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A new germination box for tree seed testing

B.S.P. Wang and F. Ackerman

Information Report PI-X-27
Petawawa National Forestry Institute

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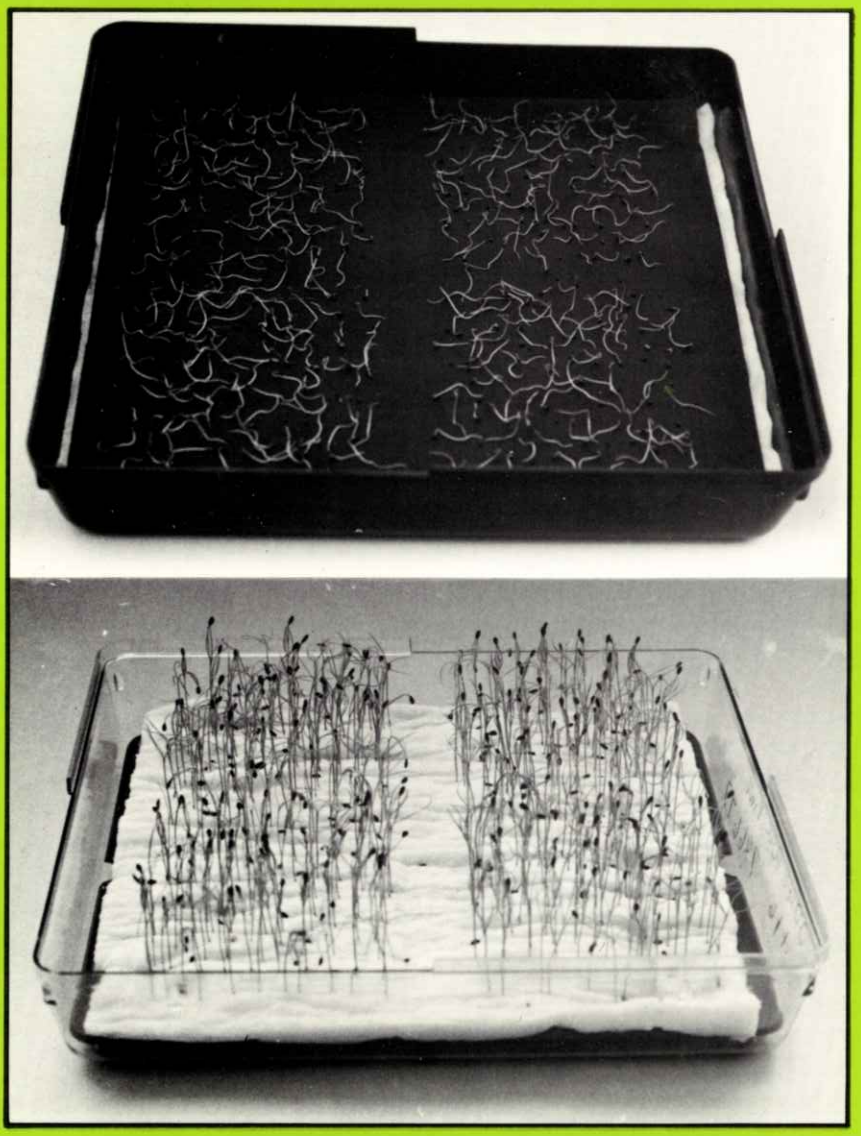

PETAWAWA NATIONAL FORESTRY INSTITUTE

The Petawawa National Forestry Institute was formed on April 1, 1979, as the result of an amalgamation of the former Petawawa Forest Experiment Station, which had existed for more than 60 years, with the Ottawa-based Forest Management and Forest Fire Research Institutes.

In common with the rest of the Canadian Forestry Service, the Petawawa National Forestry Institute has as its objective the promotion of better management and wiser use of Canada's forest resource to the economic and social benefit of all Canadians. Because it is a national institute, particular emphasis is placed on research into problems that transcend regional boundaries or that require special expertise and expensive equipment that cannot be duplicated in CFS regional establishments. Such research is often performed in close cooperation with staff of the regional centres or provincial forest services.

Research at the Institute is in two main areas:

FIRE RESEARCH AND REMOTE SENSING. Every year in Canada large areas of productive forest are destroyed by fire. Research concentrates on studies of forest fire behaviour, the development of new methods of fire control, the evaluation of fire-fighting equipment and retardants, and the development of computerized fire management systems that are rapidly finding applications with fire-fighting agencies across the country. The environmental and economic impact of forest fires and the use of fire as a silvicultural tool for intensive forest management are also studied.

In remote sensing, investigations are made into the application of modern satellite and airborne remote sensing systems to forestry problems. In this respect, the ARIES digital image analysis system is proving invaluable.

INTENSIVE FOREST MANAGEMENT. As Canada moves into more intensive mangement of its forest to meet expected increases in demand for this important resource, the role of this program will become increasingly important. An extensive reforestation program will require a steady supply of high-quality seed of the desired species. Improved growing stock, obtained through tree breeding and forest genetics research, is highly desirable. Increased emphasis is being placed on using the entire above-ground portion of the tree (biomass), but the effect on the environment of this and other forms of intensive management has to be carefully monitored. Biotechnological methods of improving yield while maintaining site productivity are being investigated.

In support of its research programs, the institute has at its disposal a 98 km^2 area of forest in the northern part of the Petawawa military reserve. Records of experiments and sample plots have been maintained since the 1920s. The forest also serves as a field laboratory for students from local schools, and a visitor centre is operated during the summer months.

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Cette publication est aussi disponible en français sous le titre Une nouvelle boîte de germination pour les tests de graines d'arbres.

Contents

Abstract

This report describes the development and evaluation of a specially designed box that provides an uniform environment for standard seed germination tests. Results of the evaluation indicated that the newly developed box is physically durable, economic, and efficient; and biologically sound for uniform seed germination.

