Lessons learnt –
Fodder tree evaluation in Galicia, Spain

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Specific group	Forage trees: protein source from Morus species for livestock feeding in Galicia, Spain
Deliverable	Contribution to Deliverable 5.14: Lessons learned from innovations related to agroforestry for livestock
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Contents

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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 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 livestock systems" participative research and development network. This report contributes to Deliverable 5.14: Lessons learned from innovations in agroforestry systems.

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 (Mosquera-Losada et al. 2016) provide background data on the evaluation of the new sources of fodder for livestock in Galicia (NW Spain).

Under free-range conditions, livestock might not always have access to a balanced diet and the introduction of new crops in the system such as *Morus alba* or *Morus nigra* could represent an economically interesting alternative, or supplementary, source of feed. Mulberry (*Morus* sp) is used as fodder in several countries around the world such as Costa Rica, Cuba and Ethiopia (Benavides 1999). The leaves of the mulberry are known for its high protein content (15-28%) with good amino acid profile, high digestibility, high mineral content, low fibre content and very good palatability (Sánchez 2000). Moreover, the high biomass yield of the plant together with its low tannin content (Patra et al. 2002) makes it an attractive fodder resource for livestock, particularly, as a supplement to low quality diets.

There are mulberry varieties for many environments, from sea level to altitudes of 4000 m (FAO 1990), and from the humid tropics to semi-arid lands, such as in the Near East with 250 mm of annual rainfall and the south-western United States (Tipton 1994). Against this background, we conducted studies with cultivars, which have high value as a feed (e.g. digestibility and protein content) but were derived from different climate and soil conditions with the objective:

- to determine the adaptation, productivity and fodder quality of four clones of mulberry (*Morus alba Criolla*; *Morus alba Tigrenda*, *Morus alba Illaverde* and *Morus nigra*) in the temperate region of northwest Spain.

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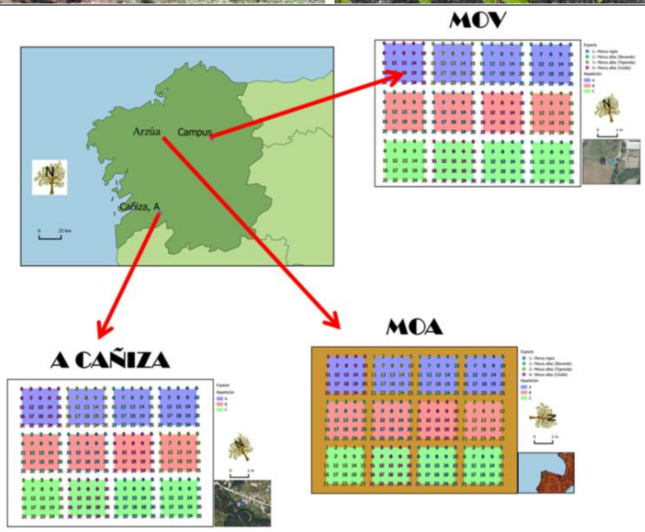
3 Methodology

The plant material consisted of four clones of mulberry (CR: *Morus alba criolla*; TI: *Morus alba tigrenda*, IL: *Morus alba illaverde* and MN: *Morus nigra*). All clones were established in vitro from explants obtained from forced shoots of branch segments. Clones CR and TI are two Cuban cultivars, from which microshoots were maintained in vitro for more than ten years (Fernández-Lorenzo et al. 2005). Clones MN and IL are Galician clones, and were established in vitro in 2010 (Costoya, 2011), and 2014 (Martínez-Cabaleiro 2017), respectively. Microshoots of the in vitro stocks of the four clones were multiplied and rooted in vitro, and finally acclimatized, in order to obtain a sufficient number of propagules to carry out the experiments in the field. Some of the plants of clones IL and CR were also obtained by cutting propagation (Martínez-Cabaleiro 2017).

A randomized block design (three blocks x clone) comprising the four clones of mulberry was set up in three sites of Galicia, NW Spain (A Cañiza, Arzúa and Lugo) with different climatic conditions, in autumn 2015. In each field plot (200 cm x 200 cm), 25 plants were planted at a planting distance of 50 cm x 50 cm

A specific description of the established experiments is provided in Table 1.

