Research and Development Protocol for Chestnut Agroforestry in Spain

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1 Context

The AGFORWARD research project (January 2014-December 2017), funded by the European Commission, is promoting agroforestry practices in Europe that will advance sustainable rural development. The project has four objectives:

1. to understand the context and extent of agroforestry in Europe,
2. to identify, develop and field-test innovations (through participatory research) to improve the benefits and viability of agroforestry systems in Europe,
3. to evaluate innovative agroforestry designs and practices at a field-, farm- and landscape scale, and
4. to promote the wider adoption of appropriate agroforestry systems in Europe through policy development and dissemination.

This report contributes to the second objective. It contributes to the initial research and development protocol (Milestone 10 (3.3)) for the participative research and development network focused on the use of agroforestry in high value tree systems.

2 Background

Agroforestry with chestnut (*Castanaea sativa* Miller) is a traditional land use system in the eastern part of the Lugo province in Galicia, in North West Spain. Although chestnut groves are rarely intercropped (due to the low understorey production) or grazed (due to the fear of tree damage), the groves create a fine-grained mosaic of land uses including cropland and forests. However pig grazing does occur in some areas during the autumn and winter, where high slopes make chestnut harvesting unprofitable. Chestnut woodlands are also one of the best habitats for the commercial production of edible mushrooms.

From the initial stakeholder meeting in Galicia (Mosquera Losada et al. 2014) three innovations were identified. These were a) the evaluation of different types of tree protection at different tree ages to allow the safe introduction of pigs on a farm, b) improved mushroom production, and c) the production and testing of grafted and self-rooted plants of selected varieties of chestnut. These are considered in turn.

3 Tree protection

3.1 Background

Large sections of the adult chestnut area belongs to the Natura 2000 network, are priority areas for birds, and are included in the recovery plan for grizzly bear populations in Galicia. These legal protection measures highlight the high natural and cultural value of the area. Moreover, the chestnuts produced in this region are recognized under the label of Protected Geographical Indication (PGI), and are mainly exported to selective markets in Europe.

3.2 Plant material

The experiment will be carried out in two stands of 50 and over 100 years old placed in the municipality of O Incio (West of Lugo province) (Table 1). The private farm occupies 16.59 ha, it is fenced and Celta pigs graze at a stocking rate of 1.5 livestock units per ha. In the first stand a comparison between protected and unprotected trees below (11 to 20 cm) and over 20 cm (21 to 30 cm) will be carried out. These stands are included within the WP7 landscape modelling therefore other sampling will be carried out and used for both activities.

Table 1. Description of the site, with soil, tree, understorey, livestock, and climate characteristics

Experimental group and site characteristics			
Group	Tree protection	Mushroom production	Varietal selection
Area Co-ordinates: Site contact: Email address:	16.6 ha O Incio, Galicia 7º 55´ 26,53´´W 43º01´57,26´´N	 O Courel, Galicia To be defined	2.5 ha 2 sites in Zone II and Zone IV, Galicia To be defined

Soil characteristics			
Soil type Soil depth Soil texture	Cambisol 50 cm Sandy	To be defined	To be defined

Tree characteristics			
Tree species Tree density (spacing) Tree protection	Chestnut *Castanaea sativa* Range of selection methods	Chestnut *Castanaea sativa*	Chestnut *Castanea sativa* varieties, and hybrids of *C. sativa* x *C. crenata* and *C. sativa* x *C mollissima* 5 m x 5 m

Understorey characteristics			
Species	Bare soil *Ulex*, *Pteridium* and *Rubus* spp	Cleared understorey Inoculation with *Boletus edulis*	

Livestock characteristics			
Livestock Stocking density	Celta pigs 5 LU ha^{-1}		

Climate data	
Mean temperature	9.8°C
Annual precipitation	1232 mm
Weather station	Corno do Boi: 43.04ºN, 7,49ºW; Altitude: 731m asl

3.3 Experimental design

The use or lack of protection will be tested in a chestnut stand (over 100 years) with trees below and over 50 cm diameter at breast height (dbh). Three replicates of 10 protected and unprotected trees will be established in each case. The effect of the protection will be measured using qualitative indicators (1: undisturbed; 2: bark affected; 3: bark destroyed and 4: root affected). The tree protection will be made of metal. In both stands, each replicate will consist of protected and unprotected trees of similar diameter.

3.4 Implementation

Pig grazing is usually carried out between April and September in the 50 years old stand and between October and December in the old stands. After and before grazing all trees will be characterized and measured (dbh).

4 Improved mushroom production

4.1 Plant material

The experiment will be carried out in the Natura 2000 network of East of Galicia, where 100-year old chestnut trees occupy most of the land. This experiment in the municipality of O Courel aims to evaluate the inoculation of an adult chestnut stand with *Boletus edulis*.

4.2 Experimental design

The mycelia will be produced in the laboratory of the University of Santiago de Compostela. The inoculum was harvested at the end of 2014. In spring 2016, the tree superficial roots of different chestnut trees of two different diameters (below and above 50 cm) will be cleaned of soil, and the inoculum will be applied with a brush. The design will be randomized blocks with three replicates. The understory will be cleared. Each experimental unit will have 25 trees (Figure 1)

X	x	x	x	x
x	o	X	o	x
x	x	X	x	x
x	o	x	o	x
x	X	x	x	x

Figure 1. Plot design. White boxes with an “o” represent the trees that will be inoculated.

Around the tree and in the area where the canopy ends the inoculum will be placed following four orientation points. 40 ml of inoculum of *Boletus* will be placed in each orientation (each tree will receive 160 ml of inoculum). Inoculation will be carried out by cleaning around 20 cm^2 at each selected point until the fine roots are exposed so that they can be painted with the inoculum.

