DORMLING, I. and IFVER, J.: Studies on frost hardiness of *Pinus contorta* DOUGL. seedlings grown in climate chambers. Studia Forestalia Suecica. Nr. 157, 47 p. (1981). — JONSSON, A. ERIKSSON, G. and FRANZEN, A.: Within-population variation in frost damage in *Pinus contorta* DOUGL. seedlings after simulated autumn or late-winter conditions. Silvae Genet. **35**: 96—102 (1986). — LEVITT, J.: Responses of plants to environmental stress. Vol. 1. Chilling, freezing and high temperature stress. Academic Press, New York. 497 p. (1980). — NAMKOONG, G.: Introduction to quantitative genetics in forestry. U.S. Dep. Agric. For. Serv. Tech. Bull. 1588, 342 p. (1979). — REHFELDT, G. E.: Cold acclimation in populations of *Pinus contorta* from the northern Rocky Mountains. Bot. Gaz. **141**: 458—463 (1980). — REHFELDT, G. E.: Adaptation of population of *Pinus contorta* to heterogeneous environments in northern Idaho. Can. J. For. Res. **13**: 405—411 (1983). — REHFELDT, G. E.: Ecological genetics of *Pinus contorta* in the Wasatch and Uinta Mountains of Utah. Can J. For. Res. **15**: 524—530 (1985a). — REHFELDT, G. E.: Genetic variances and covariances in *Pinus contorta*: estimates of genetic gains from index selection. Silvae Genet. **34**: 26—33 (1985b). — REHFELDT, G. E. and WYKOFF, W. R.: Periodicity of shoot elongation among populations of *Pinus contorta* from the northern Rocky Mountains. Ann. Bot. **48**: 371—377 (1981). — SAS Institute: SAS User's guide. Vol. 2. Cary, North Carolina, SAS Institute 548 p. (1982). — WEISER, C. J.: Cold resistance and injury in woody plants. Science **169**: 1268—1278 (1970). — YING, C. D., ILLINGWORTH, K. and CARLSON, M.: Geographic variation in lodgepole pine and its implications for tree improvement in British Columbia. *In:* BAUMGARTNER, D. M., KREBILL, R. G., ARNOTT, J. T. and WEETMAN, G. F. (eds.). Lodgepole pine: the species and its management: symposium proceedings. Washington State University, Office of Conferences and Institutes, Cooperative Extension, Pullman, p. 45—54. (1985).

Are Pollination Bags Needed for Controlled Pollination Programs with Yellow-poplar ?[1])

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Summary

Differences among trees in seed production ranged from 7% to 75% germinable seed after controlled pollination, with mean control pollinated seed set exceeding that of open pollinated seed set by 600%. Pollination bags did not have a consistent effect on filled seed percentages, or on the number of two- embryo seeds. Male parent did not have a significant effect on filled seed percentages. Removal of petals and stamens (*i.e.*, emasculation) reduced the observed number of seeds produced by insect pollination to less than 1%. Contamination of seed lots produced by controlled pollination of emasculated, non-bagged flowers will be negligible.

Key words: Liriodendron tulipifera, controlled pollination, pollination bags, emasculatin.

Introduction

Yellow-poplar is an insect-pollinated species with a large, perfect flower. The gynoecium, consisting of 60 to 100 pistils, is located in the center, surrounded by a ring of 20 to 40 stamens. The corolla is comprised of six petals, with an orange band at their base in which the nectaries are located (WILCOX and TAFT, 1969). The fruit is an elongated cone composed of 60 to 100 overlapping carpels (samaras). Individual samaras have the potential for producing two seeds, but in most cases one of the embryos aborts. Natural seed sets average only about 10 percent (BONNER and RUSSELL, 1974).

