felt that embryo growth appeared to be a simple and reliable guide to cone maturity. Therefore, we planned to repeat the study in 1951 on a layer scale. Unfortunately this proved to be impossible. We had prolonged hot weather and drought and an unprecedented forest closure. Collections could not be made as planned. Spasmodic collections were made from different areas whenever an opportunity arose. The embryo growth measurements were made on a larger sample than in 1950. A small graduated eyeglass was used instead of the binocular microscope. This proved very satisfactory.

The black line in Figure 2 shows embryo length as a percentage of seed length. It is at once apparent that there is much difference in embryo development in the two years. Here, in 1951, the embryo was fully grown, but not necessarily fully matured by the beginning of August. The germination tests in 1951 were carried out in the same way as in 1950, that is, two series of tests on stratified seeds. The results are shown by the red line on Figure 2. It is at once apparent that the trend of germination is anything but regular.

However, let me remind you that these tests were made on haphazard collections not on regular collections from the same stand. Early in August germination and embryo length seemed to be in step. Then came two drops in germination percent. I cannot explain that. Then germination rose again as in 1950. One point is apparent. The germination reached 75 percent a few days before August 20. That point seems to me to be significant. We still do not know if embryo growth is a guide to seed maturity. 1951 was a most unusual year climatically and the experiment could not be carried out as planned. But from the practical aspect we know, from three years work, that in this part of British Columbia, the cones are ready for collecting by August 20. That is the date on which our Reforestation Division usually starts cone collections. That date was based on experience over a number of years and these small studies have confirmed it.

