BIOMETRY AT INDIVIDUAL TREE AND PLOT LEVEL IN SHORT ROTATION WOODY CROPS UNDER MEDITERRANEAN CONDITIONS

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Biometry at individual tree and plot level in short rotation woody crops under Mediterranean conditions

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"Lo esencial es
invisible a los ojos"

Saint-Exupéry

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Abstract

Abstract

Developing a circular bioeconomy based on the sustainable use of biological resources appears to be the best way of responding to the challenges associated with global change. The use of biomass as a natural renewable resource is postulated as one of the key factors in generating a circular bioeconomy because of the economic effects as well as the environmental and social implications.

One of the main advantages of the use of biomass as a raw material for producing bioenergy and bioproducts is its capacity to be managed as a resource with many additional external benefits. Short rotation forest crops are an essential tool for obtaining quality biomass, with predictable periodicity and yield according to the areas of cultivation. Among the different types of short rotation forest crops, the works addressed in this thesis deal exclusively with biomass produced through lignocellulosic woody crops (mainly poplar, but also robinia) grown in short rotation high density plantations (Short Rotation Coppice, SRC). These plantations of fast growing species are managed intensively as coppice stands on cycles of between 3 and 5 years until the productivity of the stool declines, which can occur at between 9 and 14 years of age (Aylott *et al.*, 2008).

The species most commonly used as SRC crops include those in the family *Salicaceae* (*Populus* and *Salix*). Some of the most important reasons for using these species include the extensive development of breeding programs (Clifton-Brown *et al.*, 2019), supported by the broad genetic basis and also by the high capacity for vegetative reproduction and the rapid growth rate of the species, which have optimal characteristics and properties for this purpose.

The added benefits of these plantations include the provision of diverse ecosystem services, such as regulation of clean air, erosion control, climate change mitigation, increased biodiversity in agricultural environments and soil decontamination, among others. From a social perspective, woody crops contribute towards the generation of employment in rural environments. Nonetheless, important aspects such as the impact on changes in land use and water consumption in areas with hydric deficiency must be taken into account in ensuring the sustainability of these plantations.

Improving the sustainability of biomass production from SRC plantations depends on advances made in different aspects of the process, including the suitability of the genetic material, the plantation design, management activities (cultural treatments), biomass yield prediction, biomass characterization, as well as valorisation of the sustainability of the plantations and of the ecosystem services provided.

The main aim regarding these crops is to achieve high and more efficient production, maximizing the benefits and combining the economic viability with environmental sustainability. These issues are without doubt linked to the progress which is necessary in the different lines of action mentioned, since only by focusing on the situation as a whole will it be possible to guarantee the consolidation of the technology required to produce the raw material. Thus, the overall aim of the present thesis is to provide responses to aspects such as the plantation design and production prediction as well as other aspects related to sustainability. The research specifically focuses on the biometric study of these types of plantations at different scales, ranging from the individual tree level to plot level in different plantation designs.

In order to achieve the above aims, data from a large network of plots of high density, short rotation woody crops distributed throughout the areas suitable for growing these types of crops were analysed. The network encompasses a total of 32 locations in which 48 different genotypes were tested at plantation densities ranging from 5,000 to 33,333 cuttings ha^{-1}.

Regarding advances in modelling, the limited availability of **specific models** for irrigated plantations under Mediterranean conditions highlights the need to develop appropriate management tools adapted to SRC plantations grown under these conditions. This would allow biomass production to be quantified, which is essential in order to determine the availability of raw material and thus contributing to appropriate management of the biomass resources.

The direct estimation of biomass production by destructive techniques is laborious and expensive, and indirect methods are therefore usually used in practice (Picard *et al.*, 2012). The availability of tools that will enable estimation of standing biomass is therefore essential and would contribute to improving the decision making process. The use of statistical models based on a wide range of empirical data has gained importance in recent years in research on SRC production (Dickmann, 2006; Aylott *et al.*, 2008; Pérez-Cruzado *et al.*, 2011; Headlee *et al.*, 2013; Tallis *et al.*, 2013), due to the complications associated with extrapolating specific values derived from the wide variability of production to larger scales. Biometric studies at the individual tree level and at plot level are therefore important to enable the development of appropriate estimation tools.

Important advances have been made in the last decade in relation to biomass estimation in poplar plantations under SRC management. Thus, advances have been made in allometric models, and great efforts have been made to model other aspects of the growth of these plantations, such as leaf architecture (Casella and Sinoquet, 2003), root production (Berhongaray *et al.*, 2013b; Berhongaray *et al.*, 2015), and even to use process modelling with this aim (Amichev *et al.*, 2010; Headlee *et al.*, 2013; Hart *et al.*, 2014).

However, for plantations grown under Mediterranean conditions, which implies applying irrigation and consequently a different biomass allometry, specific biomass SRC models for the genus are not available as far as we know. The best approximation is the specific equation, developed within a series of general biomass equations by Montero *et al.* (2005) for planting

densities and rotations aimed at timber production and up-dated by Ruiz-Peinado *et al.* (2011). Data from the previously mentioned network of SRC plots across Spain have been used to develop territorial scale models for predicting production yields, and these have been used to create maps of possible zones of production for this type of crops (Pérez-Cruzado *et al.*, 2013). There is no information about aspects of management of the SRC plantations (which usually involves periodic coppicing) in relation to biomass prediction.

This thesis therefore focuses on evaluating the biometry of SRC plantations at different scales, ranging from individual tree level to plot level under different types of management systems. Biometric study of the trees at individual level was conducted in different ways. In **chapter 2**, the variability in commonly used models was investigated with the aim of improving both the accuracy and applicability of the models. For this purpose, some of the multiple factors that create uncertainty in the allometric relationships between biomass and the independent variables commonly used in the models, such as height and diameter (Verwijst *et al.*, 2013), were evaluated. On the one hand, factors that directly affect the biological relationship (species or genotype, shoot age or coppicing rotation period) were considered, along with factors that affect the modelling process (fitting algorithms, the size of the sample used to fit the model or the sample selection criteria according to the development stage of the crop). Some of these factors are common to forest modelling in general. However, factors such as age, the model formulation, the sample size and even the sample selection criteria are all of greater relevance in poplar SRC plantations as the above- and below-ground parts are at different stage of development after successive coppicing. In the present research, age was found to have an important influence on allometry, especially during the first year after coppicing, and different options of decreasing the model uncertainty were identified, e.g. by including independent variables such as the number of shoots per stool or even the age as a dummy variable. Finally, it was also found that a minimum of 50 trees should be sampled in order to minimize the uncertainty.

The estimation models are also particularly useful to evaluate the **sustainability of plantations and quantifying the ecosystem services** provided, which may be of great interest in relation to the predicted development of these types of plantations. Criteria and indicators of sustainability have been defined, but there remains a lack of information about many aspects based on real scale quantifications.

In **chapter 3**, equations were developed for predicting above-ground biomass at the end of the rotation. The capacity to predict production levels is particularly important in regard to evaluating the economic viability of a particular crop (Pérez-Cruzado *et al.*, 2013) or making decisions about the duration of the rotation. The fact that the biomass estimation models are site specific (Tumwebaze *et al.*, 2013) or genotype specific (Sixto *et al.*, 2013a) clearly limits their practical application as management tools for biomass prediction and/or crop management planning. Although a large part of the research related to the development of biomass models for SRC has focused on site specific and genotype specific biomass models (Verwijst and Telenius, 1999; Barrio-Anta *et al.*, 2008; Mosseler *et al.*, 2014), some models have also been

developed for higher hierarchical levels (Ben Brahim *et al.*, 2000a; Dillen *et al.*, 2007). The lack of specific models for some particular genotypes is commonly overcome by the use of models created for taxonomically proximate genotypes (Dillen *et al.*, 2007). This assumes that the biomass allometry is mainly based on taxonomic affinity due to a common progenitor species. The following chapter therefore focuses on developing specific biomass predication models for a large number of genotypes commonly used in short rotation poplar plantations, as well as on producing models for taxonomic group and general level that can be used when information about the clone is not available. A detailed study of the effect of taxonomic affinity on biomass allometry was carried out to check the validity of the assumption commonly made in order to compensate for the lack of genetic information. The study findings demonstrate that for the genotypes considered, genotypic affinity and biomass allometry were not related. Therefore, the use of prediction models at the level of taxonomic groups is not always recommended, and general models are preferable. However, although the use of general models for estimations provides greater flexibility, it can also lead to a considerable loss of accuracy. Hence, the calibration of generalized biomass models for large regions must be based upon a sample of site (Pérez-Cruzado *et al.*, 2015) and under the climatic conditions covering the range of environmental conditions considered (Muukkonen, 2007; Shaiek *et al.*, 2011). The models developed at different scales represent a valuable tool for the management and design of these types of crops.

One of the main ecosystem services supplied by these plantations, or at least the best studied to date, is the effect on reducing greenhouse gases (Verlinden *et al.*, 2013b; Berhongaray and Ceulemans, 2015; Zenone *et al.*, 2015; Berhongaray *et al.*, 2017). Quantification of the amount of carbon fixed by the plantations is one of the main indicators of sustainability considered in the last few decades. Although bioenergy is mainly considered to be important for mitigating climate change via the capture of C in the soil (Djomo *et al.*, 2011; Verlinden *et al.*, 2013b), other studies have questioned this (Don *et al.*, 2012) or have established a minimum cultivation period after which a positive C balance is produced (Pérez-Cruzado *et al.*, 2012a; Pérez-Cruzado *et al.*, 2012b; Ceulemans *et al.*, 2018).

Chapter 4 reports a study of biomass allometry for above- and below-ground fractions. Although similar values were obtained for below-ground fraction as those reported for non-irrigated plantations in Europe, the values for the above-ground fraction were much higher, with lower root-shoot ratios than in other parts of Europe, except for Mediterranean regions where irrigation is applied, for which the values were very similar.

Owing to the difficulties associated with evaluating the below-ground biomass, the availability of models for estimating this fraction is of great importance for quantifying the amount of carbon fixed by poplar plantations attributable to the root fraction. When computing the carbon, not only must the carbon fixed in the soil be taken into account (Smith, 2004), or the carbon which is gradually incorporated through leaf decomposition (Walter *et al.*, 2015), but also the carbon fixed in the root systems, which are particularly important in this type of crop. Quantification of the below-ground biomass is therefore necessary, although the difficulty

involved in extracting this biomass means that the availability of tools to estimate below-ground biomass is very limited. Despite this, some authors have focused on root biomass in SRC plantations (Block *et al.*, 2006; Berhongaray *et al.*, 2015) and have developed specific estimation models for this purpose (Coyle and Coleman, 2005; Fang *et al.*, 2007; Berhongaray *et al.*, 2015).

However, irrigation of SRC plantations under Mediterranean conditions during the summer months probably affects the root development, and specific models must be developed for these conditions. Chapter 4 reports the development of below-ground models for three of the genotypes most commonly used in plantations destined for biomass production and growing under Mediterranean conditions. As with the models for the above-ground fraction, models are also developed at different hierarchical levels. The amount of carbon accumulated in the above-ground part was estimated to be around 5 Mg C ha^{-1} yr^{-1}, while the amount of carbon accumulated in the below-ground biomass was estimated to be around 1 Mg C ha^{-1} yr^{-1}.

The information obtained by applying models to SRC poplar plantations may be of great use in helping managers to make decisions about the planting establishment and later management. It could also be useful for other parties involved in managing biomass, facilitating identification of the most suitable areas for establishing biomass consumption industries. The information could also help legislators to estimate the contribution of poplar SRC plantations to the total energy supply at national level. Analysis at individual tree level is fundamental for determining the individual performance of each of the genotypes. However, analysis at stand level, whether directly with specific tools at plot level or by extrapolation from individual tree level, is particularly important for planning and managing these crops, where the aim is to maximize production over time.

There are many other management tools available indirectly based on modelling. For example, density management diagrams (DMDs) are used to graphically illustrate the relationships between density, yield and mortality over the development of the plot (Newton and Weetman, 1994), and they include concepts such as competition, site occupation and self-thinning. In SRC poplar plantations, reducing the density is not a common management practice and due to the short rotation length, these crops rarely reach self-thinning limits. Hence, the application of DMDs would therefore be severely limited. Nonetheless, these tools are useful for SRC management, as they enable estimation of biomass stocks for different stages of stand development, as well as optimization of the initial design of the density and the rotation length to maximize production. Therefore, in **chapter 5** the basic concepts used in the construction of DMDs were applied to develop a *reference diagram* specific to SRC management. This specific *reference diagram* developed for the first rotation and using genotype 'I-214' enables the rapid, simple comparison of possible different management scenarios, in relation to both the time of planting (choice of density e.g.) and the harvesting (rotation). Such models may play an important role in helping researchers to understand the production patterns in SRC poplar. Their use may also help in evaluating energy production potential, as already done for *Eucalyptus* spp. (Pérez-Cruzado *et al.*, 2011), as well as for calculating the economic benefits and in

management decision-making regarding the ideal plantation density or the ideal time for harvesting.

Finally, and with the general aim of optimizing production, improvements in yields per unit area may be brought about by considering alternative **designs.** One of the avenues explored in this search for improvement is the use of mixtures of species which can be advantageous both for natural stands and plantations. However, scarce information exists in this regard for plantations under SRC management.

There has been a considerable increase in mixed plantations in the forest sector in the last few decades due to the associated ecological and environmental benefits, as well as increased production due to factors related to facilitation. These plantations consist of tree by tree mixtures of different species, as mixing genotypes at stand level or greater dasocratic divisions is common practice in the management of forest stands (Evans, 2009). The available resources will be used more efficiently due to complementarity and interactions between the different species within the mixture (Kelty, 1992). The use of nitrogen-fixing species is a good example of facilitation, in which the effect of the mixture increases as the N limitation in the soil increases (Bouillet *et al.*, 2013; Forrester, 2014). In SRC, the use of such species may reduce the need for fertilizer throughout the rotation, which is a key factor considered in this type of design.

Although the use of these designs has aroused some interest, publications on this topic are scarce. Few examples exist of mixed plantations of poplar with other species in SRC, although one example is the mixture of *Populus* spp. with *Alnus* spp. (Dawson and Hansen, 1983; Radwan and DeBell, 1988) along with recent interest in the mixture of *Populus* spp. with *Robinia* spp. (Gana *et al.*, 2014; Marron *et al.*, 2018). Therefore, in **chapter 6** focuses on evaluating the suitability of the mixture *Populus alba* **-** *Robinia pseudoacacia* (a known nitrogen fixing species) in SRC under Mediterranean conditions. The use of nitrogen-fixing species can reduce or eliminate the need for fertilizer (with the consequent ecological and environmental implications) and may lead to increased production. However, although recognized to be of potential interest, the use of such combinations has not yet been studied in detail. Both species (*P. alba* and *R. pseudoacacia*) are considered more tolerant of drought than other species. A similar design but using the euramerican genotype 'Dorskamp' has been tested by Marron *et al.* (2018). In the present research, the results of the first rotation demonstrated an increase in biomass production of 26.83% in the mixture including 75% poplar and 25% robinia, relative to the poplar monoculture. No clear facilitator effect has been observed in the other mixtures; however, it has been established that the distribution pattern of the species within the row is a key factor in the responses observed, due to the particular distribution in this type of design.

In summary, this thesis provides excellent modelling tools for managing poplar SRC plantations under Mediterranean conditions. The models can also be used to quantify some ecosystem services, which will be essential for the short-term evaluation of the sustainability

of this type of plantation. The constant search for improvements in sustainability is reflected in this thesis by assessing the productivity of different genotypes, improvements in the planting density and identification of the best time for harvesting, and also by the proposal of an alternative design. Mixed plantations not only provide benefits in terms of production, and they also have environmental benefits, such as increased biodiversity and a reduction in the need for application of nitrogenous fertilizers.

Despite the advances made in this research in relation to managing these types of plantations, much work remains to be done regarding the practical application of these advances in order to obtain the potential benefits.

Although abundant information has already been generated in relation to this type of plantation in many parts of the world, further research remains essential. We believe that the advances made in this doctoral research will contribute to the sustainability of SRC plantations managed under Mediterranean conditions.

Resumen

La bioeconomía circular basada en el uso sostenible de los recursos biológicos parece la vía para dar respuesta a los retos derivados del cambio global. El aprovechamiento de la biomasa como recurso natural renovable se postula como uno de los pilares clave en el desarrollo de la bioeconomía, no solo por sus implicaciones a nivel económico, sino también medioambiental y social.

Una de las principales ventajas del uso de la biomasa como materia prima para la bioenergía y los bioproductos es su alta capacidad para ser un recurso gestionado con muchas externalidades positivas añadidas. Los cultivos forestales son una herramienta fundamental para la obtención de biomasa de calidad, con periodicidad y rendimiento previsible según las áreas de cultivo. Dentro de los cultivos forestales, los trabajos de esta tesis hacen referencia a cultivos lignocelulósicos con especies leñosas (principalmente chopo aunque también robinia) en alta densidad de plantación y turno corto (Short Rotation Coppice, SRC). Estas plantaciones con especies de crecimiento rápido se gestionan bajo esquemas de gestión intensivos de monte bajo, con ciclos de recepe de entre 3 y 5 años hasta el declive de productividad de la cepa, lo que sucede en un rango de entre 9 y 14 años (Aylott *et al.*, 2008).

Entre las especies más utilizadas para este tipo de cultivos, destaca la familia *Salicaceae* (*Populus* y *Salix*). Algunas de las razones de la relevancia de estas especies se basan en el amplio desarrollo de programas de mejora (Clifton-Brown *et al.*, 2019), sustentada en una amplia base genética, pero también en la facilitad en la multiplicación vegetativa y el hecho de ser especies de crecimiento muy rápido, con características y propiedades de la madera óptimas para esta finalidad.

Pese a los beneficios adicionales de estas plantaciones concretados en diversos servicios ecosistémicos como la regulación de aire limpio, el control de la erosión, la mitigación del cambio climático, el aumento de la biodiversidad en el entorno agrario o la descontaminación de suelos entre otros, así como también los beneficios a nivel social debido a la creación de empleo en el medio rural, lo cierto es que la sostenibilidad de su implementación deberá tener en cuenta aspectos relevantes como el impacto en los cambios de uso de suelo o en el consumo de agua en zonas deficitarias.

La mejora sostenible de la producción de biomasa a partir de cultivos en SRC pasa por el avance en diferentes aspectos como son la adecuación del material genético, el diseño de las plantaciones, las actuaciones sobre la gestión (tratamientos culturales), la predicción de la producción, la caracterización de la biomasa, así como la valoración de la sostenibilidad en sí de las plantaciones y los servicios ecosistémicos que aportan, entre otros.

El objetivo principal con respecto a estos cultivos es lograr una producción elevada y más eficiente, maximizando los beneficios y combinando la viabilidad económica con la sostenibilidad ambiental. Su viabilidad económica y sostenibilidad está sin duda vinculada al necesario progreso en las diferentes líneas de actuación mencionadas, ya que solo actuar sobre su conjunto garantizará la consolidación de la tecnología en relación a la producción de la materia prima. En este sentido se justifica la presente tesis, siendo su finalidad contribuir a dar respuestas sobre aspectos relativos al diseño del cultivo, la predicción de la producción así como sobre aspectos ligados a su sostenibilidad. La presente tesis profundiza en el estudio de la biometría de este tipo de plantaciones a diferentes escalas, avanzando desde el nivel de árbol individual hasta el nivel de parcela en diferentes diseños de cultivo.

Para abordar estos objetivos se ha contado con información procedente de una amplia red de parcelas de cultivos leñosos en turno corto y alta densidad, distribuida por aquellas zonas de la península con aptitud para este tipo de cultivos. La red abarca un total de 32 localidades en las que se han testado 48 genotipos diferentes con un rango de densidades que va desde los 5,000 hasta los 33,333 pies ha^{-1}.

En cuanto a los avances en la modelización, las limitaciones en la disponibilidad de **modelos específicos** para plantaciones sometidas a regadío bajo condiciones mediterráneas remarcan la necesidad de disponer de herramientas de gestión adecuadas y adaptadas a las condiciones mediterráneas de plantaciones en SRC. Esto permitiría la cuantificación de la producción de biomasa, que será clave para conocer la disponibilidad de materia prima, contribuyendo de esta forma a una gestión adecuada.

La estimación directa de la producción de biomasa mediante técnicas destructivas es laboriosa y tiene un elevado coste, por lo que se han generalizado para usos operativos los métodos de estimación indirecta (Picard *et al.*, 2012). Disponer de herramientas que permitan la estimación de la biomasa en pie es un factor esencial para la gestión de este tipo de plantaciones, contribuyendo a la toma de mejores decisiones. El uso de modelos estadísticos basados en un amplio rango de datos empíricos ha ganado importancia en los últimos años en la investigación de la producción de los cultivos bajo SRC (Dickmann, 2006; Aylott *et al.*, 2008; Pérez-Cruzado *et al.*, 2011; Headlee *et al.*, 2013; Tallis *et al.*, 2013), debido a las complicaciones a la hora de extrapolar a mayor escala valores muy específicos derivados de la amplia variabilidad de producción de este tipo de cultivos. Por ello, el estudio de la biometría no solo a escala de árbol individual sino también a escala de parcela es importante a la hora de poder desarrollar herramientas de estimación apropiadas.

En la última década el avance en la **modelización** para la estimación de biomasa procedente de plantaciones de chopo bajo SRC ha sido importante. No solo se ha avanzado en modelos alométricos, sino que también ha habido un amplio esfuerzo en modelizar otros aspectos del crecimiento de estas plantaciones: como pueden ser la arquitectura de las hojas (Casella and Sinoquet, 2003), la producción de las raíces (Berhongaray *et al.*, 2013b; Berhongaray *et al.*,

2015) o incluso utilizar modelos de procesos con ese fin (Amichev *et al.*, 2010; Headlee *et al.*, 2013; Hart *et al.*, 2014).

Sin embargo para los escenarios de cultivo desarrollados bajo condiciones mediterráneas que impliquen aplicación de riego y por tanto una alometría diferente, no hay disponibles ecuaciones específicas de biomasa para el género *Populus* spp. La única aproximación es la ecuación específica para el género *Populus* spp., desarrollada en el marco de las ecuaciones de biomasa generales de Montero *et al.* (2005) para densidades y turnos de plantación de uso maderero y su actualización por Ruiz-Peinado *et al.* (2011). A partir de la información de la red de parcelas SRC a lo largo de la geografía española citada anteriormente si se han desarrollado modelos predictivos de la producción a escala de territorio que se han utilizado para crear mapas de posibles zonas de producción para este tipo de cultivos (Pérez-Cruzado *et al.*, 2013). Tampoco existe información sobre los efectos que tienen los aspectos relacionados con la gestión de los SRC, que suelen consistir en esquemas de monte bajo con recepados periódicos, sobre las predicciones de biomasa mediante ecuaciones específicas para primeros turnos.

Por ello esta tesis se centra en la evaluación de la biometría de plantaciones SRC a diferentes escalas, avanzando desde el nivel de árbol individual hasta el nivel de masa bajo diferentes sistemas de gestión.

El estudio de la biometría a nivel de árbol individual se ha abordado a varios niveles. En el **capítulo 2** se analiza la variabilidad de los modelos comúnmente usados con el fin de mejorar tanto la precisión como su aplicabilidad. Para ello, se han evaluado algunos de los múltiples factores que crean incertidumbre en las relaciones alométricas entre la biomasa y las variables independientes comúnmente usadas en los modelos como pueden ser el diámetro o la altura (Verwijst *et al.*, 2013). Por un lado, se establecieron factores que afectan directamente a la relación biológica (la especie o genotipo específico, la edad de la parte aérea o el turno de recepado) y por otros factores que afectan a la modelización de la relación (los algoritmos de ajuste, el tamaño de muestra usado para ajustar el modelo o el criterio de selección de la muestra en función del estado de desarrollo del cultivo). Algunos de estos factores son comunes a la modelización forestal en general. Sin embargo, factores como la edad, la formulación del modelo utilizado, el tamaño e incluso el criterio de selección de la muestra cobran mayor relevancia en plantaciones de chopo bajo SRC debido al diferente estado de desarrollo de la parte aérea y subterránea, tras los sucesivos recepados. Como resultado de la presente tesis, se ha observado una notable influencia de la edad en la alometría especialmente en el primer año tras el recepado, observando también que existen formas de disminuir la incertidumbre de los modelos, por ejemplo incluyendo algunas variables independientes como el número de brotes por cepa o incluso la edad mediante variables dummy. Por último también se ha evaluado la idoneidad del tamaño mínimo de muestra estableciendo en 50 el número de árboles a muestrear para reducir la incertidumbre.

Los modelos de estimación son también de especial utilidad a la hora de evaluar la **sostenibilidad de las plantaciones y la cuantificación de los servicios ecosistémicos** que

estas aportan, lo cual puede ser de mucho interés si consideramos las predicciones de desarrollo de este tipo de plantaciones. Se han definido criterios e indicadores de sostenibilidad pero existe falta de información sobre muchos aspectos basada en cuantificaciones a escala real.

Por ello en el **capítulo 3** se han desarrollado ecuaciones para la predicción de la biomasa al final del turno para la parte aérea. La capacidad de predecir los niveles de producción es particularmente importante cuando se debe evaluar la viabilidad económica de un cultivo determinado (Pérez-Cruzado *et al.*, 2013) o cuando se deben tomar decisiones con respecto a la duración de la rotación. El hecho de que los modelos de estimación de biomasa sean específicos del sitio (Tumwebaze *et al.*, 2013) o genotipo (Sixto *et al.*, 2013a) limitan claramente su uso práctico como herramientas de gestión predictivas de la producción de biomasa y/o de la planificación del manejo del cultivo. Si bien gran parte de la investigación relacionada con la creación de modelos de biomasa para SRC se ha centrado en el desarrollo de modelos de biomasa específica de sitio y genotipo (Verwijst and Telenius, 1999; Barrio-Anta *et al.*, 2008; Mosseler *et al.*, 2014), también hay algunos ejemplos de modelos desarrollados en niveles jerárquicos superiores (Ben Brahim *et al.*, 2000a; Dillen *et al.*, 2007). La falta de modelos específicos para algunos genotipos concretos, se aborda comúnmente mediante el uso de modelos creados para genotipos próximos taxonómicamente (Dillen *et al.*, 2007). Esto supondría que la distribución de biomasa se basa principalmente en la afinidad taxonómica debido a su especie progenitora común. Y es debido a esta premisa que el siguiente capítulo no solo se centra en facilitar modelos de estimación de biomasa específicos para un número relevante de genotipos comúnmente utilizados en las plantaciones de chopo en corta rotación, sino que también se aportan modelos a nivel de grupo taxonómico y generales a nivel de género, para poder usarlas cuando no se dispone de información para un genotipo determinado. También se ha realizado un estudio detallado del efecto de la afinidad taxonómica en la alometría de la biomasa con el fin de esclarecer si la premisa comúnmente usada para subsanar la falta de información genética es correcta. Como resultado de este trabajo se pudo comprobar que, para los genotipos estudiados, no existe relación entre afinidad genotípica y alometría de biomasa. Por lo tanto, los modelos de estimación a nivel de grupo taxonómico no siempre son los aconsejables, siendo preferible el uso de modelos generales. El uso de modelos generales para estimaciones a nivel de genotipo, al tiempo que permite una mayor flexibilidad, pueden sin embargo implicar una pérdida considerable de precisión. Por lo tanto, la calibración de modelos de biomasa generalizada para grandes regiones debe basarse en una muestra del sitio (Pérez-Cruzado *et al.*, 2015) y en las condiciones climáticas que abarca el rango de condiciones ambientales cubiertas (Muukkonen, 2007; Shaiek *et al.*, 2011). La disponibilidad de modelos a diferentes escalas constituye una herramienta de gran utilidad para la gestión y planificación de este tipo de cultivos.

Uno de los principales servicios ecosistémicos de estas plantaciones o al menos el más estudiado hasta el momento es su efecto sobre la reducción de los gases de efecto invernadero (Verlinden *et al.*, 2013b; Berhongaray and Ceulemans, 2015; Zenone *et al.*, 2015; Berhongaray *et al.*, 2017). Por ello la cuantificación del carbono fijado por la plantación es uno de los

principales indicadores de sostenibilidad considerados en las últimas décadas. Si bien la bioenergía se considera, en su conjunto, relevante para la mitigación del cambio climático entre otros motivos por la captación de C en el suelo (Djomo *et al.*, 2011; Verlinden *et al.*, 2013b), otros estudios sin embargo lo cuestionan (Don *et al.*, 2012), o bien establecen un periodo mínimo de cultivo a partir del cual se produce un balance positivo de C (Pérez-Cruzado *et al.*, 2012a; Pérez-Cruzado *et al.*, 2012b; Ceulemans *et al.*, 2018).

En el **capítulo 4** se ha realizado un estudio del comportamiento de la alometría en términos de biomasa tanto de la parte aérea como de la parte subterránea. Se ha observado que si bien la parte subterránea muestra valores similares a los encontrados en otras plantaciones europeas no regadas, la parte aérea es claramente superior, mostrando ratios raíz-tallo inferiores a los encontrados en otras zonas áreas europeas, con la excepción de la zona mediterránea con regadío donde los valores son muy similares.

Debido a la dificultad para poder evaluar la biomasa subterránea, la disponibilidad de modelos de estimación de biomasa subterránea es de gran relevancia a la hora de cuantificar el carbono fijado por las plantaciones de chopo atribuible a la fracción radicular. A la hora de realizar el computo de carbono no solo hay que tener en cuenta el carbono fijado en el suelo (Smith, 2004), o el carbono que se va incorporando con la descomposición de las hojas (Walter *et al.*, 2015), sino también el carbono fijado en los sistemas radicales que son de especial relevancia en este tipo de cultivos. Por ello la cuantificación de la biomasa subterránea es necesaria, si bien su difícil extracción hace que la disponibilidad de herramientas de estimación sea deficitaria. A pesar de la dificultad de estudio de esta fracción, algunos autores han centrado su atención en la biomasa radical de las plantaciones bajo SRC (Block *et al.*, 2006; Berhongaray *et al.*, 2015) y han desarrollado modelos de estimación específicos (Coyle and Coleman, 2005; Fang *et al.*, 2007; Berhongaray *et al.*, 2015).

No obstante, las plantaciones SRC bajo condiciones mediterráneas y aplicación de riego durante los meses estivales, probablemente condiciona el desarrollo radical, y hace necesario establecer modelos de estimación específicos para estas condiciones. En el capítulo 4 se han desarrollado estos modelos para tres de los genotipos más utilizados en plantaciones para la producción de biomasa en condiciones mediterráneas. Al igual que para los modelos de estimación de la parte aérea, se consideraron también diferentes niveles jerárquicos. Se ha estimado que el carbono acumulado en la parte aérea se encuentra en torno a 5 Mg C ha^{-1} yr^{-1}, mientras que el carbono acumulado en la biomasa subterránea es del orden de 1 Mg C ha^{-1} yr^{-1}.

La información derivada de la aplicación de modelos en plantaciones de chopo bajo SRC puede ser de gran utilidad para los gestores a la hora de la toma de decisiones, tanto en relación al establecimiento de la plantación como en relación a la gestión posterior. Igualmente puede resultar de utilidad para otros agentes implicados en la gestión de la biomasa, facilitando la identificación de las áreas más adecuadas para establecer industrias basadas en su consumo. También para los legisladores, ayudando a estimar la contribución de las plantaciones de chopo bajo SRC al suministro total de energía a nivel nacional. El análisis a nivel de árbol individual

es básico para conocer el comportamiento individual de cada uno de los genotipos plantados, sin embargo el análisis a nivel de masa, ya sea de forma directa con herramientas específicas a nivel de parcela o mediante la extrapolación de las herramientas a nivel de árbol individual, es realmente el factor más relevante a la hora de la planificación y la gestión de estos cultivos en los que se busca optimizar la producción al máximo

Existen además otras muchas herramientas de gestión que se fundamentan indirectamente en la modelización, como por ejemplo de los diagramas de manejo de la densidad, entre otros. Los diagramas de manejo de la densidad (DMDs) se utilizan para ilustrar gráficamente las relaciones entre densidad, rendimiento y densidad de mortalidad dependiente en todas las etapas del desarrollo de la parcela (Newton and Weetman, 1994), incluidos conceptos como competencia, ocupación del sitio y autoaclareo. En plantaciones de chopo en SRC, la reducción de densidad no es una práctica de manejo común y debido a la corta rotación, estos cultivos raramente alcanzan límites de autoaclareo, por lo que la aplicación de DMD estará severamente limitada. Aun así, estas herramientas son de utilidad para la gestión de SRC, debido a que permiten estimar stocks de biomasa para distintos estados de desarrollo de la masa, y optimizar rotaciones de corta para maximizar producciones. Por ello, se aplicaron los conceptos básicos utilizados en la construcción de DMDs para desarrollar un *diagrama de referencia* para la gestión de SRC. Todo ello se muestra en el **capítulo 5** de esta tesis. Este diagrama de referencia específico para la primera rotación de una plantación en turno corto y alta densidad del genotipo 'I-214' permite una rápida y sencilla comparación de las diferentes situaciones de manejo que se pueden presentar, tanto en relación al momento de la plantación (densidad) como a la hora de la corta (turno). Estos modelos pueden jugar un papel importante para ayudar a entender los patrones de producción de las plantaciones de chopo en corta rotación, ya que su adaptación puede ayudar a la evaluación del potencial de producción energética como ya se ha hecho para eucaliptos (Pérez-Cruzado *et al.*, 2011), así como en el cálculo de los beneficios económicos y en la toma de decisiones de los gestores sobre el momento idóneo de la corta.

Por último, y dentro del objetivo general de optimizar la producción, hay que considerar que la mejora de los rendimientos sobre la misma unidad de superficie pasa en parte por contemplar alternativas en el **diseño.** En esa búsqueda se ha considerado oportuno explorar alternativas mediante la utilización de mezclas de especies que resultan ventajosas tanto en masas naturales como en plantaciones, pero para las que existe poca información en plantaciones bajo un sistema de manejo SRC.

El auge de las plantaciones mixtas en el ámbito forestal ha sido muy relevante en las últimas décadas debido a los beneficios no solo ecológicos y ambientales, sino también a nivel de producción debido a factores ligados a fenómenos de facilitación. Se trata de plantaciones con mezcla de especies pie a pie, ya que la mezcla de genotipos a nivel de rodal o divisiones dasocráticas superiores es una práctica habitual en gestión de masas forestales cultivadas (Evans, 2009). La complementariedad y la interacción entre las diferentes especies dentro de la mezcla hace que estas usen de una manera más eficiente los recursos disponibles (Kelty, 1992). El uso de especies fijadoras de nitrógeno es un claro ejemplo de facilitación, incrementándose

el efecto de la mezcla a medida que las limitaciones del N del suelo son mayores (Bouillet *et al.*, 2013; Forrester, 2014). En el caso de las plantaciones SRC, el uso de estas especies podría reducir las necesidades de fertilización a lo largo de la rotación, lo que es clave a la hora de plantear este tipo de diseños.

Si bien la aplicación de estos diseños ha suscitado interés, las publicaciones al respecto son, sin embargo, bastante limitadas. Hay pocos ejemplos de plantaciones mixtas de chopo en SRC con otras especies, pudiendo destacar la combinación de *Populus* spp. con *Alnus* spp. (Dawson and Hansen, 1983; Radwan and DeBell, 1988) y el interés reciente de la mezcla de *Populus* spp. con *Robinia* spp. (Gana *et al.*, 2014; Marron *et al.*, 2018). Por ello, en el **capítulo 6** se ha explorado la posibilidad de conseguir incrementos en la producción mediante la utilización de *plantaciones mixtas* para lo que se estableció una plantación en la que se combinaron diferentes proporciones de mezcla de las especies *Populus alba* y *Robinia pseudoacacia* (conocida fijadora de nitrógeno) bajo SRC en condiciones mediterráneas. El hecho de utilizar una especie fijadora de nitrógeno que, además de reducir o incluso eliminar los aportes en fertilización (con sus consecuentes implicaciones tanto ambientales como económicas), pueda dar lugar a un incremento en la producción se postula como una combinación de interés que hasta el momento no ha sido estudiada en profundidad. Ambas especies, *P. alba* y *R. pseudoacacia*, se consideran más tolerantes frente a escenarios de posible sequía. Un diseño similar pero utilizando un genotipo euramericano 'Dorskamp' está siendo ensayado por Marron *et al.* (2018). Nuestros resultados de la primera rotación muestran un incremento de la producción de un 26.83% de la producción en biomasa de la mezcla 75% chopo y 25% robinia con respecto al monocultivo de chopo. No se ha encontrado un claro efecto facilitador en el resto de las mezclas, sin embargo si se ha establecido que la disposición de los pies dentro de la misma fila parece clave en las respuestas observadas, debido a la especial distribución en este tipo de diseño.

En resumen esta tesis proporciona herramientas de modelado excelentes para la gestión de plantaciones de chopo SRC en condiciones mediterráneas. También se considera su utilidad en la cuantificación de determinados servicios ecosistémicos, lo que será esencial a corto plazo en la evaluación de la sostenibilidad de este tipo de plantaciones. La búsqueda constante de mejoras en la sostenibilidad no solo se refleja en esta tesis en la evaluación de de la productividad de los diferentes genotipos o en la mejora de la adecuación de la densidad o del momento de corta, sino también en la consideración de un diseño de cultivo alternativo. Las plantaciones mixtas proporcionan beneficios no solo a nivel productivo sino también a nivel ambiental, como el aumento de la biodiversidad o la reducción de la aplicación de fertilizantes nitrogenados.

Sin lugar a dudas, a pesar del largo camino recorrido en la investigación de este tipo de plantaciones, todavía queda un largo camino por recorrer en el que los avances obtenidos en esta tesis doctoral serán muy útiles.

Si bien es abundante el conocimiento generado en relación a este tipo de plantaciones en buena parte del mundo, queda todavía un largo camino por recorrer. Los avances conseguidos en esta tesis creemos que contribuyen a ello y en especial a la implementación de plantaciones sostenibles bajo condiciones de manejo mediterráneo.

CHAPTER

1

Introduction: background, outline of the thesis and objectives

Based in: Oliveira, N., Pérez-Cruzado, C., Rodrígez-Soalleiro, R., Cañellas, I. and Sixto, H., 2018. Modelos de estimación de biomasa en plantaciones de turno corto y alta densidad de *Populus* spp. and Sixto, H., Oliveira, N. and Cañellas, I., 2018. Cultivo del chopo en turno corto para la producción de biomasa: Un camino a medio recorrer. Both in II Simposio del Chopo. Sociedad Pública de Infraestructuras y Medio Ambiente de Castilla y Leon S.A. (ed.), Junta de Castilla y León, Valladolid.

Introduction: background, outline of the thesis and objectives

1.1. General context

The challenges associated with climate change along with the changed paradigm as regards both economic development and the energy model are what define, in general terms, the research work addressed in this doctoral thesis.

The linear economy, based on fossil resources, needs to be redirected towards a circular bioeconomy based on the use of sustainable biological resources. This would seem to constitute the best route to dealing with the challenges arising from global change, food safety, water resource management etc., distancing economic growth from environmental degradation. Hence, the Research Strategy 20-20-20 of the European Commission, reflected in the European Directive on Renewable Energy (2009/28/CE) and its temporal horizons 2020 (COM/2010/639), 2030 (COM/2014/015) y 2050 (COM/2011/0885), as well as the Innovation Strategy for sustainable growth: A Bioeconomy for Europe (COM, 2012), establish the bioeconomy as the general framework and key factor for green, sustainable growth in Europe. In Spain, in harmony with the European strategy but also taking into account the national possibilities, published the Spanish Strategy for the Bioeconomy in 2015 (MINECO, 2015).

Therefore, development in this sense must take into consideration the potential of natural resources including, among others, biomass. The options of using existing biomass in forests, agricultural residues or the production of new biomass from crops planted specifically for this purpose are all seen as key to the development of the bioeconomy (COM, 2012), not only for economic reasons but also with environmental and social considerations in mind. In this context, biomass is a highly valued resource both for *bioenergy* production and for *bioproducts*, thus contributing towards addressing the abovementioned global challenges.

The term 'biomass', in the context of its use in energy production, has been defined in different ways. Perhaps the simplest and most general definition refers to the organic material of value as a whole. The definition in the European Directive on Renewable Energy (2009/28/EU), which is also used in the European Bioeconomy Strategy, refers to biomass as the biodegradable fraction of products, waste or residues of biological origin coming from agriculture, forests or related industries, including fishing, as well as the fraction from industries and urban solid waste. A similar definition was also contained in the RD 661/2007, repealed in 2013, as regards the regulation of electrical energy production under special regime.

Besides being a renewable resource, biomass production can be managed such that it provides other advantageous aspects in comparison to other more tried and tested energy

production technology, such as wind or solar energy. Other benefits associated specifically with biomass include, among others, greater impact on rural employment or the prevention of wildfires as a consequence of removing wood from the forests (Unión por la Biomasa, 2018).

As a resource for producing energy, the use of biomass dates back to the origins of humanity. It has accompanied humanity throughout our history, although in recent centuries its use has been more restricted to rural areas and to less industrialized countries. Even so, biomass is still the main source of energy used for cooking by approximately 41% of the global population (World Health Organization, 2015). In most areas of the planet, technological advances over the years led to the use of other sources of energy (fossil fuels) which facilitated the industrial revolution. However, under this scenario with fossil fuels at the forefront, certain geopolitical events and concern over the possible exhaustion of such fuels (oil crises of 1973 and 1979) led to the exploration of alternative forms of energy, particularly renewable energy sources (RE). However, it was the confirmation by the scientific community of anthropogenic impact on the climate (Manabe and Wetherald, 1975; IPCC, 1990) which most probably led to the increased focus on finding alternative energy sources, with renewable energy becoming increasingly prominent.

On a global scale, in 2016 renewable energy sources accounted for 13% of the total primary energy supply and 18% of the gross final energy consumption (WBA, 2018). Of the latter percentage, 13% corresponded to biomass, which is 72.22% of the total of renewable energy consumption and 10.11% of the total global energy supply (Fig. 1.1). The potential of bioenergy on a global scale, taking into consideration all biomass based resources, is expected to reach somewhere between 15 and 25% by the year 2050 (Beringer *et al.*, 2011; Rosúa and Pasadas, 2012).

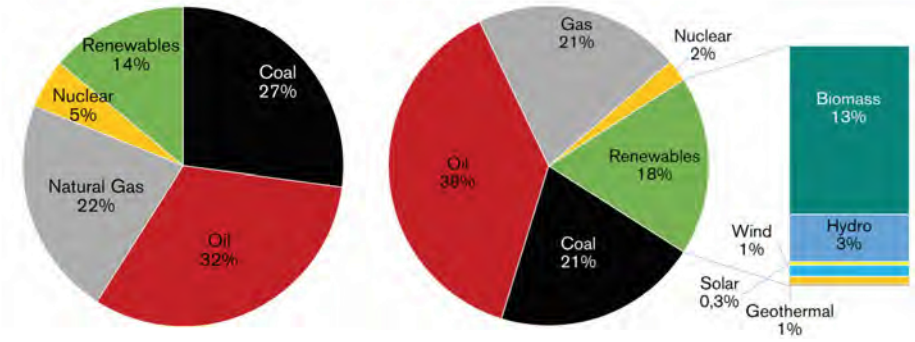

Figure 1.1. Total global energy supply in 2016 (a) and gross final energy consumption at global scale in 2016 (b) (WBA, 2018).

At European level (EU-28), the percentage of RE in relation to the gross final energy consumption in 2016 was 17%, which is double the figure for the 2004, the latter year being the first for which figures are available (Eurostat, 2018). Among the diverse sources of renewables, bioenergy currently represents the greatest percentage (60%) (Bioenergy Europe, 2018). It is likely that new agreements within the EU, which is increasingly ambitious in its

policies towards this kind of energy (32% RE by 2030), will lead to an even greater contribution of biomass to the global supply of energy.

As regards Spain, in harmony with the policies of the EU, regulations have been implemented over the past fifteen years which have promoted the use of renewables. Among such initiatives is the Plan for Promotion of Renewable Energy (PER), developed for the years 2005-2010, or the National Action Plan for Renewable Energy (PANER), updated for the period 2011-2020. The gross final energy consumption in our country is slightly higher (17.3%) than the European average, although only 30% of the RE production comes from biomass based resources, despite the availability of raw material (MINETAD, 2017). The new European objectives mentioned above and the new national laws currently being drawn up (Climate change and Energy transition law and the new Climate and Energy Plan), provide new incentives to improve the status of biomass as a source of renewable energy, particularly since the sector recognizes that these objectives cannot be achieved using only the renewable energy sources currently employed.

Apart from the use of biomass for energy production, the bioeconomy opens up a range of other uses, since biomass represents a renewable source of carbon which can be used to obtain bioproducts of interest in the chemical industry (pharmaceutical, textile, cosmetics, packaging, etc.) and which are linked to the development of biorefineries, termed integrated industries (Bioplat, 2017).

For these reasons, to develop the bioeconomy as defined above, the potential of forest resources must be considered a huge biological infrastructure (Hetemäki *et al.*, 2017). In Spain, the available potential of wood coming from forest exploitation and residues is around 19 million tons per year, and from woody crops planted on forest and agricultural land is estimated at 21.6 million tons annually according to data from Bioplat (2017). However, the estimates from numerous studies on the potential of biomass under various scenarios do not always agree.

There are various sources of forest biomass, which can be differentiated into: i) that derived from forest management activities associated with timber exploitation ii) residues resulting from silvicultural operations apart from timber exploitation iii) biomass derived from the forestry industry, and lastly iv) forest crops designed specifically for the production of woody biomass.

The first of these categories described above includes remains from harvesting and that derived from pieces of wood of small diameter which, in the same way as larger pieces of wood, is used for energy production (pellets or wood chips). The former is the type of biomass which is mainly used in Spain today, while the latter refers to residue from thinning or pruning, which only generates limited amounts of biomass. It should also be mentioned that in the case of forest crops for woody biomass production, intensive techniques commonly associated with agriculture rather than forestry, are often used. Forestry crops are seen as being of particular

importance in terms of their potential contribution to efficient diversification of biomass sources (Broeckx *et al.*, 2012a; Huber *et al.*, 2017; IUFRO, 2018).

With regard to forests in the EU, it is estimated that only 60 to 70% of the annual increment of wood is utilized. In our country there have been constant warnings within the sector regarding the underutilization of this resource at national level by the Spanish Renewable Energy Association (APPA), estimates pointing to a level of utilization of around 41% of the annual wood growth, which is notably below the average for Europe (Sancho, 2018). Furthermore, several forums highlight the positive socioeconomic balance associated with appropriate utilization of this resource (Unión por la Biomasa, 2018). However, it should be borne in mind that estimates of available forest biomass, both at European and national scale can vary substantially despite increasing efforts to harmonize the estimation criteria.

1.2. High density short rotation forest crops for the production of biomass

Within the sphere of biomass, crops destined for energy production (IDAE, 2007) or bioproducts (biopolymers, bioplastics, green chemicals, etc.) (Bioplat, 2017) have been defined in the following categories:

- Oleaginous crops for the production of oils.
- Alcoholigenous crops aimed mainly at sugar platform biorefinery.
- Lignocellulosic crops, including both herbaceous and woody crops, for thermal applications, simple electricity generation or through cogeneration, or to obtain second generation biofuels as well as for the lignocellulosic platform and synthesis gas.

The works addressed in this document deal exclusively with biomass produced through *Lignocellulosic Woody Crops* grown in short rotation high density plantations (Short Rotation Forestry). In general, fast growing species are used in such plantations employing intensive or semi-intensive techniques (Weih, 2004) and managed as coppices, with coppicing cycles of between 3 and 5 years until the productivity of the stool declines, which normally occurs between 9 and 14 years of age (Aylott *et al.*, 2008), depending on the site quality. Given the characteristics of the management applied in this type of plantation, they are defined in scientific literature as Short Rotation Coppice (SRC).

It is thought that biomass from this type of crops may become essential in the near future as an addition to that provided by forests, contributing towards satisfying the demands of European industry and assuring stability in the market (European Commission, 2014). The contribution of this type of biomass is probably linked to the necessity to find spatiotemporal complementarity in biomass resources, and this is already a commercial reality in many parts of the world (Mareschi *et al.*, 2005). The suitability of such biomass is also linked to the intrinsic characteristics of its production and management (Dickmann, 2001), such as the abundance of improved, highly adaptable genetic material, high rate of successful rooting, good juvenile growth or its resprouting capacity, among others (Ceulemans *et al.*, 1996; Laureysens *et al.*,

2005; Al Afas *et al.*, 2008a). Other characteristics associated with its end use are also deemed to be important, such as the low chorine (Cl) and sulphur (S) content, low ash content as well as high lignin content, (Zhang *et al.*, 2011; Patel and Gami, 2012; Surendran Nair *et al.*, 2012; Rodrigues *et al.*, 2018) among others.

Woody crops have been found to provide ecosystem services which in many cases have not been sufficiently quantified and contrasted. Such services include their role in air cleaning, control of erosion or flooding in certain areas of the country (Tufekcioglu *et al.*, 1998; Schultz *et al.*, 2004), mitigating the effects of climate change (carbon fixing in foliar or root biomass fractions)(Grigal and Berguson, 1998; Coleman *et al.*, 2004; Berhongaray *et al.*, 2017), increasing biodiversity in agricultural environments (Christian *et al.*, 1994; Njakou Djomo *et al.*, 2015) and soil decontamination (Rockwood *et al.*, 2004; Dipesh *et al.*, 2015), among others. From a social perspective, woody crops contribute towards the creation of employment in rural areas, given that these crops provide an opportunity to make use of poor, marginal or surplus agricultural land. Finally, the use of biomass from crops helps to reduce the pressure on natural forests in that it provides a raw material much demanded by society and therefore by industry.

