (0-10 °C) has been more beneficial than storage at high temperature. Both anatomical embryo development and an increase in germinability have been observed in Scots pine after storage of cones (Kardell, 1974). Sahlen, Bergström (1993) stated that not only temperature but also light, cone moisture content and ripening time had an effect on seed quality after artificial ripening on Scots pine seeds.

Scots pine has an important place in artificial regeneration in Turkey but there are many difficulties in cone collection from seed stands in late autumn and winter since most Scots pine seed stands are found between 1400-2000 m. It is of great importance to be able to start the cone collection early in the autumn so as to avoid the snow-hinder and to use the shortening working-days better.

The objective of this investigation was to analyze the germination characteristics of autumn-collected Scots pine seeds and to determine the effect of cone storage on Scots pine seeds collected at different stages of maturity.

MATERIALS AND METHODS

Scots pine cones were collected during the period September-December 2000 from two natural stands in Bolu, a locality in the northwestern Turkey at 40 °36' N, 31 °35' E, and 1370 and 1580 m a.s.l. The collection dates were: September 11, 30, October 15, November 2, 22, and December 14. At each time of collection cones were sampled from 5 trees (average height 20 m and age 80-100 years). The collection was spread all over the crown as far as possible and for the cone collection, the same trees were used every time. The cones were put into double plastic bags, well-sealed, the air having been sucked out of the inner one. The samples were immediately brought to the laboratory for analysis. On each collection, the sample material was divided into subplots. Each subplot was randomly allocated for germination test after the cones were kept in bags of nylon net in a cold room at 5±1 °C. For 1 month to one year from time of collection to test possible effects of cone storage on seed germination.

Cone moisture content was determined using the oven-drying method (105 °C, 16 h) for 5 cones at the time of collection, and once a month during storing. A sample of 4×50 seeds was taken by the cutting of cones in order to determine seed-water content. This was done on a fresh weight basis by weighing the samples before and after drying for 16 h at 85 °C.

The cones were always manually cut into pieces and the seeds were extracted by hand. The germination percentage, germination rate and germination value of filled seeds extracted directly after the cone collection from two elevations and after storage of cones for 1 month to one year were determined in 4 replicates of 50 seeds at a constant 20±0.5 °C with a 24 h photoperiod and light intensity of 1000 lx. The germination percentage and germination rate were also found in freshly harvested seeds from 1580 m at 10 and 20 °C and different photoperiods (0, 8, and 24 h light).

Germination counts were recorded every day for 21 days and a seed was considered to have germinated when the length of the radicle reached at least the length of the seed itself. Germination occurred in glass Petri dishes on filter paper in germination cabinets. After 21 test days, the percentage of empty and viable seeds among those ungerminated was determined by a cutting test. Seeds displaying abnormal radicles were excluded from germination counts. The number of empty seeds was calculated at the end of each germination trial and the results were expressed in terms of percentage of full seeds.

The germination data were calculated and expressed as germination capacity (GP), the percentage of seeds that had germinated normally at the end of the test and peak value (PV), an index of germination speed (Czabator, 1962); and germination value (GV), which combines germination speed and capacity into a single value (Djavanshir, Pourbeik, 1976).

Percentages were transformed to arcsin p 1/2 for statistical analysis. Results were analyzed by factorial analysis, and the means were compared with Duncan tests.

RESULTS

Analysis of variance showed significant differences between two localities and the dates of cone collection (P<0.01) (Table 1). The GP were significantly affected by the dates collected in September 30 and were the lowest for seeds collected in September 11. Later, the GP increased to more than 90% by November 2 in two localities.

The PV and GV of seeds were also significantly affected by the date of cone collection, and in two localities GV and PV were significantly affected at September 30. They reached to highest value in November and there were not significant differences among the dates of cone collection in November and December.

The GP, GV and PV were slightly higher for seeds collected in September from 1370 m than seeds from 1570 m. For seeds from later collections, there were no significant differences between two elevations.

The GP, PV and GV of filled seeds extracted directly after the cone collection and after storage of the cones for 1-4 months were shown in Table 2. Storing seeds for 1-4 months enhanced GP and PV of seeds from two collections in September 11. For longer durations of storing, the GP and PV were increased to more than 80% and 9.0, respectively. For seeds from later collections (September 30-December 14), the GP and PV increased considerably during even 1 month storing and were not changed after 2-
Table 1
Effects of cone collection dates and altitudes on germination of Scots pine seeds collected between 11 September and 14 December 2000.