THE USE OF CHEMICAL DYES FOR QUICK GERMINATION TESTS

by

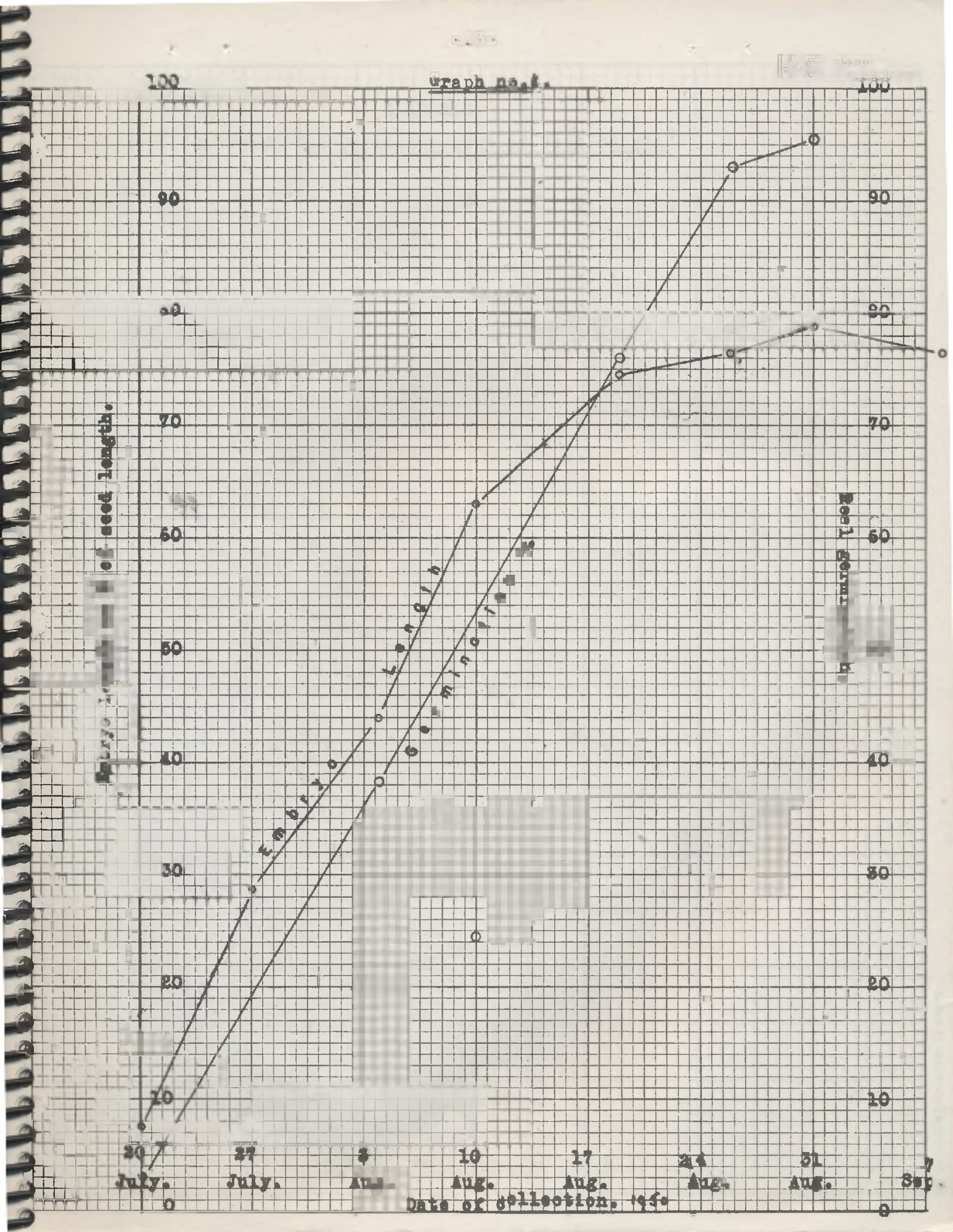
G. M. Finnis

* *

The title of this discussion is "The Use of Chemical Dyes for Quick Germination Tests." Although I intend to describe mainly one test, it may be of interest to spend 2-3 minutes discussing germination tests in general and chemical tests in particular.

The ultimate object of any method of testing seed germination quality is to provide an indication of the percentage of seeds in a given lot that may be expected to produce plants, so as to determine the amount of seed that should be sown per unit area to give a desired plant density in the field. That may seem rather obvious but please bear it in mind. I may offend the scientific gentlemen present when I say that sometimes too much time is spent on an unnecessarily accurate germination test when the original sample was not selected with equivalent accuracy and the future use of the seed will also not be within such limits of accuracy.

Now I am no expert on the subject. These are just the observations of a beginner. But I sometimes feel that the ideas and impressions of the ordinary man are of more value to the general run of us than those of the experts. With Douglas fir we have been running germination tests for 50 days on unstratified seed and for 15 days on seed previously stratified. That takes a lot of time and a certain


Graph no. 1.
100
90
80
70
60
50
40
30
20
10
0
Average length of seed length.
Leaf
Seedling
Seed
20 July.
27 July.
3 Aug.
10 Aug.
17 Aug.
24 Aug.
31 Aug.
7 Sep
Date of collection. 1915.

