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SUSTAINABLE FOREST MANAGEMENT IN POPLAR PLANTATIONS: FOREST HEALTH AND BIODIVERSITY CRITERIA

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Jorge Martín García

PhD Director Dr. Julio Javier Diez Casero (UVA-ETSIIAA, Spain)
PhD Co-director Dr. Hervé Jactel (INRA-Pierroton, France)

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GESTIÓN FORESTAL SOSTENIBLE EN PLANTACIONES DE CHOPO: CRITERIOS DE SANIDAD Y BIODIVERSIDAD

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Dirigida por:

Director Dr. Julio Javier Diez Casero
Co-director Dr. Hervé Jactel

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Contents

Abstract

Martín-García, J. 2012. Sustainable Forest Management in poplar plantations: forest health and biodiversity criteria.

Sustainable Forest Management (SFM) emerged as a response to an increase in awareness of the deterioration of the environment and, in particular, an alarming loss of forest resources. The implementation of SFM is generally achieved through criteria and indicators (C&I), and although different sets of C&I have been implemented according to country, all of them agree that forest health and biodiversity criteria must be keystones in SFM. The objective of this thesis was to test the current indicators and search for new indicators of forest health and biodiversity criteria in poplar plantations, which may be able to assess the sustainability of changing ecosystems. Towards this purpose, a wide variety of both poplar plantations and the remnant patches of natural riparian forests were surveyed. The results suggest that both abiotic and biotic stresses are associated with forest health in monoclonal poplar plantations and may therefore be used as indicators. Although no dramatic phytosanitary problems have been displayed in these poplar plantations so far, the outcomes seem to suggest that soil properties, and mainly coarse fragments, determine tree vitality. Hence, the use of classification functions developed from soil properties would be desirable to ensure that new plantations will be established on suitable lands. In addition, an assessment of 1) digital crown transparency by CROCO software, 2) *Sesia apiformis* population by a pheromone trapping system or 3) endophytes fungi may be used to evaluate the sustainability in poplar plantations. On the other hand, the studies carried out through three different taxonomic groups, including bird, vascular plant and fungi, showed marked differences on species, structural and functional diversity between poplar plantations and native riparian forests. Nevertheless, a suitable management at local level (minimising the use of harrowing) and at landscape scale (increasing the percentage of poplar plantations in relation to other crops) may favour vascular plant and bird diversity in poplar plantations. The results indicate that these taxonomic groups may be used as indicators of biodiversity for SFM in poplar plantations.

Keywords: Sustainability, *Populus*, indicator, biotic, abiotic, nutrients, pest, crown condition, endophyte, bird, vascular plant.

Resumen

Martín-García, J. 2012. Gestión Forestal Sostenible en plantaciones de chopo: criterios de sanidad forestal y biodiversidad.

La Gestión Forestal Sostenible (GFS) nació como respuesta al incremento de la conciencia ambiental de la sociedad ante el deterioro del medio ambiente y, en particular, de la alarmante pérdida de recursos forestales. La implementación de la GFS se consigue a través de criterios e indicadores (C&I), y aunque diferentes conjuntos de C&I han sido desarrollados según el país, todos ellos están de acuerdo en que los criterios de sanidad forestal y biodiversidad deben ser piezas claves en la GFS. El objetivo de esta tesis fue evaluar algunos de los actuales indicadores y buscar otros nuevos dentro de los criterios de sanidad forestal y biodiversidad, los cuales puedan ser capaces de evaluar la sostenibilidad de ecosistemas cambiantes. Para este propósito, se muestrearon una amplia variedad de plantaciones de chopo junto con bosques de ribera existentes en el área de estudio. Los resultados sugieren que tanto factores abióticos como bióticos condicionan la sanidad forestal en las plantaciones monoclonales de chopos y por lo tanto podrían ser usados como indicadores. Aunque no se han encontrado graves problemas fitosanitarios en estas plantaciones hasta la fecha, los resultados parecen sugerir que las propiedades de suelo, y principalmente los elementos gruesos, determinan la vitalidad de los árboles. Por eso, sería aconsejable la utilización de las funciones de clasificación desarrolladas a partir de las propiedades de suelo para asegurar que las futuras plantaciones se establezcan en terrenos aptos para la especie. Además, (1) la transparencia de copa calculada mediante el software CROCO, (2) las poblaciones de *Sesia apiformis* estimadas mediante un sistema de trampeo con feromonas o (3) los hongos endófitos, podrían ser utilizados para evaluar la sostenibilidad de las plantaciones de chopo. Por otro lado, los estudios llevados a cabo en tres grupos taxonómicos diferentes, incluyendo hongos, aves y plantas vasculares, apuntaron notables diferencias en la diversidad de especies, la estructural y la funcional, entre plantaciones de chopo y bosques de ribera. Si bien, un manejo adecuado a nivel de parcela (minimizando el número de gradeos) y a nivel de paisaje (incrementando el porcentaje de plantaciones de chopo en detrimento de cultivos agrícolas) podría favorecer la diversidad de aves y plantas vasculares en las plantaciones de chopo. Los resultados indican que estos grupos taxonómicos podrían ser usados como indicadores de biodiversidad para evaluar la GFS in plantaciones de chopo.

Palabras clave: Sostenibilidad, *Populus*, indicadores, bióticos, abióticos, nutrientes, plagas, condiciones de copa, endofitos, aves, plantas vasculares.

LIST OF ORIGINAL ARTICLES

The thesis consists of the following articles, which in the text will be referred to by their Roman numerals.

I. Martín-García, J. & Diez, J.J. (2012) Sustainable forest management: an introduction and overview. In: *Sustainable forest management – current research*, J. Martín-García & J.J. Diez (Eds.), 3-16 (454 pp). InTech, Rijeka (Croatia). ISBN 978-953-51-0621-0.

II. Martín-García, J., Díez, J.J. & Jactel, H. (2009) Towards standardised crown condition assessment in poplar plantations. Annals of Forest Science 66: 308-314.

III. Martín-García, J., Merino, A. & Diez, J.J. (2012) Relating visual crown conditions to nutritional status and site quality in monoclonal poplar plantations (*Populus x euramericana*). European Journal of Forest Research 131: 1185-1198.

IV. Martín-García, J., Jactel, H. & Diez, J.J. (2011) Patterns and monitoring of *Sesia apiformis* infestations in poplar plantations at different spatial scales. Journal of Applied Entomology 135 (5): 382-392.

V. Martín-García, J., Espiga, E., Pando, V. & Diez, J.J. (2011) Factors influencing endophytic communities in poplar plantations. Silva Fennica 45 (2): 169-180.

VI. Martín-García, J., Müller, M. & Diez, J.J. (2012) ITS-based comparison of endophytic mycota in twigs of native *Populus nigra* and cultivated *P. x euramericana* (cv. I214) stands in Northern Spain. Annals of Forest Science 69: 49-57.

VII. Martín-García, Barbaro, L., Diez, J.J. & Jactel, H. (2012) Contribution of poplar plantations to bird conservation in riparian landscapes. (In prep. to be submitted to Biodiversity and Conservation)

VIII. Martín-García, J., Jactel, H. & Diez, J.J. (2012) The role of poplar plantations on vascular plant diversity in riparian landscapes. (In prep. to be submitted to Biodiversity and Conservation)

INTRODUCTION

Sustainable Forest Management

The concept of forest sustainability is not a new concept and dates from centuries ago (Wiersum, 1995), although the understanding of sustainable forest management (SFM) as an instrument that harmonizes ecological and socio-economic concerns is relatively new. The change in perspective occurred at the beginning of the 1990s in response to an increased awareness of the deterioration of the environment, in particular of the alarming loss of forest resources. Nevertheless, although the rate of deforestation and loss of forest has slowed down from 16 million hectares per year in the 1990s to around 13 million hectares per year in the last decade (FAO, 2011), the loss of forest varies according to the region. While the forest area in North America, Europe and Asia has increased in the past two decades (1990-2010), it has decreased in other regions such as Africa and Central and South America, and to a lesser extent Oceania. Nevertheless, other statistics such as the disturbing decrease in primary forests[1] (40 million hectares in the last decade) and the increase in planted forests (up to 7% of the world's forests) (FAO, 2011) appear to indicate that to achieve forest sustainability, we must go beyond analysis of the changes in the total forest area worldwide.

The implementation of SFM is generally achieved using criteria and indicators (C&I). Criteria are categories of conditions or processes whereby sustainable forest management can be assessed, whereas quantitative indicators are chosen to provide measurable features of the criteria and can be monitored periodically to detect trends (Brand, 1997; Wijewardana, 2008) and qualitative indicators are developed to describe the overall policies, institutions and instruments regarding SFM (Forest Europe, 2011).

The popularity of the C&I system is evident from the effort invested in its development in recent decades and from the large number of countries that are implementing their own sets of C&I within the framework of the nine international or regional process (African Timber Organization [ATO], Dry Forest in Asia, Dry Zone Africa, International Tropical Timber Organization [ITTO], Lepaterique of Central America, Montreal Process, Near East, Pan-European Forest [also known as the Ministerial Conference on the Protection of Forest in Europe, MCPFE] and Tarapoto of the Amazon Forest). Nevertheless, although different sets of C&I have been implemented according to country, all of them agree that forest health and biodiversity criteria must be keystones in SFM.

In addition to the efforts of different states to develop C&I in the last two decades, a parallel process has been developed to promote SFM at a commercial level. This

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[1] Forest of native species where there are no clearly visible indications of human activities and the ecological processes have not been significantly disturbed (FAO, 2010)

process is termed "forest certification". Forest certification can be defined by a voluntary system conducted by a qualified and independent third party who verifies that forest management is based on a predetermined standard and identifies the products with a label. The standard is based on the C&I approach and the label, which can be identified by the consumer, is used to identify products.

Forest certification was designed as a tool whereby everyone should benefit (win-win situation): forest owners should have an exclusive market with premium prices; the forest industry should improve its green corporate image, should not be held responsible for deforestation, and should have a marketing tool available; consumers should be able to use forest products with a clear conscience; and overall, forests should be managed sustainably.

Forest health

The term "forest health" grew in popularity in the 1980s and 1990s, becoming a relevant issue for everybody (foresters, researchers and society at large). Nevertheless, although countless definitions have been provided, a consensus has not been finally arrived at. Kolb *et al*. (1994, 1995) opened Pandora's box in trying to clarify the concept "forest health" using utilitarian and ecosystem perspectives. Since then, an intense public debate has taken place (Staebler, 1994; DellaSalla et al., 1995; DellaSalla & Olson, 1996; Jenkins, 1997; MacCracken, 1997; LaSalle, 1998; Abrams et al., 2005; Warren, 2007; Marshall, 2009; Raffa et al., 2009; Turner, 2009), but there remains no universally accepted definition of forest health.

Beyond this controversy, forest health is being monitored practically all over the world through forest health monitoring programs. Thus, a forest health monitoring program has been carried out in Europe since the 1980s within the International Co-operative Programme, ICP Forest, as a result of several reports of declining forest in some parts of Europe. However, the ability of such a systematic network to capture the real health condition of the European forest has been questioned for several reasons. Firstly, a systematic sampling design based on a 16 by 16 km grid (more than 6,100 plots) is not able to monitor those small and fragmented forest types (Innes, 1988, 1993; Ferretti, 1997). This is the case of poplar plantations, which cover almost a million hectares in Europe and yet there are only 24 plots of *Populus* hybrids monitored within the European Level I Network (Fisher *et al*., 2010). Secondly, a common sampling intensity is utilised for all types of forests, but poplar, willow and eucalyptus plantations are often monoclonal, and protocols developed to assess health conditions in other types of forest may not be relevant. In particular, as the variability of traits between trees is lower, one can assume that tree clones will be similarly damaged (Braganca *et al*.; 1998; Camps, 2001) and a different sampling design, based on smaller samples of trees, would be desirable for these types of forests. Finally, quality assurance of several variables such as crown transparency and discoloration, among others, remains questionable, in spite of the fact that a considerable effort has been devoted to improve the assessment of these variables (Ferretti, 1997). Traditionally, these variables are visually estimated by observers from the ground, which implies a degree of subjectivity that impedes a proper comparison between countries and even through time within the same country (Innes, 1988, 1993; Mizoue & Dobbertin, 2003). The sources of error in the quantitative assessment of crown condition are many, including variation in the expertise of observers, weather conditions, crown appearance, tree species, tree age and social position (Innes, 1988, 1993; Innes *et al*., 1993; Ghosh *et al*., 1995; Solberg & Strand, 1999; Wulff, 2002; Redfern & Boswell, 2004).

Nevertheless, in spite of the problems listed above, indicators of Sustainable Forest Management developed by Pan-European Process (MCPFE) to assess its criterion "forest health and vitality" were essentially based on this health monitoring program and therefore it should be thoroughly re-examined. Thus, taking into account the fact that the health of forests can be subjected to numerous abiotic and biotic stressors (Ferretti, 2004) and the peculiarities of poplar plantations, the current indicators, which gauge the sustainability of this type of plantation, should be assessed and new indicators identified. This is even more urgent now that natural disturbances that have an impact on the health of forests will be altered by climate change (Kirilenko & Sedjo, 2007; Woods *et al*., 2010).

Biological diversity

The term "biodiversity", a contraction of "biological diversity", has increased in popularity within the last two decades since the Conference on Environment and Development held in Rio de Janeiro, Brazil, in 1992 (the so-called Earth Summit). Although countless of definitions can be found from the literature (Wilson, 1988; Reaka-Kudla *et al*., 1996; Maclaurin & Sterlny, 2008), the most broadly accepted is that reached at the Earth Summit "*the variability among living organisms from all sources including terrestrial, marine and other aquatic ecosystems and ecological complexes of which they are a part: this includes diversity within species, between species and of ecosystems*".

As with its definition, there are many different classifications for the subdivisions of biodiversity within the literature. Carnus *et al*. (2006) established four components of biological diversity of particular relevance on planted forests; (i) genetic diversity defined as the genetic variation within a population or a species, (ii) species diversity understood as the frequency and diversity of different species in a particular area or community, (iii) structural diversity at both horizontal and vertical level and (iv) functional diversity interpreted as the variation in functional characteristics of trees and other plant species.

It is well known that biodiversity provides numerous benefits for society, such as key natural resources for our survival, sources of medicine, maintenance of ecological processes or improvement of air and water, among others. Nevertheless, even nowadays an alarming loss of biodiversity is still taking place all over the world and mainly in tropical forests (FAO, 2010). Furthermore, although it is likely that one of the greatest threats to biodiversity is climate change (Oman *et al*., 2009; Dawson *et al*., 2011; Urban *et al*., 2012), other challenges such as economic growth should not be underestimated. The current growth in the world population (now > 7,000 million people) and the high economic growth of developing countries will lead to greater use of natural resources, which may lead to a higher loss of biodiversity. Hence, although several studies have concluded that there is no conflict between economic growth and biodiversity (Dietz & Adger, 2003; Fuentes, 2011), this aspect should be taken into account for future policies.

Fortunately, nowadays the importance of maintaining biodiversity is not just of interest to researchers, but to society at large. Thereby, new environmental and forest policies are being designed to protect biodiversity, which is evident in international conventions of biological diversity, the sustainable forest management processes implemented by most countries and the forest certification schemes.

Nevertheless, if it is accepted that biodiversity is, in one sense, everything (Wilson, 1996), then the way to measure biodiversity becomes the Achilles' heel. Obviously, the overall biodiversity in forest ecosystems cannot be measured and quantified comprehensively, hence indicators may be a relevant option (Noss, 1999). Biodiversity indicators can be based on genetic diversity, species richness, indicator species or functional diversity of one or several taxonomic groups, assuming that the diversity of these groups is correlated with other groups or that a wide range of ecological information is covered (Maes & Van Dyck, 2005; Rossi & Halder, 2010). Nevertheless, the first assumption should be tested because contradictory results are frequently reached, while some authors have identified indicator groups (Ormerod & Watkinson, 2000; Gregory *et al*., 2005; Dynesius & Zinko, 2006), others have not (Predergast & Eversham, 1997; Duelli & Obrist, 1998; Dauber *et al*., 2003; Wolters *et al*., 2006). This implies that although the overall taxonomic groups cannot be measured, a selection of them based on their mobility, mode of dispersal and their habitat requirements should be carried out to collect information at both local and landscape levels.

Most studies have focused on taxonomic groups well known by ecologists, such as birds, vascular plants, lichens, butterflies, carabids, ants, bees, etc. Among them birds are often considered as efficient indicators by playing an essential functional role in ecosystems at, or near, the top of the food chain (Ormerod & Watkinson, 2000; Gregory *et al*., 2005; Gil-Tena *et al*., 2007). Birds are also responsive to signals that accumulate across levels since bird communities typically select habitat features at multiple scales (MacFaden & Capen, 2002; Warren *et al.*, 2005; Mitchell *et al.*, 2006; Barbaro *et al.*, 2007). Likewise, vascular plants are a faithful reflection of the situation of the forests, since vegetation is directly affected by any change, including those due to both natural disturbance and human management, and their strategies of dispersal vary according to the taxon or functional type (Grime, 2001). Thereby, vascular plants are often used as a general indicator of the broad state of wildlife (Lindenmayer et al., 2000; Gray & Azuma, 2005).

On the contrary, while great efforts have been made to study these macroscopic life forms, little attention has been paid to microbial diversity (Strobel, 2003). However, the latter, and in particular endophytic fungi, may be an even better indicator of the state of biodiversity because of their high host affinity (Fisher and Petrini, 1990; Collado *et al.*, 2000).

Plantation forests

Although numerous definitions of planted forest can be found from the literature (FAO, 2003), plantation forests or planted forests can be defined as forests predominantly composed of trees established through planting and/or deliberate seedling (FAO 2010, Annex II). The Total area covered by planted forests is currently 264 million hectares, which represents 7 % of the World's forests. The global area of planted forest increased by about 5 million hectares per year in the last decade, most of this was established through afforestations (FAO, 2011). Planted forests are located mainly in East Asia, Europe and North America (75 % of the global planted forest area), although East Asia reports the greatest area accounting for about 35 %, and most are found in China (FAO, 2011).

It is well known that planted forests provide a high percentage of the wood for industrial uses, such as pulp, fibreboard, particleboard, plywood, sawn timber or bioenergy industry, which are important economically to countries because of export income and employment (West, 2006). Nevertheless, some environmental organizations denounce the replacement of primary forests with plantations in developing countries, considering that plantation forests are not real forests, from an ecological point of view, and that therefore they should not be certified (WRM, 2003, 2010). On the contrary, the two most important schemes (FSC and PECF) approve the certification of forest plantations because they believe that the promotion of wood products from plantations will help to reduce the pressure on primary forests.

Planted forests have been criticised mainly for problems regarding forest health and biodiversity, and is still a controversial subject nowadays. Although a common belief had always been that plantations, and mainly monocultures, are at high risk of damage by biotic agents, it had also been contradicted (Gadgil & Bain, 1999). However, several reviews have recently shed light on this aspect concluding that higher damages caused by pest and diseases were found in monocultures than mixed stands (Jactel *et al.*, 2005; Pautasso *et al.*, 2005).

Despite the fact that planted forests have been traditionally viewed as "green deserts", this perception is currently being challenged. Although many researchers have pointed out that biodiversity in planted forests is lower than in native forests (Hanowski *et al.*, 1997; Twedt *et al.*, 1999; Cheng, 2004; Barlow *et al.*, 2007; Makino *et al.*, 2007), several reviews have shown that plantations can provide valuable habitat for indigenous flora, fauna and fungi (Hartley, 2002; Carnus *et al.*, 2006; Stephens & Wagner, 2007; Brockerhoff *et al.*, 2008; Bremer & Farley, 2010). However, there seems to be unanimity in that natural forests are usually more suitable as habitat for a wider range of native forest species, but that plantations could contribute to biodiversity when established on degraded lands or agricultural crops.

Poplar plantations

The area covered by poplar forests represents only about 2 % of the total forest area worldwide (FAO, 2008), however its importance from an ecological and economic point of view is evident beyond statistical data. As expressed, for example, by the establishment of the International Poplar Commission (IPC) in 1947, which presently comprises 37 member countries and is aiming to transfer knowledge between managers, users and researchers.

