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Controlled Pollinations among Pine species in Greece

By D. MOULALIS, C. BASSIOTIS and D. MITSOPOULOS

Laboratory of Forest Genetics and Forest Tree Breeding
Aristotelian University of Thessalonilsi — Greece

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Introduction

The degree of relationship among pine species has been studied by several investigators and different classification systems were proposed according to the criteria used each time (SHAW 1914, PILGER 1926, DUFFIELD 1952, MIROV 1953, GAUSSEN 1955, 1960, LITTLE and CRITCHFIELD 1969). DUFFIELD (1952) was the first to use the criterion of crossability. He also proposed certain modifications to the Shaw system which is based on morphological characters. According to SHAW (1914) the Lariciones group includes among others the species *P. nigra*, *P. sylvestris* and *P. heldreichii.* These species occur naturally in Greece. The Insignes group includes among other the species *P. halepensis, P. brutia* and *P. pinaster.* The first two species occur naturally in Greece while the third does not. In DUFFIELD's modified system, *P. halepensis* (which grows naturally together with *P. brutia*, *P. pinaster* and *P. nigra)* is transferred from the Insignes group of the subgenus Diploxylon to the Lariciones group of the same subgenus.

This paper reports the results of the second phase (1967—1972) of a study which was conducted in order to obtain information on the crossability among a number of pine species growing in Greece with the ultimate purpose of exploring the possibility of transfering characters from one species to the other (because of high genetic variability in the genus *Pinus)* in order to produce superior genotypes having desirable characters and especially a high adaptability to the adverse environmental conditions of this country.

The results of the first phase (1962—1966) of this study were reported by BASSIOTIS (1972). During that phase artificial pollinations were made among pine species of the same group as well as among species belonging to the Insignes and Lariciones groups. BASSIOTIS (1972) found that most cross-pollinations between groups failed to produce filled seeds but some did produce a few. A small number of seedlings were grown from these seeds and were described by the previous author as putative between-groups hybrids. However, the overall failure of the between groups crossing program led us to reject the Duffield modifications of the Shaw system with regard to the position of *P. halepensis* and accept the Shaw system intact, at least for the pine species growing in Greece (BASSIOTIS 1972).

Materials and Methods

The failure to obtain filled seed from the between-groups crosses of the first phase (1962—1966) of the investigation forced us in the second phase (1967—1972) to limit our efforts by crossing only individuals belonging to the same group.

During this phase we repeated certain crosses of the first phase and made new ones as well. We also included *P. pinea* (pineae group), *P. nigra var. hispanica* as well as *P. mugo* as a male parent. Moreover, we backcrossed the hybrid "*P. brutia* × *P. halepensis*" with *P. halepensis* and *P. brutia*. In all, the species included in the hybridization program were:

P. pinea	*P. nigra var. austriaca*
P. halepensis	*P. nigra var. corsicana*
P. brutia	*P. nigra var. hispanica*
P. pinaster	*P. sylvestris*
P. nigra var. pallasiana	*P. heldreichii*

We also used the species *P. mugo* and *P. resinosa* as male parents. These species belong to the Lariciones group.

Crosses were repeated for a number of years because

Table 1. — *Hybridization centers.*

Areas	Latitude	Longitude	Elevation m.
1. University Forest nursery	40° 35' 41"	22° 59' 17"	9
2. Asvestochorion	40° 39' 20"	22° 59' 40"	150
3. Chalkidiki	40° 00' 30"	23° 33' 40"	150
4. Laila	41° 15' 00"	23° 27' 52"	1400
5. Metsovon	39° 51' 10"	21° 14' 10"	1500
6. Lachanas	40° 56' 12"	23° 14' 58"	450
7. Kylene	37° 55' 00"	22° 38' 00"	700
8. Vytina	37° 40' 20"	22° 10' 52"	1010
9. Thassos	40° 37' 00"	24° 41' 00"	10

results may be variable due to the provenances used (Dengler 1932, Duffield 1954 b), the biotypes (Wright 1953, Critchfield 1962) and the environmental conditions during and after pollination (Eifler 1956).

Controlled pollinations were conducted in nine centers located throughout Greece (*Table 1* and *Figure 1*). Four of these centers (1, 2, 6, and 8) were plantations while the rest were natural forest stands.

The trees which were selected as female and male parents were of young to medium age, parasite-free, vigorous and with abundand flowers. The pollen used was locally collected except in the cases of *P. mugo* and *P. resinosa* whose pollen was obtained from West Germany and the United States, respectively.

In general, the pollen was fresh. In the few cases where pollen from the previous year was used it was stored either in a desiccator at 3—5° C or in a deep freezer at —20° C. Prior to each pollination the germinability of each pollen lot (either fresh or of the previous year) was tested by the "hanging drop" method (Giordano and Bonechi 1956). Pollen with very low germinability was not used. Besides natural pollen, we used irradiated pollen (γ-^{60}Co at 400—1000r) and also a mixture of dead pollen from the female parent and natural pollen from the male parent. This mixture was used in 1971 and 1972 only.

Crosses were made according to the technique developed by Cumming and Richter (1948) and improved by Duffield (1954 a), Mergen *et al.* (1955) and Ehrenberg and Simak (1957).

Ovulate flowers were isolated when flower buds were large enough and the peduncle was just visible. Pollination bags were made of Terylene and had an opening covered

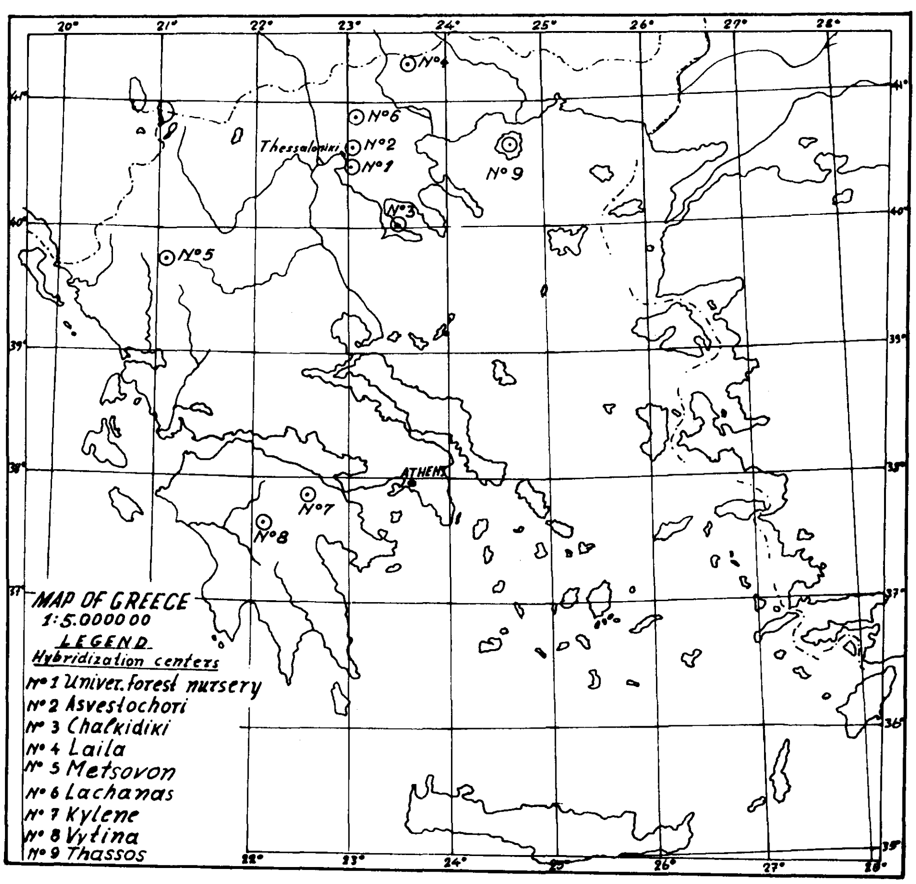

Figure 1. — Hybridization centers.

with transparent polyethylene to facilitate inspection of the flowers.

