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ACCELERATED AGING TO EVALUATE SEED VIGOUR IN EASTERN WHITE PINE (*PINUS STROBUS*) SEED*

O. Singh

Himalayan Forest Research Institute, Shimla - 171009, Himachal Pradesh, India

&

F. T. Bonner

USDA Forest Service, Southern Research Station, Starkville, MS 39759, USA

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SINGH, O. & BONNER, F. T. 2001. Accelerated aging to evaluate seed vigour in eastern white pine (*Pinus strobus*) seed. Seeds of eastern white pine (*Pinus strobus*) were subjected to accelerated aging for evaluating seed vigour in 100% relative humidity at 39, 41, and 45 °C for 24, 48, 72, 96, and 120 h. Germination and leachate conductivity before and after aging were measured. All aging treatments decreased germination and seed vigour with seed death after 72 and 48 h of aging at 41 and 45 °C respectively. The relationship of leachate conductivity to germination in the seeds of eastern white pine seemed to improve under aging conditions. Seed moisture contents and leachate conductivity increased as temperature and treatment time increased. Combining accelerated aging with leachate conductivity measurements allowed germination estimates to be concluded in three days instead of 10 weeks in this species.

Key words: Accelerated aging - leachate conductivity - germination - *Pinus strobus*

SINGH, O. & BONNER, F. T. 2001. Penuaan dipercepat untuk menilai kecergasan biji benih pain putih timur (*Pinus strobus*). Biji benih pain putih timur (*Pinus strobus*) didedahkan kepada penuaan dipercepat untuk menilai kecergasan biji benih dalam kelembapan relatif 100% pada suhu 39, 41, dan 45 °C selama 24, 48, 72, 96, dan 120 jam. Percambahan dan kekonduksian bahan larut resap sebelum dan selepas penuaan disukat. Kesemua rawatan penuaan mengurangkan percambahan dan kecergasan biji benih dengan kematian biji benih selepas 72 dan 48 jam penuaan masing-masing pada suhu 41 dan 45 °C. Hubungan antara kekonduksian bahan larut resap dengan percambahan biji benih pain putih timur didapati bertambah baik di bawah keadaan penuaan. Kandungan kelembapan biji benih dan kekonduksian bahan larut resap meningkat apabila suhu dan tempoh rawatan meningkat. Gabungan penuaan dipercepat dan kekonduksian bahan larut resap membolehkan anggaran percambahan spesies ini dibuat dalam masa tiga hari dan bukannya 10 minggu.

Introduction

A chain of physiological events begins in seeds long before the cessation of viability and it starts with the degradation of membranes and passes through energy synthesis mechanism impairment. Decline in respiration, biosynthesis, germination rate, storability, rate of growth and development, uniformity, plant resistance, yield and

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field emergence occur (Anderson 1973). All these events can take place from over a period as short as a few weeks to as long as several years. The accelerated aging technique is commonly used to obtain timely information on seed vigour. With this technique changes in the seeds at cellular level during long-term storage can be simulated within a comparatively short period of time by exposing seeds to increased temperature (40–45 °C) and high relative humidity (Delouche *et al.* 1968). The basis of this test is that higher vigour seeds tolerate the high temperature-high relative humidity treatment and thus retain their capability to produce normal seedlings in the germination test (Delouche & Baskin 1973). Accelerated aging is an accepted vigour test for many agricultural species (Anonymous 1983, Hampton & TeKrony 1995) and its use has become widespread (TeKrony 1983). This technique has been tested for some temperate conifer tree species (Blanche *et al.* 1989a, Marquez-Millano *et al.* 1991) and hardwoods (Bonner 1974, Pitel 1980), but so far, no generally accepted interpretation of the test results has been established for tree seeds.

In this paper, accelerated aging along with leachate conductivity measurements was used to measure seed vigour rapidly in eastern white pine (*Pinus strobus*). Leachate conductivity is also a common seed vigour test (Hampton & TeKrony 1995) and has shown promise with other pines (Bonner 1991) and spruces (Bonner & Agmata-Paliwal 1992).

