

## Producto: Material forestal de reproducción mejorado para la producción de resinas naturales.

**Actividad: Material forestal de reproducción mejorado para la producción de resinas naturales.**

**Entregables:**

- Guía para la producción de material forestal de reproducción mejorado para la producción de resinas naturales.



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Proyecto cofinanciado por el Programa Interreg Sudoe a través del Fondo Europeo de Desarrollo

# Proyecto Sust Forest Plus

**“Estrategia y redes de colaboración para la multifuncionalidad, la conservación y el empleo en el territorio del sur de Europa a través de la extracción de la resina”**



**Entregable 1.26.1-Guía para la producción de material forestal de reproducción mejorado para la producción de resinas naturales**



**Producto 1.7-Material forestal de reproducción mejorado para la producción de resinas naturales**

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## Guía para la producción de material forestal de reproducción mejorado para la producción de resinas naturales

### 1. Introducción

El Producto 1.7 sobre Material forestal de reproducción mejorado para la producción de resinas naturales, incluye una actividad y un entregable, de acuerdo a un plan de trabajo detallado en la descripción de la actividad.

**Producto 1.7-Material forestal de reproducción mejorado para la producción de resinas naturales -INIA (4/18 a 12/20-31/12/20)**

Actividad 1.25-Transferencia tecnológica para la provisión de material forestal de reproducción mejorado para la producción de resina-INIA (4/18 a 2/21)

Entregable 1.26.1-Guía para la producción de material forestal de reproducción mejorado para la producción de resinas naturales-INIA (31/12/20)

#### **Producto 1.7 Material forestal de reproducción mejorado para la producción de resinas naturales**

Se realizará la catalogación de materiales mejorados derivados de las actuaciones realizadas en la meseta Castellana siguiendo la guía antes mencionada. Estos materiales permitirán mejorar la producción de resinas naturales en el futuro por lo distintos interesados mediante la aplicación de la selvicultura definida para la producción de resina. El trabajo capitaliza y toma como punto de partida los trabajos realizado en el ámbito del proyecto SustForest y de las actuaciones previas del INIA sobre mejora genética en colaboración con la Junta de Castilla y León. El producto culminará con la implantación de un campo de producción de material genético de reproducción mejorado. Este producto contribuye al objetivo específico de proyecto 'Convertir las masas del sudoeste europeo en una fuente abundante y sostenible de resina a medio y largo plazo' al permitir asegurar unos materiales de calidad más productivos y por tanto más rentables para la producción de resinas naturales. Asimismo, es una 'Herramientas de gestión de la estrategia para el aprovechamiento sostenible de las resinas naturales europeas' pues permite a los interesados desarrollar materiales más adaptados a sus condiciones ambientales.

#### **Actividad 1.25. Transferencia tecnológica para la provisión de material forestal de reproducción mejorado para la producción de resina**

Fecha de inicio 3.2018 Fecha de fin 2.2021

Se procede a la transferencia tecnológica de metodologías y materiales en el campo de la genética forestal que permitirá la producción de material de reproducción mejorado con objetivos de producción resinera.



Se parte de experiencias realizadas en la meseta castellana que indican que pueden alcanzarse producciones de 7 kg/árbol, frente a las 2,5 a 3,5 de media, con la utilización de material mejorado.

Se dispone de árboles superiores genéticamente como base del programa de mejora; se cuenta con metodologías de selección en campo, y posterior evaluación genética; se cuenta con metodologías para producir materiales forestales de reproducción mejorados a partir de los árboles identificados: selección de materiales, métodos de polinización controlada, métodos de propagación vegetativa masiva, y capacidad para inscribir como material mejorado en el catálogo de material de base los materiales obtenidos. Estas metodologías y materiales serán los transferidos al sector.

**Tareas:**

1. Identificación de agentes interesados en la transferencia tecnológica para la obtención de materiales mejorados por parte de cada socio.
2. Transferencia de tecnologías de selección de árboles superiores, cruzamientos controlados y métodos de propagación vegetativa masiva.
3. Elaboración de un manual de operaciones.

Coordina INIA; se cuenta con la colaboración la DGMN de la Junta de Castilla y León, y la aportación de datos y análisis de los socios de proyecto.