Despite this, to achieve sustainability in the implementation these crops it is important to take into account aspects related to the impact of land use changes in certain areas where they may be competing for land with agricultural crops destined for human consumption, as well as aspects related to water consumption in areas with limited water resources. Thus, it is necessary to define the limitations in order to guarantee the sustainability of these crops (GBEP, 2011). Many of these aspects can probably be dealt with through the use of biotechnology to achieve improvements, or by using circular economy techniques such as wastewater reuse (De Miguel *et al.*, 2015; Marron, 2015).

Figure 1.2. General view of one of the study plots located in Peñafiel.

In Spain, the interest in producing biomass from this type of crop dates back to the mid-80's (San Miguel y Montoya, 1984; Marcos, 1985; Ciria *et al.*, 1996), coinciding to a large extent with the crisis in the oil sector. However, it was around the year 2000 when initiatives to increase sources of renewable energy at global scale and in particular in Europe, provided the impetus for our country to begin to explore the possibilities for production of biomass as a renewable resource. The climatic, edaphic and demographic characteristics in some parts of our country make them suitable for the cultivation of this type of plantation, with expected high productivity (Pérez-Cruzado *et al.*, 2013), exceeding that obtained in other European countries. However, it is also the case that, precisely because of the Mediterranean climatic conditions, such plantations are not viable without the use of irrigation during the months of summer drought. In areas where there is an Atlantic climate, the conditions for this type of short rotation forest crop are also ideal, except for the orographic characteristics, which may complicate the intensive silviculture applied in this type of plantation, or the limitations associated with certain species due to specific soil characteristics, such as the pH.

Despite the fact that there have been many studies focusing on the selection of the vegetal material best adapted to given areas of our country, there is still a long way to go as regards studying the interaction between the genotype and the environment, which is a determining factor in the success or failure of this type of plantation.

1.3. Species used. *Populus* genus: Species and hybrids

There are many tree species in the world which could potentially be cultivated for biomass production, such as *Acer* spp., *Albizia* spp., *Betula* spp., *Casuarina* spp., *Gmelina* spp., or *Eucalyptus* spp., among many others (Wang *et al.*, 1991; Walle *et al.*, 2007).

In Europe, the *Salicaceae* family (*Populus* spp. and *Salix* spp.) (the latter being mainly suited to northern European countries such as Sweden), are those which show the greatest development on an industrial level. Plantations based on species and hybrids of *Populus* are more associated with central Europe, with examples in the centre and south of Germany (Huber *et al.*, 2016), United Kingdom (Aylott *et al.*, 2008), Czech Republic (Havlíčková *et al.*, 2008), Bulgaria (Stankova *et al.*, 2016), or France (Benbrahim *et al.*, 2000b), as well as in the Mediterranean regions, mainly in Italy (Nardin and Alasia, 2004) and Spain (Sixto *et al.*, 2013). However, poplar cultivation is also attracting interest in the north of Europe, with different trials being established to evaluate its potential (Nordborg *et al.*, 2018).

To a large extent, species of this genus are at the forefront of biomass production thanks to highly efficient improvement programs in different countries, including various in Europe (Clifton-Brown *et al.*, 2019). These programs are enabled by the fact that not only is there a very broad genetic base with which to work in terms of the characteristics linked to cultivation and wood properties (Scholz and Ellerbrock, 2002; Zhang *et al.*, 2003) but also, the genome sequence is known (Tuskan *et al.*, 2006) and improvement cycles are relatively short. Other

factors contributing to the suitability of these species include a high capacity for vegetative reproduction and rapid growth rate.

The importance of cultivating *Salicaceae* for this purpose (obtaining biomass) at global scale is reflected by the abundance of information which the International Poplar Commission (statutory body within the framework of the FAO) has gathered over the last decade, covering the subject specifically in one of its working groups (FAO, 2019). The International Catalogue of Base Materials of the *Populus* genus to obtain forest reproductive material contains 358 entries, among which several are referenced specifically for biomass production ('Boiano-4', 'Baldo', 'Hunneghem' o 'Raspalje'). Many more are in the preliminary stages for inclusion and have been put forward for this use ('AF2', 'AF8', 'A4A', 'Monviso', 'Muur', 'Orion', 'Oudenberg', 'Sirio' or 'Vesten') (See Fig. 1.3). In the framework of the EU-POP project, more than 17 genotypes are currently being characterized as biomass producers in a multi-environment trial in which ten European countries are taking part, including Spain.

In our country, the main species of interest at commercial or pre-commercial level are those belonging to the genera *Eucalyptus* spp. and *Populus* spp.

The cultivation of some species and hybrids of *Eucalyptus* has been conducted in certain areas of our country, mainly by the company ENCE, with production levels of between 13.9-14.6 Mg ha^{-1} yr^{-1} in the case of *Eucalyptus globulus* Labill. and 20.4-21.5 Mg ha^{-1} yr^{-1} for *Eucalyptus nitens* (Deane and Maiden) Maiden (Pérez-Cruzado *et al.*, 2011). These rates of production are to a large extent due to the high degree of improvement achieved, resulting in well adapted material for use in plantations. ENCE has made considerable advances in the development of cultivation techniques, improvement in material aimed specifically at energy crops, irrigation techniques, fertilization, control of competing vegetation, harvesting and other stages of the process in the commercial production of biomass from crops. Unfortunately, these initiatives are now being held back by a change in the scenario of incentives to electricity generation in special regime which came into force in 2013 (Paredes-Sánchez *et al.*, 2018).

Eucalyptus has another advantage over other fast growing species, which is that it has an acceptable degree of tolerance to drought conditions (Johnson *et al.*, 2007b), although its high levels of production are associated with scenarios where droughts do not occur or where irrigation is used. However, the expansion of *Eucalyptus* to areas of the country beyond those where it is currently cultivated seems unlikely due to strong social opposition based on assumptions which are not always scientifically proven. In southern Italy there are also examples of *Eucalyptus* being cultivated for different end uses, biomass production being one of them (Facciotto *et al.*, 2009; Sgroi *et al.*, 2015).

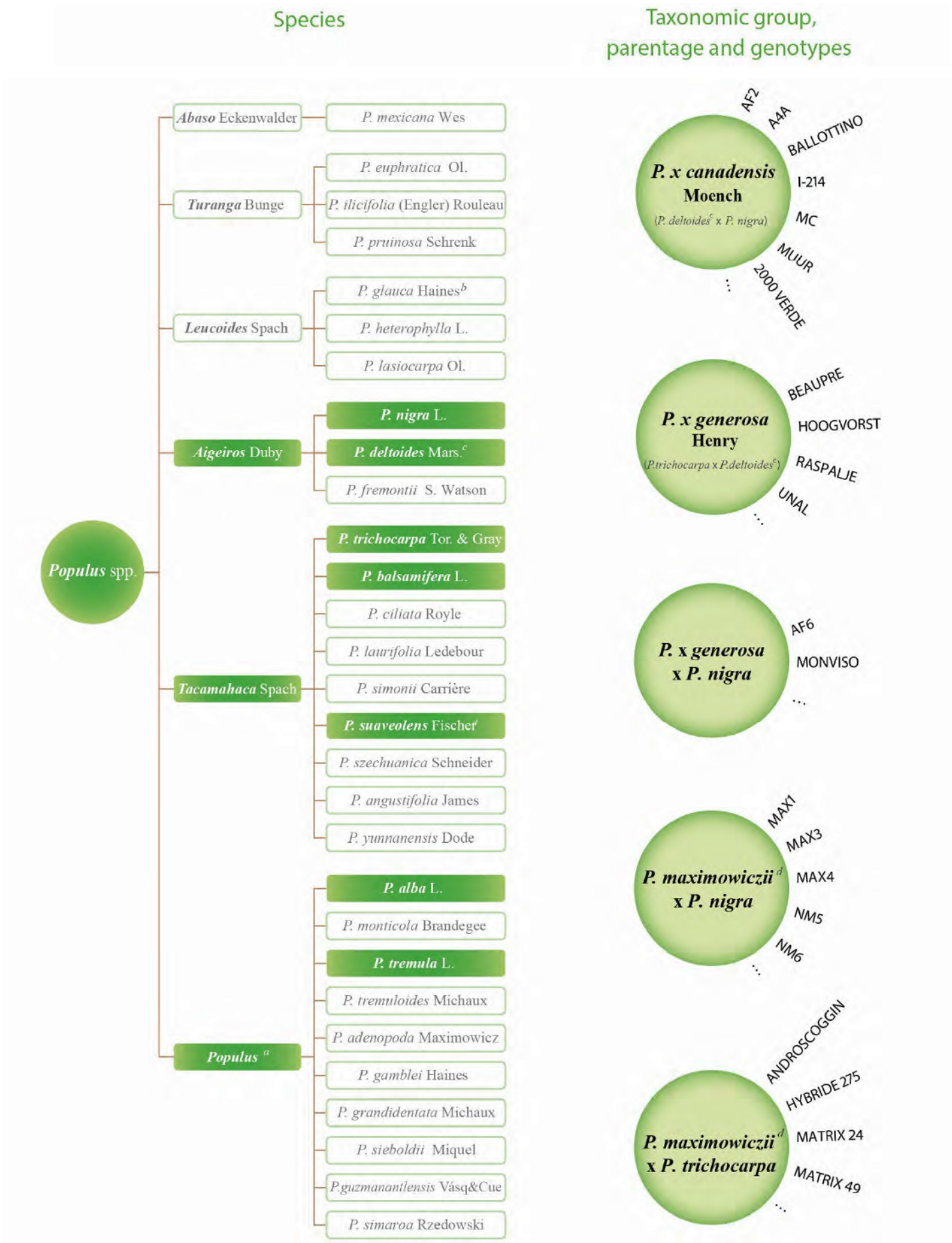
Species
Taxonomic group, parentage and genotypes
Populus spp.
Abaso Eckenwalder
P. mexicana Wes
Turanga Bunge
P. euphratica Ol.
P. ilicifolia (Engler) Rouleau
P. pruinosa Schrenk
Leucoides Spach
P. glauca Haines[b]
P. heterophylla L.
P. lasiocarpa Ol.
Aigeiros Duby
P. nigra L.
P. deltoides Mars.[c]
P. fremontii S. Watson
Tacamahaca Spach
P. trichocarpa Tor. & Gray
P. balsamifera L.
P. ciliata Royle
P. laurifolia Ledebour
P. simonii Carrière
P. suaveolens Fischer[f]
P. szechuanica Schneider
P. angustifolia James
P. yunnanensis Dode
Populus [a]
P. alba L.
P. monticola Brandegee
P. tremula L.
P. tremuloides Michaux
P. adenopoda Maximowicz
P. gamblei Haines
P. grandidentata Michaux
P. sieboldii Miquel
P.guzmanantlensis Vásq&Cue
P. simaroa Rzedowski
P. x canadensis Moench
(P. deltoides[c] x P. nigra)
AF2
A4A
BALLOTTINO
I-214
MC
MUUR
2000 VERDE
P. x generosa Henry
(P.trichocarpa x P.deltoides[c])
BEAUPRE
HOOGVORST
RASPALJE
UNAL
P. x generosa x P. nigra
AF6
MONVISO
P. maximowiczii[d] x P. nigra
MAX1
MAX3
MAX4
NM5
NM6
P. maximowiczii[d] x P. trichocarpa
ANDROSCOGGIN
HYBRIDE 275
MATRIX 24
MATRIX 49

Figure 1.3. Eckenwalder (1996) classification of the genus *Populus*. [a] Synonymous with Leuce Duby. [b] Sensu lato. Synonymous with *P. wilsonii* Schneider. [c] Synonymous with *P. sargentii* Dode and *P. wislizenii* Sargent. [d] Synonymous with *P. cathayana* Rehder, *P. koreana* Rehder, and *P. maximowiczii* A. Henry. The circles represent some of the taxonomic groups with their parentage and some of their genotypes. Some of the main genotypes planted in each country are represented on the European map.

The cultivation of species and hybrids of *Populus* for industrial use wood is well established in parts of the communities of Castilla y León, La Rioja or Navarra, among others (MAPA, 2019). The National Catalogue of Base Material for forest reproductive material of the *Populus* genus in qualified and controlled categories comprises 24 commercial quality genotypes (Order of 24/06/1992, Order APA/544/2003 of 06/032003, Resolution of 07/07/2006 of the Dirección General de Agricultura and Resolution and 07/11/2011 of the Dirección General de Recursos Agrícolas y Ganaderos). Some of this material, which is well adapted to the conditions in our country, could also be of interest for biomass production. Moreover, much of the material is being evaluated in a network of trials which will be described in this document. The potential of *Populus* is explained to a large degree by this availability of material adapted to existing conditions along with the appropriate knowledge relating to the management of the species in many parts of our country.

Figure 1.4. Detail of crowns in a high density, short rotation poplar plantation.

Furthermore, trials have taken place in recent years with other potentially usable species with differing results. In this regard there have been experimental trials with *Ulmus pumila* (Perez *et al.*, 2014), *Robinia pseudoacacia* (Böhm *et al.*, 2011; Manzone *et al.*, 2015), hybrids of *Salix matsudana* (Karp and Shield, 2008; Rosso *et al.*, 2013), *Platunus* x *hybrida* (Sixto *et al.*, 2015) or different cultivars of *Paulownia* spp. (Zuazo *et al.*, 2013; García-Morote *et al.*, 2014), the latter displaying severe adaptation problems (Hernandez *et al.* 2008). Although some of these may be of interest in terms of adding variety to the area of crop development, perhaps their main limitation stems from the fact that there is a lack of improved genetic material, necessary to allow their use in different environments.

1.4. Lines of progress: state of art

Sustainable improvement in the production of biomass from SRF crops requires advances to be made in different aspects, all of which are important to achieve the final objective.

In this section, the aim is to provide an overall vision of the current situation of each of these aspects, focusing in particular on those which are dealt with in this doctoral thesis (Fig. 1.5).

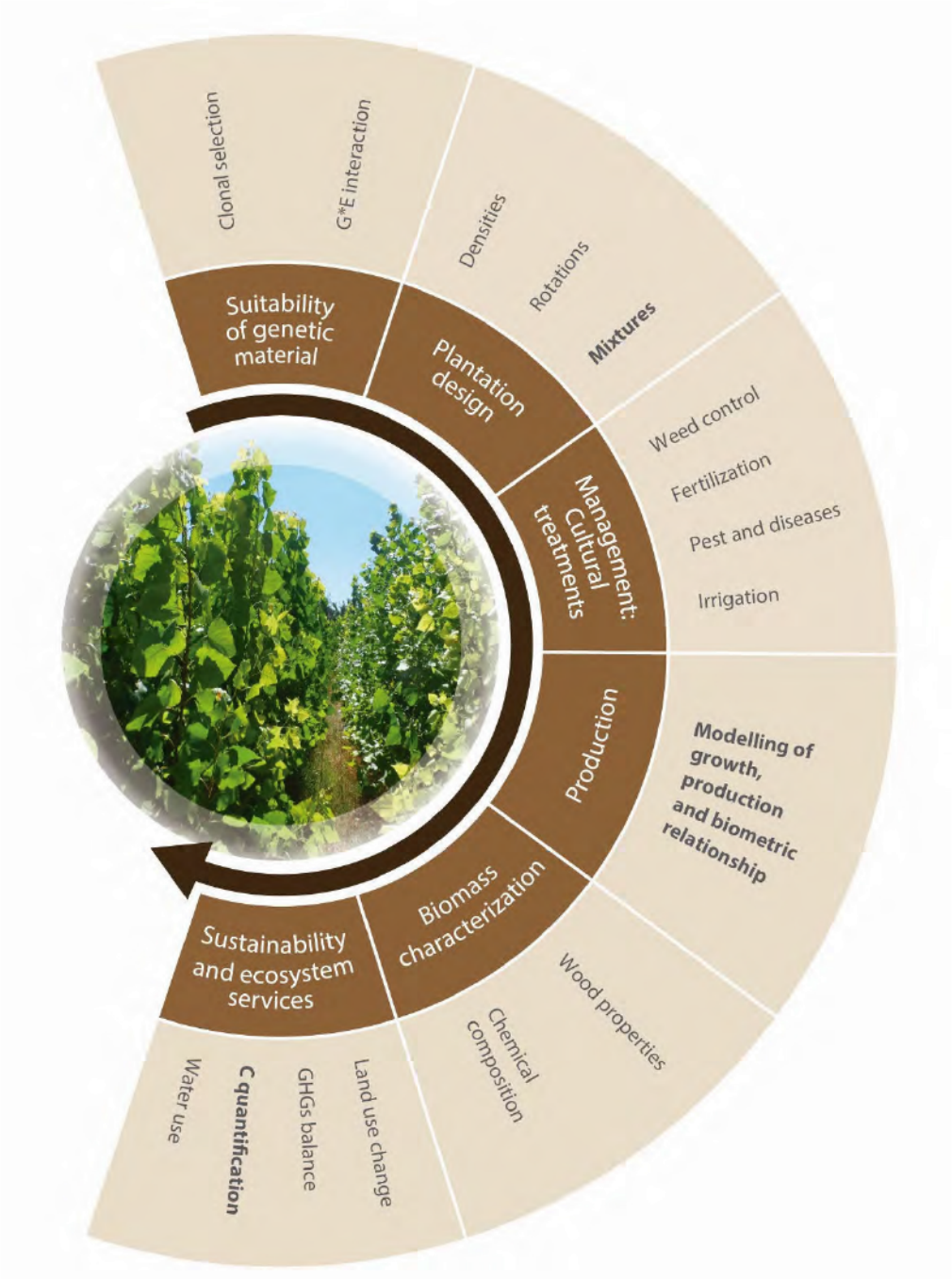

Figure 1.5. Lines of action to improve SRF managed woody crops.
*** Shaded lines of action are those addressed in this thesis.**

1.4.1. Suitability of genetic material

1.4.1.1. Clonal selection

Using the best, most adapted material when carrying out plantations helps to ensure efficient use of site resources and therefore higher levels of production. As regards the biomass, the development of genotypes aimed at achieving increased production, greater tolerance to pests and disease or specific characteristics of the wood, is ongoing through genetic improvement programs in the countries spearheading the research, the most prominent of these being the USA, Italy, France, Germany or Sweden.

Traditional approaches based on recurrent selection as a way to increase hybrid vigour (Stanton, 1995; Nardin and Alasia, 2004) are now supported by genomics and phenomic techniques which contribute to accelerating these processes (Marchadier and Sigaud, 2005; Fladung and Polak, 2012). For example, tools such as genome editing using Crispr/Cas9 technology (Fan *et al.*, 2015; Liu *et al.*, 2015; Zhou *et al.*, 2015), the development of transgenic plants (Pilate *et al.*, 2016), complete genome association analysis (Porth *et al.*, 2013), or new phenotypic tools (Ludovisi *et al.*, 2017), are now leading to important advances in genetic improvement of the *Populus* genus. An interesting review of the progress towards improved biomass quality and resilience of production using both traditional approaches and new technology is provided by (Clifton-Brown *et al.*, 2019). New avenues of research have also been opened up through considering epigenetic inheritance, also termed soft inheritance. This relates to inheritable changes to the gene expression induced by environmental changes (Gömöry *et al.*, 2017; Le Gac *et al.*, 2018).

1.4.1.2. Genotype*environment interaction

Phenotypic plasticity refers to the capacity of an organism to alter its characteristics in response to environmental conditions (Bradshaw, 1965). In short, the phenotype of an individual is conditioned by its genotype, the environment in which it develops and by their interaction (G*E interaction), this effect often being evidenced by the instability of phenotypic correlations derived from drastic differences in biomass production found in numerous studies (Mohn and Randall, 1973; Orlovic *et al.*, 1998; Zalesny *et al.*, 2009).

The G*E interaction conditions the stability of production and makes clonal recommendation more difficult, although it can also provide an opportunity to maximize production by matching the most suitable material to specific site conditions. From an agronomic perspective, genotypic stability is understood as the capacity of a cultivar to produce in accordance with the productive potential of each environment, that is, without straying from the behaviour expected for the average genotypic value (Becker and Leon, 1988). However, the strategies may differ; either attempting to find broad adaptation or optimizing the adaptation to specific site conditions, hence the necessity to characterize the material (Bergante *et al.*, 2010; Sabatti *et al.*, 2014; Li *et al.*, 2017). This characterization not only has an impact on crop management but also has repercussions on improvement strategies. In Spain, material destined

for biomass production has been characterized in recent years through analysis of this interaction (Sixto *et al.*, 2011; Sixto *et al.*, 2013a; Sixto *et al.*, 2015; Sixto *et al.*, 2016a).

1.4.2. Plantation design

The design of the plantation is also a highly influential factor for stand management and optimization of production (Fiala and Bacenetti, 2012). Over the course of the study period, many different types of design have been tested, combining different plantation densities (Cañellas *et al*. 2012), tree distributions and rotation lengths. The possibility of mixing different species or different varieties of the same species has also been put forward as a means to optimize production.

1.4.2.1. Densities

There are many examples of plantations carried out using a range of densities in order to define the optimum plantation density, from 1,000 cuttings ha^{-1} up to more than 40,000 cuttings ha^{-1} (Klasnja *et al.*, 2003; Fiala *et al.*, 2010). Even higher densities have been contemplated, albeit less frequently, as in the case of DeBell *et al.* (1993). In our country, a wide range of densities in multiple environments have been tested (Ciria *et al.*, 1995; Cañellas *et al.*, 2012). In terms of production, densities of around 20,000-25,000 stools ha^{-1} seem to be the most productive, at least for the initial rotations, although other factors such as the costs associated with the higher densities or impediments to mechanization of the plantation, mean that densities in excess of 15,000 stools ha^{-1} are not recommendable (Cañellas *et al.*, 2012).

Furthermore, for a given density, the arrangement of the stools is also an aspect which attracts controversy. Thus, it is common for trials to include both single row distributions (Rosso *et al.*, 2013; Sabatti *et al.*, 2014) and other more irregular distributions in which the stems are arranged in double rows (Aylott *et al.*, 2008; Dillen *et al.*, 2013). These differences do not appear to lead to relevant variations in production (Friedrich, 1999), although regular frameworks tend to facilitate management tasks.

Density and distribution of stools should be directly linked to the rotation lengths applied along with the maintenance and harvesting processes, always attempting to keep the production cost per biomass unit obtained as low as possible.

1.4.2.2. Rotations

The rotation is a factor closely linked to the density, as well as being influenced by the environment. Different rotations have been employed in the management of this type of plantation, from yearly to relatively long rotations coinciding with other productive objectives (Bungart and Hüttl, 2004; Nassi O Di Nasso *et al.*, 2010; Martín-García *et al.*, 2012; Truax *et al.*, 2018). In recent years, rotations of between 2 and 4 years are those most frequently used. The final harvesting age varies depending on the genotype and site conditions (Bergante *et al.*, 2010; Dillen *et al.*, 2013; Vanbeveren and Ceulemans, 2018).

Another question which has been addressed is whether or not to carry out coppicing after the first establishment year to encourage multiple shoot growth (Willebrand and Verwijst, 1993; Kopp *et al.*, 2001; Fernández *et al.*, 2016). However, in these cases, the benefits obtained through resprouting may not compensate the greater costs associated with this practice. The different management schemes are shown in Fig. 1.6.

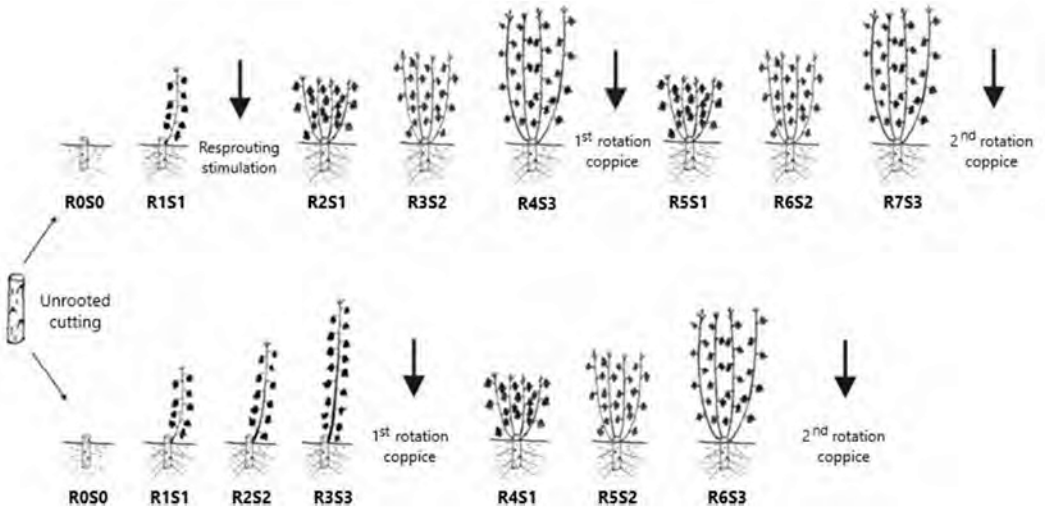

Figure 1.6. Different management schemes in SRC plantations.

1.4.2.3. Mixtures

There has been a sharp rise in the number of mixed plantations in the forestry sector over recent decades because of the perceived benefits, not only for the environment and ecology but also for production as a result of factors linked to facilitation. They are tree by tree species mixtures as mixing genotypes is common practice in cultivated forests at stand level or greater dasocratic divisions (Evans, 2009). Although applying these designs in high density, short rotation plantations has attracted interest, there is as yet very little published material. Few examples exist of mixed plantations of poplar with other species in SRC, although one example is that of *Populus* spp. with *Alnus* spp. (Dawson and Hansen, 1983; Radwan and DeBell, 1988) along with recent interest in the mixture of *Populus* spp. with *Robinia* spp. (Gana *et al.*, 2014; Marron *et al.*, 2018). One of the chapters in this thesis focuses on evaluating the suitability of the mixture *Populus alba* - *Robinia pseudoacacia* in SRC under Mediterranean conditions. A similar design but using the euramerican genotype 'Dorskamp' has been tested by Marron *et al.* (2018). The use of a nitrogen fixing species which, apart from reducing or even eliminating the need for nitrogen additions (with the consequent ecological and economic implications) can lead to an increase in production. Hence this is a possible combination of interest, which has not as yet been explored in any depth. Both species, *P. alba* and *R. pseudoacacia*, are considered to be relatively tolerant to possible drought scenarios (Grünewald *et al.*, 2009; Sixto, 2014).

Apart from mixtures of different species, complementarity is also explored in mixtures of varieties within the same genus (DeBell and Harrington, 1993, 1997). Some examples in this regard include plurivarietal plantations of *P.* x *generosa* (Miot *et al.*, 1999), of other hybrid

groups of *Populus* (Elferjani *et al.*, 2014) or of different varieties of *Salix* spp. (Dillen *et al.*, 2016), where the aim is to attain greater tolerance to biotic and abiotic stresses while also increasing productivity.

1.4.3. Management: cultural treatments

There are many aspects of plantation management which have an impact on production. The most important of these are the following:

1.4.3.1. Control of competing vegetation

Competition from weeds in the initial crop establishment stage is one of the main reasons for plantation failure. It is not only competition for water and nutrients but also for light and space, which are vital in the establishment stage. Deficits of these requirements can render the plantation unviable (Sixto *et al.*, 2007). Hence, weed control is considered a necessary practice, with treatments also being necessary after each coppicing and which can be undertaken using both chemical and/or mechanical techniques (Broeckx *et al.*, 2012b; Morhart *et al.*, 2013; Albertsson *et al.*, 2014).

Figure 1.7. Competing vegetation in the initial crop establishment stage.

1.4.3.2. Fertilization

The use of fertilizer in SRC plantations is a subject which attracts debate. There are many examples where fertilization provides no benefits (Heilman and Fu-Guang, 1994; Ceulemans and Deraedt, 1999; Scholz and Ellerbrock, 2002; Liberloo *et al.*, 2006; Bergante *et al.*, 2010) and others where there are clear visible effects on production (Van Veen *et al.*, 1981; Coleman *et al.*, 1998; Stanturf *et al.*, 2001; Coleman *et al.*, 2006; Euring *et al.*, 2012; Yan *et al.*, 2016).

The lack of response to fertilization may be due in many cases to the fact that soil fertility is optimal. However, the high nutritional requirements of poplar, especially for N (Rennenberg *et al.*, 2010; Paris *et al.*, 2015), mean that in locations where the soil characteristics are not optimal, it is beneficial to apply fertilizer to obtain the greatest possible production (Wang *et al.*, 2015). Excessive use of nitrogen fertilizers as part of conventional practice is increasingly questioned due to the environmental risks which it involves (Heller *et al.*, 2003; Gruber and Galloway, 2008; Erisman *et al.*, 2010), as well as the high economic costs associated with this practice. Therefore, the use of alternative designs such as mixed plantations with nitrogen fixing species or alternative fertilization such as sewage sludge or the use of waste water have gained prominence in recent decades (Aronsson, 2000; Sugiura *et al.*, 2008; Werner and McCracken, 2008) due to the fact that, besides not having to use traditional fertilizers, these materials are purified and/or reused and therefore the plantations act as phytoremediators (Martínez-Hernández *et al.*, 2018).

1.4.3.3. Control of pests and diseases

Newly appeared pests and diseases are one of the main problems facing agriculture in the 21st century due to the presence of extreme climatic conditions (Kollert, 2018). In poplar plantations, this risk has increased sharply as a result of the expansion of monoclonal plantations. The situation is made worse by the fact that there are only a small number of different genotypes used from one plantation to another. There has been clear progress made in this area and it continues to be one of the main objectives in efforts to improve the genus.

Figure 1.8. Caterpillar of the poplar chrysomela (*Chrysomela populi* L.).

Examples of the most serious pests and diseases affecting the leaves are those caused by *Melampsora* spp. (rusts), *Marssonina brunnea* (Ell. et Ev.) Magn., *Phloeomyzus passerinii* Sign. (woolly poplar aphid), *Venturia populina* (Vuill.) Fabr., *Chrysomela populi* L. (red poplar leaf beetle), *Leucoma salicis* L. (white satin moth); those affecting the stems and trunks are *Mycosphaerella populorum* Peck (stem canker), *Cryptorhynchus lapathi* L. (poplar and willow

borer), *Paranthrene tabaniformis* Rott. (dusky clearwing), as well as *Sesia apiformis* L., among others (Steenackers *et al.*, 1996; Newcombe *et al.*, 2001; Coyle *et al.*, 2005; Duplessis *et al.*, 2009; Broderick *et al.*, 2010). The sustainability of these plantations depends on the use of genetic material which is tolerant or resistent to these types of stress (Nordman *et al.*, 2005; Sixto *et al.*, 2007).

1.4.3.4. Irrigation

Given the marked hygrophilic nature of the *Populus* genus (Isebrands and Richardson, 2014), the availability of water in the soil is one of the limiting factors for its cultivation (Bloemen *et al.*, 2016). In Spain, therefore, due to the Mediterranean climatic conditions, it is necessary to irrigate SRC plantations during the summer drought season (González-González *et al.*, 2017). Limitations on the use of water at global scale (IPCC, 2007) have led to changes in the way irrigation is applied, moving from flood irrigation to more efficient systems such as drip irrigation, although much more can still be done to increase technical efficiency.

Water restrictions generally lead to production losses (Liang *et al.*, 2006; Hennig *et al.*, 2015), although high variability has been found in the response to drought conditions. Studies focusing on the regulation of stomatal conductance, photosynthetic rates and water use efficiency (Navarro *et al.*, 2014; Sixto *et al.*, 2015; González-González *et al.*, 2017; Navarro *et al.*, 2018b), as well as the search for genetic material tolerant to drought (Viger *et al.*, 2016), or the reuse of water from different sources (De Bustamante, 1998), have increased in number recently in an attempt to find ways to optimize production under conditions of water restriction.

1.4.4. Production

High density short rotation coppice plantations (SRC) provide a viable alternative for the production of quality lignocellulosic biomass (Makeschin, 1999). The biomass produced in SRC is characterized by predictable periodicity and yield depending on the area of cultivation. The main objective with regard to these crops is to achieve a high level of sustainable production while maximizing benefits, combining economic viability with environmental sustainability.

According to Sixto *et al.* (2007) review, related to biomass production in this type of plantation using poplar genotypes, a large quantity of literature exists on the different clonal productivity in an array of environments (374 references). Table 1.1 presents some examples of biomass production obtained in different countries, which range considerably from 1 to 37 Mg dm (dry matter) ha^{-1} yr^{-1} depending on the site characteristics, the genetic material, the design and the management scheme. In Spain, the potential production at national level is estimated at around 15.3 Mg dm ha^{-1} yr^{-1} for plantations with standard management schemes (Pérez-Cruzado *et al.*, 2013), although there is also high variability depending on the previously mentioned characteristics (Ciria *et al.*, 1995; Ciria *et al.*, 2004; Sixto *et al.*, 2013a; Sixto *et al.*, 2015; Fernández *et al.*, 2016).

Table 1.1. Biomass yield (Mg dm ha^{-1} yr^{-1}) per genotype, age and rotation in poplar short rotation plantations in Europe.

Species and poplar hybrids	Genotype	Density	Age	Country	Yield (Mg dm ha^{-1} yr^{-1}) by rotation			Reference
					1st	2nd	3rd	
P. deltoides x *P. trichocarpa*	IBW2	10,000	4	Belgium	2.5	1.6		Laureysens *et al.*, 2003 and Laureysens *et al.*, 2005
P. nigra	Wolterson				8.1	9.7		
P. trichocarpa	Columbia River				7.8			
	Fritzi Pauley				8.1	8.2		
	Trichobel				8.4	8.2		
P. x *canadensis*	Gibecq				1.6	3.5		
P. x *generosa*	Hazendans				10.8	3		
	Hoogvorst				10.1	8.2		
P. deltoides x *P.* x *generosa*	Grimminge	8,000	2 (In the 3rd rot the age is 3 yr)	Belgium	4.3	11.7	8.4	Vanbeveren and Ceulemans, 2018*
P. nigra	Brandaris				1.4	7.0	8.7	
P. nigra x *P. maximowiczii*	Wolterson				2.7	11.7	14.6	
P. trichocarpa x *P. maximowiczii*	Bakan				4.9	14.3	18.1	
	Skado				5.7	16.8	20.8	
P. x *canadensis*	Ellert				3.3	11.1	19.5	
	Hees				6.5	15.5	26.0	
	Koster				2.8	9.5	14.1	
	Muur				3.9	12.4	17.1	
	Oudenberg				3.7	13.4	14.0	
	Robusta				2.5	8.1	15.6	
	Vesten				4.7	12.6	16.1	
P. maximowiczii x *P. trichocarpa*	NE-42	2,222	3 (2nd rot: 4yr)	Czech Republic	1.0-1.4	9.4-9.8	9.1-11.4	Benetka *et al.*, 2014
			3 (2nd rot: 2yr)		8.3	15.4	18.9	
P. balsamifera x *P. tremula*	P-524	10,000	6	Czech Republic	8.1			Trnka *et al.*, 2008
P.maximowiczii x *P.berolinensis*	P-494				10.2			
P. nigra	*P. nigra*				2.6			
P. nigra x *P. maximowiczii*	J-104				11.9			
	J-105				13.9			
P. x *generosa*	P-473				9.7			
P. x *generosa*	Beaupré	3,030	3	France	1.57-11.13			Berthelot, 2001
			4		2.73-12.7			
	Boelare		3		1.37-10.53			
			4		2.85-11.6			
	Hunnegem		3		12.1			
			4		13.68			
	Raspalje		3		2.2			
			4		3.63			
P. x *generosa*	Beaupré	8,333	8	Germany	6.1			Bungart and Hüttl, 2004*
	Rap				5.8			
P. nigra x *P. maximowiczii*	Max 1				3.3			
	Max 3				3.5			
	Max 4				3			
P. maximowiczii x *P. trichocarpa*	Androscoggin				3.3			
	Hybride 275				4.2			
P. trichocarpa	Muhle Larsen				3.7			
P. maximowiczii x *P. nigra*	Max 1	8,890	2	Germany	1.0-1.59			Hartmann and Lamersdorf, 2015
		9,250			6.81			
P. maximowiczii x *P. nigra*	Max 3	17,778	4	Germany	8.6			Huber *et al.*, 2016
P. maximowiczii x *P. trichocarpa*	Androscoggin				10.5			
P. maximowiczii x *P. nigra*	Max 4	11,000	2	Germany	5.4-6.3			Kern *et al.*, 2018
P. deltoides	Baldo	5,747	2	Italy	4.75			Bergante *et al.*, 2010
		10,000			17.5			
	Dvina	10,000			11.8			
	Lambro	10,000			9.5			
	Lena	10,000			14.2			
	Lux	5,714			7.05			
		8,333			4.3			
	Oglio	10,000			14.1			
P. x *canadensis*	BL-Costanzo	5,714			3.25			
		7,142			4.45			
	Cima	5,714			4.55			
		7,142			5.7			
	I-214	5,747			4.35			
		10,000			8.4			
	Luisa Avanzo	5,714			5.55			
		7,142			6.25			
	Orion	5,747			4.46			
		10,000			17.0			
P. deltoides	Lux	10,000	2	Italy	12.38	6.99	4.99	Cabrera *et al.*, 2014
			3			7.07	14.53	

P. deltoides	Lux	10,000	1 2 3	Italy	 22.5 24.3		16.4	Nassi O Di Nasso *et al.*, 2010
P. deltoides	Lux	5,900	2	Italy	3.31-9.33	2.17-18.85		Paris *et al.*, 2011
P. maximowiczii x *P. nigra*	AF10				14.84	20.74		
P. x *canadensis*	AF2				5.66-15.77	7.54-17.01		
	I-214				3.63-11.87	4.2-18.44		
	Sirio				5.65	8.55		
P. x *generosa* x *P. nigra*	AF6				5.76-15.04	5.79-17.07		
	Monviso				6.79-17.92	9.31-23.55		
P. x *generosa* x *P.trichocarpa*	AF8				4.75-14.93	5.04-24.05		
P. x *canadensis*	AF2	6,061	2	Italy	16	17	17	Sabatti *et al.*, 2014*
	I-214				11.5	18	13.5	
P. x *generosa* x *P. nigra*	AF6				15	17.5	15	
	Monviso				17.5	23.5	17.5	
P. x *generosa* x *P.trichocarpa*	AF8				15	24	19	
P.maximowiczii x *P.trichocarpa*	NE-42	1,333	7	Poland	8			Niemczyk *et al.*, 2018
P.trichocarpa x *P.trichocarpa*	Fritzi Pauley				8.1			
P. x *canadensis*	AF2				4.1			
	Albelo				4			
	Degrosso				6.8			
	Koster				5.6			
	Polargo				4.3			
P. x *generosa* x *P.trichocarpa*	AF8				2.5			
P. nigra x *P. maximowiczii*	Max 5	11,110	3	Poland	7.8			Stolarski *et al.*, 2014
P. nigra	Lombardo Leonés	17,316	3	Spain	9.1	17.1		Oliveira *et al.*, 2014
P. x *canadensis*	A4A				20.0	12.2		
	A2A				11.8	15.3		
	I-214				22.0	30.1		
	Luisa Avanzo				17.3	27.1		
	MC				16.8	18.4		
	2000 Verde				13.64	14.25		
P. deltoindes	Baldo	10,000	3	Spain		8.59-29.94		Oliveira *et al.*, 2015
	Ballottino					11.45-19.20		
P. x *canadensis*	I-214					11.09-37.27		
	Orion					12.70-34.56		
	Oudenberg					9.66-19.96		
P. x *canadensis*	AF2	33,333	3	Spain	16.8			Sixto *et al.*, 2013a
	Guardi				12.44			
	I-214				12.21			
	MC				15.32			
	2000 Verde				15.38			
P. x *generosa*	Unal				11.13			
P. x *generosa* x *P. nigra*	Monviso				14.47			
	Pegaso				6.65			
P. deltoides	Baldo	10,000	3	Spain	12.09			Sixto *et al.*, 2015
	Ballottino				11.06			
P. x *canadensis*	I-214				11.49			
	Orion				16.11			
	Oudenberg				11.22			
P. deltoides	Viriato	13,333	3	Spain	9.55	18.20	12.82	Sixto *et al.*, 2018
P. x *canadensis*	AF2				13.47	16.66	13.99	
	Ballottino				9.48	16.75	12.27	
	I-214				9.11	13.44	15.81	
P. x *generosa* x *P. nigra*	Monviso				12.29	11.57	13.92	
P. trichocarpa	Columbia River	10,000	3	United Kingdom	6.71	6.62		Aylott *et al.*, 2008
	Fritzi Pauley				8.59	8.24		
	Trichobel				9.08	9.59		
P.trichocarpa x *P.balsamifera*	Balsam Spire				7.24	7.03		
P. x *canadensis*	Gaver				6.58	5.58		
	Ghoy				6.45	5.77		
	Gibecq				5.7	4.73		
P. x *generosa*	Beaupré				7.34	4.87		
	Boelare				6.23	4.2		
	Hazendans				7.23	7.56		
	Hoogvorst				8.84	8.12		
	Raspalje				6.69	4.66		
	Unal				7.55	5.25		
P.balsamifera x *P.trichocarpa*	Balsam Spire	10,000 4,444 10,000	5 5 4	United Kingdom	14.42 11.20 13.98			Proe *et al.*, 2002

*Estimated values from graphs.

1.4.4.1. Modelling of growth, production and biometric relationships

The use of estimation models to predict available biomass is becoming more frequent as research into this type of plantation progresses, not only because of the costs involved in carrying out direct estimates (Picard *et al.*, 2012), but also due to the greater flexibility and the possibility to extrapolate the results to larger scales when the models are based on wide ranges of empirical data.

Allometric models which relate tree diameter or another easily measurable variable to the biomass are those most commonly used in forest inventories or ecological studies (Peichl and Arain, 2007; Shaiek *et al.*, 2011). These models have also been employed in SRC plantations to estimate available biomass (Verwijst *et al.*, 2013; Mosseler *et al.*, 2014; Verlinden *et al.*, 2015). These predictions are particularly important when evaluating the economic viability of a crop (Cannell and Willett, 1976; Pérez-Cruzado *et al.*, 2013). Apart from the choice of regression model, the assumptions that underlie the regression procedures, and the data transformations used during the procedures (Ruark *et al.*, 1987), we are confronted with a large number of factors which create uncertainty and may all affect the final results. Simple equations which are valid for a wide range of conditions and plant material are usually preferred. The mathematical equation most commonly used to estimate biomass takes the form of a power function: $W = aD^b$, where W is the dry mass of the stool, D is the stool diameter, and a and b are the scaling coefficients. There are several examples of this type of model used in poplar SRC plantations (Coyle and Coleman, 2005; Liberloo *et al.*, 2006; Rock, 2007; Al Afas *et al.*, 2008a; Paris *et al.*, 2011; Di Matteo *et al.*, 2012; Rosso *et al.*, 2013; Njakou Djomo *et al.*, 2015; Vanbeveren and Ceulemans, 2018). The values of these coefficients are reported to vary according to the species, stand age, site quality, and the climate of the stands (Dillen *et al.*, 2007). The precision of such allometric equations is generally sufficient for stem biomass components (Zianis *et al.*, 2005). However, other examples of equations exist that include more predictive variables in the models, which can improve the precision of the estimations (Ben Brahim *et al.*, 2000a; Zabek and Prescott, 2006; Dowell *et al.*, 2009; Johansson and Karacic, 2011; Pérez-Cruzado *et al.*, 2013; González-González *et al.*, 2017; Huber *et al.*, 2017).

Over the last decade considerable advances have been made as regards modelling to estimate biomass from SRC poplar plantations. Not only have advances been made in allometric models. Also much effort has been directed towards modelling other aspects of growth in these plantations, such as leaf architecture (Casella and Sinoquet, 2003), root production (Berhongaray *et al.*, 2013b; Berhongaray *et al.*, 2015) or the use of process models with this aim (Amichev *et al.*, 2010; Headlee *et al.*, 2013; Hart *et al.*, 2014).

Of the very few specific biomass equations for the *Populus* spp. genus at national level, that of Montero *et al.* (2005) within the generalized biomass equations is worthy of note. In this regard, it should be borne in mind that the available equations were developed for forest stems rather than coppices, planted at lower densities and with longer rotations than those normally associated with SRC. An updated version of these equations is also available in the review

carried out by Ruiz-Peinado *et al.* (2011) in which the property of additivity is included and both the diameter and height of the tree are incorporated as independent variables (Bravo *et al.*, 2011). Using the information from the previously mentioned network of SRC plots across Spain, predictive models for production at territorial scale have also been developed and these have been used to create maps of possible zones of production for this type of crop (Pérez-Cruzado *et al.*, 2013).

The lack of specific models for SRC plantations under Mediterranean conditions highlights the necessity to develop tools for estimation which can be used both in the planning stage and in the management of this type of plantations and which are specific to the particularities of these crops. All these questions are addressed in this doctoral thesis.

1.4.5. Biomass characterization

The use of lignocellulosic biomass is not limited to the production of bioenergy. In recent years a wide range of bioproducts related options have sprung up such as biopolymers, bioplastics or sugar fermentation bioproducts, among others (Sannigrahi *et al.*, 2010; Isikgor and Becer, 2015; Bioplat, 2017).

The characterization of wood based on its physiochemical properties is vital when considering different conversion methods according to the desired end use, whether it be for thermal, electric or second generation biofuel purposes (Cai *et al.*, 2017).

Figure 1.9. Harvesting in a demonstration trial located in Faramontanos.

The poplar as a producer of lignocellulosic woodchip biomass can supply raw material for processes of thermochemical or microbiological conversion. Worthy of note among the former is combustion, not only that related to domestic plants but also those processes which are more linked to cogeneration in industrial plants or co-combustion with coal (Jenkins *et al.*, 1998; McKendry, 2002; Tharakan *et al.*, 2003; Obernberger *et al.*, 2006). Also among the thermochemical processes are: gasification in downdraft gasifiers or fluidized bed (Elder and Groom, 2011), slow pyrolysis for the production of biochar (Kwapinski *et al.*, 2010; Kloss *et al.*, 2012) or fast pyrolysis to produce biocrude oil (Elliott *et al.*, 2009; Bridgwater, 2018).

As regards the biochemical processes, the conversion to second generation bioethanol is the most studied process, although lignocellulosic biomass can also be a source of biobutanol and other second generation biofuels.

1.4.5.1. Wood properties and chemical composition

Lignocellulosic biomass is mainly composed of cellulose (42-49%), hemicellulose (16-23%) and lignin (21-29%) (Zhang *et al.*, 2011; Ibarra *et al.*, 2017). Apart from electricity production, heating (McKendry, 2002) and biofuels such as ethanol, furfural and biokerosene (Sannigrahi *et al.*, 2010; Zhang *et al.*, 2011; Krzyżaniak *et al.*, 2019), lignocellulosic biomass has many other uses. For example, cellulose is used in the manufacture of cosmetics, textiles, pharmaceutical products or tyres, among others (Doherty *et al.*, 2011; Serrano *et al.*, 2012; Krzyżaniak *et al.*, 2019). Lignin can potentially be used as a raw material in the manufacture of products with a high value added, such as vanillin, biopolymers in petrochemistry or biopesticides. Lignin can even be used as a material for soil enrichment, especial in sites with poor sandy soils (Zhang *et al.*, 2011; Krzyżaniak *et al.*, 2019).

Figure 1.10. Wood chips detail.

The heating value is a characteristic of interest in the characterization of biomass (Kenney *et al.*, 1990; Sabatti *et al.*, 2014; Álvarez-Álvarez *et al.*, 2018) and is determined by the composition (Shafizadeh, 1981). High Heating Value (HHV) includes the latent heat of water vaporization (Cai *et al.*, 2017), hence the Lower Heating Value (LHV) should be used when calculating the available energy (McKendry, 2002). The LHV value for wood from SRC is usually around 18 MJ kg^{-1} (McKendry, 2002) although it varies depending on the genotype and the age (18.36 MJ kg^{-1} (Fernández *et al.*, 2013), 17.61-18.71 MJ kg^{-1} (Monedero *et al.*, 2016) or 18.16-18.42 MJ kg^{-1} (Niemczyk *et al.*, 2018)), among others.

The quality of biomass as a solid fuel generally takes into account the humidity content (which can lead to incomplete combustion or flame instability) and the ash content. The latter parameter is negatively related to the energy value and is associated with other problems resulting from the use of wood in combustion, such as boiler corrosion.

Many other properties have attracted particular interest in recent years such as the specific density (Beaudoin *et al.*, 2007; DeBell *et al.*, 2007; Pliura *et al.*, 2007), the production of volatile materials (Monedero *et al.*, 2016), the presence of nitrogen (N) given its environmental implications, or presence of chlorine (Cl) and sulphur (S) due to their corrosive effects (Vassilev *et al.*, 2010; Monedero *et al.*, 2016; Cai *et al.*, 2017).

1.4.6. Sustainability and ecosystem services

The importance of sustainability of crops destined for biomass production has been highlighted when evaluating their future development. Much effort has been dedicated to defining criteria and indicators of sustainability in the last decade. The World Bioenergy Association (GBEP, 2011) has developed a document which includes 24 voluntary sustainability indicators related to bioenergy in general. This document is particularly important since it represents the only multilateral initiative with broad consensus among the different governments and international organizations, providing a framework for future policy development. Despite this consensus, there are many different approaches to tackling these studies, sometimes because of geographical differences, which often leads to approaches that are not always concordant. Examples of these initiatives include the analyses conducted by Dallemand *et al.* (2007) or by Dimitriou and Rutz (2014), among many others.