Résumé

Le présent rapport décrit la mise au point et l'évaluation d'un germoir spécial qui procure des conditions uniformes pour les tests normalisés de germination. Robuste, économique et efficace, le germoir favorise aussi une germination uniforme.

A NEW GERMINATION BOX FOR TREE SEED TESTING

INTRODUCTION

The National Tree Seed Centre was established at the Petawawa National Forestry Institute in 1967 to improve the quality, quantity, and effective use of tree and shrub seeds for reforestation, land reclamation, tree improvement, and urban forestry in Canada. The Centre's two projects, Research and Development and Client Services, are concerned with all aspects of production, harvesting, processing, testing, and storage of seeds. A reliable standard method for testing seed germination is essential to both projects.

Detailed prescriptions for germination conditions are provided in the international rules for testing seed (ISTA 1976). However, the Centre's experience with the testing of seeds to meet service, regulatory, and research requirements has shown that poor control of microenvironmental conditions such as aeration and moisture levels can confound test results by affecting not only the rate and total germination, but also the uniformity and reproducibility of germination results obtained under prescribed conditions. Because control of micro-environmental conditions is directly related to the kind of germination trays or boxes used, the development of trays or boxes that provide an uniform environment for germination has been a high priority task for the Centre.

This report describes the newly developed germination box, tests to determine its physical properties, and the suitability of the box for standard germination tests.

DEVELOPMENT BACKGROUND

According to the amended ISTA germination evaluation criteria for tree seed (1981), normal seedlings are those which possess all essential structures including a well-developed root system, hypocotyl, and cotyledons. This means that germinants have to be kept in germination boxes for 3 to 4 weeks depending upon seed size and species before all essential structures can be effectively evaluated. The Centre has used a variety of commercially available trays, dishes, and boxes for germination tests and found them to be generally unsatisfactory. Sizes available did not permit maximum utilization of geminator space or full development of germinants for normal seedling evaluation without removing their covers, and uniform moisture levels in the germination medium were not maintained.

Our prototype germination box was 51 cm long, 25 cm wide and 10 cm deep with a perforated false bottom, a partition divider in the middle, and a sliding lid. The box was made from 5 mm thick acrylic plastic sheet with the two ends glued to a moulded bottom. Results of a one-year trial of 100 prototype boxes in the laboratory showed that they were unsatisfactory. They were too large and heavy to handle, and became brittle, warped, and

cracked, with the glued ends coming apart. As a result of warping the lid could no longer cover the box properly and adequate moisture levels in the germination medium could not be maintained. It became apparent that both the design and acrylic plastic material were not suitable. Further modifications were focused on the physical properties of the boxes.

The second design (the subject of this report) (Figure 1) consists of two main components of different sizes: one 28 cm long x 24 cm wide x 5 cm deep bottom, and one 28 x 24 x 1 cm top (lid). Both top and bottom are moulded out of light and unbreakable clear or opaque polycarbonate plastic with a special universal locking mechanism to allow any combinations of these components. For example, a top and bottom combination forms a regular germination box of 28 x 24 x 6 cm dimension; a two bottom combination forms a regular germination box with 10 cm depth. A perforated false bottom of 26.5 x 22.5 x 0.3 cm with eight 1 cm long legs was designed to fit within the box to support the germination substrate above and to provide space below as a water reservoir.

The size and structure of the box is designed to achieve maximum utilization of space in currently available commercial seed germinators and to provide flexible depths of 6 to 10 cm to allow for development of seedlings of various species and seed sizes. The angle of the box wall was designed for nesting to save space for storage. The box is available in clear or opaque (black) plastic for germination in light or dark conditions. Four aeration holes on the side wall of the bottom component are optional.

Upon completion of the final design, financial assistance was sought and obtained from Environment Canada's Cooperative Projects with Industry (COPI) (now the National Research Council's Program for Industry/Laboratory Projects (PILP)) to fabricate a quantity of prototype germination boxes for testing. Following promising results from a market survey, Spencer-Lemaire Industries Limited of Edmonton, Alberta introduced some minor changes in the design and produced 200 boxes for testing at the Centre. The following tests were carried out.

Temperature stress test

The temperature stress test was designed to assess the effects of temperature changes on physical dimensions and structural integrity of the box by subjecting it to cycles of temperature changes considerably more pronounced than those that might be encountered in normal usage. A top and a bottom of each of a clear and a black germination box were immersed in a $65^{o}\pm1^{o}C$ constant water bath for 20 minutes and then transferred to a refrigerator at $3^{o}C$ for 30 minutes. This warm to cold temperature cycle was repeated 60 times. Width, length, and diagonal measurements of each box component were taken at the beginning and after every 10 cycles. The box components were allowed to stabilize for one hour at room temperature before measurements. The boxes remained in a refrigerator overnight and on weekends during the test period. Table 1 shows changes in dimensions of components by number of cycles.

