Lessons learnt:
Chestnut agroforestry in Galicia, Spain

Project name	AGFORWARD (613520)
Work-package	3: Agroforestry for High Value Trees
Specific group	Chestnut agroforestry in Galicia, Spain
Deliverable	Contribution to Deliverable 3.8: Agroforestry for high value tree systems: Results of innovations
Date of report	20 January 2018
Authors	Rosa Mosquera-Losada, José Javier Santiago Freijanes, Nuria Ferreiro-Domingues and Antonio Rigueiro-Rodriguez
Contact	mrosa.mosquera.losada@usc.es
Approved	Anastasia Pantera

Contents

AGFORWARD (Grant Agreement N° 613520) is co-funded by the European Commission, Directorate General for Research & Innovation, within the 7th Framework Programme of RTD. The views and opinions expressed in this report are purely those of the writers and may not in any circumstances be regarded as stating an official position of the European Commission.

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 field-, farm- and landscape scales, and
4. to promote the wider adoption of appropriate agroforestry systems in Europe through policy development and dissemination.

This report contributes to Objective 2 in that it focuses on the field-testing of an innovation within the "agroforestry for high value tree systems" participative research and development network. This report contributes, together with reports, to Deliverable 3.8: Agroforestry for high value tree systems: results of innovations.

2 Background

The initial stakeholder report (Mosquera-Losada et al. 2014), the research and development protocol (Fernández-Lorenzo et al. 2015), and the system description report (Fernández-Lorenzo et al. 2016) provide background data on the establishment of agroforestry systems under chestnut (*Castanaea sativa* Miller) in Galicia in north west Spain.

Agroforestry with chestnut (*Castanaea sativa*) is a traditional land use system in the eastern part of Lugo (Galicia, NW 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 (Rigueiro-Rodríguez et al. 2014). Chestnut woodlands are also one of the best habitats for the commercial production of edible mushrooms.

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 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. However, in this region, the use of grafted plants of selected varieties of chestnut could increase the quality and the production of chestnuts (Fernández-López 2013). 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 (Fernández-Lorenzo and Fernández-López 2005; Fernández-Lorenzo and Crecente 2010). 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.

3 Objectives

Three experiments were established to improve our capacity to manage successful agroforestry systems with chestnut trees. The objectives of the three experiments were:

- Experiment 1: to evaluate the damage caused by Celtic pigs to protected and unprotected chestnut trees to allow the safe introduction of pigs on the farms
- Experiment 2: to evaluate the inoculation of an adult chestnut stand with mushroom *(Boletus edulis)*
- Experiment 3: to produce and test grafted and self-rooted plants of selected varieties of chestnut trees

