Amélioration génétique dans le groupe Halepensis

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Le pin d'Alep et le pin brutia dans la sylviculture méditerranéenne

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Genetics and breeding in the group halepensis

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ABSTRACT

The two species of the group halepensis, *Pinus halepensis* and *P. brutia* (species complex) are valuable and irreplaceable forest species for the mediterranean area. Their natural forests, as it has been proved from provenance and progeny tests, possess considerable genetic variation, indispensible for the application of an effective breeding programme. In this respect three procedures are being recommended and discussed: establishment of seedling orchards for *Pinus halepensis*, clonal seed orchards for *P. brutia*, and mass production of artificial hybrids between the two species. Hybrids exhibit exceptional and impressive hybrid vigour for growth on a variety of sites.

RESUME

Amélioration génétique dans le groupe *halepensis.*

L'espèce *Pinus halepensis* et *P.brutia* (species complex) appartiennent au groupe *halepensis.* L'aire de répartition des deux espèces est bien distinguée et une isolation génétique, écologique et saisonnière a été observée parmi les individus du groupe.

La grande superficie qui est occupée par les deux espèces en forme de forêt dans la région méditerranéenne, ainsi que leur aptitude à s'adapter aux conditions de la sècheresse de cette région, nous permet de considérer ces espèces comme très intéressantes au niveau du produit protection, et récréation. Le pin d'Alep produit la plus grande quantité et la plus haute qualité de résine par rapport aux autres espèces forestières.

La réalisation d'un programme d'amélioration génétique basé sur la variabilité génétique bien déterminée dans le peuplement des deux espèces, est urgente et nécessaire.

L'installation des vergers à graines de semis pour le pin d'Alep et des clones pour le pin brutia est proposée d'après les données expérimentales de notre laboratoire. La sélection de pin d'Alep serait basée sur l'augmentation de la production de résine et celle de brutia sur l'amélioration quantitative et qualitative du bois. Une méthodologie particulière a été également proposée, concernant la sélection des individus convenables à la création des plantations, ou encore de formation des forêts pâturées.

Pour les régions situées hors de l'aire de répartition des espèces, on propose des hybrides artificiels. Enfin plusieurs méthodes efficaces et économiques sont présentées pour la production massive d'hybrides.

INTRODUCTION

The group halepensis, comprises two major species, *Pinus halepensis* and *P. brutia* (species complex). They are distinguished from each other by a number of morphological, anatomical and chemical characters (Panetsos 1981, Nahal 1984).

In their natural range, there is well-defined spatial isolation (Panetsos 1981, Nahal 1984), the shortest distance between the natural populations of the mountain Athos (Chalkidiki) and the island Thassos in the North Aegean sea being about 50 km. Isolated occurrences of one species inside the range of the other, have been attributed to human interferences (Panetsos 1981).

The two species, besides spatial isolation, have developed other kinds of barriers, such as prevention of fertilization when *P. halepensis* is the female partner, reduced embryo viability in hybrid seeds, low temperature tolerance, as well as differences in blooming periods. They should be considered, therefore, as two well established pine species.

In many cases, however, when they come in contact they hybridize. The extent of the hybridization and the fate of their hybrids depends upon the prevailing ecological conditions in the area (Vidacovic and Karstinic 1974, Panetsos 1975).

The distribution of the two species in the Mediterranean basin and their ability to grow in the most adverse climatic and soil conditions makes them very important for multiple purpose forestry. Moreover, on favourable sites they exhibit growth potentialities, competing successfully with the so-called fast-growing species (Matziris 1982). In the case of *P. halepensis*, and to some extent of *P. brutia* their closed cone habit makes them irreplaceable forest species in the special and delicate mediterranean ecosystem. Thererefore, not only is the initiation of a breeding programme in the section justifiable, but also an urgent necessity, in view of the fact that valuable genetic material is being lost each year due to different reasons.

VARIATION

In natural forests

A selective breeding programme, in order to be effective, should be based on existing genetic variation in the species under consideration. This variation can stem from three different sources:

a. (i) Geographic variability, which is related to the size of the species range, (ii) environmental diversity encountered within its distribution, and also (iii) the extent of the range discontinuities. This variability can be studied and determined by provenance tests. In species with these qualifications, genetic differences related to place of origin have often been found to be several times as great as those among individual trees in the same provenance.

b. Variation among stands in the same provenance. This can be attributed to edaphic factors, to elevational range, and also to selective pressures imposed on some stands by human interference, i.e. by deliberate heavy exploitation over a long period.

c. Finally, genetic variability exists between trees in the same stand, due to the predominating reproductive system in forest species, so that no two trees are of the same genotype.