Pollination was carried out during the fifth stage of the ovulate strobili development, namely, when cone scales were open and stood almost at right angles to the axis of the strobili. The procedure was repeated 2—3 days later, depending on the prevailing weather conditions. The pollen was applied into the bags by a medicine dropper bearing a rubber blower equipped with a valve. A total of 22,500 flowers were pollinated and 20,900 conelets were counted after removal of pollination bags.

Developing cones of *P. heldreichii, P. nigra var. corsicana* and *P. nigra var. austriaca* were wrapped with a cloth bag for protection against birds. These cones were collected just after maturity but before the beginning of cone dehiscence. The seeds were extracted by hand. Filled seeds were separated from empty ones by weighing and were sown in the nursery along with parental seeds for comparison.

The seedlings produced in the nursery were planted in small plastic pots and transferred to the experiment plots of the University Forest at Cholomon Mountain, Chalkidiki, and of the Forest Research Center of Vassilika, Thessaloniki.

Because in many cases crossability in interspecific crosses cannot be expressed by a number, we prefer to use in this paper two criterias namely the fertility of crosses and the viability of seeds. Fertility % is given by the ratio:

$$\frac{\text{Number of filled seeds per cone resulting from interspecific crosses}}{\text{Number of filled seeds per cone resulting from intraspecific crosses}} \times 100$$

In setting this criterion we assumed that filled seeds come from the fertilized ovules. This assumption was based on the fact that apomictic formation of seeds is negligible in conifers, particularity in the species of *Pinus* growing in Greece (Bassiotis 1972).

Viability % was calculated by the following formula:

$$\frac{\text{Germination percent of filled seed resulting from interspecific crosses}}{\text{Germination percent of filled seed resulting from intraspecific crosses}} \times 100$$

This criterion is a necessary supplement to the first one because a cross may produce filled seeds that do not germinate.

The results are reported here from all our crossing efforts and go as far as the production of seedlings. Description of putative hybrids and their comparison to representatives from each parental species will be included in a subsequent publication. It must be pointed out here that the progeny of the female parent originated from controlled crosses while that of the male parent from open-pollination.

Results

Flowers and conelets

The proportion of damaged flowers ranged between 3 and 12% with an overall mean value of 8%. This proportion must be considered small. The damage was mechanical and was done when handling the flowers during isolation and bag removal *(Table 2)*.

The percentage of conelets which developed into mature cones varied widely among the combinations of each species and reached a maximum value of 80% *(Table 2)*. The species *P. pinea* and *P. pinaster* showed the lowest values when used as female parents. This behavior of the two species was not due to the fact that they were pollinated with species of other groups because, at least with regard to *P. pinaster*, a very low cone percentage was observed not only in crosses with species from other groups but also in intraspecific crosses. In contrast, *P. nigra var. pallasiana* when crossed with species of other groups showed high cone percentages. Also, intraspecific crosses often showed markedly lower cone percentages as compared to interspecific ones. It must be pointed out that cone percentages from year to year varied markedly. On the other hand, the development of unpollinated flowers to mature cones was of no special importance as can be seen from comparisons with control flowers *(i.e.* isolated but not pollinated flowers). These flowers gave a few cones that had occasionally some empty seeds but never filled ones.

Cones

Almost all cones had quite a number of empty seeds *(Table 2)*. However in the interspecific crosses, the percentages of cones containing filled seeds were very low and in some cases negligible. Excepted were the crosses *P. brutia* × *P. halepensis, P. sylvestris* × *P. mugo, P. heldreichii* × *P. resinosa* and the backcross of the hybrid *P. brutia* × *P. halepensis* with *P. halepensis* and *P. brutia.* These five crosses showed very high percentages of cones with filled seeds; the values were mostly of the same order of magnitude with the values obtained from intraspecific crosses. Similarly, in the intraspecific crosses, a small number of cones was found without well filled seeds.

Fertility

The fertility of interspecific crosses varied generally from 0 to 37.4% *(Table 3)*. A maximum value of 108% was observed in the backcross of the hybrid "*P. brutia* × *P. halepensis*" with *P. halepensis.*

Marked differences in fertility were noted not only among combinations but also among pollination years for the same combination.

The combinations used in this study may be broadly divided, according to their fertility, into three groups. The first group includes combinations with very low or even zero fertility (0—5%). Most of the crosses conducted belong to this group. Complete failures in all the years of this study were: *P. halepensis* × *P. pinaster, P. brutia* × *P. pinaster,* the reciprocals of these two and *P. nigra var. corsicana* × *P. heldreichii.* The rest of the combinations of this group showed low fertility. These were:

P. halepensis × *P. brutia*
P. halepensis × (*P. brutia* × *P. halepensis*)
P. halepensis × *P. pinaster*
P. nigra var. pallasiana × *P. sylvestris*
P. nigra var. pallasiana × *P. resinosa*
P. nigra var. pallasiana × *P. mugo*
P. nigra var. pallasiana × *P. pinaster*
P. nigra var. austriaca × *P. heldreichii*
P. nigra var. austriaca × *P. resinosa*
P. nigra var. austriaca × *P. mugo*
P. nigra var. austriaca × *P. sylvestris*
P. nigra var. corsicana × *P. resinosa*
P. nigra var. corsicana × *P. mugo*
P. nigra var. corsicana × *P. sylvestris*
P. sylvestris × *P. n. v. austriaca*
P. sylvestris × *P. n. v. corsicana*
P. sylvestris × *P. n. v. pallasiana*
P. heldreichii × *P. n. v. austriaca*
P. heldreichii × *P. n. v. corsicana*
P. heldreichii × *P. n. v. pallasiana*
P. heldreichii × *P. mugo*
P. heldreichii × *P. sylvestris*

Table 2. — Cones, seeds and seedlings produced in crosses of certain pine species (1967—1972).