## 2. Base material for the production of improved FRM.

### 2.1. Regulation of Forest reproductive and Basic material.

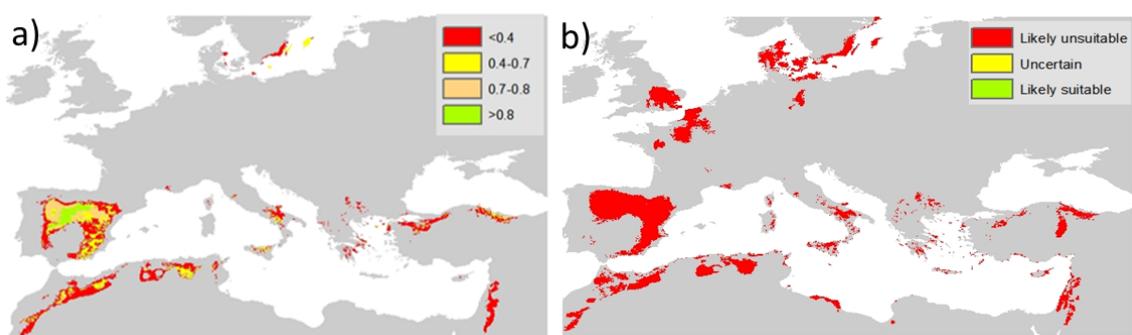
The marketing of forest reproductive material of *Pinus pinaster* is regulated by the EC195/97 directive. This directive establish the type of basic material (seed sources, stands, seed orchards, parent of families, clones and clone mixtures) where the forest reproductive material can be produced, and the categories of the forest reproductive material (identified, selected, qualified and tested). Also, establish the regulation on the production of FRM using massive vegetative propagation for some of the categories.

This proposal explore the different options to define new basic material for production of improved material for resin production, and also define the material in the area of Castilla-Leon in Spain. We also define the following steps after the approval of the basic material in the National Register of Reproductive material.

### 2.2. Breeding of *Pinus pinaster* for Resin production in Spain.

Resin production is one of the main non-timber products in the Castilian-Plateau, based on the extraction of resin from maritime pine (*Pinus pinaster*) in native forests and plantations. There have been different breeding activities for maritime pine in central Spain (IFIE). There was a selection of local plus trees (Tadesse et al. 2001c), early selection testing (Tadesse et al. 2001a), and the definition of a breeding program for the area (Auñon et al. 2001; Tadesse et al. 2001b). However, the deployment of this material has not been successfully achieved, as a result of cyclic changes in the price of the resin, and therefore in the FRM obtained.

Drought tolerance is an essential trait, as according to the predictions, the area will not be suitable for Maritime pine in the near future (Figure 1). Also, there is a clear decay of Maritime pine in the region, mainly caused by a syndrome related to the climate change.



**Figure 1. Simulated habitat suitability under current climate (a) and classified future (2050) habitat suitability (b) for the Maritime pine Genetic Group of the Castilian Plane.**

### Demand for FRM

The minimum demand for improved FRM, according to the Castilla-Leon Forest Service, is:

- Qualified or Tested FRM: 20 000 plants/year (it would be desirable to obtain 30.000 plants/year).
- Selected FRM: 5kg/seeds/year.

Therefore the activities will be based in provide this amount of material.