4 Methodology

4.1 Experiment 1: Tree protection

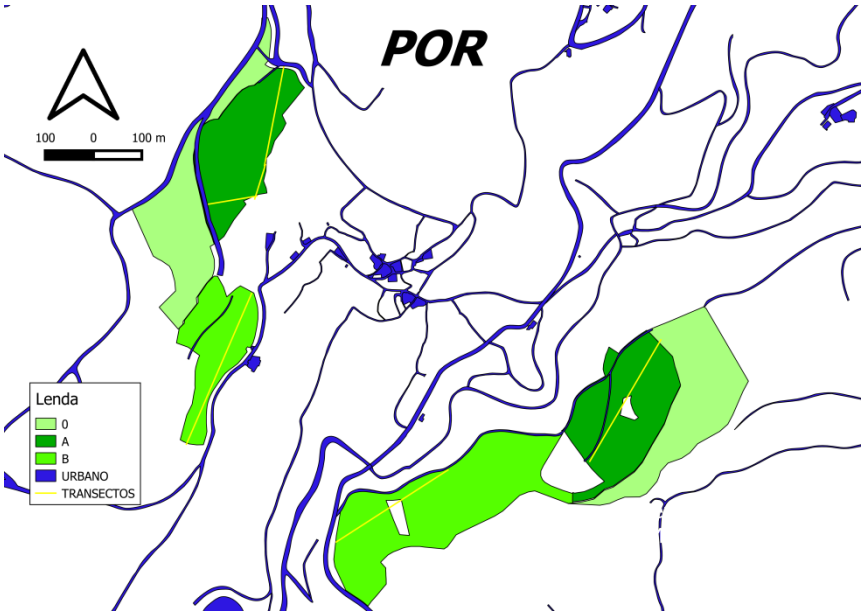
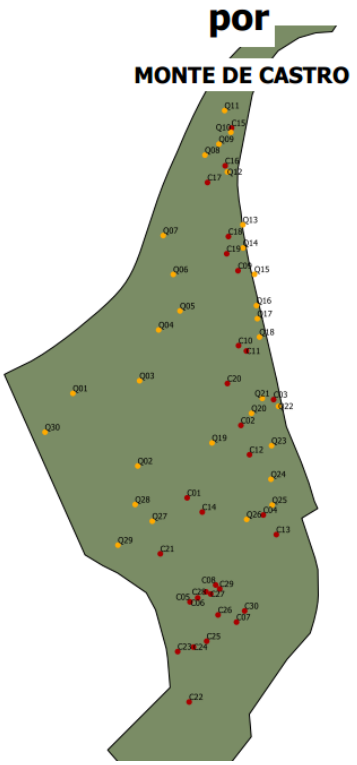
The experiment was carried out in two chestnut stands placed in the municipality of O Incio (West of Lugo province). A specific description of the established experiment is provided in Table 1.

The damage caused by the Celtic pigs to protected and unprotected chestnut trees was tested in two chestnut stands in 2016 and 2017. In each stand a total of thirty trees were evaluated. Previously the trees were classified in diameter classes according to their diameter at breast height (20-40 cm, 40-50 cm, 50-60 cm, 60-80 cm and >80 cm). However, this report only shows the results obtained in the diameter class of 60-80 cm because this is the most representative diameter class of the chestnut stands evaluated in this study. Therefore, in this experiment the treatments comprised protected and unprotected trees of similar diameter. The effect of the tree protection was measured using qualitative indicators (0: undisturbed; 1: bark/root little affected; 2: bark/root moderate affected; 3 or higher than 3: bark/root very affected). The tree protection was made of metal.

Data were analysed using ANOVA. The statistical software package SPSS was used for all analyses.

Table 1. Specific description of the experiment

Specific description of site	
Area	16.6 ha
Co-ordinates	42°40'52,11''N, 7°23'23,14''W
Site contact	University of Santiago de Compostela: María Rosa Mosquera Losada
Site contact email	mrosa.mosquera.losada@usc.es
Example photographs	

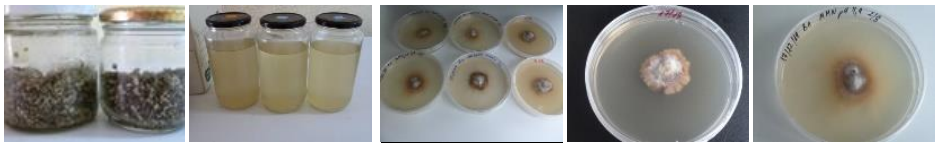
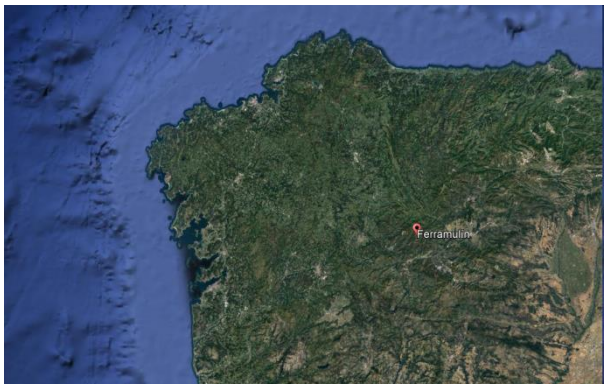
Map of system	 
Climate characteristics	
Mean monthly temperature	12.2°C
Mean annual precipitation	824 mm
Details of weather station (and data)	"Boveda" weather station http://www2.meteogalicia.gal/galego/observacion/estacions/estacionsHistorico.asp?Nest=10099

