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Mating Systems in Open-Pollinated Families of Black Locust (*Robinia pseudoacacia*)

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Abstract

The mating system of black locust (*Robinia pseudoacacia*) was investigated using six allozyme loci and the multi-locus model of RITLAND and JAN (1981). Estimated outcrossing rates averaged 0.83 across 23 seed sources, ranging from 0.46 to 1.12. Variation in outcrossing rates among sources may be due to the local patchiness of allele and genotype frequencies (Wahlund effect) and/or pollinator behavior. The estimates obtained are probably conservative because the sampling scheme employed could artificially induce Wahlund effects. The physical separation of stigmatal and antheral surfaces as well as the protogynous flowering habit encourages outcrossing in black locust. Among seed sources, germination rates were positively correlated with outcrossing rates. Low germination rates may be a manifestation of inbreeding depression in seed families with higher amounts of consanguineous mating.

Key words: Allozymes, Black locust, Outcrossing rates, Fixation indices.

1. Introduction

Non-obligate outcrossing behavior is characteristic and advantageous in colonizing, clonal entomophilous species because scattered individuals may be reproductively isolated (BAKER, 1965). Previous studies (PHILLIPS and BROWN, 1977; MORAN and BROWN, 1980; HOPPER and MORAN, 1981; YEH *et al.*, 1983; BROTSCHOL *et al.*, 1986) have shown that

outcrossing rates of entomophilous tree species are typically less than 0.90 and are sensitive to fluctuations in pollinator behavior and stand structure (BROWN *et al.*, 1989; AIDE, 1986). Black locust (*Robinia pseudoacacia*) combines several traits that might be expected to reduce effective rates of outcrossing. It is a colonizing species that spreads locally by vegetative growth and it has limited seed dispersal. It also flowers profusely and is primarily pollinated by hymenopterans. Even though black locust is economically important in Europe and ecologically important in the United States, little is known of its mating system.

From an applied standpoint, estimates of quantitative genetic parameters (i.e. additive genetic variance and heritability) important in tree breeding programs often rely on the assumption that open-pollinated progeny arrays are half-sibs. When considering entomophilous species such as black locust, it is unlikely that insect vectors randomly distribute pollen. Nearby individuals shedding pollen at the proper time probably sire a majority of the offspring of neighboring trees (WASER and PRICE, 1983). Such non-random pollen deposition creates correlated matings (SCHOEN and CLEGG, 1984; SYMTH and HAMRICK, 1984) and increases the genetic relatedness of open-pollinated progenies. Bias introduced by non-random mating behavior can lead to an overestimation of additive genetic variance, heritability, and subsequently, genetic gains (SQUILLACE, 1974).

In this study, six allozyme loci were used to estimate effective outcrossing rates for 23 geographic seed sources located throughout the natural range of black locust. Data were also used to determine whether pollen allele frequencies vary among individual trees within a geographic seed source.

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2. Materials and Methods

2.1 Experimental Material

Open-pollinated seeds were collected from (maternal) trees within 23 geographic sources located throughout the natural range of black locust (*Fig. 1*). Each seed source was represented by a circular area of 10 km radius. As a general rule, within seed sources, seeds were sampled from single trees in each of two to five stands separated by distances ranging 0.1 km to several km. This sampling scheme was employed to assure that collections were from separate clones. For this paper, seed sources were treated as a single sample, although they cannot be considered as a single panmictic population.

Six to twelve seeds (mean = 11.8) were randomly selected from each tree. The number of progeny electrophoretically assayed per source ranged from 22 (two trees) to 60 (five trees) and on average, 51 progeny were sampled in each source. Across all 23 seed sources, 1178 individuals in 100 families were assayed.

Vegetative tissue from four-week-old seedlings was used as the enzyme source. In families having more than 12 seedlings, the 12 chosen for analysis were randomly selected. Details of wick preparation and electrophoretic methods have been described previously in *SURLES et al.* (1989). A subset of six loci (isocitrate dehydrogenase [IDH-1], leucine aminopeptidase [LAP-2], malic dehydrogenase [MDH-1 and MDH-3], 6-phosphogluconate dehydrogenase [6-PGDH-5], and phosphoglucomutase [PGM-3]) were selected from the 40 loci used in *SURLES et al.* (1989) on the basis of clarity and number of alleles. Mendelian inheritance of these loci was conferred in open-pollinated families of black locust (*SURLES*, 1988). Recipes for enzyme stains and buffer systems used are found in *SOLTIS et al.* (1983).