♀	♂	Replications (years × centers)	Number of centers	Number of trees	Number of bags	Number of flowers	Number of conelets	Number of cones	%	Number of cones with filled seeds	%	Total number of seeds	Number of filled seeds	%	Number of germinable seeds	%
1	2	3	4	5	6	7	8	'	9		10	11		12		13
P. pinea	*P. brutia*	2	2	2	22	33	33	3	(9.1)	0	(0)	143	0	(0)	0	(0)
	P. pinaster	2	2	2	28	55	50	2	(4.0)	0	(0)	16	0	(0)	0	(0)
	P. n. v. pallasiana	2	2	2	20	55	54	3	(5.6)	0	(0)	19	0	(0)	0	(0)
	P. sylvestris	2	2	2	28	66	63	2	(3.2)	0	(0)	10	0	(0)	0	(0)
P. halepensis	*P. brutia*	15	3	41	408	824	753	190	(25.2)	7	(3.7)	4804	65	(1.4)	34	(52.3)
	P. pinaster	10	2	36	391	726	665	228	(34.3)	1	(0.4)	6575	4	(0.1)	3	(75.0)
	P. brut. × *P. halep.*	2	1	5	38	90	79	45	(57.0)	39	(86.7)	950	231	(24.3)	196	(84.9)
	P. halepensis	12	3	15	40	61	53	18	(34.0)	17	(94.4)	536	426	(79.5)	316	(74.2)
P. brutia	*P. halepensis*	15	3	63	841	2364	2190	934	(42.6)	746	(79.9)	23820	6243	(26.2)	4239	(17.8)
	P. pinaster	12	3	17	236	745	678	196	(29.0)	2	(1.0)	6006	2	(~0.0)	2	(100.0
	P. brut. × *P. halep.*	3	1	7	61	203	195	75	(38.5)	69	(92.0)	2214	1205	(54.4)	884	(73.4)
	P. brutia	18	3	47	111	316	286	83	(29.0)	72	(86.8)	2479	2146	(86.6)	1519	(61.3)
P. brutia × *P. halepensis*	*P. halepensis*	3	1	9	69	169	169	99	(58.6)	86	(86.9)	3068	1125	(36.7)	813	(72.3)
	P. brutia	3	1	12	91	219	215	84	(39.0)	59	(70.2)	2014	735	(36.5)	468	(63.7)
	P. pinaster	1	1	5	67	156	153	58	(38.0)	0	(0.0)	765	0	(0.0)	0	(0.0)
	P. brut. × *P. halep.*	3	1	8	37	108	105	56	(53.3)	50	(89.3)	1554	1030	(66.3)	753	(73.1)
P. pinaster	*P. halepensis*	8	2	28	361	916	875	71	(8.1)	0	(0.0)	701	0	(0.0)	0	(0.0)
	P. brutia	8	2	30	375	950	832	118	(14.2)	0	(0.0)	1272	0	(0.0)	0	(0.0)
	P. sylvestris	1	1	4	33	75	69	0	(0.0)	0	(0.0)	0	0	(0.0)	0	(0.0)
	P. n. v. pallasiana	1	1	4	34	86	83	0	(0.0)	0	(0.0)	0	0	(0.0)	0	(0.0)
	P. pinaster	8	2	14	48	104	90	6	(6.7)	6	(100.0)	459	413	(90.0)	245	(59.3)
P. nigra var. pallasiana	*P. brutia*	2	2	2	30	78	72	45	(62.5)	2	(4.4)	249	3	(1.2)	1	(33.3)
	P. pinaster	2	2	3	56	173	157	63	(40.1)	1	(1.6)	402	11	(2.7)	5	(45.5)
	P. sylvestris	20	4	40	608	1752	1670	727	(43.5)	13	(1.8)	10143	18	(0.2)	9	(50.0)
	P. resinosa	6	2	21	271	1037	1008	425	(42.2)	5	(1.2)	1836	27	(1.5)	16	(59.3)
	P. heldreichii	6	2	27	364	1129	1067	201	(18.9)	9	(4.5)	327	23	(7.1)	14	(60.9)
	P. mugo	2	2	13	134	443	426	319	(74.9)	0	(0.0)	5433	0	(0.0)	0	(0.0)
	P. n. v. pallasiana	15	3	25	109	290	277	142	(51.3)	123	(86.6)	2831	1856	(65.6)	1311	(70.6)
P. nigra var. austriaca	*P. sylvestris*	2	1	10	150	332	319	210	(65.9)	2	(1.0)	1809	10	(0.6)	7	(70.0)
	P. heldreichii	2	1	7	130	216	213	149	(70.0)	2	(1.3)	434	2	(0.5)	1	(50.0)
	P. resinosa	2	1	5	105	199	194	110	(56.7)	1	(0.9)	791	9	(1.1)	7	(77.8)
	P. mugo	1	1	3	45	88	88	60	(68.1)	4	(6.7)	983	16	(1.7)	14	(87.5)
	P. n. v. austriaca	2	1	8	35	61	58	43	(74.2)	36	(83.7)	849	339	(39.9)	175	(51.6)

(Continued from Table 2)

♀	♂	Replications (years × centers)	Number of centers	Number of trees	Number of bags	Number of flowers	Number of conelets	Number of cones	%	Number of cones with filled seeds	%	Total number of seeds	Number of filled seeds	%	Number of germinable seeds	%
1	2	3	4	5	6	7	8	9		10		11	12		13	
P. nigra var. *corsicana*	*P. sylvestris*	3	1	21	256	678	648	435	(67.1)	10	(2.3)	7955	18	(0.2)	10	(55.6)
	P. heldreichii	3	1	14	227	528	510	184	(36.1)	0	(0.0)	400	0	(0.0)	0	(0.0)
	P. resinosa	3	1	20	230	707	678	427	(63.0)	6	(1.4)	3901	14	(0.4)	13	(92.9)
	P. mugo	1	1	4	37	104	98	73	(74.5)	1	(1.4)	800	15	(1.9)	8	(53.3)
	P. n. v. corsicana	3	1	10	44	110	100	68	(68.0)	54	(79.4)	1179	880	(74.7)	587	(66.7)
P. nigra var. *hispanica*	*P. sylvestris*	1	1	1	3	10	8	6	(75.0)	0	(0.0)	123	0	(0.0)	0	(0.0)
	P. resinosa	1	1	1	10	29	28	19	(67.9)	0	(0.0)	249	0	(0.0)	0	(0.0)
P. sylvestris	*P. n. v. corsicana*	3	1	16	185	632	552	318	(57.6)	7	(2.2)	2735	76	(2.9)	44	(57.9)
	P. n. v. austriaca	3	1	16	230	758	688	218	(31.7)	6	(2.8)	1238	59	(4.8)	30	(50.9)
	P. n. v. pallasiana	4	1	32	355	1051	938	450	(48.0)	7	(1.6)	3335	70	(2.1)	13	(18.6)
	P. nigra var. *hispanica*	1	1	8	71	212	181	83	(45.9)	0	(0.0)	340	0	(0.0)	0	(0.9)
	P. mugo	1	1	7	76	309	257	106	(41.2)	59	(55.7)	1123	181	(16.1)	31	(17.1)
	P. heldreichii	4	1	23	299	1016	868	242	(27.9)	8	(3.3)	380	92	(24.2)	28	(30.4)
	P. sylvestris	3	1	12	48	144	142	43	(30.3)	42	(97.7)	844	663	(78.6)	397	(59.9)
P. heldreichii	*P. n. v. corsicana*	2	1	8	135	282	270	213	(78.9)	22	(10.4)	14006	28	(0.2)	7	(25.0)
	P. n. v. austriaca	2	1	11	135	346	308	250	(81.2)	15	(6.0)	11294	26	(0.2)	8	(30.8)
	P. n. v. pallasiana	3	1	16	274	630	574	427	(74.4)	17	(4.0)	21585	24	(0.1)	3	(12.5)
	P. mugo	1	1	4	45	101	92	47	(51.1)	0	(0.0)	2449	0	(0.0)	0	(0.0)
	P. resinosa	1	1	2	50	98	88	51	(58.0)	40	(78.4)	3426	115	(3.4)	21	(18.3)
	P. sylvestris	3	1	16	240	584	563	266	(47.3)	22	(8.3)	11216	37	(0.3)	6	(16.2)
	P. heldreichii	3	1	14	55	116	112	58	(51.8)	54	(93.1)	3051	2375	(77.8)	2034	(85.6)

Table 3. — Crossability of certain pine species (1967—1972)+.

