HEDESELSKABET

Danish Land Development Service

P.O.Box 110
DK-8800 Viborg
Denmark

Telephone
internat. 45 6 62 61 11
national 06 62 61 11
Telex 66228 danla dk

A As

DRAFT

A TREE IMPROVEMENT PLAN FOR PINUS PINASTER IN PORTUGAL

February 1988

This breeding plan has been worked out at the Estacao Florestal Nacional, INUA, with the Danish Land Development Service as consulting agency, under Programa de Investigacao Agraria, Numero 9620/86/02/17/04.

The plan has been written by Hans Roulund, Danish Land Development Service and Margarida d'Alpuim, Carolina Varela and Alexandre Aquiar, Estacao Florestal Nacional, INIA, Portugal.

Ib Smith, Forest Officer, has been responsible for the major part of the stand selection.

Messrs. H. Chaperon, AFOCEL, and Ph. Baradat are acknowledged for useful discussions about Pinus pinaster breeding.

CONTENTS Page

CONTENTS (continued) Page

ABSTRACT

With the background in the forestry situation in Portugal a tree breeding strategy is set up. The strategy concludes that Pinus pinaster has an econimic importance, which justifies a long term intensive breeding programme and the main frames of such a programme are lined out. Some general information about breeding strategy is given, too.

A short description of the breeding work up to now visualizes that the breeding activities must be intensified in order to cope with the present and expected demand for plants.

Breeding aims are defined to

- increase of stem straightness
- increase of volume per hectar
- decrease of spiral grain.

A separate programme for increase of resin content is considered. A breeding programme, which is scaled for the demand of plants is worked out and described in details.

The breeding programme ensures that genetic variability is maintained over generations, while improvement in the desired traits is achieved.

Improved seed will be available already from the beginning and larger gains will appear after twelve years.

Estimates of gentic gains for volume are made, which indicate that gains in volume between 14 and 27% may be obtained in this period and even larger gains stem form could be expected.

2. INTRODUCTION

In 1984 a proposal for an intensivation of the tree breeding of the two main species in Portugal - Pinus pinaster and Eucalyptus globulus - was put forward to the Ministry of Agriculture from the Danish Land Development Service. With the pre-membership funds from the EEC it was possible to initiate such an activity.

The proposal was further negotiated in January 1985.

In March 1987 it was finally accepted by all necessary authorities and could be put into action in April 1987.

The importance of the forestry sector in Portugal is rather large.

The export value of forestry was for 1980 20% of Portugal's total export.

It is expected that in future the forestry sector will be of increasing importance to Portugal's economy because an expansion of the afforested area is planned as well as a development of the forest industry. The potential area for further afforestation makes up approx. 2,250,000 ha.

The aims of the tree breeding are:

- To supply to the forestry with a genetic better material,
- increase the production per area unit,
- increase the quality of wood, and thereby
- improve the economy for each individual forest property and the country as such.

The importance of a programme for forest tree improvement is emphasized by Portugal's present comprehensive forest area and by the important investment now taking place in connection with new afforestations.

The objectives of this project is to work out a tree improvement plan for Pinus pinaster, alternatively for Eucalyptus globulus, and to dimension the framework of the tree improvement unit of Estaçao Florestal Nacional (Ministry of Agriculture) to solve the tasks.

A complete tree improvement programme will have to take place in two phases:

1) A planning phase where the aim is to create a breeding strategy and a detailed breeding plan, and to dimension staff and facilities necessary for the plan.

2) An operational phase with an implementation of breeding activities according to the plan.

This project deals solely with the first step.

3. TREE BREEDING STRATEGY

Tree breeding is generally considered a long-term procedure, although, with proper planning, some short-term gain may be achieved. As with all long-term projects, careful planning and implementation are essential since errors will be more difficult to correct in the later stages of the project.

Unfortunately, in the early days of tree breeding, long-term plans were often neglected, and - as experience has shown - long-term gain in these instances will be minimal.

However, since the 1970's many countries have created and implemented breeding strategies. The term "strategy" is generally used to indicate planning at a high level, that is, establishing the main parameters of the project and also including subjects and ideas not necessarily dealing directly with the topic.

A breeding strategy should address four major subjects: Time, breeding organization, the tree population structure and function, and breeding techniques, (Kang 1982). An essential consideration is, of course, which species to breed. In Portugal, at present, from an economical point of view, the most important species for breeding are the Pinus pinaster, the Eucalyptus globulus, and the Quercus suber. From the standpoint of breeding the first two species mentioned are easier to breed due to a much shorter generation turnover.

3.1. Time

From and economic point of view it would seem that the Pinus pinaster would be a good choice for a long-term breeding programme due to the great economic importance of this species in Portugal.

An illustration of a tree breeding model showing the relationship between long-term breeding, short-term activity and supportive research is shown in Figure 1 (Isreal 1987). A long-term breeding model has to be invented for Pinus pinaster.

3.2. The Breeding Organization

Most recent tree-breeding has been carried out by the tree-breeding unit at Estaçao Florestal Nacional. (See paragraph 4, page 17). In addition,

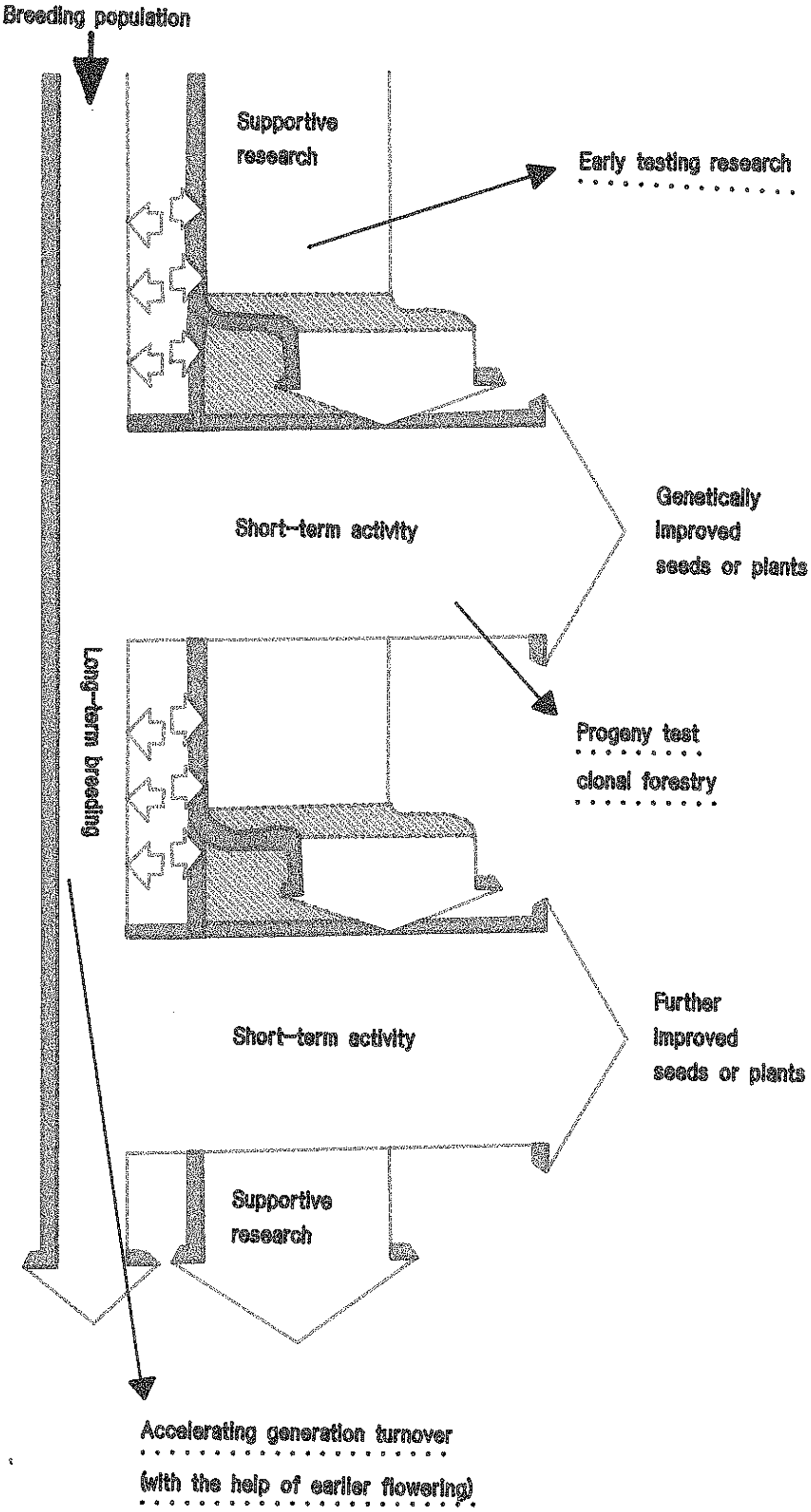


Figure 1. A tree breeding model showing the relationship between long-term breeding, short-term activity, and supportive research. From Israel, B.J., 1987: Early testing in forest tree species: a review. - Forest Tree Improvement 20: 45-78.

EFN 987A

Legend : The place of early testing is indicated by .....

some technical investigations are carried out at Instituto Superior de Agronomia in Lisbon and at Instituto Universitario de Tras-Os-Montes e Alto Douro. The Forest Service has so far made land available for field trials at five sites plus an area designated as a clonal seed orchard. In addition to this, the nurseries at Marinha Grande and Escaroupim serve in part as nurseries for the tree-breeding unit. It is within the scope of this programme to dimension a unit, which is able to handle two long-term tree-breeding programmes.