Family germination rates were determined from a prior study using 200 seeds per family (*BONGARTEN*, unpubl. data). Prior to germination, seeds were scarified by soaking in concentrated sulfuric acid for one hour. After rinsing, a water vacuum treatment (30 minutes at 40°C) was used to enhance water penetration through the seed coat. Seeds were then allowed to imbibe water for an additional 24 hours. Seeds were planted into a peat/vermiculite medium. Percent germination was recorded two weeks later.

2.2 Data Analysis

Estimates of outcrossing rates (t) and pollen allele frequencies (p) for each seed source were jointly estimated following the multi-locus procedure of *RITLAND and JAIN* (1981). The iterative procedure of *RITLAND and JAIN* (1981) uses the *NEWTON-RAPHSON* method to estimate t and the *Expectation-Maximization* method to infer p . The *NEWTON-RAPHSON* method was used because it yields statistically valid estimates of t greater than 1.0. Maternal genotypes were inferred by the *RITLAND and JAIN* procedure according to the method of *BROWN and ALLARD* (1970).

Like most mixed-mating models, the principle assumptions of *RITLAND and JAIN's* (1981) procedure are: 1) no genetic changes due to mutation or selection following fertilization; 2) no assortative mating; 3) no heterogeneity in the incorporated pollen pool; 4) outcrossing is independent of maternal genotype. Assumption 3 is often violated in plant species, especially in populations of forest trees. For this reason, a goodness of fit test with chi-square values was used to test the heterogeneity of pollen allele frequencies among maternal trees within seed sources with suf-

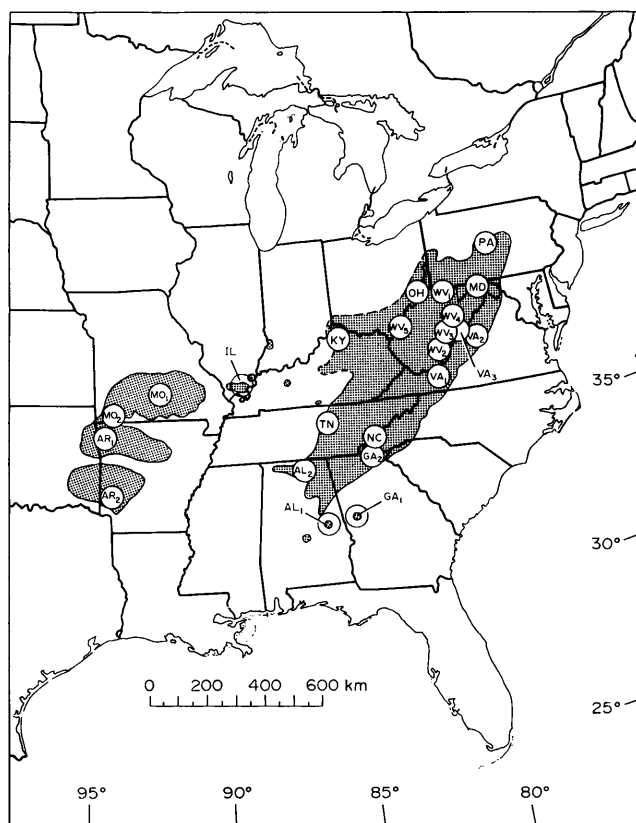


Figure 1. — Natural range and location of the 23 sampled black locust seed sources.

ficient numbers of progeny (48 per family). Sufficient progeny were available for this analysis from only five seed sources.

To investigate the compliance of seed source genotypic frequencies to *HARDY-WEINBERG equilibrium*, *WRIGHT's* fixation indices were calculated from progeny genotypic frequencies. The mean fixation index based on progeny genotypes (F_{IS}) was statistically compared to the mean equilibrium fixation index ($F_e = [1-t]/[1+t]$). If all *HARDY-WEINBERG* assumptions are satisfied and genotypic frequencies are determined solely by the mating system F_{IS} and F_e are equal (*HEDRICK*, 1983). Discrepancies between F_{IS} and F_e reflect the amount by which a population deviates from inbreeding equilibrium. A Spearman rank correlation coefficient was used to measure the relationship between outcrossing rates and germination rates.