Soil type	
Soil type	Leptosol
Soil depth	50 cm
Soil texture	Loam
Additional soil characteristics	Water soil pH: 5.3
Aspect	Umbra (opposite to light orientation – when the slope is north)
Tree characteristics	
Species and variety	*Castanea sativa*
Date of planting	The experiment was carried out in two stands of 50 and over 100 years old
Intra-row spacing	The chestnut stands come from natural regeneration, so there is not regular plantation pattern
Inter-row spacing	The chestnut stands come from natural regeneration, so there is not regular plantation pattern
Tree protection	The use and the lack of tree protection were tested as treatments
Typical increase in tree biomass	12 m^3 ha^{-1} $year^{-1}$
Crop/understorey characteristics	
Species	*Ulex, Pteridium and Rubus* spp
Management	None
Fertiliser, pesticide, machinery and labour management	
Fertiliser	None
Pesticides	None
Machinery	None
Manure handling	None
Labour	Four people to establish the experiment, two people to visit the experiment all weeks and two people to carry out the field samplings
Fencing	Not required
Livestock management	
Species and breed	Celtic pig
Date of entry to site	April in the 50 years old stand and October in the old stand
Date of departure from site	September in the 50 years old stand and December in the old stand
Stocking density	1.5 livestock units per ha
Animal health and welfare issues	Pigs need to be regularly checked to ensure health and welfare
Annual mortality rate	0
Requirement for supplementary feed	Yes, during the previous period of pig fattening. They are fed with a mean amount of concentrate consumption of 2.5 kg per pig per day
Typical level of pig production	Animals are usually killed when their weight reaches 100 kg

4.2 Experiment 2: Mushroom production

The second experiment was carried out in in two chestnut stands established in O Courel (Galicia, NW Spain). Table 2 provides a specific description of the established experiment.

Table 2. Specific description of the experiments

Specific description of site	
Area	0.5 ha
Co-ordinates	42°34'31,66''N, 7°3'16,06''W
Site contact	University of Santiago de Compostela: María Rosa Mosquera Losada
Site contact email	mrosa.mosquera.losada@usc.es
Example photograph	Mycelia produced in the laboratory of the University of Santiago de Compostela  Inoculation of the chestnut trees 
Map of system	Location of the system 
Climate characteristics	
Mean monthly temperature	11.9°C
Mean annual precipitation	905 mm

Details of weather station (and data)	"Folgoso do Caurel" weather station http://www2.meteogalicia.gal/galego/observacion/estacions/estacionsHistorico.asp?Nest=10102
Soil type	
Soil type	Leptosol
Soil depth	50 cm
Soil texture	Sandy Loam
Soil pH	Water soil pH: 4.54
Tree characteristics	
Species and variety	*Castanea sativa*
Date of planting	The experiment was carried out in two stands of 35 years old
Intra-row spacing	3 m and 5 m
Inter-row spacing	3 m and 5 m
Tree protection	none
Typical increase in tree biomass	12 m^3 ha^{-1} $year^{-1}$
Crop/understorey characteristics	
Species	Cleared understorey
Management	Inoculation with *Boletus edulis*
Typical crop yield	200 kg of boletus ha^{-1} $year^{-1}$
Fertiliser, pesticide, machinery and labour management	
Fertiliser	None
Pesticides	None
Machinery	None
Manure handling	None
Labour	Four people to establish the experiment, two people to visit the experimental site all weeks and two people to carry out the field samplings.
Fencing	Not required
Livestock management	
Species and breed	None