In controlled breeding studies with yellow-poplar, precautions are usually taken to prevent unwanted pollination. Flowers which are unopened, but reproductively mature, are emasculated by hand by removing the sepals, petals and stames. The desired pollen, collected by forcing flowers in the laboratory, is then applied to the gynoecium with a small paint brush, and a pollination bag is placed over the flower to prevent subsequent visits by unwanted pollinators. Even though the average period of receptivity of individual flowers is only 12 to 24 hours (KAEISER and BOYCE, 1962), pollination bags are usually removed several days later.

Bagging is an expensive and time-consuming process, especially if an extensive breeding or seed production effort is contemplated. However, if petals and nectaries are removed during the controlled pollination process, insects theoretically should have little reason to visit the emasculated flower, thus drastically reducing the probability of unwanted pollination, the necessity of bagging, and the time required to complete a given set of crosses.

The objectives of this study were to (1) determine the effect of the pollination bag on seed set following controlled pollination, and (2) estimate how much pollination would occur if emasculated flowers were not protected by pollination bags.

Materials and Methods

Branches bearing unopened flower buds were cut from selected trees (*Table 1*) and transported to the laboratory in an ice chest. Pollen was collected by forcing the flowers in water culture. When the anthers dehisced (generally within 24 hrs), pollen was extracted, dried at room temperature (24° C) for 30 min, and stored in vials at 4° C until needed for pollination. Pollen lots were kept separate by male parent.

Pollinations were accomplished in early June. Flower buds were selected for use if they were unopened, and a space could be detected between the petals and the gynoe-

Table 1. — Codes and location of the yellow-poplar trees used in the pollination experiment.

Tree No.	Location
1, 2, 3, 4	Ohio Agricultural Research and Development Center, Wooster, OH
5, 6	Millersburg, Ohio
7, 8	Holden Arboretum, Mentor, Ohio

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cium. Stigmas are receptive at this stage of development (TAFT, 1962). Flowers to be pollinated were emasculated by hand, removing the entire perianth and the stamens, leaving only the gynoecium; pollen was applied with a small camel hair paint brush.

After emasculation and pollination, non-woven cloth pollination bags were placed over the flowers. A strip of cotton was placed around the twig below the flower, and the pollination bag tied off around the cotton in order to exclude insect pollinators. Bags were removed 6 to 10 days later when the gynoecia were no longer receptive to pollination.

Trees #1, #2, #3, #4, #7, and #8 served as female parents, while #5, #6, #7, and #8 served as pollen parents (*Table 1*). The following treatments were imposed on each female parent: (1) selfed, with pollination bag, (2) open-pollinated (*i. e.*, insect pollinated), (3) unpollinated, emasculated, with pollination bag, (4) unpollinated, emasculated, without pollination bag, (5) control-pollinated, emasculated, with pollination bag, and (6) control-pollinated, emasculated, without pollination bag. Each treatment was replicated four times (4 flowers) on each female parent.

Cotton mesh seed bags were placed over the fruits in early September to insure against seed loss. Seeds were collected when mature, kept separate by treament and cross, and dried at room temperature on greenhouse benches until the cones disintegrated.

Filled seed percentages were determined by x-ray photography. Dewinged seeds were stuck on flexible mylar sheets, placed on 13 cm × 18 cm Kodak Industrex AA film, and x-rayed at 15 kvp, 3ma, at 71 cm for 45 sec as modified from KRIEBEL (1966).

Seed images on developed films were scored on a light table to determine percentages of filled and empty seeds, as well as the percentage of double-embryo seeds. Three 50-seed lots were scored for each treatment on each female parent tree.

All seed lots were soaked in water for 24 hr and then placed in stratification for 90 days at 2° C (BONNER and RUSSELL, 1974). Germination tests were conducted in petri dishes on two sheets of moist filter paper and germination counts taken every other day through 65 days. Each test was replicated three times.

Data were stabilized using the arcsin transformation and subjected to analysis of variance. Differences in seed set among female parents were tested by TUKEY's honestly significant difference procedure. Differences in overall means between the two treatments of greatest interest, i.e., "control-pollinated with bag" vs. "control-pollinated without bag", were tested by Student's t. Data for "open-pollinated" and "unpollinated without pollination bag" treatments were not tested for statistical significance. Table values represent actual percentage data.