3. Results

Multi-locus estimates of outcrossing rates (t) ranged from 0.46 (WV_1) to 1.12 (WV_3) with a mean of 0.83 (*Table 1*). In eight of the 23 seed sources, outcrossing rates were 0.90 or greater. No apparent geographic patterning of outcrossing rates was observed. The four sources with the lowest t values (GA_2 , NC , WV_1 , and OH) were not geographically clustered. Similarly, sources with t values greater than 0.95 were not geographically clustered. It is interesting to note that, AR_1 with an outcrossing rate of 0.96, had the second lowest observed heterozygosity (0.268), while, NC , the source with the highest observed heterozygosity (0.322) had the second lowest outcrossing rate.

If the monomorphic comparisons were excluded, 12 of the 25 tests for heterogeneity of pollen allele frequencies

Table 1. — Total number of families and progeny, outcrossing rates (t), and the observed F_{IS} and expected (F_e) fixation indices for 23 *R. pseudoacacia* seed sources.

Seed Source	Total Number of Families	Total Number of Progeny	t(s.d.)	F_{IS}	F_e^*
AR ₁	2	24	0.96(0.11)	0.024	0.020
AR ₂	5	60	0.80(0.07)	0.090	0.112
MO ₁	5	59	0.82(0.06)	0.053	0.098
MO ₂	2	22	0.90(0.12)	0.025	0.054
AL ₁	5	58	0.74(0.07)	0.077	0.147
AL ₂	5	60	0.72(0.07)	0.096	0.163
GA ₁	4	43	0.82(0.09)	0.080	0.100
GA ₂	5	60	0.66(0.07)	0.039	0.206
NC	5	60	0.55(0.07)	0.100	0.293
TN	3	28	1.02(0.10)	-0.005	-0.007
KY	3	36	0.92(0.09)	0.020	0.042
VA ₁	5	60	0.96(0.06)	0.057	0.022
VA ₂	5	60	0.95(0.06)	0.087	0.028
VA ₃	4	48	0.84(0.08)	0.033	0.089
WV ₁	5	60	0.46(0.07)	0.171	0.371
WV ₂	5	59	0.91(0.06)	0.032	0.045
WV ₃	5	60	1.12(0.04)	0.067	-0.056
WV ₄	5	60	0.84(0.07)	0.024	0.085
WV ₅	5	60	0.81(0.06)	0.006	0.106
MD	4	48	0.87(0.06)	0.076	0.070
PA	5	60	1.00(0.06)	0.109	0.033
OH	3	33	0.59(0.10)	0.177	0.260
IL	5	60	0.84(0.07)	0.052	0.087
Mean	4.3	51.2	0.83(0.03)	0.065(0.001)	0.107(0.020)

* $F_e = (1-t)/(1+t)$

Values in parenthesis for the mean fixation indices indicate standard errors.

among trees within a source were significant at the 5% level (Table 2). This indicates that each maternal tree is not randomly sampling pollen from the composite pollen available to each seed source. Such heterogeneity in pollen allele frequencies results in underestimates of the true outcrossing rate.

Germination rates, on a family basis, ranged from less than 60% to more than 90%. The Spearman rank correlation between outcrossing and germination was positive and approached significance ($r = 0.59$; $p = 0.10$).

The mean F_{IS} value for the 23 sources was 0.065, ranging from -0.005 (TN) to 0.177 (OH) indicating slight deficiencies of heterozygotes in relation to HARDY-WEINBERG expectations. As expected, sources having lower RITLAND and JAIN estimates of outcrossing also had higher inbreeding coefficients and sources having high t values had lower

F_{IS} values (Table 1). This relationship, as measured by the Spearman correlation coefficient, was significant at the 10% level. Fixation indices at inbreeding equilibrium (F_e) estimated from t values ranged from -0.056 to 0.371. In most seed sources, there were more heterozygotes than predicted under inbreeding equilibrium. The overall mean F_e was significantly larger than the mean F_{IS} value ($p < 0.05$).

4. Discussion

The mean outcrossing rate observed in this study (0.83) lies within the range estimated for other animal-pollinated tree species. For example, outcrossing rates for several *Eucalyptus* species ranged from 0.77 to 0.85 (Table 3). BROTSCHOL *et al.* (1986) found that outcrossing rates in

Table 2. — Chi-square values for testing among-tree heterogeneity in pollen allele frequencies at six loci for five seed sources of *R. pseudoacacia*.

