CANADIAN TREE IMPROVEMENT ASSOCIATION/
ASSOCIATION CANADIENNE POUR L'AMÉLIORATION DES ARBRES

Tree Seed Working Group

NEWS BULLETIN

No. 26, May 1997

WORKING GROUP UPDATE

I wish to start this edition of the Tree Seed News Bulletin with an apology. The fall/winter (Nov. 96) never hit the presses. Among my excuses include a dead hard drive without adequate backup. As the adage goes, I'll try not to let it happen again. Please note the following:

The membership mailing list is due to be updated. A number of copies of the last issue were returned. In order to help reduce mailing costs, we need a more current list. Please complete and return the page enclosed with this issue.

Plans have been formulated for the upcoming meeting of the Canadian Tree Improvement Association /- Association canadienne pour l'amélioration des arbres in Quebec City. The theme of this years' meeting is "Tree Improvement: Its contribution to sustainable development". A brief summary of the background and program for the meeting can be found later in the NewsBulletin. The Tree Seed Working Group is again holding a workshop on the Monday prior to the main session. The theme for the Workshop is "The Use of Artificial Pollination in Seed Orchards". Stephan Mercier has lined-up a good slate of speakers for what promises to be a very interesting and informative workshop. See the write-up later in the newsletter.

Last but not least, I want to thank everyone who contributed information and articles for this issue of the News Bulletin. For those who didn't quite get around to it, please take a few moments to send off a few lines for the next issue. Thanks again to Carole Leadem for the Seed trivia. How about seed trivia contributions for eastern species?

Editors Note

As many readers are probably aware, our Chairman, Guy Caron recently became seriously ill. Guy and his family wish to extend their appreciation to all of those who have offered support in this difficult time. For the time being, please address any questions, comments or concerns about the working group to the News Bulletin Editor.

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Comments, suggestions and contributions for the Newsletter are welcomed by the editor.

The Next Meeting of the CTIA/ACAA

The 26th meeting of the CTIA/ACAA will be held in Quebec City from August 18 to 21, 1997. The organizing committee has lined up an exceptional slate of speakers in what will undoubtedly be an excellent session. Both the Tree Seed and Wood Quality Working Groups will be holding workshops on Monday August 18th, and optional field trips have been organized for Thursday August 21st. A brief synopsis and description of the background for the meeting follows.

Background

People around the world are increasing in their concern about the environment. Managing our existing forests in a sustainable manner for both consumptive and non-consumptive values presents one of the greatest challenges to forest managers. The objective of the twenty-sixth meeting of the Canadian Tree Improvement Association is to foster discussion between specialists in the fields of tree improvement, resource management, and social sciences. Discussions among the speakers and the participants on how to meet the increasing demands for wood fibre while reducing rates of deforestation and natural resource depletion should prove to be most provocative and interesting.

The program will update participants on the current knowledge of breeding for quantitative characters, actual vs expected genetic gains in advanced generations, pest resistance, the composition and functioning of tree genomes, and the preservation of genetic diversity in tree improvement programs. Through the program we hope to foster discussions on how breeding programs can contribute to sustainable development from the perspectives of breeding, ecology, socioeconomics, and industry. The program summary follows:

Tuesday August 19

Current Knowledge in Tree Breeding

J. Beaulieu - Canadian Forest Service
Progress in improvement of morphological traits of forest tree species in eastern Canada.

S.E. McKeand - North Caroline State University
Actual vs expected genetic gains after two complete breeding cycles.

W.J. Libby - Zobel Forestry Associates
Levels of genetic diversity maintained in tree breeding populations.

A.D Yanchuk - British Columbia Ministry of Forests
Progress in breeding for pest resistance in forest trees

D.B. Neale - USDA Forest Service
Progress in the genome study of forest trees.

On Tuesday evening, **Pierre Charest**, Canadian Forest Service will be moderating a special workshop titled **"Intellectual property of genetically improved material: Issues related to transgenic plants".** , and their potential contributions to sustainable development will be discussed.

Wednesday August 20

Future Directions of Tree Improvement and contributions to Sustainable Development

G. Vallée - Ministère des Ressources naturelles du Québec
A tree breeder's point of view.

M.J. Lechowicz - McGill University
An ecologists's point of view.

J.-C. Mercier - Forintek Canada
A socioeconomist's point of view.

G.W. Adams - J.D. Irving
A forest company's point of view.

For further information, please contact any of the organizing committee:

Chairman
Michel Villeneuve, Quebec Ministry of Natural Resources

Vice-chairman local arrangements
Jean Bousquet , University of Laval

Vice-chairman symposium
Jean Beaulieu, CFS Laurentian Forestry Centre

Secretary
Ariane Plourde, CFS Laurentian Forestry Centre

Tree Seed Working Group Workshop

As you probably know, the theme of this year's Tree Seed Working Group Workshop is "The use of artificial pollination in seed orchards". We wish to demonstration that operational use of pollination techniques in forest seed orchards is both feasible and practicable. More specifically, we hope to foster debate on the issue of when and at what point in time is it possible/feasible to increase the genetic seed gain through artificial pollination? Among the topics on which we hope to stimulate discussions include increasing genetic gains (how much?), as well as the costs and benefits of SMP. Debates on cost/benefit raios for SMP should prove to be most interesting.

We will be again publishing the Workshop Proceedings in the Forestry Chronicle. And intend to have simultaneous translation services for the morning presentations.

The program is as follows:

Moderator: **André Rainville** (Direction de la recherche forestière)

7 h 30	Registration
8 h 30	Welcome
8 h 45	Artificial pollination in larch: from controlled crosses for breeding to seed orchard management **M. Bonnet-Masimbert, I. Balder, L. Pâques, G. Philippe**
9 h 15	Status and potential of using controlled parentage in operational reforestation. **G. W. Adams and K. J. Tosh**
9 h 45	Mass control pollination in Loblolly pine to increase genetic gains. **F. E. Bridgwater and D. L. Bramlett**
10 h 15	Break
10 h 30	Mass pollination in white spruce at Romieu seed orchard (Québec). **S. Mercier, A. Savary, J. Grenier and C. Parent**
11 h 00	T.S.W.G. business meeting
12 h 00	Lunch
13 h 30	Visit to MRN-pollen bank
14 h 30	Move to Station forestière de Duchesnay
15 h 00	Visit of indoor seed orchard, demonstration of electrostatic pollen gun an vacuum pollen collector
15 h 45	Visit to intensively managed seed orchards
16 h 30	Return to the University of Laval
17 h 00	Informal get-together

Andre Rainville

Update on Internet Discussion Group

The transfer of the TREESEED discussion group on the Internet is complete, and is up and running. Other than a couple of people who have had trouble subscribing to the Discussion Group, things seem to be running reasonably well. Traffic has not been especially busy, but some people have posted requests for information. While a number of replies under the TREESEED Discussion Group umbrella have been posted, most replies seem to have been sent directly to the individual who requested the information. This is probably the more desirable way to do business as it reduces the clutter on the electronic highway.

