



Special Newsletter

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Plant production and
restoration of yew
woods in areas of the
LIFE BACCATA project

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LIFE15 NAT/ES/000790

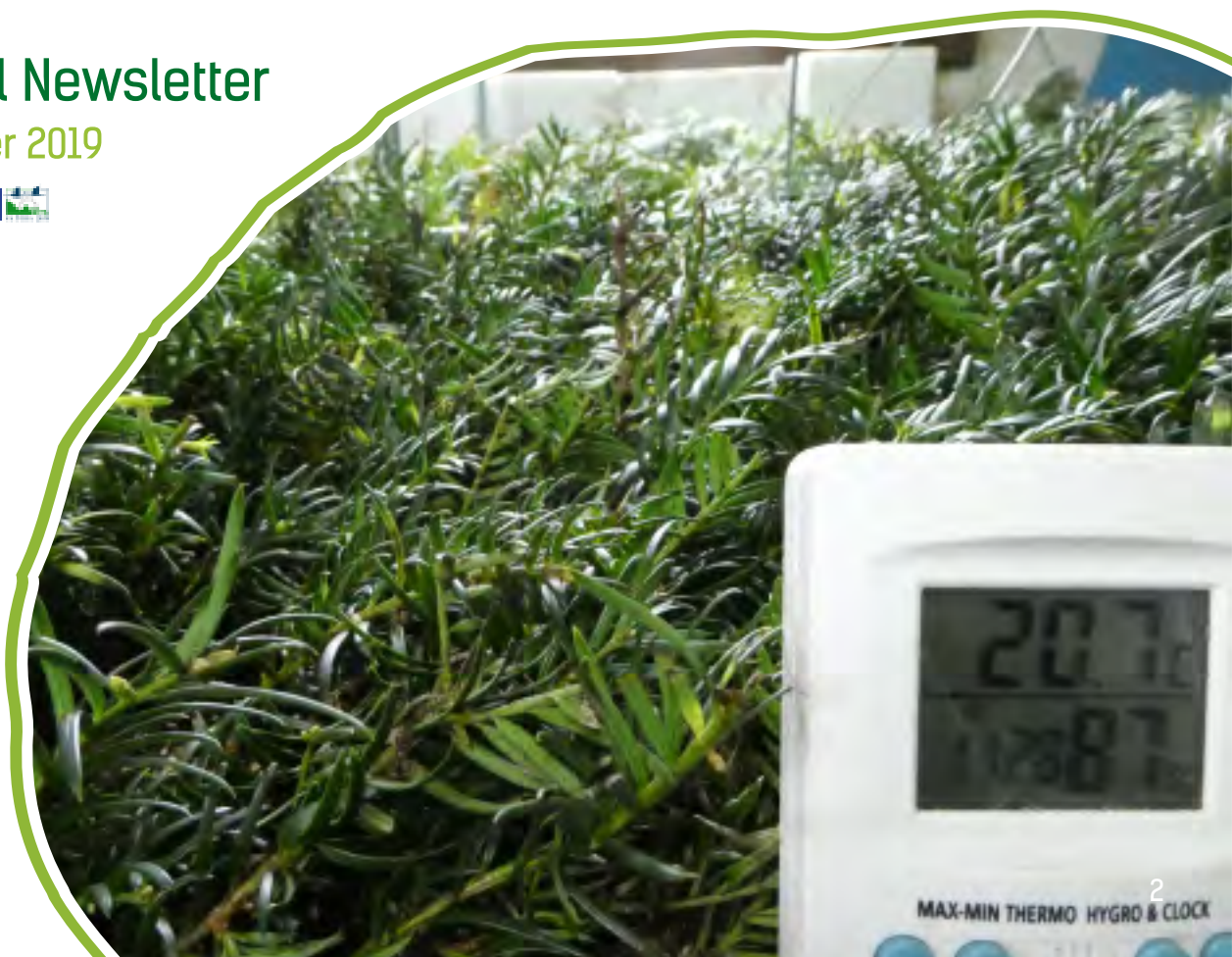
Collection, storage and growth of the 9580* habitat characteristic species

