the NEWS LETTER

of the

ASSOCIATION OF OFFICIAL SEED ANALYSTS

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THE NEWSLETTER

OF THE

ASSOCIATION OF OFFICIAL SEED ANALYSTS

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1976 MEETING

The staff of the Pennsylvania Seed Laboratory is looking forward to extending a warm welcome to Pennsylvania, the Bicentennial State. We still have a number of things to do to get ready. We hope you will pre-register even if it is past the deadline, since it will help us in our final planning.

The following is a rough outline of the program:

Friday -	June 18,	P.M. SCST Executive Board
Saturday -	June 19,	A.M. SCST Examination - AOSA Executive Board
		Evening - SCST President's Coffee Hour
Sunday -	June 20,	A.M. Joint Opening Session
		P.M. SCST Open Business Meeting
		Evening - SCST Banquet
Monday -	June 21,	A.M. SCST Research - AOSA Referee
		SCST Business Meeting
		P.M. Open House - Seed Laboratory
Tuesday -	June 22,	Symposium on Seed Vigor and Deterioration
Wednesday	June 23,	A.M. & P.M. Technical Papers
		Evening - AOSA Banquet
Thursday	June 24,	A.M. - Technical Papers
		A.M. - AOSA Business Meeting
		P.M. - Tour to Pennsylvania Dutch Country and Supper

Many open and closed committee meetings are scheduled.

There will be something planned for the ladies and children and many things they can do on their own.

If you have any questions or need information, contact:

Wendell P. Ditmer
PA Dept. of Agriculture
2301 North Cameron Street
Harrisburg, PA 17120
717-787-4894 or 5609

NOTE FROM YOUR EDITOR

The Newsletter is your organ for letting your fellow seed analysts know what is happening in your laboratory or your state. Some of the issues of the Newsletter have been very slim, and with rises in costs it is difficult to justify printing four issues a year. Not only do we need articles from laboratories but why not reports from committees? The membership likes to know what is going on in AOSA.

We would like to hear from Associate and Honorary members, members of SCST and ISTA also.

Thank you for your support in 1975-76.

DEADLINE - JULY 2, 1976

FOR RECEIVING MATERIAL FOR

AUGUST NEWSLETTER

MARK THIS DATE ON YOUR CALENDAR

CURIOUS BICENTENNIAL POEM

Editor's Note: During the Revolution, while British troops occupied Philadelphia, this double-meaning poem appeared in the Philadelphia Gazette. Read as a whole, it appears to espouse loyalty to the Crown. We have set part of it in italics, however, to show its dual meaning. When the left and right sections are read separately, the true significance of the verse emerges.

Hark, hark, the trumpet sounds, *the din of war's alarms*
O'er seas and solid ground, *doth call us all to arms.*
Who for King George doth stand, *their honor soon will shine,*
Their ruin is at hand, *who with the Congress join.*
The acts of Parliament, *in them I much delight.*
I hate their cursed intent, *who for the Congress fight;*
The Tories of the day, *they are my daily toast,*
They soon will sneak away, *who independence boast;*
Who nonresistance hold, *they have my hand and heart,*
May they for slaves be sold, *who act a whiggish part.*
On Mansfield, North and Bute, *may daily blessings pour*
Confusion and dispute, *on Congress evermore*
To North, the British Lord, *may honor still be done*
I wish a block or cord, *to General Washington*

NOTES FROM LABORATORIES AND MEMBERS

ALASKA

P. Mulligan

The Alaska Seed Testing Laboratory was established and accepted into the Association of Seed Analysts (AOSA) in April, 1975. The laboratory exists within the jurisdiction of the Alaska Plant Materials Center which operates a 285-acre farm in the Matanuska Valley near Palmer.

Our first year of existence accounted for 168 purity and germination tests, mostly on barley, wheat, oats, kentucky bluegrass, red fescue, timothy, and some tree and woody shrub work. Germination tests are also conducted to confirm label compliance on vegetable seed lots for both commercial and garden producers.

Current operations are conducted in a remodeled farm house, with construction beginning this spring on a new office/laboratory which will house both the PMC office and seed lab.

Special seed laboratory projects include one designed to determine purity and germination standards for Bluejoint (Calamagrostis canadensis), a native rhizomatous grass which has developed economic interest, due to its widespread adaptability, for revegetation along the Trans-Alaska Oil Pipeline. This small seeded grass (3.7 million/pound) offers intriguing problems in the establishment of purity and germination procedures. Any helpful hints or suggestions from AOSA members will be gratefully appreciated.

Current staffing of the lab is one seed analyst, myself, for approximately 50% of my time (mostly in the winter), the remainder being spent in initial evaluation of herbaceous plants, seed production studies, foundation grain and grass seed production, and strawberry nursery stock production.

AOSA members traveling in Alaska are urged to inspect the PMC and seed laboratory, a visit to which can only be topped by the magnificant scenery in the 49th state.

* * * * *

ARIZONA

R. A. Countryman

Seed enforcement officials continually run into the problem of dormancy when testing seed and find that the seed label reads a 90% germination, and when tested without breaking dormancy we find germinations in the 60's.

This is fine for the seed technologists and analysts to prechill and then test the seed to get a final germination, but this is not a true test. The farmer or homeowner is getting "rooked" in establishing a stand of grass or a crop where annual ryegrasses and small grains marketed in Arizona are concerned.

The buyers complain to us that their seed is not coming up; and in areas such as Arizona where lawns and crops have to be irrigated, it makes for a frustrating situation, as well as a costly one, if you want maturity of a crop at a specific time that will harvest properly or meet a market.

I believe we either are going to have to write legislation or have analysts come up with some realistic solution to this matter.

Examples concerning dormancy problems are:

1. Recently harvested and processed seed (Ryegrasses and small grains) are shipped from the northern part of the U. S. into the south and southwestern U. S. for early fall plantings.

2. Companies engaged in small grain seed increases produce a crop in the southwestern U. S., and immediately after harvesting and processing they ship it to colder climate areas in adjacent states or to the northern part of the U. S. for seed increase the same year. The lapse of time in harvesting to planting in some cases is just three weeks.

* * * * *

IOWA

L. E. Everson

Bonnie Jenkins retired February 15, 1976 after 23 years of dedicated professional service in the Iowa State University Seed Laboratory, serving as the purity analysis supervisor for most of those years. Many persons profited from the training she gave,

and all persons acquainted with Bonnie know what a fine person she is. We wish that Bonnie could have continued to stay with us but know that she much deserves to enjoy the benefits of retirement. If you would like to keep in touch with Bonnie her present address is:

Mrs. Bonnie Jenkins
2312 N. E. 37th Terrace
Kansas City, MO 64116

Before Bonnie left she wrote a poem to her many friends. Since it tells so much about her personality, we are taking the liberty of including it here.

FRIENDS

Occasionally the fire burns low
And smoldering embers lose their glow;
We throw on a log, and stir about,
To keep the fire from going out.

Just so, by a dear friend's gentle word
The lagging heart is often stirred.
Hope is restored, fears put to flight
And once again the world seems right.

I'm sure it must have been God's plan--
Knowing how weak is the faith of man--
He provides the friends that our souls require
To "throw on a log" and replenish the fire.

* * * * *

NATIONAL SEED STORAGE LABORATORY

L. N. Bass

I am pleased to announce that Dr. Phillip C. Stanwood has recently joined the staff of the National Seed Storage Laboratory as an Agronomist. His primary responsibilities are supervision of the Germination Laboratory which includes cleaning and storing incoming seed samples, as well as testing for viability.

Dr. Stanwood, age 31, was born and raised in Modesto, California, located in the heart of the Central Valley agricultural region. He received a B. S. degree in Agronomy from Cal Poly, San Luis Obispo, California, and a M. S. and Ph.D. in Crop Physiology from Oregon State University, Corvallis, Oregon.

He has served as an Administrative Assistant at the US Army Biological Research Center, Fort Detrick, Maryland; a Soil Conservationist with the USDA Soil Conservation Service, Annapolis, Maryland; and most recently as Project Leader (seed research and production), O. M. Scott and Sons, Pacific Northwest Research Station, Salem, Oregon.

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NATIONAL SEED TESTING STANDARDIZATION LABORATORY

E. W. Sundermeyer

The additional space for which we have been patiently waiting has become available, and renovation work is now going on in the new space, as well as in existing space. We are attempting to carry on among the carpenters, electricians, plumbers, and painters.

We are hopeful that by June we will have things looking more nearly like we want them to look.

We extend a hearty invitation to everyone who will be coming to Hershey for the AOSA-SCST meetings to come by Beltsville for a visit.

Twenty-two official and commercial seed analysts from eight mid-Atlantic States attended a Seed Testing Workshop at NSTSL, Beltsville, Maryland, February 2-6, 1976.

Purity and identification studies included Agropyron, Elymus, Bromus, Agrostis, Vicia, Brassica, Sisymbrium, and cultivars of Kentucky bluegrass and soybean.

Germination studies included soybean, wheat, Japanese millet, alfalfa, crownvetch, Kentucky bluegrass, tall fescue, lettuce (including physiological necrosis interpretation), endive, onion and pelleted and taped seed. Tetrazolium tests and accelerated aging tests on soybean were demonstrated and discussed. Demonstrations and discussions were also conducted on special disease problems.

Purity instructors were Mrs. Elizabeth Wiseman and Mrs. Constance Ogburn. The germination sessions were instructed by Miss Eltora Schroeder and Mrs. Jane Skinner. Mr. James F. Schoen worked with those interested in disease problems.

Dr. Miller McDonald conducted the sessions on TZ and vigor testing and Dr. Richard Payne demonstrated methods for distinguishing soybean varieties.

Guest speakers were Mrs. Vivian Toole and Dr. Charles R. Gunn, both of the Agricultural Research Service. Mrs. Toole

discussed environmental effects on seed germination. Dr. Gunn presented an illustrated lecture on distinguishing Vicia spp.

Mrs. Wiseman conducting a purity session.

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NORTH CAROLINA

G. Sullivan

"First - The Seed" will be a featured exhibit at the North Carolina State Fair in October of 1976. The exhibit will trace the important role of seeds from the beginning of civilization to their current role in our daily lives. Displays on seed improvement, production, processing, and testing will be included. The exhibit will be housed in an 80 foot geodesic dome.