Ron Smith

Seed Trivia - Did you know?

Some seeds, e.g., Pacific silver fir, western redcedar and western hemlock, have resin vesicles in the outer layers of their seed coats. It is not known what the purpose these vesicles serve, but puncturing the vesicles reduces germination. There is no direct connection between the embryo and the outer seed coat, so it is uncertain why such treatment affects germination; it might be due to phytotoxic effects from terpenes in the resin, or because the injury presents a point of entry for microbial infection.

Special article

Fluorescein diacetate: A new viability stain for tree seeds.

Fluorescein diacetate (FDA) was tested as a viability stain with seeds of green ash, jack pine, Scotch pine, red pine, eastern white pine, Austrian pine, and eastern hemlock. In five tree species tested, the intensity of FDA staining of embryos seemed to correlate well with percent germination. In eastern hemlock and Scotch pine embryos FDA staining intensity also appeared to be correlated with germination, provided the stain incubation period was lengthened from 30 minutes to overnight (18h). Compared to tetrazolium chloride, the standard viability test for tree seeds, the potential advantages of FDA were; better selectivity for living cells, faster speed of the procedure, and greater ease of interpretation. Based on these preliminary results, FDA shows significant promise as a quick and easy test for seed viability with the results being used to help evaluate seedling and seed quality for nursery and reforestation operations.

Introduction

Fluorescein diacetate was first used as a stain to indicate mammalian cell viability thirty years ago (Rotman and Papermaster 1966). These authors suggested nonfluorescing, relatively nonpolar FDA penetrated the cell membrane readily, then esterases cleaved off the acetates to form the fluorescent product fluorescein. Once it formed inside the cell, the more polar fluorescein diffused out through the cell membrane at a much slower rate than its precursor, FDA, entered the cell. Therefore, only viable cells with an intact plasmalemma and functioning esterase enzymes would have cytoplasm that accumulated enough fluorescein to fluoresce brightly (Rotman and Papermaster 1966).

Fluorescein diacetate viability staining has been primarily applied to single cell or simple organisms with a few exceptions of more complex tissues being used such as detached buds, root cortex cells and orchid seeds, which are tiny, undifferentiated protoseeds. The *objective* of this study was to try to expand the application of FDA by determining if FDA could be used as an effective viability stain for tree seeds and roots.

Materials and Methods

Green ash (*Fraxinus pennsylvanica* Marsh.), Austrian pine (*Pinus nigra* Arnold), jack pine (*Pinus banksiana* Lamb.), eastern white pine (*Pinus strobus* L.), red pine (*Pinus resinosa* Ait.*),* Scotch pine (*Pinus sylvestris* L.), and eastern hemlock (*Tsuga canadensis* (L.) Carr.) seed were used for viability staining.

Seed of all species were soaked in aerated water for 18 hours and placed in sealed 3 mil plastic bags at +2°C for stratification. Austrian pine, jack pine, red pine and Scotch pine seed were stratified for 14 days before germination. Eastern white pine and green ash seed were stratified for 60 days and eastern hemlock for 120 days before germination. Germination was conducted in growth chambers with a 16/8 day/night photoperiod and a thermoperiod of 20/30°C for 16/8(midday) hours, and 75% humidity. For eastern hemlock, germination conditions were continuous dark at 20°C and 75% humidity. Germinations were counted twice a week.

Fluorescein diacetate staining was done on 4 replications of 10 embryos for each species with the following procedure. Dry seed were soaked 18 hours in aerated deionized water. All seeds were incubated for 30 min in a solution of 0.2 g FDA in 100 ml acetone. The 18 hr seeds were immediately removed from the FDA solution and incubated in a sealed vial at room temperature (22°C). Embryos were dissected from the seeds and examined for viability with a Leitz Diaplan epifluorescence microscope, under 450-490 nm blue light (long-pass suppression filter 520 nm). They were rated '0' if no yellow-green fluorescence was present, '50' if fluorescence was pale or patchy over the embryo surface, or '100' if fluorescence was bright and uniform over the entire surface of the embryo.

Results and Discussion

The relationship between germination and average FDA staining of the embryo was strongest in green ash, white pine, and Austrian pine (Figure 1A-C). The relationship between FDA staining and germination in jack pine and red pine (Figure 1D&E) is also evident but slightly more variable. Only in hemlock (Figure 2A) and Scotch pine (Figure 2C) did FDA staining intensity seem to be much lower and different than percent germination. The weak relationship between FDA and germination in these two species may have been due to insufficient incubation time since the relationship appeared much stronger when seed and stain incubated overnight (Figure 2B&D).

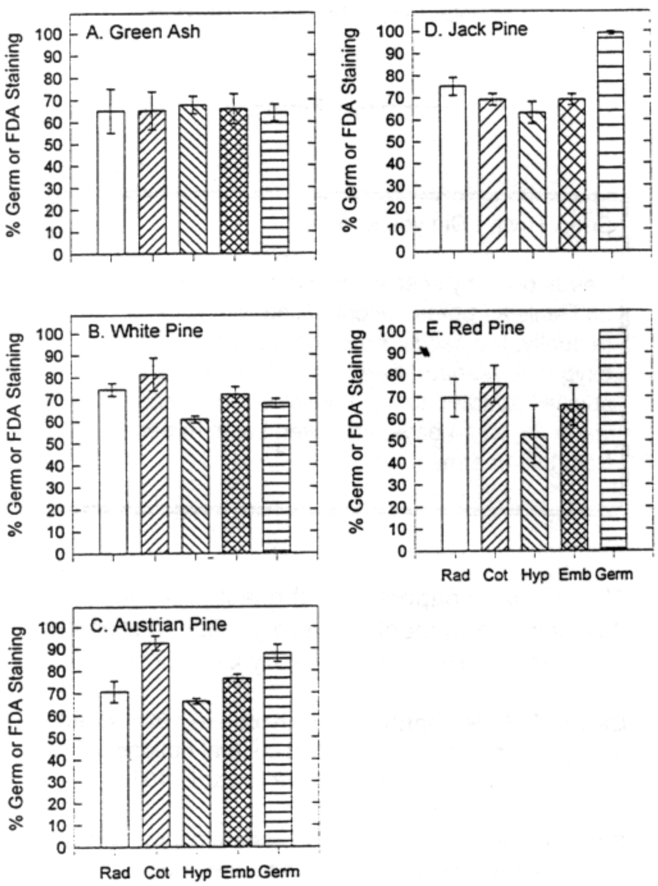

Figure 1. Fluorescein diacetate staining intensity and percent germination of A. green ash, B. eastern white pine, C. Austrian pine seed, D. jack pine, and E. red pine seed. For germination and staining n =4, mean ± standard error. Abbreviations, Rad=radicle, Cot=cotyledons, Hyp=hypocotyl, Emb=embryo, and Germ=germination