Materials and methods

The seeds used in the tests were collected from North Carolina in 1989 and had been stored at 2 °C since that time. Accelerated aging was carried out in aging cabinets and boxes of Stults Scientific Engineering Corporation according to the procedures recommended by the Association of Official Seed Analysts (Anonymous 1983). The plastic aging boxes measured 14 × 14 × 5 cm and were equipped with bronze wire-mesh seed holders. Samples of 100 seeds each were spread in a single layer over the screens suspended above 40 ml of distilled water. Temperatures of 39, 41 and 45 °C were tested simultaneously in three separate aging cabinets. Boxes were withdrawn at 24, 48, 72, 96 and 120 h of aging. Seeds not subjected to accelerated aging were used as controls. There were six replications of each time and temperature combination. Four replicates were set up for germination tests and two for moisture content determinations.

Leachate conductivity of seeds was measured following the standard procedure of Bonner (1991) for bulked samples. After samples were removed from the aging chamber, they were weighed and immersed in distilled water for 24 hours. At the end of this period, the seeds were strained and conductivity was measured with a YSI Model 32 conductivity meter. After subtracting the values of water blanks, conductivity was expressed as microsiemens per gram of seed (μS/g). These samples were then placed in cold stratification for 60 days to break seed dormancy prior to germination tests (Anonymous 1974).

Germination was tested on moist blotters in Stults Scientific germinator. The test regimes were 16 h at 20 °C in the dark and 8 h in light at 30 °C. All germination tests were conducted for 21 days and seeds were considered germinated when the radicle attained a length of 1 cm. Germination was recorded daily and expressed as germination percentage and peak value following Czabator (1962):

Peak value (PV) = Maximum mean daily germination
= Cumulative percentage of full seed germination ÷ number of days elapsed since sowing date

Seed moisture contents were measured according to the rules of International Seed Testing Association (Anonymous 1993). Samples were dried in aluminium cans at 103 ± 2 °C for 17 h. Moisture contents were expressed as percentage of fresh weight.

Results and discussion

Germination percentage and peak value for the controls were 78 and 4.5% per day respectively. Germination declined with increased aging periods and temperatures (Table 1a). All aging treatments decreased germination and there were no signs of enhancement of germination with the shorter aging treatments as noted for *Pinus elliottii* (Blanche *et al.* 1989a), *Quercus nigra* and *Carya illinoensis* (Blanche *et al.* 1989b), and *Pinus taeda* and *Liquidambar styraciflua* (Bonner 1984). No germination was recorded after 72 and 48 h at 41 and 45 °C respectively but 20% germination was observed even after 120 h at 39 °C. Assuming that electrical conductivity of seeds is related to seed quality and also a measure of membrane function, the 20% germination at 39 °C suggests that membrane function was less damaged at this particular aging temperature. These results suggest that a temperature of 39 °C was too low and that of 45 °C might be too high to measure seed viability of this species. An aging period of 48 h at 41 °C appeared to be the best in terms of decreasing performance of this moderately good seed lot. Analysis of Variance (ANOVA)/Critical Difference (CD) values of germination percentages at different periods and temperatures also suggest that germination percentage of seeds aged for 48 h at 41 °C differed significantly at the 0.05 probability level from germination percentages of 24 and 72 h of aging at the same temperature (Table 1b). Peak values also followed the same trend as germination percentages (Table 1a).

Leachate conductivity values increased sharply as aging progressed from 24 h to 120 h at all three temperatures (Table 2a). Results at the two higher temperatures suggest that germination ceased completely when conductivity reached about 150 µS/g. The increase in electrolyte leakage in deteriorated seeds is an indication

Table 1a. Germination percentage (GP) and peak value (PV) means and standard errors of *Pinus strobus* seeds after accelerated aging at three temperatures

Period (h)	39 °C		41 °C		45 °C	
	GP (%)	PV (%/d)	GP (%)	PV (%/d)	GP (%)	PV (%/d)
Control	78 ± 3.0	4.5 ± 0.6	78 ± 3.0	4.5 ± 0.6	78 ± 3.0	4.5 ± 0.6
24	54 ± 2.7	2.6 ± 0.1	54 ± 5.2	2.8 ± 0.3	23 ± 2.2	1.1 ± 0.1
48	34 ± 4.1	1.7 ± 0.2	16 ± 2.5	0.8 ± 0.1	2 ± 0.6	0.2 ± 0.1
72	28 ± 4.6	1.4 ± 0.2	4 ± 1.0	0.2 ± 0.1	0	0
96	32 ± 4.8	1.6 ± 0.3	0	0	0	0
120	20 ± 2.8	1.0 ± 0.1	0	0	0	0