3.3. Population Structure and Function

In forest tree breeding, populations are often to be named according to their function rather than to their physical subdivision. Namkoong et al (1966) were probably the first to recognize different population functions. Van Buitenen (1975-76) classified populations into four types:

- breeding populations
- progeny test populations
- seed production populations
- forest stands

Lindgren and Gregorius (1976) considered six classifications. They added the concepts of the founder population and that of the gene conservation population to the four groups already developed by Van Buitenen. Kang (1980) then introduced the concept of "index populations".

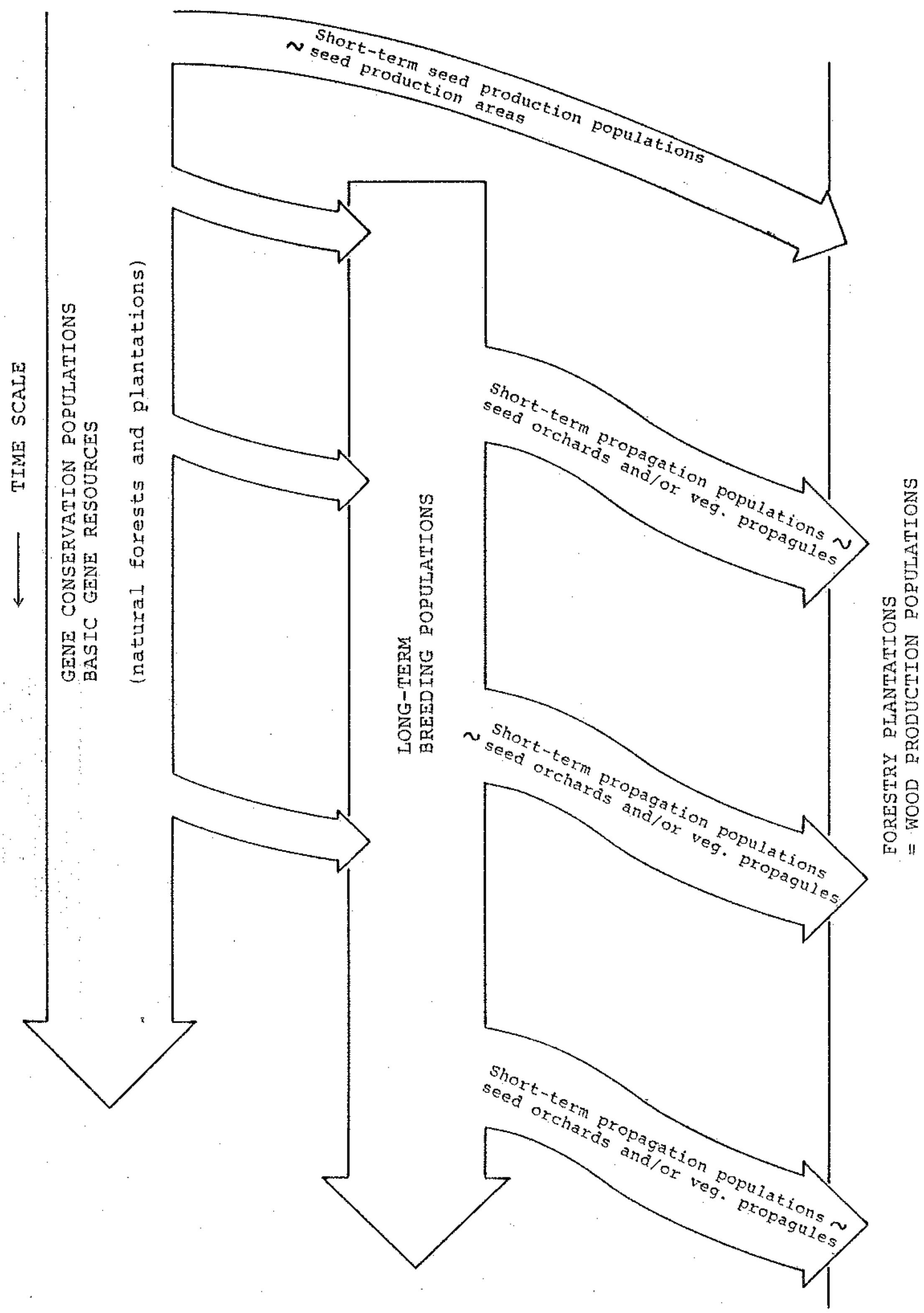
In this breeding plan for Pinus pinaster four terminologies are utilized:

- gene conservation populations
- breeding populations
- seed and plant production populations
- wood production populations

Wellendorf and Kaosa-ard (1987) give an instructive illustration of the relationship between these populations. (See Figure 2).

In most cases the four types of populations mentioned above will be found in separate stands or plantations. However, in some cases one physically isolated or separated stand may serve various functions.

Fig. 2. Forest tree populations in the tree breeding programme
(From Wellendorf and Kaosa-ard, 1987. Stencilled)



3.3.1. The gene conservation populations

Gene conservation populations are to maintain genetic variation as breeding continues. If relatively few highly bred and specific genotypes are utilized, then there is the danger of loosing genes important for adaptation and resistence to unforeseen and unknown diseases. In order to be able to go back and select and intercross such genotypes with improved ones at a later stage of the breeding procedure, it may be wise to reserve some areas for gene conservation purposes.

One might argue that Pinus pinaster has grown, and will continue to grow through natural regeneration in small farm forests, and that these stands might sufficiently serve as gene resource pools. Furthermore, Pinus pinaster is cultivated over large areas of France, Spain, and Italy, so these stands could conceivably serve as gene resources as well.

However, it will have little economic influence on wood production as such if some areas might be taken out of normal silvicultural practice and left over as areas for the purpose of gene conservation.

It is, therefore, suggested that 10-20 ha in each forest district (circumscriçao) should be registered as gene conservation area and treated as such.

3.3.2. Breeding populations

The breeding populations must have a certain amount of genetic variation to allow for change in breeding characteristics. They should also, by selection and crossing, increase their desirable traits in each generation. However, selection and crossing in a closed population system will sooner or later lead to inbreeding, resulting in inbreeding depression.

To postpone this situation the breeding population should be divided into a number of sub-populations or breeding lines. Selection and crossing should then take place within these lines in the first generations. When inbreeding becomes a problem, crossing between sub-populations will postpone it further. Incrossing of improved material from other countries may be another method to delay or prevent inbreeding.

The breeding populations will in this programme physically be in the form of clone banks and progeny tests. Seed orchards may sometimes serve this purpose also.

- Any succeeding generation, except the first two, should derive from controlled crossings.
- The mating design should be "single pair mating" or some sort of "disconnected factorial".

- Each progeny test should be replicated in at least four sites.

For details, see paragraph 9.

3.3.3. Seed and plant production populations

Seed and plant production populations should meet the national demand for seed and plants. (See paragraph 6). As soon as even a small improvement is realized, it should be made available to the forests through a seed production population. Larger gains may only be noticed after a number of years, but nonetheless utilization of smaller gains should not be excluded for that reason.

This plan will deal with the following types of seed and plant production populations:

- selected seed stands
- tested seed stands
- clonal seed orchards
- seedling seed orchards

If the technique develops further, propagation orchards for clonal propagation might also be considered.

The current practice in Portugal so far is not to register seed origin nor to keep seed lots separated.

Under this project an inventory and survey of the forest districts have been carried out. That has led to a selection of 53 seed stands. A proposal for regulations for seed collection and distribution and certification of seed has been worked out as well. (See paragraph 5 and appendices I, II & III).

The types of seed-orchards and progeny tests are described in paragraph 9 and appendix IV-VIII in more detail.

3.3.4. The wood production populations

The wood production populations are the forest stands. It is not the purpose of this project to give advice on silvicultural methods. It should, however, be stressed that a good product can only be obtained by using genetically superior plants and by astute silvicultural management. An efficient flow of information from the tree-breeding unit of the INIA to the Forest Service and private forest owners and vice versa is of prime importance in obtaining good results.

3.4. Breeding Techniques and Subjects Influencing the Breeding Process

The following breeding techniques and subjects influencing the breeding process will be considered:

- selection methods
- mating designs
- inbreeding
- juvenile-mature correlation
- genotype-site interaction
- breeding zones and seed zones
- breeding population size and structure

3.4.1. Selection methods

In this breeding plan various selection methods should be used. For selection of seed stands phenotypical selection for health, growth, stem form and branching habits should be used. A further consideration would be that stands should be selected from the entire area of cultivation and representing all altitudes. No specific measurements or scores would be taken.

First generation plus tree selection has so far been phenotypical selection with records of height, diameter, stem-form, branching habits, wood density and spiral grain. The same is recommended for the future. (See paragraph 8).

Selection of second and further generations of plus trees will be combined family and individual selection based on measurements of field trials. For the characteristics in question "independent culling level" or "index selection" will be considered.

3.4.2. Mating design

The mating design should ensure the production of unrelated famillies for coming generations and selection for general and specific combining abilities. Mating designs should be kept relatively simple. Mating designs which to some extent meet these demands are "single pair mating" and "disconnected factorials". (More details are given in paragraph 9).