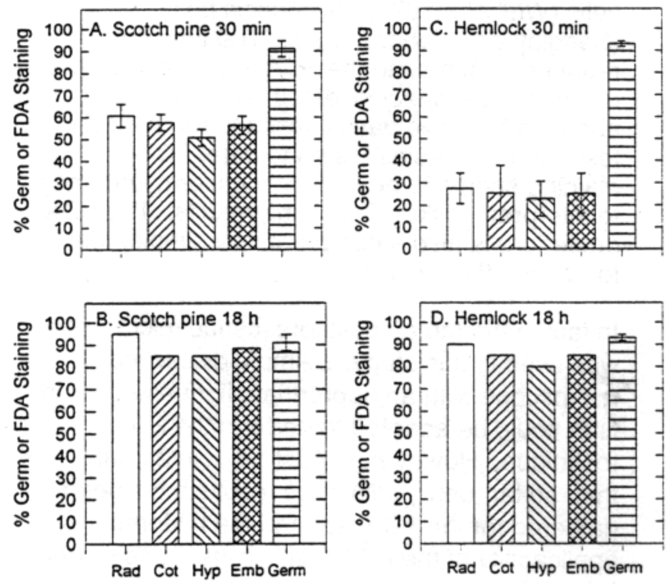

Figure 2. Fluorescein diacetate staining intensity and percent germination: Effect of incubation time. A. eastern hemlock seed 30 minute staining B. eastern hemlock seed 18 h staining C. Scotch pine seed 30 minute staining, D. Scotch pine seed 18 h staining. For germination and 30 minute staining n =4, mean ± standard error, for 18 h staining n = 1.Abbreviations, Rad=radicle, Cot=cotyledons, Hyp=hypocotyl, Emb=embryo, and Germ=germination.

The current standard viability test for dormant tree seeds like green ash and eastern white pine is the tetrazolium chloride (TZ) test (Justice 1972). Unfortunately, it can take years of practice to be able to correctly interpret the TZ test results (Bonner *et al.* 1994). Some of the problems associated with the TZ test include difficulty staining some seeds, lack of agreement between staining and germination results, especially for seed with low germination, and the lack of significance of staining intensity coupled with the critical importance of stain location on the embryo (Justice 1972; Bonner *et al.* 1994). Finally, the TZ test usually takes over 2 days to complete (Bonner *et al.* 1994).

The FDA test for seed viability offers many potential advantages over the currently accepted TZ test for seeds. Fluorescein diacetate's comparative superiority to TZ as a viability stain has been reported for both plant cells (Widholm 1972) and *Acacia* pollen (Sedgley and Habard 1993). The total time required to complete the FDA test is less than a day with most species tested so far, which is about half the two days it takes to run the TZ test. In the species tested to date, FDA staining seems to be relatively uniform and easy to interpret, unlike TZ staining results. The FDA procedure is also a comparatively simpler test to do than the TZ test.

In fact, although an epifluorescence microscope was used in this study, a simple dissecting microscope with an appropriate light source and filter could be adapted to perform the FDA technique. However, it must be pointed out that, so far, only preliminary results have been gathered for the FDA test and the uniform applicability of the FDA test to other species and for seeds of the same species with varying germination remains to be shown.

Conclusions

FDA appears to show significant promise as a viability stain for tree seeds. In all seven tree species tested, FDA staining of embryos seemed to correlate well with percent germination provided the incubation period was long enough. Although further testing will be needed to confirm the efficacy and general applicability of FDA as a viability stain for tree seeds, FDA appears to have the potential to become the superior method used to quickly determine viability.

Literature Cited

Bonner, F. T., Vozzo, J. A., Elam, W. W., and Land, S. B., Jr. 1994. Tree seed technology training course. Instructor's Manual. USDA For. Serv., Southern For. Exp. Sta., Gen. Tech. Rep. SO-106. 160 pp.

Justice, O. L. 1972. Essentials of seed testing. In: (T. T. Kozlowski, ed.) *Seed Biology* Vol III, Academic Press, New York. pp. 301-370.

Rotman, B. and Papermaster, B. W. 1966. Membrane properties of living mammalian cells as studied by hydrolysis of fluorogenic esters. Proc. Nat. Acad. Sci. USA 55: 134-141.

Sedgley, M. and Harbard, J. 1993. Pollen storage and breeding system in relation to controlled pollination of four species of *Acacia* (*Leguminosae*: *Mimosoideae*). Aust. J. of Bot. 41: 601-609.

Widholm, J. M. 1972. The use of fluorescein diacetate and phenosafranine for determining viability of culture plant cells. Stain Tech. 47: 189-194.

Seed Trivia - Did you know?

Seeds of many species die when soils are flooded and soils air pores become filled with water (actually, the seeds drown because they need oxygen to respire). Seeds of yellow-cedar, a species adapted to wet habitats, can be soaked in water for 10-14 days, and even seem to benefit from this treatment.

The following papers from the last Tree Seed Working Group meeting were published recently in volume 72(5) of the Forestry Chronicle

Caron. G.E., R. Smith, and D. Kolotelo 1996. Seed orchard management and cultural options for quality seed production - foreward. 467

Barnett, J.P. 1996. How seed orchard culture affects seed quality: Experience with the southern pines. 469-474

Adams, G.W. and H.A. Kunze 1996. Clonal variation in cone and seed production in black and white spruce seed orchards and management implications. 475-480

Edwards, G.E. and Y. El-Kassaby 1996. The biology and management of coniferous forest seeds: Genetic perspectives. 481-484.

New Members

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Book Review

Seed of forest broadleaves from harvest to sowing. Boleslaw Suszka, Claudine Muller, Marc Bonnet-Masimbert. 1994. Institut National De La Researche Agronomique (INRA) Editions, INRA Paris, France ISBN: 2-7380-0659-0.

The book entitled, Seed of forest broadleaves from harvest to sowing, is an excellent practical and much needed reference text on hardwood seeds. The collection, handling and storage of hardwood seeds can be difficult and problematic and the authors have gone into a great deal of detail addressing many of the problems associated with hardwood seeds. The book is clearly written, with numerous illustrations.