The general trend of changes in dimensions of the various box components in temperature stress tests, ranging from 0.5 to 3.0 mm, was a

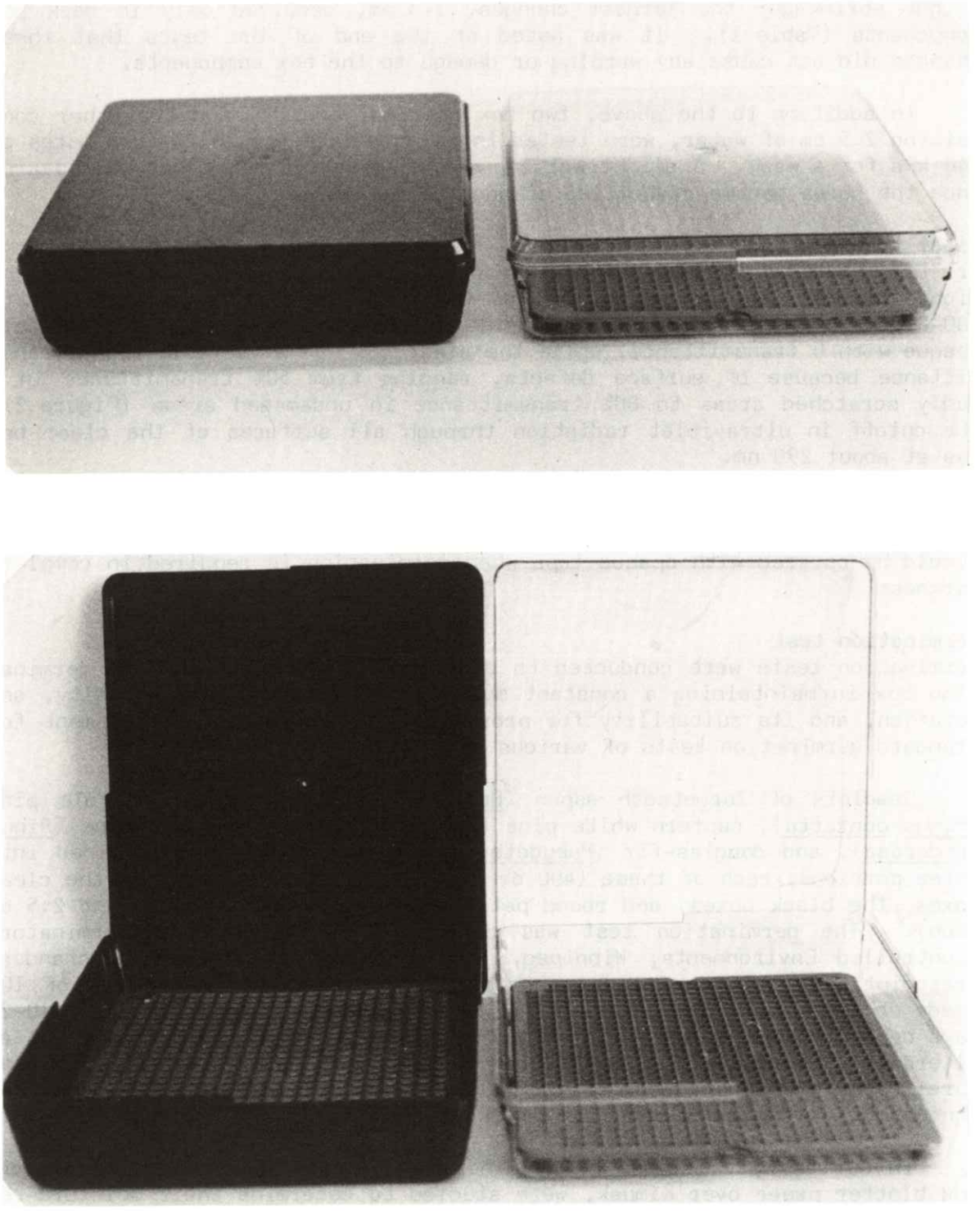


Figure 1. Newly developed clear and black germination boxes: overview (top) and inside view (bottom).

slight shrinkage; the largest changes, 2-3 mm, occurred only in dark box components (Table 1). It was noted at the end of the tests that these changes did not cause any warping or damage to the box components.

In addition to the above, two box bottoms, one dry and the other containing 2.5 cm of water, were tested in an autoclave for 20 to 60 minutes at 140 kPa for a week. A slight warping was observed at first but disappeared once the boxes became stabilized at room temperature.

Light transmission test

Dr. David Carlson of the National Research Council, Ottawa, determined the light transmission characteristics of the boxes over a wavelength range of 200-800 nm. Results indicated that the black germination box is completely opaque with 0 transmittance, while the clear box had a variable light transmittance because of surface defects, ranging from 50% transmittance in a badly scratched areas to 80% transmittance in undamaged areas (Figure 2). The cutoff in ultraviolet radiation through all surfaces of the clear box was at about 290 nm.

It should be remembered that there will be some leakage of light through the top-bottom joint of the black box and therefore the joint area should be covered with opaque tape when germination is required in complete darkness.

Germination test

Germination tests were conducted to determine the efficacy of the germination box in maintaining a constant moisture supply, relative humidity, and aeration, and its suitability for providing an uniform microenvironment for standard germination tests of various species.

Seedlots of largetooth aspen (Populus grandidentata), lodgepole pine (Pinus contorta), eastern white pine (Pinus strobus), ponderosa pine (Pinus ponderosa), and douglas-fir (Pseudotsuga menziesii) were each divided into three portions; each of these (400 or 800 seeds) was germinated in the clear boxes, the black boxes, and round petri-dishes (13.5 cm diameter and 2.5 cm deep). The germination test was conducted in Conviron G30 germinators (Controlled Environments, Winnipeg, Manitoba) according to ISTA standard prescriptions (1976). These prescriptions require four replicates of 100 seeds of the three pines, douglas-fir, and aspen to be germinated, for 10-28 days depending upon species, on top of moist blotters or filter paper at an alternating temperature of 20-30°C with 8-h light during the high temperature regime. Dormant seeds of the pines and douglas-fir have to be tested concurrently with and without 21 to 28 day cold stratification.