Table 1b. Analysis of Variance (ANOVA)/Critical Difference (CD) Values – Germination percentage of *Pinus strobus* seeds after accelerated aging at three temperatures

Temperature Period (h)	39 °C	41 °C	45 °C	Mean
24	53.75	53.75	23.25	43.58
48	33.75	16.25	0.00	16.67
72	28.25	3.75	0.00	10.66
96	31.50	0.00	0.00	10.50
120	20.25	0.00	0.00	6.75
Mean	33.50	14.75	4.65	17.63

CD Values at the 0.05 probability level. Temperature = 4.2006; Period = 3.22538; Temperature/Period = 7.2749.

of membrane deterioration of seed coat, which is a primary factor in the aging process (Mohamed-Yasseen *et al.* 1994). On the basis of germination, an aging period of 48 h at 41 °C seemed the best for this species. The leachate conductivity of the seeds aged at 41 °C for 48 h also differed significantly at the 0.05 probability level from the conductivity of 24 and 72 h-aged seeds (Table 2b).

Moisture contents of seeds also increased from about 7 to 37% with the increasing aging periods at all temperatures (Table 2a). The progressive fall in germination with the increase in moisture content following aging at different times and temperatures indicate the importance of moisture contents during storage. Increase in moisture

Table 2a. Leachate conductivity (LC) and moisture contents (MC) means and standard errors of *Pinus strobus* seeds after accelerated aging at three temperatures

Period (h)	39 °C		41 °C		45 °C	
	LC (µS/g)	MC (%)	LC (µS/g)	MC (%)	LC (µS/g)	MC (%)
Control	58.4 ± 3.4	7.29 ± 0.85	58.4 ± 3.4	7.29 ± 0.85	58.4 ± 3.4	7.29 ± 0.85
24	50.0 ± 2.8	23.91 ± 0.33	68.3 ± 7.2	24.53 ± 0.90	71.2 ± 4.6	26.44 ± 0.33
48	71.1 ± 2.1	29.56 ± 0.03	93.7 ± 8.4	32.83 ± 3.10	150.0 ± 7.5	34.04 ± 2.99
72	88.5 ± 5.8	32.99 ± 1.42	161.4 ± 12.7	34.61 ± 1.33	227.8 ± 8.5	35.13 ± 1.61
96	110.8 ± 15.3	34.50 ± 0.64	203.1 ± 12.0	36.06 ± 1.95	248.5 ± 1.4	36.42 ± 3.69
120	128.8 ± 11.1	34.97 ± 1.08	255.4 ± 11.8	37.11 ± 0.87	277.4 ± 5.2	37.17 ± 1.98

Table 2b. Analysis of Variance (ANOVA)/Critical Difference (CD) values – leachate conductivity (µS/g) of *Pinus strobus* seeds after accelerated aging at three temperatures

Temperature Period (h)	39 °C	41 °C	45 °C	Mean
24	50.01	68.27	71.21	63.16
48	71.06	93.74	150.00	104.93
72	88.53	161.37	227.76	159.22
96	110.84	203.13	248.46	187.48
120	128.83	255.38	277.42	220.54
Mean	89.85	156.38	194.97	147.07

CD values at the 0.05 probability level. Temperature = 14.5343; Period = 11.2582; Temperature/Period = 25.1742

contents with increased aging periods was also reported by various workers on tree seeds (Blanche *et al.* 1988, 1989a, b, Chaisurisri *et al.* 1993). Germination-moisture and germination-leachate conductivity curves showed similar trends of decrease in the germination of eastern white pine seeds following aging (Figure 1).

