VIABILITY AND GERMINATION IN YELLOW BIRCH¹

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ABSTRACT

Yellow birch seeds were found to vary in size directly as the strobilus on which they are borne and to increase in size within the strobilus directly as their point of origin is removed from either end. Viability was found to vary from 0% to 34% of the seeds on an individual tree. The best method of determining viability is examination of the embryo following dissection of the seed. A valid test of viability is given by 2,3,5-triphenyltetrazolium chloride, but this is not recommended because of the small size of the embryos. It is shown that the seed coats contain a water soluble substance that inhibits growth of the embryos. This substance loses its inhibiting properties when exposed to extended periods of light, but the injury to the embryo is permanent. It is suggested that trees producing few viable seeds in a seed crop may do so because this species is not functionally monoecious and that pollination difficulties would account for a small number of viable seeds on partly isolated trees.

INTRODUCTION

During a current study of mycorrhizal relationships of yellow birch (*Betula lutea* Michx. f.), the senior author has experienced considerable difficulty in securing germination of the seeds of this species under aseptic conditions. Very little has been published about dormancy, germination, and inhibitors of birch seeds, and very few articles are available that deal specifically with North American representatives of the genus. Joseph (3) discussed germination

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and vitality of birch seeds and found that they could germinate at very low temperatures, even at 0°C., after they had been stratified a long time, but fresh seeds, or seeds kept dry, required a much higher temperature. Patterson and Bunce (6) described a method of determining the percentages of fertility and sterility in seeds of yellow birch, in which they placed them over a bright light under a dissecting microscope and established the presence or absence of an embryo in each seed by the nature of the silhouette. Yellow birch in the forest was investigated by Linteau (4), who dealt specifically with seedbed conditions necessary for good germination and establishment, and silvicultural systems that would bring them about. Baldwin (1:199) referred to the results obtained by two German investigators who found that germination of birch seeds required 21 days following their exposure to suitable conditions. The United States Department of Agriculture (9:99-103) presented data on flowering, seed ripening, seed dispersal, and seed year frequency in yellow birch. It is stated that good seed crops occur every one or two years, and germinative energy varies from 6% to 48%, averaging 27%, at 20 days, and that potential germination ranges from 21% to 52% of a seed crop.

When attempts were made to secure germination under aseptic conditions, problems arose the answers to which were unavailable in the literature and it was necessary to carry out several tests for the purpose of gaining information to overcome them. Germination could not be obtained with seeds placed aseptically on filter paper moistened with distilled water. Slitting the seed coat to expose the embryo did not apparently free the embryo enough to permit it to grow. The present paper discusses the methods devised for overcoming these difficulties and obtaining yellow birch seedlings in pure culture.

METHODS AND RESULTS

All seeds used were collected from trees during the last two weeks in October and were stored dry at 0°C. for two months before any studies were begun. They were still capable of germinating 27 months after harvesting.

Difficulty was experienced in obtaining seeds that contained embryos. Seeds collected from each tree were kept separate and from each collection a sample of 500 seeds was selected, each seed being cut open to determine the presence of an embryo. They were completely absent in the seeds collected in central New Brunswick in 1951, but in 1952 embryos were easily found in the seed crop throughout New Brunswick and Nova Scotia, although their occurrence varied among trees. They were present in from 5% to 34% of the seeds produced on a tree. To determine whether the lack of embryos in yellow birch seeds was general in 1951, samples of duff from birch stands were examined in May, 1952, for germinating seeds. Two hundred seeds were recovered, but only one contained an embryo. It may possibly have been a seed of the 1950 crop, but later in the growing season new yellow birch seedlings were found at scattered points in the forests. It therefore appears that some embryos were produced in 1951. A survey of older seedlings showed that very few originated from the 1945 seed crop. There

are no records to show the size of the strobilus crop for that year, but in 1951 and 1952 there were average-sized crops.

To test viability of the seeds at hand, several methods were used. That outlined by Patterson and Bunce (6) is quite satisfactory in that it enables one to determine readily the presence of an embryo, but it fails in that it does not permit recognition of its color. Their technique may be used for selecting the seeds to be examined further. Plate 1(1) shows an embryo (right) excised from a seed compared with endosperm and two shrivelled ovules (left), the contents of another seed. Cutting the seed coats with a scalpel was found to be more satisfactory than Patterson and Bunce's method in that it permits one to see the embryo and, if it is fleshy and pure white in color, it may be considered capable of growth. In this study, it was found that those appearing more or less creamy-yellow to yellow in color were not viable. Of the number examined here, about 20% were off-color and could not be expected to grow.