Table 1. Specific description of the experiments

Specific description of sites	
Area	Total area 144 m^2
Co-ordinates	Site 1: A Cañiza (42°14'2.7'' N, 8°17'13.8''W) Site 2: Arzúa (42°58'30"N, 8°11'24"W) Site 3: Campus de Lugo (42°59'31.15"N, 7°32'47.82"W)
Site contact	University of Santiago de Compostela: María Rosa Mosquera Losada
Site contact email	mrosa.mosquera.losada@usc.es
Example Photograph	 
Map of system	

Climate characteristics	
Mean monthly temperature	Site 1 A Cañiza: 12.3°C Site 2 Arzúa: 12.6°C Site 3 Campus de Lugo: 11.5°C
Mean annual precipitation	Site 1 A Cañiza: 1421 mm Site 2 Arzúa: 1989 mm Site 3 Campus de Lugo: >1000 mm
Details of weather station (and data)	Site 1: "Queimadelos" weather station (http://www2.meteogalicia.es/galego/observacion/estacions/estacionsinfo.asp?Nest=10063&red=102&tiporede=&idprov=3) Site 2: "Boimorto" weather station (http://www2.meteogalicia.es/galego/observacion/estacions/estacionsHistorico.asp?Nest=19062&prov=A%20Coru%F1a&tiporede=automaticas&red=102&idprov=0#) Site 3: "Campus de Lugo" weather station (http://www2.meteogalicia.es/galego/observacion/estacions/estacionsHistorico.asp?Nest=19062&prov=A%20Coru%F1a&tiporede=automaticas&red=102&idprov=0#)
Soil type	
Soil type	Humic cambisol
Soil depth	Over 1 m
Soil texture	Site 1 A Cañiza: sandy loam (77.3% sand; 16.4% silt; 6.3% clay) Site 2 Arzúa: silty loam (42.3% sand; 41.1% silt; 16.6% clay) Site 3 Campus de Lugo: loamy (30.3% sand; 59.5% silt; 10.2% clay)
Additional soil characteristics	Site 1 A Cañiza: water soil pH = 5.31 Site 2 Arzúa: water soil pH = 5.25 Site 3 Campus de Lugo: water soil pH = 6.82
Aspect	Site 1 A Cañiza: North-South Site 2 Arzúa: North-South Site 3 Campus de Lugo: East-West
Tree characteristics	
Species and variety	*Morus alba criolla* (CR) from Cuba (in vitro/cuttings) *Morus alba tigrenda* (TI) from Cuba (in vitro) *Morus alba illaverde* (IL) from Lugo, Galicia, NW Spain (in vitro/cuttings) MN: *Morus nigra* (MN) from Ourense, Galicia, NW Spain (in vitro)
Date of planting	2015
Intra-row spacing	50 cm
Inter-row spacing	50 cm
Tree protection	none
Fertiliser, pesticide, machinery and labour management	
Fertiliser	None
Pesticides	None
Machinery	Machinery for soil preparation
Manure handling	None
Labour	Four people to establish the experiments, two people to visit the experimental sites all weeks and two people to harvest and process the samples
Fencing	Not required

Tree height, crown and base diameter were measured using a metric tape and a calliper, respectively, on each individual plant when the plants were established in the field (2015) and in September 2016. Plant survival checks were also done at this stage. To determine dry matter yield and protein content of leaves and stems, in the nine central plants of each plot, one shoot from each plant was taken. In the laboratory, dry matter yield was calculated after oven drying plant shoot samples at 45°C until constant weight. The crude protein concentration was determined by using the Kjeldahl method and estimated by multiplying Kjeldahl-nitrogen by a conversion factor of 6.25 (Whitehead, 1995). Data were analysed using ANOVA and differences between averages were shown by the LSD test, if ANOVA was significant. The statistical software package SAS (2001) was used for all analyses.

4 Results

4.1 Growth of the mulberry clones (total height, base diameter and shoot diameter)

The four clones of mulberry tested in this experiment showed a good survival rate ranging from 93 to 100% after a year of being planted, with no significant differences among cultivars ($p>0.05$). However, not all mulberry clones showed the same growth capacity (Table 2). In general, *M. alba tigrenda* (TI) and *M. alba criolla* (CR) presented a higher growth (total height, shoot and base diameter) than *M. alba Illaverde* (IL) and *M. nigra* (MN) ($p<0.001$). Moreover, the highest growth of mulberry clones was found in A Cañiza compared with the other sites (Arzúa and Lugo) ($p<0.001$).

Table 2. Total height growth, base diameter growth and shoot diameter growth for *M. alba criolla* (CR), *M. alba illaverde* (IL), *M. nigra* (MN) and *M. alba tigrenda* (TI) in the different sites (Arzúa, A Cañiza and Lugo). Different letters indicate significant differences between clones in each site.