The mycelia were produced in the laboratory of the University of Santiago de Compostela. The inoculum was harvested at the end of 2014. In April 2016, the first inoculation was carried out in a 35 year old chestnut stand (3 m x 3 m). The inoculum was placed around the trees and in the area where the canopy ends following four orientation points. In each orientation 40 ml of inoculum of *Boletus edulis* was placed (each tree received 160 ml of inoculum). The inoculation was applied by cleaning around 20 cm^2 at each selected point until the tree fine roots were exposed so that they could be painted with the inoculum. The inoculation was repeated in November 2016 in another chestnut stand (5 m x 5 m) of the same age. A total of six trees were inoculated in each chestnut stand.

Mushroom production in the inoculated and in non-inoculated trees was evaluated during the autumn 2016 and 2017 every week in both chestnut stands.

4.3 Experiment 3: Grafted and self-rooted chestnut of high fruit quality

The plant material used in this experiment consisted of explants (microscions/microcuttings) of five selected varieties of chestnut trees (*Castanea sativa*), obtained from forced branch segments, plantlets of four clones of hybrid chestnut (*Castanea sativa* x *Castanea crenata*), rooted in vitro used as rootstocks (Table 3), and seedlings of *Castanea sativa* germinated in growth chamber, used as rootstocks for in vivo micrografting.

Table 3. *Castanea sativa* varieties, used as scions, or tested for *in vitro* self-rooting, and hybrid (*Castanea sativa* x *Castanea crenata*; or *Castanea sativa* x *Castanea 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.

The grafting procedure was developed based on the work of Fernández-Lorenzo and Fernández-López (2005) and Fernández-Lorenzo and Crecente (2011) (Table 4):

a) Branch segments of varieties were "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 could 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 *Castanea sativa* seedlings were used as rootstocks. Scions obtained in step a) were grafted onto the rootstock using microcleft-grafting.
c) 40 to 60 days later, the elongated scions of successful grafts were 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. Implementation plan for the varietal trials

Varieties	Hybrid rootstocks
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 multiplied microshoots of the varieties were tested for self-rooting. At least two of the varieties, Parede and Loura, achieved a high proportion of successful in vitro rooting in previous experiments. Plantlets of varieties suitable for self-rooting were acclimatized.

In 2018 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 Results

5.1 Experiment 1: Tree protection

Figure 1 shows that in 2017 the damage caused by the Celtic pigs to the bark of the chestnut trees with a diameter between 60-80 cm was higher in the unprotected trees (NP) compared with the protected trees (P) ($p < 0.05$). This trend was also observed in 2016 and demonstrated the positive effect of the metal protectors on the tree protection. In each case the damage caused by the Celtic pigs to the bark of the trees was lower than 1 which means that the chestnut bark was little affected by the presence of the Celtic pigs and the trees could recover from this damage over time as was shown in other experiments. In the case of the roots, the results were not significant ($p > 0.05$). In this study, it is important to be aware that the Celtic pig is an autochthonous species which could be classified as semi-domestic and probably the damage caused to the trees by this pig species is higher than that caused by commonly used domestic species (e.g. large white). Therefore, other domestic species of pigs should be tested in the field to check its compatibility with the chestnut trees growth.

