Percent selfed seedlings: $\frac{30}{30 + 970}(100) = 3$

Percent self-fertilization: $\frac{200}{200 + 1212}(100) = 14$

Data from the 16 trees, which segregated for a total of 20 mutant forms[2] were utilized for computations such as those illustrated.

Results and Discussion

Frequency of natural self-fertilization in upper crowns averaged 7 percent. Estimates from individual trees ranged from zero to 22 percent. An estimate of 7 percent natural self-fertilization was found for the same stand by another method based on yields of filled seed (FRANKLIN, 1971).

Two aspects of the computations deserve special emphasis. First, the observed ratio of mutant to normal forms is an unbiased estimate of the actual ratio for a particular family regardless of the amount of embryonic mortality due to environmental or genetic factors. If a particular segregation ratio is assumed, such as 3 normal to 1 mutant — the classical Mendelian ratio, the undetected presence of genetic linkage, for example, would result in biased estimates of the frequency of natural selfing (SORENSON, 1967). Second, the use of germination percentages from controlled pollinations allows the estimation of actual percentages of self-fertilization, in addition to percentages of seedlings resulting from self-fertilization. Both values are necessary for a more complete understanding of the genetic structure of populations.

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[2] For descriptions of mutant forms and their segregation ratios see FRANKLIN (1969 b).

Estimates of Frequency of Natural Selfing and of Inbreeding Coefficients in Loblolly Pine

By E. C. FRANKLIN[1]

Knowledge of the genetic structure of loblolly pine (*Pinus taeda* L.) stands is needed as more sophisticated models and methods of selection, testing, and seed production are proposed for this species. Two important facets of genetic structure are the average frequency of self-fertilization and the average inbreeding coefficient of the sexually mature stand. The present experiments were conducted to estimate the frequency of natural self-fertilization in upper and lower crowns of loblolly pine trees in a natural old-field stand. Estimates of inbreeding coefficients of seed populations at the time of fertilization, and of the population of parental trees, were also sought.

Methods

Artificial self- and cross-pollinations[2] were performed on 132 trees on an old-field site in the North Carolina Piedmont in spring 1965 (FRANKLIN, 1969). Control-pollinated cones were obtained from 118 of those trees. In addition, wind-pollinated cones were collected from the upper crowns of 114 trees included in the control-pollinated group and from the lower crowns of 23 open-grown, large-crowned trees included in the wind-pollinated group.

Percentage of filled seed was found to be the best metrical trait for estimating frequency of selfing (KATSUTA, 1964).

The following relationship was used:

$$\text{Percent natural selfing} = \frac{c - w}{c - s} \times 100$$

where c, w, and s are percentages of filled seed after controlled cross-, wind-, and controlled self-pollination.

Estimates of the inbreeding coefficients cannot always be simply estimated because they are functions of the amount of inbreeding in the current generation, plus the average inbreeding coefficients of the parent trees. However, in this experiment the inbreeding coefficient of the parent trees was found to be so low (0.0003) that it could be safely ignored.[3] Therefore, the inbreeding coefficients for upper and lower crown seed lots at fertilization could be found by multiplying the percentage of self-fertilization by one-half, which is the inbreeding coefficient after one generation of selfing.

Results and Discussion

Frequency of natural self-fertilization in upper crowns was estimated to be 7 percent, and in lower crowns 34 percent. Estimated population inbreeding coefficients were as follows: upper crown at time of fertilization, 0.03; lower crown at time of fertilization, 0.17; and among parent trees in the natural stand, 0.0003.

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[2] Cross pollen consisted of a five-tree mix from a stand 10 miles distant.

[3] For estimation procedures see FRANKLIN, E. C.: Artificial self-pollination and natural inbreeding in Pinus taeda L. Ph. D. Diss. N. C. State Univ. at Raleigh, 127 pp. (1968). Available from University Microfilms, Ann Arbor, Michigan 48106. (Order No. 68-14, 653).

Seven percent natural selfing in upper crowns is insignificant for most traits, but 34 percent natural selfing in lower crowns will cause noticeable inbreeding depression in several traits, especially yield of filled seed (Franklin, 1969). For purposes of open-pollinated progeny testing, most biases due to natural inbreeding (Namkoong, 1966) can be avoided by making seed collections from upper crowns. The same applies to collections for provenance trials and to commercial seed collections. Conversely, when open-pollinated collections are used to locate trees heterozygous for recessive mutant alleles, as suggested by Snyder *et al.* (1966), chances of finding such trees are increased by collecting cones from lower crowns.