			Total height growth (cm)	Base diameter growth (cm)	Shoot diameter growth (mm)
Arzúa	*M. alba criolla*	CR	71.8 ± 7.25 a	6.5 ± 0.5 a	3.5 ± 0.3 a
	M. alba illaverde	IL	35.4 ± 4.19 b	4.7 ± 0.6 b	1.7 ± 0.3 b
	M. nigra	MN	5.7 ± 1.1 c	0.5 ± 0.2 b	1.4 ± 0.2 b
	M. alba tigrenda	TI	72 ± 5.83 a	7.2 ± 0.6 a	3.8 ± 0.3 a
Cañiza	*M. alba criolla*	CR	88.5 ± 3.66 a	9.3 ± 0.9 b	3.9 ± 0.3 b
	M. alba illaverde	IL	61.4 ± 2.7 b	5.9 ± 0.6 c	1.6 ± 0.3 c
	M. nigra	MN	26.9 ± 1.66 c	6.8 ± 0.6 b	3.7 ± 0.1 b
	M. alba tigrenda	TI	98.1 ± 7.21 a	11.8 ± 1.0 a	5.6 ± 0.4 a
Lugo	*M. alba criolla*	CR	33.4 ± 3.68 a	2.7 ± 0.4 a	1.2 ± 0.2 a
	M. alba illaverde	IL	36.4 ± 3.75 a	3.0 ± 0.6 b	1.1 ± 0.3 a
	M. nigra	MN	19.1 ± 1.67 b	5.3 ± 0.3 b	0.6 ± 0.1 a
	M. alba tigrenda	TI	29.9 ± 2.59 a	3.2 ± 0.3 a	0.7 ± 0.1 a
Means by clone	*M. alba criolla*	CR	64.6 ± 3.94 a	6.2 ± 0.5 b	2.9 ± 0.2 a
	M. alba illaverde	IL	44.3 ± 2.47 b	4.5 ± 0.4 c	1.4 ± 0.2 b
	M. nigra	MN	16.7 ± 1.32 c	4.1 ± 0.4 c	1.8 ± 0.2 b
	M. alba tigrenda	TI	59.8 ± 4.31 a	6.4 ± 0.5 a	2.9 ± 0.3 a
Means by site	Arzúa		46.4 ± 3.67 b	4.8 ± 0.4 b	2.6 ± 0.2 b
	Cañiza		65.6 ± 3.36 a	8.0 ± 0.4 a	3.4 ± 0.2 a
	Lugo		29.7 ± 1.63 c	3.6 ± 0.2 c	0.9 ± 0.1 c

4.2 Yield of the mulberry clones

Figure 1 shows that *M. alba* clones from Cuba (CR and TI) and Galicia (IL) presented significantly higher yields compared with *M. nigra* (MN) ($p<0.001$). In Arzúa, the yield of *M. nigra* (MN) is not shown because the plants were too small to carry out the harvest.

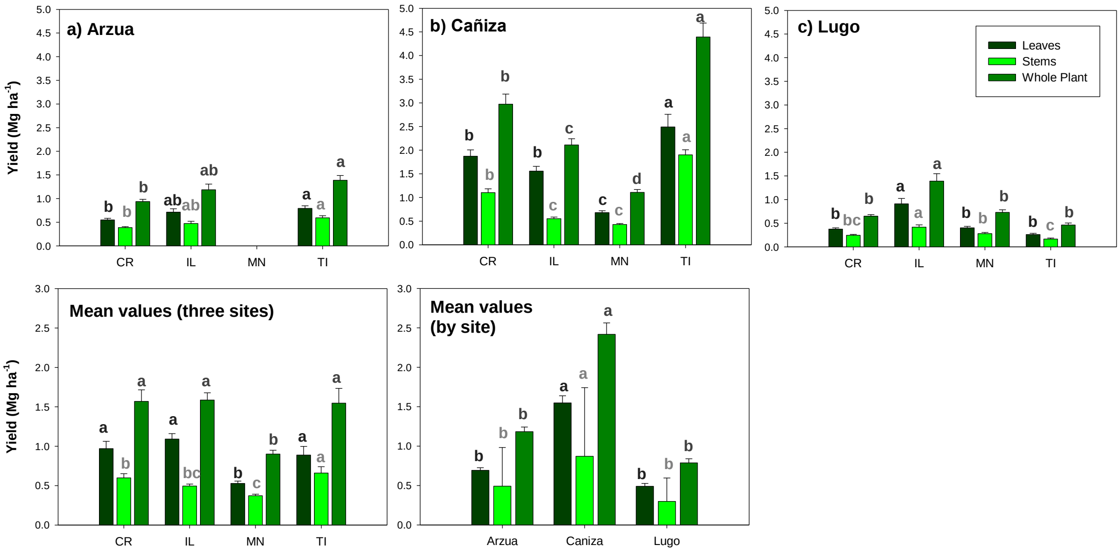


Figure 1. Yield (Mg ha^{-1}) for *M. alba criolla* (CR), *M. alba illaverde* (IL), *M. nigra* (MN) and *M. alba tigrenda* (TI) in each site (Arzúa, A Cañiza and Lugo). Different letters indicate significant differences between clones. Vertical lines indicate mean standard error.