The LIFE BACCATA Project seeks to improve the habitat 9580* conservation status in 15 SCAs (Special Conservation Areas) found in the Cantabrian Mountain Range by acting upon indicators of the conservation status: Area of occupancy, structure and functions and future prospects. In Galicia, the Project is focused on 15 ha located at the 'Monte Vecinal en Mano Común' (communal woodlands Known by the Spanish acronym MVMC) of Riocereixa (Pedrafita do Cebreiro, Lugo), within the Os Ancares – O Courel SCA (ES1120001). This area will be subject to the removal of a reforesting pine grove (Action C2) and the habitat 9580* type will be restored with a forestry plantation made up of characteristic species (Action C3). To this end, those conditions affecting the habitat type will be eliminated, its current area of occupancy will be increased and its structure, composition and connectedness will be improved, all together will contribute to improve its conservation status.

In planting habitat 9580* forestry species, and bearing in mind it is a Natura 2000 designated site, it was required to use forestry reproductive material (FRM) compatible with the local genetic pool. To ensure that compatibility, it was considered in the framework of the Project to use FRM from the Riocereixa MVMC itself. For this action, twigs and seeds from the 9580* habitat tree characteristic species were collected from the said MVMC. It was carried out according to the Galician Habitat Manual (*Taxus baccata*, *Quercus petraea*, *Fagus sylvatica*, *Betula pubescens*, *Sorbus aucuparia*, *Ilex aquifolium*, *Corylus avellana*) and then this material was stored, conserved and grown (Action C1). IBADER, from the University of Santiago de Compostela, implemented this Action, relying on some external support during some phases where necessary.

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Phase 1 COLLECTING PLANT MATERIAL

This phase took place in 2017 and 2018 and consisted of the gathering of FMR from habitat 9580* characteristic species according to the Galician Habitat Manual (*Taxus baccata*, *Quercus petraea*, *Fagus sylvatica*, *Betula pubescens*, *Sorbus aucuparia*, *Ilex aquifolium*, *Corylus avellana*). To reckon the necessary amount of plant, the overall area for reforestation and ratios of dominant and companion species of this type of habitat according to the Galician Habitat Manual were taken into account. The time schedule for the collection work was established out of critical periods for the species of conservation interest whose habitat is located within the FMR collection areas.

During the first year (2017), seeds from any characteristic species of 9580* habitat were collected since the use of sexually reproduced material allows us to ensure the genetic diversity of reforestations. This task was completed according to the following determining factors:

Collection work was manually carried out in the day time.

- No mechanical means were used.
- Seed donating feet were not shaken.
- Seed was collected from the forest floor or by accessing it with a ladder.
- Only seeds that morphologically seemed to be viable in the future were collected.
- Seed donating feet were not damaged during the collecting process and the seeds collected came from a suitable number of specimens as compared to the total available tree feet, which means that the recruitment ability of these species natural populations were not affected.
- To collect the appropriate amount of seeds per specimen, 30% of the available seeds was left on each foot to avoid an impact on the dispersal of seeds by birds.



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Thanks to this, it was possible to collect an adequate number of seeds from the entire habitat 9580* characteristic species, except for their own dominant species, that is, *Taxus baccata*. The late frost events in April of 2017 damaged the feminine flowers of local yew trees which resulted in the low production of seeds during the summer of 2017. This was not enough to meet the objectives set by the LIFE BACCATA in terms of plant production.

However this type of unexpected situations were already taken into account for the project so, during the winter and fall of 2018, the Riocereixa MCMC was visited in different occasions with the aim to collect FMR to produce *Taxus baccata* cuttings. This action will allow us to produce an adequate amount of plants to provide the target plantation figures set for the Project and therefore the successful reforestation (Action C3). To this end, a team made up of 2-4 persons went to Riocereixa MVMC and they were able, by using the appropriate tools (secateurs, trimmer, poles, rope for tying, bags, markers, etc.) to collect the *Taxus baccata* rods. The inhabitants and owners of the Riocereixa MVMC took charge of choosing the collection sites and feet. They also monitored the actions and gave advice all times as they were especially interested in the success and long-term maintenance of the reforestation the LIFE BACATTA PROJECT will carry out in their property. In this regard, they reported to IBADER team about the best feet for the intended objective as these inhabitants are the best judges of any essential aspect regarding their forests..