In any case, the criteria and indicators must be founded on scientific evidence, without assuming the validity of repeated affirmations, which are not always sufficiently verified. In this regard there are numerous aspects which need to be addressed such as life cycle and water cycle analyses, soil quality and erosion control, among many others (Zalesny Jr *et al.*, 2012). Sustainability is also necessary from an economic and energy perspective. As regards the latter, the adaptation would appear to be favourable (Nassi O Di Nasso *et al.*, 2010; Manzone *et al.*, 2014). However, this may not be the case with regard to the economic viability given the current prices of biomass and absence of subsidies (El Kasmioui and Ceulemans, 2012; Manzone *et al.*, 2014).

The review of Li *et al.* (2018), highlights the necessity to identify sustainable sources of bioenergy. With this purpose in mind, the authors of the review compile and describe biomass yield information for the five lignocellulosic crops with the greatest future potential, among which they include poplar across a whole range of locations and countries. They conclude that these crops, which only account for 3% of the bioenergy in Europe (Don *et al.*, 2012), can be grown under a wide range of climatic conditions, thus allowing direct competition with food crops to be avoided. Moreover, it has been determined that woody crops cultivated in short rotation and herbaceous lignocellulosic crops emit between 40 and 99% less NO_2 than traditional crops consequently of lower fertilization requirements and greater N use efficiency. They also sequester carbon in the biomass that remains in the soil (0.44 Mg C ha^{-1} yr^{-1}) when they are planted on disused agricultural land, although the balance is not positive when they are planted on former pastureland. Other associated ecosystem benefits include increased biodiversity (phytodiversity and zoological diversity) (Dimitriou and Rutz, 2015), erosion control and soil conservation (Rowe *et al.*, 2009), as well as the role of the crop in phytoremediation (Pandey *et al.*, 2016).

Figure 1.11. General view of the plantation at rotation age.

Among the main concerns in Europe in terms of the sustainability of crops destined for biomass production and therefore of SRC plantations, are those linked to land use change. According to some studies, the balance of greenhouse gases and C in the soil indicates that bioenergy as a whole plays a role in the mitigation of climate change (Djomo *et al.*, 2011). However, other studies question this affirmation (Don *et al.*, 2012), or point to a minimum cultivation period beyond which the C balance becomes positive (Pérez-Cruzado *et al.*, 2012a; Pérez-Cruzado *et al.*, 2012b; Verlinden *et al.*, 2013b; Ceulemans *et al.*, 2018).

The impact on water resources is also an aspect which must be rigorously managed (Rowe *et al.*, 2009). In the Mediterranean area, the need for irrigation is postulated as one of their main weakness. However, improved irrigation technology, greater water use efficiency of the new

plant material selected (Oliver *et al.*, 2014; Navarro *et al.*, 2018a), or the use of recycled water from different sources (De Bustamante, 1998; De Miguel *et al.*, 2015) provide interesting possibilities in this regard.

Hence, the importance of gathering further data which will allow us to evaluate the sustainability as well as quantify the ecosystem services of woody crops in SRC under Mediterranean conditions. With this purpose in mind, the present thesis sets out to determine the contribution of the poplar root system under SRC management to soil carbon as an initial step towards evaluating the dynamics of soil carbon under Mediterranean conditions.

1.5. Plots providing the basis for the study:

Information from a large network of plots of high density, short rotation woody crops has been used in the development of this thesis (Fig. 1.12). These plots are distributed across areas of the country where conditions are suitable for this kind of crop. The creation of this network began in February 2006 and was organized within the framework of different research and development projects under the National Plan (Agricultural Resources and Technology), the public-private collaboration (INNPACTO: Decocel and other collaborations through agreements) or backed directly by the Administration (PSE-On Cultivos, Plan E: Lignocrop).

1.5.1. Plot structure

The plot structure is partially described in Sixto *et al.* (2010), and is specified below:

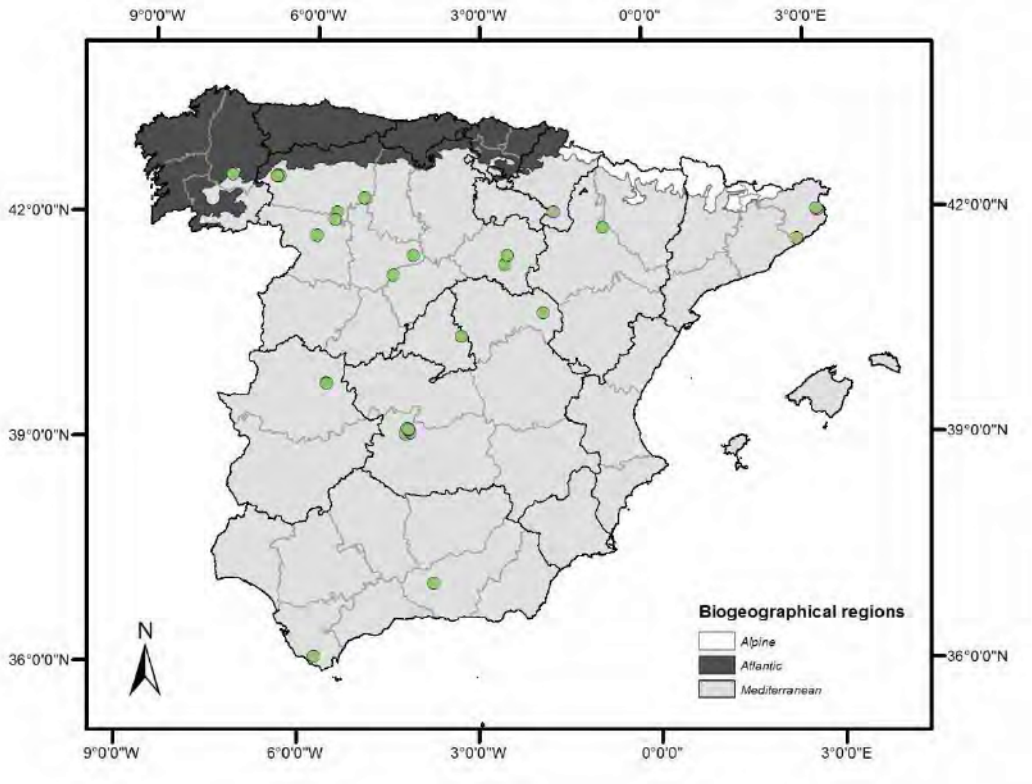

Figure 1.12. Location of the network of plots.

1.5.1.1. Demonstration plots:

The objective in these plots is to simulate the real management regime, both as regards cultural practices and particularly, the mechanization of the cultivation, which is considered essential for efficient development. A total of 30 demonstration plots were established at 23 different sites with sizes ranging from 2 to 8 hectares and variable densities from 5,000-20,000 cuttings ha^{-1}.

The genotypes used include those previously tested for SRC cultivation in other Mediterranean countries: 'Adige', 'AF2', 'AF8', 'Ballottino', 'Beaupre', 'Dorskamp', 'Grimminge', 'MC', 'Monviso', 'Muur', 'Orion', 'Oudenberg', 'Pegaso', 'Raspalje', 'Triplo', 'Vesten' and 'Viriato', as well as the genotype 'I-214', which is widely grown in Spain and therefore used as control genotype.

1.5.1.2. Experimental plots:

The objective in these plots is to test specific aspects related to the management, cultivation design, adaptation and production of the base materials etc. These plots are less than 1 ha in size. Experimental plots can be differentiated into the following trial types:

1.5.1.2.1. Density trials:

The aim of these trials is to evaluate the influence of crop density on production and to analyse the interaction between the density and the different characteristics tested. 25 plots were established at a total of 8 different sites, where the following plantation densities were tested using the genotype 'I-214':6,666, 10,000, 13,333, 15,000, 20,000, 25,000 and 33,333cuttings ha^{-1}.

1.5.1.2.2. Species trials:

15 plots with densities varying between 1,111 and 20,000 cuttings ha^{-1} were established at a total of 8 different locations. The trials included the following species: *Populus deltoides* ('Baldo'), *P.* x *candensis* ('Ballottino', 'I-214', 'Orion' and 'Oudenberg'), *Platanus* x *hispánica* ('Girona'), *Robinia pseudoacacia* ('Nyirsegi'), *Salix matsudana* x Salix spp. ('Levante'), *S. atrocinerea*, *S. trianda* x *S. viminalis* ('SA04') y *S. alba* x *S. rubens* ('SA05') y *Paulownia* spp. ('Cot 1' and 'Sun Tzu').

1.5.1.2.3. Trial with different base material from the *Populus* genus

The objective in these clonal experimentation plots is to compare different species and hybrids of the *Populus* genus in order to obtain data relating to production as well as clonal stability in the different cultivation environments. For this purpose, 39 genotypes were tested at a total of 14 different sites, with densities varying between 13,333 and 20,000 cuttings ha^{-1}. The genotypes tested were: 'A2A', 'A4A', 'AF2', 'AF34', 'AF6', 'AF8', 'Antonije', 'Beaupre', 'Brenta', 'Bruhl 7', 'B1M', 'Dellinois', 'Delrive', 'Dorskamp', 'Gazi', 'Grimminge', 'Guardi', 'Hoogvorst', 'Hybride 275', 'I-214', 'Koreana', 'Lombardo leones',

'Luisa Avanzo', 'Matrix 21', 'Max 4', 'MC', 'Monviso', 'Muur', 'Oudenberg', 'Pegaso', 'Skado', 'SV490', 'Trichobel', 'Triplo', 'Unal', 'USA 49-177', 'Vesten', 'Viriato', and '2000 Verde'.

1.5.1.2.4. Trials with autochthonous material: *Populus alba* L. and *P. deltoides* x *P. alba*

Two plots were established with a density of 10,000 cuttings ha^{-1}, one with 100% irrigation and the other with irrigation limited to 50% to test the yield of different genotypes of *Populus alba* and thus, evaluate the response of autochthonous material. The genotypes used were: 'Alba 111', 'J 1-3-18', 'PO 10-10-20', '9/8A' and '9/20A'.

1.5.1.2.5. Mixed plantations trial

To test an alternative to the traditional monospecific design, a plot was established with a mixture of *Populus alba* '111PK' and the nitrogen fixing species *Robinia pseudoacacia* 'Nyirsegi', at a density of 10,000 cuttings ha^{-1} and with different proportions of the species in the mixture.

Both the demonstration plots and the experimental plots, regardless of the specific objective for which they were designed, have allowed information to be gathered on aspects of interest related to sustainability or quantification of ecosystem services, such as litterfall and decomposition of foliar biomass, or quantification of carbon, both in the above- and below-ground fractions.

Hence, the data from the network of plots constitutes a valuable tool which has allowed and will continue to allow conclusions to be reached in the medium term with regard to the potential of the crops. Moreover, the information obtained allows us to detect the main weaknesses and determine the scenarios in which the plantations should be implemented. This Network of Plots has provided the basis for this thesis, with part of the information from the plots being used in all of the chapters.

1.5.2. Edaphic and climatic characteristics of the network of plots:

The edaphic variability of the network of plots is that of soils in areas of irrigation in Spain. The pH ranges from 5.09 to 9.82 and the percentage of organic material (%) between 0.03 and 4.59. As regards the climate, there is a high degree of macro and micro climatic variability, although the network as a whole can be encompassed under Mediterranean climatic conditions. The Mediterranean climate is characterized by mild, rainy winters and hot, dry summers, although there are considerable differences from one area to another. In fact, many of the study sites are in areas with a continental Mediterranean climate, where the winter is longer and colder. In any case, all the sites share a marked summer drought period which is the determining factor as regards the need to irrigate SRC forest crops. More information on the edaphoclimatic characteristic of the plots is provided in Sixto *et al.* (2007).

1.6. Justification for the thesis within the general context

As previously mentioned, SRC forest plantations constitute a viable alternative for the production of quality biomass, both for bioenergy purposes and to obtain a range of bioproducts in the context of the bioeconomy (Zhang *et al.*, 2011). In this regard, poplar has been identified among a small group of species with the greatest potential for the future (Li *et al.*, 2018; Clifton-Brown *et al.*, 2019).

The economic viability and sustainability of the crop is without doubt linked to the progress which is necessary in the different lines of action referred to, since only by focusing on the situation as a whole will it be possible to guarantee the consolidation of the technology required to produce the raw material. This thesis is justified in this context, its purpose being to contribute towards finding answers on aspects such as cultivation design, production estimates or sustainability. It delves into the study of the biometry of this type of plantation at different scales, advancing from individual tree level to plot level in different cultivation designs.

With regard to advances in modelling, the limited availability of **specific models** for irrigated plantations under Mediterranean conditions highlights the need to develop appropriate management tools adapted to these conditions in SRC plantations. This would allow biomass production to be quantified, which is essential in order to determine the availability of raw material.

The biomass produced in SRC is characterized by predictable periodicity and yield according to the area of cultivation. The main objective in the case of these crops is to attain high as well as efficient production, maximizing the benefits and combining economic viability with environmental sustainability. As regards the management of this type of plantation, it is essential to have tools which allow the standing biomass to be estimated, allowing improved decision making. In recent years, the use of statistical models based on a wide range of empirical data has become more frequent in research into the production of SRC crops (Dickmann, 2006; Aylott *et al.*, 2008; Pérez-Cruzado *et al.*, 2011; Headlee *et al.*, 2013; Tallis *et al.*, 2013), due to the difficulty involved in extrapolating specific values to greater scales given the wide variability in production for this type of crop. Hence, it is important to study the biometry not only at tree level but also at plot level in order to develop suitable estimation tools.

The information derived from the application of models to SRC poplar plantations can be of great use to managers for decision-making, both with regard to the establishment of the plantation and the subsequent management. It can also be of use to other agents involved in biomass management, for example making it easier to identify suitable areas to locate industries, which consume biomass or allowing legislators to estimate the contribution of SRC poplar plantations to the total energy supply at national level. Analysis at individual tree level is fundamental in order to determine the individual yield for each of the genotypes studied. However, analysis at stand level, whether directly, using specific plot level tools or through

extrapolation of the individual tree level data, will provide the most important information for the planning and management of these crops, where the aim is to maximize production.

Estimation models are also particularly useful to evaluate the **sustainability of plantations and quantify the ecosystem services** provided by these plantations. The importance of this sustainability and these ecosystem services becomes clear if we consider them in the light of predictions for future development of this type of plantation. Criteria and indicators of sustainability have been defined but there is a lack of data on many aspects based on real scale quantifications. One of the main ecosystem services associated with these plantations or at least the most studied to date is the reduction of greenhouse gases (Verlinden *et al.*, 2013b; Berhongaray and Ceulemans, 2015; Zenone *et al.*, 2015; Berhongaray *et al.*, 2017). Quantification of the carbon fixed by the plantation is one of the main indicators of sustainability used in recent decades.

When computing the carbon, not only must the carbon fixed in the soil be taken into account (Smith, 2004), or the carbon which is gradually incorporated through leaf decomposition (Walter *et al.*, 2015), but also the carbon fixed in the root systems which particularly relevant in this type of crop. Therefore, it is very important to quantify the below-ground biomass, although the difficulty involved in extracting this biomass means that the availability of tools to estimate below-ground biomass is very limited. Furthermore, in the case of SRC plantations in Mediterranean conditions, the use of irrigation during the summer months probably conditions the development of the root system, making it necessary to establish whether differences exist with respect to previous studies of plantations without irrigation.

Finally, and within the general objective to optimize production, it should be borne in mind that increased yield for the same unit area involves considering alternative **designs**. One of the avenues explored in this search for improvement is the use of mixtures of species which can be advantageous both for natural stands and plantations. However, scarce information exists in this regard for plantations under SRC management.

The complementarity and interaction between the different species in the mixture results in these species using the available resources more efficiently (Kelty, 1992). The use of nitrogen fixing species is a clear example of facilitation, the effect of the mixture increasing as soil N becomes more limited (Bouillet *et al.*, 2013; Forrester, 2014). In the case of SRC plantations where fertilization is an important factor, the use of these species could reduce the necessity for fertilization over course of the rotation period, which is a key factor when considering this type of design.

In accordance with considerations described above, we provide a breakdown of the research questions contemplated in the framework of the present doctoral thesis and which are addressed in the following chapters:

Chapter 2: Are there other factors, beyond those common to generalized forest modelling, which condition the allometry of individual trees in SRC plantations under Mediterranean conditions?

To address the design and usefulness of specific models for this type of crops, the main objective of chapter 2 is to analyse the variability of models commonly used to estimate individual tree biomass, with the aim of improving both their precision and applicability. For this purpose, some of the many factors which lead to uncertainty in the allometric relationships between the biomass and the most commonly employed independent variables such as diameter and height are evaluated (Verwijst *et al.*, 2013). On the one hand, factors which directly affect the biological relationship are considered (species or genotype, shoot age or coppice rotation period). On the other, factors which affect the modelling of the relationship (fitting algorithms, sample size used to fit the model or the sample selection criteria according to the development stage of the crop). Some of these factors are common to forest modelling in general.

Chapter 3: Above-ground biomass estimation models. In species with a wide array of available genotypes, what role is played by genotypic affinity in the allometry of individual tree biomass? Is it necessary to develop estimation models at different hierarchical levels?

It is particularly important to be able to predict levels of production when evaluating the economic viability of a specific crop (Pérez-Cruzado *et al.*, 2013) or when a decision must be taken with respect to rotation length. If biomass estimation models are site (Tumwebaze *et al.*, 2013) or genotype (Sixto *et al.*, 2013a) specific then their practical use is clearly limited as management tools to predict production and/or to plan the management regime of the crop. Although much of the research related to the creation of biomass models for SRC has focused on the development of site and genotype specific models (Verwijst and Telenius, 1999; Barrio-Anta *et al.*, 2008; Mosseler *et al.*, 2014), there are also examples of models developed for higher hierarchical levels (Ben Brahim *et al.*, 2000a; Dillen *et al.*, 2007). The lack of specific models for certain genotypes is normally addressed by using other models created for taxonomically proximate genotypes (Dillen *et al.*, 2007). This is based on the fact that the allometry of the individual tree biomass is mainly influenced by taxonomic affinity. Given this premise, chapter 3 not only focuses on providing specific biomass estimation models for commonly used genotypes in SRC poplar plantations, but also on providing generalized and taxonomic group level equations for the species, which could be used when information for a specific genotype is not available. A more detailed study of taxonomic affinity in biomass allometry was also carried out in an attempt to determine whether the premise commonly used to get round the lack of genetic information is indeed correct.

Chapter 4: Below-ground biomass estimation models. How important is the belowground biomass fraction in terms of carbon accumulation in irrigated systems?

The availability of below-ground biomass estimation models is highly important given the implications as regards the valuation of ecosystem services such as quantifying the carbon

accumulated in the soil through root biomass. Despite the technical difficulty involved in analysing this biomass fraction, different authors have attempted to do so (Block *et al.*, 2006; Berhongaray *et al.*, 2015) and have developed specific estimation models for SRC (Coyle and Coleman, 2005; Fang *et al.*, 2007; Berhongaray *et al.*, 2015). However, under Mediterranean conditions, where irrigation is necessary, no models exist to estimate root biomass in SRC poplar plantations. Hence, one of the objectives of chapter 4 is to develop below-ground biomass estimation models for the three most commonly used genotypes in plantations for biomass production in Spain. As with the models for the above-ground fraction, models are also developed at genotype and generalized levels which are applicable to any genotype growing under Mediterranean conditions.

These tools contribute to improving total carbon estimates and the influence on reducing greenhouse gases. Carbon fixation associated with the plantations could also be valued as a real sustainability factor with economic implications for plantation managers in terms of carbon trading.

Chapter 5: Modelling growth and production at plot level. How can the design and management of SRC poplar plantations be addressed under Mediterranean conditions?

Along with the biomass estimation models themselves, there are many other management tools indirectly based on modelling, such as density management diagrams (DMDs), among others. These diagrams are used to illustrate graphically the relationships between density, yield and mortality rate over the development of the plot (Newton and Weetman, 1994), including concepts such as competition, site occupation and self-thinning. In SRC poplar plantations, reducing the density is not a common management practice and due to the short rotation length, these crops rarely reach self-thinning limits. Hence, the use of DMD is restricted to the initial design of the density, the rotation length and the estimation of biomass stock at plantation level. Therefore, in this chapter the basic concepts used in the construction of DMDs were applied to develop a *reference diagram* specific to SRC management. This specific *reference diagram* (RD) developed for the first rotation and using genotype 'I-124' as the reference genotype allows a rapid, simple comparison of the different management situations that can arise, both at the time of plantation (density) and at harvesting (rotation). These models can play an important role in helping us to understand the patterns of production of these plantations, contributing to evaluate the energy production potential and optimal management in terms of density and rotation age, as is already the case for *Eucalyptus* (Pérez-Cruzado *et al.*, 2011). Furthermore, they allow the economic profits to be calculated and provide guidance to managers regarding the ideal moment for harvesting.

Chapter 6: Can mixtures of *Populus alba* and *Robinia pseudoacacia* under SRC be more productive than monocultures of these species?

In recent years, research into combined systems with two species (mixtures) has gained in importance given that such mixtures can potentially provide different benefits, not only in terms

of production but also from an ecological and environmental perspective. For this reason, the possibility of achieving increased production through mixed species SRC plantations has been explored. In this regard, a plantation was established in which different proportions of the species *Populus alba* and *Robinia pseudoacacia* (a known nitrogen fixing species) were tested. By including a nitrogen fixing species in the mixture a theoretically positive effect on the non-nitrogen fixing species was expected (facilitation) along with a reduction in the competition between the two species. The analyses in this chapter were undertaken at individual tree level in order to identify the relationships between the species, as well as at stand level to determine whether either of them was more productive in terms of biomass compared to monospecific stands typical of these plantations.

1.7. Objectives

The general objective of this thesis is the prediction and optimization of biomass production in SRC plantations of species and hybrids of *Populus* spp. under Mediterranean conditions. To achieve this objective, the different questions set out above have been addressed. Within the context referred to, the specific objectives can be summarized as follows (Fig. 1.13):

- Study of the involvement of different factors in the modelling to improve the model development methodology for more robust biomass prediction.
- Study of the above-ground biometry, as well as the similarities in biomass allometry among different genotypes.
- Study of the below-ground biometry, as well as the carbon accumulation in the above- and below-ground biomass.
- Development of a whole crop model based on biomass, mean basal diameter and stool evolution as a function of mortality.
- Evaluation of a mixed plantation as an alternative design for biomass production.

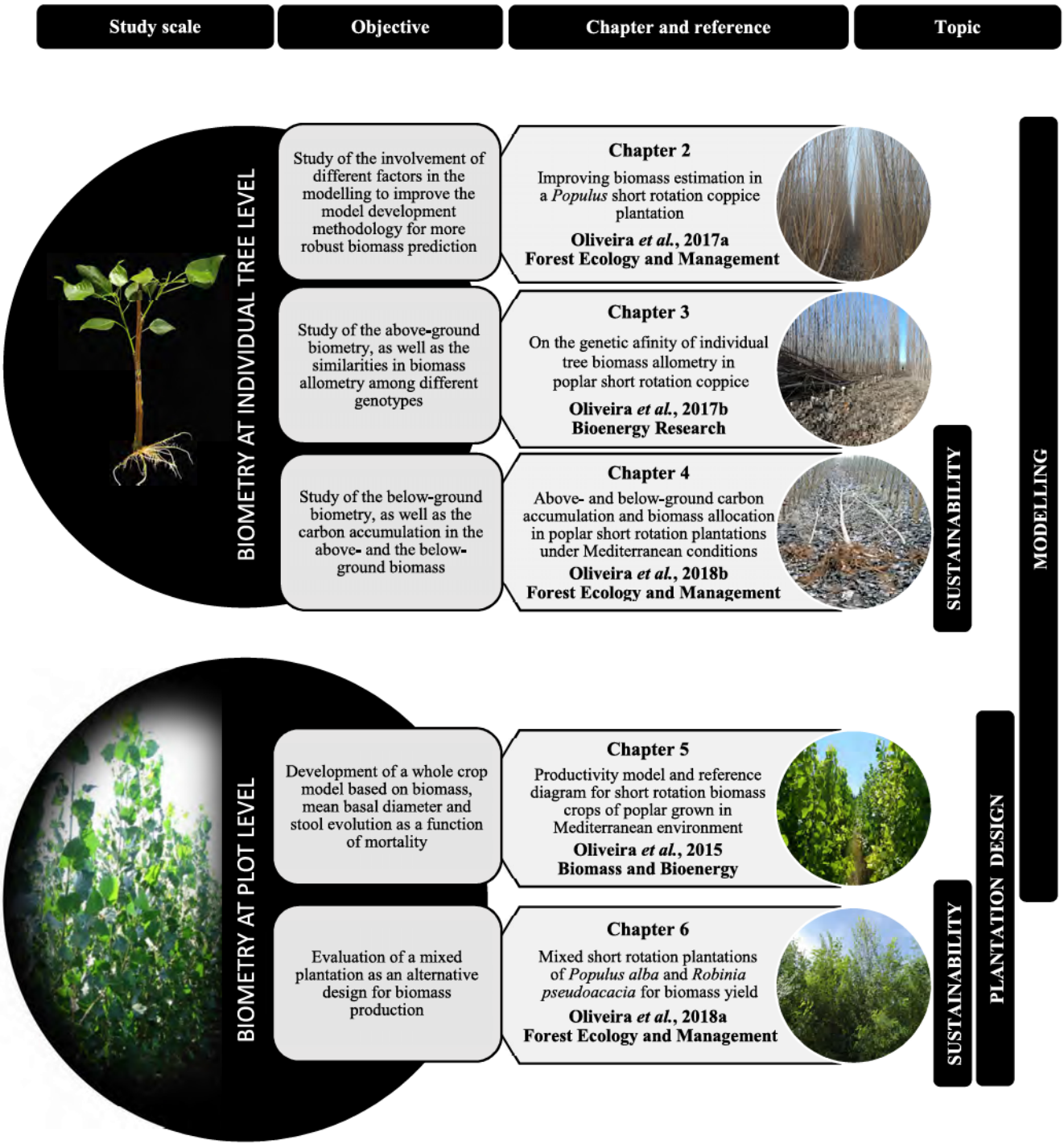

Figure 1.13. Outline of the thesis: objectives, study scale and topics addressed in Chapters 2-6.

CHAPTER

2

Improving biomass estimation in a Populus short rotation coppice plantation

Based in: Oliveira N., Rodríguez-Soalleiro R., Hernández, M.J., Cañellas I., Sixto H., Pérez-Cruzado, P., 2017. Improving biomass estimation in a *Populus* short rotation coppice plantation. Forest Ecology and Management. 391, 194-206. DOI: 10.1016/j.foreco.2017.02.020.

Improving biomass estimation in a Populus short rotation coppice plantation

Abstract

Short Rotation Coppice (SRC) plantations are important to biomass supply in the biomass market. The biomass stocks estimations should be based on robust models that allow the most accurate estimations. We have evaluated the changes in allometry for the estimation of biomass production at individual stool level in a short rotation, high density poplar plantation under a coppice system during two rotations of three years. The plantation was established in central-northern Spain, with a density of 17,316 cuttings ha^{-1}. The influence of different factors in the allometry has been evaluated to improve the methodology for more robust biomass prediction. In an SRC system, the influence of age was more pronounced in the first year after each coppicing than in subsequent years of the cycle. Our findings suggest that this feature of nature must be accommodated in the modelling procedure either through the model formulation (decreasing the parameter variability with the inclusion of age as a dummy predictor variable) or by selecting the sample according to the stage of development of the above-ground biomass (which will depend on the goals of the predictions). The minimum sample size proposed to construct a model capable of predicting biomass regardless of age in SRC was 50 trees. Furthermore, the uncertainty was quantified in two models for total above-ground dry woody biomass, the first based only on the basal diameter and the other on the basal diameter along with the number of shoots per stool; the inclusion of the latter was advisable to increase the accuracy and reduce the mean deviation in biomass predictions at different ages.

KEYWORDS: Poplar; allometric models; age-influence; mean deviation.

2.1. Introduction

In the near future, woody biomass from Short Rotation Coppice (SRC) plantations, could be an essential addition to the biomass supply from forests and related industries in the European biomass market (European Commission, 2014). The biomass produced in SRC is characterized by a precise location and a foreseeable yield, a pronounced temporality and likely yield according to the areas of cultivation. The quality of this type of biomass makes it suitable for use in the production of bioenergy through different processing methods, as well as to obtain multiple bioproducts (Zhang *et al.*, 2011). Therefore the production and use of this biomass should be viewed within the general framework of renewable energies, not only as an effective tool in the mitigation of the effects of climate change but also as a means to promote the development of the bioeconomy (European Commission, 2012). For the operational establishment of this type of plantation on a commercial scale, it is essential to maximize the benefits by combining economic viability with environmental sustainability. Species belonging to the *Salicaceae* family (*Populus* spp. and *Salix* spp.) have proved to be the most suitable for short rotation management in northern and southern Europe (Nassi O Di Nasso *et al.*, 2010; Verwijst *et al.*, 2013; Aust *et al.*, 2014) and there are many examples of commercial plantations aimed at energy production (Srirangan *et al.*, 2012).

Accurate tools for estimating standing biomass in SRC crops are an essential component for the most basic management decisions, such as determining the most appropriate rotation length, maximum volume production or the financial rotation, as well as to assess the amount of primary energy available at the end of the rotation or to estimate the nutritional balance.

Allometric models relating tree diameter or other easily measurable tree variables to any biomass component or total above-ground biomass are the most commonly used tool for estimating biomass in forest inventories and ecological studies (Peichl and Arain, 2007; Shaiek *et al.*, 2011). These methods have also been used for estimation purposes in SRC (Verwijst *et al.*, 2013; Mosseler *et al.*, 2014; Verlinden *et al.*, 2015). Apart from the variety of models that can be used to assess specific relationships, we are confronted with a large number of factors which create uncertainty in allometric relationships (Verwijst *et al.*, 2013). We should distinguish between the factors that affect the biological relationship on one hand, such as i) species or genotypes, ii) the age of the shoot or iii) the coppicing rotation, and on the other hand factors affect the modelling of the relationship: iv) the fitting algorithms, v) the size of the sample used to fit the model or vi) the sample selection criteria as a function of the stage of development. Some of these factors may be considered as general to all forest monitoring conditions. It is hypothesized that factors ii), iii) and v) have an important effect on SRC because of the differences in the stages of development of above and below-ground biomass after resprouting.

The effect of species or genotypes within species on biomass allometry has been extensively studied (Ter-Mikaelian and Korzukhin, 1997; Bond-Lamberty *et al.*, 2002). When estimating the above-ground biomass of a forest, the use of species-specific models are

preferred because there can be large differences between species in terms of tree architecture and specific gravity of the wood (Ketterings *et al.*, 2001). For poplar species and genotypes, successful generalizations have been developed, although deviations were found for certain tree components (Tuskan and Rensema, 1992).

The sample size must be large enough and distributed appropriately so that the fitted model accurately represents the underlying biological relationship (Hui and Jackson, 2007; Zapata-Cuartas *et al.*, 2012; Sileshi, 2014), although even so, the sample selection criteria will influence the model setting (Zianis and Mencuccini, 2004). The decisions as regards the model fit, such as the fitting techniques (Zapata-Cuartas *et al.*, 2012; Ahmed *et al.*, 2013) or the model formulation and the predictor variables (Van Breugel *et al.*, 2011; Tumwebaze *et al.*, 2013; Mosseler *et al.*, 2014), are an additional source of uncertainty (Van Breugel *et al.*, 2011; Sileshi, 2014). Additionally, other factors such as site quality (Ketterings *et al.*, 2001), proportion of branches (Cannell, 1984), or damaging agents such as frost (Verwijst *et al.*, 1996), leaf rust (Tuskan and Rensema, 1992) or animal browsing (Verwijst and Telenius, 1999) may also affect the allometric relationship. Various other factors such as stand density are more controversial. Although a stand density effect is assumed in some studies (Peichl and Arain, 2007), others, such as Willebrand *et al.* (1993) or Di Matteo *et al.* (2012) report that the effect is only statistically significant at certain stages of development, while other studies have not found statistically significant effects of stand density on tree allometry (Adegbidi *et al.*, 2001; Antonio *et al.*, 2007; Amichev *et al.*, 2010).

Age dependence of tree allometry is a specific issue which has been analysed on several occasions (Saint-André *et al.*, 2005; Antonio *et al.*, 2007; Peichl and Arain, 2007). The relationship between the tree variables and the biomass yield may be modified by changes in the allocation of biomass to individual components over the life cycle of the tree (Satoo and Madgwick, 1982). For this reason, the inclusion of stand age in a biomass model was found to contribute significantly to improving the quality of the fit of the model to the data, and different allometric biomass models may therefore be required depending on the development stage of the tree (Peichl and Arain, 2007). In coppice plantations, biomass allocation varies not only with the age but also with rotation, since below-ground biomass age for a given above-ground biomass age will not be the same in different rotations. Under such circumstances it is hypothesized that the effect of age on allometry is more pronounced than in single stem plantations.

The aim of this study was to improve the model development methodology for more robust biomass prediction. The main objective was to analyse the changes in allometry at stool level produced by: i) age and ii) coppicing rotation in an SRC system measured over two rotations. A second objective was to estimate the effect on parameter estimates, accuracy and mean deviation of: iii) the sample size, iv) sample selection criteria and v) model formulation. Finally, the improvement in the biomass estimation and robustness of the model by including other regressor variables together with basal diameter was evaluated.

2.2. Material and methods

2.2.1. Trial site and experimental design

The study site is located on the central plateau of Spain (latitude N 41° 28' 20.06''; longitude W 2º 32' 54.68'' and 827 m a.s.l.). The climate in this area is sub-Mediterranean with certain continental characteristics (mean annual temperature of 11.8 ºC and average annual rainfall of 561 mm). The soil has a sandy clay loam texture with moderately basic pH (8.2) and organic matter content of about 2.5%. In the spring of 2005, a clonal trial was installed using six different genotypes. Five genotypes belong to the hybrid *Populus* x *canadensis* Moench ('A4A', 'A2A', 'I-214', '2000 Verde' and 'Luisa Avanzo') and the other was a native *Populus nigra* L. genotype ('Lombardo Leonés'). The plantation had an initial density of 17,316 cuttings ha^{-1}, established in twin rows, 3 m apart with a distance of 0.5 m between single rows. A total of 1,019 unrooted cuttings of each genotype were planted in adjacent pairs of rows, including six genotypes planting consecutively. Prior to planting, 8-15-15 N-P-K fertilizer was applied. Weed control was carried out by applying Oxifluorfen at 4 l ha^{-1} after planting and by mechanical weeding in midsummer. Irrigation was applied by surface irrigation from June to September. After the first vegetative period (winter of 2006), the plantation was cut-back in order to stimulate sprouting. Hence, after resprouting, root age (R) was 2 and shoot age (S) was 1 year (R2S1). From then on, two rotations of three years were applied. Three random clusters per genotype were randomly selected in the plantation each year for destructive biomass measurement. Each cluster included 16 contiguous trees per genotype, surrounded by a buffer of two rows. The distance between the biomass sampling plots established in different years was at least 20 m.

Table 2.1. Model formulations considered in the fitting process.

Abbreviation	Model Formulation	Number Eq.
Model *d*	$W_s = a\ D_{10}{}^{b} + \varepsilon$	(2.1)
Model *dh*	$W_s = a\ D_{10}{}^{b}\ H^{c} + \varepsilon$	(2.2)
Model *dhn*	$W_s = a\ D_{10}{}^{b}\ H^{c}\ n^{d} + \varepsilon$	(2.3)
Model *dn*	$W_s = a\ D_{10}{}^{b}\ n^{d} + \varepsilon$	(2.4)
Model *h*	$W_s = a\ H^{c} + \varepsilon$	(2.5)
Model *D*	$W_s = a\ D_{10}{}^{(b_1 I_1 + b_2 I_2 + b_3 I_3 + b_4 I_4 + b_6 I_6)} + \varepsilon$	(2.6)
Model *H*	$W_s = a\ H^{(c_1 I_1 + c_2 I_2 + c_3 I_3 + c_4 I_4 + c_6 I_6)} + \varepsilon$	(2.7)
Model *Dh*	$W_s = a\ D_{10}{}^{(b_1 I_1 + b_2 I_2 + b_3 I_3 + b_4 I_4 + b_6 I_6)} H^{c} + \varepsilon$	(2.8)
Model *DH*	$W_s = a\ D_{10}{}^{(b_1 I_1 + b_2 I_2 + b_3 I_3 + b_4 I_4 + b_6 I_6)} H^{(c_1 I_1 + c_2 I_2 + c_3 I_3 + c_4 I_4 + c_6 I_6)} + \varepsilon$	(2.9)
Model *DHN*	$W_s = a\ D_{10}{}^{(b_1 I_1 + b_2 I_2 + b_3 I_3 + b_4 I_4 + b_6 I_6)} H^{(c_1 I_1 + c_2 I_2 + c_3 I_3 + c_4 I_4 + c_6 I_6)}\ n^{(d_1 I_1 + d_2 I_2 + d_3 I_3 + d_4 I_4 + d_6 I_6)} + \varepsilon$	(2.10)
Model *DHn*	$W_s = a\ D_{10}{}^{(b_1 I_1 + b_2 I_2 + b_3 I_3 + b_4 I_4 + b_6 I_6)} H^{(c_1 I_1 + c_2 I_2 + c_3 I_3 + c_4 I_4 + c_6 I_6)}\ n^{d} + \varepsilon$	(2.11)
Model *DhN*	$W_s = a\ D_{10}{}^{(b_1 I_1 + b_2 I_2 + b_3 I_3 + b_4 I_4 + b_6 I_6)} H^{c}\ n^{(d_1 I_1 + d_2 I_2 + d_3 I_3 + d_4 I_4 + d_6 I_6)} + \varepsilon$	(2.12)
Model *Dhn*	$W_s = a\ D_{10}{}^{(b_1 I_1 + b_2 I_2 + b_3 I_3 + b_4 I_4 + b_6 I_6)} H^{c}\ n^{d} + \varepsilon$	(2.13)
Model *DN*	$W_s = a\ D_{10}{}^{(b_1 I_1 + b_2 I_2 + b_3 I_3 + b_4 I_4 + b_6 I_6)}\ n^{(d_1 I_1 + d_2 I_2 + d_3 I_3 + d_4 I_4 + d_6 I_6)} + \varepsilon$	(2.14)
Model *Dn*	$W_s = a\ D_{10}{}^{(b_1 I_1 + b_2 I_2 + b_3 I_3 + b_4 I_4 + b_6 I_6)}\ n^{d} + \varepsilon$	(2.15)

Where *n* is the number of shoots per stool, *H* is the height of the dominant shoot per stool (m), D_{10} is the basal diameter of the dominant shoot per stool (mm), *Ws* is the total aboveground dry biomass per stool (g), I_1, I_2, I_3, I_4 and I_6 are the dummy variables and ε is the residual term.

2.2.2. Variables measured

In the first rotation (2006-2008) the number of shoots per stool (*n*), the dominant shoot diameter at basal height (D_{10}, mm, considering the dominant shoot as the thickest shoot per stool and basal height as 10 cm) and the total height of the dominant shoot (*H*, cm) were recorded in 16 stools for each cluster and genotype at the end of the growing season. Additionally, these stools were destructively sampled immediately, in order to quantify the biomass. The dry matter content was calculated for a subsample of one tree from the most frequent diameter class for each genotype, which was oven dried at 105ºC to constant weight. In the second rotation (2009-2011) the same evaluation procedure was followed, with the exception of the year 2010, when the shoots were measured but biomass data was not recorded. The stage of development is expressed in terms of their root and stem age, so R2S1 is the first year after coppicing, year 1 (2006), R3S2 is year 2 (2007), R4S3 is year 3 (2008), R5S1 is year 4 (2009, the first year after coppicing in the second rotation) and the last one is R7S3, which is year 6 (2011). The complete biomass sampling size was then 16 trees x 3 clusters x 5 years x 6 genotypes.

2.2.3. Statistical analysis

Biomass models were fitted using a nonlinear least-squares procedure (*nls* procedure of the *R software program* (R Development Core Team, 2014)), to relate dry biomass yield (W_s) to dominant shoot diameter at basal height (D_{10}), height of the dominant shoot of the stool (*H*) and number of shoots per stool (*n*).

2.2.3.1. Evaluation of the effect of age and model formulation

To evaluate the effect of age and model formulation on parameter estimates, three different model formulations were considered: a) a general model for all years for each genotype (hereinafter "general", equation 2.16), b) a different model for each genotype and year (hereinafter "simple", equation 2.17), and c) a model with expanded parameters through dummy variables (with a common *a* parameter, hereinafter "dummy", equation 2.18).

$$W_{ik} = a \cdot X_{ik}^{p} + \varepsilon_{ik} \qquad (2.16)$$

$$W_{ijk} = a \cdot X_{ijk}^{p} + \varepsilon_{ik} \qquad (2.17)$$

$$W_{ijk} = a \cdot X_{ik}^{(p_1 \cdot I_1 + \cdots p_j \cdot I_j + \cdots p_6 \cdot I_6)} + \varepsilon_{ik} \qquad (2.18)$$

Where *W* is the dry biomass, *X* are the predictor variables, *p* are the parameters associated to each one of the predictor variables, *I* are the dummy variables for the levels of the factor "year" and ε is the additive residual term. Using *i* to index tree, *j* to index the levels of the factor "year" and *k* to index genotype.

The aim of the parameter expansion is to take into consideration the time elapsed since the first coppicing, thus the dummy variables I_1, I_2, I_3, I_4 and I_6 take the value 1 for each corresponding year for which biomass data are available (I_1 corresponds to 2006 (R2S1); I_2 to 2007 (R3S2), I_3 to 2008 (R4S3), I_4 to 2009 (R5S1) and I_6 to 2011 (R7S3)) respectively, otherwise 0. Three variables were considered as predictive in the fitting process: basal diameter of the dominant shoot (D_{10}), the height of the dominant shoot per stool (H) and the number of shoots per stool (n). All the fitted models are shown in Table 2.1. Hereinafter the models will be denominated as the nomenclature expressed in Table 2.1, the letters of the models indicate the predictor variables included in the model and capital letters indicate that the parameter associated with that predictor variable has been expanded through dummy variables.

Kruskal-Wallis non-parametric analysis of variance (ANOVA) was used to assess differences in parameter estimates to analyse their trends among model formulation, age and rotation. This test was selected due to the lack of homogeneity in the variances among groups (without assuming them to follow a normal distribution) and the presence of more than two groups. The null hypothesis is that groups present the same parameter estimates, so small p-values lead us to reject the null hypothesis that groups share the same allometric estimates, presenting significantly differences among the parameter estimates.

Table 2.2. Statistics for the main variables measured in the 'I-214' genotype plots for each stage of development.

		R2S1 2006	R3S2 2007	R4S3 2008	R5S1 2009	R7S3 2011	ALL
n	Max [a]	25	11	9	15	17	25
	Min [b]	4	1	1	3	1	1
	Mean	9.09	3.93	4.60	7.10	7.66	6.39
	Devest [c]	4.48	2.30	1.95	3.18	3.74	3.81
***H* (cm)**	Max [a]	293	563	917	470	1031	1031
	Min [b]	70	115	249	273	331	70
	Mean	215.75	435.71	706.24	408.33	868.10	507.76
	Devest [c]	46.36	109.17	169.22	52.37	180.89	259.11
***D_{10}* (mm)**	Max [a]	18.41	57.36	96.95	29.17	96.00	96.95
	Min [b]	10.31	4.72	16.98	17.48	18.00	4.72
	Mean	15.16	37.66	60.37	25.06	66.41	39.92
	Devest [c]	1.73	12.47	20.74	3.12	20.47	24.13
***Ws* (g)**	Max [a]	692.02	3,602.31	11,768.12	2481.22	22,757.27	22,757.27
	Min [b]	16.15	100.06	245.17	155.08	152.41	16.15
	Mean	340.08	1,199.85	4,358.56	1,188.57	8,616.63	2,864.28
	Devest [c]	183.40	814.28	3,109.71	672.89	5,902.23	3,989.55
N Data		44	41	42	30	29	186

Where n is the number of shoots per stool, H is the height of the dominant shoot per stool (m), D_{10} is the basal diameter of the dominant shoot per stool (mm), *Ws* is the total aboveground dry biomass per stool (g) and *N Data* is the number of stools measured. The stage of development is expressed according to their root (R) and their stool age (S). [a] Max: maximum; [b] Min: minimum; [c] Devest: mean deviation.

2.2.3.2. Evaluation of the effect of sample size and sample selection

Effect of sample size and sample selection were analysed by considering the 'I-214' genotype. This genotype is a hybrid between *Populus deltoides* Marsh and *Populus nigra* L., and is a well-known genotype which is widely planted in Mediterranean environments because of its largely consistent productivity across sites and large yield (Cruset and Voltas, 2009; Sixto *et al.*, 2013). The descriptive statistics from the information available for this genotype are shown in Table 2.2. As model *d* is the most commonly used for biomass estimation, the effect of sample selection and sample size was evaluated by considering only this model. Model *dn* was also considered to evaluate the improvement in the residual sum of squares by adding the number of shoots as a predictor variable.

2.2.3.2.1. Effect of sample size

The full database of 186 trees for the 'I-214' genotype was randomly split to evaluate the uncertainty in parameter estimate bounds for the sample size (*ss*) considered to fit the allometric models. In order to obtain a final random sample in which all the diameter classes were represented, the database was divided into 6 groups with 31 trees per group, based on diameter range. Models with the same sample intensity and which cover all the diameter classes presented in the total database were fitted (McRoberts *et al.*, 2015). Sample size was tested from 12 (2 trees per group) to 168 trees (28 trees per group). The procedure was randomized 1000 times for each sample size to quantify the variance among estimates for different sample sizes.

A common fitting for the 186 trees was performed, obtaining confidence intervals for parameter estimates with a probability level of 99% (Venables and Ripley, 2002), in order to analyse the minimum sample size required to achieve that level of confidence. The estimated parameters in the bootstrap for different sample sizes were compared to that confidence interval, establishing the need to include the interquartile range (25^{th}-75^{th} percentile range) in that interval.

2.2.3.2.2. Uncertainty bounds for the sample selection

In order to evaluate the uncertainty in biomass estimates derived from the selection of standard trees at different ages and coppice rotations for destructive sampling, eight scenarios were defined. Bootstrapping was used to calculate the residuals using cross-validation techniques with the *statistical package R* (R Development Core Team, 2014). Each simulation was repeated 1000 times and data were randomly selected, making sure that the sample size was the same for each year. The proposed simulations were defined based on the subset of data chosen for sampling as described in Table 2.3.

The estimation uncertainty is measured on the basis of the mean relative deviation or percentage error (PE, %), the mean residuals (MRES, g) to evaluate the mean deviation and the

root mean square error (RMSE, g) to evaluate the accuracy, all of which relate to the validation data. To facilitate the interpretation of results, they were represented in box-plots.

$$PE\ (\%) = \frac{100}{x} \cdot \sum \left(\frac{y_i - \hat{y}_i}{y_i} \right) \qquad (2.19)$$

$$MRES(g) = \frac{\sum (y_i - \hat{y}_i)}{x} \qquad (2.20)$$

$$RMSE\ (g) = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{x - p}} \qquad (2.21)$$

where y_i is the observed value (g), $\hat{y}_i$ is the estimated value (g), x is the number of observations, and p is the number of parameters.

Table 2.3. Different number of simulations proposed and the sample data used to fit the models for each stage of development.

	R2S1 2006	R3S2 2007	R4S3 2008	R5S1 2009	R6S2 2010	R7S3 2011	TOTAL
Sim. 1	13		13	13		13	52
Sim. 2			26			26	52
Sim. 3	26			26			52
Sim. 4			25				25
Sim. 5						25	25
Sim. 6	26		26				52
Sim. 7				26		26	52
Sim. 8	10	10	10	10		10	50
TOTAL DATA	44	41	42	30	0	29	186

Where the simulations are abbreviated by Sim. and the number of each one, and the stage of development is expressed according to their root (*R*) and their stool age (*S*).

2.2.3.3. Evaluation of the effect of predictor variables

To analyse the improvement in the predictive ability of the models by adding another variable to the model apart from D_{10}, the process explained in the previous section was repeated. In this case, the improvement obtained by including the number of shoots in the model is tested (model *dn*). Only this variable was considered as it is hypothesized to have a great degree of importance in biomass estimation in several rotations of coppice plantations, as the values of *n* vary greatly for different rotations.

Analysis of variance (ANOVA) was performed, using the *statistical package R* (R Development Core Team, 2014) to evaluate the effect of the inclusion of the number of shoots as a predictor variable in the general model, through an F-test based on the extra sum of squares.

2.3. Results

2.3.1. Effect of the model formulation on the evolution of allometry

The model formulation can consider either simple or dummy models. The same parameter notation was used for all model formulations as follows: b is the exponent of D_{10}, c is the exponent of H and d is the exponent of n. The results of parameter estimates when simple models are applied (a different model for each age and genotype) with different combinations of predictor variables are shown in Fig. 2.1, where the years are represented on the x-axis, and the variability in the estimations of the parameters is represented on the y-axis, and the graphics included in the upper right quadrant of each parameter are zooms of specific regions of each parameter to allow a better interpretation. Overall, greater variability is observed for the estimates of parameters b and c in models that include both predictor variables (basal diameter and height (model *dh*)) and even number of shoots (model *dhn*), which is partly due to the strong correlation observed between D_{10} and H (Pearson correlation coefficient =0.92). It should also be noted that years 1 and 4 are those with the greatest variability in the estimates, revealing the important changes in stool allometry as a result of coppicing. Year 4 presents by far the greatest variability, showing a greater degree of instability in the estimates of parameters b and c when height is included as a predictor variable than when it is not included. For the harvesting years, the values tend to group, regardless of the variable included in the model.