### Production of FRM in different areas

Trees

Seed stands

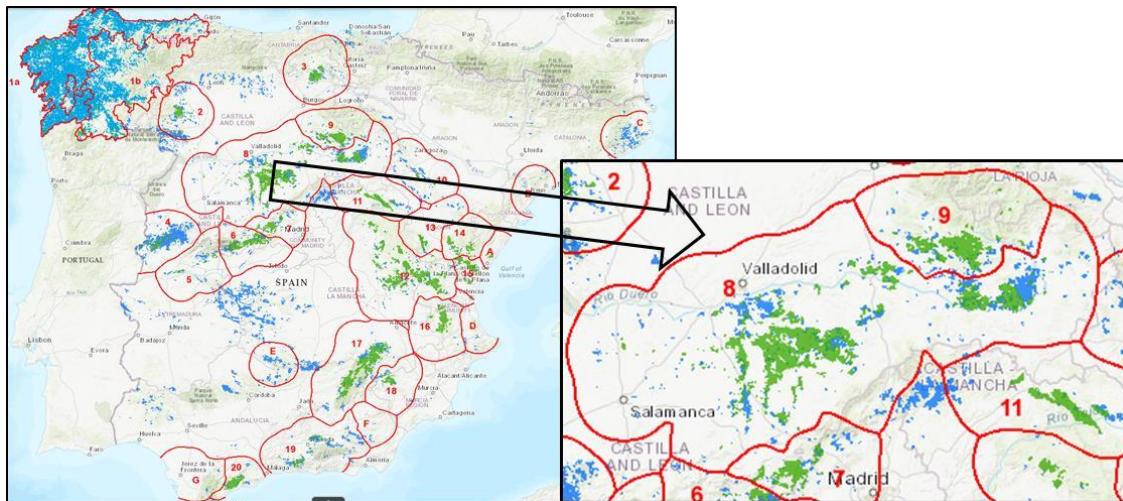
Clonal bank

Seed orchard

### Origin of the material

The target area corresponds to the Castilian Plateau. The area is characterized by extreme climatic conditions, with sandy soils consisting in Eolic dunes. The combination of these conditions results in genetically distinctive individuals in terms of tree form, growth and drought resistance (Alía et al. 1995; Alía et al. 1997), and in evolutionary history (Serra-Varela et al. 2015) in relation to their climatic characteristics. The region is the most important for resin production in Spain and includes around 40,000 ha of *Pinus pinaster* afforestation of unknown origin (probably from the same area) (Figure 2).





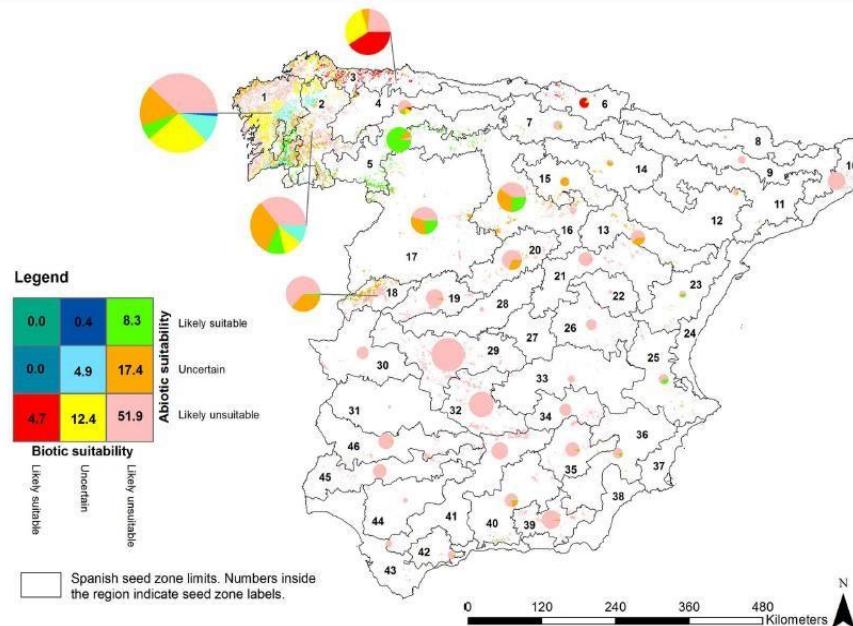
**Figure 2. Origin of the material on the Castilian Plateau. In green, natural forest; in blue, plantations of unknown origin ([www.genfores.es](http://www.genfores.es)).**

### Deployment area

The deployment area for this region, according to response in provenance tests and niche modelling, corresponds to the Deployment Regions 16 and 17, as defined in Spain. Based on provenance, provenance-progeny and clonal tests, this material could be extended to more favourable conditions, but the timber productivity of other material can be higher, compared to that of resin-production trees. Therefore, this material could be also used on more productive sites in other regions (Region 15) under the present climatic conditions.

When considering projected climatic change, the predicted area where materials should be planted has a limited range. When considering biotic and abiotic factors determining the productivity of the species in the future, this material should be used with caution.