<table>
<thead>
<tr>
<th>Cone collection date</th>
<th>Germination (%)</th>
<th>Peak value (PV)</th>
<th>Germination value (GV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1370 m</td>
<td>1580 m</td>
<td>1370 m</td>
</tr>
<tr>
<td>11 Sep.</td>
<td>39.5 Aa</td>
<td>34.5 Ba</td>
<td>2.96 Aa</td>
</tr>
<tr>
<td>30 Sep.</td>
<td>65.0 Ab</td>
<td>58.5 Bb</td>
<td>4.08 Aab</td>
</tr>
<tr>
<td>15 Oct.</td>
<td>84.0 Acd</td>
<td>81.5 Ac</td>
<td>6.15 Ab</td>
</tr>
<tr>
<td>2 Nov.</td>
<td>93.0 Ad</td>
<td>90.0 Ad</td>
<td>10.86 Ac</td>
</tr>
<tr>
<td>22 Nov.</td>
<td>96.5 Ad</td>
<td>94.5 Ad</td>
<td>11.50 Ac</td>
</tr>
<tr>
<td>14 Dec.</td>
<td>94.0 Ad</td>
<td>95.5 Ad</td>
<td>12.17 Ac</td>
</tr>
</tbody>
</table>

1 Values in the row followed by the same capital initial(s) are not significantly different (P<0.01)
2 Values in the same column followed by the same initial(s) are not significantly different at (P<0.01)

3 months cone storage. Storing the cones eliminates the differences between two elevations with regards to germination parameters. After storing the cones for 1 year, GP and PV of seeds from two elevations were still high (Table 2). The lowest GP and PV at the end of 1-year were found in seeds collected September 11. The seeds collected in September from the elevation of 1580 m didn’t germinate in darkness at 10°C (Table 3). The seeds collected in September and October germinated poorly in darkness at both temperatures. In the late-November and December collections, GP of seeds in darkness were around 30% at 10°C, and more than 80% at 20°C. For 8-h photoperiods, GP ranged from 0 to approximately 65% at 10°C, and from 18.5 to 91.0% at 20°C. For 24-h photoperiods, GP were the lowest in 11 September and the highest in November and December collections at both germination temperatures. In all collections, PV was also affected by photoperiods and found the lowest in darkness at both temperature regimes (Table 4). The PV was the highest in 22 November at 24-h photoperiods.

The natural development of the water content of the cones is shown in Fig. 1. The water content lay almost above 50% in September 11. Up to the 15th October the values fell slowly in cones collected from 1580 m, and thereafter very rapidly. In cones collected from 1370 m, the values fell rapidly in October. The low level of cone moisture content (21-23%) was reached at late November. Seed moisture content ranged between 21 and 29% during the month of September and October, and fell slowly to 14-15% in December (Fig. 2).

**DISCUSSION**

Concerning the handling of harvested cones and seeds, the process of ripening and changes in cone and seed moisture content is of basic importance. While the externally observable process of growth is finished moisture content characterizes well the ripening process inside the cone. According to Remund, Alfeldt (1973), the seed of Scots pine seems to be ripe at a water content of the cones of 40-45%, which is higher than the results reported in this study. Matyas (1973) stated that the moisture content...
Table 3
Germination percentage of seeds collected at 1580 m and incubated at two different temperatures and three photoperiods

<table>
<thead>
<tr>
<th>Collection Date</th>
<th>Germination Temperature (°C)</th>
<th>Photoperiod (h)</th>
<th>Germination %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10±1</td>
<td>20±1</td>
<td></td>
</tr>
<tr>
<td>1 Sep</td>
<td>0.0</td>
<td>8</td>
<td>0.0</td>
</tr>
<tr>
<td>0 Sep</td>
<td>0.0</td>
<td>8</td>
<td>2.5</td>
</tr>
<tr>
<td>5 Oct</td>
<td>14.0</td>
<td>24</td>
<td>81.5</td>
</tr>
<tr>
<td>Nov</td>
<td>32.5</td>
<td>75.0</td>
<td>91.0</td>
</tr>
<tr>
<td>4 Dec</td>
<td>32.0</td>
<td>75.0</td>
<td>95.5</td>
</tr>
</tbody>
</table>