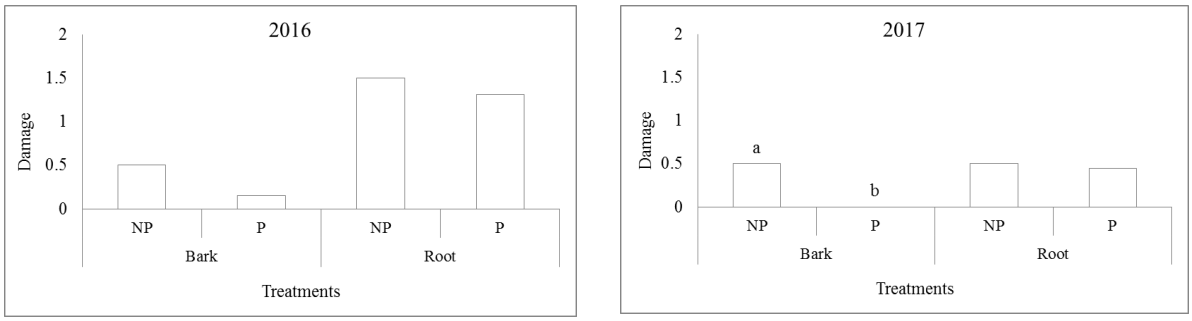


Figure 1. Mean damage caused by the Celtic pigs to chestnut trees with a diameter at breast height between 60-80 cm. NP: trees without protector and P: trees with protector. 0: undisturbed; 1: bark/root little affected; 2: bark/root moderate affected; 3 or higher than 3: bark/root very affected. Different letters indicate significant differences between protected and unprotected trees.

In Experiment 1, it was observed that the bark of more than the 80% of the chestnut trees with protectors was not damaged by the Celtic in both years after animals entered the plots, which indicate that the metal protectors are very useful to avoid bark damage on the chestnut trees by the Celtic pigs. However, in 2016 it seems that the metal protectors did not protect the tree roots from the damage caused by the Celtic pigs because about 77% (1: 38.5%; 2: 23.1% and 3: 15.4%) of the trees suffered some kind of damage in their roots. Therefore, other type of protectors should be tested to protect the tree roots when chestnut stands are grazed by Celtic pigs. This root damage is more related with trampling because of the high step of the plots. Probably the damage will be lower in flat sites.

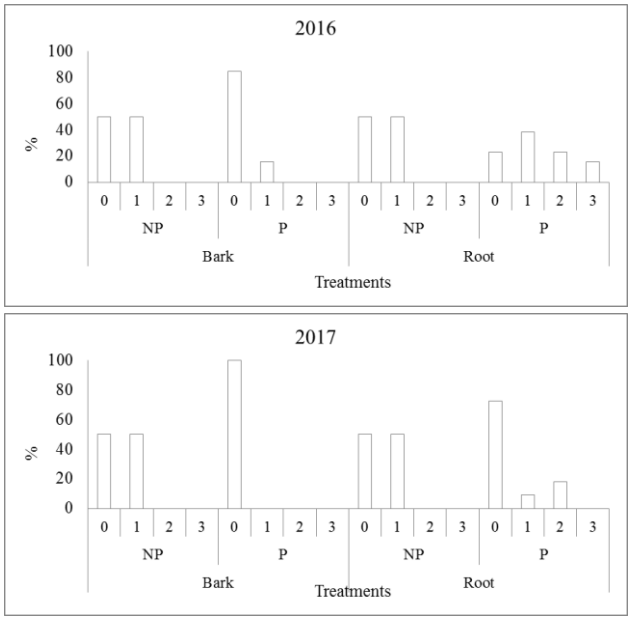


Figure 2: Proportion (%) of chestnut trees with a diameter at breast height between 60-80 cm damaged by the Celtic pigs. NP: trees without protector and P: trees with protector. 0: undisturbed; 1: bark/root little affected; 2: bark/root moderate affected; 3 or higher than 3: bark/root very affected.

5.2 Experiment 2: Mushroom production

Mushroom production was not successful probably due to the climatic conditions with long periods of drought which could have limited the mushroom growth.