The expected area for improved forest reproductive material aimed to resin production, therefore should be focused to the Deployments regions 15, 16 and 17, and at a minor scale in Regions 2, 3, 4, 5, in Spain, where this material is being tested (Figure 3).

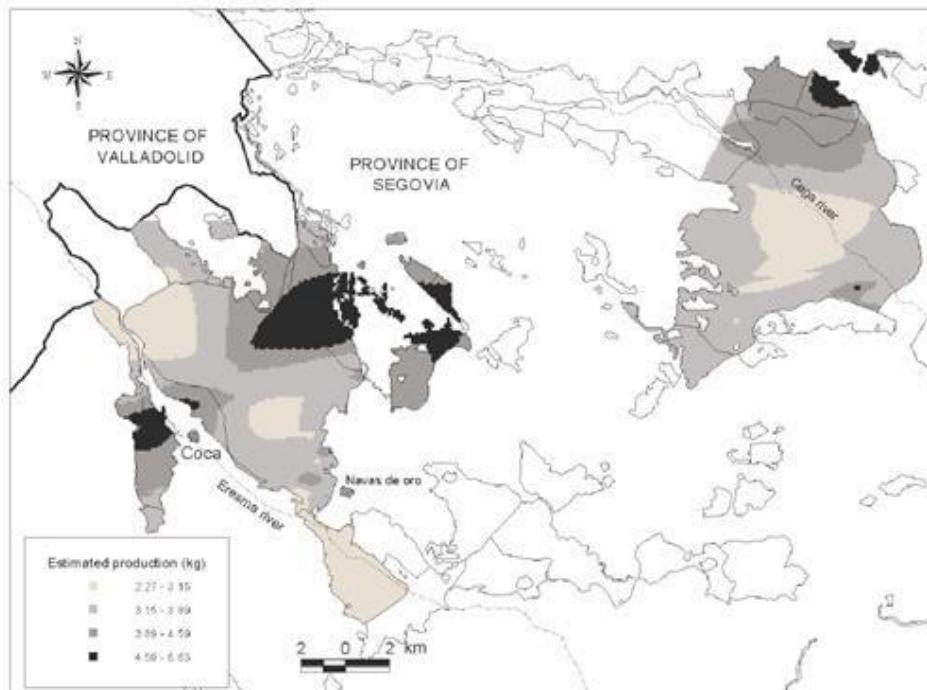


**Figure 3. Predicted deployment region of Maritime pine for resin production according to biotic and abiotic stresses (Regions 15, 16, 17 from the Castilian Plateau, and 2, 3, 4, and 5 according to evaluation tests).**

### Basic material available

The underutilized genetic resources that are available include:

- Selected stands for resin production. Resin production is quite high in the area, with large spatial differences (Figure 4).
- Plus trees selected in the area. These plus trees were evaluated in the forest for resin production for different years. From these trees, the Forest Service is collecting seeds from individual trees as OP (open-pollinated) families.
- Trees evaluated in clonal and progeny tests.



**Figure 4. High-production areas for resin production in the Castilian-Plateau (from Nanos et al. 2001).**

Some of these trees were evaluated in clonal tests, and the superiority were estimated for growth and resin production. A progeny test established in the area was evaluated for growth, reproduction and defence induction (a proxy for resin production). 20 of the trees were also evaluated in the provenance-progeny testing and clonal test of maritime pine. Finally, some trees were included from the French breeding program for resin production.

To obtain the estimates of genetic gain, we used the data from the progeny and clonal tests related to growth, resin production and drought tolerance (see study case on growth and drought tolerance in maritime pine).