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To promote diversity as much as possible, collection work was done on over 50 yew specimens ensuring that the samples collected were both, feminine and masculine and in similar proportions. At any rate, to boost the success of the final outcomes, the material is collected from those branches previously selected, according to the type of cuttings that is to be produced and by complying with the following protocol:

- Rods with 2-3 internodes were collected
- The rod baseline diameter is less than 10 mm..
- Branches with *Taxomya taxi* galls were avoided as much as possible.
- Rods were cut with sterile and well-sharpened material, applying clean cuts, without any tearing or roughness. In the process tree structure was not damaged and its biomechanics was not affected either.
- The material is prepared with rope ties that allow a convenient storage in sealed plastic bags to prevent any damage to rods.
- Transport of bags filled with FRM to the nursing was carefully done, by placing the bags in the vehicle in such a way and spots that the material could not be damaged and to avoid the direct sun rays. Furthermore, the vehicle was never parked in the sun when the material bags were inside and this way plants were not negatively affected by high temperatures.

Once the *Taxus baccata* rod-based FRM was collected and selected, it was transported to the nursing to be grown, fattened and used later on in the 9580* habitat plantations.



Phase 2. FOREST PLANT GROWTH AND FATTENING UP

Once plants collected during Phase 1 reach the nursing, storage and preparation work begins so that plants are grown and fattened up. In the case of seeds-originated species, they were first grown in 2017 and it was relatively simple. They were grown in seedbed and sown in trays so that germination took place orderly and under control (with shade netting or nursing netting, vermiculite and agri-pearlite substrate, controlled irrigation and humidity content, etc.), in order to grow effortlessly until the plant is ready to be used in the reforestation.

Concerning *Taxus baccata*, it reached the nursing in the shape of rods in February of 2018, after a first accession. Then in October of 2018, during the second time collection was done, it was used to produce cuttings and to provide plants in different phenological stages and also to compare outcomes and successful proportions. In both case and after being delivered to the nursing, this plant was stored in a cool room at 3° y 5 °C. Subsequently it was prepared as cuttings and transplanted to trays.

The first step in preparing the material was to select the best one because, despite the transportation of the material complied with the required conditions, a detailed review of the material can be done in the nursery. In this process, damaged branches or those not complying with the required conditions according to the type of cuttings to be produced can be discarded.

Secondly, material was trimmed in the shape of cuttings to fit in the forestry container. Hence each rod has 2-3 internodes, approximately 6-10 cuttings can be produced per each rod according to the following types:

Apical cuttings of approximately 15/20 cm long with a diameter of up to 5 mm and preferably lignified base.



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Heel cuttings of secondary branches with small branches of approximately 5/10 cm long (most of the produced ones).

Leaves are stripped from every cutting in the cutting's growth direction to trigger the generation of callosity. Additionally, some lateral scrapping was done to increase the cambium accessible area. Then each plant is sunken in rooting liquid hormone for 20 seconds and then inserted in a forest container with a mixture of substrate and agri-perlite which was previously hollowed out to accommodate the plant. Once this step is completed, trays were placed on the table with some background heat (15/20°C) and a controlled temperature (10/15°C) until the first plant rooting started to be at sight, months later. At this time they were transplanted to the container with commercial substrate based on a charcoal mixture.

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The whole process, that started in 2017, for seed-originated reforestation plants, and in the winter of 2018, for cutting-originated plants, and ended in the spring of 2019, allowed the production of forestry plants with a sufficient quality and quantity to ensure the reforestation of the 15 ha located in the Riocereixa MVMC that are the object of this Project. The production figures per plant species that are available for LIFE BACCATA in their Galician locality are as follows:

<i>Taxus baccata</i>	> 4.000 plants
<i>Betula pubescens</i>	> 3.000 plants
<i>Fagus sylvatica</i>	> 2.000 plants
<i>Quercus petraea</i>	> 2.000 plants
<i>Sorbus aucuparia</i>	> 1.000 plants
<i>Ilex aquifolium</i>	> 1.000 plants
<i>Corylus avellana</i>	> 200 plants

The plant produced is suitable for reforestation use hence it is in forestry containers, has an adequate height and diameter and their root development is significant, without rolling up too much because the container has been sufficiently spacy as to allow a trouble-free root growth.