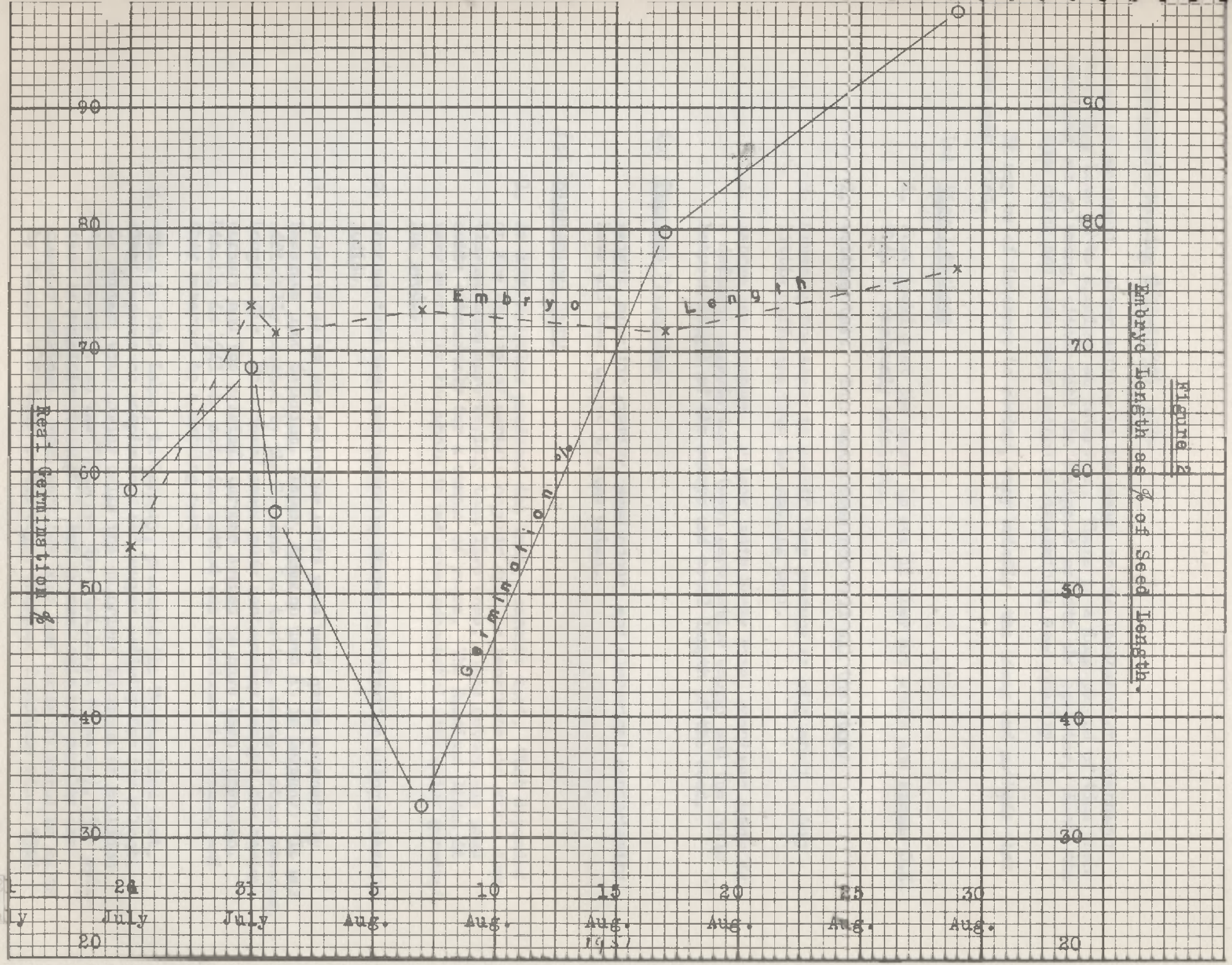

Embryo Length
Germination %
Mean Germination %
Embryo length as % of seed length.
90
80
70
60
50
40
30
20
July
July
Aug.
Aug.
Aug.
Aug.
Aug.
Aug.
1931

Figure 2

amount of equipment. After setting up the seeds in the Incubator or Copenhagen Tank, they have to be examined at intervals. There is a long period of time when something can go wrong, either with the equipment or with the operator. Hence the idea of some quick and also cheap method of determining seed quality.

It may be of interest to review, very briefly, the ideas and methods that have been tried.

(a) Visual Methods

1. Just looking at the seeds to see if they are whole and appear O.K. This is obviously primitive.

2. Cutting test. This again does not tell all the story.

3. Crushing the seeds to see if they produce an oily stain. Very little different from the cutting test.

4. Embryo ratio method. Wibeck, with Scots Pine, concluded there is a definite correlation between germinative energy and the ratio between length of the embryo and total length of the seed.

(b) Physical Properties

Weight, specific gravity, seed odour, and electrical conductivity have all been tested to some extent.

(c) Biochemical Tests

1. In 1929 Neljubov produced the Indigo carmine test. That depended on the ability of certain organic dyes to penetrate and stain dead tissues much more rapidly than live ones. This method was used quite extensively for a number of years. The Russians liked it very much. On the whole it was more successful with quick germinating seeds.

2. Another test involved measuring Starch mobilization using Potassium Iodide.

3. Selenium salts and tellurium salts were used quite extensively. Hasegawa used Selenium. Later, Eidmann used it in Germany. It was officially adopted by the Germans for testing, amongst other species, Douglas fir. Then there arose much controversy amongst the various workers and the original purpose seemed to have been forgotten. Finally in 1941 the selenium method was abandoned in Germany and they reverted to a cutting test.

4. Then in 1942 Lakon, in Germany, produced the Tegrazolium test. That depends on respiratory and enzyme activity for its effect. On contact with living cells the colorless tetrazolium solution is reduced to an insoluble red dye (triphenyl formazan). The solution is sensitive to light. But is non-toxic and results are quickly obtained. Much work has been done using Tetrazolium salts by Flemion and Poole at the Boyce Thompson Institute since 1948,

and they have used it on many species including Douglas fir. The British Forestry have conducted extensive trials with this chemical on a number of species including Douglas fir and are expecting to publish their results quite soon.