Ten embryos were removed from their seed coats and were treated with 2,3,5-triphenyltetrazolium chloride. They were split longitudinally and exposed to 0.05% solution, as outlined by Bennett and Loomis (2). Eight of the embryos selected gave a positive test for viability. These were pure white, whereas the other two were creamy-yellow. This test is not recommended for yellow birch seeds, however, because they are so small that the color reaction can be observed only under high magnification, while the cutting technique, which can yield equally accurate results, can be readily conducted under a dissecting microscope. Also, embryos must be removed from the seed coats before they can be sectioned for the chemical test.

Shortly after the selection of seed was begun, it became apparent that more of the larger seeds were viable than the smaller, that larger strobili contained larger seeds, and that, therefore, it would be advisable to collect from the largest strobili.

A group of strobili was selected and it was found that the range in length was 20 mm. to 45 mm. Five size-groups were therefore established, 21-25, 26-30, 31-35, 36-40, and 41-45 mm. long. Ten strobili were selected in each group and ten seeds were removed from the bracts at about mid-way along each strobilus. These seeds were weighed to the nearest 0.1 milligram. Weights are those of seeds stored at 98% relative humidity at 5°C. for at least two months. Table 1 gives a summary of the results, and Plate 1(2) shows the relationship between the size of seeds and the parent strobilus. Statistical analyses of the data show that the maximum diameter of a strobilus is dependent upon the length of the strobilus; that the weight of a seed is dependent upon strobilus length, but is also dependent upon diameter, which is regulated by the size of the bract on which the seed is borne. The following equation was developed by the least squares method to assist in forecasting seed size: Average weight, in milligrams, of each seed borne on central bracts of strobilus = $0.0273 (\text{length of strobilus in mm.}) + 0.0596 (\text{diameter of strobilus in mm.}) - 0.6319$. A chi-square test of this curve

shows it to fit the data very well. Germination rates of seeds and establishment of seedlings show that one may expect best results if seeds are utilized from strobili more than 3 cm. long. Seeds from central bracts were used because it was found that the weight of seeds increased as the distance of their point of origin from one or the other end of the parent strobilus, as is shown in Fig. 1.

All seeds were stored at 0°C. for two months before germination tests were made. Satisfactory surface sterilization of seeds was accomplished by covering them with a solution of sodium hypochlorite, which contained 0.5%-0.6% available chlorine, to which 1 drop of 0.5% Aerosol per 100 cc. of solution was added. After 15 minutes this solution was decanted and the seeds were washed in three changes of sterile distilled water. Any seeds still floating at the end of this period may be allowed to pour off when decanting because they will rarely contain embryos; those sinking usually do.

When seeds containing embryos were placed on filter paper and set to germinate in the dark at 22° to 25°C., only about 0.1% had germinated at the end of 30 days. When they were placed in daylight (September) the rate increased to 3.0%. Treatment with 1 normal NaOH or 95% H₂SO₄ at 25°C. for periods ranging from 1 to 60 minutes resulted in no germination after 30 days at 23°C. under either normal daylight (September) or total darkness. These treatments were discarded and an attempt was made to overcome difficulties in germination by splitting the seed coats with a sharp scalpel before the seeds were placed on filter paper. They were then placed in the dark, but again the results were very low (0.3%). Numerous embryos were removed from their seed coats and, when placed on filter paper moistened with sterile distilled water at 22° to 25°C., they grew quite satisfactorily (about 80%) after two days. Meanwhile, a few hundred seeds were placed under sterile conditions in vermiculite, which was kept well moistened with sterile distilled water. Germination rates were, in this case, very high. It was then reasoned that there must be a water soluble growth inhibitor contained in the seed coats, and an experiment was set up to investigate this possibility.

Seed coats, from which embryos were removed, and entire seeds were soaked in distilled water for 20 hours, at the rate of 20 seed coats or seeds per cc. of water. The seeds were removed and the solution rendered sterile

TABLE I.
RELATION BETWEEN LENGTH OF STROBILUS AND
THE WEIGHT OF VIABLE SEEDS IN YELLOW BIRCH

	Size classes of strobili				
	1	2	3	4	5
Length in mm.	21-25	26-30	31-35	36-40	41-45
Average length	22.9	28.2	32.8	37.7	40.8
Average diameter	15.8	18.0	19.1	21.4	21.5
Average wt. of seeds (mgs.)	0.93	1.24	1.36	1.65	1.80

FIGURE I

Relation between the weight of a yellow birch seed and the distance of the bract on which it originated from the end of the strobilus.

by passing it through a Seitz filter. This solution was used for moistening filter paper in a petri plate and 5 cc. of liquid and 20 yellow birch embryos, excised under aseptic conditions, were placed in each dish. Some were placed in continuous darkness, others in normal daylight (September) at 23°C., and growth was observed. In one case the solution was exposed to daylight for 60 hours before the embryos were placed in contact with it. Table II, which contains the results of these tests, shows that there is a water soluble substance contained in the seed coats of yellow birch seeds that prevents growth of yellow birch embryos (See Plate 1(3)) and that this substance loses its effectiveness when exposed to light. Mosheov (5) reported an inhibitor possessing similar properties in wheat seeds. Attempts were made to determine whether the substance or substances caused permanent damage to the embryo. One group of 40 embryos, after having been exposed to the aqueous extract from seeds for 54 hours, was removed, washed in sterile distilled water, and placed on filter paper moistened with sterile dis-

tilled water. None of them exhibited growth and it would appear that the damage incurred is permanent, at least after 54 hours of exposure to the extract.