4.3 Crude protein in the mulberry clones

Despite the differences in growth and yield between clones, no significant differences were found when their leaf and stem crude protein concentration was tested (Figure 2), showing thus no clear differences in fodder quality between mulberry clones ($p>0.05$). Moreover, the concentration of crude protein in *M. nigra* (MN) established in Arzúa is not shown because the plants were too small to carry out the harvest. However, the concentration of crude protein in the mulberry clones was higher in A Cañiza compared with the other sites (Arzúa and Lugo) ($p<0.001$). In any case, the leaves and stems crude protein concentrations are within the range of other studies reporting young leaves and stems crude protein values (5 to 27%) from several mulberry varieties (Sánchez 2000). The leaves crude protein values from this study are similar to other crude protein concentrations from fodder trees (e.g. black alder, ash and hazel) grown in Europe (Emile et al. 2016) and also to the values obtained (8 to 18%) during spring in pasture under silvopastoral systems in the same area (Rigueiro-Rodríguez et al. 2007). Therefore, this study shows the promising potential of using mulberry trees as a forage resource to complement pasture under temperate agroforestry systems.

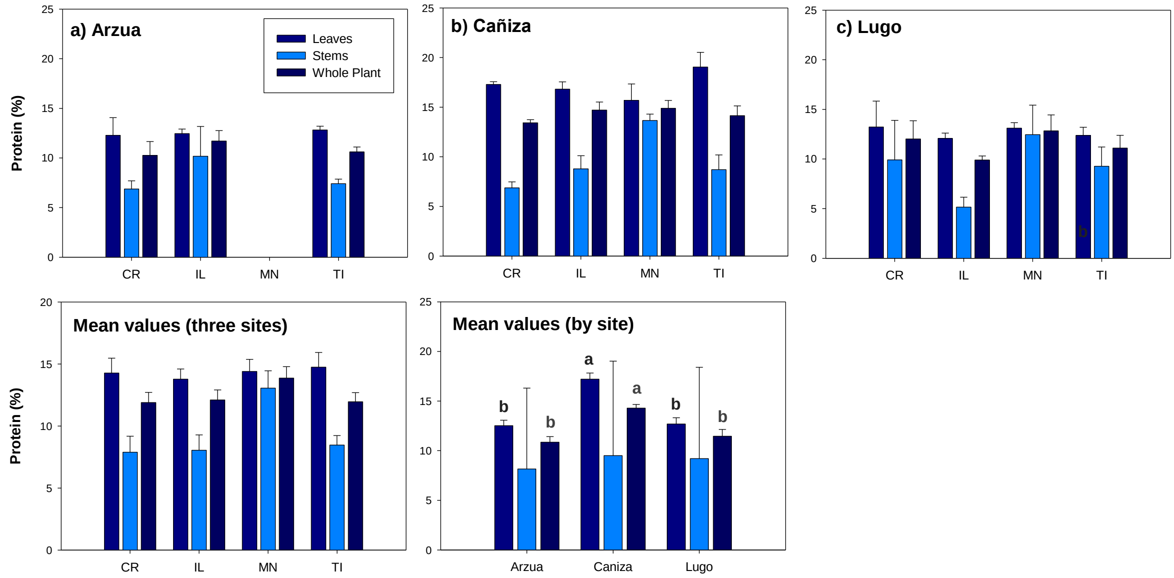


Figure 2. Crude protein (%) in the leaves, stems and whole plant of *M. alba criolla* (CR), *M. alba illaverde* (IL), *M. nigra* (MN) and *M. alba tigrenda* (TI) in each site (Arzúa, A Cañiza and Lugo). Different letters indicate significant differences between clones. Vertical lines indicate mean standard error.

The results are described in more detail in: Mosquera-Losada MR, Fernández-Lorenzo JL, Ferreiro-Domínguez N, González-Hernández P, Hermansen JE, Villada A, Rigueiro-Rodriguez A (2017) Mulberry (*Morus* spp.) as a fodder source to overcome climate change. 19th Symposium of European Grassland Federation, Sardinia, Italy.

5 Conclusions

The principal lessons learnt from the measurements and observations from Morus species for livestock feeding include:

- Cuban-source mulberries (*M. alba tigrenda* and *criolla*) presented the highest growth (total height, shoot and base diameter) during the first year of establishment when compared to Galician-source mulberries (*M. alba illaverde* and *M. nigra*).
- *M. alba* clones (both Cuban and Galician-source) presented significantly higher yields when compared to *M. nigra*.
- The different *Morus* clones showed no significant difference in protein contents (ranging from 9.8 to 21.6 % in leaves) for this first year of establishment. Significant differences only occurred found only among sites.
- Therefore, this initial study showed promising results for the use of Cuban-source mulberries as animal fodder in temperate European regions. However, increasing the duration of the field trial will provide further evidence of the best cultivar(s) for temperate climatic conditions.

6 Acknowledgements

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