The book is divided into two sections. The first section covers general aspects of seed collection, cleaning, temporary and long-term storage methods, and seed sowing methods. The second section provides detailed information on fifteen species, including alders, ash, beech, birches, hornbeam, linden, maples, oaks and wild cherry. This section provides precise recommendations for the handling, storage, and planting of each species and discusses current areas of research pertaining to these species. The information in the book pertains to growing conditions found in European countries but this information is certainly of use for other areas. This book was first published in French in 1994 and currently is available in a paper back version in English, French and Polish.

Tannis Beardmore

Second Generation Seed Production comes on Line For J.D. Irving, Limited

Second generation seed orchard establishment for black spruce and jack pine began in 1989 and the first blocks (approximately 18 hectares total) were completed in 1996. Seed production has been steadily increasing for the last couple of years. This year, over 1.7 million jack pine seeds were harvested and this will be sufficient for 1997 operational seedling production. Over 60 thousand black spruce seeds were harvested and, in 1996, GA4/7 was applied to 1100 grafts to increase production in 1997. Most of the clones in these second generation orchard are already being field tested for general combining ability thanks to the efforts of New Brunswick Tree Improvement Council members in conducting controlled pollination on the second generation selections in situ as well as the early flowering of jack pine grafts. This will allow for roguing to begin in these orchards within five years - just around the time that they will be considered to be in full production.

Other reforestation stock production is being done by rooted cuttings for black spruce. Crosses are made among tested second generation selections in the breeding hall at Sussex Tree Nursery and the resulting full-sib families are hedged for the production of cuttings. One million cuttings were produced in 1996 and the same is planned for 1997. Production increases beyond this level are currently in the planning stage. Controlled pollinations are conducted annually and the genetic quality of the crosses increases every year as more genetic information becomes available. This makes a flexible system for delivering the most genetically improved reforestation stock very rapidly. For example, by the end of 1997, a maximum of 150,000 seedlings of black spruce will have been grown since seed production started in the orchard whereas over 2.3 million cuttings will have been produced. The genetic quality of the hedge stock we are growing now is probably equivalent to a rogued seed orchard and this will continue to increase.

Greg Adams

National Tree Seed Centre

The Seed Centre is open for business! In fact we started processing orders in September. Re-establishment of the Centre went extremely well. The move afforded the opportunity to re-inventory the seedlots and update the database. The entire database was successfully converted and is running on a PC platform using Microsoft Access.

There are two sets of material in the Seed Centre. The first contains over 2200 seedlots of 200 species. This has developed over the years as the primary holding of the Centre. The second set, is a wide array of seedlots (6000 +) acquired from the breeding/genetics program at Petawawa. This material is stored at 4° C and as time permits will be germination tested and moved to frozen storage at -20° C.

Little has been done with respect to germination testing over the past 5 years. At this point the testing component appears to be a daunting task but with time I am sure it will become a routine operation.

This autumn we were busy assisting other Biodiversity Network staff with the collection of red spruce cones for the initiation of a major research project. Single-tree collections were made from 15 to 20 trees from 14 stands located in Ontario, New Brunswick, Nova Scotia, and Prince Edward Island. Additional collections will be made when cone crops occur.

Dale Simpson

Book Review

Seed Ecophysiology of Temperate and Boreal Zone Forest Trees. By R.E. Farmer, Jr.

Hot off the presses, the book **Seed Ecophysiology of Temperate and Boreal Zone Forest Trees** by R.E. Farmer represents a significant contribution to the whole area of seed ecology. I think that the following two points paraphrased from the authors' own Introduction accurately encapsulates the need for this book: 1) There <u>was</u> no book on the seed ecology of forest trees, and 2) The vast majority of published information to-date is scattered throughout the literature.

This book is a must have for undergraduate forestry students, foresters, and anyone interested in the field of seed production, dispersal and germination. It is truly written in an easy to read and understand format.

The book starts with a general chapter on seed biology. This section is not a detailed treatise of the biology and physiology of flowering and seed production, but does provide an important general overview of the biology of seed production of both conifers and angiosperms. Subsequent Chapters deal with all aspects of seed dispersal, germination, and dormancy. The author has left the technical aspects of seed germination and testing to others and covers the ecological aspects of this subject from genetics to the chemical and physical requirements for seed germination. The final chapters deal with the various components of seed ecology and the effects of natural and manmade disturbances on seed germination.

I believe that a book on this subject has been long overdue and will provide an invaluable tool to instructors, foresters, and researchers alike.

Ron Smith

Norway Spruce Breeding - 1996

The breeding strategy adopted by the NSTWIG (Nova Scotia Tree Improvement Working Group) and NBTIC (New Brunswick Tree Improvement Council) for Norway spruce involves crossing each selection with a standard 20-tree pollen mix (polycross) to determine individual general combining ability. Compared to white, red and black spruce, Norway spruce poses a significant challenge to achieve high seed set in controlled crosses.

It is believed that a number of factors are possibly responsible for poor seed set, including variation among clones with respect to phenology as well as morphology, sensitivity to high temperature within pollination bags, delicate nature of female strobili, and pollen viability.

In 1996 three separate agencies performed polycrosses in the Norway spruce breeding program. It is interesting to compare their breeding success (Table 1). For a cross to be considered successful, at least one full seed must be produced.

Table 1. 1996 Norway spruce breeding summary of the numbers of crosses (CR) and (%success), numbers of cones (NC), total numbers of full seed (FS), sound seed per cone (SSPC), and the coefficient of variation (CV) and range for SSPC.

Agency	CR	NC	FS	SSPC	CV	Range
A	26 (74)	274	5008	18.07	1.11	0.3-73.8
B	20 (65)	200	744	3.98	12.24	1.0-35.0
C	22 (79)	122	1737	14.24	3.82	9.0-55.0
Totals	68 (72)	596	7485	12.56		

As indicated by the coefficient of variation (C.V.) For seeds per cone, there are large differences in seed set among agencies. Agencies B and C used the same pollen (stored) whereas agency A used fresh pollen and pollinated all crosses twice.

Table 2 contains a summary of the number of crosses which produced no full seed. The large number of unsuccessful crosses (126 cones in the 26 unsuccessful crosses) is puzzling.

Table 2. 1996 Norway spruce breeding summary of unsuccessful crosses.

Agency	# of crosses	# cones
A	9	44
B	11	76
C	26	6
Totals	26	126

Howard Frame

Seeds of Woody Plants in the United States

For those of you who do not know, The U.S. Forest Service is in the process of revising and updating the USDA Agr. Handbook 450. "Seeds of woody plants in the United States". Dozens of new species and about 25 new genera will be added. Over half of the genus revisions / additions have been received by the Technical Coordinator (Frank Bonner) and forwarded to the Editor. Part I is also being revised, with a different chapter lineup; about half of these are done. We should be ready to print in late 1997, but that's no promise!