TABLE II.

COMPARISON OF GROWTH OF YELLOW BIRCH EMBRYOS IN LIGHT AND DARKNESS ON FILTER PAPER MOISTENED WITH DISTILLED WATER OR AN AQUEOUS EXTRACT FROM SEEDS OR SEED COATS

Filter paper moistened with	Distilled water		Aqueous extract from seeds				Aqueous extract from seed coats	
Pretreatment			Placed 60 hours in light before use		Used immediately		Used immediately	
Incubated in	light	darkness	light	darkness	light	darkness	light	darkness
No. embryos	40	821	40	40	40	80	40	40
No. showing growth	28	583	18	14	0	0	0	1
Percentage showing growth	70	71	45	35	0	0	0	2.5

Reliable evidence of dormant fungus mycelium existing beneath the seed coat of some species was presented by de Zeeuw (11), and Raynor (7) reported that mycorrhizal fungi are sometimes found within seeds. During the present study, over 1000 yellow birch embryos were excised and placed on bacto-malt-agar, but no fungus emerged from them at any time that can be considered to have been contained within the seed coat.

From the results presented, it can be stated that there are two ways by which yellow birch seedlings may be obtained in pure culture. By the first method, seeds containing embryos are placed in sodium hypochlorite, with 0.5% to 0.6% available chlorine, to which 1 drop of 0.5% Aerosol per 100 cc. of solution is added. After 15 minutes this solution is decanted and the seeds are washed in three changes of sterile distilled water. Embryos are then removed from their seed coats, under aseptic conditions, and are placed on a sterile moist substratum. Growth begins in 3 to 4 days.

PLATE I

- (1) Embryo excised from yellow birch seed (right), endosperm and two shrivelled ovules from unfertilized seed (left).
- (2) Yellow birch strobili, with ten seeds removed from their central bracts, representing arbitrarily selected size classes: A, 21-25; B, 26-30; C, 31-35; D, 36-40; E, 40-45 mm. long.
- (3) Yellow birch embryo 4 days after being placed on aqueous extract from seed coats (right). A young seedling growing from an embryo that has been 4 days in sterile distilled water (left).

The other method requires that seeds, surface-sterilized as above, be placed in a sterile, hygroscopic compound, such as vermiculite, that is kept well watered for at least 21 days. Using either method, the germination chambers should be arranged so that embryos or seeds receive light for at least part of each day. Temperatures from 23° to 25°C. are satisfactory.

DISCUSSION

A review of the literature on seed production of forest trees indicates that the percentage of viable seed produced varies from year to year. Results recorded in this paper indicate that 1945 and 1951 were years in which this percentage was very low for yellow birch in the Maritime Provinces. Baldwin (1:18) stated that these conditions may be due to weather conditions at the time of pollination. According to studies on European birches by Sarvas (8), weather conditions, by their effect on the pollen or ovum at the time of pollination, can be considered to be indirectly responsible for much of the failure in seed production. Thus, it is possible that prolonged cold could prevent pollination and, although a large crop of conelets may be produced, they would contain no viable seeds. A study of the quality of seed crops to be expected following varying weather conditions at pollination time would supply valuable information to seed collectors.

The relation of seed size to size of strobilus and also to the position of the seed along the axis of the strobilus is of importance when seeds are being collected for research or other special purposes. As stated earlier, it is inadvisable to collect strobili under 30 mm. long from yellow birch, if good germination and establishment are requirements.

Patterson and Bunce (6) found that certain birch trees produce only a few viable seeds each year, others none. This may be due to their distance from other trees of the same species. It is suggested that yellow birch trees, although morphologically monoecious, are functionally dioecious. If this should be the case, isolated trees could bear no viable seeds if pollen from other trees was unable to reach them. Sarvas (8) stated that dense birch stands were required for an abundance of viable birch seeds.

The occurrence of a water soluble inhibitor of growth in the seed coats of yellow birch is, no doubt, a protective measure, delaying germination until proper weather conditions for the establishment and survival of the seedlings prevail. The fact that the inhibiting substance is rendered ineffective following exposure to daylight, at least when in solution, explains why better germination is obtained when the duration and intensity of light on birch seed-beds are increased, as was stated by Vaartaja (10). It is possible that many tree seeds possess growth inhibitors of a similar nature.

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