In our test, two types of germination media, Kimpak (cellulose paper) and blotter paper over Kimpak, were studied to determine their moisture-retaining capacity and uniformity under conditions of high humidity (Figure 3). All germination media were initially moistened to saturation point. Germination counts were made daily and seeds with 3 mm long radicles were considered to have germinated. The criteria used for evaluating the efficiency and suitability of the newly developed boxes were: (1) rate and capacity of germination, (2) uniformity in terms of variation among

Table 1. Measurements of new germination box components after temperature stress test

Box type	Component	Dimension (mm)	Measurements (mm) of changes in dimensions by stress cycles							Maximum change (mm)
Clear box	Lid	Length	282	282	282	282	282	282	281.5	-0.5
		Width	244	244	244	243	243	243	243	-1.0
		Diagonal	361	361	361	360	360	360	361	-1.0
	Bottom	Length	282	282	282	281	281	281	281	-1.0
		Width	242	243	242	242	242	243	242.5	+1.0
		Diagonal	361	361	361	360	360	360	360	-1.0
Opaque box	Lid	Length	282	282	282	282	281	281	281	-1.0
		Width	243	243	243	243.5	243	243	243	+0.5
		Diagonal	363	360.5	360.5	361	361	360	360	-3.0
	Bottom	Length	282	282	281	281	281	281	281	-1.0
		Width	242	242	242.5	242	242	242	242	+0.5
		Diagonal	362	361	361	360	360	360	360	-2.0

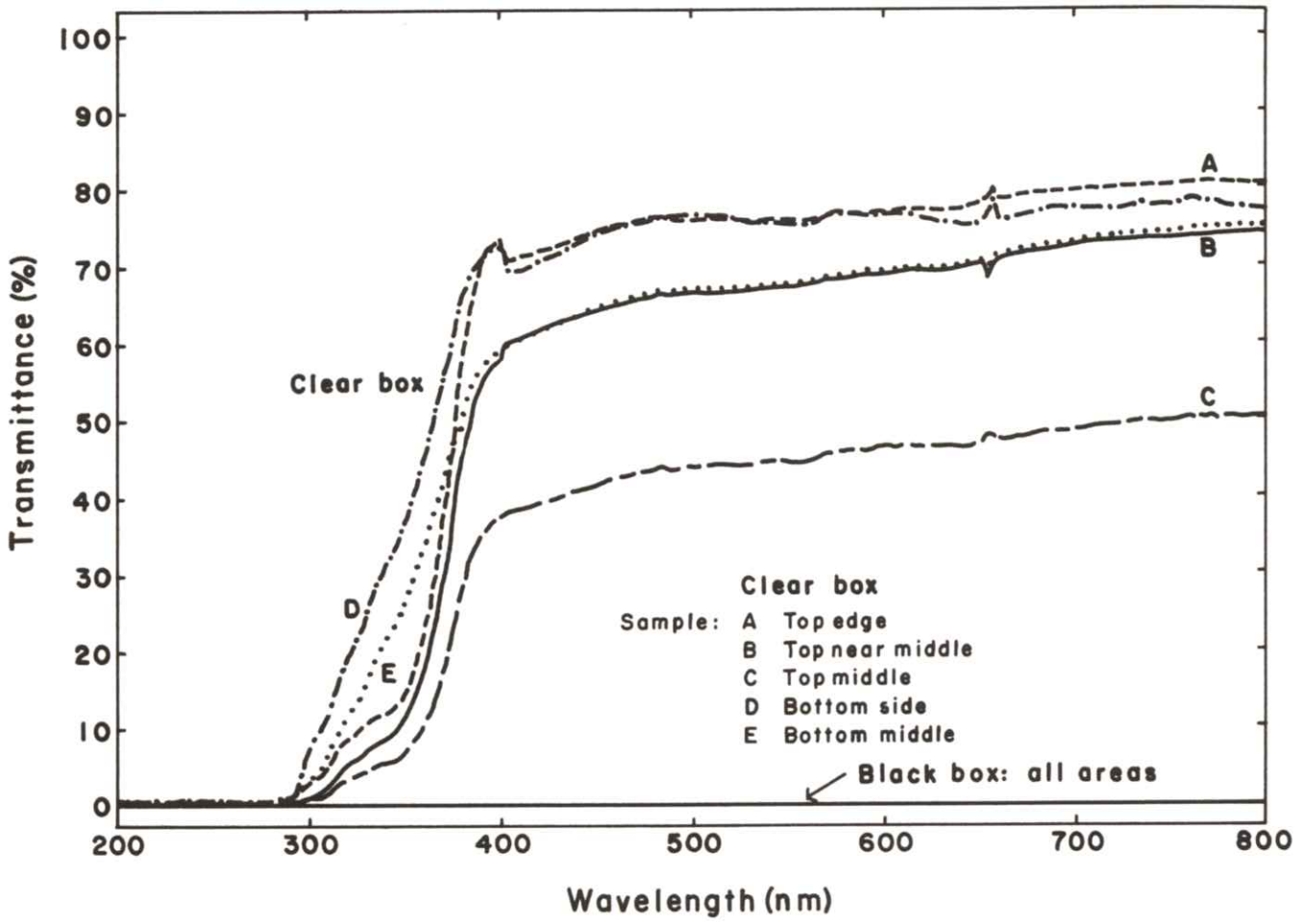
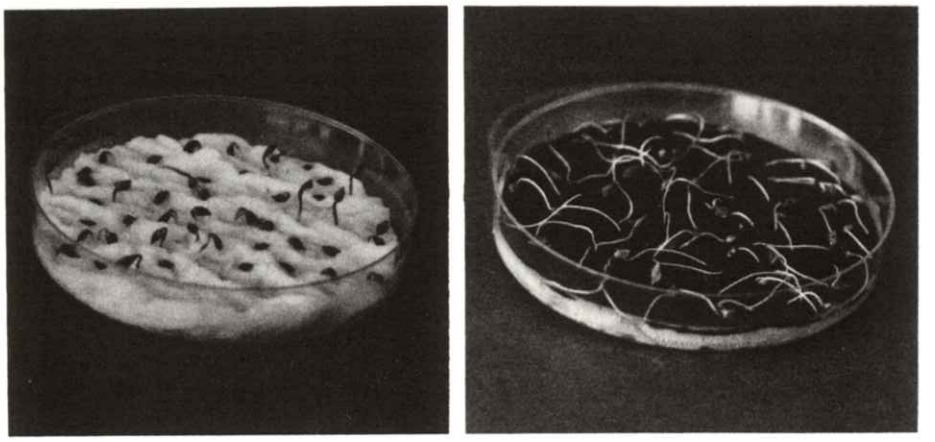


Figure 2. Light transmittance of clear and dark germination boxes.

