BIOMASS PRODUCTION AND CONCENTRATION OF ROSMARINIC ACID IN *MELISSA OFFICINALIS* L. ESTABLISHED UNDER *PRUNUS AVIUM* L.

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Abstract

The production of medicinal plants could be combined with the production of timber from high value trees as *Prunus avium* L. because many medicinal plants are characterised by its capacity to growth under a partial shade. This type of agroforestry practice called silvoarable or forest farming (depending on tree density) could increase the economic and environment benefits of farms. The aim of this study was to evaluate the effect of two tree densities (1333 and 666 trees ha^{-1}) and the fertilisation with sheep manure on the biomass production and the concentration of rosmarinic acid in *Melissa officinalis* L. established under *Prunus avium* L. in Galicia. The results of this experiment did not show a negative effect of *Prunus avium* L. shade on the *Melissa officinalis* L. biomass production. Moreover, the high tree density increased the concentration of rosmarinic acid in *Melissa officinalis* L. probably due to the delay of the flowering caused by the tree. This result is very important from a management point of view because the harvest period could be delayed without decreasing the concentration of active components in the medicinal plants, improving farmer time organization.

Keywords: intercropping; tree density; fertilisation; medical plants; active components

Introduction

About 80% of the people in the world use medicinal plants, being mostly harvested without control, which may reduce the natural populations (Rao et al. 2004). However, in the tropics, many medicinal plants are planted and its natural regeneration is carefully placed in agroforestry systems, mainly due to its capacity to grow in partial shade conditions. In this context, medicinal plants could be intercropped with high value trees such as *Prunus avium* L. This tree species is characterised by a low radiation interception for the understory and a fast growth rate with better financial returns (3000 € m^{-3}) compared with other more extended used tree species in the Galicia region (NW Spain) where this experiment was established (Horgan et al. 2003; Chifflot et al. 2006).

When medicinal plants are intercropped with trees it is important to evaluate the biomass production of the medicinal plants but also the quality and amount of the active components for which the medicinal plants are valued. The production of active components is not directly related to the plant biomass increase and depends among other factors on the edaphoclimatic conditions but also on the duration of the shade effects generated by the trees (Rao et al. 2004). The aim of this study was to evaluate the effect of two tree densities (1333 and 666 trees ha^{-1}) and the fertilisation with sheep manure on the production biomass and the concentration of rosmarinic acid in *Melissa officinalis* L. established under *Prunus avium* L. in Galicia.

Materials and methods

The experiment was established in Boimorto (A Coruña, Galicia, NW Spain) on a plantation of *Prunus avium* L. managed by the Bosques Naturales company. Bosques Naturales is a forestry company focused on the management, maintenance, monitoring and research of high-value hardwood species plantations, mainly walnut and cherry. The plantation of *Prunus avium* L. was carried out in 2008. Initially, the plantation was a mixed stand which was managed to establish *Prunus avium* L. at the final densities of 6 m x 1.25 m and 6 m x 2.5 m, equivalent to 1333 and 666 trees ha^{-1}, respectively. In November 2015, after the soil preparation, *Melissa officinalis* L. was planted in-between tree rows following a randomized block design with three replicates. Medicinal plants were planted in 1.75 m-wide alleys, at 2.12 m distance from the base of the trees. Distance between plants rows was 0.7 m and distance between plants within a row was 0.4 m. Medicinal plants were planted in one of the alleys, whilst the other alley remained uncropped to allow access for machinery for annual pruning and phytosanitary application to the trees. Moreover, medicinal plants were protected with a plastic mesh. The total number of treatments was four because the *Melissa officinalis* L. was established under *Prunus avium* L. planted at two tree densities (1333 and 666 trees ha^{-1}) without fertilisation and with fertilisation (5 t ha^{-1} of sheep manure applied at the beginning of the experiment).

Melissa officinalis L. was harvested in July 2016 and 2017. During the harvests the orientation of the plants in the plot was taking into account. In each plot the central plants and the plants with North orientation (North-Center) were separated from the plants with South orientation (South). The plants were weighed fresh in the field. The mortality of the plants was also recorded. In the laboratory, a subsample of the plants was weighed fresh, dried (36-38ºC) and weighed dry to estimate the dry matter production. The concentration of rosmarinic acid in the leaves of *Melissa officinalis* L. was determined through an UV-V spectrophotometry analysis (RFE, 2002). In this study, the medicinal plants production per hectare was calculated considering the area occupied by the trees and assuming that the medicinal plants were established in all alleys of the plot.

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.

Results

Figure 1 shows that the production of *Melissa officinalis* L. was not significantly modified neither by the tree density, nor the orientation of the medicinal plants in the plots, nor by the fertilisation ($p>0.05$).

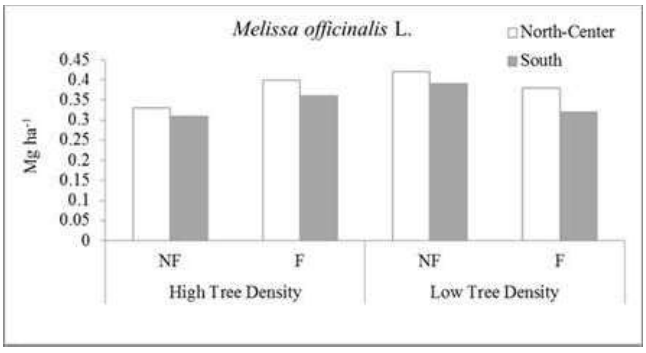

Figure 1: Biomass production of *Melissa officinalis* L. (Mg dry matter ha^{-1}) under *Prunus avium* L. established at different tree densities (high tree density: 1333 trees ha^{-1} and low tree density: 666 trees ha^{-1}) in Galicia (NW Spain) in July 2016. NF: no fertilisation, F: fertilisation with 5 t ha^{-1} of sheep manure. North-Center and South indicate the orientation of the medicinal plants in the plots.