♀	♂	Pollination year	Number of produced cones total	Number of produced cones with filled seeds	Cones with filled seeds %	Number of produced seeds total	Number of produced seeds filled	Filled seeds %	Number of filled seeds per cone*	Number of germinable seeds total	Number of germinable seeds %	Crossability** fertility %	Crossability** viability %
		1	2	3	4	5	6	7	8	9	10	11	12
Pinus halepensis	*P. brutia*	1967	39	3	7.7	756	4	0.5	0.1	2	50.0	0.3	69.4
		1970	36	0	0	820	0	0	0	0	0	0	0
		1972	31	2	6.4	1246	2	0.2	0.1	1	50.0	0.2	56.2
	P. pinaster	1967	43	0	0	816	0	0	0	0	0	0	0
		1968	40	0	0	1173	0	0	0	0	0	0	0
		1970	56	0	0	1866	0	0	0	0	0	0	0
		1972	44	0	0	1496	0	0	0	0	0	0	0
	P. brutia × *P. halepensis*	1967	15	10	66.7	270	62	23.0	4.1	51	82.3	11.5	114.3
	P. halepensis	1967	7	7	100.0	325	250	76.9	35.7	180	72.0	100.0	100.0
		1968	3	2	66.7	19	15	78.9	5.0	8	53.3	100.0	100.0
		1970	4	4	100.0	100	96	96.0	24.0	73	76.0	100.0	100.0
		1972	2	2	100.0	60	54	90.0	27.0	48	88.9	100.0	100.0
Pinus brutia	*P. halepensis*	1967	389	282	72.5	10180	2210	21.7	5.7	936	42.0	15.1	75.0
		1969	448	391	87.3	11007	3669	33.3	8.2	3058	83.4	37.4	112.4
		1972	44	31	70.4	1188	117	9.8	2.7	83	70.9	5.8	79.9
	P. pinaster	1969	42	0	0	0	1305	0	0	0	0	0	0
		1972	74	0	0	2174	0	0	0	0	0	0	0
	P. brutia × *P. halepensis*	1967	43	42	97.7	1324	630	47.6	14.7	514	81.6	39.1	145.7
	P. brutia	1967	17	16	94.1	746	639	85.7	37.6	358	56.0	100.0	100.0
		1969	51	46	90.2	1306	1116	85.4	21.9	828	74.2	100.0	100.0
		1972	6	6	100.0	289	275	95.2	45.8	244	88.7	100.0	100.0
Pinus brutia × *Pinus halepensis*	*P. halepensis*	1967	31	30	96.8	902	403	44.7	13.0	320	79.4	108.3	93.4
		1968	63	52	82.5	2074	689	33.2	10.9	480	69.7	54.5	89.2
		1971	4	4	100.0	92	33	35.9	8.3	13	39.4	66.9	155.7
	P. brutia	1967	19	13	68.4	227	110	48.5	5.8	87	79.1	48.3	93.1
		1968	41	34	82.9	1477	503	35.9	12.3	360	67.9	61.2	86.9
		1971	19	12	63.2	220	73	33.2	3.8	21	28.8	30.7	113.8
	P. pinaster	1971	30	0	0	400	0	0	0	0	0	0	0
	P. brutia × *P. halepensis*	1967	5	5	100.0	99	60	60.6	12.0	51	85.0	100.0	100.0
		1968	44	41	93.2	1331	883	66.3	20.1	690	78.1	100.0	100.0
		1971	7	6	85.7	124	87	70.2	12.4	22	25.3	100.0	100.0
Pinus pinaster	*P. halepensis*	1968	69	0	0	697	0	0	0	0	0	0	0
	P. brutia	1968	52	0	0	551	0	0	0	0	0	0	0
	P. pinaster	1968	2	2	100.0	279	261	93.5	130.5	128	49.0	100.0	100.0
Pinrs nigra var. pallasiana	*P. sylvestris*	1968	115	2	1.7	1165	2	0.2	0.02	0	0	0.2	0
		1969	144	0	0	1267	0	0	0	0	0	0	0
		1970	79	3	3.8	1279	4	0.3	0.05	1	25.0	2.0	52.2
		1971	37	1	2.7	2	2	100.0	0.05	2	100.0	0.1	114.9
		1972	92	0	0	714	0	0	0	0	0	0	0
	P. heldreichii	1968	25	3	12	53	18	34.0	0.7	14	77.8	7.8	90.6
		1969	56	1	1.8	7	1	14.3	0.02	0	0	0.3	0
		1970	106	4	3.8	262	4	1.5	0.04	0	0	0.2	0
	P. resinosa	1968	95	0	0	178	0	0	0	0	0	0	0
		1969	127	0	0	803	0	0	0	0	0	0	0
		1970	132	3	2.3	402	8	2.0	0.06	4	50.0	0.3	104.4
	P. mugo	1970	193	0	0	3315	0	0	0	0	0	0	0
	P. pinaster	1971	9	1	11.1	57	11	19.3	1.2	5	45.4	3.0	52.2
	P. n. v. pallasiana	1968	65	60	92.3	1219	603	49.5	9.3	518	85.9	100.0	100.0
		1969	18	7	38.9	145	111	76.6	6.2	56	50.4	100.0	100.0
		1970	18	17	94.4	522	383	73.4	21.3	183	47.9	100.0	100.0
		1971	11	11	100.0	510	444	87.0	40.4	386	87.0	100.0	100.0
		1972	16	14	87.5	188	133	69.0	8.3	63	47.4	100.0	100.0
Pinus nigra var. austriaca	*P. heldreichii*	1969	86	0	0	224	0	0	0	0	0	0	0
		1970	63	2	3.2	210	2	1.0	0.03	1	50.0	0.3	81.3
	P. resinosa	1969	62	1	1.6	403	9	2.2	0.15	7	77.8	2.2	188.8
		1970	48	0	0	388	0	0	0	0	0	0	0
	P. mugo	1970	60	4	6.7	983	16	1.6	0.3	14	87.5	2.9	81.8
	P. sylvestris	1969	129	1	0.8	1035	9	0.9	0.07	6	66.7	1.0	161.9
		1970	81	1	1.2	774	1	0.1	0.01	1	100.0	0.1	162.6
	P. n. v. austriaca	1969	24	18	75.0	259	165	63.7	6.9	68	41.2	100.0	100.0
		1970	19	18	94.7	590	174	29.5	9.2	107	61.5	100.0	100.0

(Continued from Table 3)