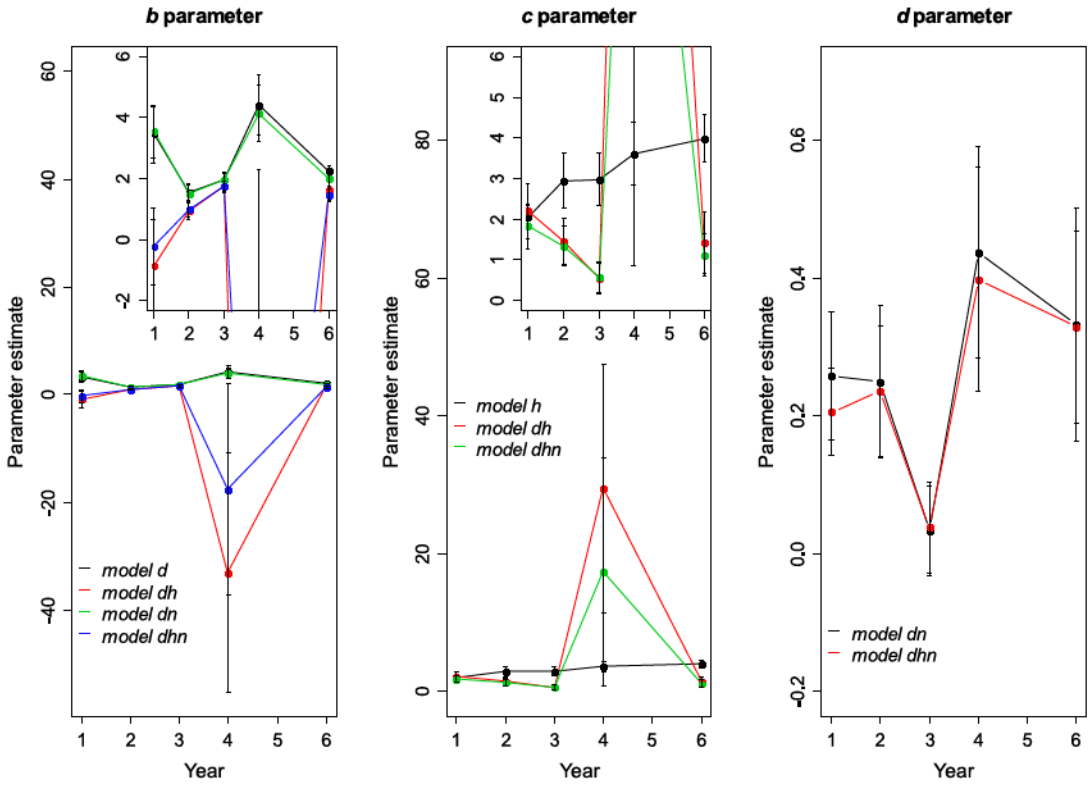

Figure 2.1. Evolution of the exponential parameters of the simple models with age: *b* parameter (left), *c* parameter (centre) and *d* parameter (right). The letters of the models indicate the predictor variables included in the model. Zooms are included in the upper right quadrant.

The estimates of parameter *b* show a decreasing trend within each rotation, always with biologically meaningful, positive values, provided that height was not included as a predictor variable (p-value<0.01for both rotation). For the simple model which includes only height, parameter *c* has an increasing trend with age (p-value<0.05). If diameter and height (and even number of shoots) are included as predictor variables, the parameter *c* estimate has a clear decreasing trend with age (p-value<0.01), maintaining positive values, thus showing a reduction in the relevance of height in explaining stool biomass. In parameter *d* (Fig. 2.1), the variability decreases with time after coppicing for the first rotation (p-value<0.05), while it remained stable within the second rotation (p-value>0.50). The data do not show an overall trend for both rotations, but the values in the second rotation appear to be greater than those estimated for the first (p-value<0.05). Within the first rotation, a decreasing trend with age is evident (p-value<0.05).

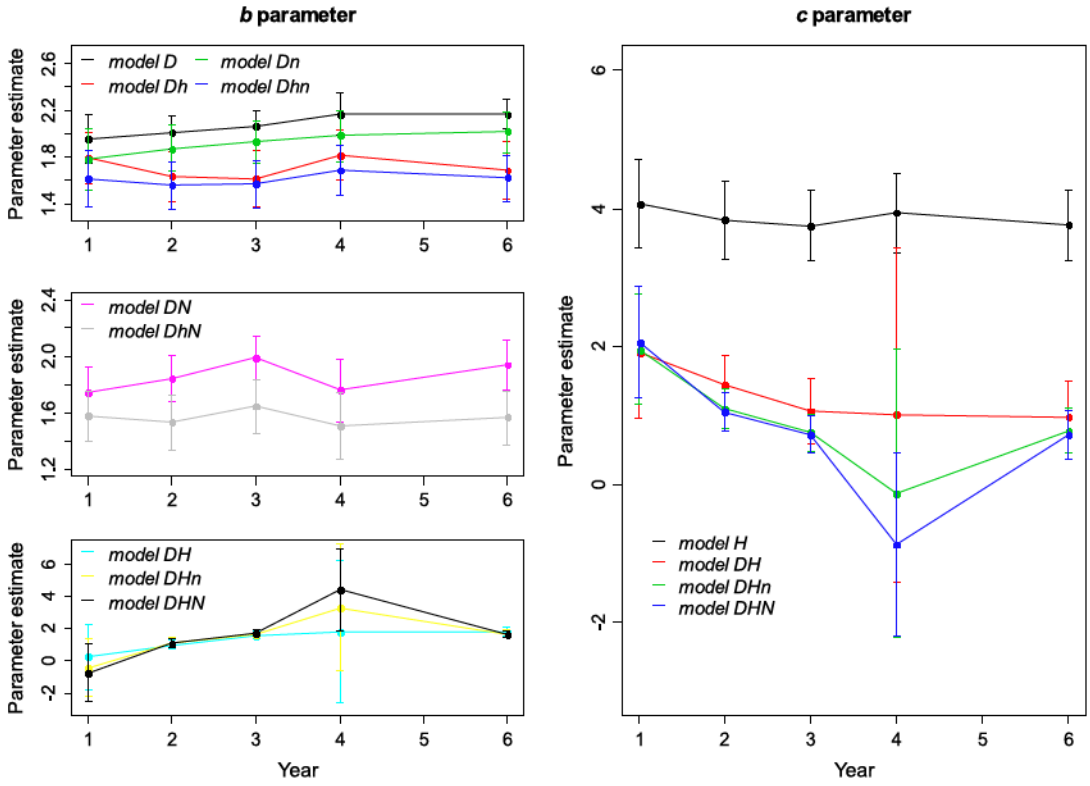

Figure 2.2. Evolution of the exponential parameters for the dummy models with age: *b* parameter (left) and *c* parameter (right). The letters of the models indicate the predictor variables included in the model. Capital letters indicate that the parameter associated with that predictor variable has been expanded through dummy variables. Zooms are included in the upper right quadrant.

Fig. 2.2 shows the evolution of the *b* and *c* parameter estimates when dummy models are fitted. Overall, if we compare it with Fig. 2.1, it can be seen that the variability between different models is smaller in dummy models, revealing more robust parameter estimation. Greater variability in parameter *b* and *c* estimates is observed for the dummy models that expand the parameter associated with height (*DH*, *DHn* and *DHN*, Fig. 2.2 bottom-left) (p-value<0.01 for both parameters *b* and *c*). Estimates of parameter *b* are much more stable for the dummy

procedures than for the simple one (even for the harvesting years), as can be seen in Fig. 2.2 (top and centre-left) for the models *D*, *DhN* and *Dhn*, as a result of using a common parameter *a* for all years (*p*-value>0.50).

The evolution of parameter *c* estimates for the dummy models *H*, which exclusively expand this parameter, shows clear stability of the values (*p*-value>0.50), in contrast to the abovementioned increasing trend found for the simple model *(p*-value<0.05). The simultaneous expansion of *b* and *c* parameters in model *DH, DHN* and *DHn* produces a decreasing trend in parameter *c* estimates (Fig. 2.2 right) (*p*-value<0.01), whereas the inclusion of number of shoots in models *DHN* and *DHn*, also produces unrealistic negative estimates for some years.

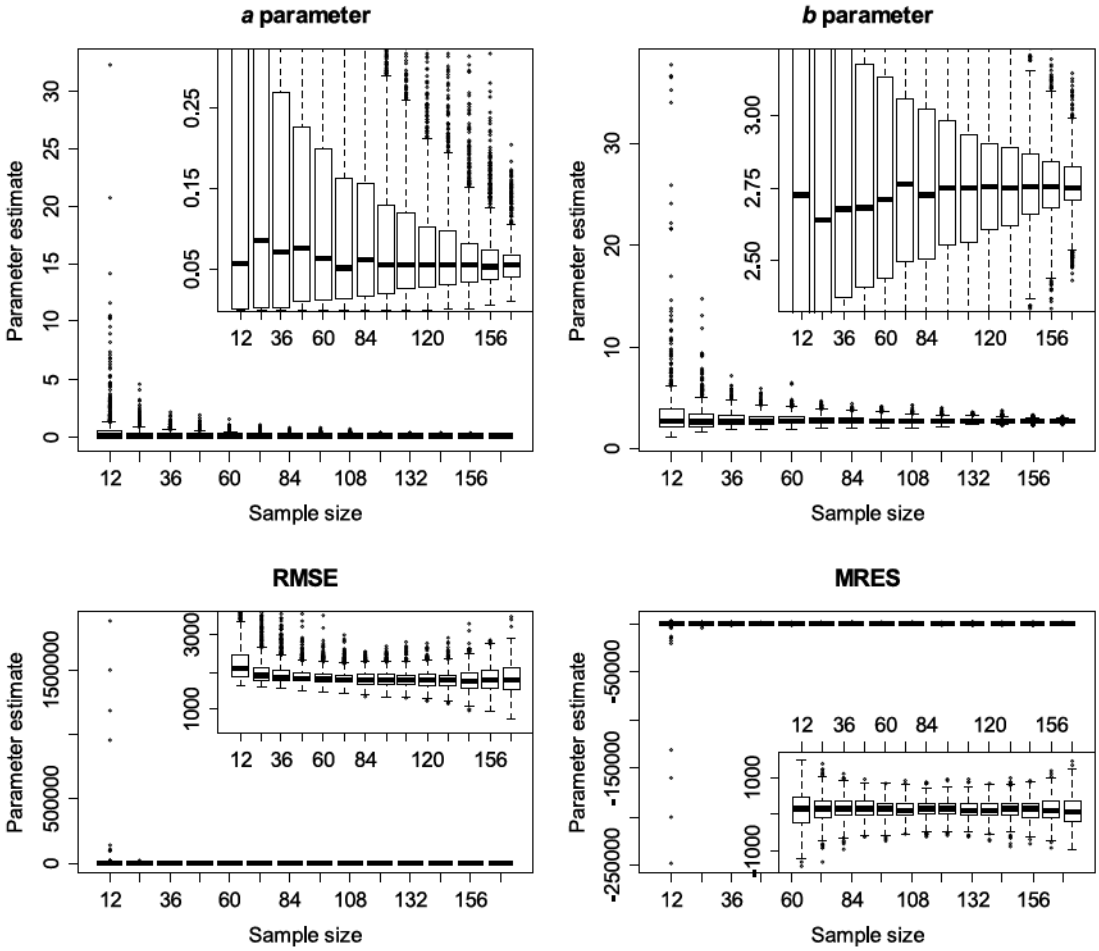

Figure 2.3. Uncertainty in parameter estimation for genotype 'I-214' and model *d* for different sample size simulations: Parameter *a* (top-left), parameter *b* (top-right), RMSE (g) (bottom-left) and MRES (g) (bottom-right).

2.3.2. Effect of sample size on the model prediction ability for the 'I-214' genotype

Fig. 2.3 shows the results of the parameter estimates for genotype 'I-214', model *d* and the simple procedure of fitting. As expected, the variability of the parameter estimation decreases as the sample size increases (Fig. 2.3). We calculated the minimum sample size necessary to assure that the interquartile range between the 25th and 75th percentile of the parameter *b*

estimates was inside the 99% confidence interval derived from the fitting of the whole data set (parameter *a* from 0.01 to 0.31 and *b* from 2.36 to 3.19 (these values were the limits in the y-axis for the zooms in Fig. 2.3). This minimum size was *ss*=48 for parameter *b* and *ss*=36 for parameter *a* when all years are considered together.

The development of an allometric model which includes basal diameter and the number of shoots per stool as predictor variables would require a similar sample size to obtain similar, stable estimations of the parameters. However, the RMSE and MRES values were greater, even when small sample sizes were used. Parameter *a* required more than 24, parameter *b* 36 and parameter *d* required 48 to obtain the same level of accuracy as a confidence interval of 99% in model *dn* for the whole data base (Fig. 2.4) (parameter *a* from 0.05 to 0.51, *b* from 2.01 to 2.58 and *c* from 0.4 to 0.61 (these values were the limits in the y-axis for the zooms in Fig. 2.4)). Estimates of parameter *d,* in samples of less than 60, presented some negative values, which denotes a negative relationship between the number of shoots and biomass yield. In samples of more than 60 this parameter is stabilized, showing only positive values.

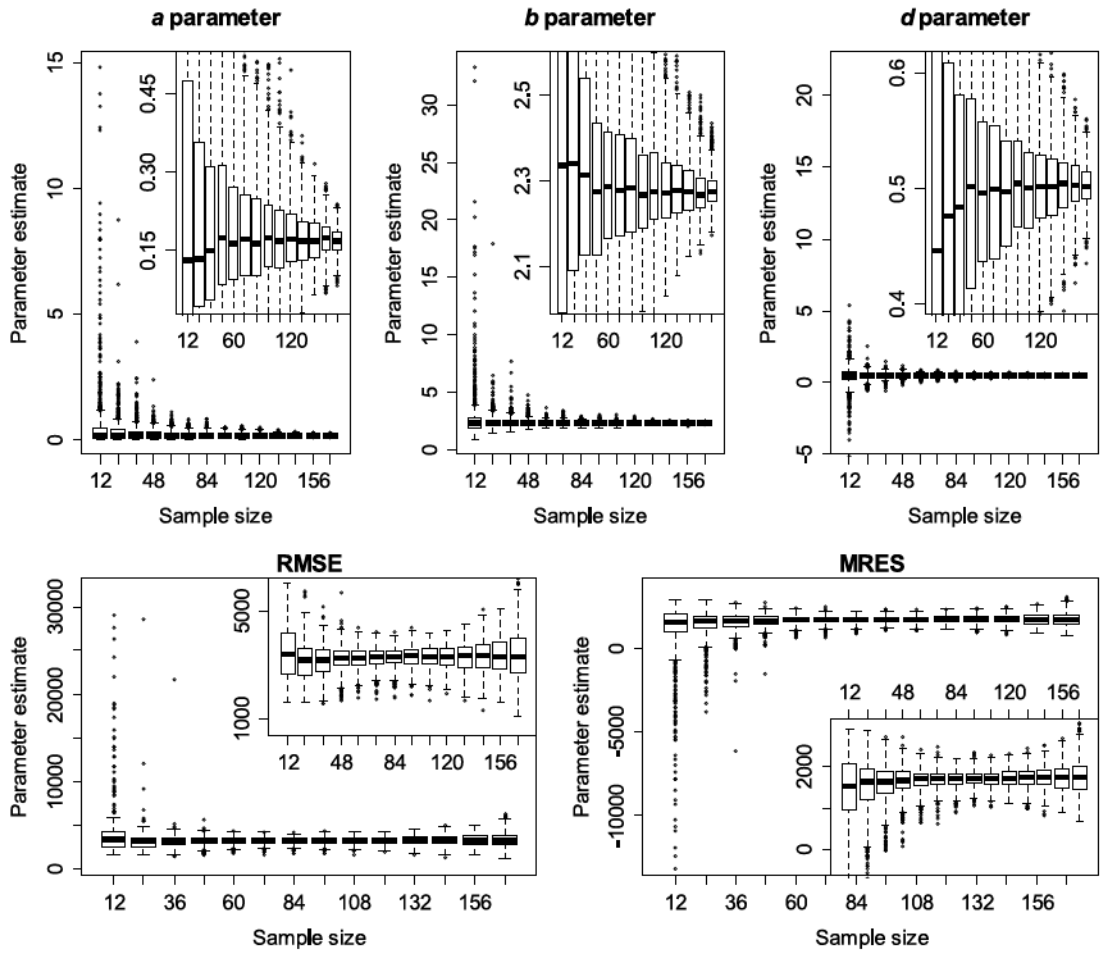

Figure 2.4. Evolution of the estimations of the parameters for genotype 'I-214' and model *dn* for different sample size simulations. Parameter *a* (top-left), parameter *b* (top-centre) and parameter *d* (top-right), RMSE (g) (bottom-left) and MRES (g) (bottom-right).

2.3.3. Model uncertainty according to sample selection criteria for the 'I-214' genotype

Fig. 2.5 presents the results in terms of PE (%) derived from the eight sample selection alternatives simulated. Sampling by considering all the data, irrespective of crop age (Sim. 8), would produce good results when used to estimate biomass at any growth stage, with a general overestimation of 7%, but would result in an underestimation of approximately 50% for the first year after each coppicing. PE in Sim. 8 decreased gradually with increasing rotation years, reaching the smallest values in the harvesting years, and with the second rotation values being smaller than the first rotation. The option where sampling was focused on cutting years (Sim. 2) provided similar PE results to those of Sim. 8 although the sampling activity is more concentrated in time.

In cases where the rotations are sampled separately (Sim. 4, Sim. 5, Sim. 6 and Sim. 7), it is clear that significant mean deviations results when data from the second rotation (Sim. 5 and Sim. 7) are used to estimate first rotation biomass, particularly at harvest (R4S3, year 3) (Table 2.4). Sampling in the first rotation (Sim. 4 and Sim. 6) is a better way to achieve lower mean deviations at age R4S3 (year 3), but leads to positive mean deviations when applied to biomass estimation in the second rotation.

Table 2.4. Mean residuals (MRES, g) (left) and root mean square error (RMSE, g) (right) at stool level for the genotype 'I-214' in model *d* and *dn* (Table 2.1), for each simulation.

		MRES						***RMSE***					
		All ages	R2S1 2006	R3S2 2007	R4S3 2008	R5S1 2009	R7S3 2011	All ages	R2S1 2006	R3S2 2007	R4S3 2008	R5S1 2009	R7S3 2011
Model *d*	Sim. 1	-211	175	-521	-1,569	646	1,381	1,755	244	951	2,461	857	3,261
	Sim. 2	-6	230	-352	-1,483	759	1,490	1,153	281	782	2,374	948	2,867
	Sim. 3	-16,252	100	-4,986	-26,105	-64	-30,292	26,228	169	6,437	34,402	396	37,483
	Sim. 4	784	219	-114	50	771	3,354	2,116	272	601	129	962	4,718
	Sim. 5	-768	168	-848	-2,694	570	-53	2,030	229	1,222	3,610	784	2,530
	Sim. 6	860	205	-169	-4	739	3,325	2,309	259	617	1,270	934	4,714
	Sim. 7	-1,210	105	-1,121	-2,900	400	-191	2,268	183	1,467	3,691	607	2,514
	Sim. 8	-33	223	-307	-1,432	760	1,477	1,842	278	813	2,469	950	3,306
Model *dn*	Sim. 1	-160	-16	-425	-511	285	397	1,265	157	805	1,980	529	2,008
	Sim. 2	-33	55	-313	-473	404	482	875	149	709	1,876	605	1,754
	Sim. 3	-9,185	43	-2,613	-13,036	-48	-19,885	15,306	132	3,283	17,045	372	25,401
	Sim. 4	842	229	-150	3.77	798	3,712	2,318	282	675	1,329	993	5,189
	Sim. 5	-458	-180	-717	-857	-2	-105	1,282	252	1,052	2,144	437	1,851
	Sim. 6	921	216	-196	-42	766	3,631	2,499	270	682	1,307	965	5,114
	Sim. 7	-551	-180	-728	-852	-23	-65	1,368	232	1,058	2,122	380	1,778
	Sim. 8	-20	54	-283	-417	415	489	1,323	169	718	1,967	620	2,119

Where the simulations are abbreviated by Sim. and the number of each one, and the stage of development is expressed according to their root (*R*) and their stool age (*S*).

Sampling exclusively one-year-old shoots after coppicing (Sim. 3) presented the greatest mean deviation, hence is not recommended.

Although PE in Sim. 1, Sim. 2 and Sim. 8 was smaller (Fig. 2.5), MRES for general models reached values of up to 1400 g per stool in the cutting years (Table 2.4), while the fittings for each rotation presented lower mean deviations both in general and in the cutting years (~ 50 g per stool in both rotations).

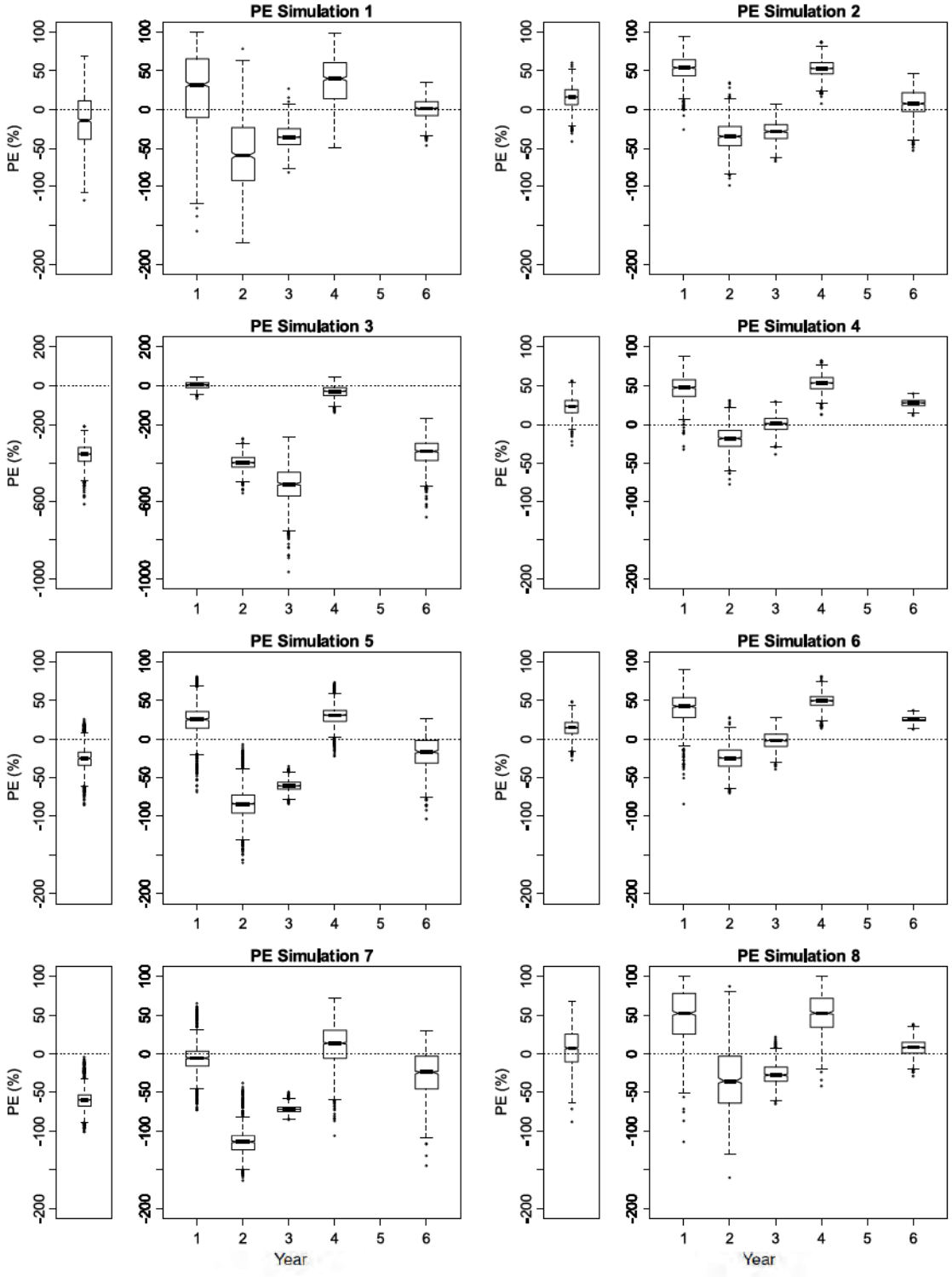

Figure 2.5. Percentage error (PE, %) at stool level for the genotype 'I-214' in model *d* for each simulation (both in general (left) and by years (right)).

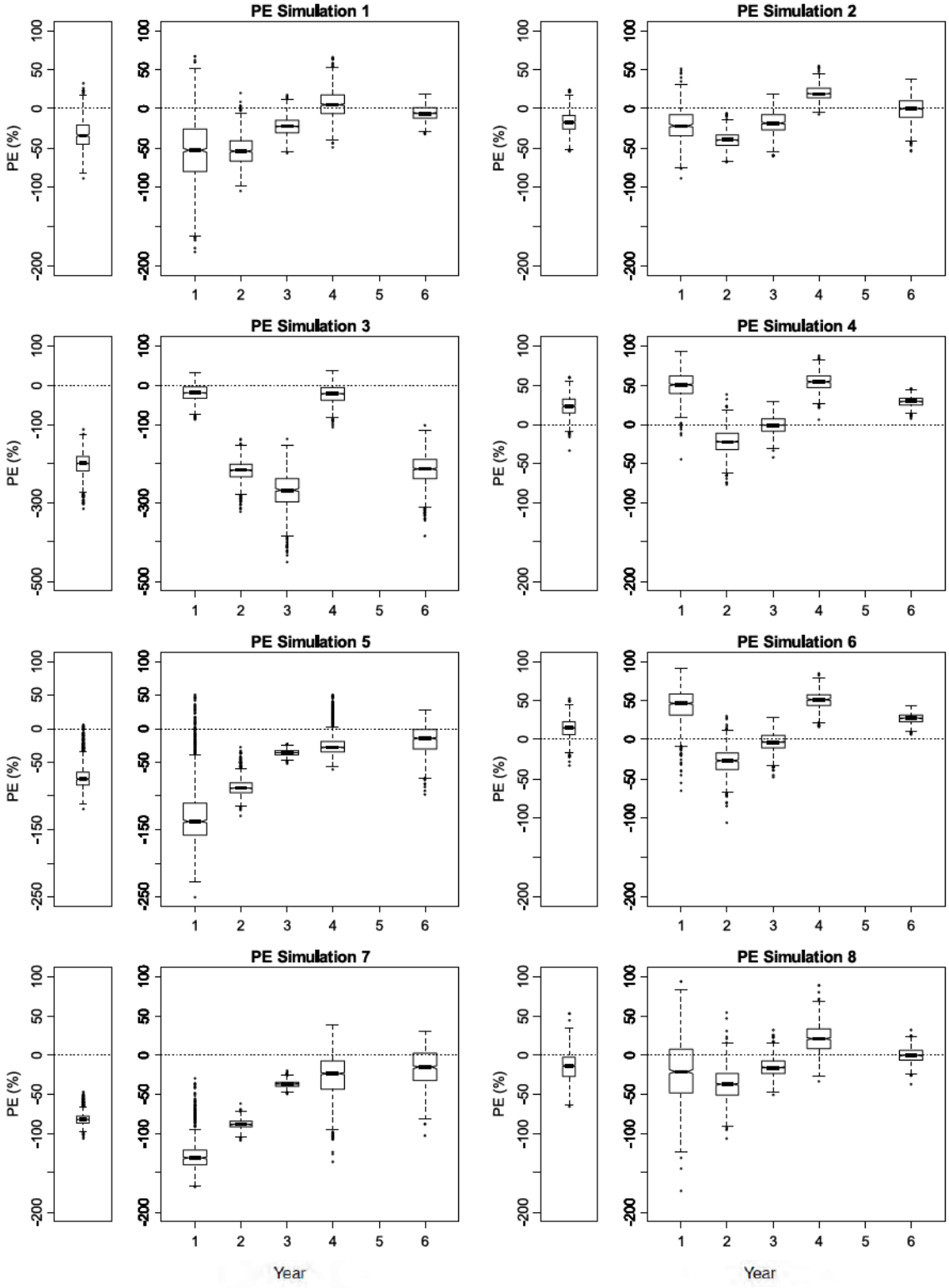

Figure 2.6. Percentage error (PE, %) at stool level for the genotype 'I-214' in model *dn*, for each simulation (both in general (left) and by years (right)).

The RMSE at stool level (g) provides values of precision for the simulations analysed. The accuracy of all models was low, the RMSE increasing over the years in all simulations. The

specific simulations for each rotation were better but the RMSE values reached up to 1,200 g per stool in the cutting year of the first rotation and up to 2,500 g per stool in the second rotation (Table 2.4).

2.3.4. The inclusion of the number of shoots per stool

The inclusion of the number of shoots per stool in the biomass model for genotype 'I-214', resulted in a significant improvement in the model *d* (p-value < 0.01). To quantify the increase in the predictive ability of model *dn* in comparison to *d*, the same analysis as that described in the previous sections was performed.

There was an improvement in the PE obtained, most notably at the end of the first rotation and in the second rotation (Fig. 2.6). The year R5S1 (year 4) showed the best response to the inclusion of the number of shoots, with the PE decreasing in nearly all simulations. However, there were no differences in the PE between model *d* and model *dn* in year R2S1 (year 1). Moreover, in Sim. 1, for example, the general PE in model *dn* was more than double that obtained with model *d* (Fig. 2.5). The best response to the inclusion of the number of shoots was observed in Sim. 2 and Sim. 8. No appreciable variations were detected in Sim. 4 and Sim. 6.

RMSE and MRES behaved very similarly to PE in the model *dn* (Table 2.4), improving general simulations (Sim. 1, Sim. 2 and Sim. 8) in relation to model *d*, while the behaviour in Sim. 4 and Sim. 6 was quite similar to model *d* although with greater mean deviation and lower accuracy.

2.4. Discussion

The variability in parameter estimations for individual tree biomass models can be a problem in forest modelling. However, the behaviour of the allometric parameters, as well as the interaction with the cutting regime in coppice stands has not been analysed in the literature as far as we know. Bearing in mind that coppice crops present a different below-ground biomass age for a given above-ground biomass age in different rotations, some of these factors may have an important impact.

A significant effect of stand age on tree allometry has been identified in our study, with more effect in the first year after harvest. By using data from only one site we are isolating the site variability in parameter estimates. The hypothesis of the authors is that there is no interaction in the trends observed in the parameters' variability with site.

2.4.1. Parameter estimate variability

Most of the published biomass models assume a constant relationship between age and allometry (Ter-Mikaelian and Korzukhin, 1997; Bi *et al.*, 2004; Pérez-Cruzado *et al.*, 2011). This implies a constant rate of relative growth of biomass and diameter regardless of age.

However, a number of studies have reported a change in the allometric relationship at a given point within the valid application range of the models. Bond-Lamberty *et al.* (2002) observed a change in the allometry beginning when diameter at breast height is ~3 cm, suggesting different allometry for saplings than for mature trees. Some authors have addressed this problem by including age as a predictor variable in the models (Saint-André *et al.*, 2005; Shaiek *et al.*, 2011).

Our study shows that there is a clear relationship between the estimated value of each parameter and the stage of development of the stand (Figs. 2.1 and 2.2), as previously reported by Saint-André *et al.* (2005) and Antonio *et al.* (2007). Thus, the application of allometric models leads to considerable systematic lack of fit in tree components biomass predictions (Peichl and Arain, 2007). This lack of fit was found to be greater in the case of the short rotation coppice plantations studied, as the development stages fall within the range where most of the parameter variation occurs.

The evolution of the parameter estimates with age showed greater variability in the first year after each coppicing than in the remaining years of the rotation. A general trend towards stabilization at the end of each rotation was also identified. This greater uncertainty in the estimation of the parameters at ages 1 and 4 (years following coppicing) may be due to the tree changes after coppicing. Such as for example, an extraordinary allocation of resources to the aerial part after resprouting or the fact that the proportion of free growth as opposed to predetermined growth (preformed leaves over-wintered in the bud) is greater after the coppicing, this proportion decreasing with age. According to our findings, the first year after each coppicing should be avoided for sampling in the biomass model construction process, unless these dimensional classes are of specific interest.

Other factors affecting parameter estimates are the predictor variables used in the model as well as the model formulation. The basic model proposed here uses the basal diameter as a predictor variable (model *d*), which is advisable for very young woody crops (Long *et al.*, 1981; Smith and Brand, 1983; Pitt and Bell, 2004). The inclusion of height in estimation models led to more variability in the parameters for each year, particularly in the first year after each coppicing. The use of height in simple models could nevertheless increase the accuracy of predictions without increasing parameter instability in the harvesting years.

The variability of parameter estimates decreased with the use of formulations that include age as a dummy variable (Fig. 2.2). Although trends were similar for all model formulations, dummy formulations produced less variability because of the inclusion of a common *a* parameter, for all years. Therefore, these models seem to be more robust and probably more suitable than the simple formulation to make consistent predictions for different years.

There are studies that support the use of a universal parameter *b,* without distinction between species, site or year, assigning for example a value of b=8/3 according to the model proposed by West *et al.* (1999) (hereinafter, WBE model), which has been widely studied

(Chambers *et al.*, 2001; Anfodillo *et al.*, 2006; Niklas, 2006). Nevertheless, it is generally accepted that there is substantial variation in parameter *b* from one species to another (Ter-Mikaelian and Korzukhin, 1997; Ketterings *et al.*, 2001), and even within the same species. Despite the fact that Zianis and Mencuccini (2004) propose an empirical value for parameter *b* (*b*=2.36), they also state that the use of a universal value for the exponent should only provide a rough estimate of tree biomass. Although the scale for parameter *b* is between 2 and 3 (Zianis and Mencuccini, 2004), for small trees (diameter at breast height (*dbh*) <15 cm) it is generally less than 2 (Duursma and Robinson, 2003). In juvenile stages, it was observed that the values of parameter *b* had greater variability, suggesting that the WBE model (West *et al.*, 1999) is only applicable in actively-growing plants (Pilli *et al.*, 2006). According to our findings, the value of the *b* parameter could be close to those observed by other authors, with some differences arising from the consideration of basal or breast height diameters.

Therefore, the acceptance of a constant value of *b* should be viewed as tentative, and applicable only for rough predictions of biomass (Zianis and Mencuccini, 2004). For more accurate predictions it is necessary to take into account differences in species, sites and ages (Picard *et al.*, 2012). In spite of this, some ecophysiological models, as the model 3PG (Physiological principles in predicting growth) (Landsberg and Waring, 1997), use a fixed value of the parameter *b* to predict the biomass from diameter or the other way round.

2.4.2. Sample size

The requirement as regards sample size to obtain an adequate description of a population by means of an empirical model is still to be determined. Heterogeneity in empirical values of the allometric parameters can result from methodological artefacts including small sample size, the regression methods used and fitting of models to parts of a dataset (Sileshi, 2014). While some studies point to the need for at least 50 observations to obtain stable estimations of the parameter values (Coomes and Allen, 2009; Sileshi, 2014), other studies observe that the effect of uncertainty in model predictions can only be considered below the sampling uncertainty when sample size is greater than 100 trees (McRoberts and Westfall, 2014). The minimum sample size necessary to achieve stable parameter estimates under the conditions studied was evaluated in this paper. The minimum sample size was only evaluated for the genotype 'I-214' as this genotype is commonly used as reference in scientific studies. For this genotype it was concluded that as sample size increased, parameter variability decreased (Figs. 2.3 and 2.4), as reported by other authors who have addressed the influence of this factor on a wide range of species (Zapata-Cuartas *et al.*, 2012). The parameters *a* and *b* display great variability when the sample size is small. Therefore, a small sample size leads to a great deal of uncertainty in the exponent values. In our case, for the genotype 'I-214', a threshold of 48 stools for short rotation coppice plantations was also proposed as the minimum sample size necessary to reduce the variability of the parameters, at least in cases where all the stages of development were included in the model. These results highlight the conclusions drawn from the simulation of different sampling schemes, as a sample size of ~50 was considered for all sampling options simulated.

2.4.3. Uncertainty analysis

Age and rotation play an important role in individual tree allometry in coppice stands. Large uncertainties, both in precision and accuracy, were observed in models fitted for conditions different to those for which they are applied, or when age or rotation was not considered in the model formulation. This suggests that the decision to prioritize different aspects of allometric model development depends on the specific research objectives (Van Breugel *et al.*, 2011) and potential use of the final models. Under such conditions, the predictive ability of the model cannot be improved by increasing the sample size but rather by stratifying the development conditions.

There are multiple disadvantages associated with using the PE criterion alone for analysing the prediction uncertainty, such as values being extremely skewed when observed biomass values are close to zero (Sileshi, 2014), which can occur in the simulations that include data from the first year after each coppicing (Sim. 1, Sim. 3, Sim. 6, Sim. 7 and Sim. 8); a heavier penalty on positive deviances than on negative deviances; and unrealistic mean values when negative and positive deviances offset each other (Sileshi, 2014). For these reasons, each simulation is evaluated using a global PE and a specific PE for each year (Figs. 2.5 and 2.6). MRES and RMSE were also calculated to contrast the PE results (Table 2.4). The RMSE also gives a relatively high weight to large uncertainties due to the squaring of errors before they are averaged (Sileshi, 2014).

Some studies have reported good predictive abilities of models (total above-ground, below-ground or total tree biomass) that depend only on diameter for entire age sequences (Peichl and Arain, 2007), which in the present study was considered in the Sim. 8 sampling option. However, our results show that this is not a good approach for coppice plantations, the inclusion of other predictor variables (such as the age or the number of shoots per stool) being advisable in these cases to achieve accurate biomass estimates. The use of development stage-specific models was found to be the option with the smallest mean deviation and greatest accuracy, as well as minimizing the sampling effort and associated costs. The latter option, however, limits the use of these models at different stages of development both within the same rotation and between different rotations. The above findings lead us to recommend the use of models with age as a predictor variable when the objective is to provide predictions for all dimensional stages, or age-specific models when the interest is focused on predictions at a specific development stage.

The fact that the accuracy of the specific fittings for the second rotation was not as good as for the first rotation may be due to the greater variability over successive rotations. The growth rate was greater during the second rotation, therefore the diameter variability was greater, although a larger number of rotations and clusters would be necessary to assess the precise evolution according to the rotations. If D_{10} alone is used as a predictor variable in the model, bearing in mind the possibility of greater diameters in successive rotations due to a more developed root system (depending on the rotation number, the site conditions and the genotype),

skewed estimations are likely to occur when such variability is not taken into account. Under these circumstances it would be advisable to introduce other variables that are sensitive to these changes in the growth pattern.

Only data from the first rotation years were used to fit the model in Sim. 3, which would be a similar option to the small trees sampling scheme (SSS) proposed by Zianis and Mencuccini (2004). It was observed that the biomass values of the smallest trees strongly affected the values of the coefficients in the allometric relationship between biomass and diameter (Chave *et al.*, 2001; Pérez-Cruzado and Rodríguez-Soalleiro, 2011). According to our results, the use of this technique causes much larger uncertainties in the cutting year, which makes it inappropriate unless the interest is focused on prediction at these specific ages.

The use of the cutting year alone to fit the predictive model for each rotation would appear to be the most accurate and unbiased option for the predictive purpose with regard to biomass crops. Despite the fact that the smallest sample size for fitting the model was that used for simulations Sim. 4 and Sim. 5 (below the limit set for the sample size), the lack of data may be of less consequence given the more specific range of values. The use of a common fit for both rotations, using only the cutting years or all the stages of development gives poorer predictions for the cutting years but better results for all the years in general. Thus, the decision as to the best option depends on the final aim of the model, bearing in mind that each choice with regard to the construction of the model implies associated uncertainty that will affect the predictions.

The inclusion of the number of shoots per stool as a predictor variable improved the biomass models under the coppice conditions studied. The inclusion of this variable had a significant influence in terms of uncertainty reduction (Fig. 2.6) and also reduced the sample size necessary to achieve the same level of accuracy (Fig. 2.4), apart from the frequency of negative parameter predictions for the c parameter. The uncertainty decreased with the inclusion of the number of shoots per stool in common models (Sim. 2 and Sim. 8), due to differences in n between the first and the second rotation. In Sim. 4, in which only the cutting year of the first rotation was used to fit the model, the inclusion of the number of shoots per stool did not improve the fitting neither in mean deviation or accuracy. However in Sim. 5 (second rotation), the inclusion of the number of shoot per stool in the model improves the accuracy but not the mean deviation in the cutting year. This fact is attributed to the greater number of shoots per stool in consecutive rotations with respect to the first rotation. Under such circumstances, there are several dominant shoots rather than a single dominant shoot, resulting in a larger biomass accumulation.

2.5. Conclusions

A greater variability in biomass prediction has been observed in an SRC plantation depending on the factors studied, such as the predictor variables used in the models, the model formulation, the sample size and even the sample selection criteria. There is a notable influence of age on allometry in the years after harvest. The implicit inclusion of age in the model as a

dummy variable, expanding the exponent of diameter, reduces the variability in parameter estimations for each year and each rotation. However, the sample selection criteria may also reduce the influence of age in the model. It is evident that different options may be employed depending on the purpose of the model, so it is advisable to carefully consider the aim of the model in order to select the most appropriate time of sampling. If the final objective is to predict biomass at different ages in an SRC plantation, the inclusion of a specific variable such as the number of shoots per stool seems appropriate since it is easy to record, reduces the mean deviation and increases the accuracy. The minimum sample size to construct the model in the case of the genotype 'I-214' is 50. However, if the final aim is to predict the biomass at the planned cutting year, only the use of cutting year data in the model construction will suffice. The greater degree of accuracy achieved by only collecting data from the cutting year means that the sample size required can be smaller and therefore the sampling activity can be more concentrated in time.

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CHAPTER

3

On the genetic affinity of individual tree biomass allometry in poplar short rotation coppice

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On the genetic affinity of individual tree biomass allometry in poplar short rotation coppice

Abstract

Woody biomass is one of our main resources available to enhance the bio-economy, but its production varies considerably depending on the species, the environment and crop management. The variability associated with these crops complicates the biomass estimation through prediction models. The specificity of environment or genotype-level limits the application of many of the models, which are often developed for use at local geographical-levels. Although generalizations involve some loss of accuracy, the inclusion of a wide range of data for a wide range of environments and genotypes can improve model applicability. A total of 11,265 data from short rotation, high density poplar plantations (from 22 sites in Spain, covering 29 genotypes belonging to 7 different taxonomic groups) were used to develop biomass prediction models under Mediterranean conditions and test whether similarities in individual-tree biomass allometry occur within the taxonomic group-level. A general model, as well as taxonomic group- and genotype-level models, were fitted using weighted nonlinear regression. The simplified model, in which only the basal diameter is included, presented the best model performance, explaining 87% of the variability. The allometric similarities among different genotypes were evaluated in order to explore the relationship between the most frequently used poplar genotypes in the Mediterranean area, and although certain groups were identified, it was not possible to relate these similarities among different genotypes to their taxonomic group affinity. This was also confirmed by comparing the performance of the general models with the taxonomic group-level models when predicting at genotype level. Although estimates made using the general models are relatively precise, the use of genotype-level models is recommended for more accurate predictions.

KEYWORDS: *Populus* spp.; biomass; short rotation forestry (SRF); allometric models; weighted nonlinear regression; generalized model.

3.1. Introduction

The use of biomass as a resource to further the development of the bioeconomy is currently one of the main avenues of research in the field of bioenergy. There are a variety of sources of biomass and among the crops used for this purpose, woody species have gained particular importance (Tallis *et al.*, 2013) and are now a commercial reality in many parts of the world (Mareschi *et al.*, 2005).

The main aim with regard to these crops is to achieve greater, more efficient production as well as sustainability and this requires a broad understanding of the factors involved in the production process. Total biomass production varies considerably, not only due to factors related to species and site conditions but also to plantation management (Sixto *et al.*, 2015).

The suitability of the *Salicaceae* family for lignocellulosic biomass production using high-density crops in short rotation coppice (SRC) is well documented (Yemshanov and McKenney, 2008; Sixto *et al.*, 2011). *Populus* spp. is considered very favourably for this purpose under Mediterranean conditions, whether unirrigated (Paris *et al.*, 2011) or irrigated (Sixto *et al.*, 2013a), depending on the area. Nevertheless, a wide genetic variability in biomass production is found among *Populus,* their hybrids and even among genotypes of the same taxonomic group (Dillen *et al.*, 2007). Additionally, site conditions, crop management and all the interactions between them also play an important role in determining the production attained (Sixto *et al.*, 2015).

The ability to predict production levels is particularly important when the economic viability of a given crop is to be assessed (Pérez-Cruzado *et al.*, 2013) or when decisions regarding rotation length have to be taken. The allometric prediction models based on empirical data allow estimates of biomass stocks and yield to be made at genotype-level or in accordance with specific plantation conditions. The fact that biomass estimation models are site-specific (Tumwebaze *et al.*, 2013) or genetic-specific (Sixto *et al.*, 2013a) clearly limits their practical use as management tools to predict biomass and plan crop management. The main constraint in this respect is the lack of models for some genotypes, although this is usually addressed by using models fitted for close genotypes according to the progenitor species (same hybrid group). This is a common procedure for biomass estimation in highly diverse natural forest, where the characteristics (such as wood specific gravity) of the genotypes of the same species, genus and even family are averaged in order to make predictions when information specific to a given species is lacking (Flores and Coomes, 2011). This procedure assumes that biomass allometry is mainly driven by taxonomic affinity due to their common progenitor species. This is also the case for biomass estimation in SRC, where biomass models for taxonomically proximate genotypes are commonly used in the case of genotypes for which there are no published biomass models (Dillen *et al.*, 2007).

However, the use of general models can involve a considerable loss of accuracy for genotype-specific estimations, while allowing greater flexibility in its use. Hence, the

calibration of generalized biomass models for large regions must be based upon a sample of site and climatic conditions, encompassing the range of environmental conditions covered (Muukkonen, 2007; Shaiek *et al.*, 2011). This is both costly and time consuming.

While much of the research related to biomass modelling for SRC has focused on developing site- and genotype-specific biomass models (Verwijst and Telenius, 1999; Barrio-Anta *et al.*, 2008; Mosseler *et al.*, 2014), there are also some examples of models developed at greater hierarchical levels (Ben Brahim *et al.*, 2000a; Dillen *et al.*, 2007). In any case, scarce research has been conducted that focuses on the loss of accuracy and precision in biomass estimation at genotype-level due to the use of widely applicable general models. Moreover, the validity of biomass models for other genotypes of the same or even other taxonomic groups has not been studied in depth.

The aims of this study are: i) to evaluate the similarities in biomass allometry among different genotypes, and ii) to evaluate the accuracy and precision associated with using biomass models fitted at different hierarchical levels:, generalized, taxonomic group and genotype-specific models fitted for an extensive dataset which includes information on different poplar genetic material and site conditions in the Mediterranean area under irrigation management.

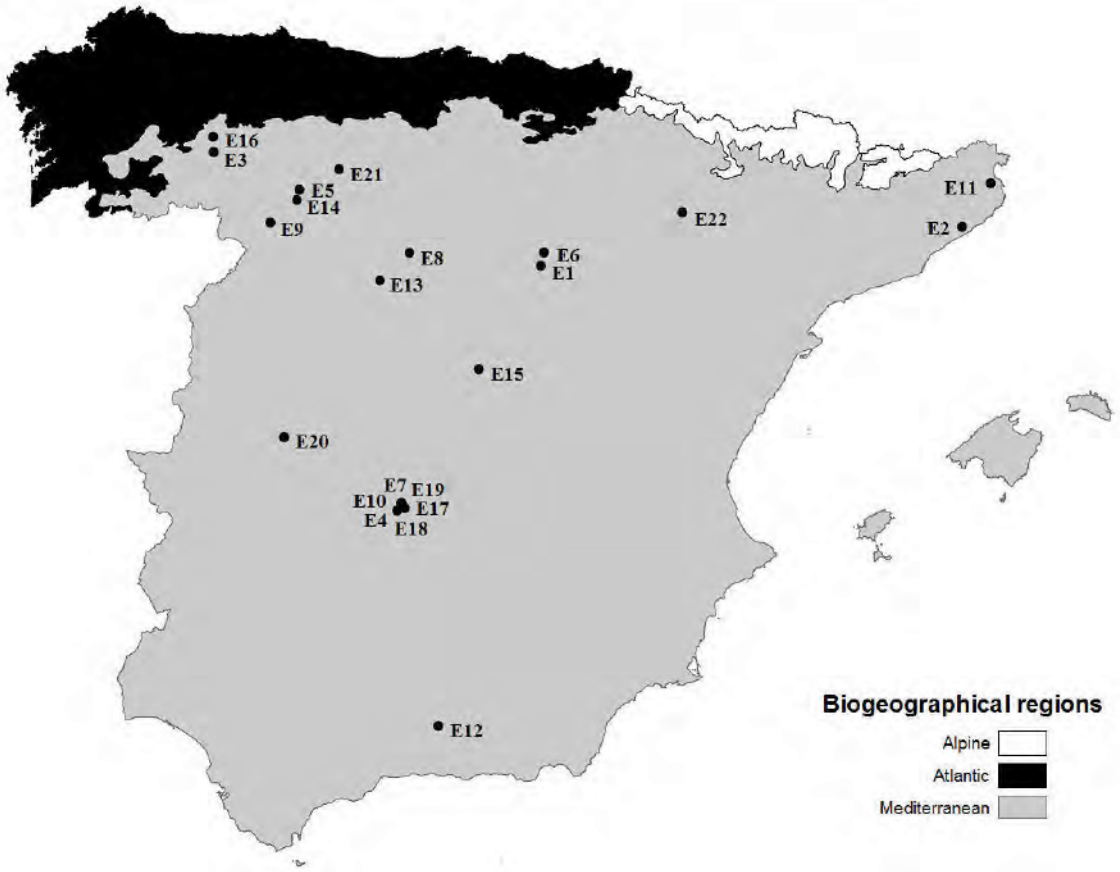

Figure 3.1 Location of the experimental sites.