A



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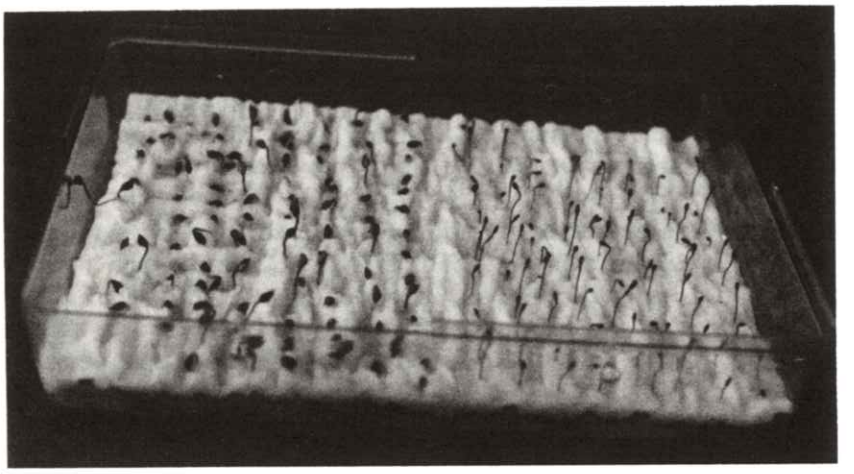

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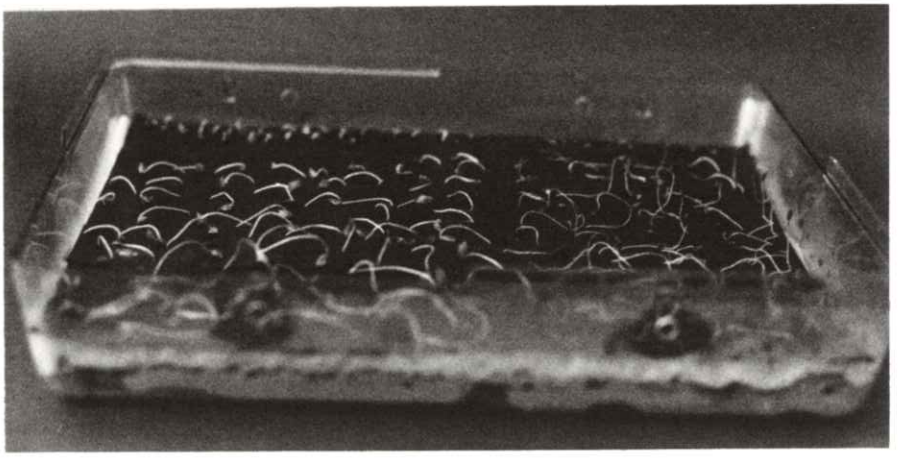

Figure 3. Germination of douglas-fir and lodgepole pine seeds on Kimpak (A left) and blotter/Kimpak (A right) in petri-dishes (A) and newly developed clear germination boxes (B-C).

replicates (4 replicates of 100 seeds each), (3) greenhouse effect, (4) degree of fungal infection and, (5) capability of uniform moisture retention.

Seeds of all species tested had similar germination rates as expressed by germination value (representing both speed of germination and total germination) (Czabator 1962, Djavanishir and Pourbeik 1976) and capacity in the germination box and in the petri-dishes (Tables 2-3). Some species germinated slightly better and faster in the new germination box than in the petri-dishes and vice versa.

Uniformity of germination was indicated by the ISTA tolerance limits expressed as the maximum difference in germination percentage between 4 replicates. Compared on this basis both the new germination boxes and petri-dishes provided equally uniform microenvironments for maximum seed germination with only two exceptions. Both exceptions were due to a high percentage of dead seed in one replicate after a three week pre-chilling treatment. When the coefficient of variation among the four replicates for all the species, germination media, and pretreatments were combined, the average uniformity was slightly better for the newly developed germination boxes than for the petri-dishes.

The greenhouse effects were evaluated, by measuring temperatures inside the boxes and in the germinator by paired thermocouples with a Speedomax Multipoint Recorder and a multi-channel thermometer, four to five times daily for four days. There was no significant difference in temperature measurements between the inside of the germination box and the germinator at any level or position within the germinator at radiation intensities normally used in the Conviron germinators.

Degree of fungal infection was evaluated on the basis of percentage of seed with mould. There was no difference in mould development between seeds germinated in the newly developed clear germination boxes and the petri-dishes (0-4%). However, black germination boxes, which were sealed by aluminum foil or tape, had variable degrees of mould development, ranging from nearly 0 to 59%. This was probably due mainly to the lack of aeration resulting from sealing.

In order to determine moisture loss, five replicates of each germination medium without seeds, in clear boxes with or without holes and with or without water reservoir were used. The same number of replicates and identical media were used in petri-dishes. All the media were cut to the same size for each type of germination box, moistened with measured quantities of water, and weighed before and after they were left in two dry germinators for 1, 2, 3, and 4 weeks at 20-30°C alternating temperature with 8-h light during the high temperature regime. This test condition represents adverse condition for seed germination.