The exhibit will include a large display of seeds. In the seed display area, emphasis will be on the variation among seeds in size, shape, color, surface texture and special dispersal means.

Will you assist in this display by sending samples of seed from your state? We need crop seed, vegetable seed, tree seed,

herb seed, flower seed, weed seed, etc. Please send a sample of each kind of seed you have available to:

Gene A. Sullivan
Crop Science Seed Specialist
North Carolina State University
P. O. Box 5155
Raleigh, North Carolina 27607

We need approximately one cup of seed in each sample. Please include the scientific name and common name of sample you send. Thanks for your help.

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SOUTH DAKOTA

R. C. Kinch

Dr. Allyn O. Lunden has just been named Supervisor of the South Dakota Seed Laboratory.

Dr. Lunden obtained his B. S. degree in Agronomy at South Dakota State University in 1952 and an M. S. from the same University in 1956 in Seed Technology, working under the direction of Professor R. C. Kinch. He received his Ph.D. degree at the University of Florida in 1960. He worked at Oak Ridge, Tennessee, with the Atomic Energy Commission for four years.

He has been leader of the sorghum and soybean projects at South Dakota State University since 1964 and is transferring to Supervisor of the Seed Laboratory July 1, upon my retirement.

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ANNOUNCEMENTS

REVISION TO "RULES FOR TESTING SEED"

The revisions of the "Rules for Testing Seeds" are ready for distribution.

All AOSA member laboratories of the Association, SCST, CSAAC and accredited ISTA member laboratories will receive one set each, free of charge, with the May Newsletter.

Extra sets of the revisions, of course, may be ordered and any person may order as many sets as required. The cost per set will be $3.00.

Orders will be billed and sent out as quickly as possible after they are released from the printers.

For copies other than those supplied free as described above, write:

R. H. Hofmann, Secretary-Treasurer, AOSA
Federal Seed Laboratory
P. O. Box 1705
North Brunswick, New Jersey 08902

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THE NEED FOR NEW SEED ANALYSTS

There is a critical shortage of people, especially young people, in seed testing technology. During the past year the Seed Branch (AMS, USDA) has been recruiting to fill positions in the NSTSL at Beltsville, Maryland, and in the enforcement section at Hyattsville. We have found what many of you know--that seed analysts are scarce, and graduate agronomists, horticulturists, or botanists with experience or training in seed testing, are hard to find. State and industry employers are experiencing this problem with the USDA. There simply are not enough people with careers in seed testing to meet the needs.

For various reasons, fewer students are working in public and private seed laboratories. Fewer seed laboratories are using student help. It's too bad. Students who work in seed labs gain valuable practical knowledge and are good part-time help. They start as trainees and develop into semi-independent analysts. They are particularly useful during the seasonal rush of many laboratories. The student employment helps to start them in careers in seed technology.

Although the seed industry is not rated as a large employer, it needs a constant supply of new skilled employees, including seed analysts. Colleges and public and private seed labs are urged to acquaint new students with the need for analysts and to use their services whenever they can. It will be of benefit to all.

* * * * *

AOSA/SCST BICENTENNIAL SYMPOSIUM ON SEED VIGOR AND DETERIORATION

Second Announcement

A Bicentennial Symposium on Seed Vigor and Deterioration will be held during the Association of Official Seed Analysts (AOSA) and the Society of Commercial Seed Technologists (SCST) joint meetings on Tuesday, June 22, 1976 at Hershey, PA.

The keynote speaker will be Dr. W. H. Bragonier who will discuss, "Seed Vigor--What Is It?". Other topics will include "Seed Trade Viewpoint on Vigor"; "Regulatory Viewpoint on Vigor", Mr. George Spain; "Measurement of Vigor", Dr. D. F. Grabe; "Development and Evaluation of Vigor Tests", L. W. Woodstock; "Maintenance and Invigoration", Dr. A. A. Khan; "Field Performance", Dr. J. S. Burris; "Standardization of Vigor Tests", Dr. J. C. Delouche; and "Genetic Aspect of Seed Vigor", Dr. W. R. Kneebone.

Those who register for the AOSA/SCST meetings will be able to attend the Symposium during the meetings. Those interested primarily in attending the Symposium should contact Dr. L. W. Woodstock, Secretary, Symposium Planning Committee, ARS, Seed Research Laboratory, Room 105-C, Building 006, BARC-West, Beltsville, MD 20705.

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SEED BRANCH AND PLANT VARIETY PROTECTION OFFICE MOVE TO WASHINGTON, D.C.

The Seed Branch and Plant Variety Protection Office of the Grain Division, Agricultural Marketing Service, U. S. Department of Agriculture, have moved to the South Agriculture Building at 14th and Independence Avenue, SW., Washington, D.C. 20250. The move from Hyattsville, Maryland, was made on March 13, 1976.

Clyde R. Edwards, Chief of the Seed Branch, will be in Room 0648, telephone number 202-447-9340. The Enforcement Section will be temporarily located in Room 0764, temporary telephone number 202-447-9323, until permanent space next to the Chief's Office is renovated. When the Enforcement Section moves into its permanent space, it will have the same telephone number as the Chief's Office. The National Seed Testing Standardization Laboratory will remain at the Agricultural Research Center, Beltsville, Maryland.

Stanley F. Rollin, Commissioner of the Plant Variety Protection Office, will be in Room 1634, telephone number 202-447-9170. The Plant Variety Protection Examiners Office is located at

the National Agricultural Library, Beltsville, Maryland, telephone number 301-344-2518.

This is the final phase of moving all of the Grain Division offices from Hyattsville to the South Agriculture Building, Washington, D.C..

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VACANCY ANNOUNCEMENT

POSITION: Purity Analyst, Iowa State University Seed Laboratory.

DATE OPEN: March 1, 1976

RESPONSIBILITIES: To conduct purity analysis on a large number of seed species including soybeans, oats, small seeded legumes, turf grasses and range grasses.
Help train beginners in purity analysis.
To provide leadership for purity analysts with less education and experience.
Aid in teaching seed analysis short courses.
Act as a host for visiting groups.
Aid in the checking of seed sample reports.

QUALIFICATIONS: High school education with some college courses desirable.
Demonstrated interest in seed technology.
Experience in seed testing, in particular purity analysis so that rapid, accurate tests of small seeded legumes and grasses can be conducted.
Ability to communicate and work with others.
Ability to train beginning analysts.
Botanical knowledge pertaining to the structure of seeds.

SALARY: Open, depending on training and experience.

FRINGE BENEFITS: TIAA and Cref annuity programs, annual vacation, sick leave, disability insurance, group medical, hospital and group life insurance optional.

APPLICATION: Send resume and letter of application to: L. E. Everson, in charge, Iowa State University Seed Laboratory, Old Botany Bldg., Ames, Iowa 50011.

FILING DEADLINE: April 1, 1976 or until position is filled.

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"PLANNING FOR THE 1980 CENSUS: WHAT ARE YOUR SUGGESTIONS?"

The decennial census is a major national undertaking, and census data are widely used in many important government, private, and community programs. You have an important stake in the decennial census, both as a member of AOSA and as an American citizen.

The Census Bureau is now actively working on plans for the 1980 census and important decisions have to be made in the relatively near future. For example, the full content of the basic census questionnaire must be determined by the spring of 1977 so that further preparatory steps can be accomplished successfully.

Although there are many constraints on the census in terms of what and how much information can be collected and tabulated, the Bureau believes that it is very important to obtain and review the recommendations of as wide a range of users and potential users of decennial census data as possible. The Census Bureau is therefore anxious to have the ideas of the members of AOSA.

If you have any suggestions, questions, or comments on the 1980 census, please send them to:

Director
U. S. Bureau of the Census
Washington, DC 20233

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ABSTRACTS OF PAPERS TO BE PRESENTED AT THE ANNUAL MEETING

Evaluation of Thin Layer Chromatography Methods for Soybean Cultivar Identification (a)

The leaf extract of single seedlings and plants grown from the breeder's seed of two soybean cultivars were studied by thin layer chromatography. Differences in the chromatogramic pattern between the spots of leaf extract within the cultivars were observed. When the extract of the progeny of these plants was investigated under these conditions (0.25 mm thick Silica Gel-G plates in HCOOH: n-BuOH: H_2O) the spots visualized by spraying with Tetraphenyl Diboroxide Ethanolamine complex were identical as those of their parent plant.

(a) N. S. Dhesi, M. Malaiyandi, R. W. Desormeaux
Authors

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Identification of Empty Seed by Radiography and by a Cutting Test.

E. W. Belcher, Jr.,

A more precise and detailed knowledge of the internal structure of tree seed is obtained by radiography. However, this increased knowledge of the internal seed structure has required some refinement of definitions. Where with a cutting test a seed is considered filled or empty, many degrees of filled or emptiness can be identified on the radiograph. Selected seed were subjected to x-ray and then to a cutting test. The results are presented and discussed in detail.

* * * * *

Laboratory procedures useful for the differentiation of soybean cultivars

Richard C. Payne

Laboratory procedures useful for differentiating soybean cultivars will be discussed. These procedures will include seed coat peroxidase activity, seedling response to germination at 25°C in the dark for 10 days, and observations of plants grown in a growth chamber with continuous high intensity lighting.

* * * * *

Scientific Names of Vascular Plants in the AOSA "Rules for Testing Seeds 1970" and Amendment 1974

Charles R. Gunn and Sara McAndrew

The six hundred and forty-eight scientific names of vascular plants in the AOSA Rules and 1974 amendment were carefully reviewed and correlated with the Federal Seed Act and the International Seed Testing Association. About twenty percent of the names were updated. Although no major review of common names was undertaken, a few changes are noted. Common names in the AOSA Rules and the Federal Seed Act (as updated in 1975) were correlated.

* * * * *

Development of a Varietal Purity Testing System for Soft Red and Soft White Winter Wheat

G. W. McKee, Diane Knight, W. P. Ditmer and W. R. Cook

Varietal purity testing systems were devised and tested for varieties of soft red winter wheat grown in the Northeastern States and for available varieties of soft white winter wheat grown

in the United States. The testing system utilized the phenol test, KOH test, coleoptile color, accelerated heading, leaf pubescence, seed length, and paper and thin layer chromatography. For each variety, authentic varietal characteristics were derived from several lots each of foundation or breeder seed. It was found that foundation seed of some lots of some varieties contained an appreciable degree of mixture. Development and use of wheat varietal purity testing systems will be discussed.