3.2. Materials and methods

3.2.1. Data

An extensive dataset of *Populus* spp. growing under SRC and established at 22 sites in Spain was used in this study (Fig. 3.1). The sites cover a wide range of site conditions (Table 3.1), and are highly representative of the areas where this crop is present ranging from temperate to warm Mediterranean climates. Twenty nine genotypes of seven different taxonomic groups were included in the network of plots (Table 3.2)(FAO, 2016).

Table 3.1. Location and site characteristics of the experimental sites.

Sites	Coordinates		Altitude (m asl)	*Tm*[a]	*pH*	*OM*[b]	Köppen climate classification
	Latitude	Longitude					
E1	41° 28´18.6´´ N	2° 32´51´´ W	947	11.2	8.42	2.08	Temperate oceanic climate
E2	41° 41´42.6´´ N	2° 40´22.3´´ E	65	16.1	6.31	0.3	Warm Mediterranean climate
E3	42° 37´25´´N	6° 38´54´´ W	556	12.7	6.21	0.85	Temperate Mediterranean climate
E4	39° 11´40.22´´ N	4° 16´57.86´´ W	670	15.0	6.1		Cold semi-arid climate
E5	42° 9´6.24´´ N	5° 33´42.37´´ W	254	11.8	6.84	0.62	Temperate Mediterranean climate
E6	41° 35´58.3´´ N	2° 30´23.7´´ W	1,097	10.2	6.4	0.57	Temperate oceanic climate
E7	39° 15´39.81´´ N	4° 13´46.6´´ W	670	14.6	6.08	1.11	Warm Mediterranean climate
E8	41° 35´29.65´´ N	4° 10´20.48´´ W	770	11.9	8.27	1.78	Temperate Mediterranean climate
E9	41° 50´11´´N	5° 54´36.1´´ W	716	12.1	5.41	1.51	Temperate Mediterranean climate
E10	39° 14´53.99´´N	4° 13´44.13´´ W	670	14.6	6.2	0.93	Cold semi-arid climate
E11	42° 04´47.2´´ N	3° 3´56.3´´ E	12	15.7	8.43	0.66	Warm Mediterranean climate
E12	37° 11´43.1´´ N	3° 46´3.7´´W	554	15.3	8.45	1.43	Warm Mediterranean climate
E13	41° 19´29.94´´ N	4° 32´4.34´´W	756	12.3	9.04	0.89	Temperate Mediterranean climate
E14	42° 3´21.9´´ N	5° 35´12.4´´ W	715	11.8	8.26	1.04	Temperate Mediterranean climate
E15	40° 30´47.4´´ N	3° 18´39.6´´ W	593	13.7	8.4	0.49	Warm Mediterranean climate
E16	42° 36´45.7´´ N	6° 39´52.6´´ W	531	12.8	6.01	1.02	Temperate Mediterranean climate
E17	39° 14´42.75´´ N	4° 13´21.88´´ W	670	14.7	6.29	1.25	Cold semi-arid climate
E18	39° 13´3.45´´ N	4° 11´38.83´´ W	670	14.7	6.18	0.94	Cold semi-arid climate
E19	39° 15´24.74´´ N	4° 13´36.87´´ W	670	14.6			Warm Mediterranean climate
E20	39° 50´56.82´´ N	5° 39´32.79´´ W	258	16.7	7.05	0.93	Cold semi-arid climate
E21	42° 21´4.78´´ N	5° 4´11.07´´ W	838	11.9	7.54	1.18	Temperate oceanic climate
E22	41° 56´59.47´´ N	0° 47´15.98´´ W	279	14.5	8.26	1.15	Warm oceanic climate

[a] *Tm* is the average annual mean temperature (°C) and [b] *OM* is the soil organic matter (%).

Plantations were established in early spring between 2005 and 2010 using hardwood cuttings of 20-30 cm in length. Densities ranged from 4,762cuttings ha^{-1} to 33,333cuttings ha^{-1} in a design consisting of single rows 3 m apart. Similar management was applied at all sites for all genotypes, including fertilization during soil tillage according to the specific soil characteristics, pre-emergence treatment with oxyfluorfen (4 l ha^{-1}) to control weeds, and drip or flood irrigation (to field capacity) during the summer months (Sixto *et al.*, 2013b). The summer drought, characteristic of the Mediterranean climate, necessitated the application of irrigation at all the sites. A rotation length of three years was established for all except three of the sites (E4, E5 and E17) where a four year rotation was determined due to poor development. In the plots established at E1, trees were cut back one year after planting in order to stimulate sprouting (Willebrand and Verwijst, 1993; Kopp *et al.*, 2001). Hence, the stages of development covered in this study at the end of the first rotation were: R3S3, R4S3 o R4S4, denoting root age as R and stem age as S.

Table 3.2. Genotypes and taxonomic groups considered on this study.

Genotype	Parentage		Section	Origin	Breeder	Number of sites where present
'2000 Verde'	*P.* × *canadensis* Mönch	D × N	Aigeiros	-	Italy, 1982	1
'A2A'		D × N	Aigeiros	-	Italy,1993	1
'A4A'		D × N	Aigeiros	(Illinois USA) × (Piamonte,Italy)	Italy,1986	6
'AF2'		D × N	Aigeiros	(Illinois USA) × (Italy)	Italy,1994	10
'B-1M'		D × N	Aigeiros	-	Italy, 1960s	1
'Ballottino'		D × N	Aigeiros	-	Italy	11
'Dorskamp'		D × N	Aigeiros	(Missouri USA) × (Italy)	Nederland, 1952	4
'Guardi'		D × N	Aigeiros	(Stonville USA) × (Spain)	Italy, 1960s	5
'I-214'		D × N	Aigeiros	('Canadesi bianco') × (Italy)	Italy, 1929	17
'Luisa Avanzo'		D × N	Aigeiros	-	Italy, 1968	1
'MC'		D × N	Aigeiros	Italy	Italy, 1954	4
'Muur'		D × N	Aigeiros	(Iowa USA × Illinois USA) × (Italy × Belgium)	Belgium, 1978	1
'Orion'		D × N	Aigeiros	(Texas USA) × (Italy)	Italy, 1983	4
'Oudenberg'		D × N	Aigeiros	(Iowa US × Illinois USA) ×(Italy × Belgium)	Belgium,1978	14
'Triplo'		D × N	Aigeiros	(Mississipi USA) × (Italy)	Italy, 1961	2
'Vesten'		D × N	Aigeiros	(Iowa USA × Illinois USA) × (Italy x Belgium)	Belgium,1978	6
'Beaupre'	*P.* × *generosa* Henry	T × D	Tacamahaca × Aigeiros	(USA) × (Iowa USA × Missouri USA)	Belgium	1
'Hoogvorst'		T × D	Tacamahaca × Aigeiros	(USA) × (Michigan USA)	Belgium, 1969	1
'Raspalje'		T × D	Tacamahaca × Aigeiros	(USA) × (Iowa USA × Missouri USA)	Belgium	3
'Unal'		T × D	Tacamahaca × Aigeiros	(USA) × (Iowa USA × Missouri USA)	Belgium,1960	5
'AF8'		(D × T) × T	(Aigeiros × Tacamahaca) × Tacamahaca	(Iowa, USA × Oregon, USA) × (Washing.USA)	Italy, 1993	8
'Grimminge'		D × (T × D)	Aigeiros × (Tacamahaca × Aigeiros)	(Michi. USA × Connec. USA) × (Wash.USA × (Iow.USA × Misso. USA)	Belgium, 1976	3
'AF6'	*P.* × *generosa* Henry × *P.nigra* L.	(T × D) × N	(Tacamahaca × Aigeiros) × Aigeiros	-	Italy	1
'Monviso'		(D × T) × N	(Aigeiros × Tacamahaca) × Aigeiros	(Iowa USA × Oregon USA) × (Piamo. Italy × Umbr.Italy)	Italy,1991	9
'Baldo'	*P.deltoides* W. Bartram ex Marshall	D	Aigeiros	-	Italy,1985	4
'Viriato'		D	Aigeiros	(Hardward,USA) × (Stoneville, USA)	Portugal,1986	5
'Gazi'	*P. nigra* L.	N	Aigeiros	-	Turkey	1
'Lombardo Leones'		N	Aigeiros	León, Spain	Spain, 1970	1
'Bruhl 7'	*P. trichocarpa* Torr. & A. Gray	T	Tacamahaca	Oregon, USA	USA,1950	1

Information obtained from the International Register of Cultivars of *Populus* L. (FAO, 2016).

Measurements were taken annually after planting. Diameters over bark at 10 cm above ground level (D_{10}, mm), accounting for the size of the shoots at the usual harvesting height, were measured using a digital caliper. Total height of the highest shoot (H, cm) was also

measured with a measuring pole. The number of shoots and the density of living trees per plot were also recorded. The above-ground woody biomass was evaluated in all plots by destructive analysis at the end of the rotation, once leaves had fallen. Total above-ground dry woody biomass (*Ws*, g dm (dry matter)) was determined for each sample from the fresh weight and the estimated wood moisture by randomly selecting whole plants from each of the plots and then oven-drying a sample randomly selected for each genotype to constant weight at 105 ºC.

3.2.2. Statistical analysis

3.2.2.1. Fitting and evaluating biomass models at different hierarchical levels

We fitted regression models at three levels: 1) a joint general model for all the genotypes, sites and management schemes (density and rotation length) included in the study, 2) taxonomic group-level models by taking into account the hybrid group of each tested genotype (see Table 3.2), excluding those taxonomic groups which were only present at one site or only contained one genotype (*P.* x *generosa* x *P.nigra* L., *P. deltoides* W. Bartram ex Marshall x *P.* x *generosa* Henry, *P. nigra* L. and *P. trichocarpa*), and 3) genotype-level models for each of 18 genotypes (after excluding those which were only present at a single site).

Due to the lack of homogeneity in the residual variances, weighted nonlinear regression was used to correct heteroscedasticity, frequently present in individual-tree biomass data (Parresol, 1999). Each observation was weighted by the inverse of its basal diameter variance ($1/D_{10}^2$). This weighting factor stabilized the variances and provided homogeneous studentized residuals.

For the general model fit, different tree-level variables were tested as regressor (*d* (3.1), *h* (3.2), *dh* (3.3), *dn* (3.4), *dhn* (3.5)) and also the different stages of development according to the root age (R) or the stem age (S) were evaluated (*dR* (3.6), *dS* (3.7)) (Table 3.3).

Table 3.3. Model formulations.

Equation	Nomenclature	Model
3.1	*d*	$Ws = a * D_{10}{}^{b} + \varepsilon$
3.2	*h*	$Ws = a * H^{c} + \varepsilon$
3.3	*dh*	$Ws = a * D_{10}{}^{b} * H^{c} + \varepsilon$
3.4	*dn*	$Ws = a * D_{10}{}^{b} * n^{c} + \varepsilon$
3.5	*dhn*	$Ws = a * D_{b}{}^{b} * H^{c} * n^{d} + \varepsilon$
3.6	*dR*	$Ws = a * D_{10}{}^{b} * R^{e} + \varepsilon$
3.7	*dS*	$Ws = a * D_{10}{}^{b} * S^{f} + \varepsilon$

Total above-ground woody dry biomass (*Ws*, g dm), diameters over bark at 10 cm of dominant shoots per stool (D_{10}, mm), total height of the highest shoot per stool (*H*, cm), number of shoots per stool (*n*), root age (*R*, yr), stem age (*S*, yr) and ε is the residual term.

In all the proposed models, parameter *b* is the power of the basal diameter (D_{10}), parameter *c* of the total height (*H*), parameter *d* of the number of shoots (*n*), parameter *e* of the root age (*R*) and parameter *f* of the stem age (*S*).

To evaluate the performance of the models, we used the proportion of variance explained by the weighted models (pseudocoefficient of determination, *pse*-R^2), the root of weighted mean square error (RMSE$_\Psi$), the error sum of squares (SSE$_\Psi$) and the total sum of squares (SST$_\Psi$):

$$SSE_\Psi = \sum_{i=1}^{n}\left(\Psi_i * \left(W_{s_i} - \widehat{W}_{s_i}\right)^2\right) \qquad (3.8)$$

$$SST_\Psi = \sum_{i=1}^{n}\left(\Psi_i * \left(W_{s_i} - \frac{\sum_{j=1}^{n}(\Psi_i * W_{s_i})}{\sum_{i=1}^{n}\Psi_j}\right)^2\right) \qquad (3.9)$$

$$pse\text{-}R^2 = r^2_{w_i\widehat{w}_i} \qquad (3.10)$$

$$RMSE_\Psi = \sqrt{\frac{SSE_\Psi}{n-p}} \qquad (3.11)$$

where $r^2_{w_i\widehat{w}_i}$ is the correlation coefficient for a linear regression between the observed and the predicted values of the dependent variable (Ryan, 1997), W_{s_i} is the observed biomass, $\widehat{W}_{s_i}$ is the predicted biomass, Ψ_i is the weighted factor, n is the number of data used in the fitting and p is the number of parameters to be estimated.

The genotype- and taxonomic group-level models were fitted by considering only the basal diameter (D_{10}) as regressor variable, as this is the most common model formulation used in biomass prediction and was the model which explained the highest variability at general-level.

3.2.2.2. Evaluation of the similarity in biomass allometry among genotypes

The similarity in biomass allometry among genotypes was evaluated by cross predicting with the genotype-level models and comparing the predictions at genotype-level with the observed values. Two genotypes are assumed to share allometry when the biomass model fitted for a given genotype produces non-deviated predictions over the other. Four non-parametric validation tests were used for evaluating the similarity in biomass allometry among genotypes (Pérez-Cruzado *et al.*, 2015). All these tests showed similar performance, and the mean *p*-values were computed pairwise for all genotypes. The null hypothesis for all tests is that both samples belong to the same population, so small *p*-values lead us to reject the null hypothesis that both samples share the same allometric model. To plot the model suitability of each genotype, a radial chart was used, which is a useful graphical tool for data comparison on a two-dimensional chart. It is also known as a radar chart or kiviat diagram (Chambers *et al.*, 1983; Gil *et al.*, 1999; Howse *et al.*, 2000).

3.3. Results

3.3.1. Allometric above-ground biomass models

3.3.1.1. General weighted fit

First, a general model was fitted which included all sites, genotypes and management variability (stand density and rotation length), and which used different combinations of

regressor variables such as basal diameter, dominant shoot height, number of shoots and age (represented by root age and stem age). The parameter estimations and fitting statistics are shown in Table 3.4.

Table 3.4. Parameter estimates and goodness-of-fit statistics for the general models, testing different combinations of regressor variables.

N°	MODEL		Estimate	Std. Error	*t* value	*p*-value (>|t|)	*RMSE* pond	*pse*-R^2	*SSE*	*SST*	DF
3.1	*d*	$\hat{a}$	0.1351	0.0053	25.6999	<0.0001	15.11	0.87	2,571,247	19,346,661	11,263
		$\hat{b}$	2.4577	0.0092	266.9371	<0.0001					
3.2	*dh*	$\hat{a}$	0.0093	0.0005	18.5732	<0.0001	18.12	0.81	3,697,895	19,346,661	11,262
		$\hat{b}$	1.8020	0.0126	142.7784	<0.0001					
		$\hat{c}$	0.8149	0.0126	64.4689	<0.0001					
3.3	*h*	$\hat{a}$	0.0005	0.0000	11.0285	<0.0001	32.62	0.38	11,982,596	19,346,661	11,263
		$\hat{c}$	2.3581	0.0135	175.2047	<0.0001					
3.4	*dn*	$\hat{a}$	0.1287	0.0050	25.7229	<0.0001	15.64	0.86	2,754,025	19,346,661	11,262
		$\hat{b}$	2.4630	0.0092	268.8941	<0.0001					
		$\hat{d}$	0.0853	0.0059	14.5741	<0.0001					
3.5	*dhn*	$\hat{a}$	0.0086	0.0005	18.6787	<0.0001	18.04	0.81	3,665,854	19,346,661	11,261
		$\hat{b}$	1.8043	0.0125	144.8650	<0.0001					
		$\hat{c}$	0.8200	0.0125	65.6383	<0.0001					
		$\hat{d}$	0.0897	0.0049	18.2734	<0.0001					
3.6	*dR*	$\hat{a}$	0.0905	0.0039	23.2819	<0.0001	22.10	0.72	5,501,217	19,346,661	11,262
		$\hat{b}$	2.4302	0.0096	254.2441	<0.0001					
		$\hat{f}$	0.4495	0.0249	18.0194	<0.0001					
3.7	*dS*	$\hat{a}$	0.0905	0.0039	23.2819	<0.0001	22.10	0.72	5,501,217	19,346,661	11,262
		$\hat{b}$	2.4302	0.0096	254.2441	<0.0001					
		$\hat{e}$	0.4495	0.0249	18.0194	<0.0001					

Total above-ground woody dry biomass (*Ws*, g dm), diameters over bark at 10 cm of dominant shoots per stool (D_{10}, mm), total height of the highest shoot per stool (*H*, cm), number of shoots per stool (*n*), root age (*R*, yr) and stem age (*S*, yr).

All the parameter estimates simultaneously contributed to statistically significant improvements in the quality of the fit of the model to the data (*p*-value < 0.0001) and the models accounted for more than 72% of the total variability in biomass yield except for the model that only included height as a predictor, which only accounted for 38% of the total variability. Model 3.1 (*d*) accounted for 87% of the total variability, which is the highest variability explained by any of the tested general models. This model had the smallest sum of squares error and $RMSE_{\Psi}$, and the highest *pse*-R^2. The sum of the weighted residual was similar when the number of shoots was included in the model, but this is not the case where only height is used as a predictor.

3.3.1.2. Genotype weighted fit

Tables 3.5 and 3.6 show the parameter estimates and fitting statistics for the models fitted at taxonomic group- and at genotype-level, respectively. The singularity of these equations is

that they include the site variability. The number of sites where each genotype is present is shown in Table 3.2.

Table 3.5. Parameter estimates and goodness-of-fit statistics for the taxonomic group-level models with the formulation $Ws = a * D_{10}{}^{b} + \varepsilon$.

TAXONOMIC GROUP		Estimate	Std. Error	*t* value	*p*-value (>|t|)	*RMSE* pond	*pse-R*^{2}	*SSE*	*SST*	DF
P. × canadensis Mönch	$\hat{a}$	0.1435	0.0062	22.9121	<0.0001	14.34	0.87	1,785,955	13,350,259	8,688
	$\hat{b}$	2.4394	0.0104	234.7719	<0.0001					
P. × generosa Henry	$\hat{a}$	0.1054	0.0135	7.8377	<0.0001	18.12	0.87	408,389.3	3,168,968	1,244
	$\hat{b}$	2.5182	0.0294	85.7138	<0.0001					
P.deltoides	$\hat{a}$	0.0496	0.0149	3.3341	0.0010	17.32	0.86	86,103	609,394	287
	$\hat{b}$	2.6940	0.0700	38.4895	<0.0001					

Total above-ground woody dry biomass (*Ws*, g dm), diameters over bark at 10 cm of dominant shoots per stool (D_{10}, mm) and ε is the residual error.

All the equations considered achieved convergence and the parameter estimates significantly improved the quality of the model fit to the data at *p*-value = 0.05, except parameter *a* for the genotype 'Baldo' (Table 3.6). It is known that the numerical value of the parameter *a* depends on the value of the *b* parameter as well as on the units of the response and regressor variables, which implies that the *a* parameter has no biological meaning and therefore there is no serious implication as regards the non-significance of this parameter (Antonio *et al.*, 2007).

The general model accounts for only 87% of the variability in biomass estimations, whereas the taxonomic group-level models account for 86 to 92% of the total variability. The genotype-level models accounted for between 82-95% of the total variability of production.

3.3.2. Similarity in biomass allometry among different genotypes

The *p*-values of the validation tests used to explore the similarity in biomass allometry among the different poplar genotypes are represented in Fig. 3.2. Only those cases where the tests detected differences in biomass allometry among genotypes (*p*-value > 0.05) are represented in Fig. 3.2. The genotypes in Fig. 3.2 are grouped in circular sectors according to the different taxonomic groups to which they belong, represented in different colours. It was found that clusters did not depend on which taxonomic group they belong to. This can be seen in the distribution of the colours for the different taxonomic groups, as all colours are distributed over the four circular sections considered. It was also observed that, for the genotypes within the same taxonomic group (for example, the dots in blue), the *p*-values in the outermost positions were located in circular sections belonging to different taxonomic groups. This means that closest allometries were more frequently observed for genotypes belonging to different taxonomic groups.

Table 3.6. Parameter estimates and goodness-of-fit statistics for the genotype-level models with the formulation $Ws = a * D_{10}{}^{b} + \varepsilon$.

GENOTYPE		Estimate	Std. Error	*t* value	*p*-value (>|t|)	*RMSE* pond	*pse-R*^{2}	*SSE*	*SST*	DF
'Guardi'	$\hat{a}$	0.1251	0.0252	4.9737	<0.0001	9.40	0.91	24,232	250,858	274
	$\hat{b}$	2.4925	0.0501	49.7590	<0.0001					
'Oudenberg'	$\hat{a}$	0.1942	0.0195	9.9478	<0.0001	11.34	0.87	203,304	1,604,185	1,580
	$\hat{b}$	2.3877	0.0249	95.8349	<0.0001					
'Vesten'	$\hat{a}$	0.1865	0.0271	6.8909	<0.0001	8.71	0.93	26,613	378,115	351
	$\hat{b}$	2.3788	0.0354	67.1952	<0.0001					
'Triplo'	$\hat{a}$	0.1404	0.0653	2.1494	0.0334	15.89	0.91	33,573	364,686	133
	$\hat{b}$	2.4126	0.1055	22.8632	<0.0001					
'I-214'	$\hat{a}$	0.0920	0.0079	11.6919	<0.0001	14.19	0.89	458,511	4,059,415	2,278
	$\hat{b}$	2.5453	0.0201	126.4736	<0.0001					
'AF2'	$\hat{a}$	0.0758	0.0130	5.8334	<0.0001	20.50	0.84	358,168	2,216,374	852
	$\hat{b}$	2.5787	0.0389	66.3466	<0.0001					
'Ballottino'	$\hat{a}$	0.1619	0.0186	8.6926	<0.0001	10.44	0.90	126,820	1,236,377	1,163
	$\hat{b}$	2.3804	0.0279	85.2810	<0.0001					
'A4A'	$\hat{a}$	0.1875	0.0446	4.2019	<0.0001	16.46	0.89	70,960	615,059	262
	$\hat{b}$	2.3869	0.0556	42.9665	<0.0001					
'Dorskamp'	$\hat{a}$	0.3048	0.0752	4.0558	0.0001	17.21	0.85	86,195	562,713	291
	$\hat{b}$	2.2766	0.0588	38.7374	<0.0001					
'MC'	$\hat{a}$	0.1915	0.0351	5.4527	<0.0001	11.13	0.88	70,847	563,988	572
	$\hat{b}$	2.3583	0.0447	52.7246	<0.0001					
'Orion'	$\hat{a}$	0.1114	0.0464	2.4013	0.0181	19.12	0.89	38,003	325,625	104
	$\hat{b}$	2.5631	0.0968	26.4910	<0.0001					
'Unal'	$\hat{a}$	0.1166	0.0176	6.6204	<0.0001	9.42	0.95	22,710	434,365	256
	$\hat{b}$	2.5004	0.0355	70.4094	<0.0001					
'Raspalje'	$\hat{a}$	0.1546	0.0319	4.8490	<0.0001	18.20	0.94	63,951	1,026,973	193
	$\hat{b}$	2.4903	0.0463	53.7515	<0.0001					
'AF8'	$\hat{a}$	0.1952	0.0426	4.5795	<0.0001	15.82	0.86	125,672	900,930	502
	$\hat{b}$	2.3317	0.0506	46.0501	<0.0001					
'Monviso'	$\hat{a}$	0.1729	0.0231	7.4882	<0.0001	15.66	0.90	205,267	1,939,761	837
	$\hat{b}$	2.4257	0.0315	76.9794	<0.0001					
'Grimminge'	$\hat{a}$	0.3257	0.0775	4.2022	<0.0001	8.88	0.90	16,012	151,701	203
	$\hat{b}$	2.2301	0.0598	37.3135	<0.0001					
'Baldo'	$\hat{a}$	0.0291	0.0186	1.5652	0.1206	23.86	0.82	59,189	314,277	104
	$\hat{b}$	2.8152	0.1463	19.2396	<0.0001					
'Viriato'	$\hat{a}$	0.0677	0.0194	3.4946	0.0006	12.07	0.90	26,353	249,441	181
	$\hat{b}$	2.6221	0.0685	38.2790	<0.0001					

Total above-ground woody dry biomass (*Ws*, g dm), diameters over bark at 10 cm of dominant shoots per stool (D_{10}, mm) and ε is the residual error.

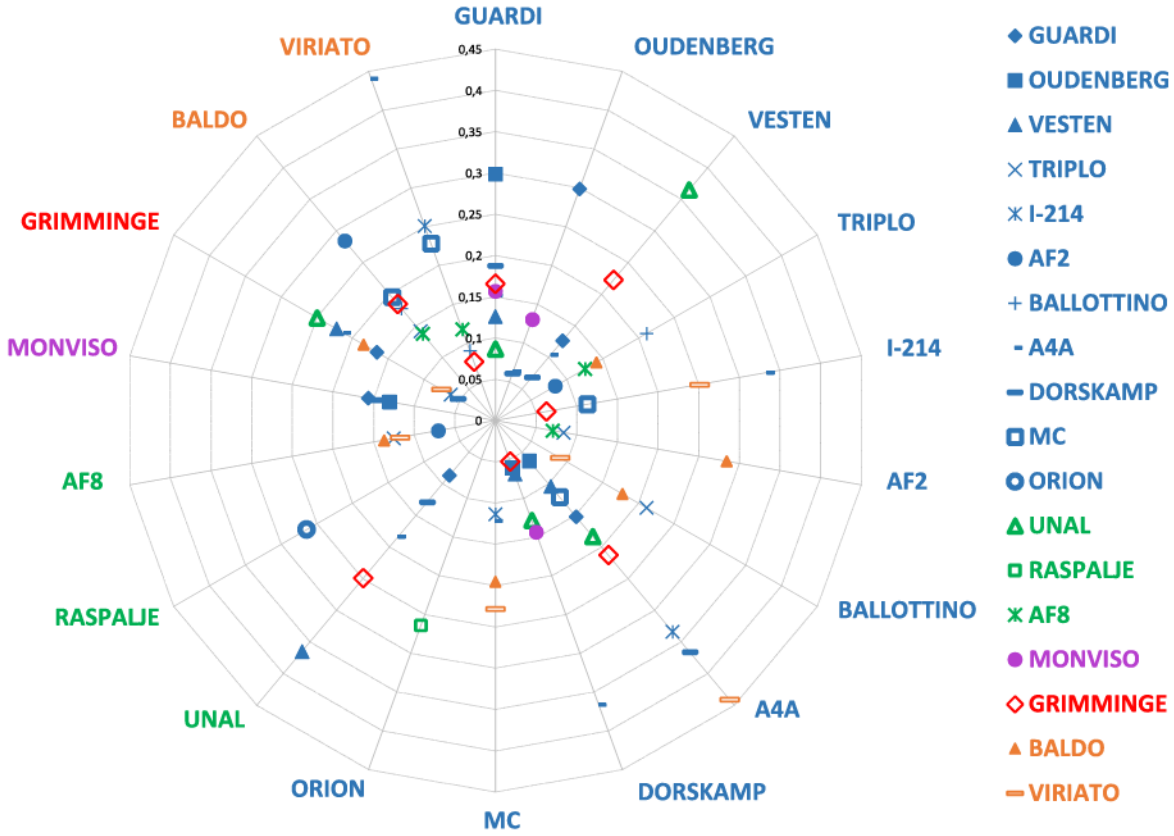

Figure 3.2. Radial chart of the average *p*-values from the four tests applied (Pérez-Cruzado *et al.*, 2015) (showing only the results with a *p*-value exceeding 0.05). The vertices are the genotype-level model and the symbols in each line represent the genotypes for which these models can be applied as a result of considering the average *p*-value.

3.4. Discussion

3.4.1. Allometric above-ground biomass models

The need for an accurate biomass estimation tool for poplar cultivation in SRC is an issue which has been extensively discussed (Ben Brahim *et al.*, 2000a; Laureysens *et al.*, 2005; Paris *et al.*, 2011; Verlinden *et al.*, 2013a). However, due to the specificity of these equations, which are often constructed using data from a very limited area, their applicability in large areas is limited (Ketterings *et al.*, 2001). In addition to the inherent limitations due to the genetic specificity (Wang, 2006), it is important to consider the genotype*environment interaction (G*E), both in the development of the equation and when applying it (Yang *et al.*, 2004).

The general model proposed in this study may provide a useful tool for poplar biomass estimations as the data used for its development cover a wide range of environments and genotypes, ensuring its applicability in Mediterranean conditions. A total of 22 different environments and 29 different genotypes belonging to 7 different taxonomic groups were used to develop the equation, attaining 87% of explained variability using model *d* (3.1) (which only includes basal diameter of the dominant shoot as predictor). Even though the model we propose

explains less variability than that developed by Ben Brahim *et al.* (2000a) (94%), our model was fitted using a larger dataset and a broader range of genotypes-sites and rotation length.

The main grouping criteria considered in biomass production is clearly related to the genetic background of the genotypic plant materials (Verlinden *et al.*, 2013a), and previous studies exist which suggest that a single equation for tree dry weight might be applied at taxonomic group-level irrespective of the environmental conditions (Dillen *et al.*, 2007). Hence, we provide taxonomic group-level models for cases where we have sufficient data (*P.* x *canadensis*, *P.* x *generosa* and *P.deltoides*). The variability explained at taxonomic group-level is within the range found by Dillen *et al.* (2007), from 0.74 to 0.99. Although these equations are expected to be more accurate than the generalized equation, this is not so in all cases. This evidences the fact that taxonomic group affinity is not a suitable clustering factor to explain individual-tree biomass allometry. When a specific equation for a particular genotype is not available or where there are several different genotypes in a given plantation, the use of general equations simplifies the estimation of biomass stocks, despite some loss of precision. The greatest precision is achieved using genotype-level models, so when this information is available, the use of such models is preferable over any of the general models. Although the genotype-level models provided in this study are specific in terms of genotype, data were collected from different environments so G*E are included and can be considered suitable for Mediterranean conditions.

3.4.2. Similarity in biomass allometry among different genotypes

The similarities or differences found with respect to the biomass allometry among genotypes do not appear to bear relation to the taxonomic group, in contrast to what might be expected (Dillen *et al.*, 2007). In fact, the results highlight that genotypes belonging to the same taxonomic group exhibit a wide range of biomass allometry. This may be evidence that the usual procedure of clustering in taxonomically-close groups does not always yield efficient results in biomass modelling under SRC. This is also a common procedure in biomass estimation in highly diverse forests, where attributes at genus, family, or higher taxonomic-levels are averaged when species-specific values are missing (Baker *et al.*, 2004; Swenson and Enquist, 2007; Phillips *et al.*, 2009). It could be expected that the geographical origin of the progenitor species for similar crossing could imply that the biomass prediction models based on allometry can transcendent taxonomic barriers. However, there are no similarities in allometry that allow common models for genotypes in which the geographical origin seems similar (For example: ‘Oudenberg’ and ‘Vesten’ or ‘A4A’ and ‘AF2’).

Given that the individual-tree biomass allometry is driven by the volume allometry and the variability in specific wood density (Nogueira *et al.*, 2008), it is possible that different genotype traits linked to architecture patterns, wood specific gravity and different carbon assimilation rates, among others, are interacting. This makes it difficult to identify which factor is causing the dissimilarities in biomass allometry within genotypes of the same taxonomic group. It would therefore be interesting in future studies to determine which factors underlie the allometric

similarities found between genotypes. This will enable the development of generalized allometric equations which depend on specific variables based on similarities between the genotypes and which would be more accurate than those based on the taxonomic group.

3.5. Conclusions

This study evaluated the similarity in biomass allometry among genotypes belonging to different taxonomic groups. It was observed that genetic origin did not explain similarities in biomass allometry among genotypes. This is a particularly important finding, since taxonomic grouping is a common procedure in biomass modelling both in short rotation woody crops as well as in other forest monitoring exercises.

Biomass models at different hierarchical levels (general, taxonomic group- and genotype-level) are provided for a wide range of site and environmental conditions under Mediterranean climate. These models were developed based on a robust and extensive database covering the geographical variability of SRC under Mediterranean conditions. The genotype-level biomass models cover the main poplar genotypes used in SRC in this area. It was observed that the taxonomic group-levels did not always perform better than the general model, which confirms the dissimilarities in biomass allometry among the genotypes of the same taxonomic-group.

Acknowledgements

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CHAPTER

4

Above -and below- ground carbon accumulation and biomass allocation in poplar short rotation plantations under Mediterranean conditions

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Above- and below-ground carbon accumulation and biomass allocation in poplar short rotation plantations under Mediterranean conditions

Abstract

Beside the production of biomass, short rotation coppice (SRC) poplar plantations can also contribute to carbon sequestration in the soil through their below-ground biomass. The present study evaluated the allocation of above and below-ground biomass at the end of the first rotation of four SRC plantations under Mediterranean conditions. The genotypes evaluated are commonly used for biomass plantations, i.e. genotypes 'AF2' and 'I-214' (*Populus* x *canadensis* Mönch), and 'Monviso' (*P.* x *generosa* Henry x *P. nigra* L.). No significant differences among genotypes were found with regard to below-ground biomass yield. The root:shoot ratio decreased in line with the growth in shoot basal diameter, with values ranging from 0.15-0.26. The accumulation of carbon in the below-ground fraction of the biomass ranged from 0.86 to 0.91 Mg C ha^{-1} yr^{-1}, whereas the above-ground carbon accumulation ranged from 3.89 to 6.48 Mg C ha^{-1} yr^{-1}. A general as well as a genotype-specific allometric model allowed to accurately predict the below-ground biomass yield using shoot basal diameter as the predictor variable. Both models provide an important tool to quantify the carbon accumulated in the below-ground fraction of the biomass.

KEYWORDS: Root biomass; Below-ground allometry models; *Populus* spp.; Root:Shoot ratio; Short Rotation Coppice (SRC).

4.1. Introduction

Research concerning poplar plantations under a short rotation regime has recently become prominent with the aim of maximizing the raw material both for bioenergy and more recently for bioproducts. Additionally, these plantations can play an important role in maintaining or sequestering carbon in the soil (Block *et al.*, 2006; Berhongaray and Ceulemans, 2015) as well as in contributing towards the reduction of greenhouse gas emissions thereby helping to mitigate climate change (Agostini *et al.*, 2015). Studies focusing on differences in yield based on the genotypes or on the management have led to much progress in this area, even though variation due to genotype-environment interactions (plasticity) makes it more difficult to attain the anticipated progress. Furthermore, the final tally in terms of carbon sequestration has also been the subject of research since the capacity of short rotation coppice (SRC) to sequester carbon remains unclear (Hillier *et al.*, 2009; Walter *et al.*, 2015). Some studies suggest a neutral carbon balance without emissions (Hansen, 1993; Johnson *et al.*, 2007a), although some recent studies reported that after successive rotations a point is reached at which the carbon balance becomes positive (Arevalo *et al.*, 2011; Verlinden *et al.*, 2013b).

SRC crops are defined as high-density plantations of fast growing trees (such as poplars or willows), managed in rotations of 2 to 8 years (Berhongaray *et al.*, 2017; Ferré and Comolli, 2018). This implies a different stage of development of the shoots (S) which regrow from the stool and the root (R) in successive rotations. Plant roots are an essential component of net primary productivity (NPP), which is the sum of visible growth and litter production of above- and below-ground components (Roy *et al.*, 2001). Poplar root systems can account for around 25-35% of the total plant biomass (Heilman *et al.*, 1994; Pregitzer and Friend, 1996), this percentage being higher (up to 63%) in juvenile trees (King *et al.*, 1999; Yin *et al.*, 2004; Block *et al.*, 2006). In SRC plantations the roots can be of even higher importance given the expected rotations, which will imply that the above-ground and the below-ground parts are of different ages over the duration of the crop. Due to the difficulties in studying the root system, advancing the knowledge in this area is far from simple (Mokany *et al.*, 2006; Jha, 2017). Despite this, some studies have focused on this fraction of the stool in relation to the whole SRC system (Block *et al.*, 2006; Berhongaray *et al.*, 2015). Although studies on poplar root systems are somewhat scarce, there are more that focus on fine roots given the ease of their extraction, as well as their importance in the assimilation of water and nutrients (Dickmann *et al.*, 1996) and their important influence on carbon turnover (Mulia and Dupraz, 2006), despite the fact that fine roots only represent a small fraction of total tree biomass (Al Afas *et al.*, 2008a; Berhongaray *et al.*, 2013b). There has not been much progress in the study of roots under irrigated short rotation plantations in Mediterranean areas, neither as regards fine roots nor as regards the radical system as a whole.

Root biomass can be directly measured using excavation techniques (Levillain *et al.*, 2011) although these methods are costly and time consuming (Addo-Danso *et al.*, 2016). A common approach for estimating not only above-ground biomass in highly diverse forests but also below-ground biomass, is to use allometric relationships based on an easily measurable variable

(Clark *et al.*, 2001; Saint-André *et al.*, 2005; Kenzo *et al.*, 2009). The use of allometric equations to predict root biomass is a generalized method (Vogt *et al.*, 1995; Kurz *et al.*, 1996; Cairns *et al.*, 1997; Snowdon *et al.*, 2000), since these relationships provide a simpler and more accurate means for estimating root biomass (Domenicano *et al.*, 2011). However, few allometric relationships are available for below-ground biomass estimations in poplar SRC (Coyle and Coleman, 2005; Fang *et al.*, 2007; Berhongaray *et al.*, 2015), particularly as regards those growing under Mediterranean conditions where irrigation determines root development.

Poplar SRC plantations for the production of biomass provide an additional benefit in the form of carbon sequestration in the soil (Smith, 2004). To quantify the potential of carbon sequestration in the soil in SRC plantations, it is important to take both litterfall and fine root turnover into account (Walter *et al.*, 2015), as well as the carbon accumulated in the below-ground woody biomass which remains in the soil during the successive coppices (Pacaldo *et al.*, 2014). Hence, the accuracy of the allometric equations used to quantify the below ground biomass is of major importance.

The present study evaluated the allocation of the above and below-ground fraction in different short rotation poplar plantations under Mediterranean conditions at the end of the first rotation. Our main objective was to contribute towards furthering our understanding of the root system, providing accurate knowledge of the root system allocation in the first rotation of this type of plantations under Mediterranean conditions, which necessitate irrigation due to the severe summer drought. Therefore four plantations in Spain comprising three commonly used genotypes and with a range of different ages were evaluated. The specific objectives were: (i) to quantify the above and below-ground biomass, as well as the root:shoot relationship at genotype level, (ii) to develop allometric relationships based on shoot basal diameter or shoot basal area, from which root biomass could be estimated, and finally (iii) to compare the total and disaggregated amount of carbon accumulated in the biomass (above and below-ground) at plantation level, considering the genotype used.

4.2. Material and Methods

4.2.1. Experimental field sites

Four experimental field sites in Spain (E1 to E4) were used in this study, covering different site conditions in the Mediterranean climate (Table 4.1). Three high-yielding genotypes were selected from the trial network. These were: 'AF2' (*Populus* x *canadensis* Mönch), 'I-214' (*P.* x *canadensis* Mönch) and 'Monviso' (*P.* x *generosa* Henry x *P. nigra* L.). The plantations were established in early spring using cuttings of 20-30 cm in length. Densities ranged from 5,555 cuttings ha^{-1} to 13,333 cuttings ha^{-1} in a design consisting of single rows 2.5 or 3 m apart. Three to four randomized replications were established for each genotype within each plantation. A similar management was applied in all plantations as regards fertilization during soil tillage according to the specific soil characteristics, weed control and irrigation (to field capacity)

during the summer months (Sixto *et al.*, 2013b). A drip irrigation system was established to optimize water application.

Table 4.1. Main characteristics of the four experimental sites (E1 to E4) in Spain including the Köppen climate classification.

Sites	Coordinates		Köppen Climate classification	Altitude (m asl)	*Tm* (°C)	*pH*	*OM* (%)	Stage of develoment	Desing (m)	Density (cuttings ha^{-1})	Replicate
	Latitude	Longitude									
E1	42º 9´ 6.24´´N	5º 33´ 42.37´´W	Temperate Mediterranean climate	254	11.8	6.84	0.62	R4S4	3 x 0.5	6,666	3
E2	42º 37´ 25´´N	6º 38´ 54´´W	Temperate Mediterranean climate	556	12.7	6.21	0.85	R3S3	2.5 x 0.3	13,333	4
E3	39º 14´ 42.75´´N	4º 13´ 21.88´´W	Cold semi-arid climate	670	14.7	6.29	1.25	R4S4	3 x 0.5	6,666	3
E4	39º 15´ 24.74´´N	4º 13´ 36.87´´W	Warm Mediterranean climate	670	14.6	6.08	1.11	R3S3	3 x 0.6	5,555	3

Tm: average annual mean temperature (°C); *OM*: soil organic matter (%); Stage of development: R4S4 indicates a root age of four years with four year old shoots, a.s.o. The number of replicates at each of the sites is also indicated.

Different rotation lengths were applied depending on the developmental status. The rotation length established at sites E2 and E4 was three years, while at sites E1 and E3 it was four years. Hence, the stages of development covered in this study at the end of the first rotation were: R3S3 or R4S4, denoting root age as R and shoot age as S. None of the stands were coppiced at the studied time; so the age in the above- and below-ground fraction was the same.

4.2.2. Above- and below-ground biomass

The sampling unit was considered the stool, which is the plant consisting of a stump, base or root from which one or several shoots are produced. Measurements of the above-ground fraction were taken at the end of each rotation on 16-25 stools per site, replicate and genotype. Shoot diameters over bark at 10 cm above ground (d_{10}, mm), accounting for the size of the shoots at the usual harvesting height, were measured using a digital calliper (Absolute, Vogel Germany, Kevelaer, Germany) (accuracy to 1 mm). The basal area (BA_{10}, mm^2) of the entire stool was calculated from basal diameters of all shoots. Total height of the highest shoot (H, cm) was also measured with a measuring pole to an accuracy of 1 cm. The number of shoots and the density of living stools were also recorded to quantify mortality. The above-ground woody biomass was assessed in all stools through destructive sampling after leaf fall. We considered above-ground woody biomass as the whole biomass above the harvesting height of these stools, which were cut back at 10 cm above the soil. Total above-ground dry woody biomass ($W_{above\text{-}ground}$, g dm (dry matter)) was determined for each sampling unit from the fresh weight and the estimated above-ground wood moisture by randomly selecting one entire stool from each of the plots and then oven-drying a randomly selected sample of each genotype to constant weight at 105 ºC.

Five stools for each site, replicate and genotype were selected for below-ground biomass measurements. They were selected by covering the different diameter classes observed for each replicate. The root systems were extracted using a backhoe with manual assistance, exploring around 1 m^2 of soil for each stool (the dimensions were adapted to the different planting design in proportion to the density). All roots that we were able to retrieve within this area (up to a diameter of 0.10 mm) were manually collected, those roots belonging to the adjacent stools were excluded, although in some cases identification was not possible. The diameter of each broken root tip in each sample was measured to estimate the remaining biomass in the soil. We considered below-ground biomass as the whole biomass below the harvesting height of these stools, so it included the stump below the harvesting height, as well as the coarse roots and fine roots. Total dry biomass of the roots ($W_{below\text{-}ground}$, g dm) was determined for each sample from the fresh weight and the estimated below-ground wood moisture that was obtained using the same procedure as for the above-ground wood moisture.

The root:shoot ratio (defined as the below-ground biomass divided by the above-ground biomass) was calculated using the mean of the individual ratio of all samples (five stools per genotype and site). Below-ground (root) was considered as all biomass below the harvesting height and above-ground as all woody biomass above the harvesting height, i.e. all shoots produced by each stool.

$$root{:}\,shoot\ ratio = \frac{W_{below-ground}}{W_{above-ground}} \qquad (4.1)$$

Dried above- and below-ground biomass was separated for subsequent carbon analyses through dry combustion using a CNS-2000 (TruSpec® CHNS, LECO, St Joseph, Michigan, USA). Above- and below-ground biomass values were converted to carbon accumulations using the average above- and below-ground carbon accumulations fractions, respectively.

4.2.3. Data analysis

4.2.3.1. Exploratory analysis of the data

The influence of the genotype, site and their interaction (G*E) was examined using a mixed model of analysis of variance (ANOVA) for each of the studied variables (above-ground biomass, below-ground biomass and root:shoot ratio). The λ value of the Box-Cox procedure was used to transform the non-normal data. The values shown in the test, as well as in the tables and figures correspond to the non-transformed data.

$$X_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + C_{k(j)} + \varepsilon_{ijkl} \qquad (4.2)$$

where X_{ijkl} is the dependent variable, μ is the intercept of the model or overall mean, α_i is the genotype effect, β_j is the site effect, $\alpha\beta_{ij}$ is the interaction between genotype and site, $C_{k(j)}$ is the random effect of the replicate within the site and ε_{ijkl} is the residual error.

Each mixed model was developed with two crossed fixed factors (genotype and environment), their interaction (G*E) and a third random factor nested in one of the fixed factors (in this case the replication nested in the environment).

To get a better understanding of below-ground biomass distribution we explored the relationship between the below- and above-ground biomass using the root:shoot ratio. The similarities in allometric relationships among genotypes were evaluated by cross-predicting with the proposed root:shoot ratio theoretical trend and comparing this trend at the different genotype levels ('AF2', 'I-214' and 'Monviso') with the observed values. Two levels were assumed to share the same allometry when the model fitted for a given level produced non-deviated predictions for the other. The α-trimmed non-parametric validation tests were used to evaluate the similarity in biomass allometry among genotypes (Pérez-Cruzado *et al.*, 2015). The null hypothesis for the test was that both samples belonged to the same population, so small p-values led us to reject the null hypothesis that both samples shared the same model. We simulated the theoretical root:shoot ratio trend using models to estimate the above and the below-ground biomass and calculating the root:shoot ratio derived from these estimations. For the below-ground biomass estimation, we used the specific genotype-models fitted in the current study, whereas for the above-ground biomass estimation we used the *ad hoc* specific genotype-models previously described (Oliveira *et al.* 2017), that were fitted for Mediterranean conditions. Both models are specific to short rotation, high density plantations, so the estimations of the below- and above-ground biomass ranged from 0 to 200 mm basal diameter.

4.2.3.2. Allometric relationships for below-ground biomass

The total below-ground biomass for each selected stool was carefully measured, but losses in the root extraction procedure could not be completely avoided had to be corrected, e.g. broken root tips. To estimate the remaining root biomass ($W_{under\text{-}estimated\ below\text{-}ground}$, g dm) beyond the broken root tip, we used the data obtained for a single root from one of each five stools selected per replicate, genotype and site. For each single root, segments of 10 cm were cut, and their diameter ($d_{broken\ tip}$, mm) and dry biomass ($W_{under\text{-}estimated\ below\text{-}ground}$) were measured. Based on this information, an allometric relationship was established between the broken tip diameter and the remaining root biomass up to the full length of the root for each genotype (Table 4.2). A non-linear allometric relationship was fitted by ordinary least squares using the *statistical programme R (R* Development Core Team, 2017).

$$W_{under-estimated\ belowground} = a * d_{broken\ tip}{}^{b} + \mathcal{E} \qquad (4.3)$$

where $W_{under\text{-}estimated\ below\text{-}ground}$ (g) is the remaining below-ground biomass beyond the broken root tip, $d_{broken\ tip}$ (mm) is the diameter of the broken root tip and, a and b are regression parameters to be estimated and $\mathcal{E}$ is the residual error.

Total below-ground biomass was determined from the sum of the root biomass extracted and the remaining root biomass estimated with the broken tip diameter. We fitted different

allometric relationships between below-ground biomass and the dominant basal diameter as well as between below-ground biomass and basal area including the basal diameter of all shoots from a stool.

$$W_{below-ground} = a * D_{10}{}^{b} + \mathcal{E} \qquad (4.4)$$

$$W_{below-ground} = a * BA_{10}{}^{b} + \mathcal{E} \qquad (4.5)$$

where $W_{below-ground}$ (g) is the below-ground biomass, D_{10} (mm) is the dominant basal diameter per stool, BA_{10} (mm^2) is the basal area obtained for the total basal diameters of all the shoots per stool and a and b are regression parameters to be estimated and $\mathcal{E}$ is the residual error.

Table 4.2. Parameter estimates and goodness-of-fit statistics for the remaining root biomass models with the equation $W_{under-estimated\ belowground} = a * d_{broken\ tip}{}^{b} + \epsilon$ for three genotypes during the first rotation.