This leads us on to our small experiments.

We heard of this new chemical late in 1949. Its full name is 2.3.4. Triphenyl tetrazolium chloride and bromide, the latter being the more successful one. Happily this substance is known under the Trade Name of Grodex. It is obtainable in Canada from May and Baker (Canada) Ltd., 400 Atlantic Avenue, Montreal, and a 1 gr. tube costs 50¢. Hence the small outlay for such experiments.

The technique may best be illustrated by examples. The seeds to be tested are soaked in water overnight. Next morning they are cut in half longitudinally. The embryo should be sliced in half lengthways or it can be excised whole. This is easiest done. One half of each embryo is using a scalpel and forceps and keeping track of the numbers with a tally register, then placed in a freshly prepared 1% solution of Grodex. After 3-4 hours in darkness at room temperature, the solution is drained off and the embryos washed in water. They are then placed on a porcelain tile and graded according to the intensity of staining. The classification is:

A. Strongly and completely stained over the whole surface.

B. Strongly stained over 2/3 of the surface.

C. Strongly stained over 1/3 of the surface or markedly stained over the whole surface.

D. Not stained.

Group A = Field germination.

Groups A and B = Germination capacity as measured in the Incubator or Copenhagen Tank.

In our first test we used 5000 seeds all from the same seed lot.

The set-up was as follows:

Method	No. of Seeds	Real Germ. %
Grodex	1,000	94.35 (94)
Selenium	1,000	85.60 (86)
Unstratified	2,000	92.98 (93)
Stratified	1,000	89.50 (90)

Those results are in close agreement. It is a debatable question what accuracy you should expect and also what accuracy you require. Probably the Nursery man will only alter his rate of sowing for quite large classes, even up to 20%.

The Grodex method obviously gave good results and was simple to perform and cheap in time and money.

So we decided to repeat it. This time leaving out the Selenium test and including a Nursery test. We had hoped to have this test completed in time for their meeting but unfortunately this is not so. We had a cold May and June and germination was slow and is still continuing, so the results are incomplete. However, the results to date are as follows. We used two lots of seed; one collected in 1950 and one in 1951.

On each lot of seed we ran the following tests:

500 seeds Stratified)		1,000
500 seeds Unstratified)		1,000
500 seeds Grodex)	X 2	1,000
4 x 500 seeds Nursery)		2,000
		5,000

That gave a total of 10,000 seeds in the test. The results to date are:

	1950 Seed			1951 Seed		
Method	Lot			Lot		
No treatment	62.6,	64.0	63.3	43.8,	59.2	51.5
Stratified	76.4,	83.2	79.8	88.0,	91.0	89.5
Grodex	85.4,	78.6	82.0	79.2,	87.0	83.1
Grodex gp:A			70.3			72.8
Field Test			53.4			60.9

The stratified seed and the Grodex test are in close agreement. I think you can safely say that either figure gives an accurate picture of the seeds in question. It will be interesting to see how the Field germination eventually finishes up. Whether it will in the end approximate to the Grodex Group A total. I think you will agree that the Grodex test offers great possibilities. The cost is small, the time required short, and the operation is simple to perform. There is a small amount of the personal element in the classification but by having a set of standards this may be overcome. This may be done by preserving all specimens so that they can be checked later by another person or by comparing them with a set of standards or by photographic sets of standards. We hope to set up some standards at a later date. If anyone is interested in trying this method I shall be only too glad to help or at any rate to keep in touch and see how the work goes.

Chairman Webster: Were your Grodex tests carried on immediately after you gathered your seed? How soon after?

Mr. Finnis: About November.

Chairman Webster: When were your stratified seed tests carried on?

Mr. Finnis: We got the seed from the extractory around the 10th of November, as far as I remember, and immediately I put one lot into the incubator, one lot into the stratification and started on the Grodex. They were all started pretty well simultaneously. The second test which was carried out was on 500. The second test carried out about January this year. All started about the same.

Mr. Cameron: Was there any appreciable difference in the test which was started in November and in January on your Grodex results?