3.4.3. Inbreeding

Inbreeding must be avoided in the "seed and plant production populations", i.e. seed stands and seed orchards. In the "long-term breeding populations" inbreeding is postponed by sublining. Some inbreeding may be allowed in the breeding population if the genotypes are outcrossing in the "seed and plant production population". If reciprocal recurrent selection is used, inbreeding can even be an advantage (Lindgren and Gregorius, 1976).

3.4.4. Juvenile-mature correlation

Juvenile-mature correlation is decisive for the generation turn-over. Progeny tests should be established and managed in a way which increases juvenile-mature correlation. A good method for calculating the optimal age for selecting in progeny tests is described by Lambett (1980). Maternal effects on height in Pinus pinaster up to an age of 9 years have been registered in France. (pers. com. Phillippe Baradat). Due to lack of experimental data, the time for selection has been arbitrarily set at 10 years from germination.

3.4.5. Genotype-site interaction

So far no knowledge about genotype-site interaction has been recorded. Pinus pinaster appears to have a great ability to adapt to a wide range of conditions, and, as mentioned earlier, seed is distributed freely over the country. However, the large climatical and edaphical variation in the regions where Pinus pinaster thrive make it likely that genotype-site interaction exists. Therefore, it should be one of the primary steps in the breeding plan to analyse this problem.

3.4.6. Breeding zones and seed zones

As mentioned earlier, no zoning for seed distribution and utilization currently exists. Therefore, as part of the breeding plan, the immediate implementation of two seed zones is recommended, these zones to be delineated according to climatical and edaphical conditions.

When the genotype-site interaction investigation has been carried out, decisions should be made regarding changing or maintaining the seed zones and also as to whether or not breeding zones should be established. (See paragraph 9 and appendix III).

3.4.7. Breeding population. Size and structure

The size of the breeding population should in the first generation be 256 plus trees with accompanying progeny tests. In the following generations it should be reduced to 128 plus trees and the necessary progeny tests. The "plus" trees should then be grafted and maintained as clones in a clone bank. (See paragraph 9 and appendix VI).

4. DESCRIPTION AND STATUS OF THE TREE IMPROVEMENT ACTIVITIES UP TO NOW

Tree breeding activities in Portugal are carried out at Estaçao Florestal Nacional under Instituto Nacional de Investigaçao Agrária (INIA). Some forest genetic research takes place at Instituto Superior de Agronomia, Lisbon, and at Instituto Universitário de Trás-os-Montes e Alto Douro.

Improvement and selection of Eucalyptus globulus has to some extent been handled by private enterprises.

In this context only work on Pinus pinaster will be mentioned.

4.1. Early Work

The most important work about improvement of Pinus pinaster dates from 1953 and was done by the great forest improvement pioneer, Domingos Pereira Machado. However, it was in 1963/64 when the Australian technician D.H. Perry came to the country to select "plus trees" in Mata National de Leiria, (as a result of tests made in Perth with Pinus pinaster from different origins) that the work on improvement of this species began to have a bigger impact in Portugal.

Perry's selection consisted essentially in choosing superior trees which were then grafted in clone banks and seed orchards, not only in Australia (with material sent directly for that purpose, by this technician), but also in Portugal, Perry (1967), Alpuim (1971), Machado and Pessoa (1972).

The selected trees were progeny tested in Australia. In these tests some progenies showed a superiority of 20% in volume and 40% in straightness of the bole (Butcher, 1977).

4.2. The Recent Breeding Plan

In the following the recent breeding plan is shortly described.

4.2.1. Breeding characteristics

The following characters have been considered important to improve:

- height

- volume
- stem form
- wood density
- absence of spiral grain
- branch characteristics.

4.2.2. Plus-tree selection

It was early realized that selection of more plus trees in different areas of Pinus pinaster was needed, (Alpium 1971, Machado 1972). Finally in 1987 it was possible to begin the selection again, thanks to the collaboration of D.G. Florestas.

Further 29 plus trees have been selected in the regions of Sao Pedro do Sul, Aveiro, Marinha Grande, Bussaco and Figueira da Foz. Together with the 86 plus trees selected in Leiria by Perry it makes a total of 115. Some of the earlier selected trees do not exist any more, but most of them are represented in the clone bank at Foja and in the seed-orchard at Escaroupim.

4.2.3. Progeny test

In 1970 progeny test from open pollination of the plus trees were started. Nursery results are published (Almeida 1978), but due to various reasons field trials were not carried out.

In 1986 nursery tests were established with 46 open pollinated progenies from the clonal seed-orchard in Escaroupim. The plants were raised in the Forest Service nurseries in Marinha Grande and Mata Nacional de Escaroupim.

In 1987 field trials with these plants were established at 5 sites, 3 in Mata Nacional de Leiria (deptm. 24, 152, 166) and 2 in Mata Nacional de Escaroupim (deptm. 5).

4.2.4. Clonal seed-orchard

The intention with the plus-tree selection was to transfer genetic superiority to plants used for reforestation. One way to do that is by means of a clonal seed-orchard. The work was started in 1970. It was intended to make a progeny test of all the plus-trees, do genetic thinnings in the seed-orchard according to the test, and to establish a new clonal seed-orchard with the best clones. Unfortunately this work was stopped in 1979 due to lack of funds and technical equipment. The first seed-orchard, 4 ha, is going to be thinned now. Due to lack of information from the progeny tests as mentioned above, thinning is carried out partly after phenotypical and phenological evaluation, partly taking equal spacing in consideration.

The two following pages give the data sheets of the two clonal seed-orchards.

4.2.5. Seedling seed-orchard

In 1980 a seedling seed-orchard with 50 half-sib families were established in Mata Nacional de Escaroupim. Unfortunately harsh climatical conditions caused high mortality, so the seed-orchards had to be given up.

Since then, there has been no conditions for a reestablishment.

4.2.6. Clone banks

Between 1967 and 1968 a clone bank in Mata Nacional de Foja was established. The clone bank contents clones selected from Leiria. Two previously established clone banks in Mata de Leiria had to be abandonned due to destruction of labels.

At the moment establishment of a new clone bank in an area of Circunscriçao Florestal de Coimbra, Viveiro da Mealhada, is planned. This should be easier to maintain than the first one.

1. *Identification* *No.* 1

Species Pinheiro bravo Pinus pinaster
(common name) (latin name)

Responsible institute ESTAÇAO FLORESTAL NACIONAL

Location Mata Nacional de Escaroupim

Latitude 39° North *Longitude* 8° West (W)

Size 4 ha *Map ref.1/25,000* 377

2. *Objectives* Production of seed for afforestation of public forests at the coastal plain, for production of wood for sawn timber.

Selection criteria Volume, height, stem form, spiral grain and branch habits.

Recommended zones for the seed Matas Nacionais do Litoral north to parallel 39.

Estimated maximum seed production 10-15 kg/ha/year

Average production of 10 years 10 kg/ha/year (average value)

3. *Composition of clones*	*propagation method*	*year of propagation*	*initial spacing*
60	grafting	1970 - 1975	4mx4m

Isolation belt of Eucalyptus globulus and Pinus pinea

4. *Base material*
Provenance Leiria *Origin*

Latitude 39.5°-40°N *Longitude* 8°-9°W *Altitude* 10-100 m

General characteristics of the base material Managed stands for ordinary rotation forestry, more than 60 years. The thinning has always been well programmed and executed.

SEED-ORCHARD REGISTER

1. *Identification* *No.* 2

Species Pinheiro bravo Pinus pinaster
(common name) (latin name)

Responsible institute ESTAÇAO FLORESTAL NACIONAL

Location *Mata Nacional de Escaroupim*

Latitude 39° North *Longitude* 8° West (W)

Size 3 ha *Map ref.1/25,000* 377

2. *Objectives* Production of seed for afforestation of public forests at the coastal plain, for production of wood for sawn timber.

Selection criteria Volume, height, stem form, spiral grain and branch habits.

Recommended zones for the seed Matas Nacionais do Litoral north to parallel 39.

Estimated maximum seed production 10-15 kg/ha/year

Average production of 10 years 10 kg/ha/year (average value)

3.

Composition of clones	*propagation method*	*year of propagation*	*initial spacing*
49	grafting	1975 - 1980	4mx4m

Isolation belt of Eucalyptus globulus and Pinus pinea

4. *Base material*

Provenance Leiria *Origin*

Latitude 39.5°-40°N *Longitude* 8°-9°W *Altitude* 10-100 m

General characteristics of the base material Managed stands for ordinary rotation forestry, more than 60 years. The thinning has always been well programmed and executed.

5. REGISTRATION OF WELL DEFINED VALUABLE OCCURENCES OF PINUS PINASTER

5.1. Natural Distribution

The natural distribution of Pinus pinaster is according to Chritchfield and Little (1966) scattered in a number of occurences.

From the northern part of Marocco to Bordeaux in France, and from Portugal through Spain to Corsica and north western part of Italy. (see map, figure 3).

It is, however, doubtful whether Pinus pinaster is indigenous or it has been planted in early days (Pinto 1939, Morais 1940, Percy and Hopkins 1967). Nevertheless, Pinaster has been grown for so long in Portugal that the Portugueese populations may be genetically different from occurences in France, Spain and Italy.

5.2. Genetic Variation Within Portugal

It is difficult to say whether there are genetic differences between stands or not.

For the past 50 years seed from coastal regions has been sown over large areas in the mountains. Seed is in general harvested on felled trees either after clearcutting or thinning. The origin is not recorded and seed of several origins may be mixed.