Genotype		Estimate	Std. Error	*t* value	*p*-value (>|t|)	*RMSE*	*pse-R²*	*DF*
'AF2'	$\hat{a}$	0.009	0.002	4.300	<0.001	8.491	0.940	194
	$\hat{b}$	2.476	0.056	43.88	<0.001			
'I-214'	$\hat{a}$	0.295	0.047	6.312	<0.001	5.414	0.831	348
	$\hat{b}$	1.549	0.049	31.43	<0.001			
'Monviso'	$\hat{a}$	0.198	0.046	4.304	<0.001	8.231	0.832	256
	$\hat{b}$	1.636	0.065	25.12	<0.001			

Std.Error: standard error; RMSE: root of mean square error; *pse*-R²: pseudo coefficient of determination; DF: degrees of freedom.

Different hierarchical levels were considered: (i) general level using all information (196 data points) and (ii) a model at genotype level.

To evaluate the goodness-of-fit of the models, we used the proportion of variance explained by the models (pseudo coefficient of determination, *pse-R²*) and the root of mean square error (*RMSE*).

$$pse\text{-}R^2 = r^2_{y_i\hat{y}_i} \quad (6)$$

$$RMSE\ (g) = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-p}} \quad (7)$$

where $r^2_{y_i\hat{y}_i}$ is the correlation coefficient for a linear regression between the observed and the predicted values of the dependent variable (Ryan, 1997), y_i is the observed value, $\hat{y}_i$ is the estimated value, n is the number of data used in the fitting and p is the number of parameters to be estimated.

The similarities in allometric relationships among genotypes were evaluated by cross-predicting with the proposed models and comparing the predictions at the different levels – i.e. at the general level using all information (196 data points) and at the genotype level ('AF2', 'I-214' and 'Monviso') – with the observed values. The α-trimmed non-parametric validation tests

were used for evaluating the similarity in biomass allometry among genotypes (Pérez-Cruzado *et al.*, 2015).

4.2.3.3. Stand extrapolation and carbon accumulation

The below-ground biomass of each stool in the plantation was estimated using the best models for each genotype provided in this study. From the above-ground biomass data collected at the end of the rotation (16-25 stools per site, replicate and genotype) and the estimated below-ground biomass data calculated with the models, the carbon accumulation in each above- and below-ground fraction was calculated using the carbon concentration percentages that were obtained by dried combustion, analysing samples of each genotype (Table 4.3).

Table 4.3. Carbon (C) concentration (in %) of the above- and the below-ground woody biomass for the three genotypes. Values in brackets correspond to the standard deviation.

	ABOVE-GROUND CARBON (%)	BELOW-GROUND CARBON (%)
'AF2'	46.53 (±0.18)	45.95 (±0.17)
'I-214'	46.75 (±0.15)	46.30 (±0.05)
'Monviso'	49.92 (±0.24)	46.36 (±0.12)

4.3. Results

4.3.1. Exploratory analysis of the data

In the case of the above-ground biomass there were significant differences among the genotypes (p-value=0.040), sites (p-value=0.001), and the interactions between them (p-value<0.045). The interactions among genotypes and environments are shown in Table 4.4. In every site, 'AF2' was the most productive genotype with a $W_{above\text{-}ground}$ of 2,441 g stool^{-1} yr^{-1}, and 'I-214' was the least productive with 1,683 g stool^{-1} yr^{-1}, with the exception of E3, where 'I-214' exhibited a special behaviour and was the most productive genotype (Table 4.4). The differences among sites were even more evident (Fig. 4.1) and the $W_{above\text{-}ground}$ ranged between 610.9 and 3,123 g stool^{-1} yr^{-1}, being the E3 the most productive and E2 the least productive in all cases.

The below-ground biomass showed neither differences among the genotypes (p-value=0.818) nor G*E interactions (p-value=0.766), but there were significant differences among the sites (p-value<0.009) (Fig. 4.1). The below-ground biomass ranged between 330.6 and 360.3 g stool^{-1} yr^{-1} for genotypes 'Monviso' and 'AF2', respectively, and between 161.6 g stool^{-1} yr^{-1} and 514.1 g stool^{-1} yr^{-1} for the different sites (E2 and E3, respectively).

There were significant differences in the root:shoot ratio among the sites (p-value=0.001), genotypes (p-value<0.001) and the G*E interactions (p-value<0.001) (Fig. 4.2). The root-shoot ratio of 'I-214' (i.e. a value of 0.29) was significantly different from the two other genotypes, which showed ratios of 0.21 and 0.18 for 'Monviso' and 'AF2', respectively. Analysing the G*E interaction, it is observed that the root:shoot ratio of 'I-214' (i.e. a value of 0.29) is higher than the two other genotypes (averages of 0.21 and 0.18 for 'Monviso' and 'AF2',

respectively), with the exception of the site E3. In this location, 'I-214' showed a low root:shoot ration due to the notable imbalance between the above- and below-ground yield. Even so, this genotype also stands out as regards the below-ground fraction in site E3 (Table 4.4).

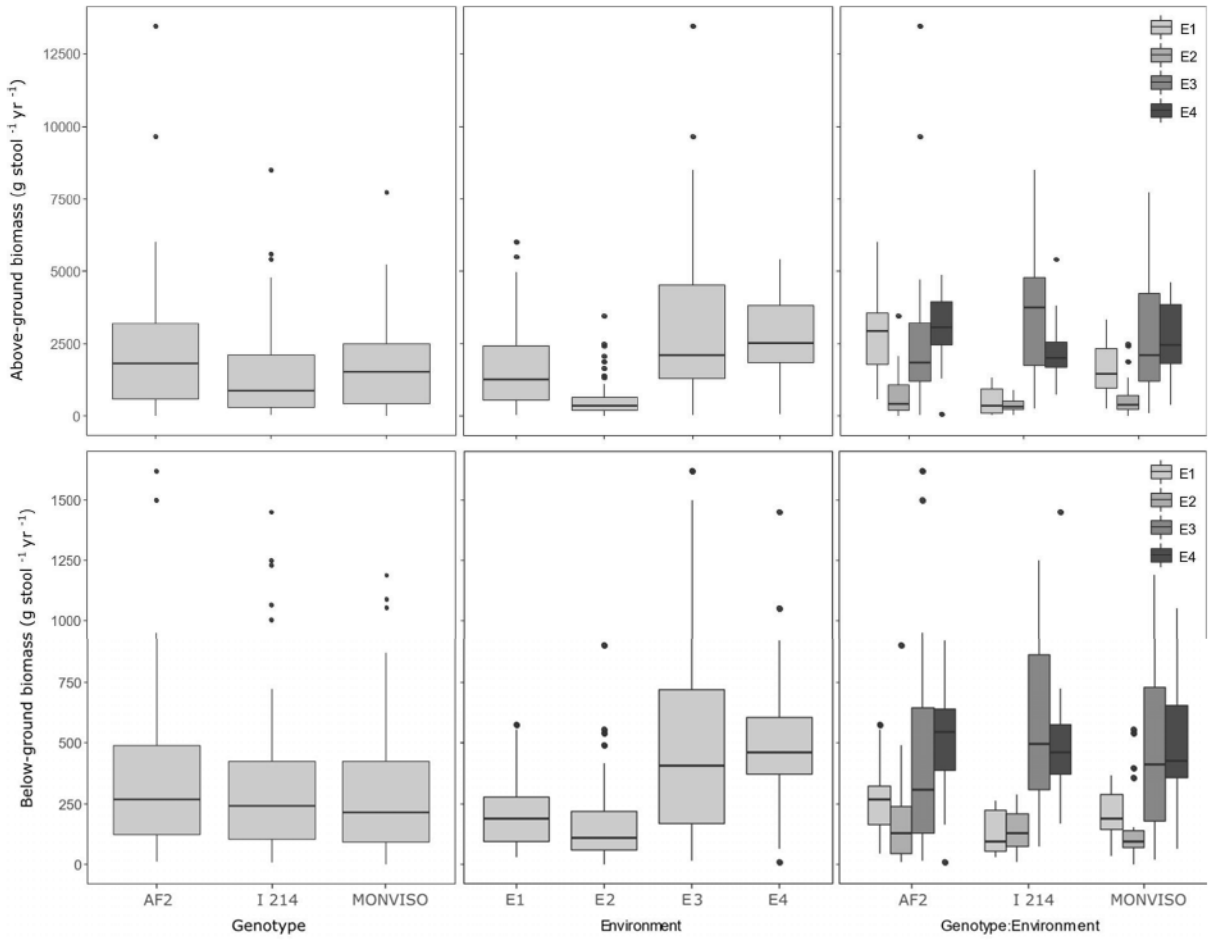

Figure 4.1. Above-ground (top) and below-ground (bottom) biomass per stool and per year at end of the first rotation (three genotypes, four sites and G*E interactions). The values have been represented by boxplots.

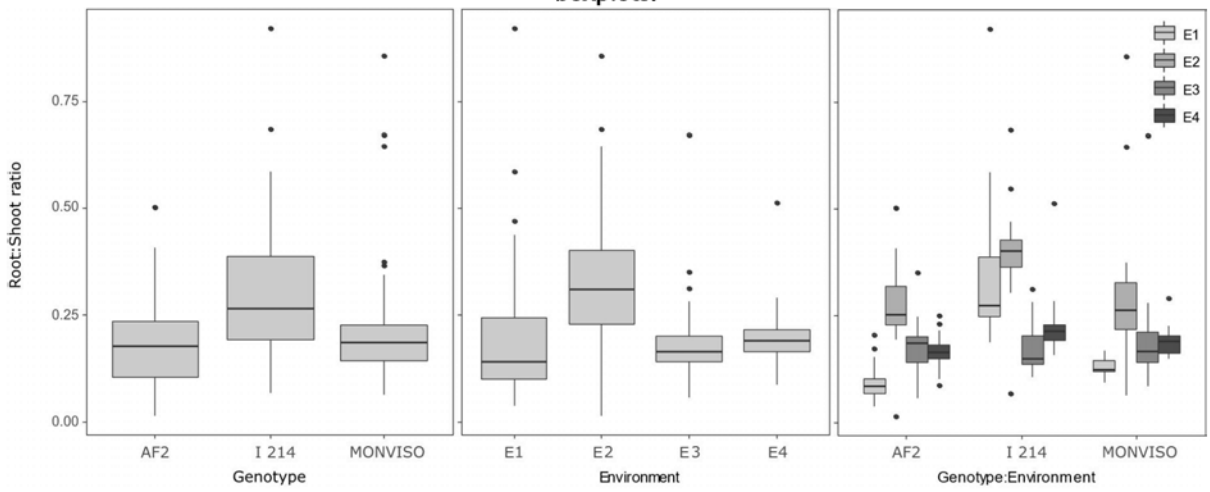

Figure 4.2. Root:shoot ratio (calculated per stool and year) at the end of the first rotation (three genotypes, four sites and G*E interactions).

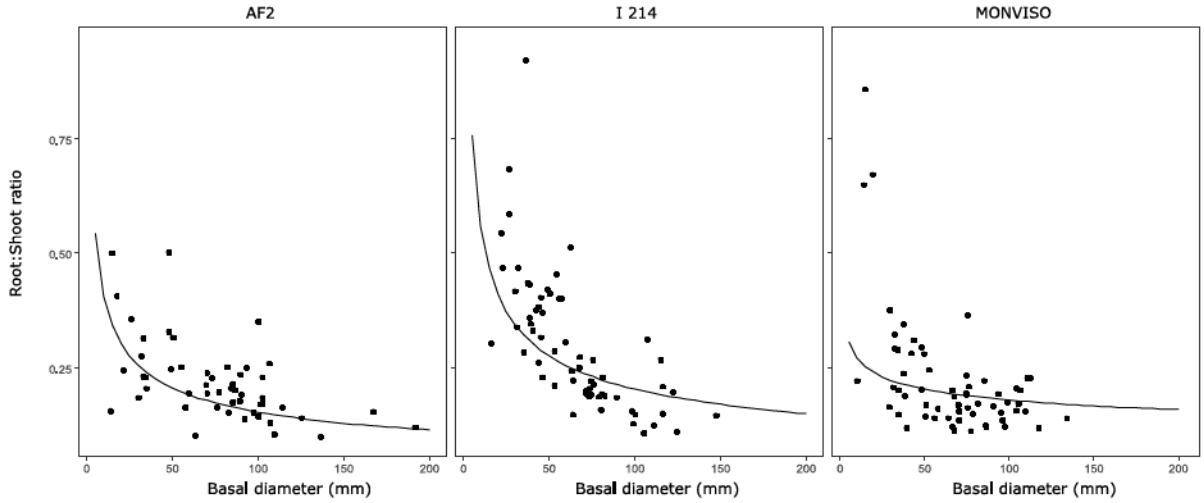

Figure 4.3. Root:shoot ratio estimated (lines) and real values (points) for genotypes 'AF2', 'I-214' and 'Monviso' respectively.

The relation between the root:shoot ratio and D_{10} for each genotype is shown in Fig. 4.3. The root:shoot ratio was simulated using the specific genotype-models fitted in this study (Table 4.4) for the below-ground biomass and the specific genotype-models for the above-ground biomass explained in Oliveira *et al.* (2017). The lines represent the trends of the ratios and the symbols represent the real values calculated in this study. We found significant differences among genotypes using the α-trimmed non-parametric validation tests (Pérez-Cruzado *et al.*, 2015). As can be seen from Fig. 4.3 the trends obtained from the models explained how the different genotypes behaved. The three genotypes included in this study showed a greater decline in the root:shoot ratio at smaller diameters, achieving a constant value as the diameter increases. Whereas 'AF2' and 'I-214' showed higher root:shoot ratios for small diameters, 'Monviso' showed similar root:shoot ratios regardless of the diameter.

Table 4.4. Genotype and total values (i.e. means and standard deviation) for the above- and below-ground biomass and the root:shoot ratio at the four experimental sites.

	SITE	'AF2'	'I-214'	'MONVISO'	TOTAL
Above-ground biomass (g yr^{-1})	**E1**	2,894.3232 (1645.88)	534.1882 (454.66)	1,650.0670 (966.90)	1,692.8595 (1,180.65)
	E2	779.3989 (862.18)	367.2629 (232.49)	685.9394 (763.67)	610.8671 (216.08)
	E3	3,099.1010 (3,725.13)	3,553.2708 (2,191.53)	2,716.6280 (2,173.30)	3,122.9999 (418.83)
	E4	2,992.0906 (1,283.14)	2,276.8529 (1,160.19)	2,593.5782 (1,440.59)	2,620.8406 (358.40)
	TOTAL	2,441.2284 (1,111.04)	1,682.8937 (1,516.74)	1,911.5532 (945.83)	2,011.8918 (1,148.08)
Below-ground biomass (g yr^{-1})	**E1**	264.1482 (153.71)	133.2521 (86.53)	208.9721 (107.22)	202.1241 (65.72)
	E2	186.4223 (217.88)	140.7222 (89.21)	157.6066 (165.90)	161.5837 (23.11)
	E3	487.9445 (506.62)	583.9806 (390.73)	470.2596 (367.14)	514.0616 (61.19)
	E4	502.6232 (247.55)	511.1325 (294.09)	485.3672 (290.92)	499.7076 (13.13)
	TOTAL	360.2846 (159.19)	342.2719 (238.92)	330.5514 (171.44)	344.3693 (175.10)
Root:shoot ratio	**E1**	0.0978 (0.05)	0.3524 (0.19)	0.1334 (0.02)	0.1946 (0.14)
	E2	0.2783 (0.11)	0.3980 (0.12)	0.3055 (0.17)	0.3273 (0.06)
	E3	0.1798 (0.07)	0.1756 (0.06)	0.2042 (0.14)	0.1865 (0.02)
	E4	0.1676 (0.04)	0.2334 (0.08)	0.1912 (0.04)	0.1974 (0.03)
	TOTAL	0.1809 (0.07)	0.2899 (0.10)	0.2086 (0.07)	0.2264 (0.09)

4.3.2. Allometric relationships for below-ground biomass

From the similarities in the allometric parameters between both below-ground biomass estimation models (general model and model at genotype level), it became clear that a specific model should be used for each genotype, at least for the first rotation that was the rotation analysed in this study (data not shown). The environmental effect included in the model derived from the four study sites made the below-ground biomass allometry different for each genotype. Whereas there were no significant differences in below-ground biomass among the genotypes for the four sites nor G*E interaction, differences were observed among genotypes in some particular sites.

Table 4.5. Parameter estimates and goodness-of-fit statistics for the root biomass models with the equation $W_{belowground} = a * D_{10}{}^{b} + \mathcal{E}$ (first rotation).

Genotype		Estimate	Std. Error	*t* value	*p*-value (>|t|)	*RMSE*	*pse*-R^2	*DF*
'AF2'	$\hat{a}$	0.080	0.048	1.669	0.010	519.1	0.825	63
	$\hat{b}$	2.156	0.123	17.53	<0.001			
'I-214'	$\hat{a}$	0.141	0.094	1.505	0.137	476.3	0.838	64
	$\hat{b}$	2.107	0.142	14.81	<0.001			
'Monviso'	$\hat{a}$	0.071	0.060	1.183	0.241	450.9	0.812	63
	$\hat{b}$	2.246	0.185	12.24	<0.001			
General	$\hat{a}$	0.217	0.082	2.658	0.009	529.4	0.787	194
	$\hat{b}$	1.981	0.080	24.90	<0.001			

where $W_{below\text{-}ground}$ (g, dm) is total below-ground dry biomass, D_{10} (mm) is diameter over bark at 10 cm for dominant shoots per stool and $\mathcal{E}$ is the residual error. Std.Error: standard error; RMSE: root of mean square error; *pse*-R^2: pseudo coefficient of determination; DF: degrees of freedom.

The below-ground biomass estimation models with shoot diameter as the predictor variable were as good as those which used the basal area, at least for this first rotation. Therefore, given their simplicity, we selected the below-ground biomass estimation models with shoot diameter as the predictor variable for this study (Table 4.5). The genotype specific below-ground biomass estimation models accounted for more than 80% of the total variability in root biomass. All considered equations converged and the $\hat{b}$ parameter estimates significantly improved the quality of the model fit to the data at *p*-value = 0.05. The general below-ground biomass estimation model explained 78% of the total variability in root biomass. It included all the variability of the different genotypes and site conditions typical of the Mediterranean climate.

4.3.3. Above- and below-ground carbon accumulation

The carbon accumulated per year in the above-ground fraction of the biomass was four to seven times larger than the carbon accumulated in the below-ground fraction. The highest amount of carbon in the first rotation was stored in the above-ground woody biomass, although between 12.38 and 17.31 % of the total carbon pool is stored in the below-ground fraction, and will remain there regardless of the fate of the above-ground biomass.

The range of genotypic variation was smaller in the below-ground fraction than in the above-ground fraction. In both fractions (above- and below-ground) genotype 'AF2' showed the highest amount of carbon accumulation (6.44 and 0.91 Mg C ha^{-1} yr^{-1}, respectively). In contrast carbon accumulation values of 'I-214' (4.28 Mg C ha^{-1} yr^{-1}) were the lowest in the above-ground fraction, while carbon accumulation values of 'Monviso' (0.89 Mg C ha^{-1} yr^{-1}) were the lowest in the below-ground fraction (Table 4.6).

Table 4.6. Above- and below-ground biomass and accumulated amount of carbon per year in the three genotypes. Values in brackets correspond to the standard deviation.

GENOTYPE	ABOVE-GROUND BIOMASS (Mg ha^{-1} yr^{-1})	BELOW-GROUND BIOMASS (Mg ha^{-1} yr^{-1})	ABOVE-GROUND CARBON ACCUMULATION (Mg C ha^{-1} yr^{-1})	BELOW-GROUND CARBON ACCUMULATION (Mg C ha^{-1} yr^{-1})
'AF2'	13.83 (±5.10)	1.98 (±0.38)	6.44(±2.37)	0.91 (±0.18)
'I-214'	9.15 (±6.15)	1.93 (±0.85)	4.28 (±2.87)	0.90 (±0.39)
'Monviso'	11.43 (±1.76)	1.92 (±0.33)	5.36 (±0.83)	0.89 (±0.15)

4.4. Discussion

4.4.1. Exploratory analysis of the data

Genetic and environmental effects on the above-ground biomass of SRC have been previously documented in Europe (Dillen *et al.*, 2007; Benetka *et al.*, 2014), including under Mediterranean conditions (Bergante *et al.*, 2010; Sixto *et al.*, 2015). Nevertheless, the results of the present study show that the differences in the above-ground biomass due to the genotype are not always reflected in the below-ground part. The factors linked to the site had a larger influence. It seems that at least under the conditions described, differences among genotypes as well as the G*E interaction in the below-ground fraction are not evident. This finding contradicts some studies that reported a strong genetic control of allometric biomass partitioning to roots (King *et al.*, 1999; Al Afas *et al.*, 2008b), although other studies reported results similar to the present ones (Dickmann *et al.*, 1996; Verlinden *et al.*, 2013b). As regards fine roots a study on poplar SRC without irrigation, reported that in spite of the genotypic differences in above-ground biomass, annual fine root productivity was similar (Verlinden *et al.*, 2013b).

The range of above-ground yield (1,683-2,441 g $stool^{-1}$ yr^{-1}) in our plantations at the end of the first rotation was larger than that reported for other poplar SRC plantations (e.g. 380-870 g $stool^{-1}$ yr^{-1} in Belgium (Berhongaray *et al.* 2015) or 118-438 g $stool^{-1}$ yr^{-1} under conditions with plenty of water and nitrogen at the end of the first growing season in the USA (Pregitzer *et al.* 1990)). However, the below-ground biomass values found in this study (331-360 g $stool^{-1}$ yr^{-1}) were very similar to those previously reported (e.g. Berhongaray *et al.* (2015): 270-400 g $stool^{-1}$ yr^{-1}; or Pregitzer *et al.* (1990): 132-375 g $stool^{-1}$ yr^{-1}). This may be highly affected by the fact that irrigation is applied, as well as by the climatic conditions of the area. As poplar is

an opportunistic rooter (Hallgren, 1989), the availability of water through irrigation means that it does not need to explore a large ground area or to develop an extensive root system. This might, however, also be explained by the limited space for development due to the high planting density. Furthermore, the guaranteed water availability, together with the high average temperature and irradiance in the Mediterranean region (Faria *et al.*, 1998), has led to a high above-ground development.

As regards the root:shoot ratio values, we found genotypic differences similar to those reported in previous studies with different poplar genotypes (King *et al.*, 1999; Yin *et al.*, 2005). The root:shoot ratios in our study (0.18-0.29) were lower than those obtained in two other studies, both with younger plantations (2 years) (0.27-0.33 or 0.45-0.70) (Friend *et al.*, 1991; Berhongaray *et al.*, 2015). However, our root:shoot ratios are consistent with those (0.16-0.23) reported for another irrigated Mediterranean plantation (Calfapietra *et al.*, 2003). These findings emphasize the difference in above- and below-ground development under irrigation, with higher growth of the above-ground fraction in relation to other plantations although with similar growth of the below-ground fraction.

The root:shoot ratio reflects – in physiological terms – the differential investment of photosynthates between the above- and below-ground fraction of the tree (Titlyanova *et al.*, 1999). The ratio may, therefore, reflect the cumulative response to biotic, abiotic and management influences, although it only represents the net effects of carbon allocation, without considering carbon losses (Mokany *et al.*, 2006).

Our results showed an exponential decrease in the root:shoot ratio values as basal diameter increased, which agrees with the findings of other authors who reported that this ratio declined significantly with stand age (Coleman *et al.*, 2004; Coyle and Coleman, 2005; Berhongaray *et al.*, 2015). The genetic background of the two groups of genotypes ('AF2' and 'I-214', versus 'Monviso') is different, but there were insufficient genotypes to support this hypothesis. The differences in the root:shoot ratio value reported by other authors (e.g. Berhongaray *et al.*, 2015) are probably explained by the differences in age (3 or 4 years old *versus* 2 years old), although the higher above-ground biomass in our plantations was also due to the better growing conditions.

When developing an eco-physiological based model (as e.g. 3PG) the root:shoot ratio can be very useful for estimating the root biomass allocation (Landsberg and Waring, 1997). The maximum fraction of NPP allocated to roots has been considered as 0.7, and the minimum as 0.17 (Headlee *et al.*, 2013). The latter authors argued that different genotypes may have different parameter values, particularly in the case of maximum allocation. In this sense, the relationship shown by this root:shoot ratio under Mediterranean conditions could provide a useful estimation parameter for the root biomass allocation.

4.4.2. Allometric relationships for below-ground biomass

Given the differences among sites but not among genotypes nor G*E interaction, it would seem rational to fit site-specific below-ground biomass models with no differentiation at genotype level. However, site- specific models have been ruled out, at least in this study, due to their limited applicability (Oliveira *et al.*, 2017). When fitting general models it is important to consider that the inclusion of all sites would result in model errors due to the significant differences associated with the site. Therefore, both a general model and genotype-specific models were developed in this study, despite the absence of significant differences among genotypes. Other authors, however, have opted for a single, general model which included all genotypes (Verlinden *et al.*, 2013b), given that no differences were found among them. In our case, the models used data from the four study sites and therefore this variability has been included in the model.

The availability of modelling tools to estimate the below-ground fraction is important when quantifying the distribution and the amount of carbon. Models for predicting below-ground biomass in poplar trees have been developed also using shoot diameter or basal area as independent variables (Heilman *et al.*, 1994; Domenicano *et al.*, 2011; Berhongaray *et al.*, 2015). However, none of them has been developed specifically for short rotation, high density poplar plantations under Mediterranean conditions. Furthermore, both methodologies (direct digging or estimations based on models) have their shortcomings due to root breakage and incomplete sampling associated with excavations of root systems (Clark *et al.*, 2001).

4.4.3. Carbon accumulation

The accumulation of carbon by SRC occurs in the soil, not only in the litterfall and fine root turnover, but also in the below-ground biomass fraction (Pacaldo *et al.*, 2014). Hence it is important to take this fraction into account in the final carbon balance. The carbon concentrations found in our plantations were consistent with previously reported values (Fang *et al.*, 2007), that ranged from 42-50% for all biomass components, with the highest carbon concentrations in shoots.

As a result of the higher biomass yield found in our studies, the values for above-ground carbon accumulation were higher (4.3-6.4 Mg C ha^{-1} yr^{-1}) than the values obtained in conditions of lower productivity (e.g. Verlinden *et al.*, (2013b): 2.5 Mg C ha^{-1} yr^{-1}). Other studies in which biomass yield were more similar to those obtained here reported similar values (e.g. Fang *et al.*, (2007): 3.1-5.75 Mg C ha^{-1} yr^{-1}). However, under Mediterranean conditions with irrigation (Central Italy), the above-ground carbon accumulation were notably higher (6.1-8.6 Mg C ha^{-1} yr^{-1}; Gielen *et al.*, (2005)) than the values obtained in our study. The below-ground carbon accumulations (0.89-0.91 Mg C ha^{-1} yr^{-1}) were very similar to those reported under non-Mediterranean conditions (Verlinden *et al.*, (2013b): 1.01 Mg C ha^{-1} yr^{-1}), although a wider range of total below-ground biomass (0.85-1.5 Mg C ha^{-1} yr^{-1}) was reported for lower density plantations (1,111 trees ha^{-1}; Fang *et al.*, (2007)). Under Mediterranean conditions with

irrigation in Central Italy the below-ground carbon accumulations reported were also similar (0.87-1.21 Mg C ha^{-1} yr^{-1}; Gielen *et al.*, (2005)) to the values obtained in our study.

The below-ground carbon accumulation would surely be larger if not only the carbon accumulated in the below-ground biomass but also in the fine roots turnover had been taken into account. However these accumulations are difficult to account for because of the high turnover rates.

4.5. Conclusions

This study provided models which may be used to estimate the biomass yield in poplar root systems in Mediterranean SRC during the first rotation. The root:shoot ratio provided useful information with regard to the allocation of the biomass in the presence of irrigation, which could be useful for the development of future ecophysiological models. Whereas the above-ground biomass yield was notably higher than that reported in other studies, the below-ground biomass yield was more similar. The accumulation of carbon in the below-ground fraction was therefore similar to other scenarios and was approximately 1 Mg C ha^{-1} yr^{-1}.

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CHAPTER

5

Productivity model and reference diagram for short rotation biomass crops of poplar grown in Mediterranean environments

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Productivity model and reference diagram for short rotation biomass crops of poplar grown in Mediterranean environments

Abstract

A *Reference Diagram* (RD) was constructed for first rotations of the Euroamerican poplar 'I-214' grown as short rotation coppice (SRC). Data from 144 plots, established in eleven sites in Mediterranean environments, were used to develop the model. The density at establishment of the plantations ranged between 6,666 and 33,333 cuttings ha^{-1}, covering the usual densities ranges used in short rotation forestry (SRF). The RD was based on a density-independent mortality model that relates the density of living stools to the average height of dominant shoot and the initial plantation density, and it includes a system of two simultaneously fitted equations relating a) quadratic mean basal diameter of dominant shoots to the average height of dominant shoot and the final density, and b) total above-ground woody dry biomass to quadratic mean basal diameter and final density. The isolines in the RD represented mortality, quadratic mean basal diameter of dominant shoots and total above-ground woody dry biomass at the end of a first rotation of three years. The final yield in terms of biomass ranged from 1 to 85 Mg dry matter ha^{-1}. The RD enables rapid and straightforward comparison of different situations, both at planting and at harvesting, and is a useful tool, based on a wide range of empirical data, for management and decision making regarding short rotation poplar crops.

KEYWORDS: Poplar; Short rotation coppice (SRC); Density Management Diagrams; Reference Diagrams; Biomass.

5.1. Introduction

Woody biomass is a renewable resource that is suitable for getting different bioproducts, including bioenergy for different applications (heat, electricity or second generation biofuels)(Kauter *et al.*, 2003; White, 2010). Use of woody biomass for bioenergy purposes represents a promising opportunity to contribute to the mitigation of climate change, to the extent that the presence of this resource may become important in the overall energy mix. The production and management of woody biomass may provide a new opportunity for economic development of rural areas (European Commission, 2008) involving many different productive sectors.

Forestry crops represent one source of supply of woody biomass, which is characterized by its spatiality and temporality (Sixto *et al.*, 2007). Such crops can be established on both abandoned agricultural land and on marginal land. Short rotation woody crops under coppicing management (SRC), such as *Salicaceae* (*Populus* spp. or *Salix* spp.), are well represented in northern and central Europe and to a lesser extent in southern Europe (Mareschi *et al.*, 2005; Yemshanov and McKenney, 2008; Verwijst *et al.*, 2013). Studies involving the production of these crops have revealed a wide range of biomass yield depending on different factors such as plant material, site characteristics, crop design (density and rotation), management regime, presence of pest and diseases, and survival after planting (Laureysens *et al.*, 2003; Pérez-Cruzado *et al.*, 2013; Benetka *et al.*, 2014).

Plantation density is an important factor to be considered in SRC and has both production and economic implications. As tree density increases, the total yield rises initially in proportion to the number of plants per area if individual plants receive sufficient resources for growth. However, the specific density at which optimal production is attained has not yet been determined, although the range within which production remains constant is known (Willebrand *et al.*, 1993). Several experimental and commercial plantations have been established to test densities ranging from 1,000 to more than 40,000 cuttings ha^{-1} (Klasnja *et al.*, 2003; Fiala *et al.*, 2010; Cañellas *et al.*, 2012).

Density Management Diagrams (DMDs) are used to graphically illustrate the relationships between density, yield and density dependant mortality at all stages of stand development (Newton and Weetman, 1994), including concepts such as competition, site occupancy and self-thinning. These biological models are mainly used for decision-making in density management, for example to determine the post-thinning density or even to display and evaluate alternative density regimes in forest management (Shaw and Long, 2007). There are many examples for different areas and species, such as *Pinus palustris* Mill. (Shaw and Long, 2007), *Quercus robur* L. (Barrio-Anta and Álvarez-González, 2005) and *Pseudotsuga menziesii* (Mirb.) Franco (López-Sánchez and Rodríguez-Soalleiro, 2009). However, as far we know, models relating the initial cutting density to yield, stool mortality or height and basal diameter growth for different developmental stages in SRC are scarce. Such models have been developed, e.g. for *Eucalyptus globulus* and *Eucalyptus nitens*, on the basis of plantations established at the usual range of initial forest densities used in southwestern Europe and managed in short rotations (Pérez-

Cruzado *et al.*, 2011). In SRC, density reduction is not a common management practice and because of the short rotation, these crops rarely reach self-thinning limits, so that the application of DMD will be severely limited. We have therefore applied the basic concepts used in the construction of DMDs to propose a *Reference Diagram* (RD) for SRC management. These models may play an important role in helping understand short rotation yield patterns in poplar plantations as adaptation of the models may assist in the assessment of energy yield potential, and optimum stand management in terms of density and rotations, as reported for eucalypts (Pérez-Cruzado *et al.*, 2011).

The SRC yield of poplar has been considered in many studies by the application of allometric equations that can estimate shoot biomass from basal diameter and shoot height, which must therefore be measured in sample plots (Pontailler *et al.*, 1997; Verwijst and Telenius, 1999; Amichev *et al.*, 2010; Headlee *et al.*, 2013; Mosseler *et al.*, 2014). As basal diameter growth is directly related to stool density, and height growth is more dependent on site properties and management (Mosseler *et al.*, 2014), the development of whole crop yield models can provide estimation tools based on easily measured crop variables, particularly height and living stool density. However, as coppicing causes large changes in shoot density and growth rate, different sets of equations must be developed for the first and subsequent rotations. This is the most important factor limiting the application of process-based models to SRC (Amichev *et al.*, 2010). Whole crop empirical statistical based models, which are powerful tools for estimating biomass, calculating economic gains and helping managers reach decisions about the timing of harvesting, are not yet available. A wide range of empirical data is required to enable development of such models (Dickmann, 2006).

The aim of the present study was to develop a whole crop model based on biomass, mean basal diameter and stool evolution as a function of mortality in a short rotation poplar crop, thus enabling subsequent development of a practical and dynamic adaptation of Density Management Diagram concept, that we call *Reference Diagram*. A further objective was to study the effect of initial density on biomass production at a rotation age of 3 years.

5.2. Materials and methods

5.2.1. Data

The study was carried out using data from a trial network of short rotation *Populus* plots established in 11 sites in Spain under Mediterranean conditions (Table 5.1). The genotype selected for development of the DMD was 'I-214' (*Populus* x *canadensis* Moench), this genotype is a hybrid between *Populus deltoides* Marsh and *Populus nigra* L. This is a well-known genotype widely planted in Mediterranean environments because of its high site plasticity and yield. This justifies use of 'I-214' as the reference genotype in the trial plots for comparison of yield and other growth-related features. Data from 144 plots were available for fitting the base equations, these data were taken from three types of experimental plots in which the aim was to evaluate the genotype effect, stocking rate and site by measuring variables related to production. Genotype 'I-214' was used in all the experiments. As the trial design took

competition effects into account, the plot size is large enough to ensure that the measured samples are not subjected to an edge effect. The trial plots include plantations ranging in surface area from 0.25 ha to 8 ha. Plots were established in early spring between 2005 and 2009, with 3 to 11 replications per site and plant densities. These densities ranged from 6,666 cuttings ha^{-1} to 33,333 cuttings ha^{-1}.

Plantations were established using hardwood cuttings of 20-30 cm in length, planted in single rows 3 m apart, either by hand or by use of adapted planting machines. Similar management was applied, including fertilization during soil tillage according to the specific soil characteristics, pre-emergence treatment with oxyfluorfen (4 l ha^{-1}) to control weeds, and drip or flooding irrigation (to field capacity) during the summer months (Sixto *et al.*, 2013b). A rotation length of three years was established in all plots. In some (32) plots, trees were cut back to a height of 0.1 m one year after planting, to stimulate sprouting (Willebrand and Verwijst, 1993; Kopp *et al.*, 2001). In these plots, root age was 4 and shoot age was 3 years (R4S3) at harvest time (Table 5.2). In the remaining plots (112), root age and shoot age were both 3 years (R3S3). In addition, 16 plots in the database had been already harvested once and were in a stage of development corresponding to R7S3; however, the data from these plots were only used to test the performance of the diagram for a second rotation and not for model development.

Table 5.1. Locations and UTM coordinates of the experimental SRC poplar plots located in Spain.

Sites	Coordinates		Altitude m (a.s.l.)	*Tm*[a] (°C)	*P*[b] (mm)	*pH*	*OM*[c] (%)	Sand (%)	Silt (%)	Clay (%)
	Latitude	Longitude								
E1	41° 28´18.6´´N	2° 32´51´´W	827	11.19	447	8.42	2.08	18.2	34.44	47.35
E2	41° 35´58.3´´N	2° 30´23.7´´W	1,090	10.19	614	6.39	0.57	85.14	5.52	9.33
E3	42° 36´45.7´´N	6° 39´52.6´´W	560	12.80	747	6.01	1.02	53.43	23.22	23.34
E4	42° 37´25´´N	6° 38´54´´W	560	12.69	476	6.21	0.84	55.36	23.88	20.76
E5	42° 10´30.5´´N	1° 40´0.8´´W	263	14.10	405	8.52	1.24	38.3	37.01	24.70
E6	37° 11´43.1´´N	3° 46´3.7´´W	738	15.3	355	8.45	1.42	40.04	35.28	24.77
E7	42° 04´47.2´´N	3° 3´56.3´´E	20	15.69	658	8.61	0.36	74.09	11.53	14.38
E8	41° 41´42.6´´N	2° 40´22.3´´E	52	16.10	677	6.31	0.29	82.59	6.03	11.36
E9	41° 19´29.94´´N	4° 32´4.34´´W	756	12.30	440	9.04	0.89	80.1	11.28	8.62
E10	41° 56´59.47´´N	0° 47´15.98´´W	279	14.50	408	8.26	1.14	54.90	23.64	20.70
E11	41° 50´11´´N	5° 54´36.1´´W	705	12.10	484	5.41	1.51	57.66	21.56	20.77

[a] *Tm*: average annual temperature; [b] *P*: annual accumulated rainfall; [c] *OM*: soil organic matter in the A horizon.

Measurements were made annually after planting. Diameter over bark at 10 cm (d_{10}, mm) and at 130 cm (*dbh*, mm) were measured using a digital calliper, once leaves had fallen. This first diameter (d_{10}) accounts for the size of the shoots at the usual harvesting height. Total height of the highest stool (*H*, cm) was also measured with the aid of a measuring pole. The number of shoots per stool and the density of living stools per plot were also recorded. The above-ground fresh biomass, excluding leaves, was evaluated in all plots by destructive analysis at the end of the third year. Total above-ground woody dry biomass (*Ws,* kg dm ha^{-1}) was calculated from the estimated dry weight of randomly selected whole plants from each of the plots after oven-drying a subsample of 1 kg for each genotype to constant weight at 105ºC.

Table 5.2. Information on the plot characteristics and the average values of the main variables evaluated.

Site	Age	Initial Density	Number of Evaluated Plots	Replicate	Average H[a] (cm)	Average Ws[b] (Mg dm ha^{-1})	Average BA_{10}[c] (m^2 ha^{-1})	Average dg_{10}[d] (cm)
1	R4S3	15,000	3	16	430.27	22.58	22.01	4.32
1	R4S3	25,000	3	16	391.17	22.95	27.42	3.73
1	R4S3	33,333	3	16	338.11	24.30	30.30	3.39
2	R4S3	17,316	3	16	617.96	66.04	48.37	5.95
2	R4S3	20,000	4	16	568.06	50.23	45.96	5.27
3	R3S3	13,333	4	25	524.41	17.50	17.82	4.06
3	R4S3	20,000	4	16	786.92	51.43	49.15	5.58
3	R4S3	20,000	3	16	774.06	51.73	42.15	5.17
3	R4S3	15,000	3	16	823.83	55.17	40.34	5.84
3	R4S3	25,000	3	16	797.27	70.17	57.08	5.38
3	R4S3	33,333	3	16	671.35	56.54	51.30	4.42
4	R3S3	13,333	4	16	481.13	13.96	16.67	3.96
6	R3S3	13,333	4	16	933.67	58.21	46.10	6.63
6	R3S3	13,333	4	25	747.65	32.83	31.98	5.51
6	R3S3	10,000	3	16	949.06	58.22	42.39	7.34
6	R3S3	15,000	3	16	909.79	62.97	49.29	6.45
6	R3S3	20,000	3	16	863.44	68.96	56.19	5.98
7	R3S3	13,333	4	16	501.18	25.27	23.36	4.70
7	R3S3	6,666	3	25	775.47	26.65	30.15	7.58
7	R3S3	13,333	3	25	686.39	27.72	34.62	5.74
7	R3S3	6,666	3	16	431.13	5.93	9.97	4.31
7	R3S3	10,000	3	16	470.45	9.22	16.77	4.54
7	R3S3	15,000	3	16	387.34	7.41	14.80	3.53
7	R3S3	20,000	3	16	423.66	14.79	22.94	3.76
8	R3S3	6,666	3	25	408.41	9.61	11.17	4.58
8	R3S3	13,333	3	25	472.83	16.73	23.37	4.71
9	R3S3	13,333	3	15	287.58	3.56	6.97	2.55
9	R3S3	13,333	4	25	170.76	1.11	3.09	1.68
9	R3S3	10,000	3	17	365.15	8.74	9.87	3.54
9	R3S3	15,000	3	16	322.97	8.77	14.53	3.50
9	R3S3	20,000	3	15	292.61	7.65	13.01	2.87
10	R3S3	13,333	3	16	634.67	27.98	27.21	5.09
10	R3S3	13,333	6	25	656.35	33.28	28.04	5.09
10	R3S3	10,000	3	17	731.86	33.13	28.85	6.05
10	R3S3	15,000	3	16	666.07	33.26	28.54	4.92
10	R3S3	20,000	3	16	737.68	49.70	40.88	5.08
11	R3S3	13,333	4	16	314.58	15.67	17.94	4.00
11	R3S3	6,666	4	25	488.52	16.20	12.33	4.81
11	R3S3	13,333	7	16	441.87	24.72	22.66	4.62
11	R3S3	10,000	3	16	500.02	21.71	20.63	5.08
11	R3S3	15,000	3	16	455.02	34.58	27.79	4.83
11	R3S3	20,000	3	16	378.76	20.66	20.59	3.61

[a] H: height of dominant shoots; [b] Ws: total above-ground woody dry biomass; [c] BA_{10}: basal area of basal diameters; [d] dg_{10}: basal quadratic mean diameter of dominant shoots.

5.2.2. Model development

The following crop variables were used for each plot: crop age (t), number of stools per hectare (N), basal area of basal diameters (BA_{10}), basal quadratic mean diameter of dominant shoots (dg_{b0}), mean height of the dominant shoot of each stool (H), total above-ground woody dry biomass (Ws) and mortality. The number of stools per hectare (N) was calculated as the difference between the initial density (N_0) and the natural mortality. The basal area of basal diameters (BA_{10}) was calculated as the sum of the basal section of dominants shoots of all the

stools in each plot. Basal quadratic mean diameter of dominant shoots (dg_{10}) was calculated as the quadratic mean diameter of the dominant shoots of each stool (Table 5.3).

Table 5.3. Statistics for the main variables measured in the plots.

	Max [a]	Min [b]	Mean	Devest [c]
H (cm)	1,025.6	153.1	557.9	214.3
dg_{10} (cm)	7.72	1.41	4.74	1.28
N (stools ha^{-1})	33 333	3750	13 328	5 680
N_0 (stools ha^{-1})	33 333	6 666	15 378	6 085
Ws (Mg dm ha^{-1})	85.3394	0.7901	29.7845	20.9524

[a] Max: maximum; [b] Min: minimum; [c] Devest: mean deviation.

A static density-independent mortality equation was fitted with data from a total of 333 plot density measurements covering the 3 years rotation established for each plot. Reduction in stool number was fitted directly without prior consideration of the probability of mortality in the plots (Dieguez-Aranda *et al.*, 2005). In this case, the age of the crop was not included in the model, as the height of the dominant shoot in the stool is the variable represented in the RD. Age was not included in the model because in short rotation crops, the relationships between age and height of the stools is highly dependent on the climatic conditions during the rotation, and therefore height is more representative (than age) of biomass yield. The equation used to predict the changes in stool density is exponential, which provides a constant rate of mortality independently of the initial density, as derived from the data:

$$N = N_0 * e^{*H} \qquad (5.1)$$

where N is the density (stools ha^{-1}), N_0 is the initial density (stools ha^{-1}), H is the average height of the dominant shoot of each stool (cm), and K is a constant parameter.

Structurally, DMDs consist of a number of functional and empirical quantitative relationships that collectively represent the cumulative effect of various underlying competition processes on tree and stand yield variables (Newton *et al.*, 2005). The RD developed in this study included a previously calculated density-independent mortality equation, and a system of two equations, the first of which is used to predict the quadratic basal mean diameter and the second of which is used to predict above-ground woody dry biomass:

$$dg_{10} = a_1 * H^{a_2} * N^{a_3} \qquad (5.2)$$

$$W_s = a_4 * dg_{10}{}^{a_5} * N^{a_6} \qquad (5.3)$$

where dg_{10} is the quadratic mean diameter of dominant shoots (cm), H is the average height of the dominant shoot of each stool (cm), N is the crop density (stools ha^{-1}), Ws is the above-ground woody dry biomass (kg dm ha^{-1}), and a_1, a_2, a_3, a_4, a_5 and a_6 are constant parameters.

The first equation is based on the relationship between average tree size, density and a productivity indicator (Goulding, 1972; Long and Shaw, 2005; Castedo-Dorado *et al.*, 2009).

The second equation relates stand productivity (expressed in terms of stand above-ground woody dry biomass) to basal quadratic mean diameter and number of stools per hectare.

Graphical representation of the model was carried out using the lattice function of the *R statistical package* (Sarkar, 2008; Sarkar and Andrews, 2011), which enables plotting of multivariate data. In this case, the xyplot function was used to produce a two-dimensional graphic. The RD was constructed by plotting mean height of dominant shoots (*H,* cm) on the X-axis and the number of stools per hectare (*N*, stools ha^{-1}) in logarithmic scale on the Y-axis. The basal quadratic mean diameter of the dominant shoot isolines were represented in the diagrams by using constant values for dg_{10} and solving equation (5.2) for *N*. The mortality isolines were represented in the diagrams by using constant values for N_0 and solving equation (5.1) for *N*. The above-ground woody dry biomass isolines were represented in the diagrams, clearing the basal quadratic mean diameter of the dominant shoots for equation (5.2), using constant values for *Ws* and solving equation (5.3) for *N*.

Bioenergy and ash content application were also provided. The energy content in above-ground woody dry biomass was calculated on the basis of the lowest heating value (*LHV*) at 0% moisture content, which corresponds to 18.36 MJ kg^{-1} for three year old shoots of the 'I-214' poplar genotype (Fernández *et al.*, 2013). The ash content (%) was calculated on the basis of the percentage of ash considered by Klasnja *et al.* (2002) for the 'I-214' genotype (5.44% for bark and 0.78% for wood), and the percentage of bark as a function of diameter at breast height (*dbh*) measured by Guidi *et al.* (2008). For application of the latter percentage, an equation relating *dbh* to dg_{10} was required:

$$dg_{10}\ (mm) = 10.666 + 1.0649 * dbh \qquad (5.4)$$

Data from 144 plots were used to fit equation (5.4). The estimated single coefficient of the model is highly significant, and the model accounted more than 90% of the total variability of basal quadratic mean diameter of dominant shoots (dg_{10}). This model was used to obtain the relationship between dg_{10} and ash content (2.25 % for dg_{10}=2.54 cm, 2 % for dg_{10}=3.47 cm, 1.75 % for dg_{10}=4.42 cm and 1.5 % for dg_{10}=8.92 cm).

For graphical representation, the dominant height axis ranged from 100 to 1,200 cm, and densities ranged from 3,000 to 35,000 stools ha^{-1} on a logarithmic scale. The basal quadratic mean diameters values ranged from 1 to 10 cm and finally the above-ground woody dry biomass values ranged from 0 to 100 Mg dm ha^{-1}.

5.2.3. Statistical analysis

Equation (5.1) was fitted with the *nls* procedure of the *R software program* (*R* Development Core Team, 2013). Although this equation can also be considered as part of a simultaneous fitting system along with equations (5.2) and (5.3), separate fitting was preferred because of the different sample size and the interest in obtaining the most accurate estimate of living density of stools.

Equations (5.2) and (5.3) define a structurally simultaneous system, in which *N* and *H* are exogenous variables whose values are determined independently of the system of equations, *Ws* is an endogenous variable, and dg_{10} is an endogenous instrumental variable. The system of equations was fitted simultaneously using the SYSTEMFIT *package* (Henningsen and Hamann, 2007) and the SUR (Seemingly unrelated regression) methodology in the *R* statistical package (*R* Development Core Team, 2013). The pseudo coefficient of determination and the root mean square error were used to check the accuracy of the models.

Analysis of variance (ANOVA) was performed, using the *R statistical package* (*R* Development Core Team, 2013), to evaluate the effect of the stage of development in terms of height growth and Mean Annual Increment (*MAI*) in biomass, and as the samples sizes were different for different stages of development, the generalized linear model procedure (GLM) was used. Tukey's HSD test was used for comparison of means when significant differences of *p*-value<0.05 were observed.

5.3. Results

5.3.1. Model fitting

The results of fitting the three equations included in the model are shown in Table 5.4. The estimate of the single coefficient of the density-independent mortality model was highly significant (*p*-value< 0.0001) and the model accounted for more than 80% of the total variability in stool number. The equation obtained can be used to estimate the number of living stools from the mean height of the dominant shoots and planting density, and it produces a fixed mortality rate, which is consistent with the observations (Fig. 5.1).