Results

Percentages of filled seeds by female parent, for each treatment as judged by x-ray analysis, are presented in *Table 1*. No filled seeds were produced by unpollinated flowers covered by pollination bags, and selfed seeds were produced by only a single tree (#8). These two treatments (1 and 3), were thus excluded from subsequent statistical analyses of the six treatments.

Open-pollinated seed sets ranged from 4% to 10% (avg. = 8.5), which is typical for this species (BOYCE and KAEISER, 1961; GUARD and WEAN, 1941). Analyses of variance indicated highly significant differences among female parent trees in control-pollinated seed sets, both with and without pollination bags, and mean control-pollinated seed set exceeded open-pollinated seed production by 600%. The effect of pollination bags on filled seed percentages was not consistent from tree-to-tree, however, and the difference between overall means for bagged versus non-bagged control-pollinated seed sets was not significant (0.05 level) as judged by either X-ray analysis (*Table 2*, $t_{5df} = 1.56$) or germination tests (*Table 4*, $t_{5df} = 0.88$).

Table 2. — Percent filled seed, by female parent, for pollination bag treatments as judged by x-ray analysis.

	Female parent tree						
Pollination Treatment[1]	#1	#2	#3	#4	#7	#8	Avg.
Selfed	0.0	0.0	0.0	0.0	0.0	21.0	3.5
Open poll.	16.0	7.0	10.0	5.0	4.0	9.0	8.5
Contr. poll. w/ bag	51.0ab[2]	43.0b	52.0ab	22.8b	27.8b (*)	79.0a (*)	45.9
Contr. poll. w/o bag	52.8a	38.8ab	56.0a	21.8b	10.5c	51.2a	38.5
Unpoll. w/o bag	1.3	0.0	0.5	1.2	0.0	2.0	0.8

[1]) Stamens and petals were removed in all treatments except open-pollinated. The "unpollinated with pollination bag" treatment produced no filled seeds, and therefore was excluded from further analysis.

[2]) Means in rows followed by the same letter are not significantly different at the 0.05 level (Tukey's test). Asterisks (*) between rows indicate significant differences between treatments, *i.e.*, controlled pollination with or without pollination bag, for female parent trees #7 and #8.

There also were significant differences (0.05 level) among trees in the percentage of seeds bearing two embryos (*Table 3*), and the overall treatment means for control pollinated with vs. without bags approached significance (0.05 level; $t_{5df} = 2.56$). As might be expected, the correlation between total percent filled seed and percent filled seed with two embryos was high (r = 0.96), but somewhat lower for the non-bagged controlled pollinations (r = 0.83). The small, consistent but non-significant increase in numbers of

Table 3. — Percent seed with two embryos, by female parent, for pollination bag treatments as judged by x-ray analysis.

	Female Parent Tree						
Pollination Treatment[1]	#1	#2	#3	#4	#7	#8	Avg.
Open poll.	2.5	0.0	1.0	0.0	0.0	0.0	0.4
Contr. poll. w/ bag	17.8ab[2]	12.2bc	10.5bc	2.8c	2.0c	29.2a (*)	12.4
Contr. poll. w/o bag	17.5a	7.8abc	7.2bc	2.0c	0.5c	13.2ab	8.0
Unpoll. w/o bag	0.0	0.0	0.3	0.0	0.0	0.0	0.1

[1]) Stamens and petals were removed in all treatments except open-pollinated. The "unpollinated with pollination bag" treatment produced no filled seeds, and therefore was excluded from further analysis. The "selfed" treatment produced no double embryo seeds and was also excluded from the analysis.

[2]) Means in rows followed by the same letter are not significantly different at the 0.05 level (Tukey's test). The asterisk (*) between rows indicates a significant difference between treatments, *i.e.*, controlled pollination with or without pollination bag, for female parent tree #8.

two-embryo seeds from bagged flowers suggests the existence of more favorable conditions for pollen germination and/or pollen tube growth inside the bags (e.g. warmer temperatures, protection from wind, rain, etc.).