It is, however, likely that some natural selection has taken place already in the first generation in the mountains. Therefore the zonations and the provenance test.

5.3. Registration Method

In order to be able to map possible genetic differences between stands, it was decided to select a number of good stands in each region of importance to Pinus Pinaster.

Each regional forest officer in the regions of Porto, Vila Real, Coimbra, Marinha Grande and Viseu was asked to show their best stands according to health, quality and production.

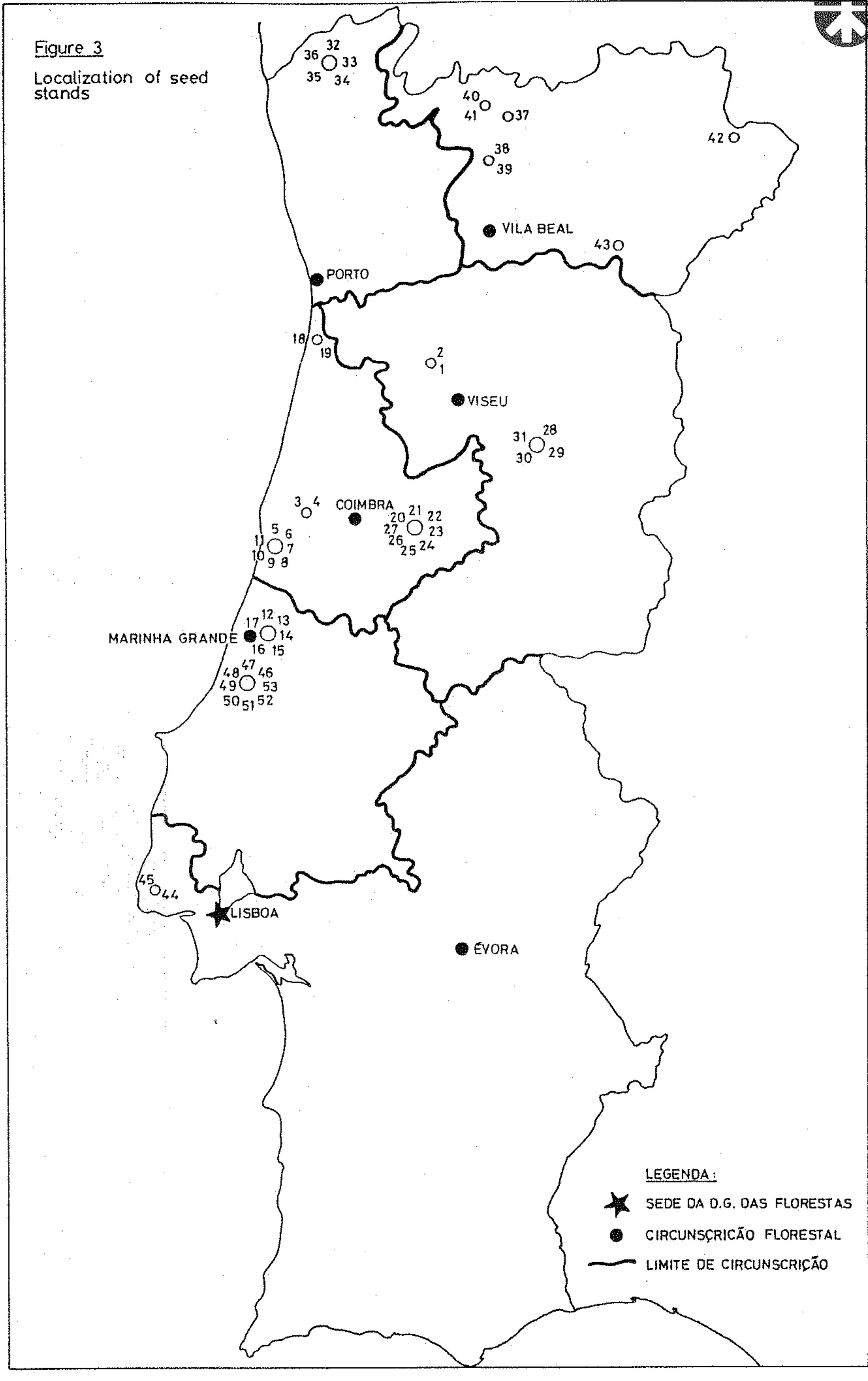


Figure 3
Localization of seed stands
PORTO
VILA BEAL
VISEU
COIMBRA
MARINHA GRANDE
LISBOA
ÉVORA
LEGENDA:
SEDE DA D.G. DAS FLORESTAS
CIRCUNSÇRICÃO FLORESTAL
LIMITE DE CIRCUNSCRIÇÃO

The stands were inspected by the staff of the tree improvement section of INIA and one or two forest officers from the Danish Land Development Service, and the best stands in each region were chosen.

To give an impression of the quality and growth of the stand a photograph was taken, and height and diameter of a dominating tree was measured. A formular was filled for each stand. The formulars are present in appendix II. Below is given a total list of all the stands selected, and their localisation are shown in fig. 4.

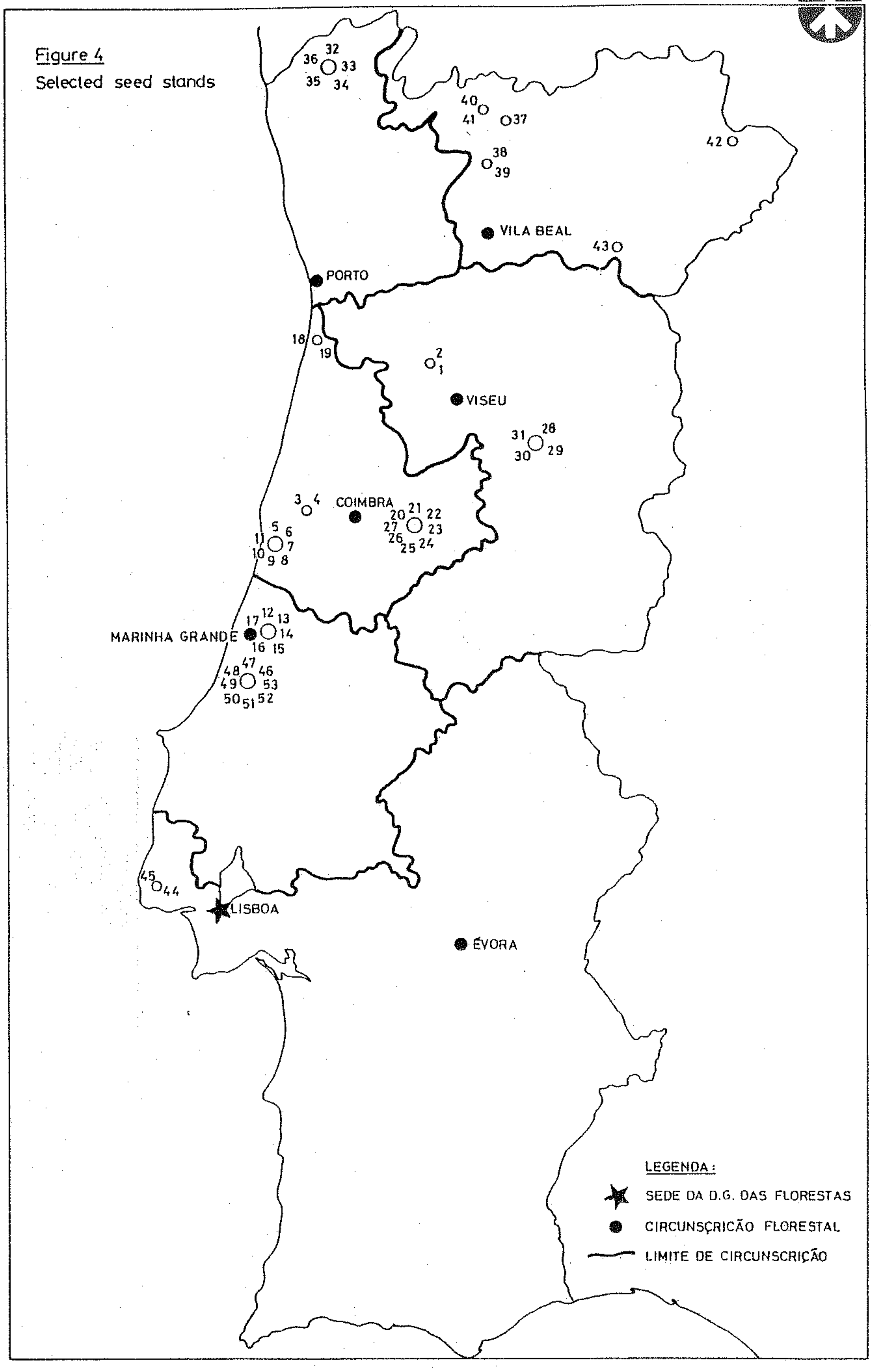


Figure 4
Selected seed stands
PORTO
VILA BEAL
VISEU
COIMBRA
MARINHA GRANDE
LISBOA
ÉVORA
LEGENDA:
SEDE DA D.G. DAS FLORESTAS
CIRCUNSÇRICÃO FLORESTAL
LIMITE DE CIRCUNSCRIÇÃO