In the two species of the section *halepensis*, there exist all the presumptions promoting the development of genetic variation of the tree sources mentioned above; extensive discontinuous distribution, accompanied by long-time isolation, due mainly to geological history of the entire region, and to some extent to the gradual changes of climate. Most of their forest, being along the coast of the Mediterranean Sea and around the centres of old civilizations, have been subjected to destructive exploitation with dramatic changes of their gene pool and frequencies. Inaccessible stands, however, as for example those inside the Gorge of Samaria on the island of Crete, present excellent growth and comprise trees with straight stem form and fine branches (Panetsos 1981). The same is true for stands in the remote region around Taurus (Turkey), as reported by Arbez (1974).

It is interesting to mention the altitudinal variation of *Pinus brutia* related to crown form. Narrow crowned trees were found in the transitional zone of this species to *Pinus nigra* on the islands of Thassos and Lesvos, as well as in the Gorge of Samaria (Crete) at high elevation (300-1000 m). This is also noted by Arbez (1974), with reference to the population of Eskibag (Turkey - elevation 950 m).

Apart from this variation, on the island of Lesvos and in the S.E. part of it, a form of *Pinus brutia* which has not been found in other forests of that species complex occurs naturally. This form develops a spherical crown, without a main leader, dense branches, small cones, seeds and needles (6-7 cm), while the stem is ramified just above the ground. This form is mixed with typical trees in various frequencies, ranging from 3-35%. It seems that in this particular area natural selection favours the establishment and expansion of the new form, as can be inferred by comparing its percentage in old stands and their progenies.

On the island Lesvos the high occurrence of trees with witches brooms is conspicuous. Seeds collected from one of them raised both normal and dwarf plants, like the ones obtained from a broom growing on an Aleppo pine tree (Panetsos 1981).

In *Pinus halepensis* natural forest, phenotypic variation is very common too, at all levels. The forests of Chalkidiki and north Euboia are distinguished from the rest of the species for their growth and form. As in *P. brutia*, narrow crowned trees can also be found, not necessarily related to elevation. In Kassandra (Chalkidiki) a stand was spotted with high frequency of narrow crowned trees, which exhibited superiority in growth compared to trees of normal form in the same stand.

Provenance and progeny tests

Besides phenotypic variations already described, provenance testing with a large number of seed lots, covering almost all the range of *P. halepensis* and a part of *P. brutia* species complex, verifies the occurrence of genetic variation of the same nature (Palmerg 1975, Bellefontaine and Raggabi 1977, Panetsos 1981). Especially in our experiments established on various sites all over Greece, significant differences were found between provenances for height, DBH, stem form, bark type, duration of juvenility, precocity and prolificity in flowering and fruiting, needle characters etc. Furthermore, progeny tests conducted with open pollinated families from a number of greek provenances revealed the existence of significant differences among families within populations for growth rate, stem form, bark type and gum production in the case of *P. halepensis*. From the total variation concerning growth rate, the largest portion (2/3) accounts for between provenances and 1/3 for families.

It is interesting and significant for breeding programmes to mention that at least for*P. halepensis* no intereaction of *genotype* x *environment* was found, regardless of site quality and climate.

From the data obtained, four races of *P. halepensis* could be distinguished: (i) East European, (ii) West European, (iii) North African, and (iv) Moroccan.

As far as *P. brutia* is concerned, on the basis of data obtained from provenance tests, it was concluded that populations of *P. brutia* on the island of Crete should be considered as an independent and distinct variety of that species. (Panetsos 1981).

Crossing experiments conducted by our Laboratory for several years have conclusively shown the absence of genetic isolation among populations of *P. brutia* or between species of its complex. It was suggested that all species of *P. brutia* complex should be reduced to the rank of subspecies or varieties: the same has been proposed by Nahal (1962, 1984), Debazac and Tomassone (1965).

Recent studies of isoenzyme variation concerning several populations of the two species, showed that in *P. halepensis* genetically distinct groups can be recognised. These findings coincide with the results obtained in our provenance test, with the exception of the Moroccan group, which probably was not included in these studies. In the same study it was found that *P. brutia*, as a group, differs from Aleppo pine, while genetic distances among the species of *P. brutia* complex are very small and should be considered as subspecies or varieties (Grunwold C., G. Schiller and M.T. Conkle 1984 report).

Preliminary results on the effect of air pollution, and especially on concentration of sulphur dioxide (Schiller 1984), showed that *P. brutia* is sensitive to low concentration (0.060-0.120 ppm), whereas Aleppo pine displays foliage injuries only at the highest level (0.300-0.400 ppm SO_2).