* * * * *

A Quick Method for Removing Coating Material from Prill-Coated Alfalfa Seed

M. S. Dhaliwal and F. J. Lewis

A quick, simple and safe technique has been developed to remove the coating material from prill-coated alfalfa seed to facilitate purity analysis and to assess seed quality, which are required for compliance with the seed regulatory standards. This technique involves washing of the coated seed with distilled water, pH 8 - 8 8.4.

*Prill-coat is a trade mark of the Prillcoat Industries, Inc., CA

* * * * *

Effect of Coating Materials on Seed Moisture Microenvironment

Eric E. Roos and Gregory Jackson

Seed coatings on carrot, lettuce, and onion from several commercial sources were found to vary widely in their hydrophobic or hydrophillic properties. Too much of too little moisture in the germination substratum drastically altered the final germination in standard tests. However, all kinds of seeds were not equally sensitive to these coating-moisture interactions. Removing the coating material prior to germination was a less desirable alternative to careful control of substratum moisture.

* * * * *

The Segmented Test Procedure

Walter F. Westrin

Seed "lots" are sampled and tested by the Michigan Dept. of Agriculture to obtain usable and timely information about seed quality for the proper enforcement of Seed Laws. The more seed lots that are sampled, tested and reported before or early in the spring

and fall seeding seasons, the more usable and timely the information obtained will be to the MDA, the seed users and the seed vendors. Obviously, when field action can be taken on lots found in violation before the seed has been dispersed, sold or planted, the more effective seed law enforcement will be and the more protection and service will be provided to the buyer, seller and user of seed.

* * * * *

Evaluation of Barley Cultivars for Seedling Growth

Loren E. Wiesner

Seed of 25 barley cultivars, which were produced at Bozeman, Montana in 1975, were evaluated for percentage seed dormancy, speed of germination, speed of elongation, speed of emergence and dry matter production.

The two cultivars which had the highest percentage seed dormancy (Steptoe and Vantage) were slowest to emerge from soil and their seedlings dry weight did not increase rapidly. Those dormant cultivars also had a low indices for speed of germination and elongation. The cultivar "Hein Flo" produced the most dry matter per 100 seedlings and ranked high in speed of emergence, germination and elongation. "Hulless Compana" an isogenic line of Compana derived from a Stamm/7*Compana cross was inferior to Compana for speed of elongation, emergence and weight per 100 seedlings.

* * * * *

A method for germinating plastic coated rape seed

B. Borrel and D. Ashton

The efficacy of several organic solvents in dissolving a thin covering of polyvinyl chloride (plastic solid) on rapeseed was investigated. Tetrahydrofuran and 1,4-Dioxane removed the plastic coating within 60 seconds. Exposure of the seeds to the solvent for 30 minutes had no adverse effect on subsequent seedling development.

Mechanical removal of the plastic coat was found impractical as a routine laboratory method while alternating freezing and thawing proved damaging to the embryo.

* * * * *

Improving Germination of Indian Ricegrass Seeds

Miller B. McDonald, Jr.

Indian Ricegrass /Oryzopsis hymenoides (Roem. & Shult.) Ricker/ seeds fail to germinate without a scarification pretreatment (conc. H_2SO_4, 35 min). The pronounced post-harvest dormancy of freshly harvested seeds was relieved by 100 uM GA_3. Control of fungal pathogens following scarification was achieved with a 40% Maneb dust. These treatments, in combination, produced 80 - 90% germination in 7 days at 30°C. The potential of these methods is discussed.

* * * * *

The Effect of Temperature and Light on Seed Dormancy in Western Wheatgrass

Quentin Schultz and R. C. Kinch

Lots of Western wheatgrass (Agropyron smithii) were tested by four methods which contrasted the standard germination procedures (darkness, alternating temperatures) to determine their effect on seed dormancy. Exposure to constant temperatures and white light significantly increased seed dormancy over the control. Short exposures to Red and Far Red light decreased dormancy of the seed lots over the standard germination procedure. Red light at four minutes exposure time gave a significant decrease in dormant seeds.

* * * * *

The detection of potentially low yield seed lots of small-grained cereals

R. Robinson

Differences in vigour and field performance are well documented for several types of crop seed, but small-grained cereal crops, such as wheat and barley, have been investigated in far less detail.

Evidence is presented to show the difference in performance which can be found between seed lots of the same varieties of wheat and barley under U. K. and U. S. A. field conditions.

Techniques have been developed to successfully detect these potentially low yielding seed lots, and the importance of this rejection system is discussed.

* * * * *

Further evidence on the relationship between vigor tests and field stands.

James F. Harrington

Ten lots of Calmar variety lettuce of commercial quality (over 90% germination) were obtained from California producing seed companies. Germination, first count, and slantboard tests in the laboratory were and are being compared with field plantings under various stress levels in lettuce growing areas in California. The results to date indicate that even with quality lots separation is possible on the basis of germination and vigor and these separations correlate significantly with field stands but not rate of emergence in field. Data on yield will be collected.

* * * * *

The Weibull Function: A New Method of Comparing Seed Vigor.

F. T. Bonner & T. R. Dell

The three-parameter Weibull function provides a mathematical model for describing frequency of seed germination. The C parameter of this function is concerned with the shape of the germination curve, and thus can be used to quantify differences in germination response between seed lots. Germination data for two tree species, sweetgum and white oak, are presented to illustrate how differences in vigor, produced by artificial aging, can be quantified with Weibull function parameters.

* * * * *

Bioassay techniques for fungicide treated seeds in Pennsylvania.

S. H. Kim

In Pennsylvania, seeds of barley, wheat, oat, sorghum, rye, squash, cucumber, sweet clover, beans, pea, beet, watermelon, mustard, spelt, spinach, corn and grasses treated with fungicides were bioassayed using two techniques. Seeds treated with captan, mercuries, carboxin, carbamates and thiocyanomethylthiobenzothiazole were bioassayed against Aspergillus niger (A. Halfon-Meiri and I. Dishon. 1964. A biological method for testing the efficiency of peanut seed treatments. PDR 48:853-854), and seeds treated with pentachloronitrobenzene were bioassayed against Rhizoctonia solani (S. H. Kim. 1976. Fungistasis and degradation of pentachloronitrobenzene by vapor. Proceedings of the American Phytopathological Society. Vol. 3). Both of the methods were applied when an unknown compound was involved. Both of the bioassays were concluded in 24 hours.

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COMMITTEE ACTIVITIES OR REPORTS

CONSTITUTION COMMITTEE
G. D. Koskinen

The following proposed changes in the By-Laws will be presented to the Association at the 1976 Annual Meeting. An affirmative vote by two-thirds of the member laboratories present and voting shall be required for adoption of the proposed changes.

1. By-Law I

   Election of Officers and Executive Board Members, Section 1

   Make the following underlined addition to the fifth sentence:

   The ballots for the election of Vice-President and Secretary-Treasurer shall include at least two, but no more than three, top available candidates for each office, <u>except in the case of a tie vote</u>.

2. By-Law II

   Membership, Section 3 (Honorary Members)

   Make the following underlined addition to the fourth sentence:

   If approved <u>by the executive board</u>, the name will be presented to the delegates at the Annual Meeting.

3. By-Law V

   Standing Committees, Section 7 (Membership Committee)

   Make the following underlined addition to sentence two:

   The committee has the privilege of submitting names for honorary membership <u>to the executive board for its approval before presentation</u> for election at the Annual Meeting.

See the A.O.S.A. Newsletter, Volume 49, No. 3, August 1975, pages 3, 4, and 5, Minutes of Executive Board Meeting, Portland, Oregon, for action taken by the Executive Board relative to the above changes.

PROPOSAL UNDER CONSIDERATION

A proposed new category of membership:

<u>Member Affiliate</u>

Requirements and qualifications yet to be defined.

Anyone having suggestions for changes to the Constitution and By-Laws, please submit the suggestions in writing to the Constitution Committee chairman. Copies of the Constitution and By-Laws are available on request from the AOSA Secretary-Treasurer or the Constitution Committee chairman.

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MEETING PLACE COMMITTEE

C. E. Christenson

Nebraska has extended an invitation to host the 1978 AOSA-SCST Meeting.

Any member laboratory that may have intentions of hosting future meetings should let their wishes be known in writing to The Meeting Place Committee.

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RULES COMMITTEE

L. E. Wiesner, Chairman
Proposals for Rules Changes

(These proposals were distributed in a loose form with the February 1976 Newsletter)

These proposals will be discussed and voted on at the AOSA meeting in Hershey, PA. Please study each proposal carefully and send your comments to the committee chairman.

Revision of "Rules" sections
2.6, 2.7, 2.9, 2.10, 2.11, 2.12, 3.1 and 4.8

2.6 Seed unit. - The seed unit is the structure usually regarded as a seed in planting practices and in commercial channels. The seed unit may consist of one or more of the following structures:

a. True seeds.

b. Seed units in the grass family:

(1) Caryopses and florets;

(2) Multiple florets and spikelets in tall oatgrass (Arrhenatherum elatius), oat (Avena), gramas (Bouteloua), rhodesgrass (Chloris gayana), barley (Hordeum) and bluegrass (Poa);

(3) Entire spikelets in Panicum, Setaria and Sorghum;

(4) Spikelet groups that disarticulate as units with attached rachis and internodes in bluestems (Andropogon), side-oats grama (Bouteloua curtipendula) and yellow indiangrass (Sorghastrum nutans);

(5) Fascicles of buffelgrass (Pennisetum ciliare) consisting of bristles and spikelets;

(6) Burs of buffalograss (Buchloe dactyloides)

(7) Bulblets of bulbous bluegrass (Poa bulbosa).

c. Dry indehiscent fruits in the following plant families; buckwheat (Polygonaceae), sunflower (Compositae), geranium (Geraniaceae), goosefoot (Chenopodiaceae), and valerian (Valerianaceae).

d. One - and - two seeded pods of small-seeded legumes, burs of the bur clovers, and pods of peanuts. (This does not preclude the shelling of small-seeded legumes for purposes of identification). Pods of legumes normally containing more than two seeds, when occurring incidentally in the working sample, should be hulled if the kind is usually hulled when marketed.

e. Fruits or half fruits in the carrot family (Umbelliferae).

f. Nutlets in the following plant families; Borage (Boraginaceae), mint (Labiateae), and vervain (Verbenaceae).

g. "Seed balls" or portions thereof in multigerm beets (Beta), and fruits with accessory structures such as occur in New Zealand spinach (Tetragonia expansa). Refer to section 2.7i and 2.10a (9).