♀	♂	Pollination year	Number of produced cones total	Number of produced cones with filled seeds	Cones with filled seeds %	Number of produced seeds total	Number of produced seeds filled	Filled seeds %	Number of filled seeds per cone*	Number of germinable seeds total	Number of germinable seeds %	Crossability** fertility %	Crossability** viability %
		1	2	3	4	5	6	7	8	9	10	11	12
Pinus nigra var. corsicana	*P. heldreichii*	1968	73	0	0	92	0	0	0	0	0	0	0
		1969	90	0	0	272	0	0	0	0	0	0	0
		1970	21	0	0	36	0	0	0	0	0	0	0
	P. resinosa	1968	138	2	1.4	1774	2	0.1	0.01	2	100.0	0.04	117.7
		1969	223	1	0.4	1784	1	0.06	0.01	1	100.0	0.06	195.7
		1970	66	3	4.6	343	11	3.2	0.2	10	90.9	2.3	218.0
	P. mugo	1970	73	1	1.4	800	15	1.9	0.2	8	53.3	2.8	127.8
	P. sylvestris	1968	109	3	2.7	2842	3	0.1	0.03	2	66.8	0.1	78.6
		1969	234	6	2.6	3535	8	0.2	0.03	3	37.5	0.3	73.4
		1970	92	1	1.1	1578	7	0.5	0.08	5	71.4	1.1	171.2
	P. n. v. corsicana	1968	15	15	100.0	636	432	67.9	28.8	367	85.0	100.0	100.0
		1969	40	31	77.5	421	352	83.6	8.8	180	51.1	100.0	100.0
		1970	13	8	61.5	122	96	78.7	7.4	40	41.7	100.0	100.0
Pinus sylvestris	*P. n. v. austriaca*	1967	63	5	7.9	584	58	9.9	0.9	29	50.0	4.5	57.5
		1970	127	0	0	653	0	0	0	0	0	0	0
	P. n. v. corsicana	1967	181	6	3.3	1046	74	4.5	0.4	42	56.8	2.0	65.3
		1970	113	0	0	966	0	0	0	0	0	0	0
	P. n. v .pallasiana	1967	167	2	1.2	1455	18	1.2	0.1	13	72.2	0.5	83.0
		1970	112	3	2.7	1311	50	3.8	0.4	0	0	2.9	0
		1972	65	0	0	118	0	0	0	0	0	0	0
	P. heldreichii	1967	86	0	0	123	0	0	0	0	0	0	0
		1970	67	0	0	136	0	0	0	0	0	0	0
		1972	78	6	7.7	94	68	72.3	0.9	26	38.2	6.2	54.2
	P. mugo	1970	93	55	59.1	1051	177	16.8	1.9	31	17.5	12.4	33.8
	P. sylvestris	1967	5	5	100.0	103	92	89.3	20.6	80	87.0	100.0	100.0
		1970	30	28	93.3	581	459	79.0	15.3	238	51.8	100.0	100.0
		1972	9	9	100.0	160	112	70.0	12.4	79	70.5	100.0	100.0
Pinus heldreichii	*P. n. v. austriaca*	1967	106	0	0	4407	0	0	0	0	0	0	0
		1968	114	15	13.2	6887	26	0.4	0.2	8	30.8	0.6	38.5
	P. n. v. corsicana	1967	119	0	0	7946	0	0	0	0	0	0	0
		1968	94	22	23.4	6060	28	0.5	0.3	7	25.0	0.8	31.3
	P. n. v. pallasiana	1968	189	16	8.5	11244	23	0.2	0.1	3	13.0	0.3	16.3
		1972	56	1	1.8	1641	1	0.06	0.02	0	0	0.1	0
	P. mugo	1967	47	0	0	2449	0	0	0	0	0	0	0
	P. sylvestris	1968	152	22	14.5	6826	37	0.5	0.2	6	16.2	0.6	20.3
		1972	56	0	0	1603	0	0	0	0	0	0	0
	P. resinosa	1968	51	40	78.4	3426	115	3.4	2.2	21	18.3	6.0	22.9
	P. heldreichii	1967	25	23	92.0	1673	1217	72.7	48.7	1115	92.4	100.0	100.0
		1968	28	26	92.9	1236	1049	84.9	37.5	839	80.0	100.0	100.0
		1972	5	5	100.0	142	109	77.0	21.8	82	75.0	100.0	100.0

+ This table includes only combinations in which the interspecific crosses and the corresponding intraspecific ones were carried out at the same time

* The filled seeds refer to the total number of cones (column 2).

** Fertility % $= \frac{\text{Shybrid}}{\text{Smother}} \times 100$, Shybrid: The number of filled seed per cone for hybrid.

Smother: The number of filled seed per cone in crosses within the maternal species (column 8).

** Viability % $= \frac{\text{Ghybrid}}{\text{Gmother}} \times 100$, Ghybrid : Germinable seed per cent for hybrid column 10).

Gmother: Germinable seed per cent in crosses within the maternal species (column 10).

The second group includes combinations with fertility of 5 to 10%. The combination *P. heldreichii* × *P. resinosa* belongs undoubtedly here. We might also list in this group the combinations *P. sylvestris* × *P. heldreichii* and *P. nigra var. pallasiana* × *P. heldreichii* but with some reservations because one cross resulted in more than 5% fertility while two others were complete failures.

Finally, in the third group (>10%) we listed the crosses *P. brutia* × *P. halepensis* (always successful), *P. sylvestris* × *P. mugo* and the previously mentioned backcrosses.

Viability

The viability of filled seeds produced by interspecific crosses was generally high. However, we noted some exceptions. Thus, the cross *P. sylvestris* × *P. nigra var. pallasiana* in 1970 produced 50 filled but nonviabe seeds. Also, the successful combination *P. sylvestris* × *P. mugo* showed low seed viability (33,8%). Finally very low viability resulted from all combinations in which *P. heldreichii* was used as the female parent.

Irradiated Pollen

Irradiation of pollen promoted crossability among pine species in a few combinations only and to a limited degree *(Table 4)*. These combinations were:

P. nigra var. pallasiana × *P. heldreichii*
P. nigra var. austriaca × *P. mugo*
P. sylvestris × *P. nigra var austriaca*

In the combinations *P. nigra var. austriaca* × *P. resinosa*, *P. nigra var. corsicana* × *P. mugo* and *P. nigra var. austriaca* × *sylvestris* by using irradiated pollen we obtained one cone only per each combination. This cone had very few filled seeds.

Irradiated pollen had the same effect on seed production as the natural pollen, in the following combinations:

P. halepensis × *P. pinaster*
P. sylvestris × *P. mugo*
P. nigra var. pallasiana × *P. sylvestris*
P. nigra var. corsicana × *P. sylvestris*

As contrasted to these combinations, the combination *P. sylvestris* × *P. nigra var. pallasiana* produced filled seeds only with natural pollen. On the other hand, in the following combinations we observed no production of filled seeds either with natural or with irradiated pollen.

P. halepensis × *P. pinaster*
P. brutia × *P. pinaster*
P. nigra var. pallasiana × *P. pinaster*

A final point to be added on the use of irradiated pollen is that, as in the case of natural pollen, its efficiency varied among years.

The use of dead pollen from the female parent had no effect on cone and seed production.

Discussion

A high percentage of conelets developed into mature cones. However, the formation of cones resulting from the crosses among the aforementioned pine species does not seem to be associated with the fertilization process itself. This is suggested by the following two observations: First, for intraspecific crosses the percentage of conelets developing into cones varied usually between 30 and 70%, *i.e.* although fertilization was complete, percentages higher than 70% were rarely noted. Second, the cone percentages for interspecific crosses were equal or higher than those for intraspecific ones although the fertilization of egg cells was incomplete. Our results do not agree with CRITCHFIELD's findings (1962). According to this investigator the combinations *P. ponderosa* × *P. palustris* and *P. ponderosa* × *P. echinata* yielded far less cones than the intraspecific crosses. It should be noted that Shaw's system lists the above three species in another group (Australes).

In contrast to fertilization, the presence of pollen on the female flowers stimulated cone formation. This becomes evident by comparing cone percentages of intraspecific and interspecific crosses, in which all female flowers were pollinated, to the cone percentages of the control flowers which were isolated but not pollinated. The percentages were 41.8 and 5.0% respectively, and refer to all the species crossed during this phase of the study.

