INTERSPECIFIC HYBRIDIZATION IN CARPINUS

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ABSTRACT.- This paper is a first report on successful interspecific controlled pollination in Carpinus, involving five species. C. tschonoskii was a tetraploid rather than diploid-(as previously reported), C. betulus was octoploid, and the other species were diploid. Female parents in all species appeared to be highly self-incompatible. Three authentic interspecific hybrid combinations were produced: C. betulus X orientalis (and reciprocal), C. betulus X tschonoskii and C. orientalis X tschonoskii. The hybrids were verified by chromosome counts on seedling root tips. Metro. Tree Impr. Alliance (METRIA) Proc. 1: 73-79, 1978.

There is a definite need for a wider range of choice in shade and landscape trees of moderate height(30-40 feet) at maturity. Among the trees in this category currently being investigated at the U.S. National Arboretum are the hornbeams of the genus Carpinus. No major insect or disease pests have been reported that might limit the culture of the hornbeams.

Although the genus may include as many as 26 species, (Rehder, 1940), few are in cultivation in the United States. The European hornbeam(C betulus L.) is extremely popular in Europe, partly because it can withstand repeated clipping for hedges and ultraformal plantings. Although modern American horticultural practice seldom includes "hedging" of trees, many.American nurseries offer C. betulus and its cultivar 'Fastigiata'. Wyman (1965) has pointed out that nomenclatural confusion has resulted from trees of both the cultivars 'Fastigiata' and 'Columnaris' being distributed under the name 'Fastigiata'..

The other available species in the genus are superior to European hornbeam in one or more landscape characteristics. A few nurseries grow our native American

hornbeam (C. caroliniana Walt.). This species, as well as C. japonica Bl. and C. tschonoskii Maxim., can develop a desirable vase-shaped-form. The Japanese and American hornbeams may also exhibit an attractive orange to russet-red autumnal leaf coloration in some climatic zones. Fall color is less characteristic of the slow-growing C. orientalis Mill., but the delicate foliage texture and interesting growth habit of this species may have merit in certain situations.

Sargent **(1896)** defined two sections in Carpinus. Of the species investigated here, only C. japonica is classified in Sect. Distegocarpus. The rest of the species belong to Sect. Eucarpinus (= Sect. Carpinus).

Wetzel (1929) studied pollen-mother-cell meiosis in C. betulus and reported n=8 chromosomes. This count was confirmed by Jaretsky **(1930).** Woodworth **(1930)** likewise found n=8 in a "normal" tree of this species but reported n=32, an octoploid number, in C. betulus var. fastigiata Jaeg. (=cv. Fastigiata). Scheerer (1940) reported n=32 for native trees in Schleswig-Holstein and Sylven, by personal communication to Scheerer (1940), found n=32 in trees of Swedish origin. Johnsson (1942) investigated native trees from seven locations in Sweden and found all to be octoploid with n=32 chromosomes. He also studied meiosis in a botanic garden specimen of C. carpinizza Kitaibel ex Host and found it to be a diploid with n=8 chromosomes. The significance of this finding is that C. carpinizza was accepted by Rehder (1940) as C. betulus var. carpinizza (Host) Neilreich. Thus, C. betulus was believed to occur in both diploid and octoploid forms, and some authors thought that "races" with intermediate numbers might exist.

For the other species used in our hybridization studies, Woodworth (1930) reported n=8 for C. caroliniana, C. japonica, and C. orientalis. Johnsson (1942) studied a botanic garden specimen of C. tschonoskii and found it to be a diploid with n=8 chromosomes.

To our knowledge, no natural or artificial interspecific hybrids have been reported in Carpinus. The purpose of the present study was to determine the crossabilities of several Carpinus species and to investigate the chromosome number of parents and progenies.

MATERIALS AND METHODS

All of the trees in this study were growing at the U. S. National Arboretum or the Plant Introduction Station at Glenn Dale, Maryland. No more than two trees of any species were available for crossing. The male catkins were the first to mature on all species. These catkins were removed from selected branches before anthesis, and the female catkins were enclosed in a terylene non-woven fabric bag. about two to three days after normal pollen shedding on each tree, we pollinated the female flowers by carefully removing the bag and applying pollen with a pipe cleaner. The bags were replaced after pollination and left on the branches for another two weeks.

Fruit catkins were harvested in late August, when the bracts had begun to turn brown. The seeds were removed manually from the bracts, and the numbers of seeds and bracts were counted. After about a week, most of the seeds had lost any green color. Seed from crosses on C. betulus and C. orientalis were immersed in a 2% solution of ethyl alcohol to distinguish full seed ("sinkers") from empty seed ("floaters"). The full seed were then stratified in moist sand in a refrigerator for 120 days before sowing. Seed harvested from C. japonica could not be separated by the flotation test. -Therefore, all seeds were stratified. An estimate of the percentage of good seed from the intraspecific C. japonica cross was made by a ctting test on a 50-seed random sample.

All of the seed from crosses on C. betulus and C. orientalis and about 80 of the intraspecific C. japonica seed were row-planted in greenhouse flats in January" The remainder of the seed from C. japonica was broadcast-sown. Germination of many of the seedlots from crosses on C. betulus in **1974** was poor or non-existent after 11 weeks. The ungerminated seed was dug up and restratified for 100 days and resown in July. All the seedlings that developed from this double-stratified seed (C. betulus X Self, C. betulus X betulus 'Cordata', C. betulus X orientalis) germinated within one week after the second sowing.