As indicated in *Tables 2* to *4*, significant differences occurred among female parent trees in the number of filled seeds produced with different treatments. In contrast, there were no significant differences in germination among the four male parents in percent filled seed, or among overall means for the control pollinated bagged vs. unbagged treatments (*Table 5*), even though the mean filled seed percentage for male trees #7 and #8 (located 80 km north) exceeded that for #5 and #6 by 51%.

The correlation between percent filled seeds as judged by x-ray analysis (*Table 2*) and by germination (*Table 4*) was similar to that reported by TAFT (1962). The correlation between the two measures was r = 0.97 for seedlots from pollination bag treatments, and r = 0.96 for unbagged pollinations. However, percent filled seed as estimated by germination tests averaged 9% less overall than that estimated by x-ray analysis. Close inspection of x-ray films (*Figure 1*) indicated that a conservative scoring of seed x-ray images would bring the two evaluations into closer agreement. Cutting tests of x-rayed seeds suggested that those seds in which endosperms had shrunken away from the seed coat even slightly were less likely to be viable. Similar results were reported by HOUSTON (1976) for white ash.

The percentage of filled seeds produced by flowers which were emasculated, and then left unpollinated and unprotected by pollination bags, was very low (*Tables 2, 4*),

Table 4. — Percent filled seed, by female parent, for pollination bag treatments as judged by germination tests.

	Female Parent Tree						
Pollination Treatment[1]	#1	#2	#3	#4	#7	#8	Avg.
Selfed	0.0	0.0	0.0	0.0	0.0	9.0	1.5
Open poll.	12.0	0.0	9.0	1.0	3.0	3.0	4.7
Contr. poll. w/ bag	42.8bc[2]	24.2bcd	47.5b	6.5d	18.0cd	75.0a (**)	35.7
Contr. poll. w/o bag	45.3ab	28.5bc	52.2a	7.0d	8.5cd	46.8ab	31.4
Unpoll. w/o bag	0.6	0.0	0.5	0.8	0.0	2.0	0.6

[1]) Stamens and petals were removed in all treatments except open-pollinated. The "unpollinated with pollination bag" treatment produced no filled seeds, and therefore was excluded from further analysis.

[2]) Means in rows followed by the same letter are not significantly different at the 0.05 level (Tukey's test). Asterisks (**) between rows indicate a highly significant difference between treatments, *i.e.*, controlled pollination with or without pollination bag, for female parent tree #8.

Table 5. — Percent filled seed, by male parent, for pollination bag treatments as judged by germination tests.

	Male Parent Tree				
Pollination Treatment	#5	#6	#7	#8	Avg.
Contr. poll. w/ bag	28.0	30.4	39.8	38.8	34.2
Contr. poll. w/o bag	23.8	26.2	43.4	41.8	34.0
Avg.	25.9	28.3	41.6	40.3	