Thus, we see that the crossed pine species formed parthenocarpic cones only when pollen was present. MCWILLIAM (1959) reached the same conclusion with regard to *P. nigra*. On the other hand the species *P. brutia*, *P. nigra var. corsicana* and *P. nigra var. pallasiana* were exceptions because they tended to form a few cones (24, 24 and 28 respectively) without pollination. BASSIOTIS (1972) observed parthenocarpic formation of a few cones in the species *P. brutia*, *P. nigra var. corsicana* and *P. hedreichii*. Parthenocarpy in the genus *Pinus* was also reported by DENGLER (1932), WETTSTEIN (1940), PLYM FORSHELL (1953), WRIGHT (1953), and by EHRENBERG and SIMAK (1957).

P. sylvestris × *P. nigra var. corsicana*
P. heldreichii × *P. sylvestris*

The stage at which conelet abortion occurred in the control flowers, due to lack of pollen stimulation, was not determined precisely. Most conelet abortion took place shortly after pollination time while some conelets remained a few months or even 1 year. In MCWILLIAM's study (1959) the unpollinated ovulate strobili whithered 3 months after

P. nigra var. corsicana × *P. resinosa*
P. heldreichii × *P. nigra var. austriaca*
P. heldreichii × *P. nigra var. corsicana*
P. sylvestris × *P. heldreichii*

isolation.

HUMMEL (1930) noted that only cones but no seeds were produced when crossing not closely related species. In our study all the crosses produced some empty seeds. We also found, within the same combination, marked differences in

P. nigra var. pallasiana × *P. mugo*
P. nigra var. austriaca × *P. heldreichii*
P. nigra var. corsicana × *P. heldreichii*

seed production from year to year. All combinations of *P. halepensis*, *P. brutia*, *P. heldreichii* and of the hybrid *P. brutia* × *P. halepensis* yielded a large number of seeds per cone which was similar to the number obtained from the intraspecific crosses. The species *P. heldreichii* used as a female parent scored the highest number of empty seeds per cone, while the lowest number was obtained from crosses in which this species was used as a male parent. Also the combinations in which *P. pinaster* and *P. sylvestris* were used as female parents produced a low number of seeds per cone. These results agree with the ones obtained by BASSIOTIS (1972).

For many interspecific crosses the number of empty seeds per cone was more or less lower than the one for intraspecific crosses *(Table 3)*. This indicates that the fertilization process had some effect on the formation of empty seeds in spite of the fact that the formation of the seed coat and the endosperm, but not of the embryo, takes place before fertilization. The presence of pollen stimulates and activates the female flowers to a different degree depending on the relationship of the crossed species. Several of the interspecific crosses produced a large number of empty seeds and a small number of filled seeds and vice-versa. Therefore, since it is the number of filled seeds which is the basic criterion of crossability between two species and since this number is not associated with the number of empty seeds we may conclude that the number of empty seeds could not be used as a criterion of crossability of the pine species. WRIGHT and GABRIEL (1958) also observed the lack of any association between empty and filled seeds in interspecific crosses of the Lariciones group. On the other hand, CRITCHFIELD (1962) who experimented with pine species of another group in South California found in many crosses a relationship between empty and filled seeds. No satisfactory explanation has so far been proposed for the different performance of these two groups of pine species. Furthermore, WRIGHT (1970) believes that the total number of seeds is not a reliable indication of the success of a cross.

Table 4. — Results of pine-crosses by the use of natural and irradiated pollen.

♀	♂	Pollination year	Treatment of pollen	Total anumber of seeds/cone	Number of produced cones with filled seeds	Number of filled seeds	Filled seeds %
1	2	3	4	5	6	7	8
Pinus halepeniss	*P. brutia*	1967	Natural	23	0	0	0
			400r.	19	3	4	0.8
			1000r.	16	0	0	0
		1970	Natural	24	0	0	0
			400r.	24	0	0	0
			1000r.	17	0	0	0
		1971	Natural	17	0	0	0
			600r.	33	0	0	0
		1972	Natural	44	1	1	0.3
			400r.	39	1	1	0.1
			1000r.	36	0	0	0
Pinus brutia	*P. halepensis*	1967	Natural	27	201	1607	22.8
			600r.	25	81	603	19.3
		1969	Natural	27	207	2078	35.0
			600r.	22	184	1591	31.3
		1972	Natural	34	18	57	7.3
			400r.	10	1	4	13.3
			1000r.	21	12	56	14.7
Pinus nigra var. pallasiana	*P. heldreichii*	1968	Natural	0.2	0	0	0
			400r.	9.8	3	18	46.2
			1000r.	11.0	0	0	0
		1969	Natural	0	0	0	0
			400r.	0.2	0	0	0
			1000r.	0.1	1	1	20.0
		1970	Natural	0.1	3	3	50.0
			400r.	4.9	1	1	0.5
			1000r.	2.3	0	0	0
	P. sylvestris	1968	Natural	35	0	0	0
			1000r.	26	1	2	0.1
		1968	Natural	9	2	2	0.6
			1000r.	11	0	0	0
		1969	Natural	8	0	0	0
			400r.	6	0	0	0
			1000r.	10	0	0	0
		1970	Natural	16	0	0	0
			400r.	16	2	4	0.6
			1000r.	9	0	0	0
		1970	Natural	18	1	2	0.5
			400r.	11	1	1	0.3
			1000r.	20	1	1	0.2
		1971	Natural	16	1	1	0.3
			600r.	12	2	2	0.8
		1972	Natural	6	0	0	0
			400r.	6	0	0	0
			1000r.	11	0	0	0
Pinus nigra var. austriaca	*P. mugo*	1970	Natural	23	1	2	0.4
			400r.	11	0	0	0
			1000r.	13	3	14	6.5
	P. resinosa	1969	Natural	7	0	0	0
			400r.	2	1	9	64
			1000r.	7	0	0	0
		1970	Natural	4	0	0	0
			400r.	10	0	0	0
			1000r.	10	0	0	0
	P. sylvestris	1969	Natural	6	0	0	0
			400r.	3	0	0	0
			1000r.	14	1	9	1.5
		1970	Natural	12	0	0	0
			400r.	7	0	0	0
			1000r.	10	0	0	0

(Continued from Table 4)

♀	♂	Pollination year	Treatment of pollen	Total anumber of seeds/cone	Number of produced cones with filled seeds	Number of filled seeds	Filled seeds %
1	2	3	4	5	6	7	8
Pinus nigra var. corsicana	*P. sylvestris*	1968	Natural	23	1	1	0.2
			400r.	31	0	0	0
			1000r.	24	2	2	0.2
		1969	Natural	15	1	1	0.1
			400r.	13	3	3	0.3
			1000r.	19	2	4	0.3
		1970	Natural	15	1	7	1.5
			400r.	19	0	0	0
			1000r.	18	0	0	0
	P. resinosa	1969	Natural	5	1	1	0.1
			400r.	7	0	0	0
			1000r.	11	1	1	0.1
		1970	Natural	6	1	4	2.3
			400r.	8	0	0	0
			1000r.	2	2	7	13.3
	P. mugo	1970	Natural	5	0	0	0
			400r.	12	0	0	0
			1000r.	13	1	15	3.1
Pinus sylvestris	*P.nigra var. pallasiana*	1967	Natural	15	2	18	2.6
			500r.	0	0	0	0
			1000r.	13	0	0	0
		1967	Natural	4	0	0	0
			500r.	8	0	0	0
			1000r.	1	0	0	0
		1970	Natural	12	3	50	4
			400r.	3	0	0	0
			1000r.	5	0	0	0
		1972	Natural	5	0	0	0
			400r.	2	0	0	0
			1000r.	1	0	0	0
	P. nigra var. austriaca	1967	Natural	12	0	0	0
			500r.	12	5	58	20
			1000r.	3	0	0	0
		1970	Natural	8	0	0	0
			400r.	6	0	0	0
			1000r.	1	0	0	0
	P. nigra var. corsicana	1967	Natural	1	0	0	0
			500r.	9	5	73	13
			1000r.	21	1	1	0.1
		1970	Natural	8	0	0	0
			400r.	9	0	0	0
			1000r.	10	0	0	0
	P. mugo	1970	Natural	18	26	113	19
			400r.	10	26	58	14
			1000r.	3	3	6	14
	P. heldreichii	1967	Natural	0.1	0	0	0
			500r.	0.4	0	0	0
			1000r.	4.0	0	0	0
		1970	Natural	0.4	0	0	0
			400r.	3.0	0	0	0
		1972	Natural	3.0	5	61	71
			400r.	0.4	1	7	88
Pinus heldreichii	*P. nigra var. pallasiana*	1967	Natural	50	0	0	0
			500r.	62	0	0	0
			1000r.	51	0	0	0
		1968	Natural	53	2	2	0.1
			1000r.	49	5	9	2.6
		1972	Natural	33	0	0	0
			400r.	30	0	0	0
			1000r.	23	1	1	0.3