Cytological studies of meiosis were made on male catkins brought into the laboratory for forcing in February. Mitotic chromosome counts were made on root tips of seedlings derived from open or controlled pollination. Standard acetocarmine squash techniques were employed for all cytological work. Measurements of mature pollen grains were based on 50 randomly selected grains, and pollen abortion was determined from examination of 100 random grains.

RESULTS AND DISCUSSION

Cytology and pollen.- Table 1 gives the chromosome numbers of the parent trees used in hybridization. One important finding was that our C. tschonoskii trees were tetraploids, with n=16, 2n=32 chromosomes, rather than diploids. All individuals of C. betulus were octoploids. Polyploids could be easily distinguished from diploids by pollen size, but there were no significant differences between tetraploids and octoploids. The number of germinal apertures in the pollen grain was 3 for diploid species, 4 for C. tschonoskii, and 4 to 5 for C. betulus. Pollen from all trees showed less than 5% abortion.

We were fortunate in having seedlings of C. betulus and C. orientalis from several known provenances in Yugoslavia. Yugoslavia is in the southern portion of the range of C. betulus, and the fact that the trees were octoploid casts some doubt on the idea of geographic cytological races in this species. I submit that C. betulus may be entirely octoploid and that an intensive cyto-taxonomic study of European Carpinus species might lead to the verification of one or more "new" species.

The differences in chromosome number among parental species allowed a simple verification of the interspecific hybrid seedlings.

Controlled pollination.-- Table 2 gives the results of controlled pollinations. Pollen origin appeared to have little effect on the percentage of female catkins that matured to interspecific pollinations, with an overall average of 79 percent. Only two interspecific combinations-- C. betulus X japonica (32%) and C. japonica X orientalis (51%) -- were significantly below this average.

The seeds (nutlets) produced in the female catkins were subtended by a large bract, and a count of the bracts indicated the potential number of seeds that could be developed. For the three species used as female parents, the bract numbers were: C. betulus, 13.9; C. japonica, 34.7; C. orientalis, 8.9.

Based on present evidence, Carpinus species appear to be highly self-incompatible. This characteristic could be an advantage to the breeder when dealing with isolated trees. We tried pollinating, without bagging, an isolated tree of C. betulus with C. tschonoskii pollen. The results indicated that such a technique could be very successful.

C. japonica used as both the male and female parent, did not produce hybrids with any other species. The taxonomic classification of C. japonica in a different section than the other species thus appears reasonable on a genetic basis.

Only three authentic interspecific hybrid combinations were produced: C. betulus X orientalis (and reciprocal), C. betulus X tschonoskii, and C. orientalis X tschonoskii. Fortunately, the three parental species differed in chromosome number and the hybrids could be verified cytologically. We did not attempt to establish morphological criteria of hybridity on the young seedlings.

The germination data in Table 2 show that more reliable methods must be found to assay seed development, determine seed germination potential, and produce good yields of control-pollinated seedlings. Without efficient seed evaluation and germination techniques, some "successful" crosses may be missed, and progeny numbers in certain seedlots may be too few for meaningful evaluation and future selection.

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Table 1. Chromosome and pollen data on *Carpinus* parents, hybrid progeny, and provenance seedlings.

Species or Cross	Chromosome Number	Pollen Size (microns)
Parents		
C. caroliniana	n=8 ,2n=16	30.2
C. japonica	n=8,2n=16	27.9
C. orientalis	n=8	28.4
C. tschonoskii	n=16,2n=32	36.3
C. betulus	n=32,2n=64	38.9
C. betulus 'Cordata'	n=32	39.6
C. betulus 'Purpurea'	n=32	36.7
Provenance Seedlings (Yugoslavia)		
C. orientalis	2n=16	____
C. betulus	2n=64	____
Hybrid Seedlings		
C. betulus X *orientalis*	2n=40	____
C. orientalis X *betulus*	2n=40	____
C. betulus X *tschonoskii*	2n=48	____
C. orientalis X *tschonoskii*	2n=24	____

Table 2. Results of controlled pollinations in Carpinus, 1973-1974,

Cross[a]	Number Catkins		Number Seed			
	Pollinated	Harvested	Potential[b]	Harvested	Full	Germinated
C. japonica						
X Self	11	11	382	382	?	2
X japonica	24	23	833	810	550[c]	192
X betulus	12	8	416	332	?	0
X b.'Purpurea'	36	31	1249	1070	?	2[d]
X orientalis	43	22	1492	760	?	0
X tschonoskii	20	18	694	571	?	0
C. betulus						
X Self	15	9	209	34	20	8
X b.'Cordata'	20	16	278	161	133	17
X b.'Purpurea'	19	15	264	143	125	0
X caroliniana	18	16	250	129	6	1[d]
X japonica	31	10	431	9	0	--
X orientalis	29	28	403	336	58	10
X tschonoskii	32	27	445	286	141	38
C. orientalis						
X Self	11	9	98	23	1	1
X betulus	29	29	258	249	24	18
X japonica	18	18	160	104	0	--
X tschonoskii	12	12	107	102	4	4

a/ Female parent listed first.
b/ Based on average number of bracts per catkin pollinated.
c/ Estimated from cting tests.
d/ Non-hybrid seedlings.