2.7 Kind or cultivar considered pure seed. - The pure seed shall include all seeds of each kind and/or cultivar under consideration present in excess of 5% of the whole. Under certain circumstances kinds and/or cultivars present to the extent of 5% or less of the whole may be considered pure seed; for example, kinds or cultivars shown on a label as components of a mixture in amounts of 5% or less. The following shall be included with the pure seed:

a. Immature or shriveled seeds and seeds that are cracked or otherwise damaged. This does not include seeds of legumes, crucifers, and conifers with the seed coats entirely removed, which shall be classified as inert matter. See section 2.10a (1).

b. Pieces of broken and otherwise damaged seed units, which are larger than one-half of the original size. For separated cotyledons of soybean, peanut, bean, vetch, lentil and cowpea seeds see section 2.10a (2). For chalcid damaged seeds refer to section 2.10a (3).

c. Insect-damaged seeds, provided that the damage is entirely internal, or that the opening in the seed coat is not sufficiently large to allow the size of the remaining mass of tissue to be readily determined. Weevil-infested vetch seeds, irrespective of the amount of insect damage are to be considered pure seed, unless they are broken pieces one-half or less the original size. This provision is not applicable to chalcid-damaged seed. See section 2.10a (3). For classification of broken pieces of seed units refer to section 2.10a (2).

d. Seeds that have started to germinate.

e. Seeds units of Cucrubitaceae and Solanaceae regardless if they are filled or not.

f. Intact fruits, whether they contain a seed or not, of species belonging to the following families: sunflower (Compositae), buckwheat (Polygonaceae), carrot (Umbelliferae), valerian (Valerianaceae), mint (Labiatae), and other families in which the seed unit may be a dry, indehiscent one-seeded fruit. For fruits which are visibly empty refer to section 2.10a (9).

g. Seed units of the grass family listed in section 2.6b (1) through (5) if a caryopsis with some degree of endosperm development can be detected in the units either by slight pressure or by examination over light. Refer to sections 2.7h and 2.10a (8) when nematode galls, fungus bodies, etc. have replaced the caryopses in seed units.

(1) Burs of buffalograss (Buchloe dactyloides) shall be considered pure seed whether a caryopsis is, or is not, present.

(2) Uniform blowing procedures should be used to determine classification of florets into pure seed or inert for Kentucky bluegrass (Poa pratensis), Canada bluegrass (P. compressa), Rough bluegrass (P. trivialis), Pensacola bahiagrass (Paspalum notatum cv saurae), and orchardgrass (Dactylis glomerata) as listed in section 2.11.

(3) Special purity procedures for chewings fescue (Festuca rubra var. commutata), creeping red fescue (F. rubra), orchardgrass (Dactylis glomerata), crested wheatgrass (Agropyron cristatum and desertorum), pubescent wheatgrass (A. trichophorum), intermediate wheatgrass (A. intermedium), and smooth bromegrass (Bromus inermis) are listed in section 2.12.

(4) For methods to determining pure seed of annual and perennial ryegrass, refer to section 3.5.

h. Diseased seed units and seed units with nematode galls or with fungus bodies such as ergot and smut bodies which are entirely enclosed within the seed unit and caryopses which are spongy or corky. Refer to sections 2.10a (8) and c (1).

i. Seed units of New Zealand apinach (Tetragonia expansa) and beets (Beta). Refer to sections 2.6g and 2.10a (9).

2.9 Weed seed. - Seeds, bulblets, tubers, or sporocarps of plants recognized as weeds by laws, official regulations, or by general usage shall be considered weed seeds. For classification of badly damaged weed seeds and undeveloped seedlike structures refer to section 2.10b. Species requiring special consideration are as follows:

a. Capsules and clusters of seeds of Juncus tenuis, and other species of Juncus having seeds of similar size, are classed as weed seeds. For individual seeds of Juncus, see section 2.10b (9).

b. For species having seeds larger than Juncus, the individual seeds are to be removed from fruiting structures such as pods, heads, etc. The seeds are placed with the weed seed and the remaining fruiting structures are placed in inert matter. Individual seeds are to be counted rather than fruiting structures.

c. Wild onion and garlic (Allium) bulblets which have any part of the husk remaining and are not damaged at the basal end are considered weed seeds regardless of size. For Allium bulblets classed as inert refer to 2.10b (5).

2.10. Inert Matter. - Inert matter shall include seeds and seedlike structures from both crop and weed plants and other material not seed as follows.

a. Seeds and seedlike structures from crop plants.

(1) Seeds of legumes, crucuifers, and conifers with the seed coats entirely removed. Refer to section 2.7a.

(2) Pieces of broken and damaged seed units, including those that are insect damaged, which are half the original size or less. See section 2.7b and c. Also included are separated cotyledons of soybean, peanut, bean, vetch, lentil and cowpea, commonly referred to as "splits", irrespective of whether or not the radicle-plumule axis and/or more than half of the seed coat may be attached.

(3) Chalcid-damaged seeds of alfalfa, red clover, crimson clover, and other legumes which are puffy, soft, or dry and crumbly. Refer to section 2.7c.

(4) Glumes and empty florets except as stated under pure seed, section 2.7g and h.

(5) Broken and unattached wings of tree and shrub seeds.

(6) Attached wings of tree and shrub seeds as follows: for Cedrus, Picea, Tsuga, and Pinus (except as noted below) the entire wing shall be detached and removed as inert matter, for Abies, Larix, Libocedrus, Pseudotsuga, Pinus echinata, Pinus elliottii, Pinus palustris, Pinus rigida, and Pinus taeda the wing shall be detached and removed as inert matter, except for that part which encloses the seed and is difficult to remove in normal seed processing without damage to the seed. The seed wing shall not be removed as inert matter for Acer, Betula, Catalpa, Chamaecyparis, Cupressus, Fraxinus, Liquidambar, Liriodendron, Platanus, Thuja, and Ulmus.

(7) Seedlike structures from trees and shrubs consisting of wings with a thickened spot instead of a seed.

(8) Diseased seed units with nematode galls or fungus bodies (ergot smut bodies or other sclerotia) protruding from the tip of the seed unit.

(9) Fruit portions or fragments of monogerm sugar beets and New Zealand spinach which visibly do not contain seeds. Refer to sections 2.6g and 2.7i.

b. Seeds and seedlike structures from weed plants, which by visual examination (including the use of light or dissection), can be definitely demonstrated as falling within the following categories.

(1) Damaged seed with over half of the embryo missing.

(2) (a) damaged grass caryopses, including free caryopses with over half the root-shoot axis missing (the scutella excluded) and undeveloped glumes and florets devoid of both embryo and endosperm;

(b) immature florets of quackgrass (Agropyron repens) in which the caryopses are less than one-third the length of the palea;

(3) Seeds of legumes and species of Brassica with the seed coats entirely removed.

(4) Undeveloped seed units, devoid of both embryo and endosperm, such as occur in the following plant families: sedge (Cyperaceae), buckwheat (Polygonaceae), morning-glory (Convolvulaceae), nightshade (Solanaceae), Punctureviné (Zygophyllaceae), and sunflower (Compositae). Cockebur (Xanthium spp.) burs are to be dissected to determine whether or not seeds are present.

(5) Wild onion and wild garlic (Allium) bulblets: (a) bulblets which are completely devoid of the husk and pass through a 1/13-inch round-hole sieve; (b) bulblets which show evident damage to the basal end, whether husk is present or absent. Refer to section 2.9c for Allium bulblets classes as weed seeds.

(6) Dodder (Cuscuta): Seeds devoid of embryos and seeds which are ashy gray to creamy white in color are inert matter. Questionable seeds should be sectioned to determine if an embryo is present. Questionable seeds, include those which may have normal color, but are slightly swollen, dimpled or with minute holes.

(7) Buckhorn (Plantago lanceolata): black seeds, with no brown color evident, whether shriveled or plump; the color of questionable seeds shall be determined by use of a stereoscopic microscope with magnification of approximately 10X and a fluorescent lamp with two 15-watt daylight type tubes.

(8) Ragweed (Ambrosia); seed with both the involucre and pericarp absent.

(9) Individual seeds of Juncus shall be left in the inert and their presence recorded under weed seeds.

c. Other matter that is not seed.

(1) Free nematode galls or fungal bodies such as ergot sclerotia and smut bodies.

(2) All inert matter such as soil particles, sand, stones, chaff, stems, leaves, flowers, cone scales, pieces of bark, pieces of resin, etc.

2.11 Uniform Blowing Method.

a. Specific instructions for use of the uniform blowing method and stained samples for blower calibration will be supplied by the Association of Official Seed Analysts through the National Seed Standardization Laboratory Rm. 215 Building 306. Agricultural Research Center, Beltsville, Maryland 20705.

b. Uniform blowing method for Kentucky bluegrass (Poa Pratensis). The uniform blowing method shall be used for the separation of pure seed and inert matter in seeds of common Kentucky

bluegrass and cultivars of Kentucky bluegrass including Merion.

c. Uniform blowing method for Pensacola bahiagrass (Paspalum notatum cv saurae). The uniform blowing method shall be used for the separation of pure seed and inert matter in seeds of Pensacola bahiagrass.

d. Uniform blowing method for orchardgrass, (Dactylis glomerata). The uniform blowing method shall be used for the separation of pure seed and inert matter in seeds of orchardgrass. In the case of mixtures with other kinds, the orchardgrass shall be separated from the other kinds before using the blowing procedure.

2.12 Special purity procedures.

a. Multiple units: The following methods shall be used for the species included in the following table when multiple units are present in a sample. These methods are applicable to the species listed when they occur in mixtures or singly.

b. Definition: A multiple unit is defined as any seed unit possessing a structure other than the rachilla which extends to or beyond the tip of the fertile floret. Any seed unit in which an attached structure is shorter than the fertile floret, shall be considered a single floret. The awn shall be disregarded when determining length of the fertile floret and attached structure.

c. Procedures:

(1) For a single species determine the percentage of single florets present, based on the total weight of single florets and multiple units. Apply the appropriate factor as determined from the table, to the weight of the multiple units and add that portion of the multiple unit weight to the weight of the single units. The remaining multiple unit weight shall be added to the weight of the inert matter.