(Continued from Table 4)

♀	♂	Pollination year	Treatment of pollen	Total anumber of seeds/cone	Number of produced cones wiht filled seeds	Number of filled seeds	Filled seeds %
1	2	3	4	5	6	7	8
Pinus heldreichii	*P. nigra var. austriaca*	1967	Natural	29	0	0	0
			500r.	41	0	0	0
			1000r.	54	0	0	0
		1968	Natural	54	7	15	0.5
			400r.	62	6	7	0.4
			1000r.	70	2	4	0.2
	P. nigra var. corsicana	1967	Natural	69	0	0	0
			500r.	66	0	0	0
			1000r.	65	0	0	0
		1968	Natural	72	13	17	0.5
			400r.	63	4	6	0.6
			1000r.	51	5	5	0.4
	P. sylvestris	1968	Natural	39	2	2	0.1
			400r.	49	6	6	0.2
			1000r.	45	14	29	1.2
		1972	Natural	35	0	0	0
			400r.	27	0	0	0
			1000r.	20	0	0	0

Based on the fertility of the interspecific crosses we distinguished three groups of combinations. The first group with 0—5% fertility includes most combinations. Some of them, as we already mentioned, failed completely while the rest should be considered very difficult. It should be noted that this first group includes also combinations of species which are closely related taxonomically *e.g. P. halepensis* × *P. pinaster, P. brutia* × *P. pinaster* and others.

Buchholz (1944) regards as the cause of the sterility, encountered in crossing some pine species, the inability of pollen tube to reach and fertilize the egg cell before it disintegrates. In McWilliam's study (1959), only a small number of pollen grains in the combination *P. nigra* × *P. resinosa* germinated and grew on the nucellar tissue but eventually no fertilization took place. This was attributed by the author to the gradual disintegration of the egg cells during the first 12 months. This disintegration occurred (mainly between the 2nd and 4th month) after pollination. According to the same author, egg cell disintegration is due to the fact that pollen grains do not germinate or they do so slowly and thus they do not provide the necessary factor for the development of the female gametophyte. The failure of pollen grains to grow vigorously or even germinate at all and reach the embryosac may be due to a particular aminoacid concentration in the two pine species (McWilliam 1959).

The second group with 5—10% fertility comprises the combination *P. heldreichii* × *P. resinosa*. This successful cross of *P. resinosa* with another pine species is noteworthy because it is very hard to cross either as a female or as a male parent (Wright 1953). The bibliography we have reviewed does not mention any other case in which such a cross was successful. According to Bassiotis (1972) 29 filled seeds were produced out of one cone. These seeds were sown and gave 12 seedings resembling the female parent with regard to morphology, anatomy and growth pattern. This resemblance and the production of a large number of filled seeds from one cone leads us to suspect that perhaps there was some technical error during pollinations. The fact, that some previous investigators did have success with certain interspecific combinations and others did not, was attributed by Eifler (1956) to differences in weather conditions during and after pollination, while other investigators (Dengler 1932, Wright 1953, Duffield 1954 b, Critchfield 1962) attributed it to crossability differences among the particular biotypes and races that were combined each time. Our data show also marked differences in the fertility of the crosses from year to year. The crosses *P. sylvestris* × *P. heldreichii* and *P. nigra var. pallasiana* × *P. heldreichii* showed the same fertility but only in one out of three attempts. Therefore we suggest that these crosses be repeated again.

The combinations *P. brutia* × *P. halepensis* and *P. sylvestris* × *P. mugo* were highly fertile (>10%). The first one was successful in all our attempts and yielded always a large number of viable seeds. The results agree with those reached by Hummel (1930), and by Moulopoulos and Bassiotis (1961). Moreover, Papajoannou (1936), reported natural hybrids betwen *P. brutia* and *P. halepensis*. Some investigators also have success with the combination *P. sylvestris* × *P. mugo*.

The species *P. heldreichii* pollinated with pollen of *P. nigra* yielded a small number of viable seeds. This was also observed by Bassiotis (1972), while Vidakovic (1963) did not succeed with this particular cross.

In general, most of the filled seeds produced by the interspecific crosses were viable. The crosses in which *P. heldreichii* was the female parent were an exception because the seeds showed low viability. Another exception was the combination *P. sylvestris* × *P. nigra var. pallasiana* which produced 50 filled seeds none of which germinated in 1970. This lack of viability was attributed by Rohmeder (1972) to a dead embryo or to complete or even partial necrosis of the endosperm.

The seedlings produced in the nursery (putative hybrids) were in all cases healthy and did not differ in growth habits from the parental seedlings during the first year.

Irradiation increased pollen activity in certain difficult to cross combinations because it resulted in the production of a small number of viable seeds while, in the same combinations, natural pollen either failed completely or produced two or three filled seeds only. It seems that irradiation may have caused some chemical changes, even mutations, which promoted the development of the female gametes. The same conclusions were reached by Vidaković (1963) and by Bassiotis (1972) with regard to this point. Furthermore, our results confirmed the findings of Vidaković (1963) whereby the use of dead pollen from the female parent in mixture with pollen from the male parent did not promote crossability.

In general, crossing various pine species of the subgenus Diploxylon is still a difficult problem for the forest tree breeder, with the exception of two or three cases where interspecific crosses are easy. However, even low fertility crosses may in practice be satisfactory for breeding programs, because by heterovegetative propagation of the few valuable hybrids we can still breed a series of clones for further use. Such partial successes are very useful because the breeder is enabled to explore various problems and provide answers to theoretical questions. Moreover, by studying and checking the few hybrids produced, we are able to evaluate them so that we can concentrate our further research on those combinations which produce hybrids clearly superior to their parents.

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Summary

A series of crosses among the following pine species of the subgenus Diploxylon were carried out in nine hybridization centers throughout Greece during 1967—1972:

P. pinea	*P. nigra var. austriaca*
P. halepensis	*P. nigra var. corsicana*
P. brutia	*P. nigra var. hispanica*
P. pinaster	*P. sylvestris*
P. nigra var. pallasiana	*P. heldreichii*

Two further species *P. mugo* and *P. resinosa* were used as male parents only. Also, the hybrid *P. brutia* × *P. halepensis* was backcrossed to the parental species.