Mr. Finnis: No. When the field test is completed, this will all be subject to statistical analysis - but not by me. I think there is a great future in Grodex. We have no legal seed certification laws at the moment, and in countries where they have them, I don't know as yet that Grodex is accepted by law, but I think that may come.

Mr. Adams: Who sells Grodex?

Mr. Finnis: I will give you the Canadian address. May and Baker (Canada) Ltd., 400 Atlantic Avenue, Montreal.

Dr. Wright: On your field tests, how did you do that?

Mr. Finnis: They were sown in ordinary seed beds. Ordinary soil, yes, to the same density of 50 seedlings per square foot.

Dr. Wright: And you based your percentage on the number that emerged?

Mr. Finnis: Yes, we had the seed bed like that, and just for tallying purposes, I divided the seed bed into 4 squares and sowed them at the standard density in each bed, and they were covered with the same covering as the other seeds in the nursery, the same treatment of shade and watering, and on emergence, I just plucked them out and counted them.

Dr. Wright: If they don't total up quite as high as the others, you might get a little better by using Chloropicrin to cut down your losses. It might happen in pre-emergence damping off that you don't get emergence out of the soil.

Mr. Finnis: Well, I wanted to do it as like our standard nursery practice as I could.

Mr. Wells: Have you done any testing of seed that was older than 2 years old?

Mr. Finnis: No.

Mr. Engstrom: What is standard density?

Mr. Finnis: 50 per square foot.

Mr. Isaac: Well, 2 years ago when Eidman was over here, with seven other European foresters, and I was with him most of the time for 2 weeks, when this color test was brought up, it was like a three-ring circus. None of them agreed with him, and they couldn't agree with each other on that selenium business, and it had then been law in Germany, but they claimed that it was not workable and not good, only under certain conditions.

Mr. Finnis: I got quite a kick reading the literature. Even in there, you could see what was going on.

Mr. Isaac: It was just like a three-ring circus when you got them started. There was one other thing I wanted to mention, in connection with your earlier statement regarding germination or viability. When that curve goes up in germination, some of that germination won't produce seedlings. The seed hasn't got viability enough to produce a seedling that will survive the season and the following winter, and I think that is particularly true of seed that is gathered a little too early, and I think that you not only have to hit that point in there where you get suitable germination, but if you want to get seed that is going to produce good vigorous stock, you have to go a little bit beyond and you would not be safe in taking the earliest point there.

Mr. Finnis: Yes.

Mr. Augenstein: Well, Dr. Waters at the University of Montana has been carrying on some tests with tetrasolium, the chloride solution, and they found that testing just the embryo probably wasn't sufficient because of the endosperm. They sliced the whole thing in two and then tested the whole works. They found there was dead tissue on the endosperm and there wasn't sufficient live tissue on that to keep the embryo alive when it was germinating. It may germinate and then die, or maybe not completely germinate, and by testing the endosperm they found quite often it had that dead tissue in there.

Mr. Taylor: Over in Iowa State, they have been studying the effects of treating injury on immature seed corn, and they have set up rather definite standards as to the extent of obtaining the endosperm that has a normal viability to produce a seedling. May I ask the question, why do you consider the bromide better than the chloride?

Mr. Finnis: I don't. I am just going on what other people say.

Mr. Cameron: I wanted to make a remark about germination tests in general that may not be in place here, but we have had considerable experience, of course, with germination tests on a good many seed lots, and have run into some very sad experiences to the effect that there will be a germination test with a very low result and in the sale of seed it can be a very important factor. That same lot of seed will be germinated by perhaps the same party or in a different laboratory and get entirely different results. On the sale or on the purchase of seed, if a germination test is going to be used, there must be several tests performed rather than just one to arrive at the germination value of the seed. Within the last two years that has affected us to the tune of a good many thousand dollars on the sale of the seed which we thought was of a low germinative power, and later turned out, on subsequent tests, was a very good germination.

Mr. Augenstein: And vice versa too, of course!

Chairman Webster: We don't feel we get a real germination test in our department the ordinary way we germinate unless the seed is first stratified. We all know you can take fresh seed right out of a cone and get practically nothing from a germination test if it hasn't gone through the pre-dormancy period.

Mr. Haddock: To what extent have you standardized your sampling methods?

Mr. Cameron: All the seed is sent for germination. A lot of seed might vary from a very small lot to a lot of several thousand pounds, but take all the samples, endeavor to mix the lot quite thoroughly and take a cross section of it with the sampling rod.