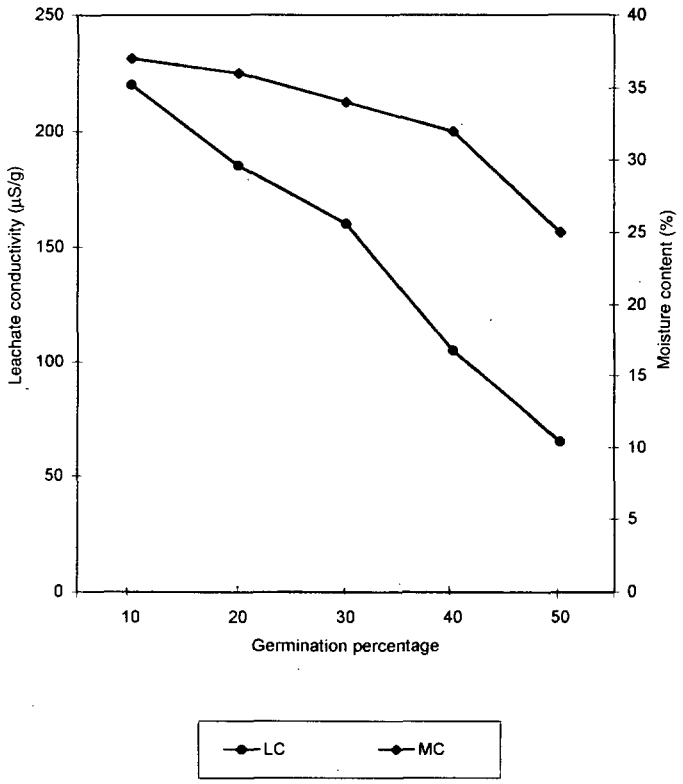

Figure 1. The relationship of leachate conductivity (LC) and moisture contents (MC) to germination percentage of aged seeds of *Pinus strobus*

The relationship between conductivity and germination in this seed lot was not linear, hence germination percentages were transformed to probits to provide linear models. Data from 39 °C were not included since this temperature was not severe enough. The linear models for 41 and 45 °C gave very good fits (Figure 2). The R values were 0.856 and 0.787 at 41 and 45 °C respectively. The equations were:

(1) probit germination percentage = 6.570 – 0.022 (µS/g) at 41 °C,

(2) probit germination percentage = 6.154 – 0.02 (µS/g) at 45 °C.

The relationship of leachate conductivity to germination holds special interest as a relatively rapid estimation of germination. Without aging, germination may be poorly correlated with leachate conductivity in some conifer seeds (Bonner 1991, Bonner & Agmata-Paliwal 1992). The present study indicates that the relationship between germination and leachate conductivity improved significantly after aging. So accelerated aging followed by leachate measurements can be used to make precise, rapid estimates of germination, since even without aging, studies on southern pines suggest that rapid estimation of seed germination can be obtained by measuring leachate conductivity of seeds ± 12% of actual germination (Bonner 1991). Bonner and Vozzo (1986) also made germination estimates by measuring electrical

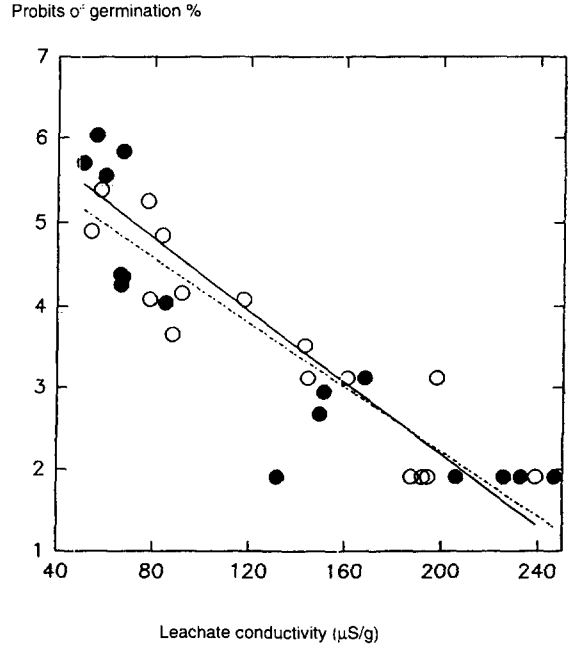

Figure 2. The relationship of leachate conductivity to the probits of germination percentage (GP) following accelerated aging at 41 °C (filled circles) and 45 °C (hollow circles)

conductivity of leachates of seeds ± 5–10% of actual germination in pines, white fir, douglas fir, engelmann spruce and blue spruce.

Laboratory germination tests for eastern white pine require at least 60 days of stratification (Anonymous 1993) followed by 21 days of test. Accelerated aging (48 h at 41 °C), followed by a leachate conductivity measurement (24 h) can provide an estimate of germination in only three days instead of 10 weeks. Hence a combination of accelerated aging and leachate conductivity can be used to monitor seed quality in the long term storage of this species, which exhibits considerable dormancy. However, these tests should not be considered equal to germination tests and should be used only when time constraints do not allow germination tests to be conducted. Similar type of research can also be conducted on other tree species, either temperate, subtropical or tropical, that exhibit considerable dormancy to get rapid estimates of germination.

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