IMPROVEMENT PROGRAMME

General aspects

An improvement programme should consider strategies and purposes. The data available on the genetic structure and the variation of the two species, for a number of characters, offer an orientation for the application of a programme. However, the most difficult task is to specify the objectives which should be accomplished.

For Aleppo pine the main aim should be combined improvement of wood and gum yield, since it is the only species in the mediterranean region capable of producing high quantities of resin. The target must be both quantity and quality. There is already available data indicating the existence of variation for wood and resin quality in the species.

Trees with narrow crown form can be subjected to special breeding for the development of cultivars, suitable for plantation in areas where we pursue a combination of pasture and wood production. In addition, various forms, encountered in natural forests, are valuable as ornamental trees for the mediterranean region and should be selected, evaluated and propagated. In this connection, resistance to air pollution is also a serious aspect for consideration.

As far as *P. brutia* is concerned, the effort for genetic improvement should be concentrated on wood quantity and quality, because resin production is restricted, compared to the yields obtained from Aleppo pine (Papamichael 1970). Having in mind, however, its remarkable plasticity and the fact that it grows on some of the most attractive islands in the Mediterranean Sea, the aspect of recreation and amenity forestry should not be neglected. Furthermore, forms like the ones which were found to grow naturally on Lesvos, could be propagated as ornamental plants for gardens and even for growing in pots.

Concluding, it should not be forgotten that both species are capable of producing fertile hybrids, naturally and artificially.

Breeding methods

There is no best procedure for all situations, the choice depends upon the species concerned and the expected gain of the characters under consideration.

In *P. halepensis*, if the main purpose of improvement is adopted, i.e. combined selection for wood and resin production, the establishment of seedling seed orchards with half-sib families, could be the best procedure for a number of reasons: (i) it is easy to perform at moderate cost, (ii) the species flowers early after planting and produces large seed crops for regeneration, (iii) progeny tests will allow selection of the best families, combining growth and resin yield, at the time the orchard will be ready to produce seeds. In Slash pine positive correlation between volume and resin production has been found (Squillace 1965). In our provenance progeny test, the same correlation is under investigation and also the variation of gum production in relation to provenance and families.

The seed will be collected from plus trees of the best provenances, and will be sown in a replicated nursery test. Then all families will be outplanted in a replicated permanent plantation on sites favourable to the species. At the same time, all plus trees will be transferred, by grafting, to a gene bank. After 5-6 years the orchard will be thinned to leave only the best trees (for growth rate and gum yield) from each of the best families. These trees will be the seed's source for afforestation. It is anticipated that as many seed orchards as the number of races determined in our provenance tests should be established (Panetsos 1981).

The follow-up will be the establishment of a second generation orchard with full-sib families, originating by crossing the best phenotypes selected in the previous step and also the best genotypes of the parental trees growing in the gene bank. This second generation orchard, which will be in full production about 15 years after the initiation of the programme, will be thinned to leave the best trees from each superior full-sib family. In the meantime, seeds for afforestation will be suplied by the first generation orchard.

In both cycles, outstanding trees from the seed orchards and the gene bank should be mass-propagated by vegetative means. This particular aspect calls for research on methods of mass vegetative propagation of both species, which will greatly accelerate genetic improvement. Methodology applied by Chaperon, Alazard and Arunet (198) to *Pinus pinaster*, might be suitable for the two low elevation mediterranean species. With respect to breeding for narrow crowned forms, it is anticipated that clonal seed orchard, accompanied by full-sib progeny test, is the most effective and promising procedure.

In *Pinus brutia*, as reported in a previous publication (Panetsos 1981), the best improvement procedure would be the establishment of clonal seed orchards. In view of the extensive and variable range of the species, and the restriction of data concerning its genetic structure, intensive selection of plus trees, from the best provenances of each physiogeographic division of the species is recommended, and establishment of corresponding orchards, accompanied by full-sib progeny tests. Narrow crowned trees can be specially selected for breeding cultivars suitable for agroforestry. The same procedure as for *P. halepensis* should be followed.

Grafting of Aleppo and Brutian pines

Experiments conducted by our laboratory and others (Iktueren 1974, Schiller 1984), have shown that there is no restrictions to vegetative propagation by grafting of the two species. In our experiments it was found that "cleft grafting" is the most efficient method, with success of almost 80%. It is worth mentioning that grafting scions of *Aleppo pine* on *P. brutia* rootstock was as successful as was grafting on rootstocks of the same species.