Another big change is that we are revising this to go on the Internet also, probably via the USFS web page. Individual genera will be in separate files, so persons interested in only one or two genera could download only those. We hope everyone will buy a copy of the entire book, of course! Many Canadians have contributed data for the revision, and at least one genus revision is being written by a Canadian. We all are looking forward to completion of this very popular book. If anyone has questions, just contact me (Frank Bonner) through my Compuserve email address:

102346.253@compuserve.com.

Frank Bonner

Newsletters at a glance

Seed and Seedling Extension Topics

The summer 1996 issue of Seed and Seedling Extension Topics was again an interesting and informative newsletter. Among the articles of potential interest to our readers include two by Dave Kolotelo which pertain directly to seed; one on the cold fungus Caloscypha on Abies spp. and a second on germination testing of western redcedar seed. For further information, please write the editor:

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Seed Trivia - Did you know?

Unlike most conifers, Pacific yews do not produce cones; instead they produce infrequent crops of small berry-like structures called arils which contain only a single seed. The seeds do not have wings, so depend on animals for their dispersal. Also, unlike most conifers, yew trees are sexually dimorphic (like humans), e.g., some are males, and some are females.

Cone Production at the Weyerhaeuser Seed Orchard, Prince Albert, Saskatchewan.

Seed production has been a problem in the past, but due to Mother Nature's cooperation, careful management, and the fact that the grafted trees are growing well, an overwhelming white spruce cone crop was collected in August 1996 (Figure 1A). All of the 40 clones in the orchard contributed to the harvest. Until recently, fewer than 50 % of the clones produced cones, and only in relatively small amounts. We now have a five-year supply of seed. In the next few years, improved seedlings from the 1996 orchard crop will provide 40 to 60% of Weyerhaeuser's reforestation stock within our FMLA. One hundred percent of our planting stock should from improved seed in the near future.

The 1996 jack pine cone harvest was also exceptional (Figure 1B). Weyerhaeuser, Saskatchewan now has a considerable amount of improved seed in storage.

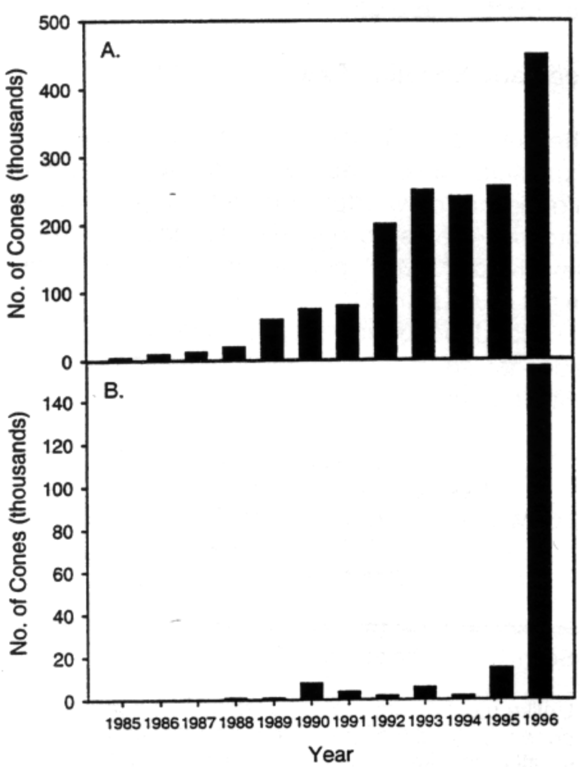

Figure 1. Summary of cone production, by year for the A. jack pine and B. white spruce seed orchards.

Louise Corriveau

Germination testing of Mountain Hemlock Seeds

After discussions with Dr.George Edwards, we tried something a little different here at Western Tree Seeds to see if we could in fact improve the germination capacity and vigour of Mountain Hemlock by simply stratifing and germinating Mtn. Hemlock in a darkened environment within our cabinet germinator.

The result we achieved was that both the germination capacity and vigour proved to be far superior than that of the test set exposed to normal prescribed standards of light. Within the germinator the temperature was set on a 20/30 split, 16 hours at 20 C. and 8 hours at 30 C.

Lighted Test	Darkened Test
91% in 35 days	97% in 28 days.

- only one seedlot however.

Method:

Each seedlot was stratified for 28 days. A blackout envelope was designed by using a double layer of 2 mil. dark green plastic to minimize the effects of light. The germination trays were placed inside the plastic envelope for the entire stratification and germination period. The only time the plastic envelope was removed was when counts needed to be done. This procedure noticeably improved the overall capacity and vigour of the seedlot.

	No. Days 7	14	21	28	35
Darkened Test	2.5	74.5	95.0	97.0	
Lighted Test	2.0	40.5	65.0	80.5	91.0

This may be something you folks may want to test a little further.

Thanks for the tip George.

Kim Creasey
Western Trees Seeds Ltd.

Editors note: Those interested in more information on this subject may wish to see the article by Edwards and El-Kassaby in the Recent Publications section of this issue.

The New Brunswick Tree Improvement Council is 20 years old!

Introduction

The New Brunswick Tree Improvement Council (NBTIC) was established in 1976 to coordinate tree improvement efforts of government and industry agencies. Since the Council's formation, 20 years ago, significant progress has been made. Seed requirements (60 million) for provincial reforestation programs are being met from orchard production. In fact, first generation seed orchards have produced almost 1 billion seeds. All producing first-generation seedling seed orchards have been rogued twice, whereas roguing is just beginning in clonal orchards. A second-generation program is well under way and seed orchards are already producing significant quantities of seed. The success of NBTIC can be directly attributed to the steadfast commitment of Council members to tree improvement in New Brunswick. The following sections will summarize and highlight the progress of NBTIC over the past 20 years.

Seed Orchards and Seed Production

The total number of plus tree selections for black spruce and jack pine was 1233 and 845, respectively. The seedling seed orchard family testing strategy was adopted for these species with a total of 80 ha of black spruce and 43 ha of jack pine established from 1978 to 1986. Seed production from orchards has been far greater than the Council anticipated. Many of the NBTIC agencies have sufficient orchard seed in storage and are not collecting from their first-generation seed orchards. Jack pine started to produce seed in 1984, followed by black spruce in 1987. Since seed production started, a total of 190 million and 583 million seed, respectively, have been collected.