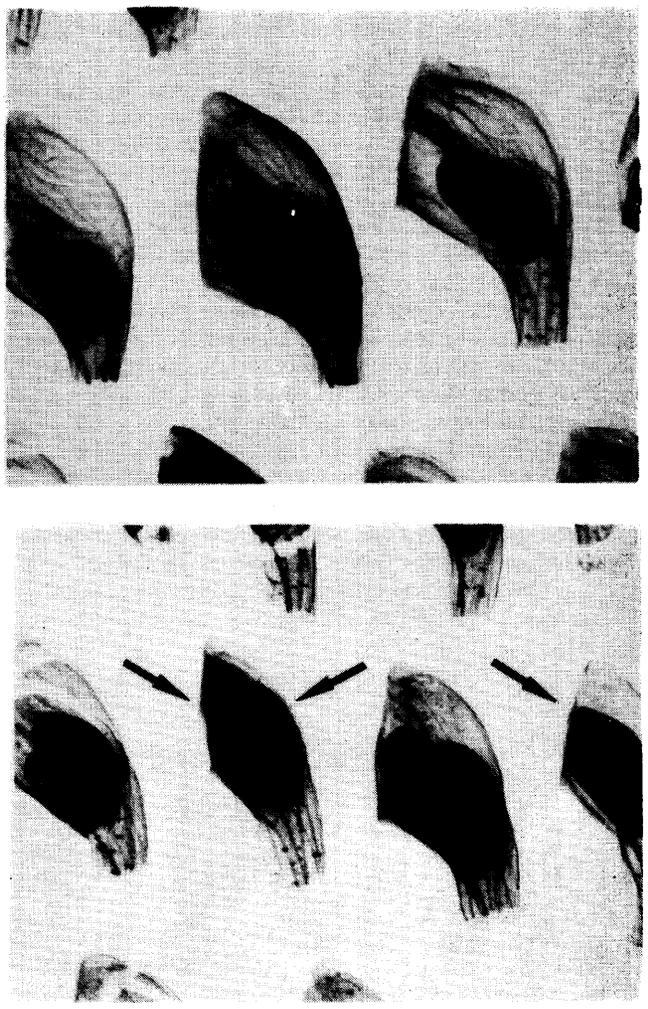

Figure 1. — X-rays of yellow-poplar samaras. A. Empty seeds. B. Filled seeds — arrows indicate endosperms which have shrunken away from seed coat and are less likely to germinate.

averaging less than 1% by both x-ray and germination analyses, and not exceeding 2% even for the apparently highly fertile tree #8. This indicates that controlled breeding/seed production programs in this species can be conducted without the necessity of using pollination bags. Removal of those flower parts known to be most attractive to insects apparently limited visits by pollinators. These results confirm the efficacy of this technique as suggested by TAFT (1962), as well as by an informal study conducted by S. G. BOYCE (reported by WILCOX and TAFT, (1969)), even though the percentage of filled seeds occurring in the emasculated, non-bagged treatment was somewhat higher in our test.

Discussion and Summary

Application of pollen to the gynoecium with a paintbrush results in very complete coverage of all stigmas, and thus the number of receptive stigmatal surfaces subsequently available to pollen from an unwanted source is greatly reduced. Because all stigmata on gynoecia in the unpollinated, unbagged treatment were available for chance pollination, it is highly likely that the extent of unwanted pollination as measured in our study would represent an overestimate. We thus hypothesize that contamination levels following controlled pollination will be considerably lower than what was actually observed in the unpollinated,

emasculated with no pollination bag treatment. If, for example, contaminants were only one-half what we observed based on germination, *i.e.*, approximately 0.3%, the maximum number of unwanted seeds produced would be about 3 per 1,000, or one seed for every 13 controlled pollinations, assuming an average yield of 25 filled seeds per pollination as found in this study.

In a *large-scale*, applied breeding program designed to produce large quantities of hybrid seed, this level of contamination would be acceptable, and generally no greater than would be expected by accident. It is certainly less than the level of contamination found in wind-pollinated seed orchards of other species. TAFT (1962) estimated that a worker can perform 15 to 20 controlled pollinations per hour using this technique. An average of 1,000 pollinations per day could thus result in the production of 35,000 to 75,000 control-pollinated seeds per man-day, of which 100 to 200 might be contaminants.

In our study the effects of environmental events such as rain following pollination were not evaluated. It is possible that such events would interfere with normal pollen germination and pollen tube growth into stigmas of gynoecia not protected by pollination bags, and may have been responsible for the significant difference between bagging treatments on trees #7 and #8, located 80 km north of the other study trees.