It is well known that although most recent models of seed germinators and incubators are usually equipped with full humidity controls, they seldom function properly. From Figures 4-5 and Table 4 it can be seen that the loss of moisture from germination media varied greatly with the type of

Table 2. Germination of lodgepole pine, ponderosa pine, eastern white pine, douglas-fir and largetooth aspen seeds after 21 days* at 20-30°C with 8-hour light by germination box type, medium, and pre-treatment

Species	Variables	Clear box				Petri-dish				Black Box			
		Kimpak		Blotter/Kimpak		Kimpak		Blotter/Kimpak		Kimpak		Blotter/Kimpak	
		Control	Chilled	Control	Chilled	Control	Chilled	Control	Chilled	Control	Chilled	Control	Chilled
Lodgepole pine	x±S.D.**	90±0.50	91±4.8	87±2.6	87±2.2	87±4.7	87±2.6	90±2.1	89±3.7	73±3.3	88±2.8	78±9.0	87±4.1
	Max. diff.***	1	11	6	5	10	6	5	9	8	6	21	10
	Max. toler.†	13	11	12	13	13	13	12	12	17	13	16	13
	Coeff. var.††	0.6	5.3	3.0	2.5	5.3	3.0	2.3	4.2	4.5	3.2	11.6	4.7
Douglas-fir	x±S.D.	87±5.4	90±5.1	69±3.2	95±3.7	75±3.9	84±7.8	79±5.9	95±1.5	-	-	-	-
	Max. diff.	12	12	7	9	9	18	13	3	-	-	-	-
	Max. toler.	16	12	18	9	17	14	16	9	-	-	-	-
	Coeff. var.	7.0	5.6	4.7	3.9	5.3	9.4	7.5	1.6	-	-	-	-
Ponderosa pine	x±S.D.	-	-	65±9.4	72±1.5	-	-	71±4.4	71±2.1	-	-	-	-
	Max. diff.	-	-	19	32	-	-	9	5	-	-	-	-
	Max. toler.	-	-	19	18	-	-	18	18	-	-	-	-
	Coeff. var.	-	-	8.47	5.37	-	-	6.16	2.91	-	-	-	-
Eastern white pine	x±S.D.	-	-	35±3.0	90±4.8	-	-	34±4.4	87±1.0	-	-	-	-
	Max. diff.	-	-	7	11	-	-	8	2	-	-	-	-
	Max. toler.	-	-	19	12	-	-	18	13	-	-	-	-
	Coeff. var.	-	-	8.5	5.4	-	-	12.8	1.2	-	-	-	-
Largetooth aspen	x±S.D.	-	-	99±1.0	-	-	-	97±2.6	-	-	-	89±3.6	-
	Max. diff.	-	-	2	-	-	-	6	-	-	-	8	-
	Max. toler.	-	-	5	-	-	-	7	-	-	-	12	-
	Coeff. var.	-	-	1.0	-	-	-	2.7	-	-	-	4.1	-

*Total germination of largetooth aspen and of those in the black box was based on germination percentage after 9 and 18 days respectively.
**Mean total germination percentage of 4 replicates of 100 seeds each.
***Maximum difference in germination percentage between the highest and the lowest among the 4 replicates.
†Maximum allowable tolerance limits based on allowable random sampling variation at 0.025 probability.
††Coefficient of variation.

Table 3. Germination value* of lodgepole, ponderosa and eastern white pine, and douglas-fir seed by germination box type, medium and pre-treatment

Species	Variables	New clear box				Petri-dish			
		Kimpak		Blotter/Kimpak		Kimpak		Blotter/Kimpak	
		Control	Chilled	Control	Chilled	Control	Chilled	Control	Chilled
Lodgepole pine	x**	59.8	80.4	61.7	75.2	55.7	70.5	60.4	76.9
	S.D.†	1.4	9.0	3.8	4.4	5.3	6.0	3.4	6.5
	Coeff. var.††	2.4	11.1	6.2	5.9	9.5	8.5	5.6	8.5
Douglas-fir	x	23.2	64.9	17.2	73.0	20.3	57.2	22.5	71.0
	S.D.	3.8	8.3	2.4	5.6	3.4	10.4	4.4	3.6
	Coeff. var.	16.6	12.8	13.7	7.6	16.9	18.2	19.3	5.0
Ponderosa pine	x	-	-	24.0	59.2	-	-	28.0	58.9
	S.D.	-	-	6.5	22.9	-	-	3.2	5.9
	Coeff. var.	-	-	27.3	38.8	-	-	11.3	10.0
Eastern white pine	x	-	-	3.4	31.6	-	-	2.8	29.3
	S.D.	-	-	0.7	5.3	-	-	1.0	0.4
	Coeff. var.	-	-	20.3	16.9	-	-	35.0	1.4

*Germination value represents both the speed of germination and total germination.
**Mean of 4 replicates.
†Standard deviation.
††Coefficient of variation.

FIGURE 4. LOSS OF MOISTURE FROM KIMPAK GERMINATION MEDIUM AFTER 4 WEEKS IN A DRY GERMINATOR.