Seed Source	Locus					
	IDH ₁	LAP ₂	MDH ₃	6PG ₅	PGM ₃	MDH ₁
AL ₂	40.07*	22.18*	0.22	17.93*	0.47	-
GA ₂	24.70*	10.20	43.57*	11.62*	0.58	2.96
VA ₃	12.99	4.66	0.18	-	-	-
MD	11.32	21.28*	1.10	0.69	7.49*	-
WV ₅	12.72	48.63*	21.80*	5.91*	10.73*	0.01

* Indicates significance at the $\alpha = 0.05$ level.

- Indicates monomorphic loci.

Table 3. — Comparisons of several tree species in outcrossing rate (t), the observed fixation index (F_{IS}), and the expected fixation index (F_e).

Species	t	F_{IS}	F_e
Angiosperms			
<i>Eucalyptus citriodora</i> ^a	0.85	0.205	0.071
<i>E. delegatensis</i> ^b	0.77	0.150	0.130
<i>E. pauciflora</i> ^c	0.84	0.121	0.087
<i>E. stoatei</i> ^d	0.82	-	0.099
<i>Liriodendron tulipifera</i> ^e			
Coastal	0.55	-	0.290
Mountain	0.86	-	0.068
<i>Alnus crispa</i> ^f	0.95	0.009	0.025
<i>R. pseudoacacia</i> ^g	0.83	0.065	0.107
Conifers			
<i>Abies lasiocarpa</i> ^h	0.89	0.105	0.214
<i>Picea engelmannii</i> ^h	0.93	0.011	0.053
<i>Pinus banksiana</i> ⁱ	0.88	-	0.064
<i>Pinus ponderosa</i> ^j	0.96	-	0.020
<i>Pseudotsuga menziesii</i> ^k	0.90	-	0.053

Sources

- a) YEH *et al.* (1983)
- b) MORAN and BROWN (1980)
- c) PHILLIPS and BROWN (1977)
- d) HOPPER and MORAN (1981)
- e) BROTSCHOL *et al.* (1986)
- f) BOUSQUET *et al.* (1987)
- g) Present study
- h) SHEA (1987)
- i) CHELIAK *et al.* (1985)
- j) MITTON *et al.* (1977)
- k) SHAW and ALLARD (1982)

Liriodendron tulipifera (Magnoliaceae), a tree often associated with black locust in the southern Appalachians, averaged 0.55 (± 0.028) in two coastal populations, while a mountain population had an outcrossing rate of 0.86 (± 0.026).

However, outcrossing rates in black locust were less than those for wind-pollinated trees. For example, *Alnus crispa*, exhibits an outcrossing rate of 0.95 (BOUSQUET *et al.*, 1987). Rates of outcrossing in wind-pollinated conifer species are also commonly greater than 0.90 (Table 3).

If pollen allele frequencies are heterogeneous among stands within sources, the sampling procedure employed will yield estimates of outcrossing rates less than those obtained from sampling within a single stand. Chi-square analysis indicated that the allele frequencies of pollen within the five seed sources tested were, in fact heterogeneous (Table 2). Because of the limitation of our sampling design, we cannot determine whether the observed heterogeneity in pollen allele frequencies among trees is due to among stand heterogeneity in allele frequencies or is representative of tree-to-tree heterogeneity in pollen allele frequencies that might occur within stands. Heterogeneity within stands is expected because pollen and seed dispersal capabilities of black locust are somewhat limited and the species root sprouts vigorously. Furthermore, pollinators tend to minimize flight distances (WASER and PRICE, 1983). Limited pollinator movement would tend to favor matings between relatives or members of the same clone. Such biparental and inter-ramet mating will increase apparent selfing and contribute to heterogeneity in the functional pollen pools. ENNOS and CLEGG (1982) suggested that the Wahlund effect in pollen allele frequencies can cause estimates of out-

crossing rates that are as much as 50% less than expected from random mating. Within-stand pollen source heterogeneity has been detected in several other tree species including *Picea mariana* (BOYLE and MORGENSTERN, 1986), *Picea glauca* (CHELIAK *et al.*, 1985), *Liriodendron tulipifera* (BROTSCHOL *et al.*, 1986), *Pinus taeda* (FRIEDMANN and ADAMS, 1985), and *Eucalyptus citriodora* (YEH *et al.*, 1983).