#### Description of the different strategies

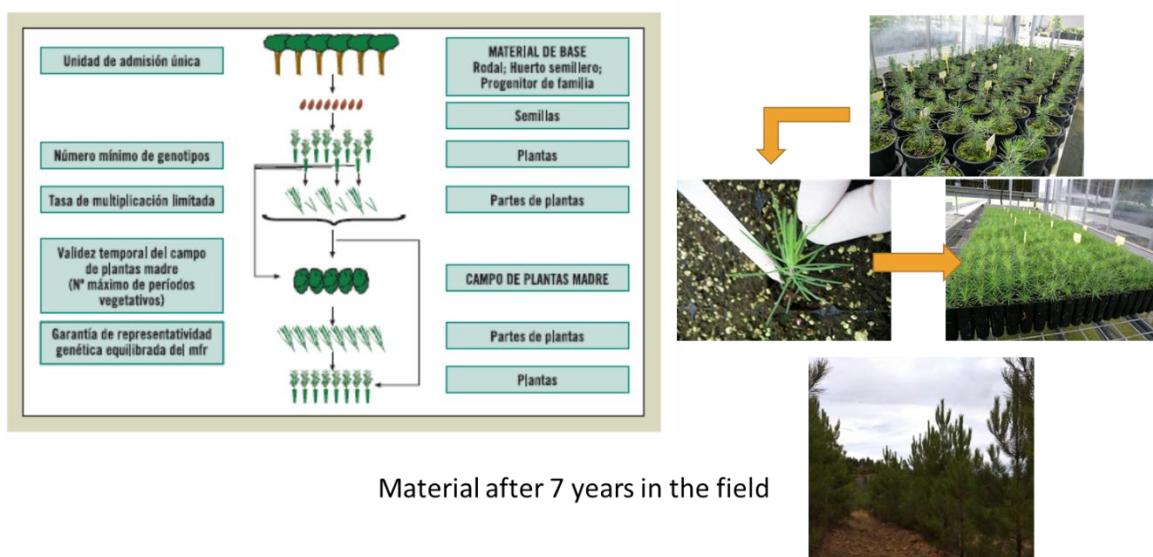
The available data were used to evaluate the following strategies to supply the required FRM (Table 1):

1. Seed stands. This strategy would be the basis for production of seeds for massive afforestation and as a backup for the other alternatives, as we can collect and store seeds quite easily for long periods of time. We can perform phenotypic selection for resin production, stem form and adaptation. At



present there are two selected stands. (characteristics), with a mean production in the last years of.. With collection by climbing, we can obtain 2.99-5.77 kg seeds/day/climber (12-20 trees/day).

2. *Parents of Families. OP in a clonal bank.* A new clonal bank should be established by using a reduced number of clones, in which OP pollination will be produced among them. The collection will be cheaper compared to the use of the existing clonal bank in the forest, as at present this clonal bank have a very limited cone production and is affected by non-selected surrounding trees. The strategy would require 20 clones, 10 ramets/clone. These clones will be tested in other environments to included as tested material at the short term.
3. *Mass propagation from CP (control-pollinated) families.* This strategy is based on mass propagation of half-sib or full-sib families from controlled crosses. In order to maintain genetic diversity, the CP will be made by polycross, using 10 mother trees and 10 fathers. From the seed produced in the clonal bank, we can select 100 seeds as donors to establish a propagation bank consisting of 100 x 4 copies/seedling for mass propagation (Majada et al. 2011). This method can produce around 2000 plants/propagation cycle and can be renovated every year (10% of the mother plants to increase the genetic diversity at the long term).



**Figure 5. Mass propagation in maritime pine.**

4. *Seed orchard.* This strategy will be based on the establishment of a qualified seed orchard with >49 clones and 4 ramets/clone.

The new clonal archive and seed orchard will be established in the Valladolid Nursery to improve seed production.

**Table 1. Strategies considered for promotion of underutilized genetic resources of high resin yielders of maritime pine**

ST	Basic material	Cross	Category	Location	Nb	Height	Stem form	Resin production	Drought tolerance	Time (years)
1	Seed stands	OP	S	Forest	3	3	3	3	0	0
2	Parent of families	OP	Q	Clonal bank	15	5	4	15	5	4
3	Mass propagation (from ST2)	OP	Q	Clonal bank	100	20	15	50	15	4+3
4	Seed orchard	OP	Q	Clonal bank	>49	10	5	15	10	7
5	Parent of families	OP	Q	Clonal bank	15	3	3	3	0	0
6	Mass propagation (from ST5)	OP	Q	Clonal bank	100	3	3	3	0	4

Stem form: measured on a scale 1-6, resin production (g/year), drought tolerance (d13C)

Based on the associated costs, available material, and cone production, we can discard strategies based in the collection of individual seed in the forest of open-pollinated families (strategy 5) or mass production based in this material (difficult to make controlled crosses in the existing clonal bank (strategy 6).