In percentage terms, the representativeness of poplar plantations (5.3 million ha, which represents around 0.1 % of the world's forests) is even much smaller. Nevertheless, a sharp positive trend of area covered by poplar plantations, estimated at around 10 % during the period 2004-2007, is currently taking place because of its high profitability, and as a result of the bioenergy sector boom. Poplar plantations are mainly located in China (4.3 million ha) and, to a lesser extent, in Europe (0.85 million ha), whereas in other parts of the world poplar plantations are irrelevant. In Europe, France with 236,000 ha accounts for the largest area of poplar plantations, followed by other countries such as Turkey, Italy, Germany and Spain, which range between 100,000 and 125,000 ha (FAO, 2008). In Spain, a positive trend has also been found, poplar plantations increasing by 5 % during the period 2004-2007 (FAO, 2008). In the same vein, it is worth noting that Castilla and León, the region with the largest area covered by poplar plantations in Spain (ca. two thirds of the total), has drawn up a first draft of a "strategy for poplar growing", the main objective of which is to double the area covered by poplar plantations in the next two decades.

Plantations of hybrid poplar are of great interest in Spain because of their high profitability as a source of plywood, which reaches between 1,200 and 2,400 €/ha/year for highly favourable sites (Díaz & Romero, 2001). Single plantations are monoclonal and although several hybrids are used in Spain, *Populus x euramericana* (Dode) Guinier clone I-214 (*P. deltoides* Marsh. ♀ x *P. nigra* L. ♂) is the most common. It is a female hybrid and covers about 70 % of the total area covered by poplar plantations (Fernández & Hernanz, 2004).

The density of poplar plantations, which is kept constant during the whole rotation, is approximately 278-400 stems/ha. The species has a deep root system and requires rich soils and large amounts of water, thereby poplar trees are placed in direct contact with the water table, usually at a depth of between 1 and 2.5 meters (De Mier, 2001; Fernández & Hernanz, 2004). Furthermore, poplar plantations are managed intensively in short rotations (12-16 years), weed control techniques (mainly surface ploughing) are used regularly during the first six years and all trees are pruned up to six meters from the ground level.

Riparian forests

In contrast with the increase in area covered by planted forests and, in particular, by poplar plantations, riparian zones have been modified or lost at an alarming rate during the past five decades in most European countries. Virgin vegetation of riparian zones was almost entirely lost when stream flow was regulated by storage reservoir and canalizations in the middle of the 20th century (Schnitzler, 1994; González & García, 2007). Such regulation was followed by a major conversion from native vegetation (riparian forests) to crops or planted forests such as poplar plantations.

In particular, the riparian zone in the Duero river basin, located in Castilla y León (NW Spain), was formerly characterized by several vegetation strips from the river to the external zone. The first vegetation strip had direct contact with the watercourse, which consisted of species, mainly a shrub stratum, with high water requirement and an ability to put up with floods. A second strip was located in alluvial meadow soils, which consisted of tree species that require a temporal water table with accessible deep (Lara *et al*., 2004). Now the first strip of vegetation in the study area is very narrow ca. 5-7 meters, and consists of a mixed tree and shrub stratum (mainly species of genus *Salix* sp. and *Alnus glutinosa*, and to a lesser extent species of genus *Populus* sp), because of a lack of periodically drastic flooding. Wetland forests of the second strip (mainly stands of alders, ash or poplars) have been replaced with agricultural crops or poplar plantations, apart from the occasional remnant patches.

AIMS OF THE STUDY

A broad consensus amongst researchers is the need to test and search new indicators adapted to each region and ecosystem, and reflect the new reality generated by global warming. Furthermore, despite the disparity of sets of C&I implemented, there seems to be unanimity in that forest health and biodiversity criteria must be keystones in SFM. In addition, the current and future increase in cover of poplar plantations as a result of national and regional policies, together with the peculiarities of the poplar plantations, due to both ecology and management, suggest the need for a more detailed study. Consequently, the main objectives are to contribute an overview of SFM and to test some current indicators and search for new indicators for forest health and biodiversity criteria in poplar plantations. To that end, abiotic and biotic stresses were studied to assess the forest health criterion, and three taxonomic groups, fungi, birds and vascular plants, were used to assess the biodiversity criterion.

More specifically the goals were:

1. To contribute an overview and identify the future challenges of Sustainable Forest Management (**I**).
2. To refine a monitoring method to assess the most commonly used forest health indicators (crown conditions) in poplar plantations quantitatively and objectively (**II**).
3. To identify the main nutrients limiting *Populus x euramericana* (clone I-214), which may explain poor growth rates and crown conditions and may therefore be used as sustainability indicators (**III**).
4. To assess the forest health of poplar plantations using an insect pest, the hornet clearwing moth, *Sesia apiformis* (Clerck), and provide a suitable monitoring tool based on a pheromone trapping system (**IV**).
5. To assess the usefulness of endophyte communities as indicators of forest health (**V**).
6. To test the effect of replacing a native tree species so threatened as the European black poplar (*Populus nigra* L.) with hybrid poplars (*Populus x euramericana*, clone I-214) on endophyte fungi diversity (**VI**).
7. To investigate the contribution of poplar plantations to bird conservation in riparian landscapes and to estimate the ecological implications of a substantial increase in the cover of poplar plantations (**VII**).
8. To investigate the influence of poplar plantations on vascular plant diversity in riparian landscapes and to estimate the ecological implications of a substantial increase in the cover of poplar plantations (**VIII**).

MATERIAL AND METHODS

Site description and experimental design.

The present study was carried out in the Duero river basin, more specifically in Palencia province (Castilla y León, NW Spain). The altitude of the study area ranges between 800 and 900 m and, in the most stands, the slope is almost flat. The average annual precipitation varies between 496 and 630 mm, and the average annual temperature, between 9 and 11.4 ºC (Ninyerola *et al*., 2005).

Thirty two *Populus x euramericana* (clone I-214) stands were selected (Figure 1), which were chosen by use of a factorial scheme with three factors: (1) stand age (young stands of 3–7 years old or adult stands of 8–14 years old), (2) understory management (harrowed or not harrowed) and (3) site quality (Figure 2). We differentiated rich sites (quality 1 and 2) or poor sites (quality 3 and 4), according to the site quality abacus developed for the *Populus x euramericana* clone I-214 in the river Duero basin (Bravo *et al*., 1995). Furthermore, the three native riparian forests found in our study area were sampled to compare bird and vascular plant diversity between poplar plantations and native forests (Figure 1). These stands consisted of an upper storey of alders (*Alnus glutinosa*), a scattered lower storey of elders (*Sambucus nigra*), common hawthorn (*Crataegus monogyna*) and common dogwood (*Cornus sanguinea*), and a forb stratum (Figures 3).

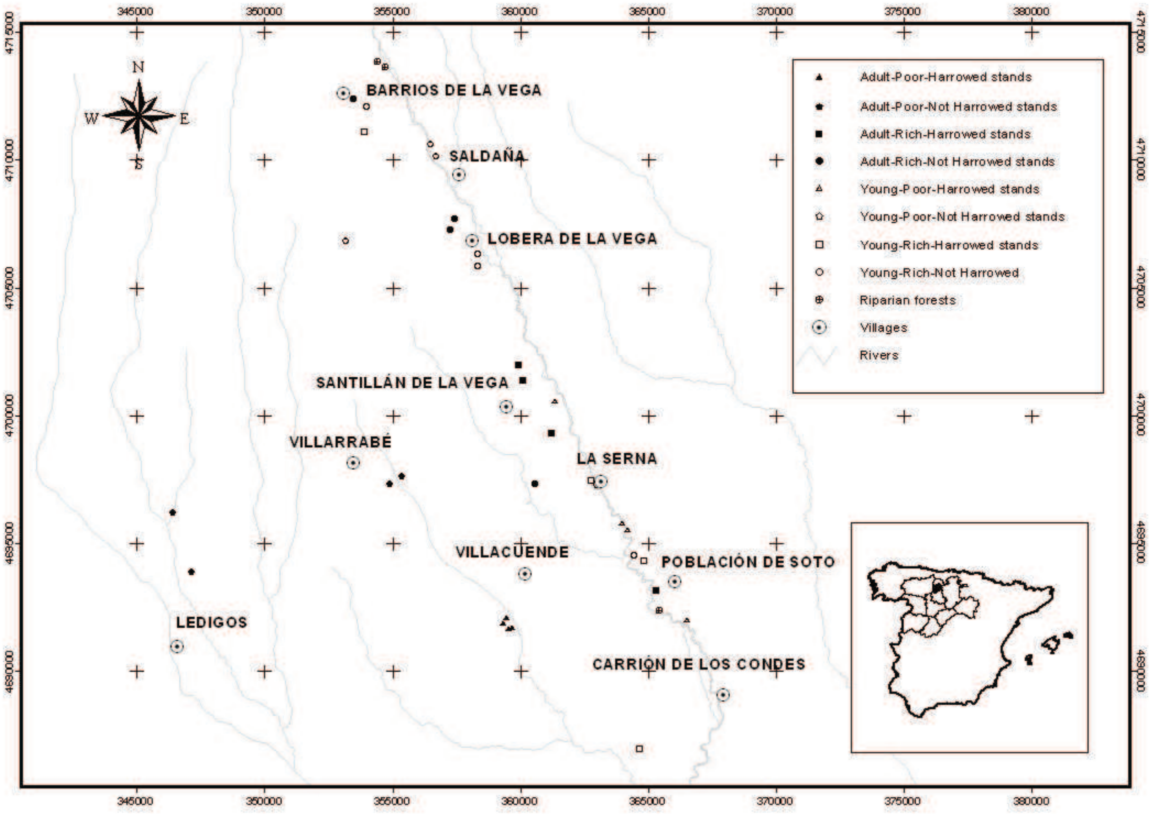

Figure 1 Geographical location of the sites surveyed

Figure 2. Appearance of young (top) and adult (bottom) stands according to management, harrowed (left) or not harrowed (right).

Figure 3. Appearance of one of the riparian forest surveyed

Because of the fact that the aim of the study **VI** was to compare the endophytic mycota of native poplar (*P. nigra*) and hybrid poplar trees (*P. x euramericana*, cv. I-214) additional stands had to be selected. Thus, three poplar plantations of clone I-214 and three native poplar stands were chosen for study, in Palencia province (Castilla y León, NW Spain) (Figure 4). The poplar plantations were 10-year-old monoclonal plantations established by the Government of the Castilla y León, whereas stands of native poplar exist as remnant patches. Native poplars of breast height diameter (10-20 cm) similar to those of the hybrid poplar sampling sites were selected for sampling.

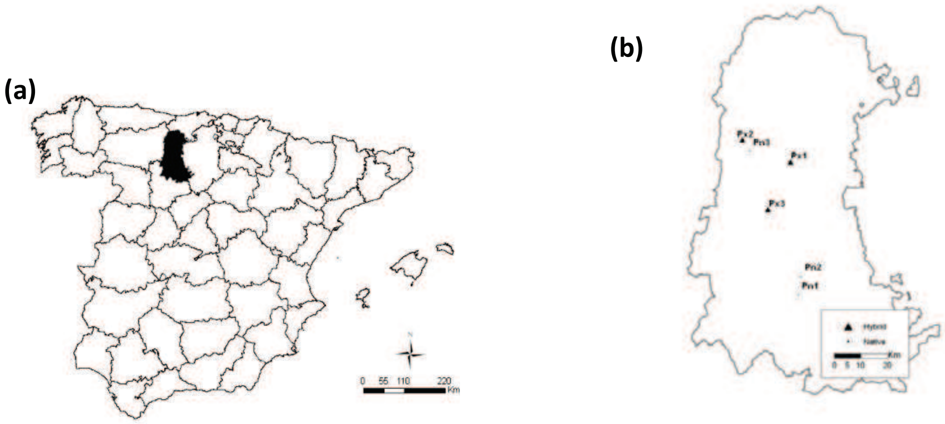

Figure 4. Geographical location of the six sites surveyed. (a) Geographic location in Spain; (b) geographic location in the province of Palencia.

Assessment of crown condition and dendrometric variables (**II, III, IV** and **V**).

In each stand, four circular subplots of 15 m radius were monitored. These subplots were located 50 m apart from each other, at the ends of a cross located in the middle of the stand (Figure 5). Within each subplot all trees (a total of 2,695 poplar trees) were marked and sampled.

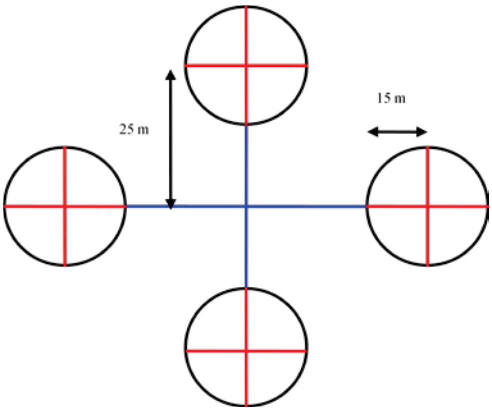

Figure 5. Sampling design of subplots

Crown transparency and discoloration were visually estimated and quantified using twenty classes of 5% width, according to Level I European network methodology (Eichhorn *et al.*, 2006). Simultaneously, all trees were photographed using a digital photo camera from the same position. Crown transparency of a subset of these trees was digitally analyzed with CROCO software according to the methodology suggested by Mizoue (2002) (Figure 6).

Figure 6. Digital photo of a crown (left) and resulting image once the thresholding algorithm win-CROCO was applied (right).

Dendrometric variables, such as diameter at breast height (DBH), total height (TH), pruned height (PH), crown diameter (CD) and crown volume (VOL) were also measured in all trees.

Sampling and analysis of soils and leaves (**III, V** and **VIII**).

Mineral soil samples were collected from the upper 30-cm soil layer in each subplot (i.e., four samples per plot). The samples were mixed, homogenized and considered as a composite sample per plot. The pH was determined potentiometrically with a pH meter, in a soil solution (1:2.5, soil/water). Organic matter was determined by the K2Cr2O7 method. Total N was determined by Kjeldahl digestion. Soil available P was extracted by the Olsen procedure and determined photometrically by the molybdenum-blue method. Soil exchangeable cations (K^+, Na^{2+}, Ca^{2+} and Mg^{2+}) were extracted with ammonium acetate and determined by atomic absorption/emission spectrometry. Particle-size distribution was determined by the Bouyoucos method (hydrometer method), and the ISSS (International Society of Soil Science) classification was applied. The Cationic Exchange Capacity (CEC) was determined by Bascomb's method (i.e., the exchange cations were displaced by Ba ions, which were then displaced by Mg ions and the remaining concentration of Mg was determined by titration against EDTA).

Foliar sampling was carried out on green leaves from a minimum of 12 trees per subplot. This sampling was carried out during the first 2 weeks of September, a period when foliar nutrients are most stable in poplar (Bengoa & Rueda, 2001). Between 12 and 15 green leaves were removed from each tree, from two main branches of the upper third of the canopy. The oven-dried (60 ºC) samples of leaves were milled (0.25 mm) and digested with HNO3, in a microwave oven. Total carbon and N in milled foliar samples were analyzed by combustion in a Leco analyzer (LECO, St Joseph, Michigan, EEUU). The total concentrations of P, K, Ca, Mg, Fe, Mn, Zn, Cu, B, Ni S, Al, Cr, As, Mo, Cd, Co, Na and Pb in the digested foliar samples were determined by ICP-EOS (Perkin Elmer, Wellesley, MA, EEUU).

Pheromone trapping survey (**IV**)

Populations of hornet clearwing moth were monitored with pheromone traps in all 32 stands (Figure 7). We used funnel traps (Uni-Trap; PHEROBANK, Wageningen, The Netherlands) baited with commercial lures (PHEROBANK) of *S. apiformis* sex pheromone. Pheromone dispensers were loaded with a racaemic mixture of (Z,Z)-3,13-octadecadienyl acetate and (E,Z)-2,13-octadecadienal, with a release rate of 0.22 lg/h at 25 ºC. Traps were assessed every fortnight for a total of six times from May to August, and lures were renewed once (after 42 days).

Figure 7. Pheromone trap and captures.

Identification of fungi (**V** and **VI**).

In the study **V**, fungal isolates were identified according to morphological characteristics, using a stereomicroscope the shape and colour of the colonies was analysed, and the main characteristics of fungal structures observed. Different taxonomic keys were used to identify the fungi (Lanier *et al.*, 1978; Von Arx, 1981; McGinnis *et al.*, 1982; Barnet & Hunter, 1987; Goidanich, 1990; Watanabe, 1994; Kiffer & Morelet, 1997).

In the study **VI**, fungal isolates were identified according to sequences of the internal transcribed spacer region of their rDNA. Total DNA was isolated according to Vainio *et al.* (1998). The internal transcribed spacer (ITS) region of the rDNA was amplified with primers ITS1-F (Gardes & Bruns, 1993) and ITS4 (White *et al.*, 1990). All amplifications were performed according to White *et al.* (1990) and Vainio & Hantula (2000). PCR products were purified with the High Pure PCR Product Purification Kit (Roche, Mannheim, Germany), according to the manufacturer's instructions. The sequences were determined in an automated sequencing apparatus (Li-Cor Global Edition IR2 system; Li-Cor Inc., Lincoln, NE, USA), by following the manufacturer's instructions. DNA fragments were sequenced in both directions at the same time. Finally, the sequences were aligned with LI-COR software (ALIGN IR ver. 2.0). Consensus sequences were identified by comparing them with sequences deposited in GenBank (NCBI) database, by means of the BLAST algorithm. The isolates were named according to the best GenBank match if at least 97% matched at least 401 bp.

Landscape metrics (**IV** and **VII**).

Landscape mapping within the study area was performed in a GIS (ArcGis 9.1, ESRI) using colour aerial orthophotographs at the scale 1: 1500, based on photo-interpretation and field cross-validation. Land cover types were assigned to each landscape patch according to the following classification: young poplar plantations (not closed canopy), adult poplar plantations (closed canopy), riparian forests, pine forests, oak forests, hedgerows, agricultural crops and roads (Figure 8). Landscape metrics were calculated within different radius circular buffers (100, 200, 300, 400, 500 y 1000 m) to capture landscape features at different spatial scales, using Fragstat 3.3 in raster version and a cell size of 2.5 m (McGarigal *et al.*, 2002). Within each buffer, we calculated the following metrics characterizing both composition and configuration of the landscape: the percentage cover of the eight land cover types, distance to river, edge density (in m ha^{-1}) and Shannon index of habitat diversity. Procedures and metrics are fully described in McGarigal *et al.* (2002).

Figure 9. Appearance of a typical riparian landscape, which consists of poplar plantations (at top left), hedgerows and crops.

Bird sampling (**VII**).

Breeding birds were sampled by the point-count method in spring 2006 (Bibby *et al.*, 1989). Unlimited distance was used within the stand, but all birds recorded were located within the limits of the sampled stands. All birds heard and seen were recorded and a semi-quantitative abundance index, with a score of 1 for singing male heard or pair or 0.5 for each non-singing bird heard or seen, was calculated (Norton *et al.*, 2000). Point counts were performed within 3 hours after sunrise and rainy days were excluded.

*Vascular plant sampling (***IV, VII** and **VIII***).*

At the centre of each stand, nine 2-m × 2-m quadrants (36 square meters) were laid out in order to quantify the understory vegetation, i.e. a total of 315 quadrants were assessed. Each vascular plant was identified (field or laboratory) and its percentage cover and characteristics were collected. The conversion of floristic data into a C-S-R signature (**VIII**) was carried out automatically by the calculator (first part of the spreadsheet tool) developed by Hunt *et al.* (2004).

Statistical analyses.

Univariate analyses: Analyses of variance and Tukey's HSD post-hoc tests (**II, III, IV, V, VI, VII** and **VIII**), Pearson's correlation matrices (**III** and **IV**), Simple linear regressions (**II, IV** and **VII**), Multiple linear regressions (**III, IV** and **VII**), Linear mixed models (**V**), Mann-Whitney U test (**VI** and **VIII**), Paired *t* test (**II**) and Wilcoxon signed rank test (**IV**).

Multivariate analyses: Correspondence analyses (CA) (**V**), Principal component analyses (PCA) (**III** and **VII**), Discriminant analyses (**III**), Canonical correspondence analyses (CCA) (**V, VI** and **VII**), IndVal analyses (**VIII**), Non-metric multidimensional scaling (NMDS) (**VIII**) and Multiple response permutation procedures (MRPP) (**VIII**).

RESULTS

The main results on each chapter are separately summarized below.