(2) For mixtures that include one or more of the species in the table; determine the percentage of single florets, based on the total weight of single florets and multiple units, for each species. Apply the appropriate factor as determined from the table, to the weight of multiple units of each species.

3.1 Noxious-weed seeds. This statement replaces paragraph two of this section.

The number of seeds per unit weight shall be based on the number of single units and individual seeds in multiple units of such species as sandbur (Cenchrus), capsules of dodder (Cuscuta), berries of horsenettle and nightshade (Solanaceae) and cockebur (Xanthium). Refer to sections 2.9 and 2.10b (4).

Factor to apply to multiple units [a]/							
Single florets in sample	Chewings fescue	Creeping red fescue	Orchardgrass	Crested wheatgrass[b]/	Pubescent wheatgrass	Intermediate wheatgrass	Smooth bromegrass
percent	percent	percent	percent	percent	percent	percent	percent
50 or below	91	80	80	70	66	72	72
50.01 - 55.00	91	81	81	72	67	74	74
55.01 - 60.00	91	82	81	73	67	75	75
60.01 - 65.00	91	83	82	74	67	76	76
65.01 - 70.00	91	84	82	75	68	77	78
70.01 - 75.00	91	86	82	76	68	78	79
75.01 - 80.00	91	87	83	77	69	79	81
80.01 - 85.00	91	88	83	78	69	80	82
85.01 - 90.00	91	89	83	79	69	81	83
90.01 - 100.00	91	90	84	79	70	82	85

a/ This factor represents the percentage of the multiple unit weight which may be considered pure seed. The remaining percentage is regarded as inert matter.

b/ Includes both Agropyron cristatum and A. desertorum.

4.8 Special procedures and alternate methods for germination.

i. Ryegrass (Lolium - Fluorescence test:

(add this statement to end of paragraph)

Distilled or deionized water should be used to moisten filter paper.

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TECHNICAL ARTICLES

A Literature Review of Laboratory Techniques Used For Cultivar Differentiation

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Currently the majority of cultivar differentiation in the laboratory is based on morphological characteristics of the

seed and to a lesser extent characteristics of germinating seedlings. This approach is becoming inadequate and will continue to be inadequate in the future for the following reasons. Many new cultivated varieties are being developed from essentially the same genetic base and therefore differ only to a slight degree from each other and existing cultivars. In addition, any one seed characteristic, at best, can be utilized to separate cultivars of a species into several groups. Evidence is also available that some seed characteristics may vary in response to environmental influence.

Several laboratory tests for cultivar differentiation, such as the phenol test and fluorescence test, have been developed and standardized. However, there is a need for the development and standardization of additional testing techniques, and unless this is accomplished, an increasing number of cultivars will have to be evaluated by growing samples to maturity in field plots.

The following literature review is an attempt to determine which techniques have been successfully utilized in differentiating varieties of cultivated species. The techniques with greatest potential for development of future testing procedures at the laboratory level will be indicated. The information contained in this review has been grouped by technique and presented by genera in a tabular form with references included. This presentation should allow seed analysts to determine which techniques would be most useful for developing a testing procedure for a plant taxa of interest.

Results of Literature Survey

Information from available literature suggests that there are several techniques which lend themselves well to cultivar differentiation at the laboratory level.

1. Electrophoresis of proteins. --There is a substantial amount of published research to indicate that electrophoresis of plant proteins has been useful in allowing researchers to divide varieties of a cultivated species into several groups (Table 1). Proteins have been extracted primarily from individual seeds and seedlings and to a lesser extent from mature leaves. The extracted proteins are separated on the basis of size and electrical charge in a gel matrix positioned in an electrical field. The proteins, having been separated into distinct bands, are stained and banding patterns representative of the cultivars tested are compared. The advantages of this technique are in the many options available to the investigator. Different classes of proteins may be extracted over a wide pH range, separated in a variety of gels that differ in pH or concentration, and stained for protein or a number of specific isozymes.

A possible drawback to this technique is the extent of technical expertise and training required. However, simplified

electrophoretic procedures are currently being developed for the analysis of serum proteins. These procedures may also prove sensitive enough for the analysis of plant proteins.

2. Germination and seedling growth under controlled conditions or stress. --A substantial amount of information concerning germination of seeds and seedling growth under controlled conditions or stress has been reported (Table 2). This approach to cultivar differentiation has, in numerous cases, allowed varieties of a cultivated species to be subdivided into several groups based on cultivar response to a particular stress. After seeds or seedlings are placed in a growth chamber and subjected to a controlled or stress condition, a number of growth measurements and observations are recorded for a designated period of time. The measurements and observations of the cultivars tested are then evaluated and specific cultivar responses compared. This approach is appealing, due to the large number of different conditions and stresses possible to employ in a testing program.

However, some difficulties would be anticipated in obtaining repeatable results among different laboratories due to variation in germination and growth chamber facilities.

3. Chromatography of flavonoids and phenols. -- Cultivar differences based on the chromatography of flavonoids and phenols extracted from mature leaves and to a lesser extent from seedlings and seeds have been reported (Table 3). Extracted compounds are separated, due to characteristic solubility properties, by their different rates of migration in a solvent system. Flavonoid and phenol spot patterns on chromatograms are detected with the aid of several chemical treatments and uv light.

The application of this technique to cultivar differentiation may be somewhat limited since difficulty in obtaining repeatable qualitative differences among cultivars and variation among seed lots have been reported (7). However, flavonoid and phenol chromatography may be of value for cultivar comparisons if applied to extracts from seeds or seedlings from certain cultivated varieties or from mature leaves in coordination with field plot or growth chamber tests.

4. Chemical treatments and fluorescence tests. --In some cases treatment of individual seeds or seedlings with a chemical or observation in uv light is sufficient to bring about color changes that are of value in cultivar differentiation (Table 4). Except for phenol, the successful use of a specific chemical treatment has usually been limited to cultivated varieties of a single species. Also, some chemicals such as HCl and NaOH have been used to accentuate pigmentation differences already present among cultivars. However, some tests such as the phenol, tyrosine and peroxidase tests are based on the change in color of an applied chemical brought about by the action of specific enzymes located in the seed

or seed coat (2,3,4,9,10). Therefore, it should be possible to develop additional tests of this type once the appropriate enzymes have been identified by protein electrophoresis and enzyme stains. For this reason, and since chemical tests can be easily administered in the laboratory by a technician, attempts should be made to expand their usage.

5. Other laboratory techniques. --Chromatography of fatty acids and quantitative chemical assays have been utilized as an aid in cultivar differentiation in several species (Table 5). However, the results are primarily quantitative and in some cases have been shown to vary due to environmental influences (1,5,6). Other laboratory techniques involving microscopy and serology have been useful in investigating cultivated varieties of only a limited number of species (Table 5).

The techniques mentioned above require considerable technical expertise and training and have not had the general application of cultivar testing that other procedures have.

Discussion

As pointed out by McKee (7), rarely can a cultivar be identified on the basis of results from one technique alone. Therefore, the use of a combination of techniques should be encouraged. The most logical approach to cultivar differentiation would be to utilize morphological seed characteristics and simple chemical tests applicable to seeds followed by evaluation of seedling characteristics. These testing procedures could then be followed by stress tests and more complicated electrophoretic and chromatographic procedures, pathological and entomological tests.

Several recent examples of this approach applied to wheat may be found in the literature. Wrigley and Shepherd (11) were successful in differentiating cultivars of wheat grown in Australia based on grain hardness (measured either by particle size or pearling resistance), phenol reaction to grain and glumes followed by protein electrophoresis. They also suggest that pathological tests would be useful. In many cases, Wrigley and Baxter (12), utilizing the same techniques as above were able to identify cultivars comprising mixed samples.

McKee (8) reported the development of a key for the identification of twelve cultivars of wheat commonly grown in Pennsylvania. He utilized the KOH test, phenol test, and coleoptile color in addition to seed size. He further advocated the use of a heading test (under accelerated growth conditions) and suggested that a test for resistance to Hessian fly would be useful.

This review is an attempt to compile information about some successfully utilized techniques available for cultivar testing

at the laboratory level. It is hoped that additional information will be added in the future by the author or other persons interested in determining cultivar identity.

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Table 1. References for staining techniques for electrophoresed proteins useful for cultivar differentiation.

Genus	Staining Technique								
	General Protein	Peroxidase	Esterase	Phosphotase	Trypsin Inhibitor	Amylase	Lipo-protein	Alcoholde-hydrogenase	Others[d]
Agrostis	1c								
Avena		2b,3b,4b,5b,6c	5b,7b						5b
Brassica	8a		8a	8a					
Cynodon	9c								
Glycine	10a,12a	11a			13a,14a,15a,16a,17a				18a,11a
Gossypium	19b		20a						
Hordeum	21a,22a	23b,3b	3b,24a,25ab,26b,27b	24a		24a,25a,28a,29a,30a			3b,31b
Lolium	32a,33c								
Lotus						34c			
Medicago	35a,36a					34c			
	34c								
Oryza		37b							
Panicum		38c	38c						
Phaseolus	40a			41c					
Pisum			23b						
Poa	1c,43c	44b,39b							
Secale	45a,46a								
Sorghum	47a	47a	47a						
Trifolium			48a				34c		
Triticale	45a,46a								
Triticum	49a,50a,51a,52a,53a,19a,54a,55a,42a,45a	56b,57b							
Zea		58b,59ab	59ab,60a,61b 62b	58b,62b 60b		63b		61b,62b,60b,65ab	59a,64ab

a-seed; b-seedling; c-leaf; d-other staining techniques (5b,59a-Peptidase; 3b,64ab-Catalase; 31b-B-Glucan Hydrolase; 18a-Urease; 11a-"Int"-Oxidase).

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Table 2. References for controlled conditions or stress tests useful for cultivar differentiation.