In July 2016, the concentration of rosmarinic acid in the leaves of *Melissa officinalis* L. was higher in the high tree density (1333 trees ha^{-1}) compared with the low tree density (666 trees ha^{-1}) ($p<0.05$) (Figure 2).

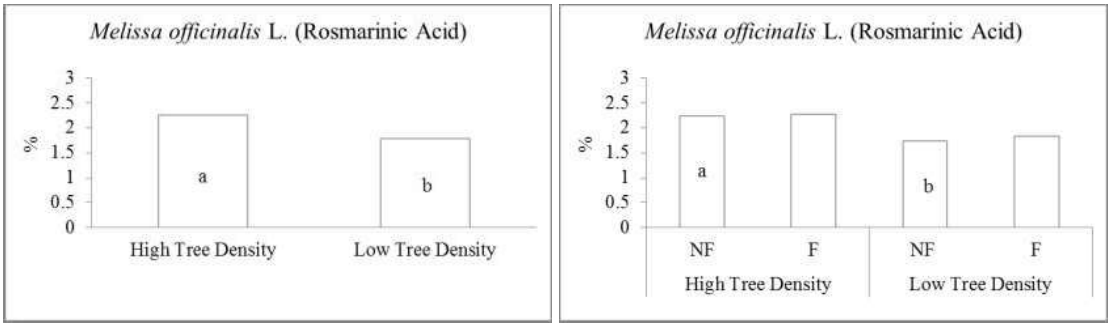

Figure 2: Concentration of rosmarinic acid in *Melissa officinalis* L. (%) under *Prunus avium* L. established at different tree densities (high tree density: 1333 trees ha^{-1} and low tree density: 666 trees ha^{-1}) in Galicia (NW Spain) in July 2016. NF: no fertilisation, F: fertilisation with 5 t ha^{-1} of sheep manure. North-Center and South indicate the orientation of the medicinal plants in the plots. Different letters indicate significant differences between treatments.

The results obtained in the harvest of July 2017 are not shown because the production was very low probably due to drought registered during 2017, being this year the driest year of the last 20 years.

Discussion

The biomass production of *Melissa officinalis* L. obtained in this study (0.31-0.42 Mg DM ha^{-1}) in July 2016 was lower than the biomass production estimated by Douglas (1993) in different areas of New Zealand (0.8 Mg DM ha^{-1}) and by Mihajlov et al. (2013) (0.5 Mg DM ha^{-1}) in the first harvest after the establishment of this medicinal plant in a region of Macedonia. The differences found between our experiment and the studies of these authors could be explained because the experiments were carried out in areas with different climate conditions but also because in our study the land occupied by the trees was discounted.

In this experiment, the biomass production of *Melissa officinalis* L. was not modified by the tree density and the orientation of the medicinal plants in the plots probably because this species is well adapted to partial shade (Canter 2003). These results indicate that this medicinal plant could be intercropped with high value trees as *Prunus avium* L., as long as the management of the plantation, mainly the pruning of branches, is adequate to allow the light inputs to the understory. Moreover, the biomass production of *Melissa officinalis* L. was not affected by the fertiliser applied to the soil probably due to the fertiliser dose was very low to modify the chemical soil properties which remained to be very poor in all plots because the Galician soils are characterised by its low fertility. The pH of the natural Galician soils is generally below 5 when fertilisers and lime are not applied to the soil, mainly due to the rainfall regime and crop extraction (Mosquera-Losada et al. 2017).

Finally, in this study the concentration of rosmarinic acid in *Melissa officinalis* L. (1.28-2.78%) was higher than the minimum required by the European Pharmacopoeia (1%) to process the plant. Moreover, the values of rosmarinic acid were within the interval defined in previous studies (0.5-6.8%) in which it is described that the concentration of this active component in *Melissa officinalis* L. varies with the geographical area and the harvest time (Lamaison et al. 1990; Zgorka and Glowwiak 2001; Wang et al. 2004). In any case, the rosmarinic acid was higher in the high tree density (1333 trees ha^{-1}) compared with the low tree density (666 trees ha^{-1}) probably due to the delay of the flowering period caused by the shade conditions generated by trees. If a delay in flowering happens generally the concentration of this active component is higher because there is more time to accumulate it. This result is very important from a management point of view because the harvest period could be delayed without

decreasing the concentration of active components in the medicinal plants, improving farmer time organization.

Conclusion

No negative effect of *Prunus avium* L. shade was found on the *Melissa officinalis* L. biomass production, which makes high value tree plantation an optimum place to combine with medicinal plants. The higher concentration of rosmarinic acid in *Melissa officinalis* L. associated to the high tree density could be explained by the delay of the flowering which improves the farmer time organization to harvest the different plots because the harvest period can be delayed without decreasing the concentration of active components in the medicinal plants.

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