I. Sustainable forest management: an introduction and overview.

Numerous sets of C&I have been implemented in a large number of countries, nevertheless three of these processes stand out against the others, namely the ITTO, MCPFE and Montreal processes. ITTO has developed C&I for sustainable management of tropical forest, whereas C&I for boreal and temperate forests were implemented by European (MCPFE) or non-European countries (Montreal process). Although the different processes have very different origins and have developed their own criteria, there are some similarities between the three major SFM programs

Several initiatives at international and national levels gave rise to numerous forest certification schemes, such as Forest Stewardship Council (FSC), Programme for the Endorsement of Forest Certification (PEFC, previously termed Pan European Forest Certification), the Canadian Standards Association (CSA), the Sustainable Forestry Initiative (SFI) and the Chile Forest Certification Corporation (CERTFOR), among others. Nevertheless, only two schemes (PEFC and FSC) have been used for most of the forest currently certified throughout the world. Furthermore, almost 90% of forests certified by the two major programs (FSC and PEFC) are located within Europe and North America. More than half (54%) of the forests in Europe (excluding the Russian Federation) have already been certified, and almost one third of the forest area in North America has been certified. On the contrary, only about 1.5% of the forests in Africa, Asia, and Central and South America have been certified, despite the fact that more of half of the world's forests and almost 60% of primary world forests are located in these countries (FSC, 2011; PEFC, 2011).

II. Towards standardized crown condition assessment in poplar plantations.

Poplar crown conditions were better in young stands and rich sites, harrowing understory vegetation improved tree health on poor sites. Comparison of crown condition (visual crown transparency and discoloration) mean values calculated from different combinations of one, two or three subplots did not show any significant differences, indicating that samples of 20 trees per stand provided the same information as 60 trees. Calibration curves of digital crown transparency estimates were successfully fitted against visual crown transparency estimates. Thus, based on a sample of seven trees selected along a linear gradient of crown transparency, such as proposed by Mizoue (2002), the calibration curve between visual estimates and digital estimates (values obtained with CROCO software) showed a very good fit with a simple regression ($R^2 = 0.97$, $p < 0.0001$). Similar regression, made with data from 265 trees selected across a longer gradient of visual crown transparency values, showed a good fit too ($R^2 = 0.64$, $p < 0.0001$).

III. Relating visual crown condition to nutritional status and site quality in monoclonal poplar plantations (*Populus x euramericana*).

High contents of coarse fragments (> 30 %) implied low growth rates, poor conditions and deficiencies mainly in Fe and B, and to a lesser extent N, P and K. Suitable and unsuitable lands were discriminated according to soil properties or foliar nutrients. In the first model, the stands were classified correctly in 81.25 % of cases on the basis of the percentage of coarse fragments and the C/N ratio in the upper mineral soil horizon, reflecting the main soil properties that determine nutrient availability. On the other hand, the second model was able to discriminate the two groups of stands with different tree growth on the basis of the foliar concentration of Fe and B (stands were classified correctly in 96.43 % of cases). Visual crown conditions were related to growth rates and nutritional status. Models showed that about 60 % and 80 % of the variability in crown condition can be explained by age and coarse fragment content, and by age and foliar Fe concentration, respectively. Nevertheless, other variables that seem to influence crown condition, such as P, K, B, Zn or Cu, were not added in the models because multiple stepwise regressions avoid the use of correlated variables.

IV. Patterns and monitoring of *Sesia apiformis* infestations in poplar plantations at different spatial scales.

The percentage of attacked trees by *Sesia apiformis* per stand varied from 0 % to 71 % with an average (± SE) of 25.5 ± 4.3 %. There were significantly more attacked trees in older stands, whereas in young stands the percentage of infested trees increased with the percentage cover of understory vegetation. There was no significant effect of site quality on the rate of infestation and no difference in tree height, diameter or crown conditions between attacked and un-attacked trees within each stand. A positive correlation between trap capture and percentage of attacked trees in a radius of 100 m around the pheromone trap was found. However, this regression was improved when the area of other poplar plantations within a distance of 600 m was incorporated in the model.

V. Factors influencing endophytic communities in poplar plantations.

A total of forty-three species of morphological types were isolated from 576 plant fragments (288 plant fragments for each tissue, leaves and twigs), of which the most frequent were *Ulocladium* spp. and *Cladosporium herbarum* (Pers.) Link. ex S.F.Gray. On the other hand, *Glonium* spp., *Pestalotia* spp., *Trichotecium roseum* (Persoon) Link. Es S.F.Gray, and several unidentified Deuteromycetes and sterile mycelia occurred at lower frequencies. Fungal species richness and relative isolation frequencies did not differ significantly between site qualities or between tissues, but did differ between ages (richness was higher in young stands than in adult stands). Moreover, the differences in richness between ages depend on the site quality, with the lowest richness values observed in old stands located on poor sites. At stand level, endophyte assemblages varied among stands according to site quality and, to a lesser extent, stand age. Likewise, crown discoloration, total height and several foliar concentrations (Fe, Zn and N/P ratio) were associated with endophytic communities in poplar plantations.

VI. ITS-based comparison of endophytic mycota in twigs of native *Populus nigra* and cultivated *P. x euramericana* (cv. I-214) stands in Northern Spain.

A total of 13 species were found. Six species originated from *Populus nigra* only [*Stemphylium vesicarium* (Wallr.) E.G. Simmons, *Alternaria* spp., *Aureobasidium pullulans* (de Bary) G. Arnaud, *Fimetariella rabenhorstii* (Niessl) N. Lundq., *Plagiostoma fraxini* (Redlin & Stack) Sogonov and *Cryptodiaporthe salicella* (Fr.) Petr.] and four from *P. x euramericana* only [*Cytospora chrysosperma* (Pers.: Fr.) Fr., *Athelia bombacina* Pers., *Biscogniauxia mediterranea* (De Not.) Kuntze and Unidentified sp. 1], whereas three were found on both types of trees [*Lewia infectoria* (Fuckel) M.E. Barr & E.G. Simmons, *Alternaria alternata* (Fr.) Keissl. and *Epicoccum nigrum* Link]. Species richness and diversity, as expressed by the Shannon index of isolates from natives poplar, were higher than those for isolates obtained from hybrid poplar, although the differences were not significant ($p = 0.10$ and $p = 0.07$, respectively). Furthermore, richness and diversity accumulation curves predicted a low increase in number of species and diversity with increasing number of trees sampled. A CCA showed that fungal assemblages differed according to type of poplar ($p < 0.01$), which explained the 17.4 % of the total variation in species.

VII. Contribution of poplar plantations to bird conservation in riparian landscapes.

Comparison between riparian forests versus poplar plantations reflected differences in species richness, total abundance and bird assemblages. In particular, riparian forests showed higher number of species and total abundance (18.3 ± 1.45 and 39.0 ± 0.29, respectively) than poplar plantations (11.7 ± 0.67 and 22.2 ± 2.91, respectively). Moreover, whereas riparian forests were characterized by several specific bird species, such as *Aegithalos caudatus*, *Cettia cetti*, *Columba palumbus*, *Cuculus canorus*, *Garrulus glandarius*, *Parus major*, *Phylloscopus collybita*, *Regulus ignicapillus* and *Serinus serinus*, poplar plantations did not show any specific species.

When focusing on the dataset of poplar plantations, a prominent influence of landscape variables on species richness, total abundance and bird assemblages was found. Our outcomes showed that the landscape matrix more influential in poplar plantations lies between 300 and 400 m and that the variable most important to explain species richness, abundance and bird assemblages was the percentage of poplar plantations in the surrounding landscape. Furthermore, our results showed that local scale was also a key factor influences for bird diversity. Indeed, understory vegetation determined bird assemblages in poplar plantations, since only poplar stands with high understory cover were associated to bird species typical of natural riparian forests, such as *Cettia cetti*, *Aegithalos caudatus*, *Parus major*, or *Regulus ignicapillus*.

VIII. The role of poplar plantations on vascular plant diversity in riparian landscapes

Comparison between riparian forests versus poplar plantations did not reflect differences in species richness (N = 6, Z = 0.44, *p* = 0.66). Nevertheless, Non-metric multidimensional scaling (NMDS) using species frequency revealed a complete turnover of vascular plant assemblages from poplar plantations to riparian forests. In the same vein, NMDS ordination test reflected a clear differentiation between riparian forests and poplar plantations according to functional diversity estimated by CSR strategy. Particularly, NMDS ordination showed that plant communities in riparian forests mainly consisted of Stress-Tolerant Competitors species (SC), whereas a clear gradient towards Ruderal species (R) was found in poplar plantations.

A total of 133 species were recorded from the thirty-two poplar plantations, of which 44 occurred in only one stand. Mean species richness in young stands was significant higher than adult stands (ca. 23 and 16, respectively), and harrowed stands showed a lower species richness than non harrowed stands (ca. 13 and 26, respectively). IndVal analysis detected 25 indicator species at several levels of the typology, but no indicator species were found in harrowed stands. On the other hand, NMDS ordination and MRPP tests reflected a differentiation in the CSR strategies in poplar plantation according to age (A = 0.06, *p* = 0.02) and site quality (A = 0.03, *p* = 0.04). Furthermore, NMDS retained two significant environmental variables (number of harrowings [*p* = 0.04] and percentage of coarse fragments [*p* = 0.04]). While, the latter was associated with a gradient from Competitors (C) to Stress-Tolerant (S) strategies, the number of harrowings did the same with the pattern Competitors (C) to Ruderal species (R).

DISCUSSION

Current and future challenges of sustainable forest management and forest certification.

Although utility of indicators is still controversial and several weakness have been pointed out (Bradbury, 1996; Michalos, 1997; Bass, 2001), there is general agreement that the advantages of the approach outweigh these limitations and that researchers should focus their efforts on testing the current indicators and searching for new indicators. Even more so in the current changing environment when the sustainability concept and indicators must be revised to reflect the new reality generated by climate change. Among other initiatives, the FORSEE project was carried out in order to shed light on usefulness of the proposed indicators and develop new indicators in the Atlantic regions of Europe (Tome & Farrell, 2009).

There are two key challenges involved in improving the C&I system, a harmonization of the sets of C&I and the use of a suitable scale of each indicator. Thus, advances in harmonization will minimise costs (avoiding duplication and preventing overlap), facilitate comparisons between countries and, overall, improve the credibility of SFM. Likewise, the use of a suitable scale would avoid additional charges as a result of correlations of several different scales and facilitate the interpretation of each indicator. On the other hand, the scientific community must search for new indicators. Gaps in knowledge have been identified, and as these mainly involve ecological aspects, researchers should go further in investigating the relationships between type of forest management and ecological and socioeconomic functions.

Forest certification has became very popular, mainly because it is regarded it as a tool whereby everyone should benefit (win-win goal). This situation would be based on an economic balance, where forest owners and the forest industry place sustainable products on the market in the hope that consumers will be willing to pay the extra cost implied by SFM. Nevertheless, forest certification is still far from reaching its initial goal (win-win), since the expected price increases have not occurred (Cubbage *et al.*, 2010; Gafo *et al.*, 2011). In practice, only consumers and the forest industry have benefited; consumers use certified forest products with a clear conscience, and the forest industry has ensured market access without any great extra cost because this has mainly been assumed by forest owners.

This leads to a difficult question, namely, are forests benefiting from forest certification? As already noted, forest certification began in tropical forests with the aim of decreasing deforestation. However, nowadays almost all certified forests are located in developed countries. Furthermore, most of these forests are productive forests, such as single-species and even-aged forests or plantations, in which only small changes must be made to achieve forest certification, while primary forests have largely been ignored. The fact that foresters are able to place certified products from productive forests on the market, with a small additional charge compared to the extra charge involved in certifying products from primary forests hinders certification of the latter, which are actually the most endangered forests. Moreover, this disadvantage may favour unsustainable management, such as illegal logging or in extreme cases conversion of forest land to agricultural land, to favour market competitiveness.

The viability of tropical forest certification will depend on forest owners obtaining premium prices that at least cover the certification costs, taking into account that these costs vary according to the type of forest (primary forest, plantations, etc.) and that consumers' willingness to pay premium prices will also differ. It should be possible for consumers to distinguish the origin of each product, and in other words different labels are required. Nevertheless, the use of different eco-labels is controversial, since many labels may confuse rather than help consumers (Teisl *et al*., 2002). On the other hand, with an absence of standardization and a powerful information campaign, most environmentally concerned consumers will probably demand wood from sustainably managed forests, without taking into account the type of certification label, and will choose the least expensive product (Teisl *et al*., 2002). This may entail a new associated problem, since producers and industries will probably also choose the bodies that certify forests most readily and at the lowest cost. This may lead to a situation where the certification schemes would tend to compete with each other and standards would be reduced to attract producers, as pointed out by Van Dam (2001).

Indicators of forest health in poplar plantations.

The health of forests is determined by numerous abiotic and biotic stressors, which vary according to type of forest and region, making it more difficult to assess (Ferretti, 2004). Researchers initially focused on crown condition as a good indicator of forest health, based on the theory that impact in forest caused by natural or antropogenic stresses would be observed in the tree crowns (Zarnoch *et al*., 2004). Nevertheless, although the usefulness of crown conditions as indicator of forest health is beyond doubt, several limitations have been found, such as a lack of objectivity in the assessment or the difficulty of identifying its relationship with stresses factors.

Different shapes and sizes of sampled plot have been proposed for forest health surveys, like four subplots of six trees located 25 m from a fixed point by the ICP-Forest in Europe (Eichhorn *et al*., 2006), four subplots spaced 36.6 m apart with a fixed radius by the Forest Health Monitoring in the U.S. (Zarnoch *et al*., 2004) and one circular plot of 24 trees in Spain (SPCAN-DGCN, 2002). In this regard, our results (**II**) shows that the assessment of only one subplot of fixed radius (exactly 15 m, i.e. approximately 18 trees in plantations with planting distance 6 × 6 m) seems to be sufficient to assess crown condition in monoclonal poplar plantation. The discrepancy with the results obtained by Innes and Boswell (1990) in even-aged stands of conifers, who demonstrated significant variations amongst the 4 subplots of each plot, may be due to the higher homogeneity of trees within monoclonal plantations. On the other hand, an objective assessment of crown transparency in poplar plantations may be available if CROCO software is used and our models are implemented.

Our outcomes support the influence of abiotic stressors on forest health. Thus, nutrient status showed an influential pattern on growth and crown condition (**III**). In particular, a coarse fragments content higher than 30 % was associated with unsuitable stands and poor crown condition, possibly due to the small volume of soil available for the development of roots, the lower amount of available nutrients per unit of soil volume or a lower soil water-holding capacity. Likewise, the soil C/N ratio appears to be a limiting factor only in young plantations, which may due to an increase in soil N with age of the stand as a result of the return of some nutrients in soils through litterfall (Das & Chaturvedi, 2005) or enhancement of N mineralization because of a well-developed root system (Browaldh, 1997). Moreover, although several foliar nutrients, such as N, P, K, Fe and B, seem to affect tree growth and crown conditions in these poplar plantations, Fe and B appear to be the most limiting elements (**III**). These deficiencies seem to be associated with high contents of coarse fragments, which would imply lower availability of these nutrients as well as a lower moisture-holding capacity in superficial soil horizons, since moisture is required for uptake of the nutrients. On the other hand, the suitability of lands could be predicted (with low cost and high probability of success) using the classification functions developed (**III**) from soil properties to ensure that new plantations will be established on suitable land.

Our results also demonstrated that forest health in poplar plantations are related to biotic factors. In this vein, the study (**IV**) displayed that poplar plantations of *P. x euramericana* can sustain high infestation rates of *S. apiformis*, up to 71 % of attacked trees per stand. This is consistent with other studies that found *P. x euramericana* to be more susceptible to *S. apiformis* than other poplar species such as *P. nigra*, *P. trichocarpa* and *P. alba* (Arundell & Straw, 2001; Straw *et al.*, 2007). Our outcomes also showed that understory management may be used as a tool for the control of the *Sesia* populations. The fact that *Sesia* attacks were positively related to the percentage cover of understory vegetation in young plantations, may be due to vegetation would provide eggs and egg-laying females with more protection against adverse weather condition (de Tillesse *et al.*, 2007) and predation (Coleman & Boyle, 2000). Both key aspects in young trees in which the eggs would be laid unprotected by bark cracks.

Nevertheless, the implementation of an insect pest as indicator of forest health requires an efficient and accurate monitoring method. In this regard, a pheromone monitoring system based on a single trap could provide reliable information to estimate *S. apiformis* attacks in poplar plantations (**IV**), whereas a minimum of three traps were necessary to properly estimate pine processionary moth (*Thaumetopoea pityocampa*) populations in maritime pine plantations (Jactel *et al*., 2006). This discrepancy is likely to originate from differences of stand homogeneity, as poplar plantations are based on monoclonal stands with regular spacing of trees. Furthermore, our outcomes support the hypothesis that the active radius of attraction by pheromone traps, baited with *S. apiformis* sex pheromone at a release rate of 0.22 µg/h, is about 100 m. Nevertheless, managers should take into account the fact that *S. apiformis* male moths could fly around 600 m and should therefore be considered when interpreting pheromone trap captures. Thus, our model could be implemented to correct the effect of surrounding poplars, and then pheromone trapping would offer a reliable monitoring tool for hornet clearwing moth population which can be easily applied on a larger scale than the usual sampling method based on exit hole counts.

Although many authors have recognized the importance of endophytic communities in forest health (Bettucci & Alonso 1997; Bettucci *et al*., 1999; Gennaro *et al*., 2003; Ragazzi *et al*., 2003; Santamaría & Diez, 2005; Zamora *et al*., 2008; Botella *et al*., 2010, 2011), it has not yet been studied in poplar plantations. Our study (**V**) displayed that several variables related to forest health (discoloration, total height and the concentration of several nutrients) determined the fungal communities in poplar plantations. In particular, fungal assemblages differed mainly according to site quality of the stands, which seems be due to a stress factor caused by nutrient deficits in poor site qualities. Thus, deficiencies in Fe, which is one of the most nutrient limiting in these poplar plantations (**III**), appears determine directly to discolorations and low growths, and indirectly endophyte communities. Furthermore, it is well known that some endophyte species, such as *Periconiella* spp. (Collado *et al*., 1999) and *Cytospora* spp. (Bettucci & Alonso, 1997; Callan, 1998), require trees to be exposed to stress conditions before colonization. On the other hand, it should also be underlined that human factors could determine forest health in poplar plantations. Thus, the occurrence of weak pathogens, such as *C. chrysosperma*, on only hybrid poplars (**VI**) may be explained by the origin of the hybrid poplar seedlings. The hybrid poplars in each sampled stand probably originated from cuttings of a single ortet, which may have been infested by the pathogen and it is therefore possible that all cuttings used in the plantation already carried this fungus as a latent infection.

In short, our studies show that both abiotic and biotic factors determine vitality in monoclonal poplar plantations and seem to point out that a lower sampling intensity, as compared to mixed forests, would be necessary to assess forest health, due to its homogeneity. Nevertheless, although it could be considered as a positive point for SFM by minimizing sampling costs, it should also be seen as a warning. Thus, although no dramatic phytosanitary problems have been displayed in poplar plantations so far, a diversification of clones at least at landscape level and a verification of the provenance of the seedlings used for plantation and its phytosanitary status would be desirable to avoid future phytosanitary problems.

Indicators of biodiversity in poplar plantations.

As already noted, effect of planted forests on biodiversity is still a controversial topic. It is even more noteworthy in Mediterranean riparian zones, where the interface between terrestrial and aquatic environments plays a key role on biodiversity (Schnitzler, 1994). Hence, it is essential to investigate the role of poplar plantations on biodiversity in riparian zones and to estimate the ecological implications of a substantial increase in the cover of poplar plantations.

Our outcomes (**VI, VII** and **VIII**) displayed that poplar plantations should not be used as surrogates of native riparian forests from the point of view of biodiversity and native riparian forests should therefore be preserved and increased as far as possible. In particular, the community structure of twig endophytes of the native *P. nigra* and the hybrid *P. x euramericana* (cv. I-214) differed greatly (**VI**), which is noteworthy as clone I-214 is a 50 % descendant of *P. nigra*. In previous studies, distinct differences between endophytic communities were found on comparison of different tree species (Fisher & Petrini, 1990). Nevertheless, taking into account the fact that *P. x euramericana* can be considered as a partly exotic species (the other parent species *P. deltoides* originates from North America), may therefore carry resistance factors (genes), which would be new to the fungal flora in Spain. Moreover, the hybrid poplar is known to grow considerably faster than the native poplar, which is the main reason for its popularity among foresters. The speed of growth may affect the mycobiota associated with the tree, as shown by Korkama *et al.* (2006) in studies on the mycorrhizal diversity of various spruce clones. Similarly, bird (**VII**) and vascular plant (**VIII**) assemblages also differed between riparian forest and poplar plantation. While poplar plantations hosted mainly generalist bird species, riparian forests were associated with specific bird species typical of well preserved riparian forest areas with a dense and unmanaged understory. In turn, most of the indicator vascular plant species found only in natural forests have been previously identified as species with high requirements in shade and/or edaphic moisture, even temporal flooding in some cases, which is typical of riparian zones.