Genus	Stress								
	Nutrient	Photoperiod	Osmotic or Saline	Toxic Compounds	Photoperiod and Temperature	Temperature	Planting Depth	Hormone	Moisture Regulation
Agrostis		68							
Avena	2,3,69								
Brassica		6,7,8,9							
Cicer			10						
Coronilla					70				
Dactylis		11			12,13				
Festuca				13,14	13,15				
Glycine	3,77	17	19	71,77	18,16,20,77	21	18	22	
Hordeum	25,64	30		5,23,24, 27,28	26,69		30	31	
Lactuca				32				32	
Lolium	33,65	65		3,13,33, 35	13,29,72	13,34			
Lotus	69	37		13,38	36,66				
Medicago			41,42	74	39,43,44, 47,66,73				40
Oryza			75	45		46		49	
Phalaris			48						
Phaseolus				50		51			
Phleum		13		13,52	33				
Poa	13,62,63, 69	53	3,13		3				
Sorghum			76						
Trifolium		54			66				
Triticale								67	
Triticum	5	5,13,56,61	13	5,24	26,55,57, 59,60		61	5,24,57	58

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Table 3. References for flavonoid and phenol chromatography useful for cultivar differentiation.

Genus	Tissue		
	Seeds	Seedlings	Leaves
Avena	3	1,2	2
Citrullus		5	
Cucumis		5	4
Festuca	6		
Glycine			3,7,8,9
Hordeum			10,11
Lolium			3
Medicago	12		
Melilotus			13,14
Phalaris			23
Oryza	15,16		
Phaseolus	17	5	18,19
Pisum	19		19
Poa			20
Secale			21,22
Triticale			21
Triticum			21
Vicia	19		19

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Table 4. Reference for chemical treatment and fluorescence test useful in cultivar differentiation.

Genus	Treatment							
	Phenol	uv Light	HCl	KOH or NaOH	Peroxidase Test	$K_2Cr_2O_7$	NaOCl or KCl	Other
Agropyron						1a		
Avena	2a,3a,6a	6a,7a,8a, 9a,3a,10a	2a,4a, 40a,3a	4a				4a
Beta		11b						
Brassica		5a	12a,13a, 6a	5a			6a	5a,14b
Festuca		6b,5b						5b
Glycine		15b,16b			17a,30a,46a			
Hordeum	2a,6a, 18a	2a,20a	2a,19a		40a		6a	
Lens		21b						
Lolium		6b,22b, 23b						
Lotus								24b
Lupinus		6a					6a	2a,6a
Medicago		26a	25b					
Melilotus								27a
Oryza	28a,6a			29a	28a			
Pisum		2ab,6a, 31b,42b		2a		2a		
Poa	6a,32a,44a,	45a						
Secale		2a						
Triticum	2a,6a,33a, 34a,35a,36a, 37a,18a,38a, 39a*,42a, 43a			2a,5a,34a, 40a,43a				
Vicia	6a	2a,30b, 41a,42b						

a-seed; b-seedling; c-other treatments - (4a-HNO_3; 5a-Chloral Hydrate; 14b-Lactic Acid; 5b-Ammonia; 24b-Sodium Picrate; 2a-Alkaloid Test; 6a-KI; 27a-Cupric Hydroxide); *-tyrosine.

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Table 5. References for other laboratory techniques useful for cultivar differentiation.

Genus	Technique					
	Quant. Assay	F.A.Chrom-atography	Microscopic Tech.		Stem Anatomy	Serology
			Epidermal	Cell Type		
Andropogon						1a
Agrostis			2c			
Brassica		5a,6a	3a	4a		
Bromus			9c,31c			
Carthamus		7a,8a				
Cynodon					10	
Glycine		11a	12c,13a			
Hordeum			14c			
Medicago			16c			15a
Oryza	17a					
Pisum				18a,19a		
Poa			20c,21c			
Sorghum	22a,24b,23a			25a		
Triticale			26c			
Triticum	27abc,28a,29a 30a					
Vicia				19a		
Zoysia					10	

a-seed; b-seedling; c-leaf.

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The Use of Ethrel To Break Dormancy Of Sunflower Seeds In A Germination Test

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Dr. L. E. Clark, Texas A & M University, reported in the "1971 Proceedings of The Association of Official Seed Analysts" on a method of breaking dormancy in peanuts by using the chemical Ethrel. Ethrel is a brand name of Amchem Products, Inc. for (2-chloroethyl) phosphonic acid. Dr. Clark used Ethrel at a 10^{-3} molar concentration to moistened paper towels. After 1 to 3 days the seeds were transferred from the Ethrel moistened rolled towels to towels moistened with water only to complete the test.

In the August 1973 issue of "The Newsletter of the Association of Official Seed Analysts", Harry L. Smith, Virginia State Seed Laboratory, reported on using 10^{-4} molar concentration of Ethrel to break dormancy in peanuts. This is one-tenth the concentration used by Dr. Clark. By this method the seeds were left in the Ethrel moistened rolled towels for the full length of the test. This concentration was effective in breaking dormancy of peanuts. Mr. Smith calculated the 10^{-4} molar concentration to be equivalent to 0.6 ml of Ethrel in 10,000 ml of water.

Our problem has been to find a suitable method to break dormancy of sunflower seed. In the fall of 1973 we tried the following methods to test sunflowers for germination: The normal prescribed method of germinating sunflower seed in water moistened rolled towels; Pre-chilling the seed sample for 3 days at 5°C before planting in water moistened towels; pre-chilling seed planted in water moistened rolled towels for periods of 1 day, 5 days and 7 days before placing in a warm germinator; using Ethrel solutions of 1.2 ml per 10,000 ml of water and 0.6 ml of Ethrel per 10,000 ml of water to moistened towels. Each test was completed in a germinator at the alternating temperature of 20°C and 30°C for 7 days.

We found that the most effective method for breaking dormancy in sunflowers was to plant seed in towels moistened with 0.6 ml of Ethrel in 10,000 ml of water and placed in germinator for 7 days at the alternating temperature of 20°C and 30°C.

Of the many tests made the most extremely dormant sample had a germination of 14% with use of water moistened towels, whereas it was 90% with the use of Ethrel. The average germination of the dormant samples was 55% while these had 86% with use of Ethrel.

The use of Ethrel solution does cause some abnormal sprouts or seedling development: The hypocotyl and radical are shortened from the effect of the Ethrel. These and only these abnormalities should be ignored in making germination counts. Where there was no dormancy in sunflower seed, in no case did the Ethrel solution result in a lower germination than with towels moistened with only water.

We found that pre-chilling the seed sample at 5°C for 3 days before planting in water moistened rolled towels did have some promise in breaking dormancy.

It should be noted that when Ethrel is mixed with water, it loses some of its effectiveness after 24 hours. Therefore, a new stock solution should be made each day one intends to test sunflowers which have some dormant seeds. Since 0.6 ml of Ethrel in 10,000 ml of water is a very large quantity, the amount can be reduced to mixing 0.233 ml of Ethrel in 1 gallon of water.

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Laboratory Germination Testing of Hippophae rhamnoides (Common seabuckthorn)

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As reported in the Journal of Forestry in 1968 (2) and more recently in 1972 (3) the writer has tested and studied the germinative characteristics of several hundred tree and shrub seeds in addition to those kinds on which testing methods have been prescribed either in our AOSA Rules (1) or the ISTA Rules (4). The writer has plans to publish these summarized findings on many more of these less common or exotic species within the next year or two and then summarize these findings in tabular form for the benefit of seed analysts, seed collectors and dealers in these species.

Within the last 10 years the writer has been requested to test Hippophae rhamnoides seed in the laboratory either for sale or planting purposes. This species belonging to the Elaeagnus family is a native of Europe and Asia. It is a very hardy deciduous shrub or small tree and used mainly for ornamental purposes or erosion control and food for wild life. Other common names of this species are sandthorn or swallow-thorn.

Summary of Findings

The seed of some lots were tested under several temperatures from a low of 15°C to the standard warm alternating 20-30°C. Extreme alternating temperatures such as 10-30°C were attempted along with the effect of light and prechill treatments prior to the standard germination test. Substratum moisture requirements were also observed during the course of these many tests.

It was found that the seed was not extremely sensitive to temperature as reported on many kinds in several past articles by the writer but most lots responded more rapid and somewhat higher at the warm 20-30°C alternating temperature with light. It was not definitely established to the writers satisfaction that light was essential to maximum response but it does appear advisable to test the seed in light. A good supply of moisture on the substratum was essential for maximum performance. If a germinator has low humidity it is suggested that the seed should be tested in closed dishes in order to insure an adequate moisture supply.

Although the seed has been reported to be dormant in some of the literature none of the sources or lots tested or studies by the writer required a moist prechill treatment for maximum germination of all viable seed within a 28 day test duration. Thus the germination potential was secured in 28 days while a prechill treatment would require a 50-60 over-all test duration. The average germination response of 5 lots covering a period of 7 years under 4 different conditions are summarized as follows:

Temperature and Treatment	Germination Percent in Days			
	7	14	21	28
20 - 30°C Light - no prechill	29	58	61	62
20°C Light or dark " "	18	43	53	58
15°C " " " "	2	15	27	36
20 - 30°C Light - Prechill 21 days at 3-5°C	58	60	60	60

Recommended Testing Method

From these several years of testing and observations it is recommended that Hippophae rhamnoides (common seabuckthorn) seed be tested at 20-30°C in light with a good moisture supply on the substratum for a duration of 28 days. No prior seed treatment appears essential for maximum germination of all viable seed.

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The Effect of Tap Water vs. Deionized or Distilled Water In Fluorescence Tests of Ryegrasses

R. H. Hofmann and C. S. Ogburn

The problem concerning fluorescence in ryegrass as detailed in the AOSA News Letter by F. T. Emmett (1) was pursued by the Federal Seed Laboratory at North Brunswick, New Jersey to determine if the fluorescence in ryegrasses could be greatly affected by unknown substances in the water supply in different areas of the country. This continued investigation of the problem arose because complaints were received on interstate shipments claiming misleading labeling of the ryegrass seed which had been released by this laboratory.

Six lots of seed were selected from those lots that had been tested by each of four laboratories which had submitted samples. Fluorescence tests were made on these six lots, using the "Maryland" method. Each lot was tested in upright rolled filter paper with both tap water and deionized water (distilled water was not available in this laboratory). Each test consisted of four 50-seed replicates. Tests were conducted at a temperature of 15° - 25°C. for fourteen days.