Selected Stands

Stand No.	Forest district	Adm. deptm.	Area	Origin
1	Viseu	S. Pedro do Sul	5	
2	-	-	125	
3	Coimbra	Figueira Foz	12	
4	-	-	3	
5	-	-	8	
6	-	-	5	
7	-	-	6	
8	-	-	22	
9	-	-	20	
10	-	-	16	
11	-	-	18	
12	Marinha Grande	Marinha Grande	18	
13	-	-	32	
14	-	-	23	
15	-	-	33	
16	-	-	36	
17	-	-	36	
18	Coimbra	Aveiro	20	
19	-	-	28	
20	-	Lousa	120	
21	-	-	120	
22	-	-	145	
23	-	Arganil	60	
24	-	Lousa	30	
25	-	-		
26	-	-	10	
27	-	-	40	
28	Viseu	Manteigas	120	
29	-	-	863	
30	-	-	135	
31	-	-	53	
32	Porto	Monçao	33	
33	-	-	25	
34	-	-	27	
35	-	-	22	
36	-	-	12	
37	Vila Real	Pedras Salg.	5	
38	-	Mondim Basto	9	
39	-	-	40	
40	-	Montalegre	40	
41	-	Mondim Basto	90	
42	-	Bragança	300	
43	-	Macedo Caval.	30	

Selected Stands (continued)

Stand No.	Forest district	Adm. deptm.	Area	Origin
44	Marinha Grande	Sintra	20	
45	–	–	95	
46	–	Marinha Grande	36	
47	–	–	35	
48	–	–	30	
49	–	–	30	
50	–	–	28	
51	–	–	12	
52	–	–	36	
53	–	–	36	

6. STATEMENT OF ACTUAL AND EXPECTED ANNUAL REQUIREMENTS OF SEED AND PLANTS

The total area of Pinus pinaster in Portugal is about 1,300,000 ha and is planned to be extended with another 100,000 ha during the next 10 years.

(Personal communication, Eng. Lucilio Martins Direçao Geral Florestal)

With an average rotation period of 45 years including losses due to fires etc. the annual area to be planted during the next 10 years will be about 40,000 ha per year at an average.

The average seed crop per ha is 30-50 kg per year.

Usually 2,000 plants are planted per ha..

To obtain 2,000 plants ready for transplanting to the field 4,000 seeds are needed.

1 kg of seed is equal to about 15,000 seeds.

About 0.3 kg of seed is needed per ha.

The annual demand of seed is then 40,000 x 0.3 = 12,000 kg. The area of seed stand should then cover between 240-400 ha.

7. BIOLOGICAL INFORMATION NECESSARY FOR THE BREEDING PLAN

A number of biological factors is decisive for the breeding plan. The characteristics listed below are based partly on experience of the tree breeding section at Estaçao Florestal Nacional, partly by investigations of Varela (1986) and Aguiar (1986).

Flowering:

- The number of years to the first flowering is about 5, but there is a rather large between tree variation. For planning purposes it should be set at 7 years.

- Pinus pinaster usually flowers every year. Good seed years give about four times as much seed as bad ones.

- Flowering occurs between the end of February and the middle of April. There are big clonal differences in flowering.

- The receptivity of the female flowers lasts for one week, and the pollen dispersion has the same duration.

Seed:

- The average number of seed per cone is 130, with a range from 70-180.

- Viability is approximately 85%.

- Survival in the nursery after 5th month is from 35% to 50%.

Growth:

- Rotation age is 60-80 years, but forest fires influence the average living time of a stand to about 45 years.

- Age for evaluation of progeny tests is expected to be 10 years, which in most cases means a height between 6 and 8 m.

8. DEFINITION OF BREEDING AIMS

The breeding aims in a long-term breeding programme must in a biological sense ensure that the adaptation of the bred varieties to the climatical conditions are not lost or, if necessary, improved.

Besides that the recent and future most important technical traits must be defined. If the species in question are injured by insects or fungi resistance breeding may pay better off than breeding for any technical characteristic.

It is possible to breed for a number of characters, but it must be remembered that if the characters are not positively correlated, the gain in one character will be diminished if a new character is subject to selection too.

8.1. Adaptive Characters

In the case of Pinus pinaster in Portugal adaptation seems to be good except from damages caused by snow in elevations above 700 m. These areas are small compared with the total area of Pinus pinaster, and should preferably be cultivated with other species.

8.2. Resistance to Insects and Fungi

Pinus pinaster seems even when grown on poor soil or harsh condition to be free from severe attacks of insects and fungi. It is in a way astonishing since the cultivation method is monoculture over many square kilometers and even aged stands up to sometimes 300 ha or more.

It looks as if we are dealing with a species which is well adapted and, so far, without many aggressive enimies.

It is, therefore, not recommended to do any resistance breeding before a problem shows up.

8.3. Technical Aspects

The use of wood and other products from the trees changes. It is, therefore, important that the overall breeding aim is rather wide, so a change in a specific character is possible in a later stage in the breeding process. Pinus pinaster gives, however, two important raw materials i.e. wood and resin.

From a national economic point of view the wood production is so far most important.

From a social economic point of view the resin is equally important. A large number of farmers and other people living in the countryside have an income from resin tapping. A big resin content lowers the wood quality, both for sawn material, chipboards and pulping. (Personal communication from Eng. A. CARVALHO).

If both wood production and resin production should be favoured, the breeding population should be split up into two separate populations after the first progeny test.

8.3.1. Overall aims

An overall aim for the two populations is suggested as follows:

Population 1: To maximize dry matter production per area unit and improve external and internal quality of the wood.

Population 2: To improve resin production per area unit and maintain a reasonable wood production and quality.

8.3.2. Selection criteria

The overall aims are not operational in the way.

Therefore more specific characters which can be measured must be defined and together fulfill the overall aim. A large number of characters have been dicussed. Wood density, or specific gravity is a character, which influences both the dry matter production and the strength of the wood. It is therefore considered an important character. In a number of conifer species, wood density is negatively correlated with growth. A number of investigations in Pinus pinaster show that in this species wood density is either not correlated or positively correlateed with growth, (Polge and Illy 1967, pers. comm. Henry Chaperon and Philippe Beradat). Therefore wood density is not chosen as a specific breeding character in this breeding plan. The following have been considered the most important:

Plan 1: Stem form
Height (diameter)
Spiral grain

Plan 2: Resin production
Height (diameter)
Stem form

9. THE BREEDING PLAN

9.1. Plan 1. Wood Production

9.1.1. Explanations of the Breeding Diagram

The breeding plan is shown in a diagram (folded in the end of the paragraph).

It is shown as a number of boxes and arrows. Black arrows indicate the direction from one action and unit to the next. Red ones indicate flow of information. Green ones indicate production.

In the following each box in the diagram will be explained using the reference number given at the right hand side of the box. THE RED FIGURES ON THE LEFT HAND SIDE OF THE BOX REFER TO THE CALCULATION OF THE GENETIC GAIN GIVEN IN PARAGRAPH 10.2.

1. Stand selection

In order to get a more organized seed supply including seed of good and known origin, 53 stands were selected. These stands will serve as a seed source until more improved material is available. (See paragraph 5 and appendices II and III).

2. Plus-tree selection - 1st generation

In the old programme 60 plus-trees are already present in a clonal seed orchard and 46 of these are under test. 1988 more plus-trees will be selected. Selection will take place in the seed stands according to the breeding criteria. (See paragraph 8.3.2.). The plus-trees should have a distance of at least 100 m, so that open pollinated progeny will, most likely, be unrelated. Seed will be harvested from the plus-trees, and measures of height, diameter, stem form, branching, and spiral grain will be recorded. The trees will be marked and mapped.

3. Open pollinated stand test

Equal amount of seed from 20 representative trees from each stand will be harvested and bulked. Two seed stands will be nominated as "standards" and large quantities of seed will be harvested from these and stored. This seed will then be used as standards in all successive trials. Field trials for Pinus pinaster will be established at 8 sites covering 2 marginal and 6 typical sites. The aim of the test is to analyse genotype-site interaction and to eventually delineate seed zones, as well as to rank the seed-stands. Proposal for design of field trial is given in appendix IV.

4. Open pollinated progeny test

Open pollinated progeny tests will be carried out for the 196 new plus-trees, the 14 old trees not yet in test, and 5 of those already in test. This will make ranking possible for all the trees. Some difficulties in ranking will occur because the open pollination of the old plus-trees is from the clonal seed orchard and the new from stands. The progeny test will be separated into two parts due to the large number of progenies. Design is shown in appendix V.

5. Evaluation of stand tests

The evaluation should show if there is significant genotype-site interaction at population level. If so, then delineation of breeding zones after the method of Wellendorf et al (1987) should be considered. The breeding population may be kept as one, i.e. without divergent selection criteria, whereas the seed and plant production population must be split up. According to the results of the test the 20% best for each seed zone will be selected as seed-stands.

6. Evaluation of progeny test

The evaluation of the progeny test will indicate genotype-site interaction of open pollinated trees at the family level, estimate family and single tree heritabilities, breeding values of the parent trees, and also rank the families. This information will then be used to select plus trees for the next generation's breeding population, also to select trees with the highest breeding value for the seed and plant production population, and to make genetic thinning in the first generation of seedling seed-orchard and clonal seed-orchard II.

7. Plus tree selection - 2nd generation

To create next generation breeding population the best tree in each family is selected. (See page 40). This is estimated to give a genetic gain of approx. 8-16%.

8. Controlled pollination within lines

The controlled crosses are carried out in a factorial design where each tree takes part in two combinations. This gives possibilities for slight family selection for 3rd generation breeding populations. (See page 41).