Furthermore, when focusing on the dataset of poplar plantations, our studies showed that diversity could be influenced by both local and landscape scales. At local scale, although poplar plantations hosted mainly ruderal species, minimising the disturbances would favour Stress-Tolerant Competitor plant species, which is a plant strategy closer to native riparian forests, in detriment to Ruderal species (**VIII**).

Moreover, plant communities seem to be determined by the frequency of disturbance (number of harrowings) rather by the time since the last disturbance (years since the last harrowing). In the same vein, Decocq *et al.* (2004) reported that some vascular plant species were mainly limited by the frequency of disturbance and not by its severity. Our results (**VII**) also point out that an increase in understory cover would favour to bird species typical of natural riparian forests, such as *Cettia cetti*, *Aegithalos caudatus*, *Parus major*, or *Regulus ignicapillus* (Jubete, 1997).

On the contrary, other local factors, such as dendrometric variables, do not appear to determine the bird and vascular plant assemblages. In the case of birds, it could be due to the fact that in our study even the oldest poplar plantations were "young" (14 y.o.) from a natural forest perspective. Trees may not be old enough, for example, to provide birds with suitable micro-habitats such nesting cavities. Likewise, dendrometric variables did not influence vascular plant communities either. This could be due to the fact that the structure of poplar plantations (a low density of plantation around 278-400 stems/ha and that all trees are pruned up to 6-8 meters above ground level) allowing enough light to be transmitted to the understory. Similar results were reported by Chen *et al.* (2004) in *Populus tremuloides* stands of northern British Columbia and by Klinka *et al.* (1996) in several tree species on West of Vancouver Island (Canada).

Furthermore, our outcomes (**VII**) showed a prominent influence of landscape variables on species richness, total abundance and bird assemblages in poplar plantations. This study shows that the landscape matrix more influential in poplar plantations lies between 300 and 400 m and that the variable most important to explain bird diversity was the percentage of poplar plantations in the surrounding landscape. This probably involves a mechanism of landscape supplementation (Dunning *et al.*, 1992), since the small size of poplar plantations in our study area (average 6.37 ha, ranging between 0.97-19.71 ha) may not allow species-rich bird communities to survive. Furthermore, other poplar plantations in the surrounding could be used by birds as corridors or stepping stones for dispersing across a complex landscape matrix with habitats as diverse as riparian forest and agricultural crops, thus acting like hedgerow networks in other areas (Parish *et al.*, 1994; Baudry *et al.*, 2000; Hinsley & Bellamy, 2000; Fuller *et al.*, 2001). Indeed, only poplar stands with a high cover of surrounding plantations were associated with bird species typical of natural riparian forests, such as *Cettia cetti*, *Aegithalos caudatus*, *Parus major*, or *Regulus ignicapillus* (Jubete, 1997). Whereas landscapes predominantly agricultural (i.e. with lower percentage of surrounding poplar plantations) showed bird species associated to open areas, such as *Carduelis cannabina, Carduelis carduelis* or *Miliaria calandra* (Jubete, 1997).

In short, poplar plantations should not be used as surrogates of native riparian forests from the point of view of diversity. Nevertheless, a suitable management at local level (minimising the use of harrowing) and at landscape scale (increasing in the percentage of poplar plantations in detriment to crops) would favour to bird and vascular plant diversity.

CONCLUSIONS

1. Criteria and indicators (C&I) of sustainable forest management should be updated continuously to be able to cope with the climate change challenge and assess sustainability of changing ecosystems. Furthermore, harmonization of C&I processes would be the most desirable outcome, since this would improve the credibility of the schemes. Forest certification has failed to avoid deforestation and has got two main challenges; (1) to certify the forests that are most important in ecological terms and that are most susceptible to poor forest management, such as tropical forests and, to a lesser extent, non productive forest in boreal and temperate regions, and (2) to achieve a market with premium prices, in which the win-win concept will prevail.

2. CROCO methodology is particularly relevant to crown transparency monitoring in monoclonal poplar plantations because it can be used with small tree sample size, such as one fixed 15 m-radius plot, and because it can be applied with no special expertise in tree pathology.

3. Soil properties, and mainly coarse fragments and C/N ratio, seem to determine tree vitality in poplar plantations and may therefore be used as an indicator of forest health. Furthermore, growers should be encouraged to use the classification functions developed from soil properties to ensure that new plantations will be established on suitable land.

4. A pheromone monitoring system based on a single trap could provide reliable information to estimate *S. apiformis* infestations in poplar plantations, provided that our model is implemented to correct the effect of surrounding poplars.

5. Fungal communities were related to several variables of forest health, such as discoloration and concentration of several limiting nutrients, which confirms that endophyte fungi are potential indicators of forest health.

6. A lower sampling intensity would be necessary to assess forest health in poplar plantation, as compared to mixed forests, due to its homogeneity. Although this fact could be considered as a positive point for SFM by minimizing sampling costs, it should also be seen as a warning. Thus, despite no dramatic phytosanitary problems have been displayed in poplar plantations so far, a diversification of clones, at least at landscape level, and a verification of the provenance of the seedlings used for plantation and its phytosanitary status would be desirable to avoid future phytosanitary problems.

7. The results show that the endophyte community of poplar differs between native (*P. nigra*) and hybrid poplars (*P. x euramericana* clone I-214), as only three out of 13 observed species were found on both poplars.

8. Bird diversity differs between native riparian forests and poplar plantations. Nevertheless, bird communities occurring in poplar plantations can still accommodate rich communities of forest bird species, providing that a high percentage of poplar plantations are held in a buffer of 400 m and a high understory cover is kept.

9. Vascular plant diversity varies according to type of habitat (native vs. poplar plantations). Disturbance, mainly in the form of a high number of harrowing, provided numerous Ruderal species (R) in detriment to Stress-Tolerant Competitor species (SC). Thus, minimising the use of harrowing would be desirable in order to favour Stress-Tolerant Competitor species, which are plants utilising a strategy closer to native riparian forests.

10. Results of the three taxonomic groups, fungi, bird and vascular plant, indicate that poplar plantations should not be used as surrogates of native riparian forests from a conservation point of view. Thus, native riparian forests should be preserved or restored as far as possible. Nevertheless, poplar plantations could contribute to diversity when established on degraded lands or agricultural crops, with a suitable management strategy at local level (minimising the use of harrowing as long as it does not affect tree growth and vitality through competition) and at landscape scale (increasing in the percentage of poplar plantations in detriment to crops) employed.

11. The results suggest that soil properties, digital crown transparency, pest populations and fungal communities may be used as indicators of forest health for SFM in poplar plantations. Moreover, endophytic fungi, bird and vascular plant, may be used as indicators of biodiversity, since they are responsive to changes in habitat and forest management.

CONCLUSIONES

1. Los criterios e indicadores (C&I) de gestión forestal sostenible (GFS) debieran ser revisados continuamente para adaptarse al reto que supone el cambio climático y poder evaluar la sostenibilidad en ecosistemas cambiantes. Además, una armonización de los distintos conjuntos de C&I sería aconsejable para mejorar la credibilidad del proceso. En la actualidad, la certificación forestal no ha conseguido evitar la deforestación y por lo tanto tiene dos retos fundamentales; (1) certificar los bosques de mayor valor ecológico y susceptibles de una mala gestión forestal, tales como los bosques tropicales y en menor medida los bosques no productivos de las regiones boreales y temperadas, y (2) lograr un valor añadido en el precio de mercado de los productos certificados, de modo que todas las partes implicadas en el proceso de certificación se vean beneficiadas.

2. El uso del software CROCO es de especial utilidad para evaluar la transparencia de copa en plantaciones monoclonales de chopo, ya que se podría utilizar un pequeño tamaño muestral (una única parcela circular de 15 m de radio), pudiendo ser aplicado por personal sin una formación específica en sanidad forestal.

3. Las propiedades del suelo, y principalmente el porcentaje de elementos gruesos y el ratio C/N, parecen determinar la vitalidad de los árboles en las plantaciones de chopo y por lo tanto podrían ser utilizados como indicadores de sanidad forestal. Además, los populicultores deberían utilizar las funciones de clasificación desarrolladas a partir de las propiedades del suelo para asegurarse que las futuras plantaciones se establezcan en terrenos aptos para la especie.

4. Un sistema de seguimiento basado en una única trampa cebada con feromona sexual podría aportar información fiable para estimar el grado de ataque de *S. apiformis* en las plantaciones de chopo, siempre y cuando nuestro modelo sea implementado para corregir el efecto de las plantaciones circundantes.

5. Las comunidades fúngicas fueron relacionadas con varias variables de sanidad forestal, tales como decoloración y concentración de varios nutrientes limitantes, lo cual confirma el potencial de los hongos endófitos como indicadores de sanidad forestal

6. Para evaluar la sanidad forestal en plantaciones de chopo sería necesaria una menor intensidad de muestreo, en comparación con bosques mixtos, debido a su homogeneidad. Aunque esto pudiera ser visto como una ventaja para la gestión forestal sostenible al minimizar los costes de muestreo, también

podría ser considerado como una advertencia. Así, aunque no se han encontrado graves problemas fitosanitarios en las plantaciones de chopo hasta la fecha, sería deseable una diversificación de clones (al menos a nivel de paisaje) y una comprobación de la procedencia de los plantones usados y de su estado sanitario, para evitar futuros problemas fitosanitarios.

7. Los resultados demuestran que la comunidad de hongos endófitos difiere entre el chopo autóctono (*P. nigra*) y el clon híbrido (*P. x euramericana* clon I-214). De las 13 especies observadas tan sólo tres de ellas (todas generalistas) fueron encontradas en ambas especies.

8. La diversidad de aves difiere entre bosques de ribera y plantaciones de chopo. Si bien, estas últimas pueden albergar una rica comunidad de aves forestales, siempre y cuando una alta cobertura de sotobosque sea respetada y un alto porcentaje de plantaciones de chopo tenga lugar en un radio circular de 400 m.

9. La diversidad de plantas vasculares varía según el tipo de hábitat (bosque de ribera frente plantaciones de chopo). Perturbaciones, y principalmente el número de gradeos favorecen a las especies ruderales (R) en detrimento de las especies con una estrategia intermedia tolerante al estrés y competidora (SC). Así, minimizar el uso del gradeo sería deseable para favorecer especies SC, las cuales son típicas de bosques de ribera.

10. Los resultados de los tres grupos taxonómicos, hongos, aves y plantas vasculares, indican que las plantaciones de chopo no deberían reemplazar a los bosques de ribera desde un punto de vista de la conservación. Así, los bosques de ribera deberían ser preservados y restaurados en la medida de lo posible. Sin embargo, las plantaciones de chopo podrían contribuir a mejorar la biodiversidad siempre y cuando fuesen establecidas sobre áreas degradadas o cultivos agrícolas, y una adecuada gestión fuera llevada a cabo en la parcela (minimizando el número de gradeos en la medida que no afecte al crecimiento y la vitalidad de los árboles por competición) y sobre el paisaje (incrementando el porcentaje de plantaciones de chopo en detrimento de los cultivos agrícolas).

11. Los resultados sugieren que las características del suelo, la evaluación digital de la transparencia de copa, las poblaciones de plagas y las comunidades fúngicas podrían ser indicadores de sanidad forestal para la GFS en plantaciones de chopo. Además, los hongos endófitos, las aves y las plantas vasculares podrían ser usadas como indicadores de biodiversidad, ya que responden a cambios en el hábitat y a la gestión forestal.

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ARTICLES

ARTICLE I

Martín-García, J. & Diez, J.J. (2012) Sustainable forest management: an introduction and overview. In: *Sustainable forest management – current research*, J. Martín-García & J.J. Diez (Eds.), 3-16 (454 pp). InTech, Rijeka (Croatia). ISBN 978-953-51-0621-0

RESUMEN

Gestión forestal sostenible: una introducción y visión global

La gestión forestal sostenible está evolucionando con la conciencia ambiental de la sociedad y el conocimiento científico y por lo tanto el concepto de sostenibilidad debe ser revisado para reflejar la nueva realidad generada por el cambio climático, donde una referencia pasada no debería ser considerada como el objetivo de la gestión. Por lo tanto, los criterios e indicadores (C&I) deberían ser revisados continuamente de modo que fueran capaces de adaptarse al reto que supone el cambio climático y lograsen evaluar la sostenibilidad en ecosistemas cambiantes. Además, una armonización de los distintos conjuntos de C&I sería aconsejable para mejorar la credibilidad del proceso. La certificación forestal no ha conseguido evitar la deforestación y por lo tanto tiene dos retos fundamentales; (1) certificar los bosques de mayor valor ecológico y susceptibles de una mala gestión forestal, tales como los bosques tropicales y en menor medida los bosques no productivos de las regiones boreales y temperadas, y (2) lograr un valor añadido en el precio de mercado de los productos certificados, de modo que todas las partes implicadas en el proceso de certificación se vean beneficiadas.

Palabras clave: Sostenibilidad, criterios, indicadores, certificación, deforestación.

1

Sustainable Forest Management: An Introduction and Overview

Jorge Martín-García[1, 2] and Julio Javier Diez[1]
[1]*Sustainable Forest Management Research Institute,*
University of Valladolid – INIA, Palencia
[2]*Forestry Engineering, University of Extremadura, Plasencia*
Spain

1. Introduction

It is well known that forests provide both tangible and intangible benefits. These benefits may be classified according to ecological values (climate stabilization, soil enrichment and protection, regulation of water cycles, improved biodiversity, purification of air, CO_2 sinks, potential source of new products for the pharmaceutical industry, etc.), social values (recreational and leisure area, tradition uses, landscape, employment, etc) and economic values (timber, non wood forest products, employment, etc.). Although forests have traditionally been managed by society, it is expected that the current growth in the world population (now > 7,000 million people) and the high economic growth of developing countries will lead to greater use of natural resources and of forest resources in particular.

2. Global forest resources

The total forest area worldwide, previously estimated at 4 billion hectares, has decreased alarmingly in the last few decades, although the rate of deforestation and loss of forest from natural causes has slowed down from 16 million hectares per year in the 1990s to around 13 million hectares per year in the last decade (FAO, 2011). Nevertheless, the loss of forest varies according to the region, and while the forest area in North America, Europe and Asia has increased in the past two decades (1990-2010), it has decreased in other regions such as Africa and Central and South America, and to a lesser extent Oceania (Fig. 1)

There is growing public concern about the importance of the environment and its protection, as manifested by the fact that the total area of forest within protected systems has increased by 94 million hectares in the past two decades, reaching 13% of all the world's forests. Moreover, designated areas for conservation of biological diversity and for protection of soil and water account for 12 and 8% of the world's forests, respectively (FAO, 2010, 2011). Nevertheless, other statistics such as the disturbing decrease in primary forests[1] (40 million hectares in the last decade) and the increase in planted forests (up to 7% of the

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[1] Forest of native species where there are no clearly visible indications of human activities and the ecological processes have not been significantly disturbed (FAO, 2010)

world's forests) (FAO, 2011) appear to indicate that to achieve forest sustainability, we must go beyond analysis of the changes in the total forest area worldwide.

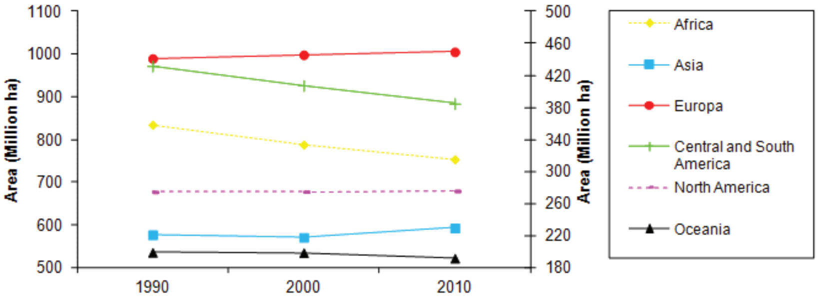

Fig. 1. State of World's Forests 2011 – subregional breakdown (Source: FAO, 2011). Africa, Asia, Europe, Central and South America and North America are represented in the left axis and Oceania in the right axis.

3. Sustainable forest management

The concept of sustainability began to increase in importance at the end of the 1980s and at the beginning of the 1990s with the Brundtland report (1987) and the Conference on Environment and Development held in Rio de Janeiro, Brazil, in 1992 (the so-called Earth Summit), respectively. Nevertheless, the need to preserve natural resources for use by future generations had long been recognised.

The negative influence of past use of forest resources, as well as the needs for continued use of these resources for future generations was already noted as early as the 17th century (Glacken, 1976, as cited in Wiersum, 1995). However, it was not until the 18th century that the concept of sustainability was specifically referred to, as follows: "every wise forest director has to have evaluated the forest stands without losing time, to utilize them to the greatest possible extent, but still in a way that future generations will have at least as much benefit as the living generation" (Schmutzenhofer, 1992, as cited in Wiersum, 1995). This first definition was based on the principle of sustainable forest yield, with the main goal being sustained timber production, and it was assumed that if stands that are suitable for timber production are sustained, then non wood forest products will also be sustained (Peng 2000). This assumption focused on the sustainability of the productive functions of forest resources, while other functions such as ecological or socio-economic functions were largely overlooked. This occurred because social demands for forests were mainly utilitarian. However, increased environmental awareness and improved scientific knowledge regarding deterioration of the environment have changed society's values and the global structural policy, which in turn have significantly influenced forest management objectives in 20th century (Wang & Wilson, 2007). Nevertheless, nowadays more and more researchers think climate change is changing the paradigm and sustainability shouldn't be referred to what we had before.

Although there is no universally accepted definition of SFM, the following concepts are widely accepted: "*the process of managing permanent forest land to achieve one or more clearly specified objectives of management with regard to the production of a continuous flow of desired forest products and services without undue reduction of its inherent values and future productivity* *and without undue undesirable effects on the physical and social environment*" (proposed by International Tropical Timber Organization: ITTO, 1992), and "*the stewardship and use of forests and forest lands in a way, and at a rate, that maintains their biodiversity, productivity, regeneration capacity, vitality and their potential to fulfill, now and in the future, relevant ecological, economic and social functions, at local, national, and global levels, and that does not cause damage to other ecosystems*" (proposed by the second ministerial conference for the protection of the forest: MCPFE, 1993). The latter concept harmonizes ecological and socio-economic concerns at different scales of management and for different time periods. Nevertheless, both concepts are just refining the definition of sustainable development gave by the Brundtland Commission (1987) "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" to apply it to forests.

4. Criteria and indicators

The implementation of SFM is generally achieved using criteria and indicators (C&I). Criteria are categories of conditions or processes whereby sustainable forest management can be assessed, whereas quantitative indicators are chosen to provide measurable features of the criteria and can be monitored periodically to detect trends (Brand, 1997; Wijewardana, 2008) and qualitative indicators are developed to describe the overall policies, institutions and instruments regarding SFM (Forest Europe, 2011).

Different studies have pointed out the main characteristics of a good indicator. Thus, Prabhu et al. (2001) suggested seven attributes to improve the quality of indicators (precision of definition, diagnostic specificity, sensitivity to change or stress, ease of detection, recording and interpretation, ability to summarize or integrate information, reliability and appeal to users), whereas Dale & Beyeler (2001) established eight prerequisites to selection (ease of measurement, sensitivity to stresses on the system, responsive to stress in a predictable manner, anticipatory, able to predict changes that can be averted by management actions, integrative, known response to disturbances, anthropogenic stresses and changes over time, and low variability in response).

Although several criticisms have been launched against the C&I system (Bass, 2001; Gough et al., 2008; Poore, 2003; Prabhu et al., 2001), the popularity of the system is evident from the effort invested in its development in recent decades and from the large number of countries that are implementing their own sets of C&I within the framework of the nine international or regional process (African Timber Organization [ATO], Dry Forest in Asia, Dry Zone Africa, International Tropical Timber Organization [ITTO], Lepaterique of Central America, Montreal Process, Near East, Pan-European Forest [also known as the Ministerial Conference on the Protection of Forest in Europe, MCPFE] and Tarapoto of the Amazon Forest). Nevertheless, three of these processes stand out against the others[2], namely the ITTO, MCPFE and Montreal processes. The first set of C&I was developed by ITTO (1992) for sustainable management of tropical forest, and subsequently an initiative to develop C&I for sustainable management of boreal and temperate forests took place in Canada, under the supervision of the Conference on Security and Cooperation, in 1993. This first initiative reached a general consensus about the guidelines that should be

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[2] Together, these three international C&I processes represent countries where more than 90% of the world's temperate and boreal forests, and 80% of the world's tropical forests are located.

followed by all participating countries. It was then decided that the countries should be split into two groups: European would establish the MCPFE and non-European countries the Montreal processes. The MCPFE process adopted a first draft of C&I in the first expert level follow-up meeting in Geneva in June 1994, which took shape in Resolution L2 adopted at the third Ministerial Conference on the Protection of Forest in Europe held in Lisbon (MCPFE, 1998), and improved at the subsequent Ministerial Conference held in Vienna (MCPFE, 2003). On the other hand, the Montreal process established its set of C&I in the Santiago Agreement (1995), with Criteria 1-6 improved at the 18th meeting in Buenos Aires, Argentina (TAC, 2007) and criterion 7 improved at the 20th meeting in Jeju, Republic of Korea (TAC, 2009).