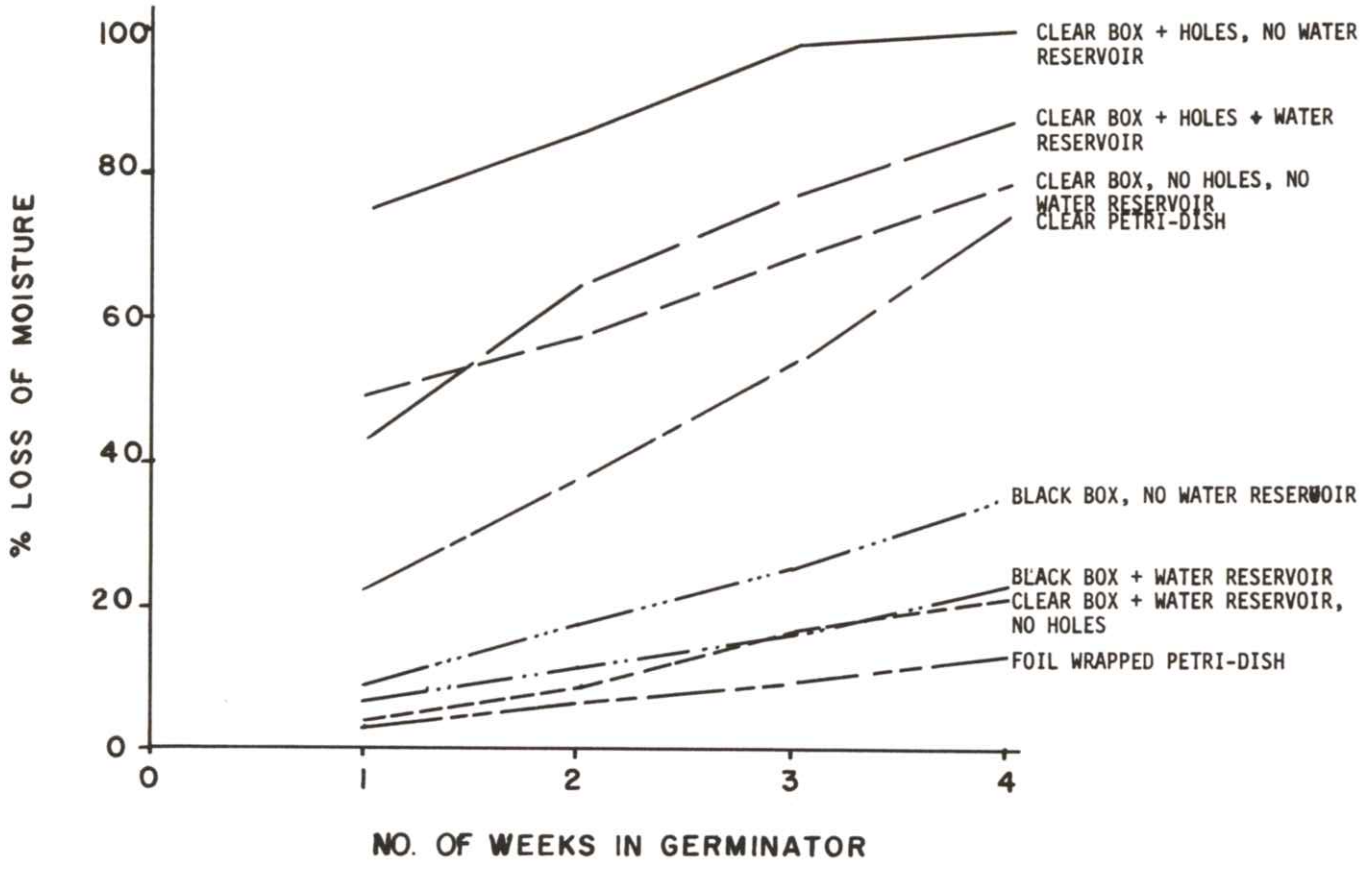

Figure 4. Loss of moisture from Kimpak germination medium after 4 weeks in a dry germinator.

FIGURE 5. LOSS OF MOISTURE FROM BLOTTER/KIMPAK GERMINATION MEDIUM AFTER 4 WEEKS IN A DRY GERMINATOR.