9. Establishment of clone bank

To ensure a certain number of plus trees and to make multiplication of a genotype possible as well as to make controlled pollination in larger scale possible, a clone bank will be established. To begin with, a traditional clone bank is planned. The clone bank should contain 300 clones, each of them represented by 6 grafts. Design is shown in appendix VI. The clone bank should at all times contain the number of unrelated clones selected for the breeding population in each generation. The rest of the clones will be the top ranking according to the latest evaluation. After each progeny test, clones will move in and out of the clone bank.

Another type of clone bank with the grafts in 25-50 l containers in connection with a breeding hall should be considered, but this technique has to be developed and tested first.

10. Controlled pollinated progeny test

To test the crossings of 2nd generation breeding population and to make selection for 3rd generation possible, a progeny test is established at four sites. For design see appendix VIII.

11. Evaluation of progeny test

As in point 6. Exceptions will be that genotype-site interaction at full-sib family level and specific combining ability will be estimated. The information from the progeny test will be used in the plant production population to create new combinations of pairs for controlled pollination and bulk propagation.

12. Plus tree selection. 3rd generation

In order to have unrelated trees within each line, the best trees in the two best families of each group of four are selected. There will then be 8 unrelated plus trees in each line.

13. Controlled pollination within lines

In order to bring the progeny tests down to a more convenient size but yet to continue to maintain four unrelated families, a "single pair" mating design is recommended. This will not allow family selection in the breeding population, but only within family selection.

14. Change in clone bank

The new set of unrelated plus-trees will replace the trees with the lowest values in the 2nd generation. Furthermore, an overall ranking of plus trees (clones) regardless of relationship will rearrange the rest of the clone bank.

15. Controlled pollination progeny test

Same as in point 10, except that the design of the field trial should favour family selection.

16. Evaluation of progeny test

As in point 11.

17. Plus tree selection

It is now possible to select four unrelated plus trees per line, only. At this time one may consider allowing some relationship and making crossings between lines.

18. Clonal seed-orchard I

The first clonal seed-orchard was established in 1970 - 1975. (See page 19). Due to the size of the trees, the seed-orchard has to be thinned. Since no data on breeding values are available, no genetic thinning will take place. Average seed yield is 40-60 kg. per year.

19. Clonal seed-orchard II

The second clonal seed-orchard will be finished in 1990. It will consist of 49 of the 60 clones in seed-orchard I. 46 of these clones are now under tests established this year. Genetic thinning will take place in the year 2000.

20. Genetic thinning

The seed-orchard design should allow a genetic thinning down to 10 clones. This would give an approximate gain of 9-15%.

21. Selected seed stands

The selected seed stands will, for a long period, be the most important seed source as far as quantity is concerned. The gain in the beginning will probably not be large, but after the test of the seed stand progenies a reasonable gain is expected.

22. Tested seed stands

When the open pollinated stand test is evaluated, it should then be decided whether there should be one breeding zone or two. If genotype-site interaction is significant, then there should be two breeding zones and the seed and plant production population will be split up into two parts, and their size adjusted to the size of the zones. Selection will be made according to the average results in the two zones. Two stands, which represent the average value, should be selected as standards and be used as such for as long as possible. The rest of the stands should be discarded and new stands included in testing.

23. Seedling seed-orchard I

The seedling seed-orchard I is the fourth output to forestry from the breeding programme. It should be split up into three separate orchards of 20 ha. Each orchard should be well isolated from other stands of Pinus pinaster. The terrain should be reasonably flat and there should be easy access to the seed-orchard. The layout is shown in appendix VIII.

24. Genetic thinning - within family selection

After 6 years the best tree in each plot is selected. This is to ensure full crown development and to avoid inbreeding between half-sibs in the first cone crops.

25. Genetic thinning - family selection

Based on the evaluation of the progeny test (6), the best 20% of the families can be selected. Trees belonging to other families must be thinned away. This will result in unequal distribution of trees over the area, but only in extreeme cases should trees from unselected families be left. The genetic thinning will improve the value of the seed from the orchard.

26. Pair crossings

Based on the evaluation of the progeny-test, the 20 best unrelated trees in the seedling seed-orchard should be selected and pair-crossings carried out.(The clone bank has not reached the flowering stage yet). Therefore a limited number of flowers will be available. One should however try to create as many seeds as possible.

27. Seedling seed-orchard II

Seedling seed-orchard II will recieve only within family selection. The design will therefore be slightly different.

28. Thinning within families

A within family selection will take place, leaving only one tree per family plot.

29. Bulk propagation

27 of the old plus-trees selected by Perry and present in clonal seed-orchard I, are under progeny testing in Western Australia for more than 20 years. Provided results from these tests could be obtained, and genotype x site interaction not is too big, for example with respect to stemform. pair crossings of the best plustrees should be made.

Seed from the pair crossings should be multiplied by vegetative propagation. A method of propagation of Pinus pinaster is developed at AFOCEL, France. This method is beeing adapted to Portuguese conditions and a rolling system of paircrossings and bulk propagation should be practised.

9.2. Plan 2. Resin production

The plan for resin production will follow the same pattern as the plan for wood production.

A test method for resin production has to be developed. This may influence the field designs considerably.

10. ESTIMATION OF GENETIC GAIN

10.1. Genetic parameters

Besides the description of the seed-orchards, clone banks, progeny tests etc., the time scale and selection methods a breeding plan ought to contain estimates of the genetic gain expected in the various steps. Such estimates are often based on experience from similar situations. If information on the necessary parameters is available, it is possible to make a more precise estimate. Using the formular

$$G = i \times h^2 \times \sigma_P$$

where G = genetic gain

h^2 = narrow sence heritability

σ_P = the phenotypic standard deviation

it is possible to calculate an estimate. In this situation the problem is that there are no experiments from which the heritability and phenotypic standard deviation can be calculated or measured. One possibility is to calculate a low and a high estimate based on hypothetic values based on experience from the French breeding work and from heritability estimates in generel.

The following parameters will be used:

Selection intensities according to Becker: Mannual of Proceedures in Quantitative Genetics, 2. edition

Phenotypic standard deviation at age 10:

Stands (provenances)	age 10	: 1.0	m	12.50%
Families	age 10	: 1.5	m	18.75%
Individuals	age 10	: 2.0	m	25.00%
Individuals	age 40	: 3.0	m	15.00%

Heritabilities:

Stands (provenances)	low estimate	: 0.40
	high estimate	: 0.80
Families	low estimate	: 0.40
	high estimate	: 0.80
Individuals	low estimate	: 0.10
	high estimate	: 0.20

10.2. Gain estimates

10.2.1. The breeding population

The numbers in the following sub paragraphs refer to the red figure at the diagram.

1. Stand selection

Phenotypic selection of stands under various climatic conditions and on various soils are not expected to give any gain in growth. The environmental influences are so large that it is very difficult to get an expression of genetic variation based on the phenotypic variation.

The aim is to avoid a negative selection with respect to growth, and to get some gain with respect to stemform.

2. Plus-tree selection, 1st generation

Selecting the 10% best

$$G = i_i \times h_i^2 \times \sigma_{P_i}$$

Low estimate :	= 1.749	x	0.10	x	0.15	=	0.03
High estimate:	= 1.749	x	0.20	x	0.15	=	0.05

3. Plus-tree selection, 2nd generation

Selection according to the mating design the best family of two and according to the field design the best plus-tree out of 10.

$$G = i_f \times h_f^2 \times \sigma_{P_f} + i_i \times h_i^2 \times \sigma_{P_i}$$

Low estimate : 0.564 x 0.40 x 0.188 + 1,539 x 0.10 x 0.25 = 0.08

High estimate : 0.564 x 0.80 x 0.188 + 1,539 x 0.20 x 0.25 = 0.16

4. Plus-tree selection, 3rd generation

Prevailing the variation between families are the same, the estimated gains will be the same as for the 2nd generation.

The accumulated gain is:

Low estimate : 0.03 + 0.08 + 0.08 = 0.19
1st gen. + 2nd gen. + 3rd gen.

High estimate: 0.05 + 0.16 + 0.16 = 0.37

10.2.2. The Production population

5. Clonal seed-orchard I

The gain will origin from the plus-tree selection.

Low estimate : 0.03

High estimate: 0.05

6. Clonal seed-orchard II

The unthinned orchard will have the gain from the plus-tree selection.

Low estimate : 0.03

High estimate: 0.05

After genetic thinning an additional gain can be calculated

$$G = i_f \times h_f^2 \times \sigma_{P_f}$$

The seed orchard is expected to be thinned down from 46 clones to 22 for height and for stemform.

Low estimate : 0.03 + 0.819 x 0.40 x 0.188 = 0.09

High estimate: 0.05 + 0.819 x 0.80 x 0.188 = 0.18

8. Selected seed stands

No response according to height growth. Some response expected with respect to stemform.

9. Tested seed stands

After testing the seed stands, the best can be selected. If the 25 out of 60 tested stands (53 selected + 7 additional) is selected for height growth and 12 of the 25 selected for stemform, the gain for growth can be estimated:

$$G = i_s \times h_s^2 \times \sigma_{P_s}$$

Low estimate : 0.924 x 0.40 x 0.125 = 0.05

High estimate: 0.924 x 0.80 x 0.125 = 0.09

10. Seedling seed orchard I - genetically thinned

In the within family thinning the two best trees in each plot according to stemform will be selected. The most fast growing of these two will be the only tree to represent the family in each plot.

