sistance is a threshold character sometimes overcome under field conditions particularly favorable for infection. The same threshold-type resistance seems to hold in seedling progenies. We are convinced that with repeated artificial inoculations under ideal conditions for infection we can induce infection on the most "resistant" seedlings available. Resislance is thus relative. Probably we shall never produce completely immune western white pine.

Blister rust resistance is known to exist in both eastern and western white pine (PATTON and RIKER 1958; BINGHAM et al. 1960), but so far there has been little concrete evidence concerning the numbers or kinds of genes involved in the resistance system. Both HEIMBURGER (1962) and BINGHAM (1963) have suggested that resistance is controlled by polygenes. The fact that resistance is seated in both foliage and bark tissues, and has quantitative-like inheritance (see Table 1, BINGHAM et al. 1960), indicates that more than one partially dominant gene is involved. The performance of selfed lines further strengthens this hypothesis by showing that control is not by single or multiple recessive genes.

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Germination of Blue Spruce and Ponderosa Pine Pollen After Eleven Years of Storage at 0° to 4° C[1])

By GILBERT H. FECHNER and ROBERT W. FUNSCH[2])

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Pollen of blue spruce (Picea pungens ENGELMANN), ponderosa pine (Pinus *ponderosa* LAWSON), lodgepole pine (Pinus *contorta* DOUGLAS), and limber pine (Pinus *flexilis* JAMES) was stored in 1954 at each of two temperatures (0 to 4 degrees C. and 25 to 27 degrees C.) and under each of three relative humidities (0, 25, and 50 percent). Periodic estimates have been made of the germination of this pollen after 24 hours of culture in vitro at room temperature on two culture media: agar (1.2 percent plus 2.0 percent sucrose), and a 10 percent aqueous sucrose solution shown by preliminary tests in 1954 to give the highest germination and/or longest pollen-tube growth of fresh pollen.[3]) The procedures used for culturing the pollen and estimating germination have been described (FECHNER, 1938). After six years of storage at 25 to 27 degrees C., the pollen of none of the species studied germinated, regardless of the relative humidity of storage (FECHNER, et al., 1960). This paper presents the results of cultures made during June, 1955 of the pollen which had been stored for approximately eleven years at 0 to 4 degrees C. and under the three relative humidities mentioned.

Samples of blue spruce and ponderosa pine pollen which had been stored for eleven years at 0 to 4 degrees C. and 50 percent relative humidity were also cultured on six aqueous sucrose solutions (0, 5, 10, 15, 20, and 25 percent concentration) in each of two Petri dish culture chambers to determine whether any change in the sucrose requirement for germination had occurred during storage.

Results

Pollen of ladgepole pine and limber pine which had been stored for eleven years at 0 to 4 degrees C. did not germinate on either culture medium used, regardless of the relative humidity at which it had been stored. Blue spruce and ponderosa pine pollen stored for eleven years at 0 to 4 degrees C. germinated as much as 30 and 70 percent, respectively. Highest germination of blue spruce pollen was obtained when the pollen was stored at 50 percent relative humidity; germination of ponderosa pine pollen was highest when the pollen was stored at 25 percent relative humidity but not significantly higher (as determined by the F-test) than that stored at 50 percent relative humildity. The germination of blue spruce pollen was not significantly affected by the medium on which the pollen was cultured, but germination of ponderosa pine pollen was approximately two to three times greater on the 10 percent sucrose modium than on the agar medium at each storage relative humidity (Table *1).*

Table 1. — Germination of blue spruce and pondeross pine pollen after 24 hours of culture at room temperature, following eleven years of storage at 0 to 4 degrees C.

Species	Storage Relative Humidity, Percent		
	0	25	50
	Germination, Percent		
Blue spruce	0.5	19.0	29.5
Ponderosa pine			
10 percent sucrose	19.0	71.0	63 0
1.2% agar + 2.0% sucrose	6.0	33.0	30.0

Pollen of blue spruce which had been stored for eleven pears at 0 to 4 degrees C. and 50 percent relative humidity showed the highest germination on a 15 percent sucrose solution, when different aqueous concentrations were compared (Figure 1). There was no significant difference in germination percent between Petri dish culture chambers in any of the concentrations tested. Following eleven years of storage at the same temperature and relative humidity conditions, however, germination of ponderosa pine pollen was significantly affected (5 percent level of probability) by the concentration of sucrose in aqueous solution, greatest germination occurring on a 5 percent solution (Figure *1).*

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[3]) The aqueous medium used for limber pine was distilled water.