Relative to anemophilous species, entomophilous species are expected to have higher variation in outcrossing rates among populations (BROWN *et al.*, 1988). Outcrossing rates in these sources of black locust varied from 0.46 to 1.12. Variation observed in outcrossing rates among seed sources may reflect differences in the magnitude of Wahlund effects or differences in pollinator behavior due to stand density and/or the physical environment. If black locust is self-compatible, t values in stands with few clones would be lower than in stands composed of many clones. Furthermore, higher outcrossing rates are characteristic of populations with lower densities because pollinator flight distances are maximized. When pollinator flight distances are maximized, the frequency of matings among non-related individuals is also maximized (ELLSTRAND *et al.*, 1978).

The mating systems of animal-pollinated tree species may also vary spatially and temporally because of differences in pollen vector behavior (AIDE, 1986) associated with variation in the environment (SCHMITT, 1983). For example, severe fluctuations in temperature may reduce the activity of insect pollen vectors, and thus increase selfing rates. The 23 seed sources in this study represent elevations of 170 m to 945 m above sea level; latitudes and longitudes vary from 32.84° N to 40.80° N and 77.92° W to 94.13° W, respectively. Furthermore, seeds from different

sources were collected over a period of three years. Variation in climate during the pollination season in the sites and years represented by the study material should contribute to the observed variation in outcrossing rates.

Sixteen of the 23 seed sources had heterozygote excesses relative to expected numbers based on the mating system (i.e. $F_{IS} < F_e$) (Table 1). Typical explanations for heterozygote excesses after correction for the mating system are negative assortative mating and heterotic selection between fertilization and seedling analysis. Negative assortative mating is not likely to operate in black locust due to the degree of genetic substructuring present in populations of this species (BONGARTEN, unpubl. data). Selection cannot be ruled out as a partial explanation for this discrepancy. However, an outcrossing rate of only 88% is necessary to reach inbreeding equilibrium ($F_e = 0.065$). In our opinion, the Wahlund effects associated with sampling a population from non-contiguous stands could account for this difference.

Sources with relatively high germination rates tended to have high outcrossing rates and ones with low germination rates tended to have low outcrossing rates. In forest trees, inbreeding is known to reduce seed set, germination rates, and seedling vigor (FOWLER, 1965; FRANKLIN, 1970; SQUILLACE and KRAUS, 1963). The negative correlation between inbreeding and germination rates may be a manifestation of inbreeding depression.

The question of self-incompatibility in black locust still remains unanswered. The rare occurrence of albino seedlings indicate that the mating system of black locust may not be completely self-incompatible. Under experimental conditions, approximately 0.1% of germinated black locust seedlings are albino (BONGARTEN, unpubl. data). However, albino seedlings can result from the exposure of rare deleterious recessive genes from other forms of consanguineous matings as well.

A preliminary investigation of the relative maturity of anthers and stigmas in a black locust population near Franklin, North Carolina indicated that the flowers were protogynous. In addition, the stigmatal surface usually protrudes slightly outside the keels, away from the anthers. These mechanisms should act to discourage selfing and enforce a relatively high frequency of outcrossing, but would still allow occasional selfing. A flexible breeding system such as this should be ideal for a colonizing tree species.

5. Conclusions

This work demonstrates that the mating system of black locust is highly outcrossed ($t = 0.83$), despite the presence of possible correlated mating, clonal structuring, and Wahlund effects. The flower structure of black locust favors a relatively high frequency of outcrossing. Variation in outcrossing rates observed among the seed sources (0.46 to 1.12) may be due to varying magnitudes of Wahlund effects and differences in pollinator behavior influenced by stand density and environments particular to each seed source. Near neighbor mating interacting with limited seed dispersal and clonal structuring are expected to produce significant genetic patchiness within stands of black locust. Therefore, some open-pollinated progeny arrays may not have a half-sib relationship, but a relationship closer to the level of full-sibs.