Table 4
Peak value (PV) of seeds collected at 1580 m and incubated at two different temperatures and three photoperiods

<table>
<thead>
<tr>
<th>Collection Date</th>
<th>Germination Temperature (°C)</th>
<th>Photoperiod (h)</th>
<th>Peak value (PV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10±1</td>
<td>20±1</td>
<td></td>
</tr>
<tr>
<td>Sep</td>
<td>0.0</td>
<td>8</td>
<td>0.36</td>
</tr>
<tr>
<td>1 Sep</td>
<td>0.0</td>
<td>8</td>
<td>1.69</td>
</tr>
<tr>
<td>5 Oct</td>
<td>0.1</td>
<td>24</td>
<td>3.33</td>
</tr>
<tr>
<td>Nov</td>
<td>0.68</td>
<td>3.40</td>
<td>5.31</td>
</tr>
<tr>
<td>4 Dec</td>
<td>1.71</td>
<td>3.57</td>
<td>10.36</td>
</tr>
</tbody>
</table>

The content of Scots pine cones decreased only slightly until October and fell suddenly from 80 to 20-30% by the end of December, and the low level of cone moisture content (20-25%) was reached during the month of December. In the present study, cone moisture content was below 30% in November and December and the low level of seed moisture content (15-23%) was reached during the months of late November and December.

The effect of incubation temperature was obvious; seeds germinated more slowly at 10°C than at 20°C. The GP and PV were low in darkness but enhanced at both 8-hour and 24-hour photoperiods. The GP and PV were, however, gradually enhanced during autumn so that seeds collected in November and December were able to germinate readily even at +10°C. Light requirement for germination interacted with cone-collection date. As autumn proceeded, a greater proportion of seeds were able to germinate in darkness.

Remrod, Aljforden (1973) stated that Scots pine seed in Sweden seems to be fully developed about 15 October and storage of the cones at +10°C during at least one month before seed extraction seems to allow cone collection one month earlier. In the present study, the GP were significantly affected at seeds collected September 30 and were the lowest for seeds collected in September 11. Later, the GP increased to more than 90% by November in two altitudes. PV and GV of seeds were also significantly affected at September 30 in two altitudes. They reached to highest value in November and there were no significant differences among the dates of cone collection in November and December.

Cones collected on late September and stored at +5°C for at least 1 month before seed extraction, gave seed with the same good properties as the seed from cones ripened under natural conditions on the trees in this study. According to Nygren (1987) Scots pine seed seems to anatomically fully develop in the beginning of September and physiological maturity was attained around mid-October for seeds from different localities (Nygren, 1987; Sahlen, Wiklund, 1995), which corresponds with the results reported here.

The elevation difference of 200 m has an influence on the seed germination percent and germination rate at the samples of September but this effect had disappeared in later collections. Also storage of seeds collected in September at least one month eliminates this difference. According to Boyidak (1981), the elevation difference of 350 m has an influence on the seed germination percentages of Scots pine if cones were collected during August and September. The attainment of anatomically maturity has also been shown to vary considerably between individual trees in Scots pine (Matyas, 1973; Remrod, Aljforden, 1973; Tilki, 2002). It can be concluded that microclimatic conditions can affect seed development and germination.

In the present study, autumn harvested Scots pine seeds kept their high germination percent and germination rate for one year when they stored at 5°C. According to Boyidak (1981) Scots pine seeds collected after 15th September nearly kept their higher germination capacity and rate at the end of 5-5.5 years cold storage, and Boyidak (1984) also found that Scots pine seeds kept their germination capacity at the
end of 11 years cold storage. However, long term storage (3 years) of early harvested Scots pine seeds was not recommended in the study done by Matyas (1973).

On the basis of the germination percent, germination rate, germination value and moisture content studied in this investigation, the seed seems to be fully developed in late October. Storage of the cones at +5 °C during at least one month before the seed extraction seems to allow cone collection about one month earlier. The seeds of Scots pine collected in mid-September could be stored for one year without any risk for low germination percent and germination rate.

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