The clonal seed orchard progeny testing strategy was used for white spruce and tamarack with a total of 414 white spruce and 268 tamarack plus trees. Forty-one hectares of white spruce and 23 ha of tamarack clonal seed orchard were established. White spruce clonal seed orchards started producing significant amounts of seed in 1991 and to date over 166 million seeds have been collected. Tamarack seed production started in 1991, with a total seed production of 24 million. Several agencies have started to conservatively rogue their clonal orchards based on information from 5-year-old progeny tests.

Genetic Testing

Planting of family and progeny tests is a major part of the Council's program. The first major outplanting occurred in 1978. Over the 20-year period, 235 tests have been planted on 274 ha. These tests provide data for several purposes, including roguing orchards, quantifying gain in various traits, and identifying superior trees for next generation improvement. Family tests of black spruce and jack pine were established at the same time as the seed orchards and was completed in 1987. Progeny testing of white spruce and tamarack using a polycross is 87% and 78% complete respectively. Pair-mating of white spruce is 90% complete. Seedlings are planted in selection tests. Superior families will be identified based on progeny test data and individual trees selected in the selection tests based on field assessment.

Second Generation

Selection of trees for second-generation breeding populations, which was started in 1989, is based on 10-year family test data for black spruce and 7-year data for jack pine. Top performing black spruce families were identified based on height growth, while for jack pine stem straightness and height were used. The goal was to form second generation breeding populations containing 400 trees. Jack pine selection was completed in 1994 while black spruce will be completed in 1997. Clonal seed orchards were established using clones from the top-ranking 40 families from the three oldest series of family tests. Seed production, which began in 1992, has shown a steady increase.

Breeding of second-generation selections began in 1990. As for white spruce, a complementary breeding program is being conducted. Over 50% of the black spruce and jack pine selections are now progeny tested. This rapid progress was possible due to excellent cooperation from all members in assisting with the *in situ* breeding and regular cone crops. Breeding has also commenced in the sublines for each species to generate full-sib progeny. Good progress has been achieved with 20% of the total number of crosses completed for both the jack pine and black spruce clones.

Gains

A series of realized gain tests was established for black spruce and jack pine in 1991. Seedlots consisted of rogued and unrogued orchard and unimproved stand checklots. These tests were measured when 5 years old. The unrogued black spruce orchard seedlot was the same height as the average checklot, however, the rogued orchard seedlot was over 6% taller which can translate into a potential volume gain of 18 to 20%. For jack pine, the unrogued seed orchard seedlot was 3% taller than the checklots, while the seedlot from the same orchard following a second roguing was over 4% taller. This has the potential for a 12 to 15% gain in volume. Stem straightness is an important quality trait for improvement in jack pine. The seedlot from the seed orchard rogued twice was 28% straighter than the checklots. Although these are early results, they certainly demonstrate the potential for increases in wood volume and improvement of stem straightness.

The Future

The NBTIC program has enjoyed a successful and productive 20 years. A continued strong commitment and cooperation among the membership will ensure constant growth for the program. Second-generation breeding programs are well underway for black spruce and jack pine and will commence for white spruce within 5 to 10 years. Although the total number of seedlings planted is significantly less than when the Council's program began, this situation is likely to change as seed of increasing genetic quality is produced and forest management strategies evolve. NBTIC looks forward to another 20 years of continued success.

Kathy Tosh
Dale Simpson

Seed Trivia - Did you know?

Whitebark pine seeds are very dormant, but seem to germinate more frequently in caches; this is seen in the clustering habit of whitebark pines growing in alpine regions. This special relationship between a corvid (member of the crow family) and a stone (hard-seeded pine) has evolved in two other sub-alpine regions of the world, in the Alps and in Russia.

Upcoming Meetings

Somatic Cell Genetics and Molecular Genetics of Trees

Joint Meeting of the IUFRO Working Parties S.04-07
and S.04-06
Quebec City
August 13 to 16, 1997
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The Tenth Annual Maritime Seed Orchard Managers Workshop

October 15-16, 1997
Fredericton, N.B.

For further information contact
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Diversity and Adaptation in Oak Species

IUFRO Working Party on Genetics of Quercus
2.08.05
October 12-17, 1997

For further information contact
Dr. Kim Steiner
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Recent Publications

Beardmore, T., S. Wetzel, D. Burgess, and P.J. Charest 1996. Characterization of seed storage proteins in *Populus* and their homology with *Populus* vegetative storage proteins. Tree Physiol. 10:833-840.

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Smith, R.F. and L.D. Yeates 1996. Proceedings of the Eighth Annual Maritime Seed Orchard Managers Workshop. Truro, N.S. Oct. 17-18, 1995. Nat. Res. Can., Can. For. Serv. Atlantic Region, 27 p.

Sorensen, F.C. 1996. Effects of seed chilling period and sowing date on family performance and genetic variainces of Douglas-fir seedlings in the nursery. New Forests 12:187-202.

Uniyal, R.C. and A.R. Nautiyal 1996. Physiology of seed development in *Aesculus indica*, a recalcitrant seed. Seed Sci. & Technol. 24: 419.

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Canadian Tree Improvement Association / Association canadienne pour l'amélioration des arbres

Tree Seed Working Group

News Bulletin Mailing List

The mailing list for our Working Group was last updated about 10 years ago. We are therefore due for an update. Please complete the information below and return to the Newsbulletin Editor at the address given below.

We will update the mailing list this coming fall after the next issue of the Newsbulletin.

Thank you for your cooperation.

Ron

✂_ ✂ _ ✂

Current mailing address:

Please continue to send me the Tree Seed NewsBulletin Yes___ No___

My mailing address above is correct: ________

Address corrections ______________________________

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Please return this form to:

This is your last chance !!!

Our records indicate that you have not updated your address to be included on our new mailing list. Please do so if you wish to continue receiving the 'Tree Seed News Bulletin'

In Memoriam - Guy Étienne Caron

The forest research community lost a dear friend and colleague on December 26, 1997. Our most recent past-Chairman, and long-time active member of our Tree Seed Working Group, Guy Caron passed away recently after a prolonged illness. For the many of us who knew him, Guy was the consummate gentleman, always wore a smile, had a positive outlook, and possessed a level of sincerity that was refreshing. At the University where he taught, Guy was well-respected by both students and colleagues. He genuinely enriched the lives of all around him and he will truly be missed. Our heartfelt condolences go to his family.

ERRATUM

Please replace pages 3/4 and 5/6 from the May 1997 Issue of the NewsBulletin with the attached pages.