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Actions taking by the Central Forestry Nursery of the Castilla y León regional government for yew plant production using seeds

The Central Forestry Nursery (VFC by its Spanish acronym) of the Regional Ministry of Public Works and Environment was involved in the production of quality plants in the Framework of the LIFE BACCATA Project. Their genetic origin was considered based on their genetic characterization carried out at the site where planted and within the LIFE A1 action. Therefore different actions took place in the VFC, ranging from surveying and collecting seed from the region natural yew woods to sowing and producing plants in forestry containers and including pre-treatments on seed germination.

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COLLECTING ON-SITE YEW TREE FRUIT

Usually yew tree fruit is manually collected directly from small branches during October and November months. Fruit may also be collected from the forest floor by previously knocking them down with poles or by shaking branches. Collection work can be challenging because this species, apart from growing in inaccessible sites most of times, they alternate bearing and it is necessary to collect fruit from a sufficient number of varied genotypes of each population. Therefore seed was collected from 2016 to 2017 to produce yew seedlings that are implanted in 2019 and 2020. Cuttings were also collected however the difficulties arose in subsequent stages prevented any production from this material.

Usually the collected material (the reddish aryl with seed) is introduced in small cans which are properly identified with double labels to guarantee its traceability and transported to the VFC facilities to be processed.

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Figure 1, 2 and 3. FRM collected from the forest site.

FRUIT RECEPTION

The forest reproductive material received as fruit at the VFC is temporarily stored in cold rooms at a 3-4°C / 37-39°F with a relative humidity of 90% until it is processed.

In order to assure the quality and traceability of seeds, batches received should meet different technical and documentary requirements, e.g. bearing identification labels to control its origin, the suitability of packaging that should be properly closed. In addition to these, a visual inspection of the product is carried out to ensure there is no damage by external pathogens.

All batches delivered to the VFC are registered in a database where the related information is entered (e.g. material origin, weight, collection date and date of delivery to the nursery). A single and unchangeable registration number is assigned during the time the material remains in the nursery. Any information gathered from the new accession (shipments, lab test, etc.) will be associated to this number.

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Figures 4 and 5. FMR (fruit and cuttings) delivered to the nursery.

SEED EXTRACTION AND CLEANING

Considering seeds and their enveloping fleshy aryl are the most commonly collected material from the forest they are subject to different extraction and cleaning operations until the right seed for use and storage is obtained. This process is aimed at getting the largest amount of cleaned and viable seed.

As far as yew tree concerns, the extraction procedure done by the VFC includes the manual pulp removal to take the aryl apart and some water rinsing. Subsequently water-based decanting, sieving and winnowing processes allow the separation of dead fragments and empty seeds.

Once seeds are cleaned, the material is permanently labelled and then stored in the seed bank.

SEED ANALYSIS AND CONSERVATION

The VFC seed lab is in charge of analysing a representative sample of a seed batch and this task includes the following: Analyses of the moisture content and purity, the specification of 1000 pure seeds, a germination test and a viability study. These tests comply with the ISTA International Rules and are carried out for both, new incoming seeds as well as long-term kept batches that can be used in the future and therefore its evolution may be relevant.

Suitable conditions should be in place for yew seed preservation so that its viability and germination ability remain the same until it is potentially used. For this reason, it is preserved in the Seed Regional Bank of the VFC which provides several cool rooms equipped with temperature and humidity control (3°C/37°F and 60%, respectively).

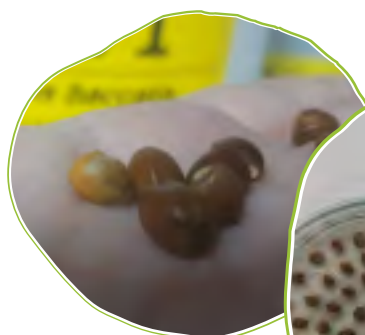


Figure 6. A batch of conserved cleaned seed



Figure 7. A germination analysis of a yew seed batch



Figure 8. Yew seed batch in germoplasm conservation bank

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PLANT PRODUCTION

Yew plant is usually produced in seed starting trays during spring. This also can be achieved through vegetative propagation from cuttings which results in the production of plant specimens that are identical to each other.