The original tests made by laboratories B and C used tap water. The original tests made by laboratories A & D used distilled and deionized water respectively. It was found that when samples of lots received from laboratories B and C, which had moistened their tests with tap water, were retested in the North Brunswick laboratory (D), using deionized water, fluorescence showed significant increases ranging from 25 - 70 percent.

Our findings, shown on the following summary sheet, confirm the comments made by Fred Emmett in the August 1974 AOSA News Letter that fluorescence in ryegrass could be affected by unknown substances in the water supply in different areas of the country.

This information is submitted in support of the proposed rules change to section 4.8(i) to moisten filter paper with only distilled or deionized water, prior to planting, and if necessary,

	Laboratory	Original Lab Results		North Brunswick Retests on Original Samples Using Tap Water		North Brunswick Retests on Original Samples Using Deionized Water	
		Annual	Perenn.	Annual	Perenn.	Annual	Perenn.
Lot 43	A	90.89%	8.36%	80.19%	16.83%	87.61%	8.91%
	B	62.03%	37.53%	83.24%	11.40%	88.69%	7.43%
	C	24.22%	74.65%	86.15%	11.45%	91.63%	4.98%
	D	92.65%	6.60%	-	-	92.65%	6.60%
Lot 30	A	90.15%	9.33%	83.74%	13.87%	91.17%	6.44%
	B	54.69%	44.88%	85.84%	11.28%	85.35%	10.30%
	C	24.81%	74.58%	79.66%	15.06%	84.03%	7.77%
	D	81.92%	17.08%	-	-	81.92%	17.08%
Lot 29	A	91.14%	8.48%	85.98%	10.44%	86.98%	8.45%
	B	55.27%	44.36%	82.75%	12.46%	83.75%	10.97%
	C	44.07%	55.45%	81.26%	12.96%	84.25%	11.47%
	D	91.28%	7.89%	-	-	91.28%	7.89%
Lot 16A	A	90.83%	8.75%	91.17%	7.43%	87.70%	7.43%
	B	62.83%	36.89%	89.69%	7.93%	86.71%	10.90%
	C	49.43%	49.87%	83.57%	8.46%	90.05%	2.49%
	D	94.04%	5.56%	-	-	94.04%	5.56%
Lot 14A	A	88.77%	10.82%	76.19%	14.94%	83.17%	11.95%
	B	56.73%	42.59%	81.18%	9.90%	82.67%	5.94%
	C	25.30%	74.57%	83.24%	10.90%	83.74%	8.42%
	D	82.76%	15.58%	-	-	82.76%	15.58%
Lot 14	A	88.36%	11.04%	80.84%	11.48%	80.84%	6.99%
	B	50.29%	49.38%	75.92%	11.83%	85.29%	7.89%
	C	27.02%	72.86%	79.84%	11.98%	82.83%	6.49%
	D	85.10%	14.50%	-	-	85.10%	14.50%

during the test period.

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(1) AOSA News Letter, Vol. 48, No. 3, National Seed Testing Service, F. T. Emmett

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2 C. S. Ogburn, Botanist, Federal Seed Laboratory, North Brunswick, New Jersey.

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Germination Studies of two Lychnis species: Lychnis alba (Evening Lychnis) and Lychnis Flos-cuculi (Ragged Robin)

Waldo C. Lincoln, Jr.,-Mary Lou McGuigan

Germination tests were run in pretty much a standard manner. Small petri dishes were used, and four hundred seed tested in replicate of one hundred seed per dish, with four replicates. Two pieces of standard blotting paper were used per dish and the paper moistened with tap water. Several temperature ranges were selected and modified over the three years of testing. Seed was collected in the Amherst, Massachusetts, area, and tested two to five months after harvest. The seed of Lychnis Flos-cuculi was tested immediately after harvest as well as six months after harvest.

Below are the tables with the data on the species of Lychnis tested.

Lychnis alba - Evening Lychnis

	1973 Seed Tested 5 Months After Harvest			1974 Seed Tested 3 Months After Harvest			1975 Seed Tested 2 Months After Harvest		
	1st Count Days	Final Count Days	Final %	1st Count Days	Final Count Days	Final %	1st Count Days	Final Count Days	Final %
15°C Light				6	13	68.75	6	10	72.75
15°C Dark	15	20	60.50	6	13	44.25	6	10	44.50
20°C Light				6	13	60.25	5	9	88.75
20°C Dark				6	13	48	6	10	60.50
15°C-25°C Light	8	20	79	6	13	88.50	-	10	88
15°C-25°C Dark				6	13	88.75	-	10	67.50
20°C-30°C Light	8	20	75.75				6	10	90

20°C-30°C Dark							6	10	66.25
PC-60 Days 15°C-25°C Light	30+7	30+11	65						
PC-60 Days 20°C-30°C Light	30+7	30+11	55.50						

Lychnis Flos-cuculi - Ragged Robin

	1974 Seed Fresh Seed Tested			1975 Seed Fresh Seed Tested			1975 Seed-Tested 6 Mo. After Harvest		
	1st Count Days	Final Count Days	Final %	1st Count Days	Final Count Days	Final %	1st Count Days	Final Count Days	Final %
15°C Light							12	20	19.50
15°C Dark		73	0				12	20	10.00
20°C Light							8	20	71
20°C Dark							8	20	25.50
15°C-25°C Light	11	44	16.50	9	34	59.33	8	20	65.75
15°C-25°C Dark				9	34	49.66	8	20	48.75
20°C-30°C Light	9	42	27.50	6	36	73.75	6	15	91.25
20°C-30°C Dark				6	36	62.75	6	15	75.50
PC-30 Days 15°C-25°C Light	30+7	30+34	23.50						
PC-30 Days 20°C-30°C Light	30+7	30+34	23.75	30+6	30+40	70.25			
PC-60 Days 20°C-30°C Light				30+7	30+33	61			

No tests were made on fresh seed of Lychnis alba. Fresh seed tests, however, were made on Lychnis Flos-cuculi seed. Pre-chilling for thirty and sixty days at 5°C-8°C did not increase germination. Dormancy does not seem to be a problem. From the above tables it can be seen that both species of Lychnis did not behave in quite the same manner. It can be said that after a two or three month period Lychnis alba seed showed good response at both the alternating temperatures of 20°C-30°C and 15°C-25°C. Lychnis Flos-cuculi, however, seemed to be more selective to temperature. The length of test until full potential is reached is also slightly different with the two species tested. Both species seemed to be somewhat sensitive to light.

Recommendations for the germination of Lychnis alba, Evening Lychnis and Lychnis Flos-cuculi, Ragged Robin: Germinate Lychnis alba at either the alternating temperature of eight hours at 25°C and sixteen hours at 15°C or eight hours at 30°C and sixteen hours at 20°C. First count should be made in six days and the final count in ten days. Light should be used for the shorter eight hour period.

Germinate Lychnis Flos-cuculi at a temperature of eight hours at 30°C and sixteen hours at 20°C. Light should be employed for the shorter eight hour period. The first count should be made in six days and the final count in fifteen days.

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Seed Studies and Laboratory Germination Testing of Pinus brutia (Calabrian pine) and Pinus eldarica (Afghanistan pine)

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Within the last 10 years there has been great interest in the pines which are native to the Mediterranean region from Spain east to Turkey, Greece, Lebanon, Syria, etc., and also including Afghanistan, Iran and the Quetta region of Pakistan. There is much confusion regarding the scientific and common names of these pine species in localized areas especially in the Eastern countries. The writer will not attempt to cover the literature on the confused nomenclature and published information but interested analysts can refer to literature citations (1,2,4 and 8).

Both Pinus brutia and Pinus eldarica are closely related in some respects to Pinus halepensis which is known as Aleppo pine or Jerusalem pine and is found growing from Spain and Morocco to Turkey and Jordan. Calabrian pine is listed as Pinus halepensis var. Brutia even though the seed is distinctly different (Figure #1). Also Afghanistan pine is listed as Pinus brutia var. eldarica and some authors consider it simply a synonym of Pinus brutia. The writer has also received seed for testing in the laboratory labeled Quetta pine and with affixed common names with no scientific justification. Within the last year the writer has had personal correspondence and a memo from W. B. Critchfield of the U. S. Forest Service Institute of Forest Genetics at Placerville, California regarding this confusion and seed identification. From a genetic standpoint he is not sure whether P. eldarica is sufficiently distinct from P. brutia to warrent specific status and that Pinus quetta is an invalid local name.

It is also stated in his memo that Pinus halepensis has much smaller seed than the strains in the P. brutia group and can be definitely eliminated as a possible source of the Afghanistan pine. He also has reported that within the P. brutia group, P. eldarica has the largest, heaviest seeds and P. brutia has the smallest. When correctly labeled the writer has also found this fact to be true although not extremely different in this respect.

Germination Testing Requests

Both seed dealers and nurserymen have requested germination tests on the various sources of these two pines and data has been compiled, observations taken both on seed size, characters and germination requirements for most rapid, complete response in the shortest period of time. These pines are causing much excitement among foresters and nurserymen in the Southwest, namely New Mexico, Arizona and California because of their extreme fast growth rate and excellent symmetry. They would not be considered hardy in our Northeast region although the writer has a few 5 year old Pinus brutia trees which have withstood the Geneva winter conditions to date. Most of the requests for seed tests have come from dealers or nurserymen in Arizona and New Mexico. It has also been found that many of these imported seed lots from Pakistan have been of poor germination and low vitality so that it was difficult to secure comparative germination results under various temperatures and other conditions.

Size of Seed Studies

Size of seed and seed per pound data have been recorded on most seed lots tested during the last 10 years of these three pines namely P. halepensis (Aleppo pine), Pinus brutia (Calabrian pine) and P. eldarica (Afghanistan pine). The range in these figures and the average seed per pound for these three pines are as follows:

	Range in Seed Per Lb.	Average Seed Per Lb.
P. halepensis (Aleppo pine)	19,800 - 29,000	25,800
P. brutia (Calabrian pine)	7,300 - 8,000	7,630
P. eldarica (Afghanistan pine)	6,120 - 6,560	6,360

A close-up photo of 2 seed sources of Aleppo pine and one each of Calabrian pine and Afghanistan pine are shown in figure #1. These photos definitely exhibit the close characteristics of the two latter pines and also show the extreme difference in both size and shape of the Aleppo pine. Thus Aleppo pine should not be considered to have any genetical closeness to the other two pines on seed characters as reported by Critchfield (4). However, on seed characters alone it is almost impossible to identify the other two species except for a small difference in seed size. Both of these pines are 3-4 times larger than normal Aleppo pine seed. It is hoped that these photos and seed per pound figures will assist analysts in correctly identifying these three pine species when seed samples may be submitted for germination testing which may be not properly labeled.