The extremely low inbreeding coefficient for parent trees indicated that a very small proportion of selfed seedlings became established and reached sexual maturity in this stand, despite appreciable levels of inbreeding at fertilization. This trend was expected because severe inbreeding depression was found in controlled self-pollinated families from this same stand (Franklin, 1969). These results firmly support the general assumption that mature stands of loblolly pine are largely outbred, even though appreciable amounts of self-fertilization occurs.

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An Instance of Clonal Incompatibility in Grafted Pinus radiata Seedlings

By R. J. Cameron and Gail V. Thomson

Introduction

In tree improvement work at the New Zealand Forest Research Institute, a method has been developed for tip cleft grafting scions collected from adult trees of *Pinus radiata* on to seedling root-stocks (Thulin and Faulds, 1966). Normally a rate of success better than 90 per cent can be expected. It has been observed, however, that scions taken from one particular tree, FRI Clone 104, show a high failure rate. The grafted seedlings appear healthy at first and grow quite vigorously, but soon after the seedling stocks are defoliated, usually early in January, a considerable proportion of the grafts become yellow, the needles droop and may fall, and many of the graft combinations die. Of the 50 per cent or so of grafted seedlings that do manage to survive the first growing season, many more die during the following spring.

The symptoms suggest that vascular transport between scion and stock is impaired. The movement of water and nutrients upwards through xylem tissues appears to be adequate for scion growth during the first growing season, but the dependence of some of the grafted seedlings, for healthy growth, upon the presence of live, functional foliage on the stock below the graft union gives the impression that in such plants photosynthates cannot pass from scion to stock. Under these conditions food reserves are soon exhausted in the stock which then, with its roots, dies of starvation. Death of the scion is concomitant. The second period of mortilaty, which occurs during the following spring when height growth is at a maximum, suggests, that in plants so affected the xylem connections in the graft are in some way inadequate; the stresses imposed at this time are too great and the plant dies.

The purpose of the present investigation was to study translocation of photosynthates within the grafted plants and thus investigate further the reason for graft failure.

The movement of photosynthates labelled with carbon-14 was used to follow phloem translocation in grafted seedlings.

From 200 plants of Clone 104 scion grafted on to 8-month-old seedling stocks in August 1966, 25 individuals were selected in February 1967, for detailed study. These comprised:

Group A: 5 apparently healthy grafted seedlings from which the foliage on the stocks had been removed four weeks previously.

Group B: 5 unhealthy grafted seedlings (foliage yellow or drooping) from which the foliage on the stocks had also been removed four weeks previously.

Groups C, D, and E: Each group consisting of five grafted seedlings, from which the foliage on the stock below the graft had not been removed.

For administration of $^{14}CO_2$ foliage zones of each grafted seedling were localised using polyethylene plastic bags, secured against the stem at open ends or ends with tight ties over cotton-wool pads, thus providing a firm seal without damaging the stem or restricting translocation. For Groups A, B, and E the polyethylene bags covered only the scion and were tied above the graft union; for Group C the whole plant stem, both stock and scion, was covered; and for Group D only foliage of the stock was covered using polyethylene tubes tied immediately below the graft union and again at ground level.

$^{14}CO_2$ was generated in the laboratory by the action of concentrated H_2SO_4 on $BaCO_3$. One cubic centimetre of a $^{14}CO_2$/air mixture, containing approximately 25 microcuries of carbon-14, was injected into each bag at 3 p.m. on 28 February and the injection hole sealed. At 9 a.m. the following day the bags were removed and foliage samples taken for later analysis, to ensure that in each plant there had occurred a reasonable uptake of $^{14}CO_2$. The results are given in *Table 2.* A check measurement on samples of foliage taken from outside the bag showed no signs of radioactivity at this stage. Earlier measurements had shown that it takes approximately 72 hours after administration of $^{14}CO_2$ for photosynthates labelled with carbon-14 to attain maximum concentration in the growing tips of roots (see *Figure 1).* Excepting the plants comprising Group E from which only small samples of foliage and roots were taken and the seedlings then transplanted for further ob-