Hybridization

Hybridization in forestry breeding work usually refers to the crossing of different species (interspecific), or different subdivisions within a species (intraspecific). In agriculture, hybridization between different strains of the same species is a tool constantly used by the agricultural crop plant breeders. In forestry this type of crossing is not common, but as more becomes known from geographic variation experiments, intraspecific hybridization is likely to become an important way of producing new trees having special combinations of desirable traits.

In hybridization work between forest species, the main object is to obtain hybrid vigor, i.e. production of F1 generation, growing faster than the parents. Another goal of this breeding method is the production of hybrids combining desirable traits of the parental species.

Large-scale use of F1 hybrids must fulfill either one or both of the above mentioned requirements, and must also be capable of being mass-produced. Furthermore, possibilities for practical use of the hybrids should exist, which means available habitats for their successive adaptation and growth. Usually, hybrids manifest hybrid vigor in hybrid habitats, i.e. in intermediate or disturbed environments, because, as has been postulated, the pure species are usually well adapted to the environments in wich they evolve (Anderson E. 1953).

Pinus brutia and *P. halepensis* have been subjected to long-time studies, with respect to artificial and natural hybridization. As pointed out, they cross readily but with one restriction: *P. brutia* should always be the female parent (Moulopoulos and Bassiotis 1961, Moulalis, Bassiotis and Metsopoulos 1976). Extensive studies of natural and artificial populations showed that the two species, when they come in contact, hybridize (Panetsos 1975): this same paper discusses extensively the possibilities and limitations of their hybridization, and particularly the available areas where the hybrids can be better adapted and grown.

In artificial hybridization experiments, it was found that the production of F1 interspecific hybrids can be obtained quite easily by conventional crossing methods; also that seed set was comparable to the pure species, but percentage of sound seed obtained was about 25%, whereas in pure species or intraspecific crosses it was about 75-95% (Panetsos 1981). Fertility improved considerably in subsequent generations (F2 and BC's : especially in F2, sound seed percentage was 61-70%, not much lower than pure species. The only exception occurred when *P. halepensis* was used as female parent in crossings with F1. In this particular case the percentage of sound seed was about 24%, high enough to raise a large number of offspring.

The percentage of sound seed obtained from crossing experiments should be considered as acceptable for mass-production of hybrids, if, of course, they meet the requirements previously mentioned in this chapter.

Recent evaluation of F1 hybrids and other combinations (F2 and BC's) 15 years after planting in four different sites in north Greece, showed that F1 hybrids possess hybrid vigor in all habitats planted (Panetsos *et al* 1983). Their superiority in growth over the pure species varied from 5-190%, related to planting site, and to particular trees of the parental species which were crossed to produce the hybrids. Hybrid vigor was manifested in intermediate and disturbed habitats, or outside the natural distribution of the pure species, but always within their ecological range, F1 proved to be more resistant to minimum temperatures than *P. halepensis*, and it appears that *P. brutia* behaves as dominant in this particular character. F2 and BC's were always inferior in growth, compared to F1 hybrids, at least in the environments in which testing was performed.

These results, concerning vigor and adaptation in connection with the areas available for plantation in Greece and elsewhere in the Mediterranean region, shows that F1 hybrids could be put into direct practical use. The main concern however, is the production of hybrid seed in large quantities at reasonable cost. In a previous paper (Panetsos 1981) different schemes were discussed for mass-production of open pollinated F1 hybrid seed. New findings, however, show that the most important factor for success is not only the production of seed by crossing selected plus trees, but the crossing of parents exhibiting special combining ability.

The fulfilment of this requirement can be achieved in different ways. One approach could be the establishment of several biclonal seed orchards, a method applied by Hyun (1976) for mass production of Pitch pine x Loblolly pine hybrids.

Another approach which might also be considered, is the establishment of a multiclonal plantation of *P. brutia*, by keeping each clone in separate block. The same applies to selected and grafted plus trees of *P. halepensis*; both orchards being isolated by distance from each other and trees of the same species. When *P. brutia* starts flowering, male flowers will be removed by hand, while females will be pollinated by using a pollen-dispersing device. In this way all seeds will be of hybrid origin, and each clone will be pollinated with pollen of desirable clone (s) of the other species.

Information of specific combining ability can be obtained by raising full-sib families from controlled pollination of each selected *P. brutia* tree with several males. It is anticipated that if crossing is performed at the time of plus tree selection, enough data will be available by the time the orchard starts flowering.