Mr. Isaac: Mike, I would like to have Mr. Finnis tell us something about the excised embryo test that Mr. Fleming and Dr. Barton and Boyce Thompson have been working with. I think he knows about it.

Mr. Finnis: I have never tried it, I am afraid. I have only read about it, but I gather what they do is cut up their seed and excise the embryo, and then put that excised embryo straight into the germinator and by that way they can get results in a matter of 4 or 5 days.

Mr. Isaac: It is all done in 4 or 5 days?

Mr. Finnis: Yes, compared to the standard tests which may be 50 days. I have never tried it myself.

Mr. Haddock: I have done it a little bit but have not correlated it with standard germination methods. Even on sugar pine it is a lot of work to excise those embryos but even though you may get done with the test, I think this would be a lot quicker and should be just as accurate.

Mr. Lanquist: We did the same thing. We excised the embryos and put them in a pewter dish in the incubator, because we got a good test there, but what we got was perhaps germinative capacity instead of germinative energy, and that is probably what you will get under 96% or something close to that.

Mr. Corson: I would like to go back to your test for the ripeness of the cones and ask you this question: how much of a job is it for the field man to make the measurements of the embryo to determine ripeness?

Mr. Finnis: That is a thing I forgot this morning. In 1950, we used a binocular microscope, which is, of course, an expensive piece of equipment, and isn't knocking around everywhere, and roughly, I used to go out in the morning and gather the cones, about a 20-mile drive, climb up and pick them, get back and after lunch I would start cutting them up and by -- I was going to say tea time -- I would have those 32 readings. In 1951, instead of that, I borrowed from our service division this little device which has a graduated scale in millimetres on here. You can plunk it down on a piece of white paper anywhere and read it straight off, and the only tools required are just a scale peeler or a pen knife is a good thing. A pocket knife is a good thing when you are pulling cones apart, and a pair of tweezers, a scale peel and this, that is all the equipment you need. It is a bit laborious, but it is nothing difficult.

Mr. Nagle: Just one question, these tests that you have illustrated here in this table, were they half seeds or were they excised embryos - the Grodex tests?

Mr. Finnis: That was a half embryo.

Mr. Nagle: That is, you just cut through the whole seed?

Mr. Finnis: I cut through the whole seed.

Mr. Nagle: Did you take the half embryo?

Mr. Finnis: Threw one half into here, and the way I used to do it, I would sit down at the desk and cut them in half on this plate, and as I tossed one into the pot, I ticked on the tally whack and threw the other away.

Mr. Nagle: But the embryo and the rest of the seed were still together, that is, the half embryo?

Mr. Finnis: The half embryo and half endosperm goes in here.

Mr. Cameron: Does the water separate one from the other?

Mr. Finnis: Some of them fall apart, so you have to pick up.

Mr. Haddock: On this basis, then, your endosperm doesn't stain in this particular time?

Mr. Finnis: It does stain, but I have never taken any account of that. Maybe we should. There is an awful lot we don't know. It is only a new system.

Mr. Rindt: You have to manipulate your lights so you can see what you are doing and still keep the solution dark?

Mr. Finnis: I just put this in the drawer of the desk and after 3 hours I take it out and tip off the solution. You can leave it out then; it won't stain any more.

Mr. Rindt: You put them in water and then you add the chemical?

Mr. Finnis: I just put a wee drop in there, just to keep them moist, that is all.

Mr. Augenstein: You have to watch after it has been out for a while. The air action will start the whole works turning red. You start the enzymes working - fermentation.

Chairman Webster: Thanks, Mike, for your very fine contribution to our meeting. I am sure we will want to refer back to your papers.

In appreciation for the hospitality that has been extended to us here by the British Columbia Forest Service, some of us talked this noon and decided it would be well if we would take up a contribution of, say, $5.00 apiece, something like that, and buy something to leave here at the training school. Now, we are not asking Harold whether we should do this or not; we are telling you. I will appoint a committee to work out recommendations for a gift and to make the collection of $5.00 apiece, if that is what the committee decides. I am going to appoint Ernie Wright as chairman of that committee, and Wally Engstrom and Verne McDaniel.