5.3 Experiment 3: Grafted and self-rooted chestnut of high fruit quality

The first results showed that micrografting success ranged from 40 to 75%, depending on the variety/hybrid combination. When using wild chestnut seedlings for serial grafting, grafting success was near to 100% in many cases. The potent for production of grafted chestnuts is extremely high: grafting cycles of 60 days and an average yield of four scions per cycle permit one single initial scion to be used to produce more than 1 x 10^6 grafted plants after 20 months under growth chamber conditions. In addition, there is a need for suitable preservation systems for chestnut seeds, for seedlings to be available all along the year to be used as rootstocks. Plants from some varieties, as 'Loura' and 'De Parede' have been successfully produced from microcuttings, but their further performance in the field is still unknown. Field tests of all these materials will enable the evaluation of possible longer term problems of delayed incompatibility when using hybrid rootstocks, and to reveal if the low height of the grafting point in micrografting, which will be very near to the soil, can have any incidence in the risk of infection of the grafted variety by *Phytophthora* spp. (causing 'ink disease').

6 Conclusions

The principal lessons learnt from the measurements and observations include:

- Experiment 1: Tree protection
  Metal protectors protected the bark of the chestnut trees from damage caused by the Celtic pigs. However in the case of the roots other type of protectors should be tested. Probably if the plots were flat and the pig species introduced in the chestnut stands were a domestic species instead of Celtic pig the damage observed would have been much lower. It is important to repeat this experiment with other pig species and in flatter areas.

- Experiment 2: Mushroom production
  The production of *Boletus edulis* depends among other factors on the climatic conditions. The two years of the experiment before harvesting included two of the most severe periods of drought ever recorded and this limits the evaluation of the results in this experiment. This could mean that mushroom production in chestnut stands will be reduced if severe droughts become more common because of climate change. Irrigation could be provided to enhance production but its cost-effectiveness would need to be determined.

- Experiment 3: Grafted and self-rooted chestnut of high fruit quality
  The production in the laboratory of grafted chestnut plants and self-rooted chestnut plants has been successful. Field tests of all these materials can be used to evaluate possible longer-term problems related with the risk of infection of the chestnut varieties by *Phytophthora* spp.

7 Acknowledgements

The AGFORWARD project (Grant Agreement N° 613520) is co-funded by the European Commission, Directorate General for Research & Innovation, within the 7th Framework Programme of RTD, Theme 2 - Biotechnologies, Agriculture & Food. The views and opinions expressed in this report are purely those of the writers and may not in any circumstances be regarded as stating an official position of the European Commission.

8 References

Fernández-Lorenzo JL, Fernández-López MJ (2005). Reinvigoration of mature Castanea sativa by micrografting onto a juvenile clone. Acta Horticulturae 693: 293-298.

Fernández-Lorenzo JL, Crecente S (2010). In vivo serial micrografting of Castanea sativa in short cycles. Acta Horticulturae 866: 291-296

Fernández-López, JL (2013). Variedades para os soutos novos. Ed. Xunta de Galicia. 38 pp.

Fernández Lorenzo JL, Rigueiro Rodríguez A, Ferreiro Domínguez N, González Hernández P, Burgess P, Mosquera Losada MR (2015). Research and Development Protocol for Chestnut Agroforestry in Spain.
http://www.agforward.eu/index.php/en/chestnut-agroforestry-in-galicia-spain.html

Fernández Lorenzo JL, Rigueiro Rodríguez A, Ferreiro Domínguez N, González Hernández P, Mosquera Losada MR (2016). System Report: Chestnut Agroforestry in Spain.
http://www.agforward.eu/index.php/en/chestnut-agroforestry-in-galicia-spain.html

Mosquera Losada MR, Ferreiro-Domínguez N, Fernández Lorenzo JL, González-Hernández P, Rigueiro Rodríguez A (2014). Initial Stakeholder Meeting Report Chestnut agroforestry in Galicia, Spain. http://www.agforward.eu/index.php/en/chestnut-agroforestry-in-galicia-spain.html

Rigueiro-Rodríguez A, Santiago-Freijanes JJ, Ferreiro-Domínguez N, González-Hernández MP, Mosquera-Losada MR (2014). Celtic Pig production in Chestnut extensive systems in Galicia. 2nd European Agroforestry Conference. Cottbus, Germany.