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Parental Balance in Douglas-fir Seed Orchards – Cone Crop vs. Seed Crop*)

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Abstract

Parental balance based on seed-cone and filled-seed crops were estimated and compared for 30 Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO] trees from a coastal clonal/seedling seed orchard. Estimates based on cone crop differed from results obtained from filled-seed production. It was demonstrated that parental balance based on seed-crop data provide a more accurate assessment of the genetic diversity and the family representation in the resultant seed crop than those based on cone crop. Individual clone or open-pollinated family cone harvest and seed extraction are recommended.

Key words: Douglas-fir, seed orchard, parental balance, cone and seed crop.

Introduction

Parental balance, the equality of male and female strobili production, and reproductive synchrony are fundamental pre-requisites for the production of seeds that reflect the genetic diversity present in seed orchards. It has been demonstrated that these two pre-requisites were not fulfilled in many seed orchards of different species (POLK, 1966; ERIKSSON *et al.*, 1973; JONSSON *et al.*, 1976; GRIFFIN, 1982, 1984; O'REILLY *et al.*, 1982; SCHMIDTLING, 1983; EL-KASSABY *et al.*, 1984, 1986, 1988; BYRAM *et al.*, 1986; SCHOEN *et al.*, 1986; FASHLER and EL-KASSABY, 1987).

Several seed orchard management practices have been proposed to alleviate that departure from expectations. These include: the use of over-head cooling treatment (FASHLER and EL-KASSABY, 1987), supplemental mass pollination (EL-KASSABY *et al.*, 1986, 1988), cultural and/or hormonal treatments (ROSS, 1978; PHARIS *et al.*, 1980; ROSS *et al.*, 1985; WHEELER *et al.*, 1985), and where possible, the mixing of crops of different years (EL-KASSABY *et al.*, 1988).

Parental balance in seed orchards commonly is assessed by cumulative cone-yield curves (GRIFFIN, 1982). In this method the seed orchard's genetic entities (clones or open-pollinated families) are ranked from high to low cone yield and cumulative percentage calculations are plotted against the number of clones or open-pollinated families censused. This method assumes that reproductive energy is equal to reproductive success (i.e., the number of filled seeds per cone is equal across the different parental

groups). In this study, the parental balance of 30 Douglas-fir [*Pseudotsuga menziesii* (MIRB.) FRANCO] trees from an 18-year-old clonal/seedling orchard is examined for cone production versus filled-seed production.

Materials and Methods

Canadian Pacific Forest Products Limited's 3.4 ha, "high-elevation" coastal Douglas-fir seed orchard located at the Saanich Forestry Centre near Victoria, B. C. (lat. 48°35' N, long. 123°24' W) provided the material for the study. The orchard consists of a combined clonal/seedling breeding population with 61 clones and 37 open-pollinated families. The trees are planted in a randomized/incomplete block design at 4 × 6 m spacing replicated 16 times (blocks). The ages of the clonal propagules and seedlings are 22 and 19 years, respectively.

The 1987 cone crop (good cone year) was managed with a combination of over-head cooling (FASHLER and DEVITT, 1980) and supplemental-mass-pollination (SMP) treatments. The over-head cooling has proven to reduce pollen contamination levels (EL-KASSABY and RITLAND, 1986), improve panmixis (FASHLER and EL-KASSABY, 1987), and reduce insect and frost damage (MILLER, 1983; FASHLER and EL-KASSABY, 1987). SMP involved blowing on dry pollen mixes representing equal volumes from at least 12 different donors (i.e., open-pollinated families and/or clones). To account for within-tree variation, receptive trees were pollinated two to three times and at two levels: the upper crown, reached by manlift, and the lower crown, reached by ground crews.

During harvest (September 1987), seed-cone crops were collected completely from 30 trees representing 30 open-pollinated families (i.e., one tree/open-pollinated family). The number of seed cones was recorded and, where possible, a sample of five to eight cones was taken at random from each tree for seed extraction. Cone samples were air dried at room temperature, seeds were extracted, de-winged and cleaned by hand. The average number of filled seeds per cone was estimated for each tree by dividing the total number of filled seeds by the number of cones. Filled and empty seeds were determined by cutting each seed individually. The total number of filled seeds per tree was estimated by multiplying the average number of filled seeds per seed-cone by the number of cones harvested per tree.

The relationship between seed-cone crop and filled-seed production was assessed using Pearson's product-moment correlation and Spearman's rank correlation (SOKAL and ROHLF, 1969).

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