A total of 22,500 flowers were pollinated with natural and irradiated pollen (γ^{60}Co, 400—1000r) and 20,900 conelets were counted during removal of pollination bags.

A high percentage of pollinated flowers developed into mature cones in contrast to the failure of nonpollinated flowers to do so, because of cone abscission during the first 12 months after pollination time.

Cones resulting from interspecific crosses nearly always had some empty seeds, but only a small percentage of these cones gave a few filled seeds. Exceptions to this general pattern were first the combinations *P. brutia* × *P. halepensis, P. sylvestris* × *P. mugo* and *P. heldreichii* × *P. resinosa* and secondly, the backcrosses of the hybrid *P. brutia* × *P. halepensis* to *P. halepensis* and *P. brutia* which produced filled seeds in a very high percentage of cones.

Based on the degree of fertility observed, three groups of crosses were distinguished, namely, first (0—5%), second (5—10%), third (>10%). The first group included most of the combinations tested. In particular the combinations *P. halepensis* × *P. pinaster, P. brutia* × *P. pinaster,* and their reciprocal as well as the combinations *P. nigra var. corsicana* × *P. hedreichii* failed completely (zero fertility). The combination *P. heldreichii* × *P. resinosa* belongs to the second group. The third group includes *P. brutia* × *P. halepensis, P. sylvestris* × *P. mugo* and the backcrosses of the hybrid *P. brutia* × *P. halepensis* to *P. halepensis* and *P. brutia.*

Viability of the filled seeds produced by interspecific crosses was generally high. However, *P. sylvestris* × *P. mugo* and combination in which *P. heldreichii* was used as a female parent produced low viability seeds.

None of the 50 seeds produced by crossing *P. sylvestris* with *P. nigra var. pallasiana* in 1967, were viable.

Pollen irradiation promoted viable seed production in a few combinations only, while in *P. sylvestris* × *P. nigra var. pallasiana* it had no effect.

A mixture of dead pollen from the female parent with natural pollen of the male parent did not promote cone and seed formation.

Key words: Controlled pollinations, Pine species, Crossability, Viability, Irradiated Pollen.

Zusammenfassung

In 9 Gebieten Griechenlands wurden in den Jahren 1967—1972 Kreuzungsversuche zwischen folgenden Kiefernarten des Sub-Genus Diploxylon durchgeführt: *P. pinea, P. halepensis, P. brutia, P. pinaster, P. nigra (var.: pallasiana, austriaca, corsicana, hispanica), P. silvestris, P. heldreichii* sowie den *P. mugo* und *P. resinosa* als männliche Partner. Darüber hinaus wurden auch Rückkreuzungen von *P. halepensis* und *P. brutia* mit dem Hybriden *P. brutia* × *P. halepensis* ausgeführt.

Insgesamt wurden 22 500 weibliche Blüten bestäubt und 20 900 Zäpfchen bei dem Austüten gezählt.

Ein hoher Prozentsatz der bestäubten Blüten entwickelte sich zu reifen Zapfen. Unbestäubte Blüten dagegen entwickelten sich nicht zu reifen Zapfen; sie fielen im ersten Jahre ab.

Die Zapfen aus den interspezifischen Kreuzungen enthielten in fast allen Fällen Hohlkörner und nur in einigen Fällen Vollkörner, wobei letztere vorzugsweise auf die Kombinationen *P. silvestris* × *P. mugo* und *P. heldreichii* × *P. resinosa* sowie die Rückkreuzungen von *P. halepensis* und *P. brutia* mit dem Bastard *P. brutia* × *P. halepensis* beschränkt waren. In diesen Kombinationen konnten auch relativ hohe Vollkornprozente beobachtet werden.

Je nach der Kreuzungsfertilität *(Tab. 3)* wurden sie in 3 Gruppen (0—5%, 5—10%, 10% und höher) eingeteilt.

Die erste Gruppe schließt die meisten Kombinationen ein. Die Kombinationen *P. halepensis* × *P. pinaster, P. brutia* × *P. pinaster* und die Gegenkreuzungen, wie auch die Kombination *P. nigra var. corsicana* × *P. heldreichii* schlugen völlig fehl (Fertilität 0%).

Der zweiten Gruppe gehört die Kombination *P. heldreichii* × *P. resinosa* an.

Der dritten Gruppe, mit einer Fertilität 10% und höher werden die Kreuzungen *P. brutia* × *P. halepensis* und *P. silvestris* × *P. mugo* sowie die Rückkreuzungen von *P. halepensis* und *P. brutia* mit ihrem Bastard *P. brutia* × *P. halepensis* zugeordnet.

Die Keimfähigkeit der bei den Artkreuzungen erzeugten Vollkörner war allgemein hoch *(Tab. 3)*. Geringe Keimfähigkeit zeigten die Kreuzung *P. silvestris* × *P. mugo* und die Kombinationen mit der Panzerkiefer als Mutterbaum. Aus der Kombination *P. silvestris* × *P. nigra var. pallasiana* des Jahres 1967 gingen keine keimfähigen Vollkörner hervor.

Die parallel durchgeführte Bestäubung mit bestrahltem Pollen (γ-Strahlen ^{60}Co, 400—1000 r) förderte die Kreuzbarkeit der verschiedenen Pinusarten nur in geringem

Maße. In der Kombination *Pinus sylvestris* × *P. nigra var. pallasiana* konnten nur mit natürlichem Pollen Vollkörner erzielt werden.

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Introgressive Hybridization in the West Himalayan Silver Firs

By K. K. JAIN

Department of Bio-Sciences, Himachal Pradesh University, Simla- 171005; India.

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Introduction

Silver fir *(Abies* MILLER) is the most widespread conifer in the western Himalayas, especially in the higher ranges. Altitudinally it appears at 2,150 m and ascends upto 3,900 m; thus having an elevational range of about 1,800 m. No other conifer in the Himalayas has such a wide elevational range.

Foresters dealing with the Himalayan firs have long been intrigued regarding the number of species occurring in the western Himalayas (GAMBLE, 1902; BRANDIS, 1906; TROUP, 1921; PARKER, 1940). The problem has recently been resolved by JAIN (1975) who made an extensive survey of the *Abies* populations in various parts of the western Himalayas and studied the morphological and anatomical characteristics of the different taxa encountered. He observed that two species of *Abies* occur in the western Himalayas, separated by altitude, and that a putative hybrid population is found between them. The high altitude species is *A. spectabilis* (D. DON.) SPACH. and the low altitude one is *A. pindrow* ROYLE. In order to reveal the true nature of the hybrid populations the author attempted sampling a transect from the lower species, through several altitudes in the hybrid zone, to the upper species. A statistical analysis of this transect sampling has been presented in the present article.

Materials and Methods

To reveal the hybrids and the extent of influence of the parents in the expression of the phenotypes of the hybrids, ANDERSON's (1949) method of "hybrid-index" was adopted for analysis. Fifteen characters were selected. The data in respect of these characters were collected from the Kalpa range in Himachal Pradesh (approximately 31.5^0 N. lat., 78^0 E. long.). This site was chosen because both species together with their putative hybrids are found in this area along an elevational range between 2,400 m and 3,900 m. Random samples were collected along the transect between 2,600 m and 3,900 m and consisted of from 40 to 50 mature trees between every 300 m interval along the transect. Measurements were made for the quantitative characters; whereas qualitative characters, which could not be measured, were recorded in relative terms *(Table 1)*.

In the present investigation the index is based on scoring