The final number of families will be 15, which will give an average space of 64 m² per tree at the end. Selection due to stemform will reduce the number of families from 60 to 30 and selection from growth from 30 to 15.

The estimated gain is:

$$G = 1/2 \times i_f \times h_f^2 \times \sigma_{P_f} + i_i \times h_i^2 \times \sigma_{P_i}$$

The accumulated gain is:

Low estimate : 0.5 x 0.03 + 0.777 x 0.40 x 0.188
+ 0.564 x 0.10 x 0.25 = 0.09

From plus-tree selection.

High estimate: 0.5 x 0.05 + x 0.777 x 0.80 x 0.188
+ 0.564 x 0.20 x 0.25 = 0.09

11. Bulk propagation

The pair crossing for the bulk propagation could consist of 10 trees from each of two families, without having inbreeding. It is however most likely that the best trees are distributed to more families. Let us in this example say 5. The situation is ideal for index-selection, but will be illustrated as independent cutting level selection made stepwise, first family selection and then within family selection. Selecting 20 families out of 60 for stemform and then 5 out of the 20 for growth. The within family selection could be 8 out of 10 for stemform and 4 out of 8 for growth. The estimated gain will then be:

Low estimate : $0.03 + 1.214 \times 0.40 \times 0.188 + 0.725 \times 0.10 \times 0.25 = 0.14$

High estimate: $0.05 + 1.214 \times 0.80 \times 0.188 + 0.725 \times 0.20 \times 0.25 = 0.27$

12. The seedling seed orchard II

The seedling seed orchard should consist of ten unrelated full-sib families deriving from pair crossing the best tree in the 20 best families. The best 35 families out of the 60 are selected for stemform and the best 20 of the 35 are selected for growth. Within family selection is carried out by selecting 5 out of 10 for stemform, and then the best out of the five for growth. An additional gain comes from the thinning of the seed orchard. In this case only within family selection is carried out with 2 out of 4 for stemform, and the best of the 2 remaining for growth. The estimated gains will be:

Low estimate : $0.03 + 0.672 \times 0.40 \times 0.188 + 1.163 \times 0.10 \times 0.25 + 0.564 \times 0.10 \times 0.25 = 0.12$

High estimate: $0.05 + 0.672 \times 0.80 \times 0.188 + 1.163 \times 0.20 \times 0.25 + 0.564 \times 0.20 \times 0.25 = 0.24$

The above estimated gains for height are calculated under the assumption that selection for stemform is carried out with the same intensity. Since stemform usually has a higher heritability the gain in stemform is supposed to be higher.

The gains estimated here refer to age 10. For height growth it is likely that a gain in height at age 10 corresponds to the same gain in volume over the whole rotation.

Similar estimates based on diameters are somewhat higher.

The gain in stemform has considerable economic importance at many levels such as logging operations, transport, sawmilling and quality due to diminished compression wood.

The gain in growth and volume has an economic effect more than just the quantity, because the trees at an earlier age obtain timber size.

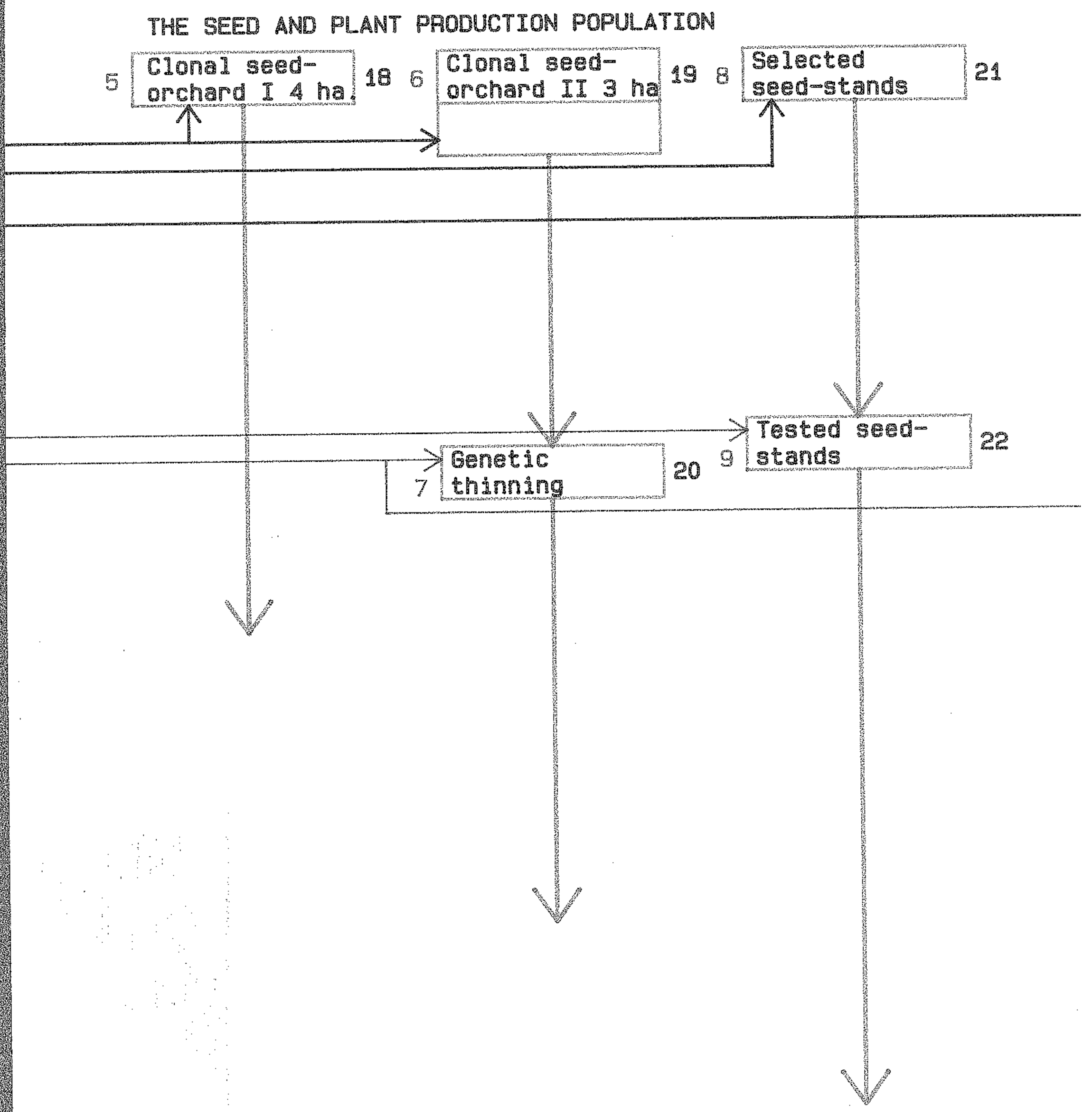
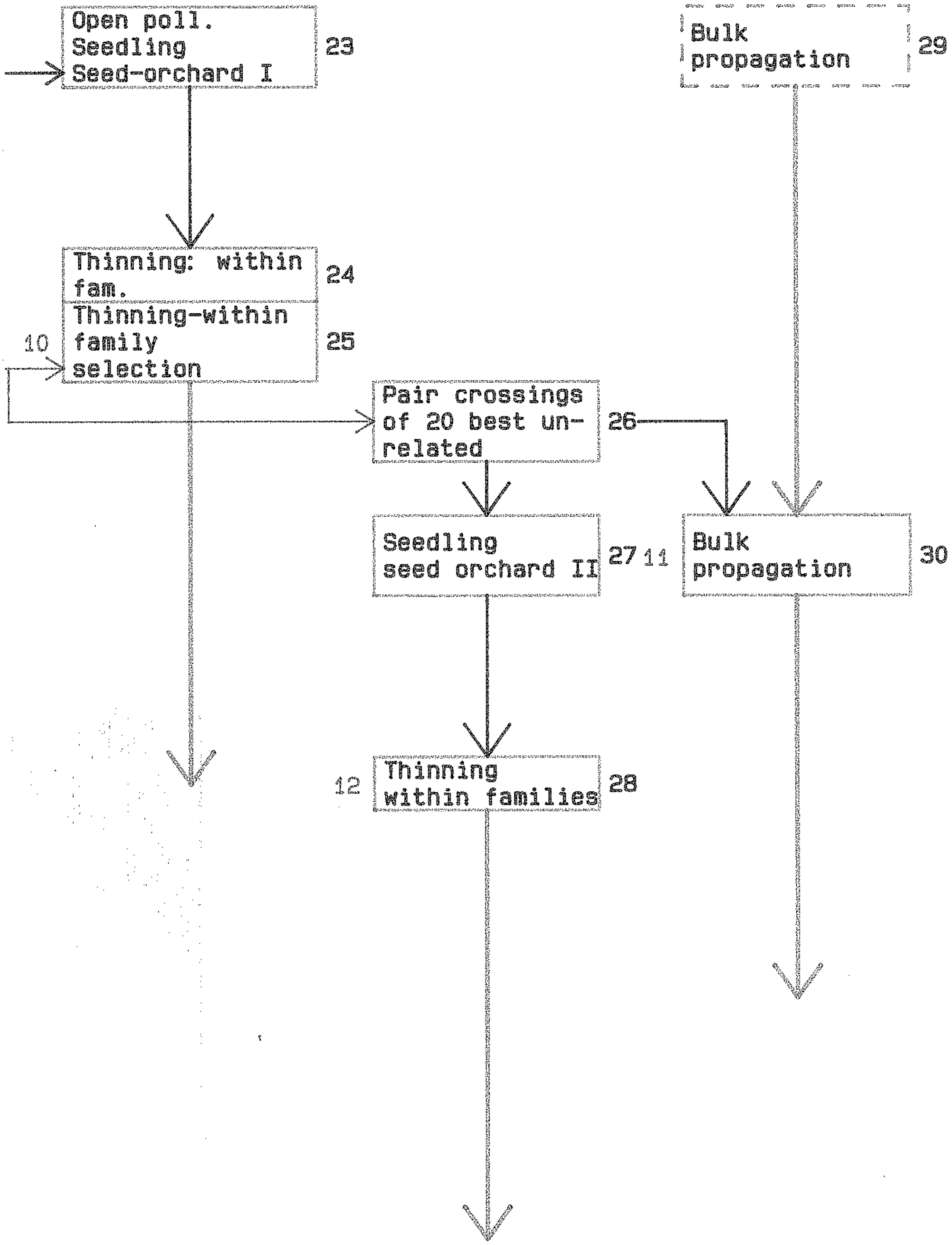