Although the different processes have very different origins and have developed their own criteria, there are some similarities between the three major SFM programs (Table 1). The main difference concerns criterion 7, developed by the Montreal process (Legal, policy and institutional framework), which was imbedded within each of the criteria in the MCPFE process (McDonald & Lane, 2004) and the concept of which is similar to criterion 1 in the ITTO process (Enabling condition). One important difference between ITTO and the other two processes is that the former does not consider maintenance of the forest contribution to global carbon cycles.

ITTO process	MCPFE process	Montreal process
C1. Enabling condition	C1. Maintenance and appropriate enhancement of forest resources and their contribution to global carbon cycles	C1. Conservation of biological diversity
C2. Extent and condition of forests	C2. Maintenance of forest ecosystem health and vitality	C2. Maintenance of productive capacity of forest ecosystems
C3. Forest ecosystem health	C3. Maintenance and encouragement of productive functions of forests (wood and non-wood)	C3. Maintenance of forest ecosystem health and vitality
C4. Forest production	C4. Maintenance, conservation and appropriate enhancement of biological diversity in forest ecosystems	C4. Conservation and maintenance of soil and water resources
C5. Biological diversity	C5. Maintenance and appropriate enhancement of protective functions in forest management (notably soil and water)	C5. Maintenance of forest contribution to global carbon cycles
C6. Soil and water protection	C6. Maintenance of other socioeconomic functions and conditions	C6. Maintenance and enhancement of long-term multiple socio-economic benefits to meet the needs of societies
C7. Economic, social and cultural aspects		C7. Legal, policy and institutional framework

Table 1. Criteria for sustainable forest management: comparison of three major programs

Other differences in indicators developed by the different processes have become apparent, and e.g. Hickey & Innes (2008) established more than 2000 separate indicators using the context analysis method. There are also substantial differences as regards the three major processes: the MCPFE process has 52 indicators (MCPFE, 2003), whereas the Montreal process has reduced the number of indicators from 67 (Santiago Agreement, 1995) to 54 (TAC, 2009), and the ITTO process has reduced the number of indicators from 66 in the first revision (ITTO, 1998) to the 56 considered at present (ITTO, 2005).

In light of the proliferation of C&I processes, the need to achieve harmonization has been widely recognised (Brand, 1997; Castañeda, 2000). Although the concept of harmonization is subject to several interpretations, harmonization should not be mistaken for standardization (Rametsteiner, 2006). Köhl et al (2000) has claimed that "harmonization should be based on existing concepts which should be brought together in a way to be more easy to compare, which could be seen as a bottom up approach starting from an existing divergence and ending in a state of comparability". Although there is not yet a common approach, considerable efforts have been made since the first expert meeting on the harmonization of Criteria and Indicators for SFM, held in Rome in 1995 (FAO, 1995), towards the search for a harmonization/collaboration among C&I processes through the Inter-Criteria and Indicator Process Collaboration Workshop (USDA, 2009). Advances in harmonization will minimise costs (avoiding duplication and preventing overlap), facilitate comparisons between countries and, overall, improve the credibility of SFM.

Although indicators are increasingly used, their utility is still controversial. Some authors have pointed out several weaknesses of the indicators, e.g. that they are often highly idealistic (Bass, 2001; Michalos, 1997), that they are a pathological corruption of the reductionist approach to science (Bradbury, 1996) or even that the same indicator may lead to contradictory conclusions according to the criterion and the scale. Nevertheless, there is general agreement that the advantages of the approach outweigh these limitations and that researchers should focus their efforts on testing the current indicators and searching for new indicators.

There are two key aspects involved in improving the current and future indicators, the use of a suitable scale and the establishment of a specific interpretation of each indicator. Although these have mainly been implemented at a national level, sub-national and forest management unit (FMU) levels are essential to assess SFM (Wijewardana, 2008). The FMU level has been considered as the finest scale in C&I processes. However it is well-known that for some indicators (mainly biodiversity indicators), another subdivision within this level may be necessary, such as plot, landscape and spatial levels, for correct interpretation (Barbaro et al., 2007; Heikkinen et al., 2004). In light of this level of precision and the fact that values of indicators are sometimes correlated with several different scales, managers and researchers should establish the most effective scale in each case, to avoid additional charges. Moreover, good indicators are not always easy to interpret in terms of sustainability, because most indicators do not exhibit a clear distinction/threshold between sustainability and unsustainability. In such cases, the achievement of sustainability should be considered on the basis of relative improvement in the current status of the indicator in question (Bertrand et al., 2008).

On the other hand, the scientific community must search for new indicators. Gaps in knowledge have been identified, and as these mainly involve ecological aspects, researchers should go further in investigating the relationships between type of forest management and ecological and socioeconomic functions. Thus, managers and researchers, with the support of scientific knowledge and public consultations, should be able to determine feasible goals, from socioeconomic and scientific points of view, since goals that are too pretentious may lead to a situation whereby SFM will not be promoted (Michalos, 1997). Only then can successful selection of new indicators of SFM be achieved.

5. Forest certification

In addition to the efforts of different states to develop C&I in the last two decades, a parallel process has been developed to promote SFM. This process is termed "forest certification". Forest certification can be defined by a voluntary system conducted by a qualified and independent third party who verifies that forest management is based on a predetermined standard and identifies the products with a label. The standard is based on the C&I approach and the label, which can be identified by the consumer, is used to identify products. Therefore, the two main objectives of forest certification are to improve forest management (reaching SFM) and to ensure market access for certified products (Gafo et al., 2011).

The first certification was carried out in Indonesia in 1990 by the SmartWood programme of the Rainforest Alliance (Crossley, 1995, as cited in Elliot, 2000). However forest certification became popular after The Earth Summit in Rio de Janeiro in 1992. Although important advances were reached at this summit, the failure to sign a global convention on forestry led environmental and non-governmental organizations to establish private systems of governance to promote SFM. In 1993, an initiative led by environmental groups, foresters and timber companies resulted in creation of the Forest Stewardship Council (FSC). Subsequently, other initiatives at international and national levels gave rise to many other schemes, e.g. the Programme for the Endorsement of Forest Certification (PEFC, previously termed Pan European Forest Certification), the Canadian Standards Association (CSA), the Sustainable Forestry Initiative (SFI), the Chile Forest Certification Corporation (CERTFOR) and the Malaysian Timber Certification Council, among others.

The area of certified forest increased rapidly in the 1990s and from then on more gradually, reaching 375 million hectares in May 2011 (UNENCE/FAO, 2011), which represents almost 10% of the global forest area. Although many forest certification systems were developed in the 1990s, only two schemes (PEFC and FSC) have been used for most of the forest currently certified throughout the world. The FSC scheme was established in 1993 to close the gap identified after the Earth Summit, and with more than 140 million hectares is the first program in terms of number of certified countries (81 countries) and the second system in terms of certified area at the moment (FSC, 2011). The PEFC scheme was established in 1999 as an alternative to the FSC scheme, and was led by European forest owners, who considered that FSC standards mainly applied to large tropical forests, but were inappropriate for small forest owners of European temperate forests. The PEFC scheme has gained importance because it endorses 30 national forest certification systems (Australian Forestry Standard, CSA, SFI, CERTFOR, etc.), and with more than 230 million hectares of certified forests is currently the largest forest certification system (PEFC, 2011). Although several authors have reported significant differences between FSC and PEFC (Clark & Kozar, 2011; Rotherham, 2011; Sprang, 2001), detailed analysis has revealed that FSC and PEFC are highly compatible, despite having arrived at their C&I by different routes (ITS Global, 2011).

Although forest certification began in tropical forests, the trend has changed and the scheme is now carried out in boreal and temperate forests. Almost 90% of forests certified by the two major programs (FSC and PEFC) are located within Europe and North America (Figure 2). More than half (54%) of the forests in Europe (excluding the Russian Federation) have already been certified, and almost one third of the forest area in North America has been certified (Figure 3). On the contrary, only about 1.5% of the forests in Africa, Asia, and Central and South America have been certified (Figure 3), despite the fact that more of half of the world's forests and almost 60% of primary world forests are located in these countries. The FSC and PEFC schemes display similar patterns of certification, since both mainly certify forests in Europe and North America. However, although the percentage of forest area certified by FSC in Africa, Asia, and Central and South America is only 16% of all certifications carried out by this scheme, this represents 75% of the forest areas certified in these regions. Furthermore, almost all certifications carried out in the Russian Federation are carried out by the FSC, whereas the PEFC has certified very few forests in this region. On the other hand, most forest certifications in Europe (excluding the Russian Federation) and North America have been carried out by PEFC (Figure 2).

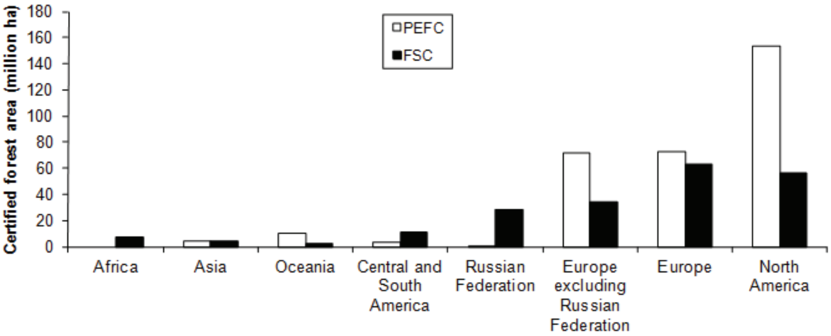

Fig. 2. Global FSC and PEFC certified forest area November 2011 – subregional breakdown (Source: FSC, 2011; PEFC, 2011)

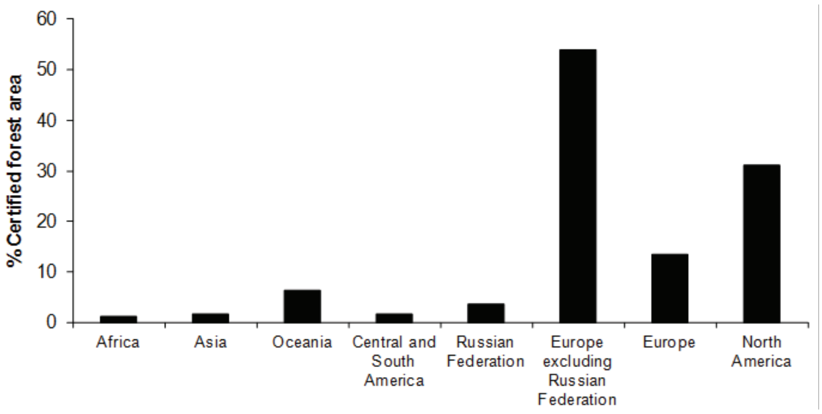

Fig. 3. Percentage of certified forest area, by both FSC and PEFC schemes, November 2011 – subregional breakdown (Source: FSC, 2011; PEFC, 2011)

Forest certification has became very popular, mainly because it is regarded it as a tool whereby everyone should benefit (win-win situation): forest owners should have an exclusive market with premium prices, the forest industry should improve its green corporate image, should not be held responsible for deforestation, and should have available a market tool, consumers should be able to use forest products with a clear conscience, and overall, forests should be managed sustainably.

The concept of forest certification is based on an economic balance, where forest owners and the forest industry place sustainable products on the market in the hope that consumers will be willing to pay the extra cost implied by SFM. Nevertheless, forest certification is still far from reaching its initial goal (win-win), since the expected price increases have not occurred (Cubbage et al., 2010; Gafo et al., 2011). In practice, only consumers and the forest industry have benefited; consumers use certified forest products with a clear conscience, and the forest industry has ensured market access without any great extra cost because this has mainly been assumed by forest owners.

This leads to a difficult question, namely, are forests benefiting from forest certification? It appears logical to believe that forest certification is beneficial to forests, since forest owners must demonstrate that the forests are being managed sustainably. Nevertheless, in depth-analysis reveals a different picture. As already noted, forest certification began in tropical forests with the aim of decreasing deforestation. However, nowadays almost all certified forests are located in developed countries. Furthermore, most of these forests are productive forests, such as single-species and even-aged forests or plantations, in which only small changes must be made to achieve forest certification, while primary forests have largely been ignored. The fact that foresters are able to place certified products from productive forests on the market, with a small additional charge compared to the extra charge involved in certifying products from primary forests hinders certification of the latter, which are actually the most endangered forests. Moreover, this disadvantage may favour unsustainable management, such as illegal logging or in extreme cases conversion of forest land to agricultural land, to favour market competitiveness. Against this background, other initiatives beyond of forest certification has been implemented, such as the FLEGT (Forest Law Enforcement, Governance and Trade) Action Plan of the European Union that provides a number of measures to exclude illegal timber from markets, to improve the supply of legal timber and to increase the demand for wood coming from responsibly managed forests (www.euflegt.efi.int) or the REDD (Reducing Emissions from Deforestation and Forest Degradation) initiative of the United Nations to create a financial value for the carbon stored in forests, offering incentives for developing countries to reduce emissions from forested lands and invest in low-carbon paths to sustainable development, including the role of conservation, sustainable management of forests and enhancement of forest carbon stocks (www.un-redd.org).

In addition, some environmental organizations now consider that plantations should not be certified, since they consider that plantations are not real forests. Such organizations also denounce the replacement of primary forests with plantations in developing countries (WRM, 2010). Although the replacement of primary forests with plantations is a damaging process, replacement of degraded areas such as abandoned pasture or agricultural land provides obvious advantages from economic and ecological points of view (Brockerhoff et al., 2008; Carnus et al., 2006; Hartley, 2002). The two most important schemes (FSC and PECF) approve the certification of forest plantations because they believe that the promotion of wood products from plantations will help to reduce the pressure on primary forests. The

FSC has added another principle (Principle 10: Plantations) in an attempt to ensure SFM in plantations, while the PECF considers that its criteria and indicators are sufficient to ensure the sustainability of planted forests. The FORSEE project was carried out in order to test the suitability of MCPFE indicators (which are used as the basis for PEFC certification in Europe) for planted forests at a regional level in eight Atlantic regions of Europe (Tomé & Farrell, 2009). This project concluded that with few exceptions, the MCPFE criteria and indicators appear suited to assess the sustainable management of forests, although it was pointed out that they should be considered as a blueprint for true SFM and adaptations are needed at the local level (Martres et al., 2011).

The viability of tropical forest certification will depend on forest owners obtaining premium prices that at least cover the certification costs, taking into account that these costs vary according to the type of forest (primary forest, plantations, etc.) and that consumers' willingness to pay premium prices will also differ. It should be possible for consumers to distinguish the origin of each product, and in other words different labels are required. Nevertheless, the use of different eco-labels is controversial, since many labels may confuse rather than help consumers. Teisl et al (2002) noted that consumers "seem to prefer information presented in a standardized format so that they can compare the environmental features between products" and highlighted "the need for education efforts to both publicize and inform consumers about how to use and interpret the eco-labels". Both of these are difficult tasks when different certifiers are rivals in the market place.

Without standardization and a powerful information campaign, most environmentally concerned consumers will probably demand wood from sustainably managed forests, without taking into account the type of certification label, and will choose the least expensive product (Teisl et al., 2002). This may entail a new associated problem, since producers and industries will probably also choose the bodies that certify forests most readily and at the lowest cost. This may lead to a situation where the certification schemes would tend to compete with each other and standards would be reduced to attract producers, as pointed out by Van Dam (2001).

6. Conclusion

Sustainable forest management is evolving with public awareness and scientific knowledge, and the sustainability concept must be revised to reflect the new reality generated by climate change, where a past reference point shouldn't be considered. Therefore, C&I should be updated continuously to be able to cope with the climate change challenge and assess sustainability of changing ecosystems. Furthermore, harmonization of C&I processes would be the most desirable outcome, since this would improve the credibility of the schemes.

On the other hand, forest certification has failed to avoid deforestation and has got two main challenges;

(1) to certify the forests that are most important in ecological terms and that are most susceptible to poor forest management, such as tropical forests and, to a lesser extent, non productive forest in boreal and temperate regions, and (2) to achieve a market with premium prices, in which the win-win concept will prevail. This will require educational campaigns and a higher level of credibility for labels. Moreover, parallel initiatives, such as FLEG and REDD, considering outside forest sector drivers leading to deforestation should be taking into account to limit this process.

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ARTICLE II

Martín-García, J., Díez, J.J. & Jactel, H. (2009) Towards standardised crown condition assessment in poplar plantations. *Annals of Forest Science* 66: 308-314

RESUMEN

En búsqueda de la estandarización en la evaluación de las condiciones de copa en las plantaciones de chopo

El objetivo del presente estudio fue desarrollar nuevas herramientas capaces de evaluar los indicadores de sanidad forestal en plantaciones de chopo. Treinta y dos plantaciones monoclonales de chopo fueron seleccionadas según un diseño factorial con tres factores: edad de los árboles, calidad de estación y la selvicultura aplicada al sotobosque. La transparencia de copa y decoloración fueron visualmente estimadas en todos los árboles de cuatro subparcelas circulares de 15 m en cada una de las plantaciones. Para poder comparar las estimaciones visuales y digitales de la transparencia de copa se evaluaron un subconjunto de árboles a partir de fotografías digitales usando un sistema semiautomático de análisis de imágenes (el software CROCO). Las condiciones de copa de las choperas fueron mejores en las plantaciones jóvenes y en aquellas establecidas en óptimas calidades de estación. El gradeo del sotobosque mejoró la vitalidad de los árboles en las peores calidades de estación. El muestreo de la transparencia de copa y decoloración de 20 árboles por parcela aporta la misma información que una intensidad de muestreo de 60 árboles. Las curvas de calibración para estimar digitalmente la transparencia de copa se ajustaron adecuadamente. Los efectos de la edad y la calidad de estación también pudieron ser detectados con la evaluación digital de la transparencia de copa. La evaluación de fotografías digitales mediante el software CROCO en alrededor de 20 árboles por parcela es recomendado para evaluar de forma precisa y objetiva las condiciones de copa en las plantaciones monoclonales de chopo.

Palabras clave: Chopera, indicador, sanidad forestal, condiciones de copa, CROCO

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Original article

Towards standardised crown condition assessment in poplar plantations

Jorge MARTÍN-GARCÍA[1]*, Julio Javier DIEZ[1], Hervé JACTEL[2]

[1] Department of Plant Production and Forest Resources, University of Valladolid, Avenida de Madrid 44, 34004 Palencia, Spain
[2] Université de Bordeaux, INRA, UMR 1202 Biodiversity, Genes and Communities, 33612 Cestas Cedex, France

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Abstract

• This work aims at developing new tools for rapid assessment of forest health indicators in poplar plantations.

• Crown transparency and discoloration were visually evaluated in all trees of four 15 m-radius subplots in 32 poplar clonal plantations, which were chosen according to a factorial scheme with three factors: tree age, site quality and understorey vegetation management. A subset of trees was assessed using digital photos processed with a semi-automatic image analysis system (the CROCO software) in order to compare visual and digital crown transparency estimates.

• Poplar crown conditions were better in young stands and rich sites. Harrowing understorey vegetation improved tree health in poor sites. Samples of 20 trees per stand provided the same information about crown transparency and discoloration as 60 trees. Calibration curves of digital crown transparency estimates were successfully fitted against visual crown transparency estimates. The same effects of stand age and site quality could be detected with digital crown transparency as response variable.

• The use of digital photos processed with CROCO in ca. twenty trees per stand is therefore recommended to accurately and objectively monitor crown condition in clonal poplar plantations.

Mots-clés :
peuplier /
indicateur /
santé des forêts /
houppier /
CROCO

Résumé – Améliorations en vue d'un suivi standardisé de l'état sanitaire des houppiers en plantations monoclonales de peuplier.

• Une étude a été réalisée pour développer des méthodes rapides et standardisées de suivi de l'état sanitaire des houppiers en plantation de peuplier.