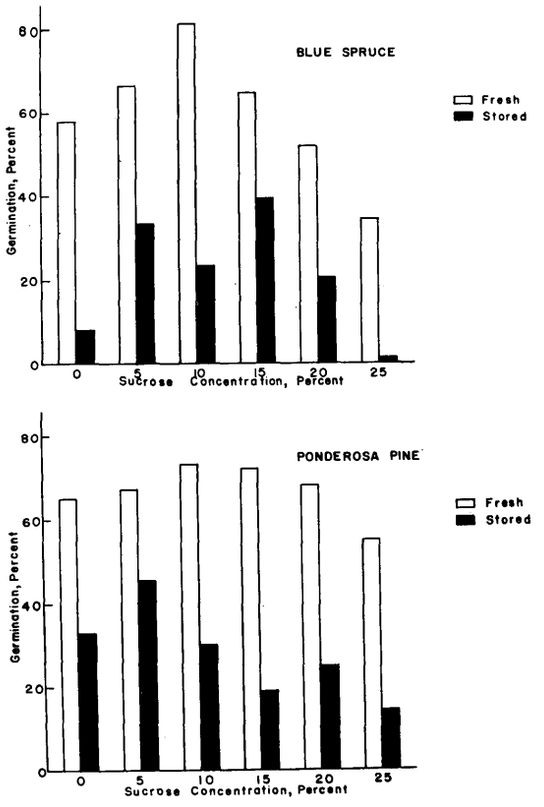

Figure 1. — Germination of fresh pollen (1954) and pollen stored for 11 years (1965) at 50 percent relative humidity and 0 to 4 degrees C. on different sucrose solutions after 24 hours of culture at room temperature.

Significant differences in germination were observed however, between Petri dish culture chambers on the 0, 20, and 25 percent sucrose concentrations.

Discussion

Most authors who have studied stored coniferous pollen, e. g., Duffield and Snow (1941), Johnson (1943), Cumming and Righter (1948) and Duffield (1954), have found that its viability, as determined by subsequent germination on artificial media, is enhanced if the pollen was stored at approximately 0 to 4 degrees C. and 25 to 50 percent relative humidity. The results of this study show that the viability of blue spruce and ponderosa pine pollens may be retained for as long as eleven years under these temperature and humidity conditions. However, that this is not always true has been shown by Stanley *et al.* (1950) and Stanley and Poostchi (1962), who found up to 77 percent germination of ponderosa pine pollen stored at 10 percent relative humidity but no germination of that stored at 25 percent or higher relative humidity after 15 years of storage at 5 degrees C. Germination of blue spruce pollen used in the present study was not significantly affected by storage relative humidity until after the first year of storage (Fechner, 1955), but the germination of ponderosa pine pollen has been greatly affected by relative humidity of storage throughout the eleven-year period.

The optimum culture medium for the germination of pollen *in vitro* apparently varies not only with species but also with the length of the storage time. No significant difference was found in 1965, nor at any other observation during the eleven-year storage period, between the germination of blue spruce pollen on the agar medium versus the sucrose medium. Although germination of ponderosa pine pollen was not significantly affected by culture medium after one and three years of storage, it was significantly affected by culture medium after six and eleven years of storage, when the sucrose solution produced higher germination than the agar medium (Fechner, 1955; Fechner, 1958; Fechner *et al.*, 1960).

Furthermore, the optimum sucrose concentration in aqueous solution changed for both species during storage. In 1954, fresh blue spruce pollen germinated significantly higher (1 percent level of probability) on a 10 percent solution than on the other concentrations tested, but pollen from the same lot stored for eleven years germinated best on a 15 percent solution. For ponderosa pine, the 5 percent concentration of sucrose appeared best in 1965, whereas in 1954 fresh pollen from the same lot germinated about equally well on all concentrations tested, although pollen tube length attained was greatest on a 10 percent solution then. Kühlwein and Anhaeusser (1951) observed that the sucrose concentration necessary for the germination of pine pollen increased from two to 20 percent after six months of pollen storage. Schoenike and Stewart (1963) found that germination and tube growth of moderately-aged pine and spruce pollen was enhanced by the presence of 5 to 10 percent sucrose in the culture medium. Stanley and Poostchi (1962) found higher quantities of free glucose and oligosaccharides in viable pollen than in non-viable pollen following 15 years of storage, and they suggested that the mechanism by which pollen cells retain their viability is related to the intracellular roles of respiration during the storage period. Precisely how these roles may be played, however, is still unknown.