For the simultaneous fit, all the coefficient estimates were highly significant (*p*-value<0.0001), except for parameter a_4 of biomass equation (5.3). This has not serious implications, as this parameter does not have any biological meaning (Antonio *et al.*, 2007). The models accounted for more than 85% of the total variability in both the quadratic mean basal diameter and the biomass yield, (equations 5.2 and 5.3). It is usually difficult to obtain accurate prediction of diameters because of the dependence on changes in density over time when longer rotations are considered.

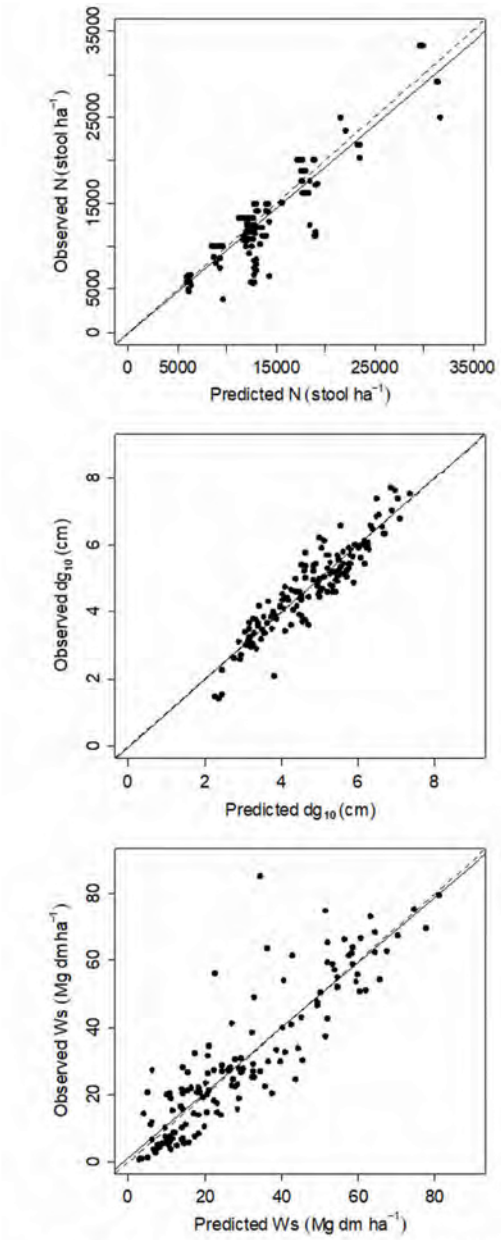

Figure 5.1. Relationship between observed-predicted density values stools ha^{-1}) in the mortality equations, basal quadratic mean diameters values (cm) and dry biomass values (Mg dm ha^{-1}) in the stool system of equations.

Table 5.4. Non-linear regression coefficients and statistics obtained from the mortality equation and simultaneous fitting of the system of 2 equations predicting basal quadratic mean diameter and total above-ground woody dry biomass.

Equation		Parameter estimates		*pse*-R^2	RMSE	N data	DF
(5.1)	$\hat{R}$	-0.0001689	<0.0001	0.8059	2 566	350	349
	$\hat{a}_1$	0.41903566	<0.0001				
(5.2)	$\hat{a}_2$	0.65089060	<0.0001	0.8553	0.488	144	139
	$\hat{a}_3$	-0.17734437	<0.0001				
	$\hat{a}_4$	0.05115256	0.1250824				
(5.3)	$\hat{a}_5$	2.39193578	<0.0001	0.8569	7,927	144	139
	$\hat{a}_6$	0.99484093	<0.0001				

where *pse*-R^2: pseudo coefficient of determination; RMSE: root of mean square error and DF: degrees of freedom.

5.3.2. Reference diagram

The range of values represented by the axes and the isolines in the RD were similar to the ranges included in the database used to construct the diagram (Table 5.3). The RD shows the isolines for basal quadratic mean diameter (dg_{10}), above-ground woody dry biomass (Ws) and mortality, as well as the observed values of H and N in the plots used for fitting (Fig. 5.2).

The isolines for stool density decrease gradually with increasing dominant height, derived from a continuous reduction in stool number of 1.67% for each metre of increment in H. The diagram can be used either by considering the initial density to predict the living stool density for a given dominant height or by directly using the actual stool density recorded in the plots. In both cases, the values of the final densities recorded in the plots ranged widely, from 3,735 to 33,333 stools ha^{-1}, and therefore the model can account for large differences in stool density. The dominant height ranged from 153 to 1,026 cm, and did not increase with initial density (Fig. 5.3).

The isolines for biomass yield are curves that tend to be vertical for low dominant heights, indicating a small effect of stool density at such stages of crop development. As dominant height increases, these isolines become more horizontal and less separated, showing strong differences depending on density and height growth (Fig. 5.2). The isolines represented a range from 5 to 100 Mg dm ha^{-1}, according to the values measured in the plots, shown in Table 5.3. The crops are most productive, as far as the first rotation is concerned, at densities higher than 15,000 stools ha^{-1}. These isolines can be used to define biomass thresholds for harvesting, which should be assessed in terms of stool density and dominant height, with the possibility of lengthening the rotation if necessary.

The isolines for basal quadratic mean diameter show a strong dependence on stool density, with very variable values (isolines considered from 2 to 10 cm, according to the observed values in the plots, Table 5.2). These isolines are rather uniformly separated and are representative of harvesting conditions and biomass quality (Fig. 5.2). The isolines tend to become more horizontal as dominant height increases. The basal area of basal diameters, a variable not included in the diagrams but used to obtain the basal quadratic mean diameter, ranged between 0.70 and 22.81 m^2 ha^{-1} yr^{-1} (Fig. 5.3) and tended to increase with density.

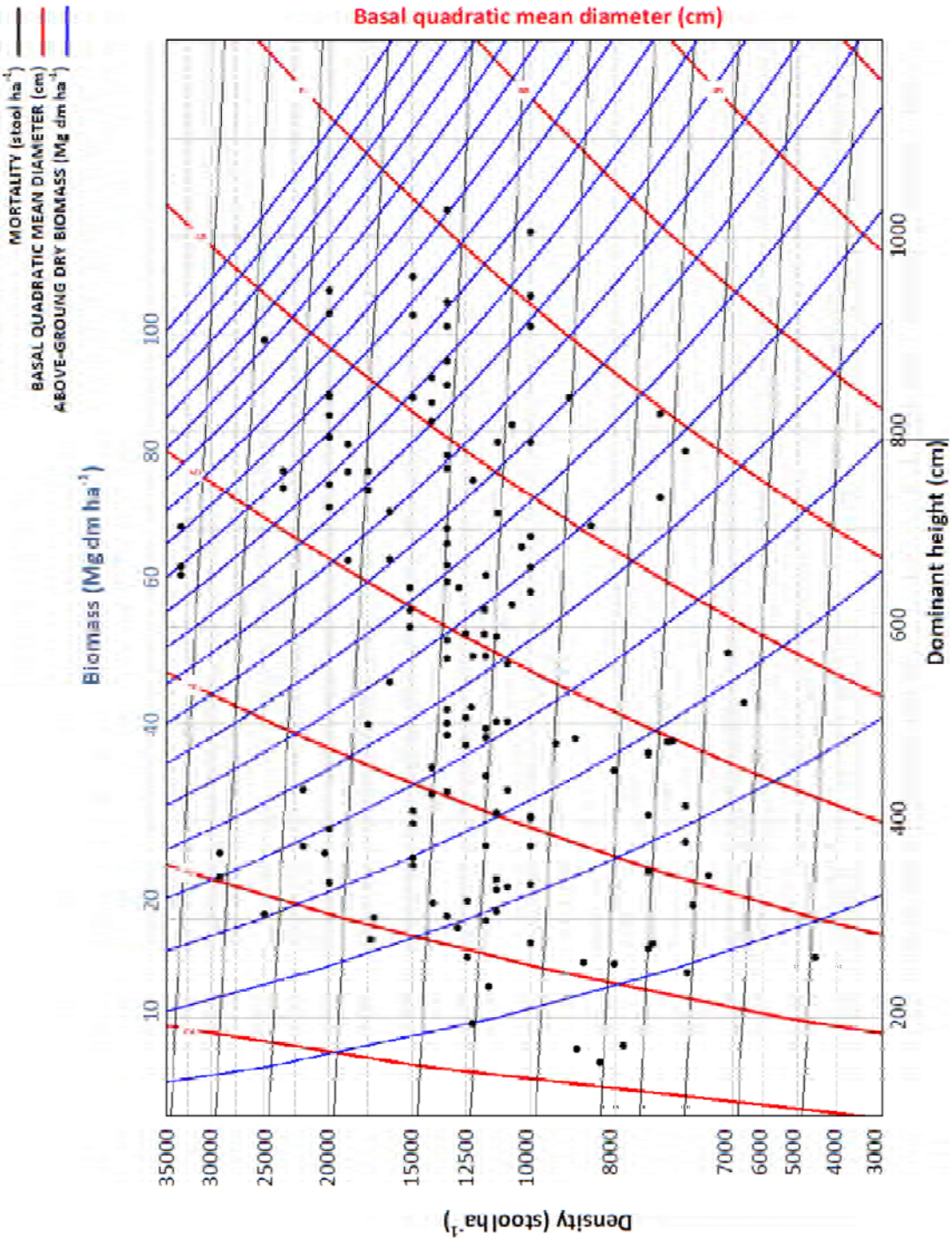

Figure 5.2. ***Reference diagram*** **for 'I-214' poplar genotype with isolines for mortality (stools ha^{-1}), basal quadratic mean diameter (cm), aboveground dry biomass (Mg dm ha^{-1}) and sample plots.**

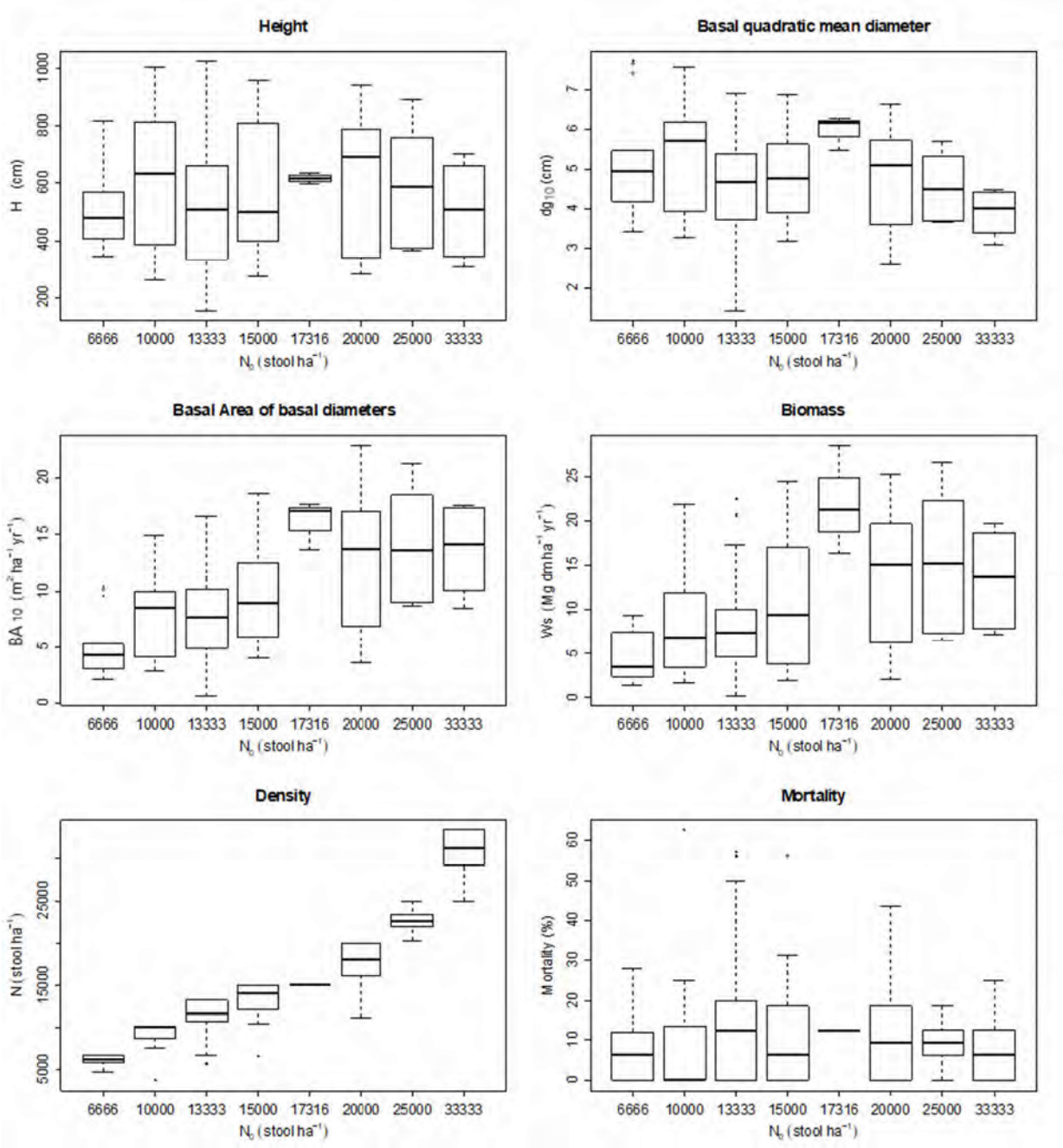

Figure 5.3. Boxplot of the variables height, basal quadratic mean diameter, basal area of basal diameters (all referring to dominant shoot per stool), and biomass, density and mortality as a function of the initial density (stools ha^{-1}).

In the database, sixteen of the plots are at developmental stage corresponding to R7S3. This information was used to test the behaviour of the diagrams for a second rotation (Fig. 5.4) and it was noted that significant biases occur both in the case of diameter and biomass.

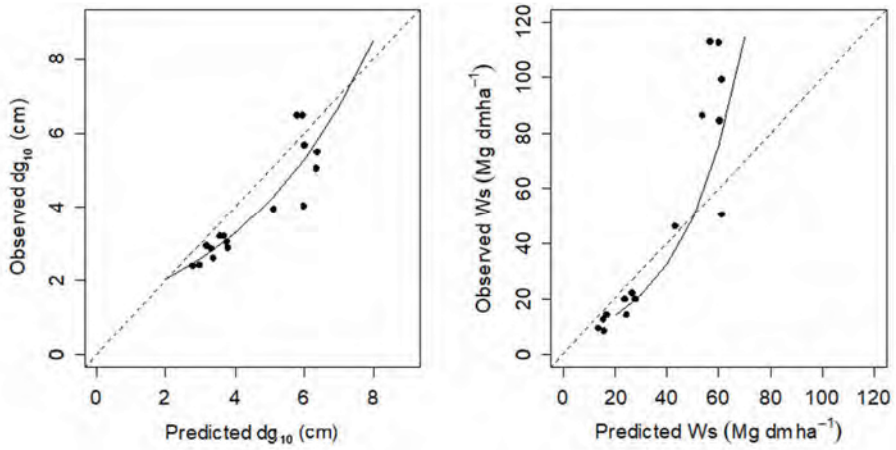

Figure 5.4. Relationship between observed-predicted dry biomass values (Mg dm ha^{-1}) and basal quadratic mean diameters values (cm) for the second rotation, in the stool system of equations for first rotation used in the diagrams.

5.3.3. Example of RD use to determine energy production

As a practical example (Fig. 5.5), we simulated an initial planting density of 10,000 cuttings ha^{-1} and a final basal quadratic mean diameter of 8 cm (which allows efficient harvesting of the crop by a chip harvester). The total aboveground woody dry biomass yield was approximately 60 Mg dm ha^{-1}, the final density was less than 9,000 stools ha^{-1} and the dominant height obtained was about 11 m.

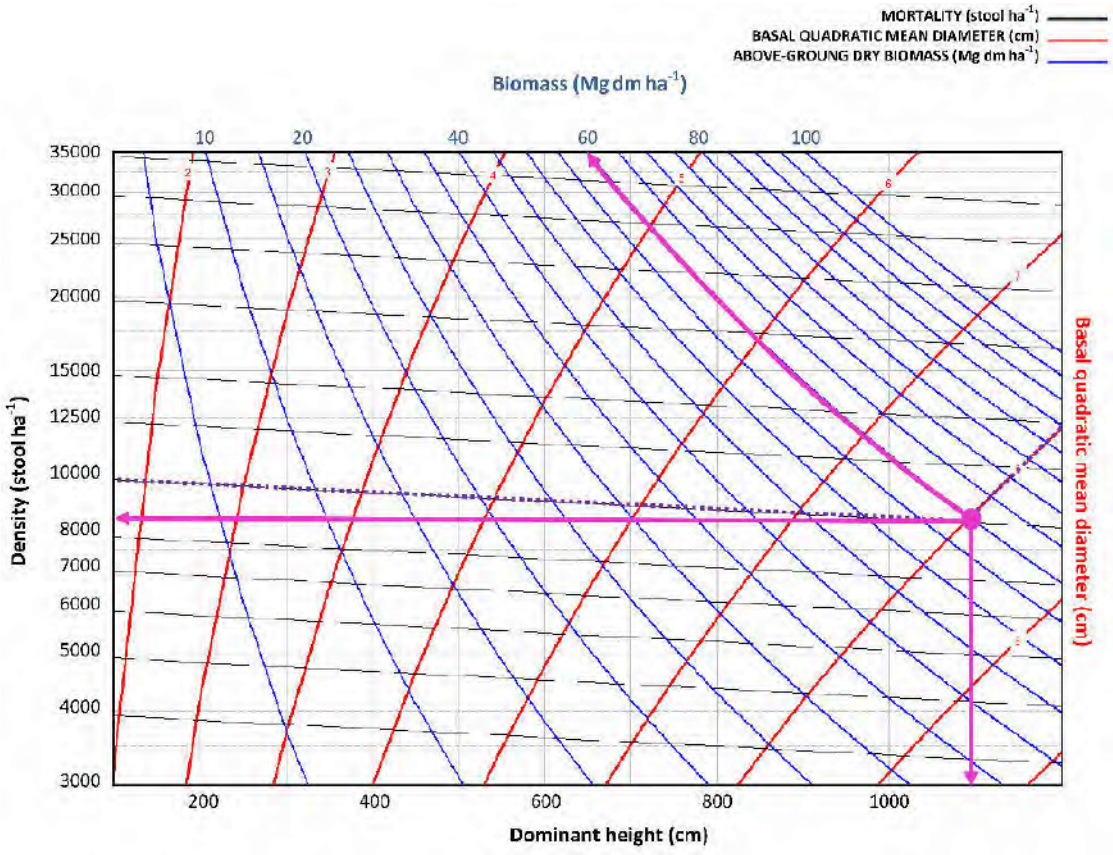

Figure 5.5. Practical example of the use of the *reference diagram*.

As an additional guide to exploring the possible values of height over time, the observed values of the quartiles for this variable are represented in Fig. 5.6, along with the predicted quartiles for R4S4 and R5S4. Significant differences in height according to the stage of development (p-value < 0.0001) were observed with increasing root age, although not in the case of shoot age. This was also found for Mean Annual Increment (*MAI*) in biomass (p-value <0.0001), except in the first year shoots, in which this variable did not vary significantly (Fig. 5.7).

The rotation for calculating *MAI* is considered as being the period between planting and harvesting (root age), and the biomass produced is considered as the sum of the yield at harvest time and the small quantities obtained during the first coppicing at age 1 year (R1S1) in the corresponding cases.

The total energy production for this simulation was 1,100 GJ ha^{-1} and the ash content approximately was 1.53 %. Therefore, the energy production rate would be 366.6 GJ ha^{-1} yr^{-1}. These values indicate that an area of 81.8 ha covered by poplar under SRC would be needed to supply a municipal power plant of 5 MW, with a performance of 1,500 hours per year and a thermal efficiency of 90% (typical dimension for several public buildings and a network of heat to various individual homes). This indicates the possibility of promoting SRC for thermal use in areas where the current biomass supply is low.

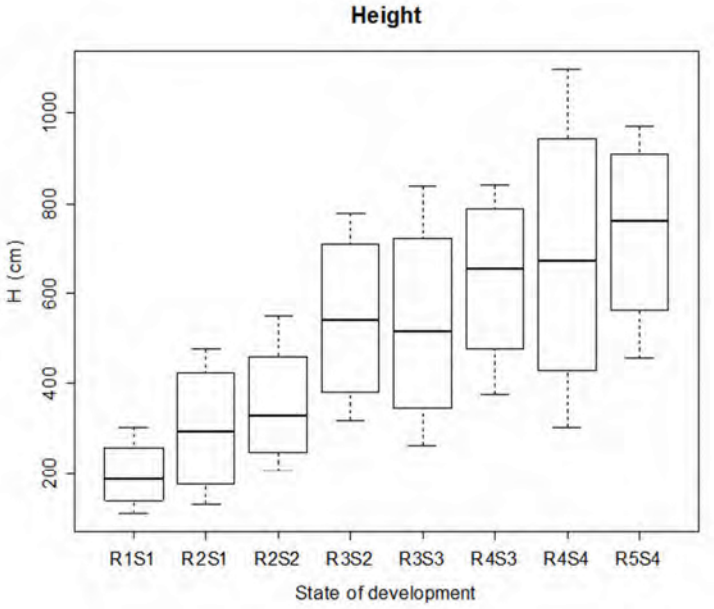

Figure 5.6. Quartiles height as a function of the stage of development.

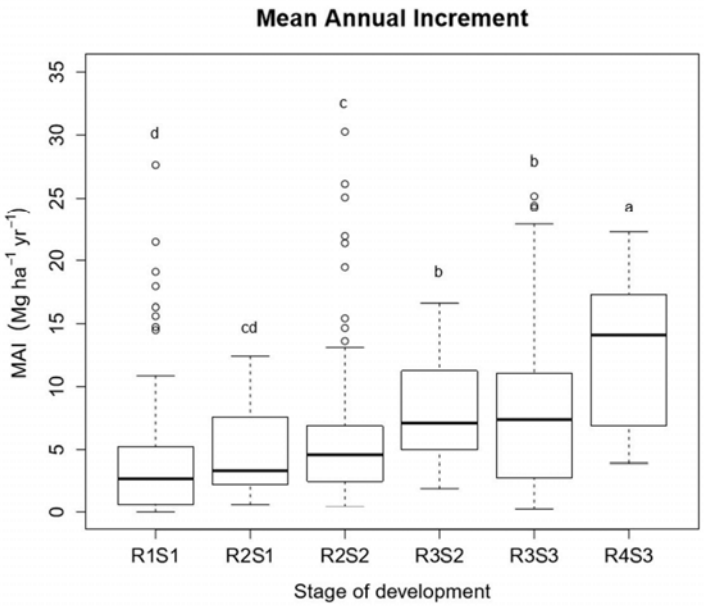

Figure 5.7. Mean annual increment in biomass as a function of the stage of development. The letters represent the results of Tukey's HSD post-hoc comparisons of group means (p-value<0.05).

5.4. Discussion

5.4.1. Model utility and limitations

The system of related equations presented enables accurate estimation of crop yield in terms of oven dry biomass and total energy, while also providing the predicted changes in stool number, which is a useful tool for decision making. A density-independent mortality model was used rather than the density-dependant model usually used for DMDs because no different trends in mortality rate for different planting densities were observed. This may be because the development stage of the plantations under study may not have reached the self-thinning threshold, as indicated by the low mean mortality rate (< 15 %) across planting densities. The two main variables included in the diagram and used for decision-making were the initial cutting density and the rotation age, represented by the increase in mean height of the dominant shoots with age, depending on site properties and the crop management. The mean height of dominant shoots can be determined easily by measurement with telescopic rods in a sample plot or from LiDAR information.

The pseudo coefficient of determination in the case of the biomass model is, however, slightly lower than the values obtained by other authors for pine or oak stands (Barrio-Anta and Álvarez-González, 2005; Barrio-Anta *et al.*, 2006; Castedo-Dorado *et al.*, 2009). This may be because in the present study, fitting was at the stool level, without inclusion of additional variables such as the shoot number per stool. The average shoot number per stool is often highly correlated with total biomass; however, for the information of first rotation managed in this study, the values obtained were low and did not improve the models. Nevertheless, this variable may have a major impact in models developed for subsequent rotations.

The variability in basal quadratic mean diameter explained by this model can be considered high relative to that reported by other authors (Pérez-Cruzado *et al.*, 2011), probably because in short rotations crops, basal diameter is more directly dependent on initial stool density. This variable may be important for considering the harvester limits and harvesting time. Chip harvesters specifically used for these types of crops may be limited by basal diameters greater than 15 cm, while multi-cutter harvesters may be more cost effective for basal diameters between 15 and 20 cm (Berhongaray *et al.*, 2013a). Tools such as that obtained in this study may provide a basis for comparing the management options for overcoming these limitations, not only by using appropriate densities but also choosing the best harvesting time. On the other hand, knowledge of the market value of the biomass as well as the costs associated with different management practices may also enable economic analysis to obtain the optimal planting density of poplar SRC. However, the models provided here are only valid for the first rotation. The predictable vigorous sprouting after the first coppice (Stanturf *et al.*, 2014) would promote rapid canopy closure and full site occupancy, reducing the differences in biomass yield between initial density levels.

Comparing the results obtained for the second rotation to those predicted by this model, we confirmed the need to develop a different set of equations for the second rotation, as basal

quadratic mean diameter is overestimated by the model and there is a strong tendency for biomass yield to be underestimated for high observed values of biomass, this was expected due to the higher growth of this type of crop in successive rotations (Sennerby-Forsse *et al.*, 1992; Di Matteo *et al.*, 2012; Dillen *et al.*, 2013); the effect of an already well-formed root system, which is able to absorb more nutrients and water, and the ability of the stool to provide multiple shoots together favour higher production after coppicing, and therefore the prediction for the second rotation with an equation fitted with first rotation data causes significant bias. This emphasizes the importance of developing similar models to those proposed here for future rotations.

The basal area of basal diameters tended to increase with density. However, by decreasing the density, the diameter of trees increased due to the decreased competition for resources, which results in a large average tree size (Hamilton, 1969; Assmann, 1970; Curtis, 1970; Dean and Long, 1992), thus improving the quality of the biomass arising from lower bark content, lower ash content and greater lower heating value (Bergante *et al.*, 2010).

High ash content of wood is less desirable for fuelwood as it is non-combustible and reduces the heat of combustion (Goel and Behl, 1996; Klasnja *et al.*, 2002). As the share of bark depends on the age of wood (Guidi *et al.*, 2008), and the ash content in the whole tree is mainly related to the amount of bark (Steenari and Lindqvist, 1997; Tharakan *et al.*, 2003), the ash content will also depend on the age of wood. Bark percentage decreases rapidly with increasing *dbh*, although it is thought that at *dbh*> 5 cm, the percentage of dry bark stabilizes at around 7% of dry matter and the decrease in ash content thus slows down (Guidi *et al.*, 2008). Previously reported values were used instead of an equation (Guidi *et al.*, 2008) to estimate the ash content because the basal quadratic mean diameter in the diagram ranged between 2-10. The ash values derived from the procedure described here were lower than the ash content observed for 'I-214' genotype by Fernández *et al*. (2013) at the end of a three year rotation (i.e. about 2.4 % on a dry weight basis).

5.4.2. Comparison with data from other studies

The values obtained in the present study in relation to the annual above-ground woody dry biomass for 'I-214' genotype in function of their densities showed a similar yield range to those reported by other authors, such as Facciotto *et al*. (2006) (15 and 35 Mg dm ha^{-1} yr^{-1}), Liberloo *et al*. (2006) (20 and 25.8 Mg dm ha^{-1} yr^{-1}) and Paris *et al*. (2011) (7.25 Mg dm ha^{-1} from a low soil quality site and 21.86 to 23.74 Mg dm ha^{-1} for medium and high soil quality sites). Under the Mediterranean conditions of the Iberian Peninsula, the observed range was between 19 and 8 Mg dm ha^{-1} yr^{-1} (Sixto *et al.*, 2013a). However, 'I-214' has been identified as stable in terms of yield for a given density (Cruset and Voltas, 2009; Sixto *et al.*, 2013a), i.e. without departing from the expected behaviour estimated from its average genotypic value.

Although the energy obtained from *LHV* is a theoretical value that can only be achieved at 0% moisture, the information derived from this product may also be useful for determining the energy value of the biomass produced in the different management scenarios. In fact, the

following equations enable calculation of biomass, lower heating value and bulk density for chips with a moisture content (h %, wet basis), considering a wood and bark basic density of 0.35 Mg m^{-3} (data not show) and a ratio of 0.38 solid m^3/apparent m^3:

$$W_h = W_s * \frac{100}{100-h} \quad (5.5)$$

$$LHV_h = LHV_0 * \frac{100-h}{100} - 0.02443 * h \quad (5.6)$$

$$BD_h = 0.35 * 0.38 * \frac{100}{100-h} \quad (5.7)$$

where W_h is the biomass weight at a humidity content (h %, wet basis), W_s is the above-ground woody dry biomass obtained from the diagrams, LHV_h is the low heating value (MJ kg^{-1}) and BD is the bulk density (Mg m^{-3}). The application of such equations for the usual humidity content at harvest time (55%) provides BD_{55}=296 Mg m^{-3}. Drying chips to h=30% would provide a biofuel classified as *BD150* (BD_{30}=190 Mg m^{-3}), *Q11* (LHV_{30}=12.1 MJ kg^{-1}), A3.0 (ash percentage always less than 3%) and thus class B for non industrial uses, according to the European standard EN14961-4.

The predicted values for the energy production at the end of the rotation ranged between 100-2,000 GJ ha^{-1}, which is higher than the values reported for poplar (173-259 GJ ha^{-1}; 10-15 Mg dm ha^{-1} yr^{-1}) grown as a short rotation woody crop (McKendry, 2002). Mean plot values ranged from 4.83 to 522.27 GJ ha^{-1} yr^{-1}, which includes the range of values obtained by other authors (165.51-311.78 GJ ha^{-1} yr^{-1} (Fernández *et al.*, 2013)) and even reached as high as the values for second rotations, which are expected to be higher (e.g. 351-537 GJ ha^{-1} yr^{-1} (Luo and Polle, 2009)).

5.5. Conclusions

Reference diagrams are useful because of their simplicity and the fact that the inputs required are easily obtainable. These features make this *Reference Diagram* an excellent tool for stand management, adapted to short rotation coppice of poplar. The extensive network of heterogeneous plots used to develop this RD confers the model a wide range of performance, whether for planning plantation, as a tool to examine the different options to achieve an end goal (i.e. to achieve a specific production to supply a plant or not to exceed certain stage of development that can be limiting at harvest time), and at the end life of the plantation, for calculating the optimal cutting time for a final goal. In both cases, this type of diagram proved to be a very useful tool for planning different management according to the constraints of the desired product and optimizing resources to maximize the performance.

Although the translation of dominant height data to age would depend on site conditions and management, the models developed are useful for estimating the amount of biomass accumulated at a certain stage of development and initial planting density and to determine the maximum developmental stage that allows the use of available machinery to harvest the crop.

These models are also useful for economic optimization of the initial density of planting in relation to production, although this requires models that are sensitive to the possible effect of the crop origin in relation to the initial density of the following rotations. Future research in this field of work should consider all these aspects.

Acknowledgements

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CHAPTER

6

Mixed short rotation plantations of Populus alba and Robinia pseudoacacia for biomass yield

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Abstract

The possibility of using mixtures of species in an attempt to increase biomass yield in plantations managed in short rotation coppice (SRC) has received little attention in scientific literature. The hypothesis is that mixtures of two complementary species under SRC are more productive than monocultures. The objective of this study was to compare the growth and yield of mixtures and monocultures at the tree and stand levels. A mixed plantation of *Populus alba* L. genotype '111PK' (P) and the nitrogen-fixing species *Robinia pseudoacacia* L. genotype 'Nyirsegi' (R) was established in 2012 in the centre of Spain, at a density of 10,000 cuttings per hectare (spacing 0.4 m x 2.5 m). The experimental design included pure as well as mixtures with different proportions of the species (100P:0R; 75P:25R; 50P:50R; 25P:75R and 0P:100R) following a random block design with 36 trees per plot. There was a positive effect of the mixture 75P:25R in terms of biomass yield, achieving a total yield of 13.66 Mg ha^{-1} yr^{-1} (26.83% higher than that of the *P. alba* monoculture and 89.99% higher than the *R. pseudoacacia* monoculture), with both species achieving a greater biomass yield per tree. In the other mixture treatments, there was no significant positive effect of the nitrogen (N) fixing species on the non-N-fixing species. Within-row interactions were more important than between row-interactions. There was a positive effect on the yield of *R. pseudoacacia* when the neighbouring trees (within a row) on both sides were *P. alba*, while *P. alba* responded better with a *P. alba* tree on one side and a *R. pseudoacacia* tree on the other. The results point to the presence of competitive reduction or facilitation as the mechanisms for increased biomass yield under certain mixture ratios, and indicates the importance of mixing within the same row rather than row-by-row, in this type of plantation.

KEYWORDS: Poplar; Black locust; Mixture; Short Rotation Coppice (SRC); Biomass.

6.1. Introduction

The need to address the consequences of climate change (COP21) as well as to generate economic wealth from biological resources (bio-economy) is becoming an important global challenge (European Commission, 2012). One approach to achieving this is to optimize the production of lignocellulosic biomass as feedstock for multiple uses (energy and bioproducts) (Zhang *et al.*, 2011). Typical forest management cannot meet the global demand for biomass. Hence, forest plantations constitute an essential complement in the biomass market, providing an alternative use for abandoned land and contributing to rural development (BIC, 2013). Short Rotation Coppice (SRC) plantations, consisting of woody cultivation in short cycles (3-7 years), provide a suitable means to obtain this raw material in short periods of time, and therefore SRC cultivation is becoming more widespread globally (Srirangan *et al.*, 2012).

Species belonging to the *Salicaceae* family (*Populus* spp. and *Salix* spp.) have proved to be one of the most suitable for SRC management in Europe (Nassi O Di Nasso *et al.*, 2010). Monoclonal (Verlinden *et al.*, 2015) as well as multi-clonal willow and poplar plantations (Aylott *et al.*, 2008) are common in many countries (Yemshanov and McKenney, 2008; O'Neill *et al.*, 2010), both in northern and central Europe (Aust *et al.*, 2014), as well as in the Mediterranean region of Europe (Weih, 2004; Paris *et al.*, 2005).

In forestry, many studies have shown that mixtures of species are more productive than monocultures because complementary traits and species interactions increase resource availability and enable them to use a higher proportion of the site resources or to use them more efficiently (Kelty, 1992; Forrester, 2014). These processes and interactions are often described as facilitative, where the growing conditions are improved; or competitive reduction, where intense intra-specific competition is replaced with a less intense inter-specific competition (Kelty, 1992; Brooker *et al.*, 2008; Forrester and Bauhus, 2016). Competitive reduction and facilitation can occur simultaneously and their effects are difficult to separate, therefore, both effects, as well as competition, are often collectively described as complementarity (Forrester, 2014). A classic example of facilitation is the effect of nitrogen-fixing species on the growth of non-nitrogen-fixing species, this effect increases as soil nitrogen becomes more limiting (Bouillet *et al.*, 2013; Forrester, 2014). The response of mixtures containing fast-growing species such as *Eucalyptus* and nitrogen-fixing species, such as *Acacia*, has received a lot of attention (Forrester *et al.*, 2006; Bouillet *et al.*, 2008; Laclau *et al.*, 2008; Nouvellon *et al.*, 2012; Zhang *et al.*, 2012). Similarly, Sayyad *et al.* (2006), evaluated plantations of *Populus deltoides* Bartram ex. Marshall and the nitrogen-fixing species *Alnus subcordata* L. using different mixing ratios. They found that poplar was positively affected by the presence of alder.

The mixtures containing *Eucalyptus* or *Populus* described above were planted at relatively low densities (< 2500 trees ha^{-1}), whereas SRC plantations are typically planted at much higher densities (> 5000 trees ha^{-1}). Many studies have shown that stand density can modify the size of mixing effects, with some studies reporting that the mixing effect was greater as density increased (Boyden *et al.*, 2005; Amoroso and Turnblom, 2006; Condés *et al.*, 2013; Forrester

et al., 2013) while others point to reduced effects (Garber and Maguire, 2004; del Río and Sterba, 2009) depending on which resources were limiting growth and how the change in density influenced the interactions for those resources (Forrester, 2014). It remains unclear, whether the balance between facilitation and competition might shift more towards competition in high density SRC plantations. For example, while many studies have shown facilitative effects of *Acacia* in *Eucalyptus* plantations, high densities of *Acacia* weeds (1,300–20,000 trees ha^{-1}), in *Eucalyptus* plantations (with 1,000 *Eucalyptus* trees ha^{-1}), can cause net competitive effects on *Eucalyptus* growth, even though soil nitrogen increases with *Acacia* density (Hunt *et al.*, 1999).

Despite their potential, less information exists with regard to mixed-species plantations in SRC, an example of high density SRC plantations include *Populus* and *Alnus* (Dawson and Hansen, 1983; Radwan and DeBell, 1988). A potentially useful mixture in the Mediterranean is that of *Populus* with *Robinia*, planted at high density and in short rotation (Gana *et al.*, 2014). Poplars and their hybrids are generally suitable for high density cultivation and also for coppicing (Dickmann, 2006). Among these, *Populus alba* L. (white poplar) is a species with a broad natural distribution, a strong presence in the Mediterranean basin and it is suitable for SRC (Rosso *et al.*, 2013). The hardiness and plasticity of *Populus alba* justify its use in marginal areas affected by dry climates, high temperatures or salinity (Sixto *et al.*, 2005; Di Lonardo *et al.*, 2011). It is also an important species for improving adaptive traits in poplar cultivation (Alba and Agúndez, 2000). *Robinia pseudoacacia* L. (black locust) is also considered a fast growing species of interest for biomass yield in SRC (Annighöfer *et al.*, 2012; Crosti *et al.*, 2016). It displays good resprouting capacity after coppicing (Luken *et al.*, 1991) and is a nitrogen fixing species (Grunewald *et al.*, 2009), which makes it a particularly good candidate for mixed-species plantations. Both poplars and black locust are considered of interest from the point of view of lignocellulosic biomass production in the south of Europe.

The objective of this study was to compare the growth and yield of mixtures and monocultures of *Populus alba* and *Robinia pseudoacacia* at the tree level, species level and stand level using a replacement series experiment with five mixture compositions. The main hypothesis was that mixtures of these two species under SRC are more productive than their monocultures as a result of the theoretically expected positive effect of nitrogen-fixing on non-nitrogen species and of competitive reduction between the two species. The specific objectives were therefore to answer the following questions: (1) Does the mixture composition in SRC influence stand growth and yield? and (2) Does it influence growth and yield of the individual trees of the two species?

6.2. Materials and methods

6.2.1. Trial site and experimental design

Short-rotation (3 years) mono-specific and mixed-species plantations established at high density (10,000 cuttings ha^{-1}; spacing 0.4 m × 2.5 m) were evaluated in an experimental trial in

central Spain (40º 28' N, 3º 22' W). The land, which was previously used for cereal production, is situated at an elevation of 595 m asl. The annual mean temperature is 14 ºC and the soil has a silty loam texture with a pH of 8.1.

The plantation was manually established in the spring of 2012. Immediately after the cuttings were planted, Oxifluorfen herbicide (4 l ha^{-1}) was applied over the whole area in order to avoid weed competition during the establishment period. The whole area was watered regularly to field capacity using a drip system between June and August of each year. Under Mediterranean conditions, SRC plantations need to be watered due to the severe summer drought. By irrigating all plots to field capacity, it is assumed that irrigation will influence all treatments in the same way.

The plantation included two species, the genotype *Populus alba* '111PK' and the genotype *Robinia pseudoacacia* 'Nyirsegi'. We used unrooted cuttings for the *P. alba* (0.40 m long) and bare rooted cuttings of the same length for *R. pseudoacacia*. The plant material was previously hydrated in water for 48 hours to facilitate rooting.

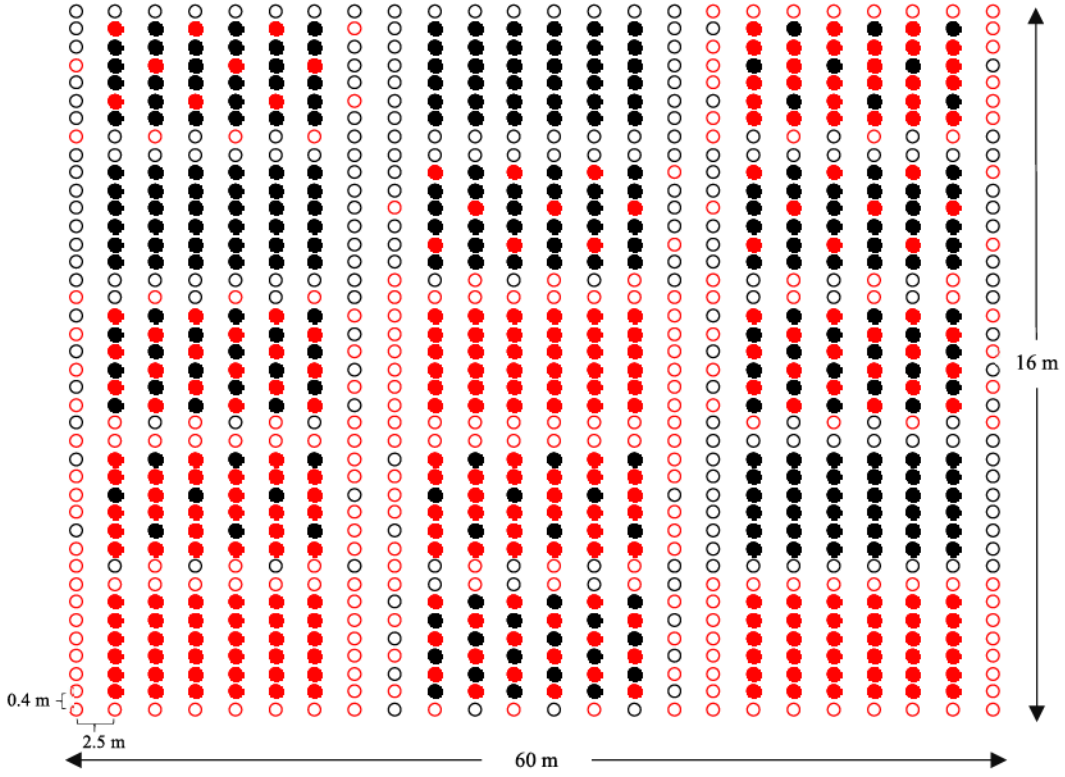

Figure 6.1. Experimental design scheme. Red dots represent *Robinia pseudoacacia* trees and black ones the *Populus alba* trees. Solid dots correspond to the measured trees and the empty dots are the buffer trees of each treatment. The three columns represent the blocks 1, 2 and 3, respectively.

A randomized complete block design (3 blocks) was applied, including five different proportions of the two species in each one (Fig. 6.1). Species were systematically mixed

following a similar replacement series design to that proposed by Kelty and Cameron (1995). The combinations of the five different species compositions were:

100 % *Populus alba* (100P:0R)
75% *Populus alba* + 25% *Robinia pseudoacacia* (75P:25R)
50% *Populus alba* + 50% *Robinia pseudoacacia* (50P:50R)
25% *Populus alba* + 75% *Robinia pseudoacacia* (25P:75R)
100 % *Robinia pseudoacacia* (0P:100R)

The number of trees per plot was 64 but only the inner 36 trees were evaluated to avoid border effects. The design of the experimental plot was consistent with commercial plantations in terms of the spacing.

6.2.2. Variables measured

The number of shoots per tree (n), the number of branches on the dominant shoot of the tree (br), stem diameter of each shoot at basal height (d_{10}, mm) and at breast height (d_{130}, mm) as well as total height of the dominant shoot (H, cm) were recorded for 36 trees for each plot at the end of each of the three growing seasons, comprising a single rotation. The basal area of each tree (ba, $cm^2\ tree^{-1}$) was calculated from the diameter at basal height (d_{10}) of all the shoots, the current annual basal area increment (ba_{yr}, $cm^2\ tree^{-1}\ yr^{-1}$) was also calculated to evaluate the effect of age on growth. These variables were also expressed per hectare (BA, $m^2\ ha^{-1}$), and per hectare and year (BA_{yr}, $m^2\ ha^{-1}\ yr^{-1}$).

Fresh biomass data were collected at the end of the rotation (third year) after the leaves had fallen. A subsample of each species in each block and composition was oven-dried to constant weight at 100 ºC to obtain the dry weight (dm). All biomass information is presented as dry biomass per tree at the end of the rotation (bi, kg dm $tree^{-1}$), as mean annual biomass increment per tree (bi_{yr}, kg dm $tree^{-1}\ yr^{-1}$) or as the mean annual biomass increment per hectare (BI_{yr}, Mg dm $ha^{-1}\ yr^{-1}$).

6.2.3. Statistical analysis

The data were analysed at the stand and individual tree levels considering the different species compositions (100P:0R, 75P:25R, 50P:50R, 25P:75R and 0P:100R) as levels of the treatment in an analysis of variance.

To study the response on growth and yield, data were analysed using analyses of variance (ANOVA) at both tree- and stand-levels. The Student-Newman-Keuls-test (SNK) was used to identify the means which were significantly affected by treatment. The time effect (three growing seasons) on growth variables was evaluated using an analysis of variance for repeated measures using mixed models. The effect of *complementarity* at the species level was calculated using the following equation, which uses mean growth or yield values for the given species:

$$Complementarity\ (\%) = \left(\frac{growth\ or\ yield\ in\ mixture - growth\ or\ yield\ in\ monoculture}{growth\ or\ yield\ in\ monoculture}\right) * 100 \qquad (6.1)$$

One characteristic of SRC plantations is that the high density is mainly achieved by narrow spacing within rows (0.4 m), since a much higher minimum distance between rows (2.5 m) is necessary to optimize biomass harvesting. Preliminary analyses, where the growth of individual trees was described as a function of the basal area of neighbouring trees, showed that most of the neighbour effects on growth were described when the neighbourhood size was a circle with a radius of 0.4 m from the target tree (data not shown), and therefore that within row competition was much more important than between row competition. Therefore, in order to analyse whether the spatial distribution of species influences overyielding, we also explored complementarity, considering the species distribution within rows regardless of the species composition of the stand. Therefore, taking into account the influence of the surrounding trees within the row, three neighbourhood situations exist (Fig. 6.1): trees surrounded by the same species (neighbourhood situation 1), trees surrounded by both species (neighbourhood situation 2) or trees surrounded by the admixed species (neighbourhood situation 3).

6.3. Results

6.3.1. Does the mixture composition in SRC influence stand growth and yield?

Survival for both species (*P. alba* '111PK' and *R. pseudoacacia* 'Nyirsegi') in monocultures or mixed plantations was 100%, revealing that competition between these two species did not influence their mortality rates during the first rotation. Mean annual biomass increment (BI_{yr}) and current annual basal area increment (BA_{yr}), at the end of the rotation (Fig. 6.2) were, however, significantly different for the different species compositions (p-value<0.0001), while no difference was detected among blocks (p-value=0.1299 and 0.7490, respectively).

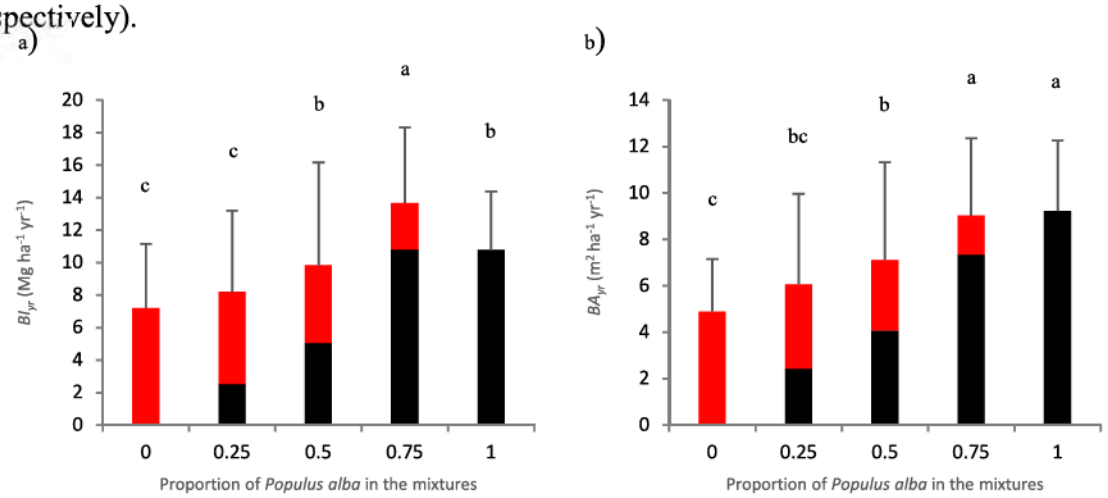

Figure 6.2. Growth at stand level by species at the end of the rotation in terms of a) mean annual biomass increment (BI_{yr}) and b) current annual basal area increment (BA_{yr}). *Populus alba* is represented by black bars and *Robinia pseudoacacia* by red bars. The letters represent the results of SNK post-hoc comparisons of group means (p-value<0.05). The proportions of *P. alba* are as follows: 0 corresponds to the species composition 0P:100R, 0.25 to 25P:75R, 0.5 to 50P:50R, 0.75 to 75P:25R and 1 to 100P:0R.