• La transparence et la décoloration des houppiers ont été estimées visuellement sur tous les arbres de quatre placettes d'un rayon fixe de 15 m, dans 32 plantations monoclonales de peuplier choisies selon un plan factoriel complet croisant trois facteurs : l'âge des plantations, la fertilité du site et la gestion du sous-bois. La moyenne des notes de transparence et de décoloration des houppiers a été calculée sur un nombre décroissant de placettes par plantation afin de tester l'influence de la taille de l'échantillon sur la qualité de l'estimation. Des photographies numériques du houppier d'un sous-échantillon de peupliers ont été également prises puis traitées à l'aide du logiciel CROCO afin de comparer les estimations visuelles et numériques de la transparence du houppier.

• L'état sanitaire des houppiers était meilleur dans les jeunes plantations de peupliers et les sites les plus fertiles. Le hersage de la végétation du sous-bois a permis d'améliorer l'état sanitaire des peupliers dans les sites les plus pauvres. Les valeurs moyenne de transparence et de décoloration du houppier par plantation, calculées à partir des données d'une seule placette de 15 m de rayon – soit une vingtaine d'arbres – ne différaient pas significativement de celles calculées à partir des données de trois placettes et permettaient de détecter les mêmes effets des caractéristiques du peuplement et du site. Des courbes de calibration de la transparence du houppier estimée à partir de photos numériques et du logiciel CROCO ont pu être ajustées aux observations de transparence. Les effets de l'âge des plantations, de la fertilité du site et de la gestion du sous-bois ont pu également être détectés avec ces valeurs numériques calculées.

• Il peut donc être recommandé d'utiliser des photos numériques traitées à l'aide du logiciel CROCO pour estimer la transparence du houppier d'une vingtaine d'arbres afin d'évaluer rapidement, précisément et objectivement l'état sanitaire des plantations monoclonales de peuplier.

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* Corresponding author: jorgemg@pvs.uva.es

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1. INTRODUCTION

Since the United Nations Conference on the Environment and Development (UNCED, Rio de Janeiro, 1992), the sustainable forest development (SFM) has become an essential concept worldwide (Castañeda et al, 2001). To assess the implementation of SFM different criteria have been elaborated. For example, the Ministerial Conferences for the Protection of Forests in Europe (MCPFE) has proposed a set of six criteria that relate to key elements of sustainability. In particular the second criterion deals with the maintenance of forest ecosystems health and vitality. Because policy makers and forest managers require instruments to evaluate progress towards implementing sustainable forest management, quantitative and qualitative indicators have been developed for each criterion. A considerable effort has been paid to improve SFM indicators as they need to be accurate and reliable but also sufficiently straightforward and cost effective to be documented over time (Hunsaker, 1993). For example crown transparency and crown discoloration are often used as indicators of tree health and vitality (Zarnoch et al., 2004), because they comply with these desirable indicators characteristics.

A forest health monitoring program has been carried out in Europe since the 1980s within the International Co-operative Programme, ICP Forest (Level I European network). It comprised more than 6100 plots, where both tree crown transparency and crown discoloration were visually estimated on ca. 20 trees per plot each year. However, the ability of such a systematic network to capture the real health condition of small and fragmented forest types remains questionable (Ferretti, 1997). This is the case of poplar plantations, which cover about 800 000 ha in Europe and yet there are only 23 plots of *Populus* monitored within the European Level I Network (Lorenz et al., 2005). It is therefore desirable to increase the number of sampled plots and one way to reach this objective for the same effort is to minimize the number of sampled trees per plot. Because poplar, willow or eucalyptus plantations are often monoclonal, protocols developed to assess health conditions in other types of forest may not be relevant. In particular, as the variability of traits between trees is lower, one can assume that tree clones will be similarly damaged (Braganca et al., 1998; Camps, 2001; Sierra, 2001) and that smaller samples of trees can be representative for the whole stand condition.

Another option to increase the reliability of forest health assessment while keeping the sampling effort to a minimum is to develop automatic, standardized methods. Considerable effort has been devoted to improve the assessment of tree crown transparency and discoloration (Ferretti, 1997). In the European Network Level I, these variables are visually estimated by observers from the ground, but due to the subjectivity of human assessment, data quality and comparability across countries have been questioned (Mizoue and Dobbertin, 2003). The sources of error in the quantitative assessment of crown condition are many, including variation in the expertise of observers, weather conditions, crown appearance, tree species, tree age and social position (Ghosh et al., 1995; Innes et al., 1993; Redfern and Boswell, 2004; Solberg and Strand, 1999; Wulff, 2002). Such sources of error make it difficult to compare patterns between countries, and even across time within the same country. They may also mask the relationship between forest decline and explanatory factors such as pest pressure or site conditions. Researchers have tried to solve these problems, combining field and control team assessments (Ghosh and Innes, 1995), using data from cross-calibration courses to estimate correction factors for between countries differences (Innes et al., 1993), using reference photographs (Solberg and Strand, 1999) or standard sets of two-dimensional silhouettes representing various degrees of foliage density (Frampton et al., 2001). Nevertheless, these improvements sometimes are not enough and proposals have been made to replace visual ground assessment by digital photo (Čermák et al., 2007; Mizoue, 2002; Sang-Mook et al., 2003) or by remote sensing techniques (Coops et al., 2004; Solberg et al., 2004; Stone et al., 2003). In particular, Mizoue (2002) developed a semi-automatic image analysis system, called CROCO, to assess crown transparency from digital photographs.

The main objective of this study was therefore to refine a monitoring method to assess two important forest health indicators in poplar stands quantitatively and objectively. To achieve this objective the following two questions were addressed:

1. Is a sample of 20 trees large enough to detect the effects of age, site and management on crown transparency and discoloration in poplar stands?
2. Can visual crown transparency be substituted by CROCO estimates to detect the same effects of site and management in poplar stands?

2. MATERIALS AND METHODS

2.1. Site description and experimental design

The present study was carried out in Castile and Leon (Spain) where there are about 45 000 ha of poplar plantations, the most common species grown is *Populus × euramericana* hybrid (*P. nigra × P. deltoides*). Several clones are used, but the clone I-214, covering about 70% of the total poplar plantation area (Fernández and Hernanz, 2004), is the most representative. The density of trees in clonal poplar plantations is kept constant during the whole rotation, at about 278–400 stems/ha, according to the planting distance of 6 × 6 or 5 × 5 m, respectively. Traditional management of poplar stands is only applied during the first six years of the plantation: understorey vegetation is controlled with disc harrowing every year until canopy closure and all trees are pruned up to six meters from the ground level. Poplar plantations are clear-cut at fourteen years.

The experimental design consisted of a factorial scheme with three factors: (1) stand age (young stands of 3–7 years old or adult stands of 8–14 years old), (2) understorey management (harrowed or not harrowed) and (3) site quality. We differentiated rich sites (quality 1 and 2) or poor sites (quality 3 and 4), according to the site quality abacus developed for the *Populus x euramericana* clone I-214 in the river Duero basin (Bravo et al., 1995). Four I-214 clonal plantations as replicates of each factors combination were sampled. A total of 32 poplar stands were therefore sampled in the north of the Palencia province, within the Carrion river basin (from 346 405 to 366 495 and 4 686 275 to 4 712 381, latitude and longitude respectively in UTM coordinates).

2.2. Assessment of crown condition variables in poplars

In each stand, four circular subplots of 15 m radius were monitored. These subplots were located 50 m apart from each other, at the ends of cross located in the middle of the stand. Within each subplot all trees were marked and sampled. All sub-plots had the same number of sampled trees in a given stand (21 on average). A total of 2 695 poplar trees were assessed during the summer of 2005 (within the first two weeks of July) as recommended in the ICP protocol. Crown transparency and discoloration were visually estimated and quantified using twenty classes of 5% width, according to Level I European network methodology (Eichhorn et al., 2006). Simultaneously, all trees were photographed using a digital photo camera of 8 mega pixels (EOS 350D, Canon), from the same position. Biotic damages in the crown were also recorded but there were so few that they have not been further taken into consideration.

Subsequently, according to the methodology suggested by Mizoue (2002), 7 trees with contrasted percentages of crown transparency were selected and photographed. Then the same crown images were analysed by one expert observer from the Spanish field crew of the European Level I network to provide an estimate of crown transparency.

A subset of 265 trees was also used to compare visual and digital crown transparency estimates. The 2 695 trees were split into four groups corresponding to stand age and site condition combinations: 601 trees from "young and poor" stands, 609 from "young and rich", 847 from "adult and poor" and 638 from "adult and rich" stands respectively. Trees were sorted according to crown transparency values within each group and then one to ten trees were randomly selected within each crown transparency class in each of the four groups.

Values of DSO index of crown transparency were calculated using the CROCO method (Mizoue and Dobbertin, 2003). In CROCO an automatic thresholding algorithm is used to obtain crown silhouette images, where foliage and branches are transformed to black pixels and background sky to white pixels (Mizoue and Inoue, 2001). CROCO calculates two fractal dimensions to estimate the crown transparency of the tree silhouette (Ds) and outline (Do). DSO is calculated as the difference between Ds and Do indicating the index of crown transparency. DSO values decrease with increasing crown transparency. To implement this method we first cut out a rectangular region of interest (ROI) in each crown photograph, including the part of the crown exposed to the sunlight, but excluding the parts overlapping with adjacent trees. At the same time, the overlap rate (OR) was visually categorized into 8 classes (no overlap, 25, 50, 75, 100% overlap on one side of the crown and 25, 50, 75% overlap on both sides). Second, an automatic thresholding algorithm was applied to the blue-filtered grey scale image to generate a crown silhouette image. Finally, DSO values were calculated from the crown silhouette images using fractal analysis (Mizoue, 2001). Trees that overlapped have positively biased DSO values (underestimation of crown transparency). This was corrected using the simple linear regression model developed by Mizoue (2002). The corrected DSO provided an estimate of crown transparency, called Digital Crown Transparency (DCT). CROCO uses Scion Image for Windows (available for free at the http://www.scioncorp.com/) and image processing software (in this work we used Adobe Photoshop).

2.3. Statistical analyses

Paired t tests of mean % Visual Crown Transparency (VCT) and mean % Visual Crown Discoloration (VCD) per stand were carried out to compare dependent variable mean values (after angular transformation, arcsin $\sqrt{(x)}$, Peña, 2002) in the same stand but calculated from different sample sizes, i.e. combinations of one, two or three sub-plots per poplar stand. Only comparisons that did not involve the same sub-plot were tested to respect data independency. For the same reason the combination of four sub-plots was not tested as all other combinations would have had shared trees.

Analyses of variance (ANOVAs) and Tukey's HSD post-hoc test of VCT and VCD mean values per poplar stand from 4 sub-plots (2 695 tree data) or 1 subplot (757 tree data) were carried out after angular transformation (arcsin $\sqrt{(x)}$, Peña, 2002) to test whether the sample size used to calculate mean values can influence the detection of the effect of site quality, stand age and understorey management on both indicators.

Simple linear regressions were used to fit calibration curves of DCT against log VCT with data from 7 trees such as proposed by Mizoue (2002), and from 265 trees to test the fit with a higher number of trees. Finally, another ANOVA was carried out to check whether DCT would permit to detect the same effects of site quality, age and understorey management than VCT, using the dataset of 265 trees.

All analyses were performed using Statistica 6.0 package (StatSoft, 2001).

3. RESULTS

3.1. Effect of sample size on crown conditions estimation in poplar stands

Comparison of VCT and VCD mean values per stand (N = 32) calculated from different combinations of one, two or three sub-plots did not show any significant differences (Tab. I), indicating that mean VCT and VCD values were similar when the sample size equalled ca. 21, 42 and 63 trees respectively.

Analyses of variance for VCT and VCD mean values per stand (N = 32), using trees from four subplots or from only one subplot, showed exactly the same significant effects of the factors on dependent variables (Tab. II). Visual crown transparency (VCT) and visual crown discoloration (VCD) mean values were similarly and significantly affected by site quality (Tab. II). Crown conditions were always of poorer quality (higher VCT and VCD values) in poor than in rich sites (Figs. 1 and 2). The interaction between site quality and stand age was significant for both VCT and VCD whereas the interaction between site quality and understorey management was only significant for mean visual crown transparency. This indicates that the pattern of crown conditions responded to both stand age and understorey management depending on site quality. Tukey's HSD tests (Figs. 1 and 2) revealed that in rich sites there was neither significant difference in VCT or VCD between old and young stands nor between harrowed or not harrowed stands. By contrast in poor sites, a significant increase of damage was observed in old stands (higher VCT and VCD values) as well as in harrowed stands (only for VCT).

Table I. *P* values of paired *t* tests ($N = 32$) comparing means of % Visual Crown Transparency or % Visual Crown Discoloration (*in italic*) of poplar trees calculated with different combinations of one, two or three sub-plots per stand, after angular transformation ($\arcsin\sqrt{(x)}$).

	1	**2**	**3**	**4**	**1 + 2**	**1 + 3**	**1 + 4**	**2 + 3**	**2 + 4**	**3 + 4**	**1 + 2 + 3**	**1 + 2 + 4**	**1 + 3 + 4**	**2 + 3 + 4**
1	▓	*0.964*	*0.549*	*0.978*	░	░	░	*0.861*	*0.771*	*0.947*	░	░	░	*0.906*
2	0.618	▓	*0.373*	*0.941*	░	*0.881*	*0.555*	░	░	*0.967*	░	░	*0.458*	
3	0.346	0.513	▓	*0.452*	*0.250*	░	*0.258*	░	*0.185*	░	░	*0.141*		
4	0.414	0.863	0.559	▓	*0.773*	*0.982*	░	*0.827*	░	░	*0.849*			
1 + 2	░	░	0.335	0.488	▓	░	░	░	░	*0.636*				
1 + 3	░	0.996	░	0.784	░	▓	░	░	*0.747*					
1 + 4	░	0.797	0.375	░	░	░	▓	*0.464*						
2 + 3	0.438	░	░	0.851	░	░	0.521							
2 + 4	0.505	░	0.451	░	░	0.941								
3 + 4	0.351	0.650	░	░	0.347									
1 + 2 + 3	░	░	░	0.749										
1 + 2 + 4	░	░	0.431											
1 + 3 + 4	░	0.965												
2 + 3 + 4	0.536													

Numbers in lines and columns indicate sub-plot numbers and combinations. Light grey cells indicate inappropriate comparisons, those in which the two compared tree samples were not independent (i.e. sharing common trees).

Table II. Analyses of variance of Visual Crown Transparency and Visual Crown Discoloration mean values per poplar stand ($N = 32$) calculated from 4 sub-plots (2 695 tree data) or 1 subplot (757 tree data) to test the effect of site quality, stand age and understorey management.

$N = 32$		Visual Crown Transparency				Visual Crown Discoloration			
		4 sub-plots		1 sub-plot		4 sub-plots		1 sub-plot	
Source	*d.f.*	*F*	*Pr > F*	*F*	*Pr > F*	*F*	*Pr > F*	*F*	*Pr > F*
Quality	1	108.2	< **0.01**	73.4	< **0.01**	20.6	< **0.01**	21.9	< **0.01**
Management	1	1.8	0.19	2.7	0.11	0.2	0.69	0.1	0.73
Age	1	94.8	< **0.01**	50.2	< **0.01**	11.9	< **0.01**	11.5	< **0.01**
Quality × Management	1	12.1	< **0.01**	7.2	**0.01**	0.7	0.41	1.2	0.28
Quality × Age	1	46.2	< **0.01**	30.8	< **0.01**	10.9	< **0.01**	8.9	< **0.01**
Management × Age	1	< 0.1	0.99	< 0.1	0.98	< 0.1	0.87	< 0.1	0.77
Quality × Management × Age	1	0.3	0.62	< 0.1	0.85	0.1	0.78	0.2	0.66

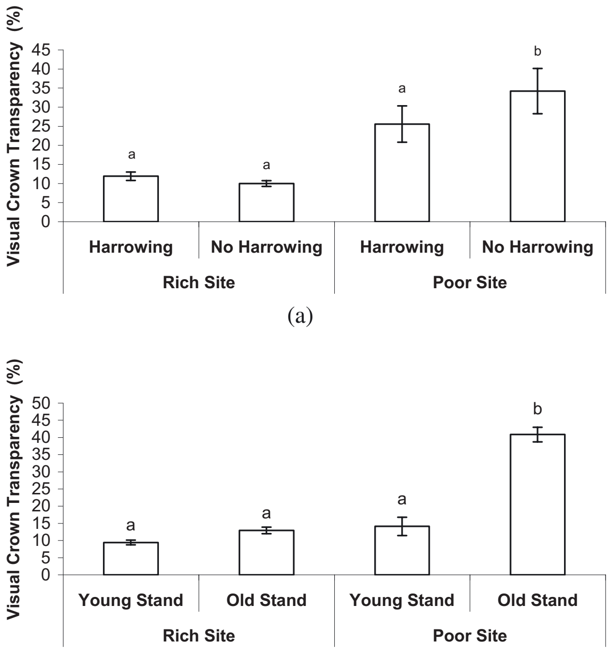
3.2. Ability of CROCO to replace visual crown transparency assessment

Based on a sample of seven trees selected along a linear gradient of crown transparency, the calibration curve between visual estimates (log VCT values) and digital estimates (DCT values obtained with CROCO) showed a very good fit with a simple regression ($R^2 = 0.97$, $P < 0.0001$) (Fig. 3). Similar regression, made with data from 265 trees selected across a longer gradient of VCT values, showed a good fit too ($R^2 = 0.64$, $P < 0.0001$) (Fig. 4).

Analyses of variance for VCT and DCT mean values per stand ($N = 32$), using the subset of 265 trees, showed similar effects of the factors, although slightly less significant for the Digital Crown Transparency dependant variable (Tab. III). Furthermore the significant effects of factors and of interactions between factors were consistent with those obtained when mean values were calculated from all trees (Tab. II). The only discrepancy concerns the moderate significant effect of understorey management on mean VCT calculated with 265 trees (Tab. III).

4. DISCUSSION

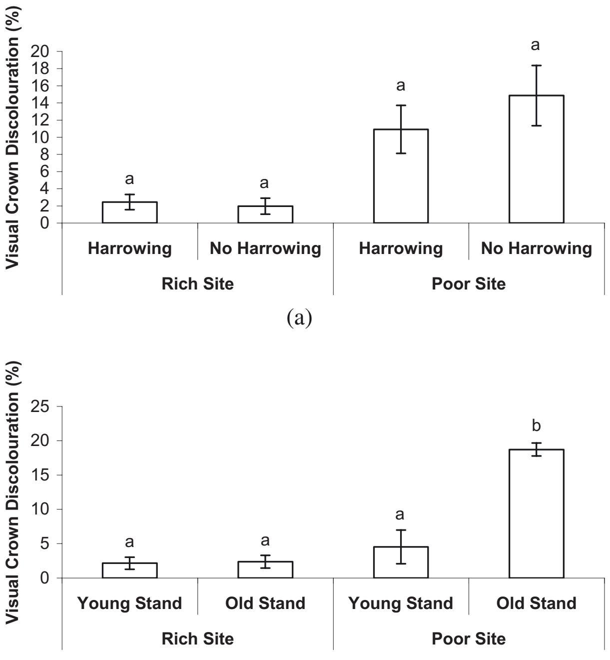
Several studies have analysed the effect of different factors, such as stand age and site conditions on crown condition in trees (Bussotti et al., 2002; Hendriks et al., 2000; Innes, 1993; Klap et al., 2000; Solberg, 1999). Stand age has often been considered as a factor potentially affecting crown transparency. Hendriks et al. (2000) observed that crown transparency significantly increased with tree age in oak and Scots pine but not in Douglas fir. In the present study crown transparency was higher in older clonal poplar plantations, particularly in poor sites. The elongation of branches in older trees may have given the impression that crown is more transparent (Metzger and Oren, 2001). The susceptibility of trees to biotic agents may also increase with age (Solberg, 1999), as well as stress factors such as drought or nutritional deficiency thus resulting in a cumulative detrimental effect with age. In this study results also showed that crown conditions in poplar stands improved with site quality, which is corroborated by several authors (Ferretti, 1998; Solberg, 1999). It is likely that nutritional stress in poor quality sites had a detrimental effect



(a)

(b)

Figure 1. Mean (± S.E.) Visual Crown Transparency (VCT) values per poplar stand for each site quality type according to (a) understorey management and (b) stand age. Within each site quality, bars with different letters indicate significantly different means (Tukey's post-hoc test, $\alpha = 0.05$).