Chairman Webster: Now Mr. Shaw is here from the "Lumberman" and he wants to take a picture of this group, I believe just outside - is that correct? When we leave the door, we will assemble just outside and have the picture taken. Then we will turn the meeting over to Tom Wells, who will take us through the nursery. Tom will have men from the British Columbia Forest Service who will enter into the program, but I will turn the meeting over to you Tom, just as soon as we have the picture taken.

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GREEN TIMBERS NURSERY

August 13, 1952

The meeting was opened by the Chairman at 9:00 A.M.

Chairman Webster: Ernie Wright, I think we should have a report from you now on your committee and the work they have done.

Mr. Wright: Well, you fellows have certainly been most generous and easy men to collect a donation from. We now have a total of $90.00 and we made a little window shopping tour last night in Vancouver and we think that will be sufficient to cover the cost of a nice chimes clock for the lounge in the administration building, with a little dedication on it, which is the toughest part of the job. I have written up a little dedication here, which I will read to you hoping that you fellows can think of a way to shorten it: "To The British Columbia Forest Ranger School, 1952. Presented by the Nursery Practice Committee, Western Forestry and Conservation Association, in appreciation of Courtesies extended to the Nurserymen's Convention."

All right, I will proceed to turn the money over, and I will do it here before all these witnesses.

Mr. McDaniel: Mike, I was asking Ernie -he is Chairman of this committee - if we could pull a buck out of every one of these fellows who is eating lunch here and staying outside. We feel we should contribute a little something.

Chairman Webster: If you want to do it, that is fine.

Mr. Pedley: I would like to take this opportunity of thanking you very, very much indeed for this appreciation, and I am sure the boys coming down here in the future will get a kick out of it, too. Apart from this, naturally enough, at this particular time you have been very, very welcome indeed, and we have been very glad to have you, and we will certainly be glad to have you back again.

Chairman Webster: Well, fellows, we are going to start right into the program now. We really have too much on the program for today and we may not have quite as much time for our discussions as we had yesterday on some of our topics, but they are all so interesting we didn't know where to cut down in trying to arrange this program. Every one of these subjects is quite interesting to at least most of the group, so that is why we have a little heavier program today than we did yesterday. There are some of the fellows that want to catch ferries tonight, some of them want to get down as far as Seattle, and I am going to try to hold our schedule, if I possibly can, so we can get through at three o'clock. If I happen to cut you off a bit short today, please be a little tolerant, it is because of the fact that we want to hold to our schedule as closely as we possibly can.

Now the first topic on the program today is going to be handled by Charlie Rindt. Charlie and I talked this over together. It is the subject of "Low Temperature in Improving Storage Conditions for Forest Tree Seed." We thought it would be well if we could get some outside person, so to speak, into our meeting this year, and we asked Paul Rudolf of the Lake States Experiment Station, or his superiors, if Paul could come out here and attend our meeting. Because of travel money, they couldn't send Paul, but he did agree to prepare a paper. I feel that Charlie Rindt is just as well qualified to prepare the paper and do the whole thing from the ground up, but the way it turned out, Charlie is going to handle the situation anyway. We are giving Charlie all the leeway he wants. He can read the paper of Paul Rudolf, or handle it any way he wishes. Charlie, will you take over now, please.

Mr. Rindt: I think what I will do, fellows, is read this paper - it isn't very long - and then devote the rest of the time to discussion. It is to me a very interesting subject and an important one, and I think that a lot of people here today realize that the storage of seed is something that we are going to have to know a lot about, because we are in the pinch right now of having a very short supply. We in the Forest Service would like to have several thousands of pounds of seed this year that we don't have because we have areas that we would like to do direct seeding on, in addition to raising trees in our nurseries. There is no seed this year in either Washington or Oregon of any species with the possible exception of some Noble fir that still is of doubtful quality. We are going to run on to these conditions in the future and certainly the only way that we can overcome them is to have seed in storage, and we want to be able to keep the seed in storage without loss of viability, because a loss of only a small percentage on many thousands of pounds means a lot of money. Of course, the seed dealers, I think, feel the same way. I know that they would like to sell us seed at a reasonable price, and certainly they can't collect seed in a year like this at a reasonable price. If they could carry it over, they could supply the market, and we would all be a lot happier and a lot better off.

Well, many of you know Paul Rudolf, and certainly many of you know him from things he has written. He prepared the paper, and I will read it as he wrote it.