4.3 Field tests

Mushroom production in both inoculated and not inoculated trees of different diameter will be evaluated during the autumn 2016 and 2017 every week. All carpophores of the plot will be harvested, weighed in humid and dry and identification of the place of harvesting will be noted.

5 Field tests of grafted and self-rooted chestnut of high fruit quality

5.1 Background

Chestnut trees are authocthonous in Galicia. Grafted chestnuts which can produce high quality fruits were expanded by monks during the Middle Ages. Chestnuts occupied most of this region until the nineteenth century and were a key source of carbohydrate for human beings. However ink disease (*Phytophthora cambivora* or *Phytophthora cinnamomi*) destroyed most of the trees in the lower latitudes of Galicia. In the recent years, the high value of the product and the environment benefits of chestnut trees have increased the demand for new plantations in low latitudes. The profitability of these new plantations could benefit from understorey management and grazing animals.

In this region, the use of grafted plants of selected varieties of chestnut could increase the quality and the production of chestnuts. Moreover, the farmers chestnut association and industrial chestnut processors have already provided their preferences from an economic perspective. Some of the selected varieties have created interest among farmers, but which varieties are most suitable for which areas is still unknown. The technique of micrografting (both in vivo and in vitro) could permit the production of a great number of grafted plants in short time periods. On the other hand, the use of self-rooted plants of varieties with a good rooting ability could help rapid establishment and carbon sequestration in ink-disease-free areas. This innovation is in line with measure 222 of the Rural Development Programme dealing with the establishment of agroforestry systems.

Hence the objective of the third set of experiments is to produce and test grafted and self-rooted plants of Galician chestnut varieties that have been selected for fruit quality.

5.2 Plant material

The plant material used for this experience consists of explants (microscions/microcuttings) of five selected varieties of chestnut (*Castanea sativa*) trees, obtained from forced branch segments, plantlets of four clones of hybrid chestnut (*Castanea sativa* x *C. crenata*), rooted in vitro to be used as rootstocks (Table 2), and seedlings of *C. sativa* germinated in growth chamber, to be used as rootstocks for in vivo micrografting.

Table 2. *Castanea sativa* varieties, used as scions, or tested for *in vitro* self-rooting, and hybrid (*Castanea sativa* x *C. crenata*; or *C. sativa* x *C mollissima*: clone 7521) rootstocks (resistant to ink disease), to be used in the experiment.

Varieties	Hybrid rootstocks
Famosa	7521[3]
Inxerta	111[3]
Parede	3[4]
Negral[1,2]	7810[4]
Longal	
(Loura)	

[1]Self-compatible variety; [2]Used both as fruit producers and pollinizers; [3]proved compatibility for grafting; [4]positive evidence of compatibility for grafting.

Grafting procedure: The following grafting procedure has been developed based on the work by Fernández-Lorenzo and Fernández-López (2005) and Fernández-Lorenzo and Crecente (2011) (Table 3).

a) Branch segments of varieties will be "forced" in a growth chamber in order to obtain shoots suitable for i) in vitro multiplication and rooting and ii) grafting as scions. Alternatively, shoots will be directly taken from plants growing in the field to be used as explants.
b) 1-2 month old acclimatized in vitro rooted hybrids or 1-2 month old *C. sativa* seedlings will be used as rootstocks. Scions obtained in step a) will be grafted onto the rootstock using micro-cleft-grafting.
c) 40 to 60 days later, the elongated scions of successful grafts will be cut into new portions (3-5) and regrafted onto new rootstocks, in a cyclic process which maximises the number of grafted plants within a limited time period.

Table 3. The implementation plan for the varietal trials

Task/Event	Date
Acquisition of plants and seeds. Storage of seeds	Oct 2013-Feb 2014
Collecting and forcing of branch segments (to get explants/scions)	Feb-Apr 2015
In vitro introduction	Mar-June 2015
Seed sowing and Collecting and forcing of branch segments (to get scions)	Feb-May 2015
Availability of acclimatized hybrids and seedlings as rootstocks/ First grafting	June-Aug 2015
Serial grafting	Aug-Dec 2015
Availability of acclimatized self-rooted varieties	Aug-Dec 2015
Establishment in the field	Jan-April 2016
Data collection	Aug 2016-Dec 2017

In vitro rooting of varieties: in vitro multiplied microshoots of the varieties will be tested for self-rooting. At least two of the varieties, Parede and Loura, have achieved a high proportion of successful in vitro rooting in previous experiments. Plantlets of varieties suitable for self-rooting will be acclimatized.

Field tests: both grafted plants and self-rooted plants will be tested in the field by establishing pilot plantations, both in sites with high (varieties grafted on hybrids) and low risk (varieties grafted on seedlings and self-rooted varieties) of ink-disease infection.

5.3 Experimental design

The experiment will take place across 2 sites: one in Zone II (Interior North; medium risk) and one in Zone IV (eastern mountains; low risk).

- Maximum of 30 combinations (20 combinations variety/hybrid + 5 combinations variety/seedling + 5 combinations self-rooted varieties)
- 30 (6 x 5) plants (each representing one combination)/block in 15 random blocks: each combination is represented 15 times per site
- Planting distance: 5 x 5 m
- Block surface: 750 m^2
- Plantation surface per site (15 blocks)= 11250 m^2 (1.125 ha)

5.4 Measurements

Measurements are planned for the laboratory and the field (Table 4).

Table 4. Measurements planned for the laboratory and the field

Location	Planned measurements
Laboratory	Proportion in vitro rooting (%); Proportion survival after acclimatisation (%) Proportion of successful grafts (%) Duration of serial grafting cycle Mean number of scions per cycle.
Field	Graft compatibility, Growth in scion length Growth in diameter of scion and rootstock after 6, 12 and 22 months.

6 Acknowledgements

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7 References

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