Germination Testing Results

The writer has reported findings on germinative characteristics of 10 exotic Pinus species in 1967 (5) and 4 more tropical pines in 1973 (6) along with laboratory testing methods for these 14 pines most of which have been added to our AOSA Rules (3).

Of the three pines discussed and reported in this article only Pinus halepensis (Aleppo pine) is presently listed both in our AOSA (3) and the ISTA Rules (7). The recommended temperature is 20°C with a test duration of 28 days although most lots have completed their germination within 21 days.

As reported by Mirov (8) the writer prefers to list these two pines as Pinus brutia (Calabrian pine) and Pinus eldarica (Afghanistan pine) but for comparative testing purposes they were combined because of identical visual seed characteristics and duplicate or double labeling as the seed lots were submitted for testing purposes.

Temperature and Light Factors

Preliminary findings on individual lots of these two pines indicated the seed was sensitive to high temperatures during laboratory testing and that optimum germination was not obtained at the standard 20-30°C in light which is also true of the close relative Pinus halepensis (Aleppo pine). The writer reported this same characteristic of temperature sensitiveness with 5 other pines in 1967 (5) namely Pinus Canariensis (Canary pine), P. patula (Jelecote pine), P. pinaster (Cluster pine), P. pinea (Italian stone pine), and R. radiata (Monterey pine) all of which have been added to our AOSA Rules.

Various seed lots were germinated at both constant and alternating temperatures including 15°, 20°, 25°, 20-30°C. Some lots were moist prechilled at 3-5°C for 21 days and germinated at both 20°C and 20-30°C. There was no indication that a prechill treatment was necessary for maximum germination of all viable seed, a typical characteristic of Aleppo pine and most of the pines reported on in 1967 (5) and 1973 (6). The average germination of 5 typical lots was 66% without any prechill treatment and 60% with a prechill treatment of 21 days at 3-5°C prior to testing the seed at the optimum 20°C.

The sensitiveness to the warmer temperature of these two pines is demonstrated in the following comparative germination percentages based on average results of 15 seed lots:

Germinated at 20° - 30°C by Days			Germinated at 20°C by Days		
14	21	38	14	21	28
28%	39%	45%	56%	64%	68%

Light did not appear to be essential for maximum germination of all viable seed. This fact was also true of studies conducted by the writer many years ago with Aleppo pine prior to our present AOSA Rules adoption. Comparative results were always within tolerance when lots were tested in light and in dark at the optimum 20°C. The average germination of 7 lots tested under these two conditions were as follows:

Days - Light - 20°C			Days - No Light - 20°C		
14	21	28	14	21	28
53%	61%	64%	53%	63%	65%

It should be pointed out that all of these seed lots were imported and no tests were conducted on strictly fresh harvested seed although some seed lots were of high quality within the 80-90% germination range and thus it is felt these findings would be true for all seed lots of these two new pine species.

Recommended Testing Method

It is recommended that both Calabrian and Afghanistan pine seed be tested at 20°C with a good moisture supply on the

substratum for a test duration of 28 days. Neither light nor a pre-chill treatment is necessary for optimum germination. Soaking these large seed for a few hours or over night may be beneficial to remove some of the superficial fungi or where the germinators may be of low humitidy.

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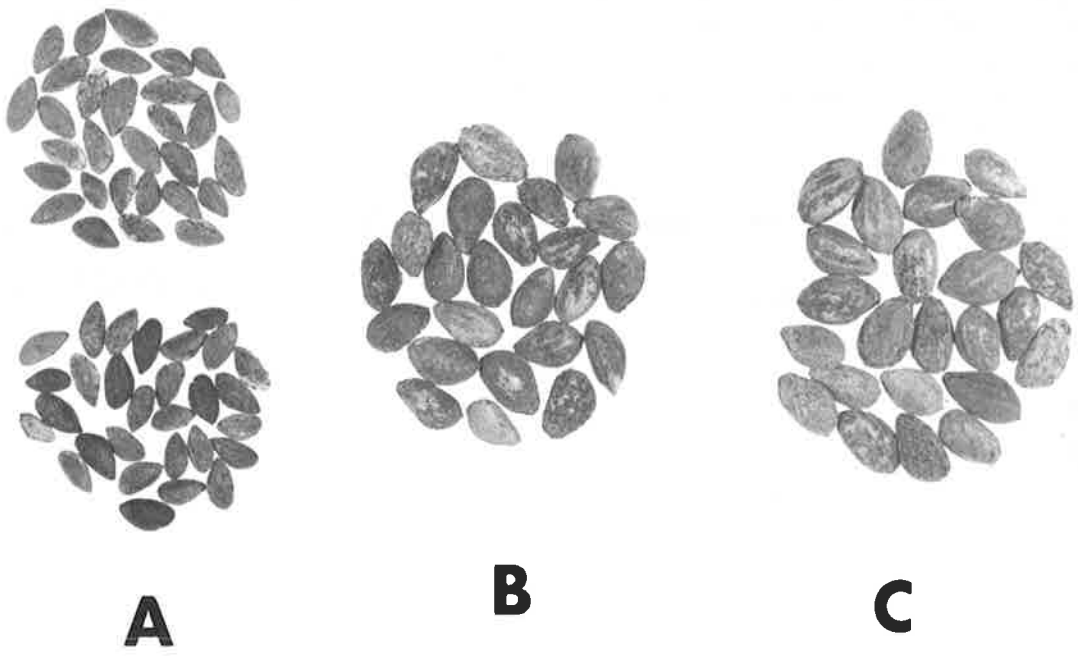

Figure #1. Comparative size and shape of three pine species as discussed in article. A - Two typical sources of Pinus halepensis (Aleppo pine). B - Pinus brutia (Calabrian pine). C - Pinus eldarica (Afghanistan pine).

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Germination Studies of *Ranunculus abortivus* and *Ranunculus bulbosus*

Waldo C. Lincoln, Jr. & Mary Lou McGuigan

Germination studies were made on two Ranunculus species: *Ranunculus abortivus* (Small-flowered Buttercup) and *Ranunculus bulbosus* (Bulbous Buttercup). Tests were made on both species using seed harvested in 1974 and 1975. The seed was collected in the Amherst, Massachusetts area. Four hundred seeds were used per test, replicated four times with one-hundred seed per dish in large petri dishes. The seed was placed on two pieces of standard germination blotting paper moistened with tap water. Counts were made as deemed necessary. Following are tables for the two Ranunculus species tested.

1974 Seed *Ranunculus abortivus* (Tested three months after harvest.)

Germination Conditions	1st Count Days	Final Count	Final %
15°C Dark	28	28	47
15°C-25°C Light	21	28	94
20°C-30°C Light	15	28	97
PC-30 Days, 15°C-25°C Light	42	60	93.25
PC-30 Days, 20°C-30°C Light	42	60	93.25

1975 Seed *Ranunculus abortivus*

	Tested Immediately After Harvest			Tested 4-Months After Harvest		
Germination Conditions	1st Count Days	Final Count Days	Final %	1st Count Days	Final Count Days	Final %
15°C Light	45	62	84			
15°C Dark	45	62	10.50			
20°C Light				19	33	98.50
20°C Dark				19	42	39.50
15°C-25°C Light				14	28	98.75
15°C-25°C Dark				14	28	100
20°C-30°C Light	61	68	97	14	18	98
20°C-30°C Dark	61	68	99	14	18	99

From the above tables it can be seen that *Ranunculus abortivus* seed has a dormant period of three to four months. With the 1974 seed prechilling at 5°C to 8°C for thirty days followed by another thirty-eight days at 20°C to 30°C will give complete germination.

The 1974 seed lot shows that after four months the seed will germinate completely in eighteen days at 20°C-30°C. Both lots of seed showed that at the constant cooler temperature of 20°C and 15°C the seed was light sensitive but not sensitive at the higher and alternating temperatures of 15°C-25°C, and 20°C-30°C.

Recommendations for germination of Ranunculus abortivus: Fresh seed should be prechilled at 5°C-8°C for thirty days followed by alternating 20°C-30°C light, for 38 days with the first count thirty days after removal from prechill. Seed three months after harvest can be germinated at 20°C-30°C with light. The first count made in fourteen days and the final count in eighteen days.

Germination Ranunculus bulbosus (Bubous Buttercup)

	1973 Seed % Germ.	1974 Seed % Germ.	1975 Seed Fresh Seed % Germ.	1975 Seed 3 months old % Germ.
15°C Light			74.75	84
15°C Dark	83	74.75	54.50	75.50
20°C Light				83.75
20°C Dark				71.75
15°C-25°C Light	87	71.25	80	88
15°C-25°C Dark			77.50	80.50
20°C-30°C Light	87	61	54.50	85.25
20°C-30°C Dark			47.50	83
PC-30 Days, 15°C-25°C Light		9.50	44	
PC-30 Days, 15°C-25°C Dark			33.50	
PC-30 Days, 20°C-30°C Light		4		
PC-60 Days, 15°C-25°C Light	15.50			
PC-60 Days, 20°C-30°C Light	17.50			

The tests for Ranunculus bulbosus were run in the same manner as for Ranunculus abortivus. From the table it can be seen that fresh seed should be germinated at 15°C-25°C for a period of 45 days. Seed three months old can be germinated in either the 15°C light or alternating 15°-25° light for a period of thirty-six days. The fresher the seed the more sensitive it is to light. Prechilling at a temperature of 5°C to 8°C for a period of thirty or sixty days tends to throw the seed into a deeper dormancy than if not prechilled at all. After three months the 1975 seed germinated equally well at all temperatures used, provided light was employed.

Recommendations for the Germination of Ranunculus Bulbosus: Germinate fresh seed in large petri dishes at an alternating temperature of 15°C-25°C; 16 hours at 15°C and 8 hours at 25°C. Light should be employed at the 25°C temperature and the eight hour time period. Length of test forty five days. For seed three months or older the germination condition should remain the same but the time for germination can be shortened to thirty-five days.