Literature Cited

BONNER, F. T. and RUSSELL, T. E.: *Liriodendron tulipifera* L. Yellow-poplar. In: SCHOPMEYER, C. S. (Tech. Coord.). Seeds of Woody Plants of the United States. USDA For. Serv. Agric. Handb. No. 450: 508—511 (1974). — BOYCE, S. G. and KAEISER, M.: Why yellow-poplar seeds have low viability. USDA For. Serv. Cent. States For. Exp. Sta. Tech. Pap. 186. 16 p. (1961). — GUARD, A. T. and WEAN, R. E.: Seed production in the tulip poplar. J. For. 39: 1032—1033 (1941). — HOUSTON, D. B.: Determining the quality of white ash seed lots by x-ray analysis. Tree Planters' Notes 27 (2): 8. 23 (1976). — KAEISER, M. and BOYCE, S. G.: Embryology of *Liriodendron tulipifera* L. Phytomorphology 12: 103—109. — KRIEBEL, H. B.: Technique and interpretation in tree seed radiography. In: Proc., Second Genetics Workshop of the Society of American Foresters and the Seventh Lake States Forest Tree Imp. Conf., USDA For. Serv. Res. Pap. NC-6: 70—75 (1966). — TAFT, K. A., Jr.: The effect of controlled pollination and honeybees on seed quality of yellow-poplar (*Liriodendron tulipifera* L.) as assessed by x-ray photography. N. Car. State Coll., School of For., Tech. Rep. No. 13. 21 p. (1962). — WILCOX, J. R. and TAFT, K. A., Jr.: Genetics of yellow-poplar. USDA For. Serv. Res. Pap. WO-6. 12 p. (1969).

Genetic Diversity in Holm-Oak (Quercus ilex L.): Insight from Several Enzyme Markers

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Summary

Genetic variation using three alloenzyme markers (respectively loci PGI1, ADH1 and IDH1) was studied in the holm-oak (*Quercus ilex* L.), an anemophilous tree which is characterized by high phenological variability. At a regional scale, the results from genetic distance analyses showed that the closer the population sites, the higher the gene flow between the populations. The determination of pollen flow seems to depend essentially on inter-site phenological divergence.

At a larger scale (which corresponds to a substantial part of the whole distribution of this species), analysis of the genetic differentiation using F-statistics revealed, on one hand, that one or very few genenations would have passed since the time of the original founding of the populations and, on the other hand, that these populations were probably differentiated from one another a long time ago.

Key words: *Quercus ilex* L., genetic differentiation, phenology, F-statistics, allozymes.

Zusammenfassung

Anhand dreier alloenzymischer Merkmale (Enzymloci PGI1, ADH1 und IDH1) wurde die genetische Diversität der Stech-Eiche (*Quercus ilex* L.) untersucht, einem anemophilen Baum mit großer phänologischer Variabilität. In einem regionalen Rahmen erwiesen genetische Distanzanalysen, daß der Genfluß zwischen den Populationen umso bedeutender ist, je dichter deren Standorte beieinander liegen. Der Pollen-Fluß scheint wesentlich durch die phänologische Divergenz zwischen den Standorten bestimmt.

In einem weiteren Rahmen (der einen guten Teil der gesamten Verbreitung dieser Art erfaßt) erwies die Analyse der genetischen Differenzierung mittels F-Statistik zum einen, daß erst eine oder nur sehr wenige Generationen seit der Gründung der Populationen vergangen sind, zum anderen, daß diese Populationen bereits seit langer Zeit voneinander differenziert waren.

Introduction

Theoretical studies on population genetic structure predict generally the occurrence of higher intra-population variability in species that are distributed continuously than in those which are made up of small isolated populations; these, on the other hand, would possess a higher inter-population variability (WRIGHT, 1931; KIMURA and CROW, 1964; NEI *et al.*, 1975). WRIGHT (1946, 1965) underlines the importance of the reproductive system on the genetic structure of populations and shows that limited gene-flow can generate consanguinity and thereby genetic structuring within those populations. These theoretical concepts were also supported by experimental studies. Thus, from the survey of 110 plant species, HAMRICK *et al.* (1979) showed that those species which have a wide distribution, long life-span and are allogamous as well as wind-pollinated, possess the greatest intra-population genetic variability.