Duffield and Callaham (1959) found that pine pollen which had been initially dried over calcium chloride, quick-frozen at −23° C., and subsequently stored for 10 months in a deep freezer had about the same germinative capacity as fresh pollen. Controlled pollinations with pollen so stored yielded approximately the same percent of sound seed as fresh pollen did, although the number of sound seeds per cone was reduced. Stanley (1962), however, found that pine pollen stored for 15 years at 10 percent relative humidity and 5° C. produced only hollow seeds, but high germination of pollen from the same lot was obtained on artificial media. Thus, germination of pollen on artificial media may or may not indicate its ability to produce viable sperm nuclei *in vivo*.

It appears from the results of this study, as well as those of other authors, that caution should be exercised in interpreting results of germination tests of pollen *in vitro*. Germination may be affected by the extraction relative humidity (Duffield, 1954), the temperature and relative humidity of storage, as well as the length of storage time, although the effect is not always the same. The optimum culture medium also appears to vary with species and the conditions under which the pollen was stored. The magnitude of variation in pollen germination that may occur between culture chambers is not presently clear, but this variation may imply that a large number of replications is required. Furthermore, the relationships between *in vitro* and *in vivo* germination of pollen, pollen-tube growth, and production of viable sperm nuclei is not fully understood.

Summary

Pollen of four conifers stored for eleven years at 0, 25, and 50 percent relative humidity and 0 to 4° C. was cultured in Petri dish chambers on an agar medium (1.2 percent

plus 2.0 percent sucrose) and on a 10 percent aqueous sucrose medium. Pollen of lodgepole pine and limber pine failed to germinate. Highest germination of blue spruce pollen was obtained from pollen stored at 50 percent relative humidity; culture medium had no significant effect. Highest germination of ponderosa pine pollen was obtained from pollen stored at 25 to 50 percent relative humidity and cultured on the aqueous sucrose medium.

In a second experiment, pollen stored at 50 percent relative humidity and 0 to 4° C. for eleven years was cultured on 0, 5, 10, 15, 20, and 25 percent aqueous sucrose solutions. Optimum concentration for germination of blue spruce pollen was 15 percent; 5 percent for ponderosa pine pollen. In 1954, optimum concentration for germination of fresh pollen from the same lots was 10 percent for both species.

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Progeny Test Designs for Pinus patula in Rhodesia[1])

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Introduction

Afforestation with exotic pines in Rhodesia had been in progress for 30 years and some 70,000 acres had been established before tree breeding was initiated in 1958. By then well over half the planted area comprised stands of *Pinus patula* while *P. elliottii* and *P. taeda* accounted for most of the remainder. The importance of *P. radiata* had diminished because of its susceptibility to disease. It was evident that the growth rate and health of the first three species was satisfactory, provided sites were carefully selected, but that improvements were desirable in other respects. The phenotypic variation in stem, crown and timber characteristics appeared to warrant the establishment of an improvement programme (HODGSON and BARRETT, 1962).

Initially, plus trees were selected for the establishment of clonal seed orchards. Forty such trees of *P. patula* have so far been selected from 3,600 acres of local stands more than 12 years old. Their frequency is estimated at 1 in 31,500 trees remaining in stands selectively thinned to an average of one-third of the original density. Plus tree clones have been randomised in suitably isolated seed orchard areas at altitudes between 5,500' and 6,100'. An additional seven clones have been acquired from other countries and most of these have been incorporated in the seed orchards.

It was provisionally intended to assess the general combining abilities of selected clones by a polycross test, to be followed in subsequent years by diallel crosses among the better combiners (HODGSON and BARRETT, 1962; HODGSON, 1963). The limited number of progenies planted to date are those derived from early work undertaken to gain practical experience in controlled pollination techniques.

In 1964 it was realised that, particularly in the early stages of the breeding programme, the progeny testing method used should not only identify the best general and specific combiners, but should also yield information on population genetics. It was therefore decided to review the existing proposals.

The progeny test plan described here is the outcome of this review in which it was possible for a forest geneticist and a biometrician to participate with local forest research staff.

The Intended Function of Progeny Testing in Rhodesia

The improvement programme must provide for a local seed source that will yield continuous and adequate quantities of seed representing the maximum improvement attainable at the time of supply.

The respective merits of clonal and seedling seed orchards have been much discussed elsewhere (see for example the first issue of *Silvae Genetica* 13, 1964, devoted to this subject). It is necessary here only to record the local reasons for the choice of progeny tested clonal seed orchards as the intended source of improved *P. patula* seed.

The continual acquisition of new plus trees, changing utilisation requirements and different rates of improvement in the various traits, all imply that seed orchards require periodic reconstitution. Routine progeny testing is essential in order to determine the optimum constitution at any given time. Clonal seed orchards have the advantage that

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