Tree Seed Working Group Workshop

As you probably know, the theme of this year's Tree Seed Working Group Workshop is "The use of artificial pollination in seed orchards". We wish to demonstration that operational use of pollination techniques in forest seed orchards is both feasible and practicable. More specifically, we hope to foster debate on the issue of when and at what point in time is it possible/feasible to increase the genetic gain through artificial pollination? Among the topics on which we hope to stimulate discussions include increasing genetic gains (how much?), as well as the cost/benefit ratios of SMP. Debates on cost/benefit ratios for SMP should prove to be most interesting.

We will again be publishing the Workshop Proceedings in the Forestry Chronicle. We intend to have simultaneous translation services for the morning presentations.

The program will be as follows:

Moderator: **André Rainville** (Direction de la recherche forestière)

7 h 30 Registration

8 h 30 Welcome

8 h 45 Artificial pollination in Larch: from controlled crosses for breeding to seed orchard management.
M. Bonnet-Masimbert, I. Balder, L. Pâques, G. Philippe

9 h 15 Status and potential of using controlled parentage in operational reforestation.
G. W. Adams and K. J. Tosh

9 h 45 Mass control pollination in Loblolly pine to increase genetic gains.
F. E. Bridgwater and D. L. Bramlett

10 h 15 Break

10 h 30 Mass pollination in White spruce at Romieu seed orchard (Quebec).
S. Mercier, A. Savary, J. Grenier and C. Parent

11 h 00 T.S.W.G. business meeting

12 h 00 Lunch

13 h 30 Visit of MRN-pollen bank

14 h 30 Move to Station forestière de Duchesnay

15 h 00 **Visit of indoor seed orchard, demonstration of electrostatic gun and a vacuum pollen collector**

15 h 45 **Visit of intensively managed seed orchards**

16 h 30 **Move to University of Laval**

17 h 00 **Informal get-together**

André Rainville

Update on Internet Discussion Group

The transfer of the TREESEED discussion group on the Internet is complete, and the discussion group is up and running. Other than a couple of people who have had trouble subscribing to TREESEED, things seem to be running reasonably well. Traffic has not been especially busy, but some people have posted requests for information. While a number of replies under the TREESEED Discussion Group umbrella have been posted, most replies seem to have been sent directly to the individual who requested the information. This is probably the more desirable way to do business as it reduces the clutter on the electronic highway.

Ron Smith

Seed Trivia - Did you know?

Some seeds, e.g., Pacific silver fir, western redcedar and western hemlock, have resin vesicles in the outer layers of their seed coats. It is not known what the purpose these vesicles serve, but puncturing the vesicles reduces germination. There is no direct connection between the embryo and the outer seed coat, so it is uncertain why such treatment affects germination; it might be due to phytotoxic effects from terpenes in the resin, or because the injury presents a point of entry for microbial infection.

Special article

Fluorescein diacetate: A new viability stain for tree seeds.

Fluorescein diacetate (FDA) was tested as a viability stain with seeds of green ash, jack pine, Scotch pine, red pine, eastern white pine, Austrian pine, and eastern hemlock. In five tree species tested, the intensity of FDA staining of embryos seemed to correlate well with percent germination. In eastern hemlock and Scotch pine embryos FDA staining intensity also appeared to be correlated with germination, provided the stain incubation period was lengthened from 30 minutes to overnight (18h). Compared to tetrazolium chloride, the standard viability test for tree seeds, the potential advantages of FDA were better selectivity for living cells, faster speed of the procedure, and greater ease of interpretation. Based on these preliminary results, FDA shows significant promise as a quick and easy test for seed viability with the results being used to help evaluate seedling and seed quality for nursery and reforestation operations.

Introduction

Fluorescein diacetate was first used as a stain to indicate mammalian cell viability thirty years ago (Rotman and Papermaster 1966). These authors suggested nonfluorescing, relatively nonpolar FDA penetrated the cell membrane readily, then esterases cleaved off the acetates to form the fluorescent product fluorescein. Once it formed inside the cell, the more polar fluorescein diffused out through the cell membrane at a much slower rate than its precursor, FDA, entered the cell. Therefore, only viable cells with an intact plasmalemma and functioning esterase enzymes would have cytoplasm that accumulated enough fluorescein to fluoresce brightly (Rotman and Papermaster 1966).

Fluorescein diacetate viability staining has been primarily applied to single cell or simple organisms with a few exceptions of more complex tissues being used such as detached buds, root cortex cells and orchid seeds, which are tiny, undifferentiated protoseeds. The *objective* of this study was to try to expand the application of FDA by determining if FDA could be used as an effective viability stain for tree seeds and roots.

Materials and Methods

Green ash (*Fraxinus pennsylvanica* Marsh.), Austrian pine (*Pinus nigra* Arnold), jack pine (*Pinus banksiana* Lamb.), eastern white pine (*Pinus strobus* L.), red pine (*Pinus resinosa* Ait.), Scotch pine (*Pinus sylvestris* L.), and eastern hemlock (*Tsuga canadensis* (L.) Carr.) seed were used for viability staining.

Seed of all species were soaked in aerated water for 18 hours and placed in sealed 3 mil plastic bags at +2°C for stratification. Austrian pine, jack pine, red pine and Scotch pine seed were stratified for 14 days before germination. Eastern white pine and green ash seed were stratified for 60 days and eastern hemlock for 120 days before germination. Germination was conducted in growth chambers with a 16/8 day/night photoperiod and a thermoperiod of 20/30°C for 16/8(midday) hours, and 75% humidity. For eastern hemlock, germination conditions were continuous dark at 20°C and 75% humidity. Germinations were counted twice a week.

Fluorescein diacetate staining was done on 4 replications of 10 embryos for each species with the following procedure. Dry seed were soaked 18 hours in aerated deionized water. All seeds were incubated for 30 min in a solution of 0.2 g FDA in 100 ml acetone. The 18 hr seeds were immediately removed from the FDA solution and incubated in a sealed vial at room temperature (22°C). Embryos were dissected from the seeds and examined for viability with a Leitz Diaplan epifluorescence microscope, under 450-490 nm blue light (long-pass suppression filter 520 nm). They were rated '0' if no yellow-green fluorescence was present, '50' if fluorescence was pale or patchy over the embryo surface, or '100' if fluorescence was bright and uniform over the entire surface of the embryo.

Results and Discussion

The relationship between germination and average FDA staining of the embryo was strongest in green ash, white pine, and Austrian pine (Figure 1A-C). The relationship between FDA staining and germination in jack pine and red pine (Figure 1D&E) is also evident but slightly more variable. Only in hemlock (Figure 2A) and Scotch pine (Figure 2C) did FDA staining intensity seem to be much lower and different than percent germination. The weak relationship between FDA and germination in these two species may have been due to insufficient incubation time since the relationship appeared much stronger when seed and stain incubated overnight (Figures 2B&D).