(a)

(b)

Figure 2. Mean (± S.E.) Visual Crown Discoloration (VCD) values per poplar stand for each site quality type according to (a) understorey management and (b) stand age. Within each site quality, bars with different letters indicate significantly different means (Tukey's post-hoc test, $\alpha = 0.05$).

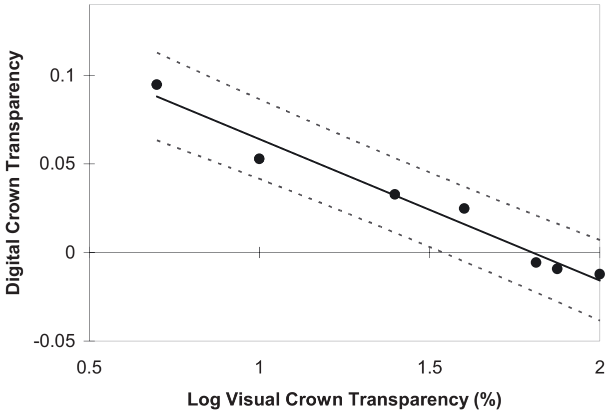


Figure 3. Relationship between Visual and Digital Crown Transparency values obtained with the CROCO computation in 7 poplar trees of contrasted crown transparency. Continuous line indicates the regression fit; dotted line indicates the confidence interval (95%) ($N = 7$, $F_{6,1} = 152.0$, $P < 0.0001$, $R^2 = 0.97$).

on tree physiology, resulting in a proportional reduction of foliar biomass or increased the susceptibility of trees to biotic hazards that damaged tree crowns. To our knowledge, the effect of forest management on crown conditions had never been studied in poplars. Our results showed that harrowing understorey vegetation, the main management practice in poplar plantations, had a beneficial effect on crown conditions in poor sites. Harrowing may reduce competition between understorey vegetation and trees for water and nutrient supply. Furthermore harrowing may also improve the soil structure and facilitate the incorporation of nutrients into the litter, thus resulting in a gain of stand fertility. These advantages should be more important in poor sites because competition for water and nutrient is higher. Crown discoloration seems to respond in the same manner to the same stand factors although slightly less significantly. This may result from less accurate VCD estimates since crown discoloration is known to be more difficult to be assessed objectively (Ferretti, 1998; Wulff, 2002).

Different shapes and sizes of sampled plot have been proposed for forest health surveys, like four subplots of six trees located 25 m from a fixed point by the ICP-Forest in Europe (Eichhorn et al., 2006), four subplots spaced 36.6 m apart with a fixed radius by the Forest Health Monitoring in the U.S. (Zarnoch et al., 2004) and one circular plot of 24 trees in Spain (SPCAN-DGCN, 2002). In this study there was no significant difference between the mean values of crown condition variables calculated from one, two or three subplots of 15 m radius. Moreover, mean values calculated from only one subplot of ca. 21 trees could be used to discriminate the same effect of driving factors than those computed from four subplots. Contrary to the results obtained by Innes and Boswell (1990) in even-aged stands of conifers, who demonstrated significant variations amongst the 4 subplots of each plot, the assessment of only one subplot of fixed radius (exactly 15 m,

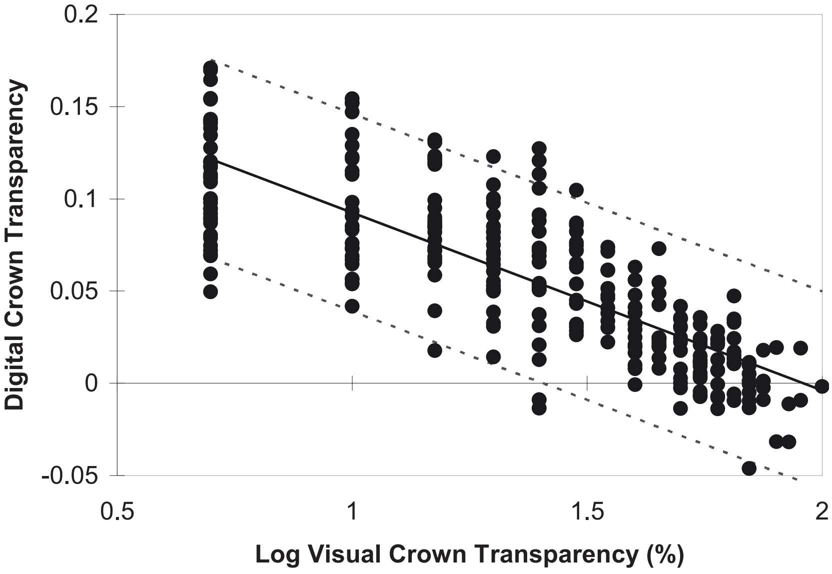


Figure 4. Relationship between Visual and Digital Crown Transparency values obtained with the CROCO computation in 265 poplar trees of contrasted crown transparency. Continuous line indicates the regression fit; dotted line indicates the confidence interval (95%) ($N = 265$, $F_{264,1} = 467.0$, $P < 0.0001$, $R^2 = 0.64$).

Table III. Analyses of variance of Visual and Digital Crown Transparency mean values per poplar stand ($N = 32$) calculated with 265 trees data to test the effect of site quality, stand age and understorey management.

$N = 32$		Crown Transparency			
		Visual		Digital	
Source	*d.f.*	*F*	*Pr > F*	*F*	*Pr > F*
Quality	1	75.1	**< 0.01**	25.7	**< 0.01**
Management	1	7.2	**0.01**	0.3	0.61
Age	1	43.4	**< 0.01**	7.4	**0.01**
Quality × Management	1	16.5	**< 0.01**	4.9	**0.04**
Quality × Age	1	33.9	**< 0.01**	11.9	**< 0.01**
Management × Age	1	< 0.1	0.87	0.9	0.3
Quality × Management × Age	1	3.6	0.07	1.3	0.3

i.e. approximately 18 trees in plantations with planting distance 6 × 6 m) seems to be sufficient to qualify forest health in poplar plantation. This discrepancy may be due to the higher homogeneity of trees within monoclonal plantations.

Because crown transparency can be considered as a relevant indicator to assess tree crown condition in poplars, our next aim was to tune up a methodology to estimate it objectively. We used the CROCO methodology developed by Mizoue (2002) and with a sample of 7 trees of contrasted crown conditions, we obtained a highly significant correlation between visual and digital crown transparency estimates ($R^2 = 96.18\%$). This value was comparable to those obtained with a similar approach in Norway spruce, silver fir, Scots pine, larch, beech, pedunculate oak, sycamore and ash, for which R^2 varied between 93 and 99% (Mizoue and Dobbertin, 2004).

We could also successfully fit a significant linear model relating visual and digital crown transparency estimates in a larger sample of 265 poplar trees, indicating that operational calibration curves, with more reference points across a longer range of VCT values, could be delivered to end-users. The use of trees from a single clone (I-214) probably made this result possible (Al Afas et al., 2007). Moreover, we demonstrated that DCT values were accurate enough to allow detecting the driving factors of crown transparency in poplar stands.

We therefore believe that the CROCO method is a valuable tool to precisely assess the "crown transparency" indicator in poplar plantation. Problems due to the subjectivity of visual assessment may be then solved, thus facilitating the investigation of biotic agents or abiotic factors responsible for changes in crown transparency (Hendriks et al., 2000) and allowing comparison between surveys results over time (Mizoue and Dobbertin, 2003). It is possible that in the future, national forest inventories have to record more variables than the traditional dendrometric features, for example tree health status. In this context, we have shown that the CROCO methodology is particularly relevant to crown transparency monitoring in poplar stands because it can be used with small tree sample size, such as one fixed 15 m-radius plot, and because it can be applied with no special expertise in tree pathology.

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ARTICLE III

Martín-García, J., Merino, A. & Diez, J.J. (2012) Relating visual crown conditions to nutritional status and site quality in monoclonal poplar plantations (*Populus x euramericana*). *European Journal of Forest Research* 131: 1185-1198

RESUMEN

Relación de las condiciones de copa, el estado nutricional y la calidad de estación en las plantaciones monoclonales de chopo

La superficie cubierta por plantaciones de chopos híbridos en Europa ha incrementado notablemente debido a su alta rentabilidad económica. Sin embargo, su productividad varía considerablemente según el estado nutricional de la parcela, y por lo tanto es esencial identificar los nutrientes limitantes. Los objetivos de este estudio fueron (1) identificar las principales deficiencias nutricionales que condicionan el crecimiento y vitalidad de las plantaciones monoclonales de chopo (*Populus x euramericana*, clon I-214) y (2) desarrollar un método para la pronta detección de problemas nutricionales basado en la evaluación de las condiciones de copa. El estudio fue llevado a cabo en una de las áreas más idóneas para el cultivo del chopo en el sur de Europa. Treinta y dos plantaciones de chopo con diferentes grados de vitalidad fueron seleccionadas para evaluar el crecimiento de los árboles, el estado nutricional (características del suelo y concentraciones de nutrientes foliares) y las condiciones de copa. Las plantaciones establecidas en suelos con un algo porcentaje de elementos gruesos mostraron bajo crecimiento de los árboles, pobres condiciones de copa y deficiencias en Fe, B, N, P, K, y en menor medida Zn y Cu. Las deficiencias se agravaron con la edad de la parcela, probablemente debido a una mayor demanda nutricional en los árboles adultos. Las condiciones de copa se relacionaron con el crecimiento de los árboles y el estado nutricional de la parcela. Modelos predictivos fueron desarrollados para relacionar las condiciones de copa al estado nutricional de la parcela, el cual puede ser evaluado mediante un estudio de las propiedades del suelo y las concentraciones foliares de los nutrientes.

Palabras clave: Chopera, estado nutricional, indicador, sanidad forestal, condiciones de copa.

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ORIGINAL PAPER

Relating visual crown conditions to nutritional status and site quality in monoclonal poplar plantations (*Populus* × *euramericana*)

Jorge Martín-García · Agustín Merino ·
Julio Javier Diez

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Abstract The area covered by plantations of hybrid poplar in Europe is increasing greatly because of the high profitability of these trees. However, the productivity varies widely depending on nutritional status, and it is therefore essential to identify the limiting nutrients. The aims of this study were (a) to identify the main nutrients limiting the growth and vitality of monoclonal poplar (*Populus* × *euramericana*) plantations and (b) to develop a means of early detection of nutrition-related problems in growth, based on visual crown conditions (crown transparency, VCT, and visual crown discoloration, VCD). The study was carried out in one of the most suitable areas for this species in Southern Europe. Thirty-two *Populus* × *euramericana* (clone I-214) stands displaying different levels of decline were selected for study, and tree growth, nutritional status (soil properties and foliar nutrient concentrations) and crown conditions were assessed. The stands, which were growing in soils with high contents of coarse fragments, displayed low growth rates, poor crown conditions and deficiencies in Fe, B, N, P, K, and to a lesser extent, Zn and Cu. The deficiencies increased with the age of the stand, presumably because of the higher nutrient demand in older trees. Visual crown conditions were related to growth rates and nutritional status. Predictive models were developed to relate crown conditions to the nutritional status identified by analysis of soil properties and foliar concentrations of nutrients.

Keywords Poplar · Nutritional status · Indicator · Forest health · Visual crown conditions

Introduction

The area covered by plantations of hybrid poplar in Europe, currently around 900,000 ha (Ball et al. 2005), is increasing greatly because of the high profitability of these trees (up to 2,400 € ha^{-1} year^{-1}; Díaz and Romero 2001). The countries where most poplars are planted are France, Italy and Spain (Ball et al. 2005), and the main use for the timber is as a source of plywood.

In Southern Europe, poplar plantations are monoclonal, and although several clones are used, clone I-214 is the most common, and covers about 70% of the total area planted (Fernández and Hernanz 2004). Poplar plantations are managed intensively in short rotations (12–16 years), and weed control techniques (mainly surface ploughing) are used regularly during the first 6 years. The density of poplar plantations, which is kept constant during the whole rotation, is approximately 278–400 stems/ha. The species has a deep root system and requires large amounts of water. Poplar trees are therefore placed in direct contact with the

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J. Martín-García (✉) · J. J. Diez
Sustainable Forest Management Research Institute, University of Valladolid, INIA, Avenida Madrid, 57, 34004 Palencia, Spain
e-mail: jorgemg@pvs.uva.es; jorgemg@unex.es

J. J. Diez
e-mail: jdcasero@pvs.uva.es

J. Martín-García
Forestry Engineering, University of Extremadura, Avenida Virgen del Puerto 2, 10600 Plasencia, Spain

A. Merino
Department of Soil Science and Agricultural Chemistry, Escuela Politécnica Superior, Universidad de Santiago de Compostela, Campus Universitario, 27002 Lugo, Spain
e-mail: agustin.merino@usc.es

water table, usually at a depth of between 1 and 2.5 m (De Mier 2001; Fernández and Hernanz 2004).

Although sites are selected on the basis of their suitability for the species, the productivity of poplar plantations is very variable, ranging from stands with growth rates higher than 25 m^3 ha^{-1} $year^{-1}$, to those with growth rates lower than 10 m^3 ha^{-1} $year^{-1}$ (Fernández and Hernanz 2004). Preliminary studies have identified nutritional status as the major parameter determining the productivity of various *Populus* species, which is consistent with findings in other areas (Brown and van den Driessche 2002, 2005; Leech and Kim 1981; McLennan 1996; van den Driessche 1999; van den Driessche et al. 2003). However, despite the important economic implications, the main factors involved in the poor growth of these plantations have not yet been established.

On the other hand, crown conditions (normally expressed as visual crown transparency, VCT, and visual crown discoloration, VCD) are estimated with the purpose of identifying stress due to biotic or abiotic agents. Although several methods can be used to estimate the crown condition objectively, such as digital photography (Martín-García et al. 2009; Mizoue 2002) and remote sensing techniques (Coops et al. 2004; Stone et al. 2003), crown conditions are visually estimated in various forest health monitoring programs carried out in Europe, including the International Co-operative Programme on the Assessment and Monitoring of Air Pollution Effects on Forest, ICP Forest (Level I European network), among others. Crown conditions could therefore be used by managers for early assessment of stand status. However, these parameters reflect a complex reaction to physiological stress imposed on trees, such as ozone exposure, drought, hard frost, nutrient imbalances and fungal infections (Ewald 2005; Horsley et al. 2000; Seidling 2007; Simon and Wild 1998), which makes it difficult to identify the main causes of forest decline. In addition, there are several sources of error due to the subjectivity of the technique, which makes accurate assessment of the visual symptoms difficult.

Several studies have attempted to relate foliar symptoms to nutritional status in different tree species (Audley et al. 1998; Cape et al. 1990; Ewald 2005; Hallett et al. 2006; Musio et al. 2007). However, few predictive models have been developed, because of the environmental complexity and the high variability within species (Ewald 2005; Horsley et al. 2000; Ke and Skelly 1994). The use of health status of monoclonal plantations may provide a more accurate assessment because these stands are less variable and the trees display similar responses to environmental stresses, such as nutritional deficiencies.

Previous studies in monoclonal poplar plantations in Southern Europe have shown a close relationship between crown conditions and tree growth (Martín-García et al. 2009). Although crown conditions may also be influenced by biotic damage (caused by e.g. insect attack and fungal leaf infections), this is not an important driver of defoliation dynamics (Martín-García et al. 2011). The current data therefore suggest that the observed decline in many plantations may be due to nutritional imbalances.

The main aims of the present study were (1) to identify the main nutrients limiting *Populus* × *euramericana* (clone I-214) in Southern Europe, which may explain poor growth rates and crown conditions and (2) to develop a rapid method of assessing poplar plantation nutrition, based on visible canopy symptoms. The information thus obtained could be used to develop predictive models to estimate the suitability of areas for poplar plantations, from analysis of soil properties and foliar nutrients.

Materials and methods

Site description and experimental design

The present study was carried out in the Duero river basin (Castilla y León, NW Spain). Thirty-two *Populus* × *euramericana* (*P. deltoides* Bartr. × *P. nigra* L., clone I-214) stands were selected for study. The altitude of the study area ranges between 800 and 900 m and, in most stands, the slope is almost flat. The average annual precipitation varies between 496 and 630 mm, and the average annual temperature, between 9 and 11.4°C (Ninyerola et al. 2005). The stands are located on fluvial terrace (22 stands) or alluvial terrain (10 stands), and the soils are mainly Entisols (25 stands) and Inceptisols (7 stands).

The planting distance in these stands was 5 × 5 m or 6 × 6 m, and the average density, 312 stems ha^{-1}. Stands were pruned annually, until the age of 5 years (to 6–8 m from ground level). None of the plots were fertilized.

The stands were chosen by use of a factorial scheme with two factors:

1. Stand stage: prior analysis showed that certain parameters change after canopy closure. Since this takes place 7–8 years after plantation establishment, two groups were distinguished: young stands of 3–7 years old (16 stands) and adult stands of 8–14 years old (16 stands).
2. Site quality: suitable stands with optimum growth rates (site qualities I and II), and poor land, unsuitable for growth (site qualities III and IV). Site quality was determined by the model based on curves developed for *Populus* × *euramericana* clone I-214 in the Duero river basin (Bravo et al. 1995). These site indices include basimetric area to 10 years old. The specific values of the site indices are 20.21, 16.77, 13.31 and

$9.87\ m^2\ ha^{-1}$ for site qualities I, II, III and IV, respectively. In an initial estimation, all four site qualities established were considered, although subsequent analysis was not able to discriminate intermediate site qualities, probably due to low number of stands analyzed in the study. Two categories of stands were therefore established on the basis of tree growth: stands with adequate growth rates (site qualities I and II, which are suitable for poplars) and stands with the lowest growth rates (site qualities III and IV, unsuitable for poplars).

3. Eight I-214 clonal plantations were therefore selected as replicates of each combination of the previous two factors (young-suitable land, young-unsuitable land, mature-suitable land and mature-unsuitable land).

The range of diameter at breast height (DBH) and total height of trees varied considerably between treatments. On one hand, the mean DBH in young stands ranged from 10.95 to 15.55 cm in unsuitable and suitable land, respectively. Mean DBH in adult stands ranged from 15.95 to 31.77 m in unsuitable and suitable stands, respectively. On the other hand, mean total heights in young stands ranged from 11.22 to 13.72 cm in unsuitable and suitable sites, respectively, whereas mean total height in adult stands ranged from 14.26 to 26.68 m in unsuitable and suitable sites, respectively.

Growth measurements and assessment of crown condition variables

In each of the 32 stands, four circular subplots of 15 m radius were established for taking tree measurements, collecting samples of soils and plants and assessing the health status of the forest. The subplots were located 50 m apart from each other, at the ends of a cross located in the middle of the stand. All trees within each subplot were marked and sampled. Diameter at breast height (dbh) and total height were measured in an average of 84 trees per stand (ranging from 68 to 112 trees per stand).

Forest health was evaluated by visual assessment of crown conditions (crown transparency and crown discoloration). Visual assessment was used because it is currently applied in monitoring programs in Europe (ICP forest), so that any tools developed in the present study could be extrapolated to these monitoring programs.

In each stand, all trees within each subplot were assessed, in accordance with the ICP protocol, during the summer (within the first 2 weeks of July) of 2005. Crown transparency and discoloration were estimated visually by only one operator, according to Level I of the European network methodology (Eichhorn et al. 2006). Trees were assessed from two directions and quantified in twenty classes of 5% interval width (so that the higher the values of the crown condition parameters, the poorer the health of the trees) based on an absolute reference tree (with 0% crown transparency and discoloration). Prior to sampling, the operator took part in an intercalibration session with the Spanish field crew of the European Level I network. Parts of the crown directly influenced by interactions between crowns or by competition were excluded; trees were assessed from a distance of about one tree length, with the observer taking care to avoid looking into the sun (Eichhorn et al. 2006). Biotic damage in the crown was also recorded, but there were so few instances of such damage that it was not considered further.

Sampling and analysis of soils and leaves

Mineral soil samples were collected from the upper 30-cm soil layer in each subplot (i.e., four samples per plot). The samples were mixed, homogenized and considered as a composite sample per plot. The pH was determined potentiometrically with a pH meter, in a soil solution (1:2.5, soil/water). Organic matter was determined by the $K_2Cr_2O_7$ method. Total N was determined by Kjeldahl digestion. Soil available P was extracted by the Olsen procedure and determined photometrically by the molybdenum-blue method. Soil exchangeable cations (K^+, Na^{+2}, Ca^{2+} and Mg^{2+}) were extracted with ammonium acetate and determined by atomic absorption/emission spectrometry.

Particle-size distribution was determined by the Bouyoucos method (hydrometer method), and the ISSS (International Society of Soil Science) classification was applied.