Therefore we will apply:

- 1- Selected: Stands for the production of selected material.
- 3- Qualified: Mass propagation from open pollinated parents of families from a clonal archive designed to this objective.
- 5- Tested: Similar to the previous strategy but with additional testing
- 6- Qualified/Tested: Seed orchard from a wide base of plus trees.

The use of FRM for resin production in central Spain is reduced to a limited amount of seedlings, and therefore the FRM production strategy should be cost-effective. The existing information provides the

basis for tested materials, so that expected gains can be obtained by mass propagation from CP families.

The strategy also considers drought tolerance as an essential trait, as the model predictions suggest a dramatic reduction of the maritime pine distribution in the area.

### 3. Register of Basic material. Parent of families

Based on the previous information, we will focus in the parent of families as the improved material of higher quality that can be established based on the existing data. The information on the early testing and field measurement (table 1) provide the basis for selecting the 15 best performing trees.

**Table 2. Superior trees. Parents of families of Pinus pinaster for resin production.**

Clon	Selection intensity	1999					2000				
		Nb ramets	Mean	std	cv	Nb ramets	n	Mea	adjusted Mean	std	cv
1	2.58	4	141.58	35.85	25.32	5	199	200.87	54.03	26.9	
4	0.73	5	84.02	22.63	26.94	5	163	165.24	52.04	31.5	
6	1.89	4	121.91	50.62	41.52	4	167	169.31	35.54	20.99	
7	3.04					4	149	150.82	34.64	22.96	
10	0.89	3	150.51	49.46	32.86	4	214	215.19	44.35	20.61	
12	3.83					3	228	229.26	76.04	33.17	
15	3.04	7	157.5	44.71	28.39	2	181	182.1	74.86	41.11	
20	2.48					7	192	194.23	54.65	28.14	
21	2.48					4	237	239.37	199.06	83.16	
25	0.67	4	99.97	36.49	36.5	5	128	129.96	60.75	46.75	
27	1.83	5	168.51	64.34	38.18	4	239	240.76	116.03	48.19	
29	2.04					5	221	223.22	100.82	45.17	
31	0.69	3	113.86	39.85	35	3	234	235.11	111.96	47.62	
36	0.99					3	171	173	65.88	38.08	
44	4.42					4	161	162.95	70.44	43.23	
82	0.41	5	84.52	29.12	34.46	4	143	145.37	78.25	53.83	
104	1.08					3	63	66.21	16.59	25.05	
Control	0					10	107	110.72	25.89	23.39	



This material are being installed via clonal banks in the Castilla y Leon central nursery for the production of seeds by open-pollination among superior trees (figure 6).



**Figure 6. Clonal propagation of superior trees from the clonal bank (Picture: J. Tranque, JCYL)**

This material are being installed via clonal banks in the Castilla y Leon central nursery for the production of seeds by open-pollination among superior trees (figure 6).

At present the objective is the practical application of minicuttings for the production of improved plants from open-pollinated seeds collected in the new clonal banks.



**Mini-cuttings: an effective technique for the propagation of *Pinus pinaster* Ait.**

Juan Majada · Celia Martínez-Alonso · Isabel Feito · Angelo Kidelman · Ismael Aranda · Ricardo Alfa



**Figure 7. Massive vegetative propagation of superior trees from the clonal bank (Picture: J. Tranque, JCYL)**

## 4. Perspectives

The existing material already selected would fulfill the needs of improved material for resin production in the Castilla y Leon region for the next years. This material is being selected for the use in the same region. However, there are new areas where *Pinus pinaster* for resin production could be of interest. Therefore, we need to expand the genetic evaluation of this material to the future area of interest (Extremadura, Galicia, in Spain) to test the adaptability and production of the material in these areas.

Also, an existing progeny tests in the area (Carbonero, Segovia) has been recently evaluated for resin production. Based on this information we can select the best performing individuals from the best families to advance in the breeding program of the species, and to provide improved material with higher genetic gains than those obtained using the existing basic material.