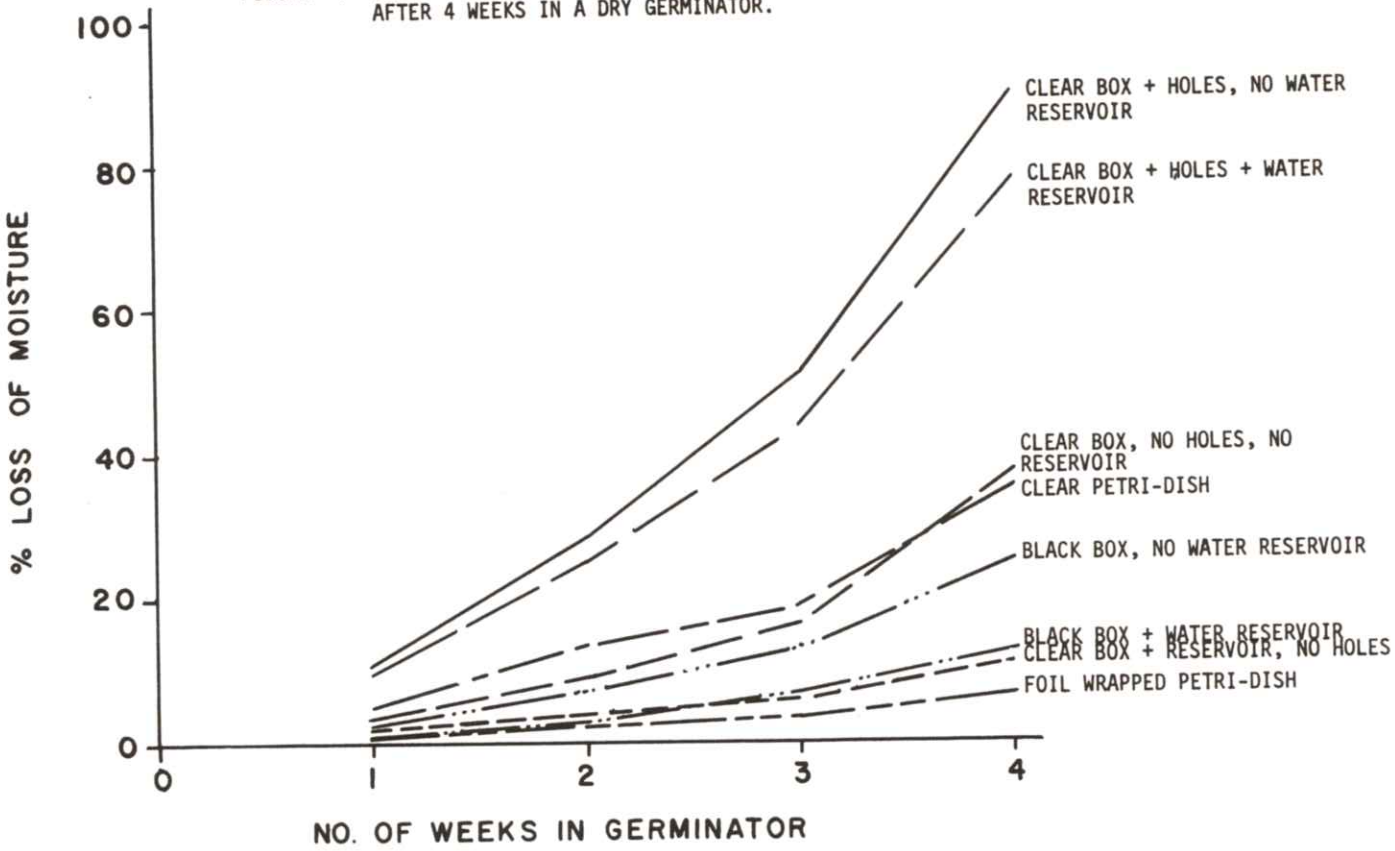

Figure 5. Loss of moisture from Blotter/Kimpak germination medium after 4 weeks in a dry germinator.

Table 4. Loss of moisture from germination media in new germination boxes and petri-dishes after 4 weeks in 2 dry germinators

Box type*	Medium**	Mean loss of moisture (%) and maximum range by week							
		1		2		3		4	
		x†	Maximum range††	x	Maximum range††	x	Maximum range††	x	Maximum range††
CB + W - H	K	4.0	2.3	8.5	5.2	16.2	20.1	21.7	22.0
	B/K	1.5	1.2	4.1	2.3	5.5	2.5	10.9	6.9
CB + W + H	K	42.9	36.6	64.0	52.8	76.7	51.1	86.9	34.7
	B/K	9.9	12.8	25.3	10.4	44.0	45.4	77.9	33.7
CB - W - H	K	48.9	88.5	57.3	80.0	68.2	71.4	78.7	51.4
	B/K	3.2	1.5	9.2	4.2	16.4	8.0	37.6	34.0
CB - W + H	K	74.8	57.7	85.7	38.3	97.3	13.2	99.9	0.3
	B/K	11.6	9.4	28.7	6.5	51.1	14.0	89.1	4.8
BB + W	K	6.5	3.1	11.5	2.3	16.2	2.7	22.8	3.3
	B/K	1.3	0.4	3.7	0.7	6.9	1.5	12.8	4.9
BB - W	K	8.8	6.0	17.2	9.7	25.0	11.3	34.8	14.4
	B/K	2.4	1.8	7.3	3.1	13.2	6.6	18.3	18.3
PD - C	K	22.0	24.0	37.5	24.7	53.5	27.5	73.9	30.4
	B/K	4.9	1.5	13.5	2.9	18.6	3.6	35.8	29.1
PD - F	K	3.4	4.0	6.3	3.0	9.5	4.0	12.7	5.0
	B/K	1.2	0.7	2.7	0.8	3.8	1.5	6.6	1.5

* CB + W + H, CB + W - H: clear box with water reservoir, and with or without aeration holes; CB - W + H, CB - W - H: clear box without water reservoir and with or without aeration holes;
BB + W, BB - W: black box with or without water reservoir; PD - C, PD - F: clear petri-dish or petri-dish wrapped with aluminum foil.

** K - Kimpak, B/K - blotter over Kimpak.

† Mean loss of moisture from germination medium of 5 replicates.

†† Maximum range of moisture loss among the 5 replicates.

germination box, germination medium, aeration, and water reservoir.

Both the newly developed clear and black germination boxes with water reservoir and without aeration holes, and the aluminum foil wrapped petri-dishes, retained much of their original moisture levels in the media throughout the 4 week test period (78-93%). The loss of moisture from the blotter/Kimpak germination medium was slow, and small in quantity (6-7%), after 3 weeks in germination boxes with water reservoir and without aeration holes. This should be compared to the boxes without aeration holes and water reservoir (13-16%) or with aeration holes and water reservoir (44%), and the clear petri-dishes (19%). It was surprising to note not only how fast moisture loss from the Kimpak germination medium occurred in a germinator without humidity, but also the large variations, expressed as the maximum range, in moisture loss among the five replicated clear boxes without aeration holes and water reservoir (51 to 89%) (Table 4). This variation was lowest with the blotter/Kimpak medium in both clear and black germination boxes with water reservoir and without aeration holes, and in the aluminum foil wrapped petri-dishes (Table 4). The blotter paper over Kimpak appeared to be far superior to the Kimpak in terms of the amount of moisture loss, and the uniformity of moisture loss among replicates.

The above findings point to the risk and possible confounding effects on germination test results by germinating seeds in open trays or improperly designed boxes, or in germinators and incubators without humidity. It also highlights the importance of adequate initial moisture in the germination medium and the continuous supply of moisture throughout the test period. The water reservoir in the newly developed box appeared to be an important factor in maintaining adequate moisture for maximum and uniform seed germination.

CONCLUSION

The test results indicate that the newly developed germination boxes are physically durable, economic, and efficient. They retain maximum uniform moisture in the medium, and provide adequate aeration and moisture required for uniform seed germination.

It was noted that improved protection of box surfaces from scratching or rubbing in the production process is needed.

The boxes are now available commercially from Spencer-Lemaire Industries, Edmonton, Alberta. Users of the boxes are invited to provide comments and suggestions for future improvement.

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