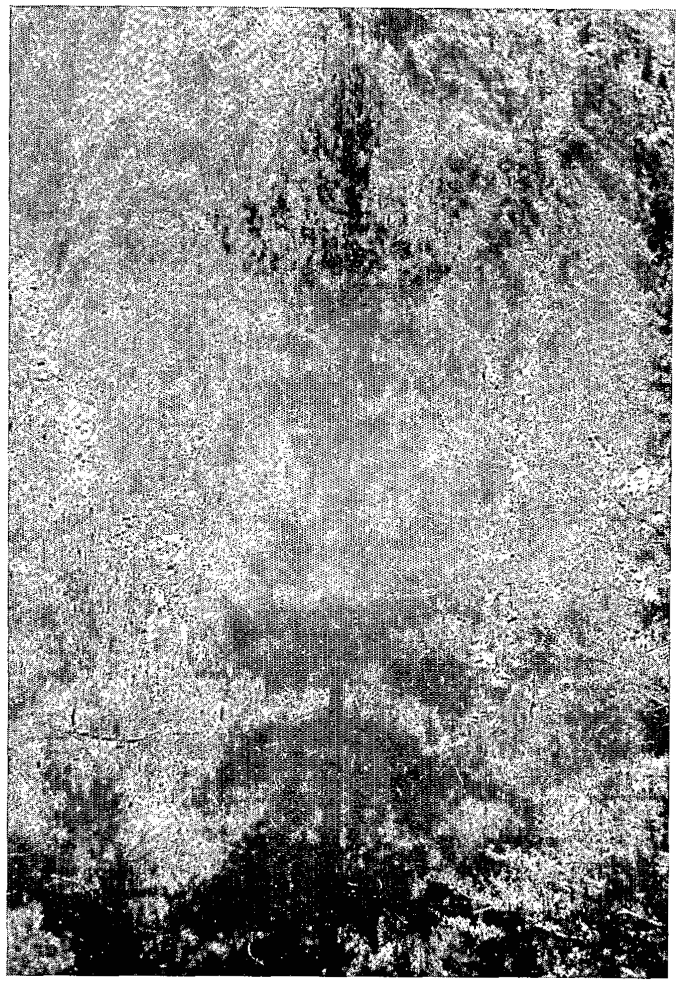

Figure 1. Narrow crowned P. brutia tree in the gorge of Samaria (Crete).

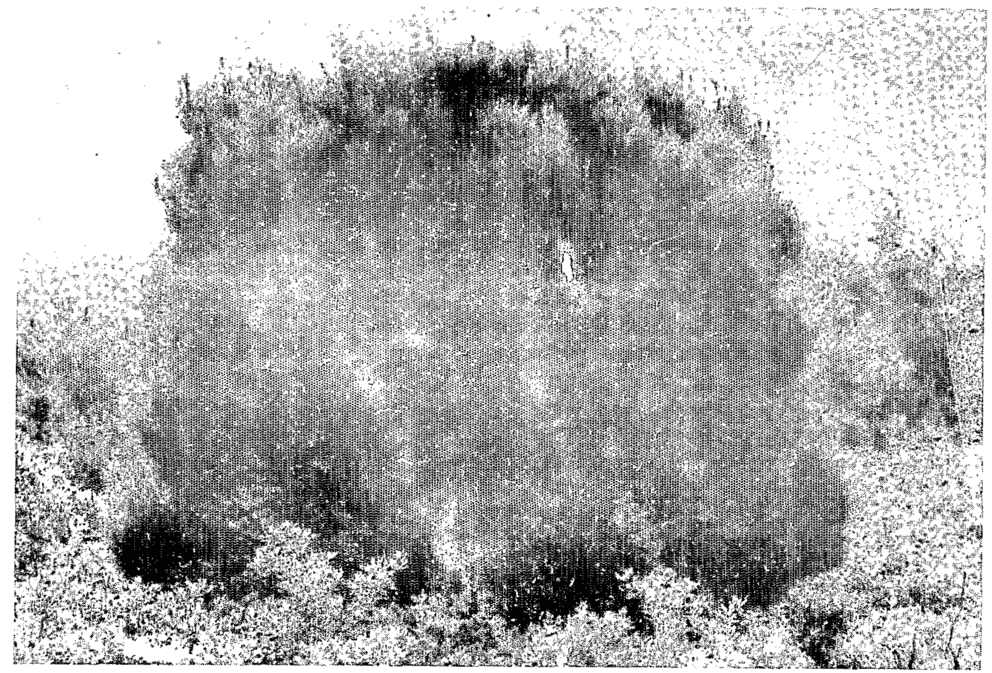

Figure 2. Form of P. brutia growing naturally in the island of Lesvos.

Figure 3. P. brutia tree, with a well developed witches broom.

Figure 4. Narrow crowned P. halepensis tree.

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