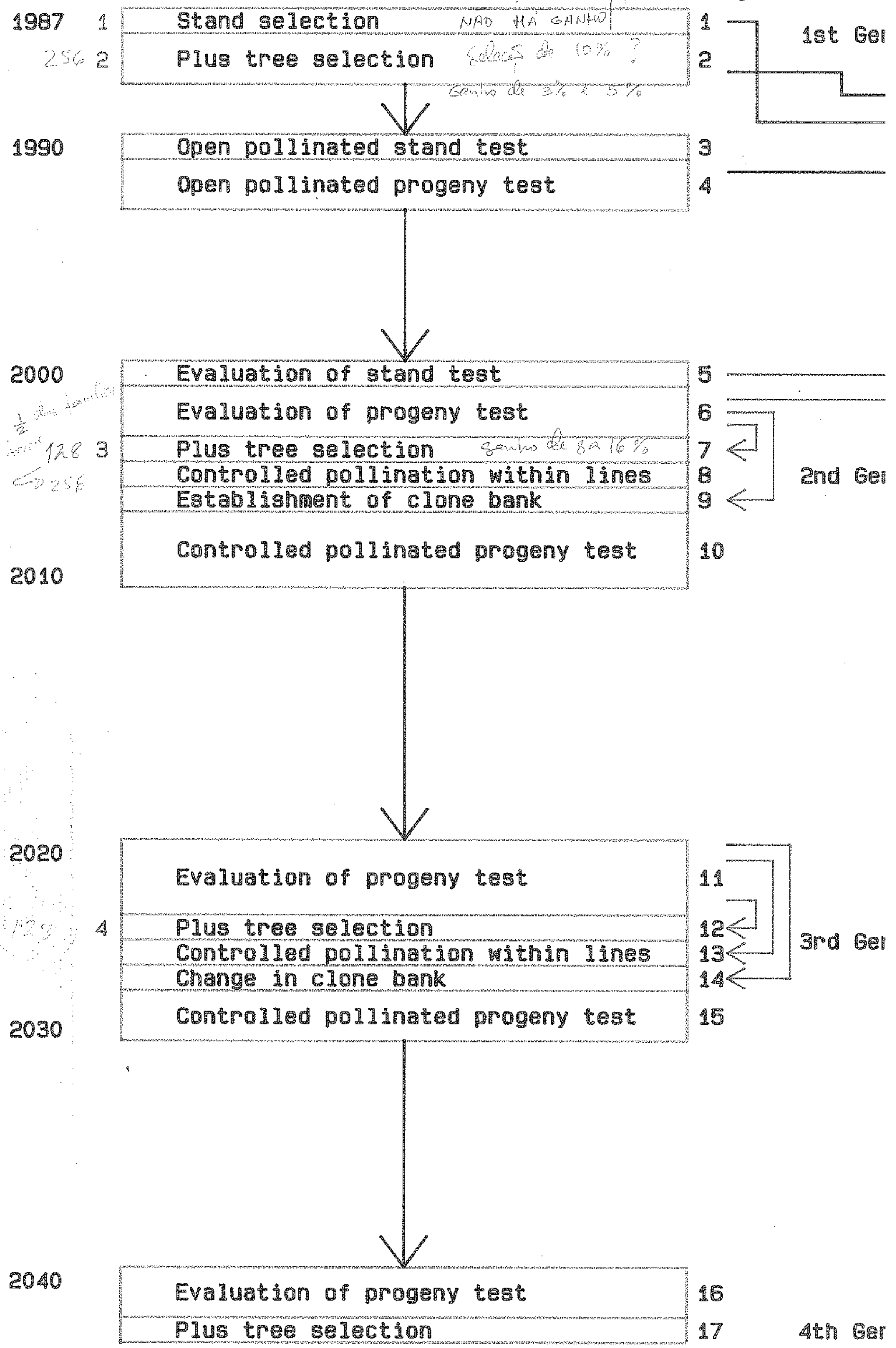
DIAGRAM OF THE BREDING PLAN

For details - see text

THE SEED AND PLANT PRODUCTION POPULATION



THE BREEDING POPULATION



11. REFERENCES

1: Aguiar, A. P. M. A.:1986. Contribucao para metodologia da colheita, manipulaçao e armazenamento da semente de Pinus pinaster AIT. - Comunicacao au congresso florestal nacional.

2: Almeida, M. H. R.: 1978. Relatorio da actividade de estagiario do curso de engenheiro silvicultor.

3: Alpuim, M.: 1971. Algumas consideraçoes sobre o melhoranento genético do Pinheiro bravo. - Estudos Informacao. D. G. F., No. 257.

4: Anonymous, 1986: Programa de Accao Florestal (PAF). - Republica Portuguesa, Ministério da Agricultura, Pescas e Alimentacao. Direccao - Geral das Florestas. 152 pp.

5: Becker, W. A., 1967: Manual of Proceedures in Quantitative Genetics. - Wash. State University. 129 pp.

6: Butcher, T. B. 1977: Gains from the Pinus pinaster AIT. Improvement Programme in Western Australia. - Third World Consultation on Forest Tree Breeding, Canbarra. 7 pp.

7: Chritchfield and Little: 1966. Geographic distribution of the pines in the world.

8: Hopkins, E. R.: 1060. Variation in the growth rate and quality of Pinus pinaster AIT. in Western Australia - Forest Dept., Perth, Western Australia. Bulletin No. 67. 33 pp.

9: Israel, B. J., 1987: Early testing in forest tree species: a review. - Forest Tree Improvement 20: 45-78.

10: Kang, H., 1980: Designing a tree breeding system. - Proceedings of 17th meeting of the Canadian Tree Improvement Assoc. part 2. pp. 51-66.

11: Kang, H., 1982: Components of a tree breeding plan. IUFRO Joint Meeting of Working parties on Genetics about Breeding Strategies Including Multiclonal Varieties. - Sensenstein; P. 119-135.

12: Lambett, C. C., 1980: Juvenile - mature correlations in Pinaceae and implication for early selection. - Forest Science, 26(4): 571-580.

13: Lindgren, D. and Gregorius, H. R., 1976: Inbreeding and coancestry. - Proceedings of Joint meeting on advanced generation breeding. Bordeaux. pp. 49-55.

14: Machado, D. P. and Machado, C. S.: 1961. Da Possibilidade de Novas Tecnicas de enxertia para o estabelicimento de Pomares Probutores de Semente Selectionada do Pinheiro Bravo. - Estudos e Informacaos, D.G.F., No. 143.

15: Machado, D.P. and Pessoa, J. S.: 1972. O Melhoramento genetico do pinheiro bravo. - Estudos e Informacaos, D. G. F., No. 251.

16: Morais, T.: 1940. Novas areas de Fitogeofrafia Portuguesa. Bol. Soc. broteriana 14.

17: Namkoong, G., Snyder, E. B., Stonecypher, R. W., 1966: Heritability and gain concepts for evaluating breeding systems such as seedling seed orchards. - Silvae Genetica 15: 76-84.

18: Percy, D. H. and Hopkins, E. R.: 1967. Importation of Breeding Material of Pinus pinaster AIT. from Portugal - Forest Dept., Perth, Western Australia. Bulletin No. 75. 66 pp.

19: Pinto, A. A.: 1939. O Pinhal do Rei. 2 vols. Alcobaça.

20: Polge, H. and Illy, G.: 1967. Héritabilité de la densité du bois et corrélations avec la croissance étudiées à l'aide de tests non destructifs sur plants de Pin maritimes de quatre ans. - Silvae Genetica. 173-181.

21: Rolctae, J.: 1986. relatório de um estágio em França.

22: Van Buitenen, J. P., 1975: Advanced generation breeding. - Proceedings of 13th Southern Forest Tree Improvement Conference; Raleigh, NC, pp. 63-72.

23: Varela, M. C.: 1986. Estudo quantitativo da floracão do Pinus pinaster AIT. No pomar clonal produtor de sementes. - Comunicacao au congresso florestal nacional.

24: Wellendorf, H. and Kaosa-ard, A.: 1986. Proposed Tree Improvement Strategy for Teak in Thailand, Stencilled, 27 pp.

D.G.F.
PROGRAMA DE ACÇÃO FLORESTAL

ESCALA 1/2000000

REGIÕES-PLANO