The most productive mixture in terms of biomass (BI_{yr}) was the proportion 75P:25R (p-value<0.0001) with a yield of 13.66 Mg ha^{-1} yr^{-1} (10.80 Mg ha^{-1} yr^{-1} of *P. alba* and 2.86 Mg ha^{-1} yr^{-1} of *R. pseudoacacia*). The least productive was the *R. pseudoacacia* monoculture (0P:100R) with 7.19 Mg ha^{-1} yr^{-1} (Fig. 6.2a). It is worthy of note that there were no differences in basal area between the 75P:25R and *P. alba* monoculture 100P:0R, the rest of compositions showing significantly lower basal areas (Fig. 6.2b).

The complementarity in relation to basal area for *P. alba* was positive in 25P:75R and 75P:25R, while the complementarity for *R. pseudoacacia* increased with the percentage of *P. alba* in the mixture (Fig. 6.3b). This trend was also observed for the biomass (Fig. 6.3a). While the complementarity in terms of biomass for *R. pseudoacacia* increased with a greater presence of *P. alba*, the latter species only benefited in the mixture with the lowest proportion of *R.* pseudoacacia (75P:25R).

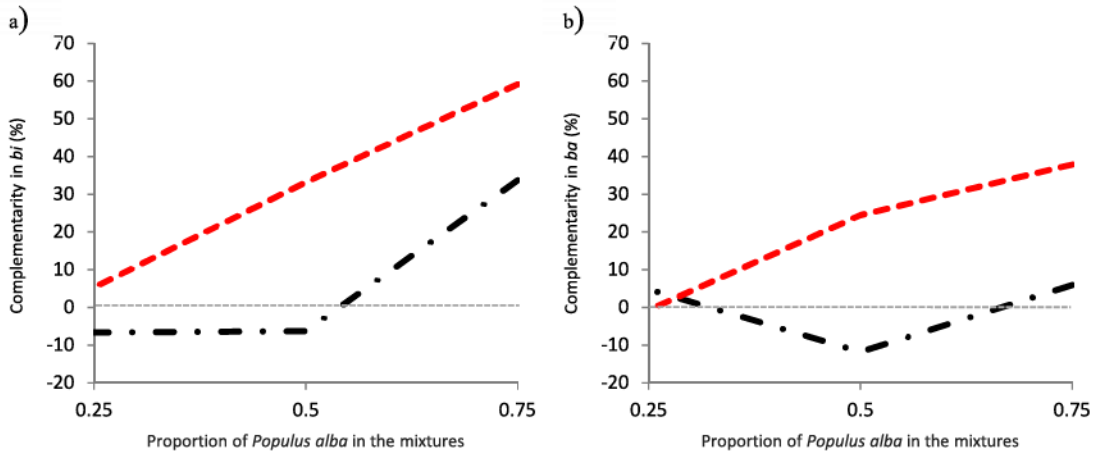

Figure 6.3. Relationship between complementarity (in %) and proportion of species in the mixture at the end of the rotation, in terms of a) biomass and b) basal area. Complementarity (%) was calculated using Eq. (6.1), with the mean value per species and mixture. *Populus alba* is represented by the black dashed line and *Robinia pseudoacacia* by the red dashed line. The proportions of *P. alba* are as follows: 0.25 corresponds to the species composition 25P:75R, 0.5 to 50P:50R and 0.75 to 75P:25R.

6.3.2. Does the mixture composition in SRC influence growth and yield of the individual trees of the two species?

The biomass yield per tree (*bi*) ranged from 3.02 to 4.32 kg tree^{-1} for *P. alba* and from 2.16 to 3.43 kg tree^{-1} for *R. pseudoacacia*, presenting significant differences (p-value<0.0001) in both species among species compositions (Fig. 6.4a). The basal area at the end of the rotation (*ba*) also presented significant differences between the different species compositions (mixtures and monocultures) for *R. pseudoacacia* (p-value=0.0002). The only exception was that the individual tree basal area (*ba*) of *P. alba* did not differ between mixtures and monocultures (p-value=0.0944) (Fig. 6.4b).

Table 6.1. Analyses of variance (ANOVA), mean and standard deviation at tree level for the number of shoots per tree, the number of branches per tree, the dominant height (*H*, cm), the current annual basal area increment (ba_{yr}, cm² tree⁻¹ yr⁻¹) and the mean annual biomass increment per tree (bi_{yr}, kg dm tree⁻¹ yr⁻¹). The letters represent the results of SNK post-hoc comparisons of group means (p- value<0.05). The proportions of *P. alba* are as follows: 0 corresponds to the species composition 0P:100R, 0.25 to 25P:75R, 0.5 to 50P:50R, 0.75 to 75P:25R and 1 to 100P:0R. No data was available for the biomass growth during the first two years.

	Variable	Species	*p*-value	100P:0R	75P:25R	50P:50R	25P:75R	0P:100R	100P:0R	75P:25R	50P:50R	25P:75R	0P:100R
Year 1	Nº Shoots	*P. alba*	0.4790	-	-	-	-	-	3 ± 1	3 ± 1	3 ± 1	3 ± 1	
		R. pseudoacia	0.0696	-	-	-	-	-		3 ± 2	3 ± 1	3 ± 1	3 ± 1
	Nº Branches	*P. alba*	<0.0001	a	a	b	b		16 ± 8	14 ± 7	8 ± 6	8 ± 10	
		R. pseudoacia	0.1450	-	-	-	-	-		9 ± 7	9 ± 6	9 ± 8	7 ± 5
	H	*P. alba*	<0.0001	a	a	b	b		271.81 ± 35.30	279.26 ± 43.81	248.00 ± 50.97	239.07 ± 60.18	
		R. pseudoacia	0.0003		a	b	b	b		213.85 ± 31.76	198.19 ± 40.63	190.41 ± 34.98	182.47 ± 34.21
	ba_{yr}	*P. alba*	0.0288	a	a	ab	b		399.49 ± 140.58	396.67 ± 128.90	352.67 ± 156.47	324.25 ± 161.61	
		R. pseudoacia	<0.0001		a	b	c	c		550.83 ± 201.78	460.03 ± 155.10	376.58 ± 155.36	352.06 ± 116.10
Year 2	Nº Shoots	*P. alba*	0.0504	-	-	-	-	-	3 ± 1	3 ± 1	2 ± 1	3 ± 1	
		R. pseudoacia	0.0015		a	b	b	b		3 ± 1	2 ± 1	2 ± 1	2 ± 1
	Nº Branches	*P. alba*	<0.0001	a	ab	c	bc		82 ± 36	69 ± 34	53 ± 30	63 ± 39	
		R. pseudoacia	0.0023		a	b	b	b		54 ± 22	42 ± 22	38 ± 19	40 ± 17
	H	*P. alba*	<0.0001	a	a	b	b		467.61 ± 51.04	477.40 ± 61.88	424.07 ± 73.91	419.00 ± 87.91	
		R. pseudoacia	0.0003		a	a	a	b		344.93 ± 53.04	327.70 ± 54.72	333.20 ± 46.54	306.12 ± 55.63
	ba_{yr}	*P. alba*	0.6810	-	-	-	-	-	631.94 ± 244.38	613.78 ± 307.05	582.97 ± 248.67	647.08 ± 309.93	
		R. pseudoacia	0.6850	-	-	-	-	-		697.75 ± 292.98	662.32 ± 527.23	630.20 ± 280.55	618.52 ± 269.87
Year 3	Nº Shoots	*P. alba*	0.0336	a	a	ab	a		3 ± 1	3 ± 1	2 ± 1	3 ± 1	
		R. pseudoacia	0.0008		a	b	b	b		3 ± 1	2 ± 1	2 ± 1	2 ± 1
	Nº Branches	*P. alba*	<0.0001	a	a	b	b		43 ± 9	40 ± 10	33 ± 11	35 ± 11	
		R. pseudoacia	0.2490	-	-	-	-	-		34 ± 11	38 ± 12	39 ± 11	36 ± 11
	H	*P. alba*	<0.0001	a	a	b	b		773.15 ± 102.85	777.48 ± 124.08	666.69 ± 166.07	680.04 ± 169.96	
		R. pseudoacia	<0.0001		a	b	b	b		541.93 ± 80.09	497.39 ± 102.13	497.05 ± 69.10	460.62 ± 90.94
	ba_{yr}	*P. alba*	0.0485	ab	ab	b	a		1,592.92 ± 590.59	1,790.37 ± 680.94	1,513.23 ± 1,058.23	1,946.62 ± 1,235.82	
		R. pseudoacia	0.0615	-	-	-	-	-		789.65 ± 619.65	742.56 ± 708.15	524.40 ± 528.50	646.50 ± 451.10
	bi_{yr}	*P. alba*	<0.0001	b	a	b	b		1,077.11 ± 359.41	1,440.31 ± 414.53	1,009.10 ± 682.45	1,005.59 ± 705.03	
		R. pseudoacia	<0.0001		a	b	c	c		1,144.34 ± 540.27	957.40 ± 584.08	757.15 ± 397.80	718.89 ± 394.00

A more in depth analysis of the behaviour of the different species and species compositions (mixtures and monocultures) was performed to evaluate the temporal development during the rotation.

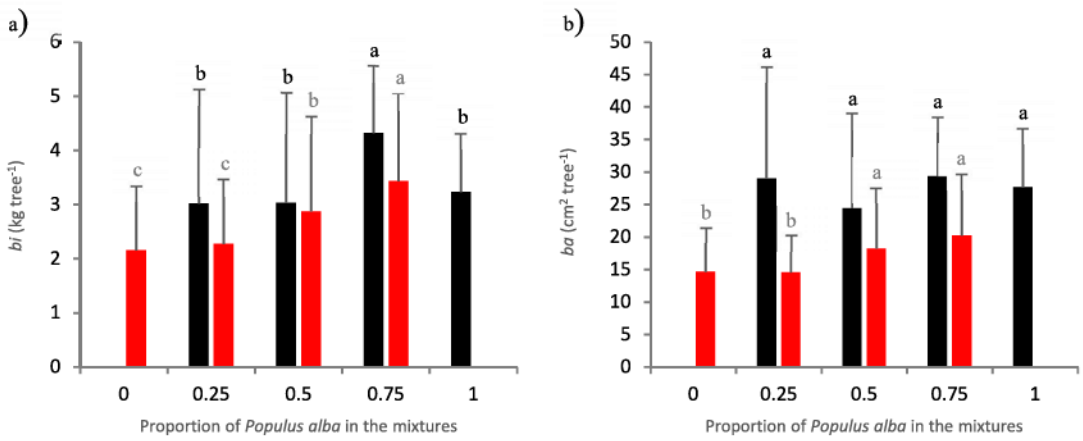

Figure 6.4. Growth at individual tree level, by species, at the end of the rotation in terms of a) biomass (*bi*) and b) basal area (*ba*) per tree. *Populus alba* is represented by black bars and *Robinia pseudoacacia* by red bars. The letters represent the results of SNK post-hoc comparisons of group means (*p*-value<0.05). The proportions of *P. alba* are as follows: 0 corresponds to the species composition 0P:100R, 0.25 to 25P:75R, 0.5 to 50P:50R, 0.75 to 75P:25R and 1 to 100P:0R.

By the end of the rotation, *P. alba* had higher values than *R. pseudoacacia* for all variables except for the number of branches (Table 6.1). For all variables, the differences between species increased over time, except for the number of shoots per tree, for which the relative difference between species remained relatively constant.

At the tree level, there was no significant difference in ba_{yr} for *P. alba* between the mixtures for the second year (*p*-value=0.6810), but there were significant differences in the first year (*p*-value=0.0288) and at the end of the rotation (*p*-value=0.0485). For *R. pseudoacacia,* there were significant differences in ba_{yr} between mixtures only for the first year of the rotation (*p*-value<0.0001). In terms of height, there were significant differences in each year of rotation for *P. alba* (*p*-value<0.0001), with the monoculture (100P:0R) and the 75P:25R mixture showing greater height growth than the 50P:50R and 25P:75R mixtures. For *R. pseudoacacia,* the first and the last years of the rotation presented significantly higher growth in the 75P:25R mixture (*p*-value=0.0003 and *p*-value<0.0001). In the second year however, all the mixtures grew more than the monoculture (0P:100R) (*p*-value=0.0003) (Table 6.1).

To evaluate whether the neighbourhood composition explained the overyielding better than the species composition, the complementarity was evaluated considering the three different within-row neighbourhood situations that were presented under this replacement series experiment. At this tree neighbourhood level within a given row, the positive complementarity effect of *R. pseudoacacia* on *P. alba* only occurred when there was an individual of each species on each side within the same row (neighbourhood situation 2), and as the proportion of *R. pseudoacacia* increased, this complementarity effect disappeared (Fig. 6.5). Notice that

neighbourhood situation 2 is only presented when the percentage of the target species is 25%. However, in the case of *R. pseudoacacia,* the complementarity increases with the presence of *P. alba*. For both species, these patterns of complementarity were stronger for biomass than for basal area.

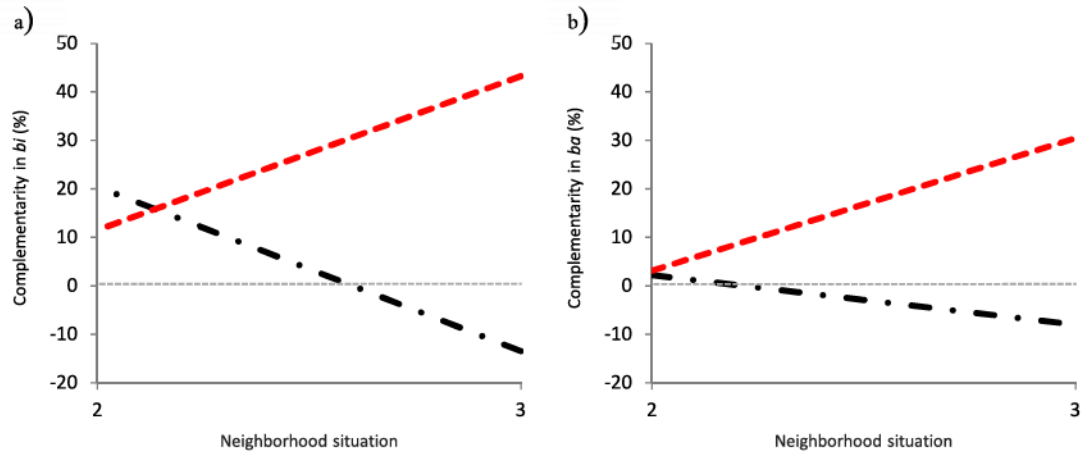

Figure 6.5. Relationship between complementarity (in %) and the neighbourhood situation at the end of the rotation, in terms of a) biomass and b) basal area. Complementarity (%) was calculated using Eq. (5.1), with the mean value per species and situation. *Populus alba* is represented by the black dashed line and *Robinia pseudoacacia* by the red dashed line. Situation 2 corresponds to a tree with a *P. alba* on one side and a *R. pseudoacacia* tree on the other and situation 3 to a tree with the opposite species neighbouring on both sides.

6.4. Discussion

Despite the greater growth often found in mixtures compared to monoculture plantations (Forrester *et al.*, 2005; Pretzsch and Schütze, 2009), there are also some studies in which this benefit has not been detected (Pretzsch *et al.*, 2010; Bouillet *et al.*, 2013). Whether the greater biomass yield can be attained by mixing species in very short rotations and at very high densities, such as in Short Rotation Coppice (SRC) plantations, is of great interest with regard to satisfying the demand for biomass. Our results point to a benefit for both species in terms of stand biomass (*BI*) in the case of the 75P:25R mixture at the end of the first rotation, which resulted in a 26.83% greater yield than that achieved with the more productive species, i.e. *P. alba*, or transgressive overyielding (Pretzsch and Schütze, 2009). This corroborates our main hypothesis that mixtures under high density short rotation plantations can improve productivity.

In the presence of facilitation, as a consequence of nitrogen fixation by *R. pseudoacacia,* a constant or increasingly positive response of *P. alba* would be expected as the proportion of *R. pseudoacacia* is increased (Binkley, 1992; Bouillet *et al.*, 2013). However, while a positive response was observed for the 75P:25R mixture, it was not found for the other mixing proportions. This could be due to confounding effects of competitive interactions associated with *R. pseudoacacia,* which change disproportionately with the nitrogen fixation effect. Competition, competitive reduction, and facilitation mechanisms frequently occur simultaneously (Forrester, 2017) and their relative importance often varies not only with abiotic

conditions (Callaway, 1998; Forrester, 2014; del Río *et al.*, 2014), but also with species proportions (Binkley, 1992). However, no data were available to help decipher the underlying mechanisms.

Therefore, the greater yield obtained in the 75P:25R mixture may not be due exclusively to the influence of the nitrogen fixing species on the non-fixing species, but also to the different competitive interactions occurring between the two species in that mixture. Certain studies have shown that N in the roots of N fixing species can be transferred to the non-N-fixing species in the first or second year (Van Kessel *et al.*, 1994; Parrotta *et al.*, 1996; Khanna, 1997). However, other studies report that the facilitation effect produced by the nitrogen fixing species may not be effective until some years after planting, as suggested in studies concerning tropical *Acacia* and *Eucalyptus* (Bouillet *et al.*, 2008), or *Alnus* and *Pseudotsuga* (Binkley, 1992).

The biomass yield in *P. alba* was only significantly higher than expected, based on monoculture yield, when growing with a small proportion of *R. pseudoacacia*. By contrast, *R. pseudoacacia* always benefited as the proportion of *P. alba* increased, which points to competitive reduction for this species in mixed plantations. A greater presence of *R. pseudoacacia* leads to less height growth in *P. alba* as well as a lower number of branches for all the mixtures except 75P:25R, suggesting that competition for aboveground resources may occur between the two species, with a positive net effect for *R. pseudoacacia*. However, at the end of the rotation, the height of *P. alba* was still higher due to its greater growth rates, as can be seen in the monocultures. Some studies have also found that, on the one hand, height growth of *P. alba* is faster than that of *R. pseudoacacia* when these two species grow together and on the other, that *R. pseudoacacia* is unlikely overgrow *P. alba* in mixed stands (Rédei *et al.*, 2006).

We verified that the design of these plantations (high density but different planting distances between rows and within the rows) had a notable influence on the observed overyielding effects, indicating the importance of analysing mixing effects at different levels, from tree to stand levels (Forrester and Pretzsch, 2015). For example, the neighbourhood level analyses indicate the importance of the within row (nearest neighbour) interactions as opposed to the between row interactions in these high density mixtures. In the 50P:50R mixture, the neighbours on each side of all the trees are of the other species (neighbourhood situation 3), this being the best neighbourhood situation for *R. pseudoacacia* and the worst for *P. alba*. In this mixture, the higher mortality of shoots per tree in the case of *P. alba* along with the lower diameter and height growth points to a clear negative interaction for this species. For the 75P:25R mixture, 25% of the trees are *P. alba* with other *P. alba* trees on both sides (neighbourhood situation 1), 50% of the trees are *P. alba* with another *P. alba* on one side and a *R. pseudoacacia* on the other side (neighbourhood situation 2) and the last 25% are *R. pseudoacacia* with *P. alba* trees on both sides (neighbourhood situation 3), in accordance with the best situation for each species.

This pattern, with the 75P:25R presenting the greatest yield, coupled with the low yield and growth in the 50P:50R mixture, highlights the importance of the spatial pattern beyond the mixing ratio. Therefore, for short rotation mixed plantations for biomass production, it is desirable that the mixing is considered within the same row (where the distance between trees is smaller than that between rows). In any case, the complexity of this design, in which the distance between trees in the same row (0.4 m) is much less than the distance between rows (2.5 m), is increased by the presence of multiple shoots per tree, which makes it even more difficult to analyse the positive and negative effects of the different mixtures.

It is important to highlight the different behaviour observed between the biomass yield and the basal area growth. In contrast to the positive mixing effects on biomass for the 75P:25R mixture (considering together *Populus alba* and *Robinia pseudoacacia*), there was no such effect on the current annual basal area increment at individual tree level (ba_{yr}). Similarly, Sayyad *et al.* (2006) found no significant differences in basal area growth between planted monocultures and mixtures of *Populus* and *Alnus*. Biomass evaluation takes account of all the variables involved in the growth of the tree, whereas the analysis of basal area ignores the height and the number of branches. Species interactions can also trigger differences in the density of wood (Zeller *et al.*, 2017), which would be ignored by basal area analyses. Our results show the importance of analysing biomass yield, since basal area provides biased estimations of productivity in these types of plantations. Accordingly, this suggests the need to develop specific biomass allometry equations for this kind of mixed plantations, which consider the effects of species interactions (Forrester and Pretzsch, 2015).

Different factors, such as the light efficiency derived from the canopy architecture for a given composition, the possible differences in root stratification in the soil profile or the N-effect on both the above- and belowground growth should be studied further in order to gain a better understanding of the behaviour of this mixture. This study illustrates the complexity of the interactions in mixtures in SRC plantations. Furthermore, an evaluation of progressive rotations and the effect of N-fixing species will be required to obtain a more complete impression of any changes in species after the roots are fully developed.

6.5. Conclusions

A clear benefit in terms of biomass per hectare was detected for the mixture 75P:25R at the end of the first rotation. This mixture was the most productive in terms of biomass yield at both tree- and stand- levels. The 50P:50R proportion was less productive. *P. alba* growth was lower for both basal area and biomass at the tree level when the neighbouring trees on both sides were *R. pseudoacacia*. *R. pseudoacacia* benefited from a higher presence of *P. alba*, which suggests that the inter-specific competition from *P. alba* was less intense than the intra-specific competition from other *R. pseudoacacia* trees.

We found a different trend when the response was evaluated using basal area growth compared with biomass production, suggesting that basal area provides biased estimations of productivity in these types of plantations.

The distribution pattern of the species within the row seems to be very relevant. The growth response was positive in the case of the 75P:25R mixture and negative for the 50P:50R mixture, showing that growth was largely affected by competition within the same row and not between rows due to the specific design of these plantations. This suggests that it would be more appropriate to consider the mixing of these species within rows rather than row by row in the case of SRC plantations.

We concluded that SRC mixtures with certain proportions could provide an interesting alternative to SRC monocultures in terms of biomass yield at the end of the first rotation. However, several aspects must be taken into account when designing mixed plantations, not only the complexity of the species interactions, with positive and negative effects acting simultaneously, but also the restrictions in the spatial distribution of trees as a result of the complex establishment of the plantation and the harvesting requirements. Additionally, an evaluation of subsequent rotations must be considered in order to gain a greater insight into the behaviour of the mixtures and the final production which may be achieved with each one.

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CHAPTER

7

Discussion

Discussion

This doctoral research was motivated by the need to optimize and quantify the sustainable production of lignocellulosic biomass in high density short rotation plantations of fast growing forest species under Mediterranean conditions. The optimization process can be studied using different approaches, some of which have been applied in this research. Particular emphasis was placed on the need for more accurate biomass estimates and thus on developing appropriate management tools for this type of plantation. Other aspects which may be of interest in relation to increasing the sustainability of the plantations, such as improving the plantation design and quantifying ecosystem services, were also considered.

In Spain, great advances have been made in recent years in relation to the selection of poplar hybrids grown under intensive management (Sixto *et al.*, 2015; Sixto *et al.*, 2016a), with the aim of improving biomass yields (Pérez-Cruzado *et al.*, 2015). Physiological and molecular characterization of attributes that are relevant to cultivation in marginal zones has been conducted (Sixto *et al.*, 2016b), and advances have been made in the use of biotechnology to develop new materials (Moreno-Cortés *et al.*, 2017), which should lead to increased production in the future. The sustainability of the plantations has been evaluated from different angles (González-García *et al.*, 2014; San Miguel *et al.*, 2015) and progress has been made in regard to characterizing biomass, preferentially for energy production (Monedero *et al.*, 2016; Ibarra *et al.*, 2017).

This thesis reports important information on the factors that affect the modelling of growth and production in this type of plantation. It has emphasized on the differences in performance of the commonly used genotypes in relation to modelling the production of both the above-and below-ground parts and their capacity to fix carbon, as well as in relation to the plot level performance of monoculture plantation designs and of an alternative design consisting of mixtures including a nitrogen-fixing tree species. Advances have been made in elaborating specific management tools for SRC plantations under Mediterranean conditions, at both individual and plot level.

The need for accurate tools for estimating biomass in poplar SRC plantations has been widely studied (Ben Brahim *et al.*, 2000a; Laureysens *et al.*, 2005; Paris *et al.*, 2011; Verlinden *et al.*, 2013a). However, to date the advances made have not been investigated in detail in relation to plantations grown under Mediterranean conditions.

Information about individual tree level biometry is key to developing accurate biomass estimation models. Such information would enable projection of estimates to larger scales such as plantation level. The availability of accurate tools for estimating biomass will enable decisions to be made on the basis of these estimates, with the aim of optimizing the plantations

and achieving maximum benefits by taking into account the economic viability and environmental sustainability.

This thesis takes into account two different approaches. The first part considers individual tree level biometry and provides models for predicting above- and below-ground biomass. The second part focuses on plot level biometry, provides a *reference diagram* applicable at national scale and includes an in depth study of an alternative management design for mixed plantations of poplar and a nitrogen-fixing tree species (*R. pseudoacacia*). Advances have been made in biometry, and new tools are provided for planning and management that can be used to optimize the sustainability of these plantations. Each of these factors will be analysed in further detail below.

The present chapter discusses the results obtained in the different parts of the research. In elaborating this chapter, the findings of each part were considered jointly, with the aim of providing a more in depth, overall analysis of the findings. Thus, in the first subsection of this chapter, we discuss general aspects of SRC. In the second subsection, we discuss the aspects that represent advances in the modelling of biomass production in SRC. In the third subsection we focus on the ecosystem services provided by SRC, with particular emphasis on C sequestration. In the fourth subsection, we propose an alternative management system to traditional SRC monoculture, i.e. mixed plantations including poplar and a nitrogen-fixing tree species. In the final subsection we summarise the future perspectives.

7.1. General aspects of SRC

Obtaining biometric information about SRC plantations is particularly important, as some of the characteristics of these plantations affect the modelling process.

First, as the plantations are managed on short rotations, of between 3 and 4 years, the changes in allometric relationships are greatly affected by the rapid rate of development of these juvenile stages. The distribution of assimilates is therefore strongly affected by ontogenetic factors and by the type of vegetative reproduction generally used to establish these types of plantations (chapter 2). Throughout the life cycle of trees, allometric relationships are modified by changes in distribution of biomass among the individual components (Satoo and Madgwick, 1982). In SRC plantations, the distribution varies with both age and rotation, as the age of the below-ground fraction differs from the age of the above-ground fraction throughout the rotation. The effect of a well developed root system that is capable of absorbing greater amounts of nutrients and water, together with the capacity of the stool to produce multiple shoots, favours enhanced production in successive rotations, up to a certain limit. These characteristics hinder data collection and modelling as they require that all of these combinations of factors must be included in the sample, as considered in chapter 2.

Although in the present study most of the models developed have been fitted to first rotations, in some of the plots the plantation design included the initial coppicing in the first

year after establishment. This initial coppicing was carried out with the aim of stimulating the production of multiple shoots (Willebrand and Verwijst, 1993; Kopp *et al.*, 2001) and thus eventually obtaining higher yields. This type of management is not usual in the plantations sampled in this study, as the benefits obtained by this early coppicing do not outweigh the economic cost. Moreover, the presence of multiple shoots complicates the modelling procedure. Thus, although the use of variables such as the quadratic mean diameter of all shoots provides data on all shoots on a stool that can be included in the model (as explained in chapter 4), inclusion of this variable greatly reduces the practical application of the model. However, as this variable is important in many of the models developed in this research, the total number of shoots per stool was included as an independent variable (chapters 2 and 3).

The plantation design includes different potentially important factors, such as the distribution of the trees in single or double rows, as well as the possible use of more than one species (mixtures) and different spacing designs. In this type of plantation, the density is a specific factor to consider in the modelling process. In the plantations sampled in the present study the density ranged from 5,000 to 33,333 stools ha^{-1}. Despite the high density, no signs of strong competition for resources were observed, and the mortality rate was relatively low (below 15%). Adequate land preparation and the use of irrigation help prevent direct competition for available resources at these early stages. Nonetheless, the effects of competition were observed in the small shoots, and the mortality of these generally increased throughout the rotation (probably due to competition for light), although it was very dependent on genotype (Laureysens *et al.*, 2005).

Irrigation is a relevant factor in this type of plantation growing under Mediterranean conditions, as the availability of water throughout the growth period is one of the main factors with the greatest effect on productivity (Bergante *et al.*, 2010). In general, the availability of water can only be guaranteed under these conditions by the use of irrigation systems. Although Mediterranean conditions generally favour growth, they also imply extreme drought in the summer months. Because of the use of irrigation systems, many of the models used for similar conditions are not applicable to these plantations.

In addition to these specific management factors in SRC plantations, other factors are also important in relation to modelling biomass production, such as e.g. the effects of genotype (Wang, 2006; Dillen *et al.*, 2007), site (Bergante *et al.*, 2010) and the genotype*environment interaction (Benetka *et al.*, 2014; Sixto *et al.*, 2015).

The observed differences in production of above-ground biomass on the basis of genotype in the plantations sampled in the present study, as well as in many other studies (Broeckx *et al.*, 2012b; Euring and Polle, 2016; Vanbeveren and Ceulemans, 2018), highlight the need to develop specific models at the genotype level (chapter 3), owing to the differences in plant architecture and shoot production (Stanturf *et al.*, 2014), among other factors. The wide range of base material available depends on the desired objective facilitates the selection. However, although genotype selection is important as the performance can also be affected in different

ways by both the environment itself and by the genotype*environment interaction (Yu and Pulkkinen, 2003; Zalesny *et al.*, 2009; Sixto *et al.*, 2011; Sixto *et al.*, 2016a).

Regarding the environmental effects, both biotic and abiotic factors must be taken into account. The specific biotic factors that affect production in the plantations include the occurrence of pests and diseases, as well as the presence of competing vegetation (of particular relevance at stand establishment (Sixto *et al.*, 2007)). Moreover, abiotic factors are also essential for understanding tree biometry. The soil acts as a reservoir of water and nutrients and also provides stability to the plant roots, and other factors such as precipitation, light and other climate-related factors interact with the plants and determine their growth (Bergante *et al.*, 2010; Pérez-Cruzado *et al.*, 2013).

Selection of the most appropriate genotypes with the aim of producing biomass in different environments remains a long-term goal. The effect of the genotype*environment interaction has been investigated in different studies (Yang *et al.*, 2004; Sixto *et al.*, 2015) and is considered one of the key factors in the sustainable development of SRC plantations. Some genotypes that provide more stable responses have been identified. From a management perspective, this concept encompasses the capacity of a cultivar to perform according to the productive potential of each environment, i.e. without departing from the expected behaviour estimated from its average genotypic value (Becker and Leon, 1988). Thus, even for these genotypes, an extremely high level of variability in productivity is observed throughout the Iberian Peninsula. This factor should also be taken into account when interpreting the predictions, although in some cases the effect has been minimized by using extensive databases, enabling the variability to be taken into account during model development.

The accuracy of predictive models should be maximized, and any potential bias should be minimized, with the final aim of providing predictions that are as realistic as possible. The use of these models goes beyond the simple estimation of yields. They are also of interest for planning future plantations and for making decisions about management of these (see chapter 5), and they can also be used in planning costs to adjust the final profitability. Furthermore, they are also valuable for evaluating the economic sustainability (Faasch and Patenaude, 2012). They can even be used to assess the environmental sustainability in many cases, as they enable quantification of some ecosystem services, previously not taken into account in sufficient detail (Jose, 2009; Berhongaray and Ceulemans, 2015).

Valorisation of ecosystem services provided by this type of plantation is increasingly important and is considered an improvement in optimization of the crop for the added external benefits, by inclusion of services such as regulation of clean air, erosion control and flooding control (in some zones), as well as increased biodiversity or climate change mitigation. Thus, although SRC plantations have an evident commercial objective, their mere existence and use promote the generation and consumption of renewable energy. Despite the intensive production schemes, these plantations generate ecosystem services derived from carbon sequestration and substitution of fossil fuel emissions. Carbon quantification (Verlinden *et al.*, 2013b) is a good

example of the use of the models for quantifying ecosystem services. In order to calculate the total amount of carbon in the plantation, and specifically the soil organic carbon (SOC), it is important to take into account not only litterfall and turnover, but also to quantify the biomass accumulated in the below-ground fraction (chapter 4). The models are especially important in relation to the below-ground parts, due to the difficulty in the direct estimation of this component.

7.2. Advances and improvements in modelling methods

Empirical models are generally considered sufficiently accurate for well-defined conditions (Verwijst and Telenius, 1999), although they show some limitations in relation to short rotation crops as they do not take into account some potentially important features. Thus, the research reported in chapter 1 evaluated the uncertainty associated with parametrization of these models and identified some of the factors affecting the modelling of short rotation crops, with the aim of assessing the suitability of the models for biomass estimation in this type of plantation.

Age had a significant effect on parameter estimation, and the effect was stronger after coppicing. The assumption of a constant ratio between biomass and diameter, which is often used in model fitting, cannot be applied to these young plantations as the changes in allometry with stage of tree development are particularly rapid at the initial stages, as pointed out by other authors Saint-André *et al.* (2005) and Antonio *et al.* (2007). The uncertainty is particularly high in the first year after harvesting and decreases as the rotation reaches its end. The results obtained indicate that the data for the year following coppicing should be excluded from biomass estimation models, as the inclusion of these data increases the associated uncertainty.

The variables that are included in the model also influence parameter estimation. We found that the variability is lower when certain variables related to the number of shoots per stool are included or when specific formulations that include the age are used, e.g. models in which age is included as a dummy variable. These models are more robust for making predictions for the different years of the rotation, not only the year of harvesting. In addition to the model used or the regression method selected, the sample size also influences the parameter estimation. According to the results obtained for genotype 'I-214', the minimum sample size required to minimize the error is 50 trees. As expected, the accuracy of the estimations increased with the sample size. However, for sample sizes greater than 50 trees, the errors occur within the 95% confidence interval for estimation of all data, and we therefore established this as the minimum sample size required.

The large variability in parameter prediction of biomass estimation models, which is further enhanced by the successive rotations of SRC plantations, must be taken into account in developing biomass estimation models. Therefore, depending on the final aim, specific methods, variables, and the sample selection criteria as a function of the stage of development that are consistent with the aim should be chosen. As shown in chapter 1, the high degree of

variability in parameter estimation gives rise to greater or lower accuracy and bias. Therefore, the factors that affect the estimation must be taken into account in interpreting the estimates provided by the models fitted. After all, the models provide estimates that are as realistic an approximation as possible of what may be encountered in the field.

Taking into account the influence of age in the variability of parameter estimation, in the following chapters only the data from the final years of the rotation were used. The end of the rotation is the period of interest for estimating biomass stock, and therefore consideration of only the final years of the rotation does not diminish the practical utility of the models.

Model accuracy is also determined by the specificity. Although the use of valid models for various genotypes implies a certain loss of accuracy, the applicability of the models is a key factor. Thus, models are not only developed at genotype level (considering different sites), but also at taxonomic group level and general (genus *Populus* spp.) level (considering different sites and different genotypes). The general availability of this type of tool for biomass estimation is of great use, as already mentioned the loss of accuracy may be considerable. In order to reduce the loss of accuracy, a large database (11,265 data) covering a total of 22 different sites and 29 different genotypes belonging to 7 taxonomic groups were considered.

Models were developed at taxonomic group level for the Mediterranean zone, as this scale has frequently been used for assessing biomass production in other areas (Dillen *et al.*, 2007; Verlinden *et al.*, 2013a). However, the results reported in chapter 3 shown that we have not found similarities in biomass allometry at this level, and therefore this is grouping is not suitable for explaining the variation in biomass allometry. Indeed, although the best results were obtained using genotype-level models, due to the marked differences in biomass allometry of the above-ground parts, if the information required is not available or a joint estimate for different genotypes is required, use of the generalized model is often preferred to the use of specific models for the taxonomic groups.

The effects of genotype and of the environment on production of above-ground biomass in SRC plantations have been widely documented (Dillen *et al.*, 2007; Benetka *et al.*, 2014), even under Mediterranean conditions (Bergante *et al.*, 2010; Sixto *et al.*, 2015). However, information regarding root biomass is very scarce (Coyle and Coleman, 2005; Berhongaray *et al.*, 2015; Jha, 2017). In the studies carried out in this doctoral thesis, the differences in the woody fraction in relation to genotype were not evident in the roots, although the environment clearly influenced this fraction.

One of the main characteristics of the study plots was the greater development of the above-ground fraction than of the roots. Thus, the root:shoot ratios were lower than reported for other parts of Europe (Friend *et al.*, 1991; Berhongaray *et al.*, 2015), except in some Mediterranean zones, where the ratios were quite similar to those observed in the present study (Calfapietra *et al.*, 2003). This finding can be attributed to many different factors, including stand age, as the root:shoot ratio tends to decrease exponentially with stand age, although the similarities to other

Mediterranean zones indicate that one of the most plausible explanations is the climatic conditions in the study area and the consequent need for irrigation. As water is supplied (by irrigation) throughout the vegetative growth period, the roots do not need to extend very far to obtain water, and the plants can invest a greater proportion of the resources available in developing the above-ground fractions. Other hypotheses indicate that development of the root fraction is restricted by the limited space due to the high plantation density, as explained in chapter 4.

As in chapter 3, chapter 4 describes the models developed for estimating the below-ground biomass at different levels: a general model and genotype-level models. Measurement errors must be considered as an additional level of uncertainty in these models as measurement of this fraction is less accurate than measurement of the above-ground fraction due to the complexity of extracting the root fraction.

The availability of management tools, as reported in chapters 3 and 4, is a key factor in managing this type of plantation. The models developed enable calculation of existing stocks, and thus facilitate decision-making about e.g. the duration of the rotation and the benefits that can be obtained at different times depending on the market demands. Although knowledge of individual tree-level biometry is essential to enable these calculations to be made, in practice determining the stock at plot level is of greatest interest because of the variability between trees, between environments, between genotypes and because of the effect of the genotype*environment interaction. Therefore, in chapters 3 and 4 plot level estimates were made from tree level estimates.

Regarding the practical application of the models at plot level, a tool commonly used in the forest sector for managing the density of different stands was adapted for the study plots, as reported in chapter 5. Density management diagrams are generally used with the aim of managing stands, particularly the density of the stands. However, the concept was adapted with the overall aim of managing the poplar plantations on short rotations, with the aim of planning the harvesting time as well as prior planning of the plantation by using a *reference diagram* to relate final biomass production and density, basal quadratic mean diameter and height, with the aim of indirectly including the age.

The tool developed can be used in a simple procedure to plan and manage short rotation poplar plantations in relation to the final objectives desired. As this tool was developed using a large network of highly heterogeneous plots, it is applicable at national level. Use of the model provides biomass estimates, and the simple equations provided in the chapter also enable calculation of the % ash content or even the final amount of energy obtained, which could be used in negotiations at the harvesting moment.

7.3. Ecosystem services: quantification of accumulated carbon

Valorisation of ecosystem services increases the value of forest plantations and favours the sustainability of these. To date, quantification of the products obtained in SRC plantations has been limited to the final biomass stock. However, the calculations should go beyond quantifying the biomass or the future potential for making bioproducts of interest, and all of the services provided by the plantations throughout the rotations should be taken into account. In this thesis we have made an initial approach of one of the services that has attracted most attention to date, i.e. accumulation of carbon as a means of decreasing CO_2 emissions. However, many other ecosystem services have not yet been considered and should be quantified in the near future with the aim of making final calculations regarding the crop (Townsend *et al.*, 2018).

Quantification of many of these services requires information about the biomass of each of the different fractions. For this purpose, as we have seen in previous sections, prediction models are fundamental. However, some fractions are more difficult to evaluate than others. Quantification of root biomass is difficult, mainly due to the difficulty in extracting this fraction, which is, moreover, much smaller than the above-ground fraction. Both the direct extraction and estimation based on predictive models have drawbacks due to breakage of the roots and the difficulty in removing all of the fraction by excavation (Clark *et al.*, 2001). In chapter 4, we estimated that the amount of carbon accumulation was around 4.3-6.4 Mg C ha^{-1} yr^{-1} in the above-ground woody biomass and around 0.89-0.91 Mg C ha^{-1} yr^{-1} in the below-ground biomass.

The below-ground biomass fraction contributes, along with litterfall, to the accumulation of carbon in the soil (Pacaldo *et al.*, 2014). The availability of these values, obtained from the previously described models, is of great use for analyzing the total carbon sink associated with SRC plantations. It has previously been assumed (almost by intuition) that the carbon balance in this type of plantation is neutral. However, recent studies aimed at the objective quantification of biomass indicate negative balances, where the inputs of carbon are greater that the outputs as the rotation proceeds (Verlinden *et al.*, 2013b; Ceulemans *et al.*, 2018).

7.4. Alternative design: mixed plantations

Alternative plantation designs, i.e. mixed plantations, can be used as a means of increasing biomass production. These designs are based on strategies of complementarity and/or facilitation between species. If one of the species used is also a nitrogen fixing species, the impact on the sustainability of the crop due to the reduction in the need for fertilizer should justify the use of this approach.

In the forestry sector, interest in mixed plantations has increased in recent years because of the expected benefits (Forrester *et al.*, 2006). However, implementation of this type of plantation under short rotation at high density has not been explored sufficiently. The possibility of increasing production by establishing mixtures rather than the commonly used monocultures

also provides many other environmental benefits. Although we did not observe the expected facilitation effect, which should lead to an increase in biomass production due to the presence of a nitrogen-fixing species (*Robinia pseudoacacia*) in the mixture, one of the proportions of the mixture used (75P:25R) had a positive effect on the final production. This was quantified as a yield increase of 26.83% relative to *Populus alba* monoculture and of 89.99 % relative to *Robinia pseudoacacia* monoculture. The increased biomass production in this proportion of mixture may be explained by the distribution of trees within each row, resulting from the particular design of the SRC plantations, with the distribution pattern in this proportion being most favourable. Other factors such as stratification of the roots or the efficiency of light capture due to differences in crown architecture are plausible explanations, although they cannot be confirmed with the data available. However, the results of a preliminary analysis indicate that the benefit is not maintained in successive rotations (Oliveira *et al.*, 2018). The results of other studies involving longer rotations (Marron *et al.*, 2018) also indicate negligible increases in production. This phenomenon may be explained by the simultaneous occurrence of different competitive interactions (competition, reduced competition and facilitation) (Forrester, 2017), together with the environmental effects and even the importance of the plantation design in mixed stands (distribution of the trees) outlined in chapter 6. On the other hand, this type of plantation may be considered as an alternative design for marginal land in which the nutritional characteristics of the soil are not optimal and where the inputs of nitrogen derived from the presence of *Robinia pseudoacacia* may be maximal (Bouillet *et al.*, 2013; Epron *et al.*, 2013).

Despite the effects made in recent years to evaluate the different alternatives, much more information is required about this type of design to enable valid conclusions to be reached.

7.5. Future perspectives

The EU has increased the target for the amount of energy produced by renewable resources, establishing that it should reach 32% by 2030, with consumption of forest biomass representing 40% of the total renewable resources. This appears to indicate positive expectations for this market. However, many points remain to be resolved before renewable resources will become profitable. In Spain in particular, the price of biomass is much lower than in surrounding countries, so that some of the biomass is being exported.

In relation to modelling, the inclusion of effects derived from the genotype*environment interaction will enable much more accurate estimates of the available stocks to be made and thus more reliable planning and management to be carried out. One of the main strengths of SRC biomass is that is a highly manageable resource, and therefore any improvements in the weak points in the management will be priority targets.

The means of incorporating some of the factors considered in this research in the overall prediction involves the use of models that combine the best features of empirical and of process models. The suitability of hybrid models that combine the advantages of process models (Korzukhin *et al.*, 1996; Johnsen *et al.*, 2001) and empirical models (Bruce and Wensel, 1988;

Barrio-Anta *et al.*, 2008) is widely supported (Mäkelä *et al.*, 2000; Landsberg, 2003; Valentine and Mäkelä, 2005; Pinjuv *et al.*, 2006). The inclusion of ecophysiological variables that enable prediction of individual tree level biometry under particular conditions, and improvements in determining the most important variables are some of the future objectives of modelling SRC plantations under Mediterranean conditions. In addition, as water restrictions represent a real threat, this factor is also being considered in the development of models that enable simulation of different climate change scenarios.

Regarding the effects on production of modifying the plantation design, although the preliminary results of using mixtures do not appear particularly encouraging, many different alternatives remain to be explored. Moreover, the application of different types of fertilizers, the utilization of sewage sludge and the search for more efficient materials regarding water use are some examples of fields where advances can be made. Moreover, although improvement of the plant material is one of the most widely studied factors, further advances are expected as a result of the use of new molecular technologies (genome editing, linkage maps etc.) aimed at producing material that is resistant to pests and diseases, tolerant to drought or high salinity or that can adapt better to specific soil and climate conditions (Clifton-Brown *et al.*, 2019).

Finally, the process of quantifying ecosystems is still being developed, particularly the quantification of the economic repercussions. Ecosystem services are likely to become more important in the near future, and the potential for increasing the sustainability and profitability of SRC plantations is encouraging. As indicated throughout this thesis, considerable advances have been made regarding the information obtained and the improvements in the sustainability of these plantations. However, the continuous improvements in many of the disciplines involved and the continuous environmental changes that imply the need for constant adaptation suggest that sustainable development of these plantations remains a distant goal. Nonetheless, the development of new technologies contributes to indicating an encouraging future for high density short rotation plantations within the context of the use of vegetation in developing a new bioeconomy.

CHAPTER

8

Conclusions

Conclusions

There is a need to evaluate the viability of high-density short rotation forest crops for biomass production in different areas. This thesis addresses the requirement to optimize and quantify the sustainable production of lignocellulosic biomass obtained through the cultivation of fast-growing forest species, particularly poplar, under Mediterranean conditions.

Biometry was evaluated at individual tree level and at plot level, employing modelling as the general framework and considering aspects linked to the design, management, sustainability and ecosystem services that these plantations provide. The conclusions drawn in chapters 2-6 are summarized below:

Chapter 2

- These plantations present certain peculiarities that highlight the need for *clear modelling purpose* and a *sample selection criterion* to be established prior to the development of the estimation model.

- The wide variability found in the parameter estimation can be approached from different perspectives depending on the final purpose of the model.

- The *years after harvest* have a notable influence on the allometry. Additionally, the inclusion in the model of certain variables such as *'number of shoots per stool'* reduces the uncertainty in the models.

- The minimum sample size needed in the development of the model to reduce the uncertainty is set at *50 trees*.

Chapter 3

- The availability of specific above-ground biomass estimation models for Mediterranean conditions makes it possible to estimate biomass yield in a more precise way, hence models have been provided at *different hierarchical levels*.

- The allometric relationships between the different genotypes *are not related to the taxonomic group* to which they belong. Therefore, this level will not always be the most suitable where genotype level information is not available; the general model being better than the taxonomic group model in most of the cases analysed.

Chapter 4

- The availability of specific estimation models for below-ground biomass in irrigated plantations facilitates the estimation of carbon accumulated in the biomass remaining in the soil.

- In poplar SRC plantations under Mediterranean conditions, while the below-ground biomass provided similar or even lower values in comparison to other European data, the above-ground biomass is clearly higher than the average, with root:shoot ratio values being lower than those reported in other European areas except for the Mediterranean, for which they are very similar.

- The average carbon accumulated in the above-ground biomass after one rotation is around 5 Mg C ha^{-1} yr^{-1}, while in the below-ground biomass it is about 1 Mg C $ha^{-1}yr^{-1}$.

- The availability of these models is a key to quantifying sequestered carbon, which is one of the ecosystem services provided by these plantations.

Chapter 5

- The application of estimation models at plot level through a *reference diagram* is a tool of particular interest both for planning density and for calculating the optimal harvesting time.

- The rapid, visual results obtained from the *reference diagram*, such as the mean square diameter, the height, the biomass obtained, or the final density facilitate decision-making.

Chapter 6

- The evaluation of an alternative design in order to optimize and increase both the sustainability and the final production of these plantations by mixing *Populus alba* with a nitrogen-fixing species such as *Robinia pseudoacacia* has revealed that the mixture 75P:25R results in an increase in biomass of 26.8% with respect to monoculture production in the first rotation.

- The arrangement of the trees within the same row seems to be a key factor, due to the specific distribution of this type of design.

- However, a clear facilitating effect was not identified in the rest of the mixtures.

This thesis provides updated modelling tools for poplar short rotation coppice plantation management under Mediterranean conditions. In some cases, we also consider their usefulness for the quantification of ecosystem services, which will be essential in the short term to evaluate

the sustainability of this type of plantations. The constant search for improvements in sustainability is not only reflected in this thesis in the evaluation of genotype productivity, the choice of planting density or crop rotation, but can also be observed in the evaluation of an alternative designs. Mixtures provide benefits not only at productive level but also at environmental level by increasing biodiversity for example or reducing the need for nitrogen fertilization.

Although much has already been achieved through research into this type of plantations, there is still a long way to go and the advances obtained in this doctoral thesis will be very useful.

CHAPTER

9

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High density short rotation coppice (SRC) crops constitute a specialised option for supplying biomass destined for the production of renewable energy as well as other bioproducts within the context of the circular bioeconomy. The objective of this thesis is to provide detailed information on the factors affecting the process of modelling production in SRC poplar plantation, the differences in the process related to differing performance of the genotypes commonly used in these plantations, the biomass production from the woody above-ground and below-ground parts, and the carbon-fixing capacity of the latter. Furthermore, the productive performance of monoculture plantations is evaluated at stand level and compared to that of mixed plantations including a nitrogen-fixing tree species as an alternative to monoculture plantations. The advances attained are reflected in the development of specific management tools for these plantations under Mediterranean conditions at both individual tree level and plot level.

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