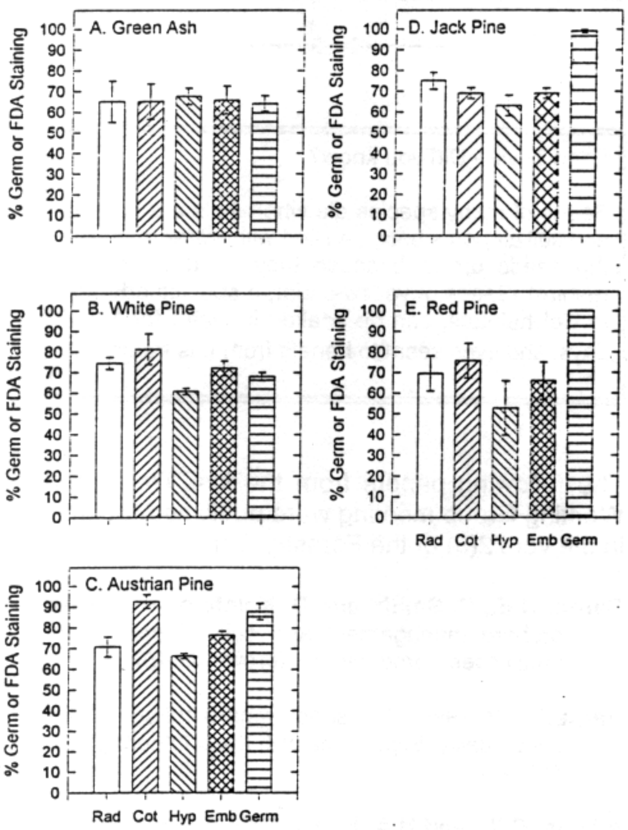

Figure 1. Fluorescein diacetate staining intensity and percent germination of A. green ash, B. eastern white pine, C. Austrian pine seed, D. jack pine, and E. red pine seed. For germination and staining n =4, mean ± standard error. Abbreviations, Rad=radicle, Cot=cotyledons, Hyp=hypocotyl, Emb=embryo, and Germ=germination

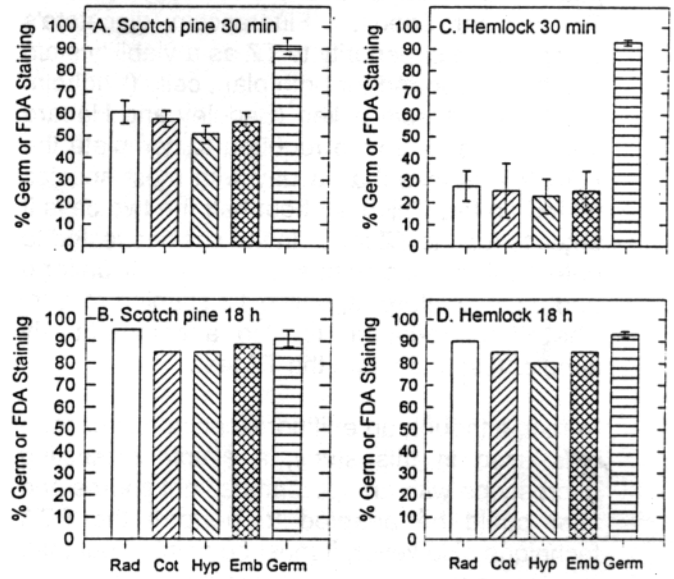

Figure 2. Fluorescein diacetate staining intensity and percent germination: Effect of incubation time. A. eastern hemlock seed 30 minute staining B. eastern hemlock seed 18 h staining C. Scotch pine seed 30 minute staining, D. Scotch pine seed 18 h staining. For germination and 30 minute staining n =4, mean ± standard error, for 18 h staining n = 1.Abbreviations, Rad=radicle, Cot=cotyledons, Hyp=hypocotyl, Emb=embryo, and Germ=germination.

The current standard viability test for dormant tree seeds like green ash is and eastern white pine is the tetrazolium chloride (TZ) test (Justice 1972). Unfortunately, it can take years of practice to be able to correctly interpret the TZ test results (Bonner *et al* 1994). Some of the problems associated with the TZ test include difficulty staining some seeds, lack of agreement between staining and germination results, especially for seed with low germination, and the lack of significance of staining intensity coupled with the critical importance of stain location on the embryo (Justice 1972, Bonner *et al* 1994). Finally, the TZ test usually takes over 2 days to complete (Bonnet *et al* 1994).

The FDA test for seed viability offers many potential advantages over the currently accepted TZ test for seeds. Fluorescein diacetate's comparative superiority to TZ as a viability stain has been reported for both plant cells (Widholm 1972) and *Acacia* pollen (Sedgley and Habard 1993). The total time required to complete the FDA test is less than a day with most species tested so far, which is about half the two days it takes to run the TZ test. In the species tested to date, FDA staining seems to be relatively uniform and easy to interpret, unlike TZ staining results. The FDA procedure is also a comparatively simpler test to do than the TZ test.

In fact, although an epifluorescence microscope was used in this study, a simple dissecting microscope with an appropriate light source and filter could be adapted to perform the FDA technique. However, it must be pointed out that, so far, only preliminary results have been gathered for the FDA test and the uniform applicability of the FDA test to other species and for seeds of the same species with varying germination remains to be shown.

Conclusions

FDA appears to show significant promise as a viability stain for tree seeds. In all seven tree species tested, FDA staining of embryos seemed to correlate well with percent germination provided the incubation period was long enough. Although further testing will be needed to confirm the efficacy and general applicability of FDA as a viability stain for tree seeds, FDA appears to have the potential to become the superior method used to quickly determine viability.

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Thomas L. Noland and Gina H. Mohammed
Ontario Forest Research Institute

Seed Trivia - Did you know?

Seeds of many species die when soils are flooded and soil air pores become filled with water (actually, the seeds drown because they need oxygen to respire). Seeds of yellow-cedar, a species adapted to wet habitats, can be soaked in water for 10-14 days, and even seem to benefit from this treatment.

The following papers from the last Tree Seed Working Group meeting were published recently in the vol 72(5) of the Forestry Chronicle

Caron. G.E., R. Smith, and D. Kolotelo 1996. Seed orchard management and cultural options for quality seed production - foreward. 467

Barnett, J.P. 1996. How seed orchard culture affects seed quality: Experience with the southern pines. 469-474

Adams, G.W. and H.A. Kunze 1996. Clonal variation in cone and seed production in black and white spruce seed orchards and management implications. 475-480

Edwards, G.E. and Y. El-Kassaby 1996. The biology and management of coniferous forest seeds: Genetic perspectives. 481-484.