Yew seeds can be very slow to germinate because they have a waterproof coat and deep physiological dormancy and therefore it requires some pre-germination treatments. In line with this, warm-humid stratification for 6-7 months followed by cold-humid stratification for 3-5 months and 1-year stay in the seedbed at a later stage are recommended. Nevertheless, warm-cold stratification cycles are being modified in the VFC to reduce these time frames. Furthermore, attempts are made to avoid the seedbed step providing that pre-germinated seed is available.

Following seedbed nascence, seedlings are transplanted into forest containers of 300-400 cm³, which are filled with a mixture of substratum and agri-perlite content. The obtained batches are identified with a registry number and entered in the database.

The objective for the following week is to achieve plants development and acclimation through fertirrigation and plant health monitoring according to each period.

Plants are finally used to increase the populations of natural yew woods through forest plantations, which will be accomplished during 2019 and 2020.



Figure 9. Lab pre-germinating yew seed



Figure 10. Yew plant in forest container

Figures 11. Yew plant from seeds at forest site.

REGENERATED YEW TREE TRASLOCATION AND HEALING IN

In parallel to the production of seed-originated FRM in SCAs of Leon Central Mountains where yew regeneration is thriving, translocation and healing in using forest containers took place for their plantation in spring or fall of 2020. Altogether they are approximately 100 seedlings and this way it is expected to complete the production of seed-originated FRM.

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Figures 13 and 14. Seedlings from regenerated yew trees subjected to healing in using forest container and pots.



Translocation of Pagoeta yew plant to promote connectivity of yew woods in Aralar

According to findings from the characterization surveys conducted in the Basque Country, yew trees presence and density are significant in Pagoeta, with a well-connected representative population and abundant regeneration.

Actually, yew woods of Pagoeta may be making up a metapopulation with other yew tree woods located outside the SCA area, as well as in other Natura 2000 sites close by, such as Hernio Gazume and Izarraitz.

On the contrary, yew stands in Aralar are further apart to each other and, on top of that, the younger specimens are missing and regeneration is hardly seen. It seems that the herbivorous effect and the land-use regime shift that reduced forest canopy in the past are the cause.

Apparently this scenario has not affected negatively the genetic structure of populations in Aralar and probably this is because it is too early to tell although this is clearly a negative condition for the habitat long-term conservation.

This situation, along with the preliminary outcomes of the genetic study, makes the translocation strategy of Pagoeta yew seedlings to reinforce and interconnect Aralar populations especially appealing.

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The following factors were considered in addressing[1]:

- The effect on donor population. Translocation practice should not have any impact on these donor stands in Pagoeta. To this end, seedlings were dispersedly removed all times without leaving any bald areas in the regenerated population of two types of areas:
 - High-density regenerated areas under yew trees of certain size. These seedlings will not be viable because they live under another specimen but they feature high densities, from 1 to 10 plants per square meter..
 - “Golpes de tejo” (Holes of yew trees) that include many plants with their viability at jeopardy because density itself. A number of 5 plants per square meter is always retained.
- Genetic relation. The relation between the donor and the host populations needs to be adequate from the genetic point of view. To this end, the genetic study outcomes were taken into account.
- Genetic diversity. To avoid negative genetic effects, seedlings from several donor stands will be translocated with the aim of providing the utmost genetic diversity to the translocated set.
- Viability in host conditions. For a successful translocation, translocated seedlings should be able to develop in Aralar or in other areas. To do so, translocations should be carefully implemented (extraction, nursery fattening up and plantation stages) and the causes that lead to the current scenario that continues showing active effects –namely herbivores– should be dealt with.

Taking this design as a basis, 350 seedlings were removed from 6 stands in Pagoeta. These were selected according to their genetic diversity and relation with the Gipuzkoa populations.

Removal took place in March 2019 by trying to keep as much seedling root apparatus as possible and transferring them on site to tray-containers with peat.

They were first irrigated in the same location and most of them were moved to the Diputación Foral of Gipuzkoa Arizmendi Nursing, in 2 days at most. Some of them were directly planted in Aralar in an experimental way, as early as possible.

Plants brought to the nursery were treated with fungicides. Subsequently, they remained under controlled conditions during a 6-8 month growth phase until being planted in the fall-winter of 2019, in fenced areas to be protected from herbivores

[1] Adapted from “Guidelines for Reintroductions and Other Conservation Translocations. IUCN 2013”

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