The Cationic Exchange Capacity (CEC) was determined by Bascomb's method (i.e., the exchange cations were displaced by Ba ions, which were then displaced by Mg ions and the remaining concentration of Mg was determined by titration against EDTA).

Foliar sampling was carried out on green leaves from a minimum of 12 trees per subplot. This sampling was carried out during the first 2 weeks of September 2005, a period when foliar nutrients are most stable in poplar (Bengoa and Rueda 2001). Between 12 and 15 green leaves were removed from each tree, from two main branches of the upper third of the canopy, with a telescoping tree pruner.

The oven-dried (60°C) samples of leaves were milled (0.25 mm) and digested with HNO_3, in a microwave oven. Total carbon and N in milled foliar samples were analyzed by combustion in a Leco analyzer (LECO, St Joseph, Michigan, EEUU). The total concentrations of P, K, Ca, Mg, Fe, Mn, Zn, Cu, B, Ni S, Al, Cr, As, Mo, Cd, Co, Na and Pb in the digested foliar samples were determined by ICP-EOS (Perkin Elmer, Wellesley, MA, EEUU).

Foliar nutrient balance was initially analyzed by DRIS indices calculated for N, P, K, Ca and Mg from norms developed by Leech and Kim (1981). However, preliminary results showed that micronutrients were the most influential variables, and the resulting DRIS indices were not encouraging. Moreover, norms developed for macro and micronutrients in the stands under study are not recommended because of the small number of stands (32 stands) and the highly variable concentrations of micronutrients, which may mask the response of macronutrients. The foliar nutrient balance was therefore finally analyzed by several ratios.

Statistical analyses

The effect of nutritional status (soil properties and foliage nutrients) on stand growth was analyzed by means of stepwise discriminant analysis, with the forward selection procedure (P enter < 0.05 and P removed > 0.05). Two categories of stands were established on the basis of tree growth: (a) suitable stands (site qualities I and II) and (b) unsuitable stands (site qualities III and IV).

Two classification functions were thus developed for each model (soil properties and foliar nutrients) and used to determine the group to which each case probably belongs. Each function allows us to compute classification scores for each case for each group. Once the classification scores were computed for a case, the case was classified as belonging to the group for which it has the highest classification score. The classification functions can therefore be used to compute classification scores directly for some new observations when growers are interested.

Analyses of variance (ANOVAs) and Tukey's HSD post hoc tests were used to test the effect of age and site quality on several soil properties, foliar nutrients and crown condition. Correlation coefficients were calculated in order to evaluate the relationships between parameters in soils and green leaves.

A three-step approach, based on principal component analysis, Pearson's correlation matrices and multiple stepwise regressions with the forward selection procedure (P enter < 0.05 and P removed > 0.05), was used to examine the relationships between nutritional status (soil properties and foliar nutrients) and crown condition (Visual Crown Transparency and Visual Crown Discoloration). All analyses were performed with STATISTICA 6.0 (StafSoft 2001).

Results

Soil properties and foliar nutrient concentrations

Data on selected soil properties in the 32 stands studied are shown in Table 1. One of the most important features is the wide range of coarse fragment contents and texture. The coarse fragment contents varied between less than 1% to more than 80%, and the textural classes ranged from sandy loam to clay. Organic matter and N contents were low in many soils, in most cases lower than 3 and 0.15%, respectively. In some stands, the C/N ratios were higher than 20, normally in soils with pH higher than 8.0.

Most of the soils were alkaline (higher than 7.0) and some were moderately acidic (7–5.9). The values of cationic exchange capacity (CEC) were moderate and partly related to soil organic matter ($r = 0.36$, $P < 0.05$). The exchangeable Ca contents were high in relation to the high pH values found in many soils (pH-extractable Ca: $r = 0.73$, $P < 0.001$), whereas the values soil of available P were always low (< 6 mg kg^{-1}: pH-extractable P $r = -0.63$, $P < 0.001$).

The data on the foliar nutrient concentrations of the plantations under study are shown in Table 1. The foliar levels of Mg were related to the availability of that element in the soil ($r = 0.47$, $P = 0.01$). By contrast, the concentrations of other foliar nutrients such as N, P, K and Ca were not correlated with the concentrations in the soil. With the exception of S, N and Ca, the concentrations of all the nutrients analyzed were significantly related to the percentage of coarse fragments in the soil. The relationships were negative for P, K, Fe, B, Zn and Cu ($r = -0.44$, $P = 0.02$; $r = -0.37$, $P = 0.05$; $r = -0.39$, $P = 0.04$; $r = -0.43$, $P = 0.02$; $r = -0.49$, $P < 0.01$ and $r = -0.39$, $P = 0.04$, respectively). By contrast, foliar Mg and Mn concentrations were positively related to coarse fragments ($r = 0.61$, $P < 0.01$; $r = 0.52$, $P < 0.01$, respectively).

The foliar N and Mn concentrations were negatively related to soil pH ($r = -0.48$, $P = 0.01$; $r = -0.61$, $P < 0.001$, respectively). High C/N ratios were always found in soil with pH higher than 8. Finally, foliar K levels were negatively related to excess concentration of Mg, as revealed by the negative correlation ($r = -0.54$, $P = 0.001$).

Influence of forest nutrition on tree growth

Stepwise discriminant function analysis was used to determine the effects of soil properties and forest nutrition on site index. The classification functions are shown in Table 2, and the models are shown in Fig. 1.

The analysis revealed that stands with different growth patterns (suitable compared with unsuitable lands) were discriminated on the basis of the percentage of coarse fragments (previously transformed by the Common logarithm to achieve the necessary assumptions in the discriminant analysis) and the C/N ratio (previously transformed by the Neperian logarithm) in the upper

Table 1 Summary of some selected soils properties, concentrations of nutrients in green leaves and crown condition in the 32 poplar stands under study, in relation to age and site quality

	All stands		Young/suitable		Young/unsuitable		Adult/suitable		Adult/unsuitable	
	Mean (± S.D.)	Range	Mean (± S.D.)	Range	Mean (± S.D.)	Range	Mean (± S.D.)	Range	Mean (± S.D.)	Range
Soil properties										
Coarse fragments (%)	28.0 (24.2)	0.64–82.1	21.0 (26.6)	1.5–66.2	23.1 (14.5)	1.8–40.4	12.6 (11.3)	0.6–28.8	55.4 (18.5)	30.7–82.1
Sand (%)	68.1 (7.0)	50.9–83.7	67.7 (10.5)	51–83.7	68.6 (4.1)	61.3–72.7	65.3 (8.1)	50.9–73.6	70.8 (3.1)	65.5–76.4
Silt (%)	17.5 (5.5)	9.6–32.1	17.4 (7.5)	9.6–32.1	18.4 (4.0)	13.6–24.4	19.7 (6.0)	13.1–27-1	14.7 (3.1)	9.8–20.6
Clay (%)	14.3 (3.1)	6.7–23.7	14.9 (4.4)	6.7–20.9	13.0 (2.0)	10.5–15.4	15.1 (3.6)	11.8–23.7	14.5 (1.5)	12.3–16.9
pH	7.5 (0.93)	5.9–8.6	7.5 (0.95)	6.3–8.5	8.1 (0.92)	5.9–8.6	7.5 (0.75)	6.4–8.5	7.0 (0.92)	6.1–8.2
OM (%)	2.2 (0.94)	0.84–5.1	1.6 (0.57)	0.84–2.4	2.6 (0.97)	1.1–4.0	2.5 (0.70)	1.4–3.4	2.1 (1.2)	1.4–5.1
N (%)	0.11 (0.04)	0.05–0.22	0.09 (0.03)	0.05–0.15	0.09 (0.03)	0.05–0.14	0.15 (0.05)	0.09–0.22	0.11 (0.04)	0.08–0.18
C/*N*	12.1 (4.4)	6.2–25.9	10.4 (2.8)	6.2–16.1	17.2 (5.5)	10.7–25.9	9.9 (0.61)	9.0–10.6	11.0 (2.4)	8.4–16.5
P (mg kg^{-1})	9.5 (8.8)	0.1–34.8	9.5 (11.2)	0.1–34.8	6.4 (8.4)	0.1–6.5	14.2 (9.0)	4.4–31.1	7.9 (4.8)	0.1–15.2
K (mg kg^{-1})	105.9 (85.3)	25.0–374.2	75.7 (51.9)	25.0–169.0	98.1 (115.7)	37.0–374.2	148.0 (112.3)	56.1–363.7	101.6 (19.3)	84.3–142.1
Ca ($cmol_c$ kg^{-1})	9.8 (5.4)	2.8–19.4	8.3 (4.1)	4.0–17.2	11.1 (5.5)	3.9–16.7	11.2 (5.7)	4.2–17.4	8.5 (6.4)	2.8–19.4
Mg ($cmol_c$ kg^{-1})	0.61 (0.21)	0.2–1.13	0.55 (0.26)	0.2–1.05	0.46 (0.15)	0.34–0.79	0.64 (0.12)	0.48–0.82	0.79 (0.15)	0.64–1.13
CEC ($cmol_c$ kg^{-1})	13.9 (3.1)	8.8–22.2	13.1 (2.5)	10–16.1	13.3 (1.5)	11.9–15.6	15.6 (3.8)	10.3–22.2	13.6 (3.8)	8.8–19.5
Foliar nutrients										
S (mg g^{-1})	2.5 (0.70)	1.5–4.3	2.5 (0.57)	1.6–3.2	1.9 (0.51)	1.5–3.0	2.9 (1.1)	1.9–4.3	2.8 (0.49)	2.1–3.6
N (mg g^{-1})	22.1 (5.8)	15.2–36.2	28.1 (7.1)	16.3–36.2	19.8 (4.1)	15.2–28.4	18.9 (2.1)	15.9–20.7	19.8 (1.6)	17.6–22.5
P (mg g^{-1})	3.2 (1.5)	1.8–6.6	5.0 (1.4)	1.9–6.6	2.5 (0.64)	1.8–3.5	2.4 (0.35)	2.1–2.8	2.4 (0.58)	1.9–3.7
Ca (mg g^{-1})	26.4 (9.8)	11.9–46.5	23.4 (7.6)	11.8–30.8	18.0 (4.2)	13.9–27.0	36.5 (9.6)	23.5–46.5	32.7 (7.8)	24.0–45.3
Mg (mg g^{-1})	3.6 (1.1)	2.2–5.9	2.7 (0.40)	2.2–3.2	3.1 (0.36)	2.6–3.6	3.4 (0.78)	2.3–4.1	5.0 (0.58)	4.2–5.9
K (mg g^{-1})	10.8 (5.0)	3.0–22.9	13.9 (2.6)	11.2–17.3	10.0 (4.9)	3.8–18.2	13.9 (7.0)	7.8–22.9	7.0 (3.1)	3.0–12.9
Mn (μg g^{-1})	203.2 (175.6)	21.7–589	156.6 (126.3)	21.7–379.7	83.3 (110.5)	22.3–354.2	168.7 (140.1)	67.8–376.1	386.8 (155.8)	149.7–589.0
Fe (μg g^{-1})	80.3 (37.4)	43.7–177.7	95.1 (36.0)	55.6–163.0	65.3 (18.7)	46.0–107.0	135.6 (37.2)	98.8–177.7	52.9 (6.7)	43.7–62.5
B (μg g^{-1})	58.7 (44.3)	10–174.1	113.5 (44.0)	32.4–174.1	29.7 (18.2)	10.0–61.7	53.1 (5.5)	48.3–59.0	35.8 (17.0)	18.8–66.3
Zn (μg g^{-1})	94.2 (48.8)	28.3–225.2	125.3 (58.5)	54.7–225.2	103.5 (46.2)	61.5–194.3	78.8 (36.8)	28.3–110.5	61.5 (22.0)	30.5–93.0
Cu (μg g^{-1})	6.6 (2.2)	3.6–11.7	8.5 (1.9)	5.2–11.7	6.0 (1.5)	3.6–8.0	6.5 (1.3)	4.7–8.0	5.2 (2.3)	3.6–10.6
N/P	7.6 (1.8)	3.5–10.6	5.9 (1.6)	3.5–8.4	8.1 (1.5)	6.2–9.8	8.1 (1.7)	5.8–9.5	8.5 (1.5)	5.2–10.6
P/Zn	37.9 (15.9)	17.1–73.5	44.7 (17.3)	25.9–68.6	26.4 (7.4)	171.1–38.8	37.9 (23.8)	24.9–73.5	42.7 (12.2)	30.6–64.8
P/Cu	494.6 (153.6)	185.7–854.6	587.4 (141.5)	371.0–854.6	432.3 (111.2)	267.3–561.3	384.6 (134.7)	306.9–586.4	518.9 (170.2)	185.7–744.5
Crown condition										
VCT	19.4 (2.4)	6.5–50.7	9.5 (2.0)	6.5–12.1	14.3 (7.6)	9.2–31.9	13.0 (2.7)	10.0–18.1	40.9 (5.9)	32.6–50.7
VCD	6.9 (1.4)	0.0–24.4	2.0 (2.5)	0.0–5.8	4.6 (6.9)	0.0–20.5	2.5 (2.6)	0.0–5.0	18.8 (2.7)	15.5–24.4

VCT visual crown transparency, *VCD* visual crown discoloration

Table 2 Classification functions in relation to soil properties and foliar nutrient concentrations

Type of analysis	Classification functions	Classification probabilities (%)
Soil properties	Suitable = −41.909 + 6.997 Lg(CF) + 33.093 Ln(*C*/*N*)	81.25
	Unsuitable = −56.925 + 9.672 Lg(CF) + 37.937 Ln(*C*/*N*)	
Foliar nutrient concentrations	Suitable = −47.151 − 29.129 Ln(Fe) − 10.446 Ln(B)	96.43
	Unsuitable = − 78.895 − 36.813 Ln(Fe) − 14.506 Ln(B)	

CF percentage of coarse soil fragments

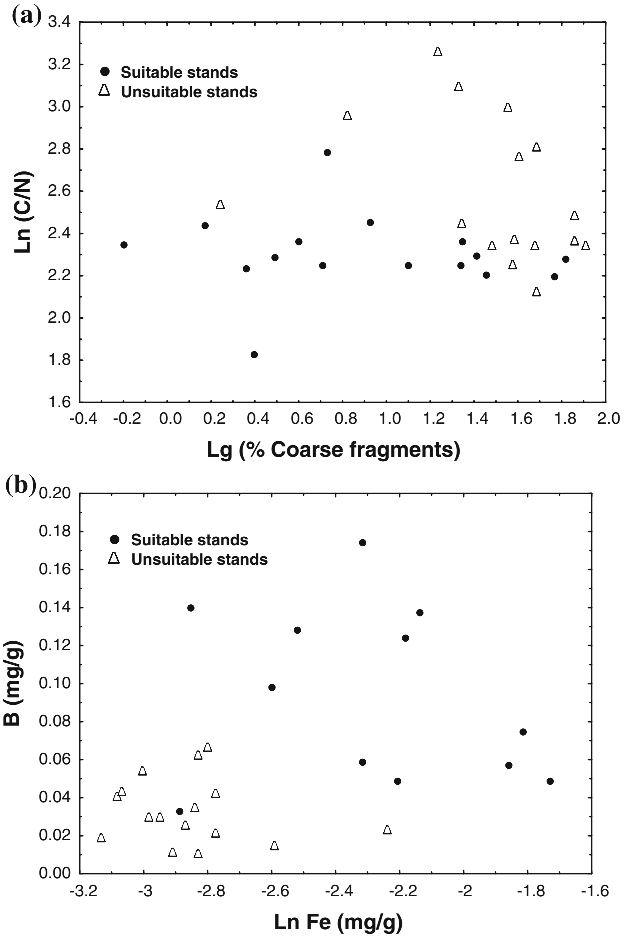

Fig. 1 Results of the discriminant analysis applied to **a** soil properties and **b** foliar nutrient contents

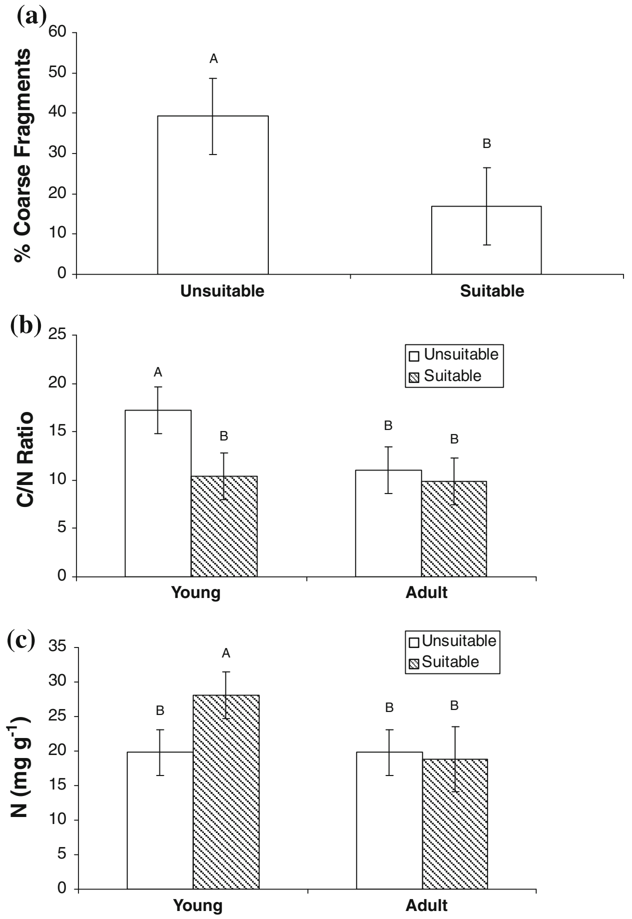

Fig. 2 Mean values (±95 confidence intervals) of soil properties **a** coarse fragments, **b** *C*/*N* ratio and **c** N (%) according to age and site quality. *Bars* with *different letters* indicate significantly different means (Tukey's post hoc test, $\alpha = 0.05$)

mineral soil horizon (Wilks' lambda = 0.562, approx. F (2.29) = 11.292, $P = 0.0002$) (Table 2 and Fig. 1a), reflecting the main soil properties that determine nutrient availability. Stands were classified correctly in 81.25% of cases. The structure of the canonical variables factor (pooled-within-groups correlations) revealed that the *C*/*N* ratio and coarse fragments were similar in terms of their importance in discriminating the two groups of stands (−0.573 and −0.561, respectively). According to the results of this analysis, suitable stands were associated with low percentages of coarse fragments and/or lower *C*/*N* ratio. The opposite was found for the unsuitable stands (Fig. 1a).

The results of the ANOVA showed that the coarse fragment content was a key factor for growth, since unsuitable stands were associated with soil containing large amounts of coarse fragments. Thus, tree growth rates were low in stands growing on soil with more than 30% coarse fragments (Fig. 2a). No significant effect of age or of the interaction between age and quality site was found for coarse fragment content (Table 3). Nevertheless, a significant interaction between age and site quality was found for the *C*/*N* ratio (Table 3). Tukey's HSD tests (Fig. 2b) revealed that in young stands, the *C*/*N* ratio was higher in

Table 3 Analyses of variance of several soil properties and foliar nutrient concentrations, to test the effect of age and site quality

Independent variables	df	Soil properties						Foliar nutrients			
		Coarse fragments		*C/N* ratio		N (%)		N (mg g^{-1})		P (mg g^{-1})	
		F	*Pr* > *F*	F	*Pr* > *F*	F	*Pr* > *F*	F	*Pr* > *F*	F	*Pr* > *F*
Age	1	1.27	0.268	7.18	**0.012**	7.23	**0.012**	4.71	**0.040**	10.92	**0.003**
Site quality	1	10.53	**0.003**	12.09	**0.002**	2.37	0.135	3.01	0.095	8.63	**0.007**
Age * site quality	1	3.07	0.09	5.61	**0.025**	1.30	0.263	6.05	**0.022**	9.14	**0.006**

Independent variables	df	Foliar nutrients									
		K (mg g^{-1})		B (μg g^{-1})		Fe (μg g^{-1})		Mg (mg g^{-1})		Cu (μg g^{-1})	
		F	*Pr* > *F*	F	*Pr* > *F*	F	*Pr* > *F*	F	*Pr* > *F*	F	*Pr* > *F*
Age	1	0.81	0.378	0.87	0.362	0.88	0.358	42.88	**<0.001**	3.71	0.066
Site quality	1	10.58	**0.003**	21.12	**<0.001**	35.14	**<0.001**	23.27	**<0.001**	6.36	**0.019**
Age * site quality	1	0.83	0..372	5.17	**0.032**	7.22	**0.013**	9.68	**0.005**	0.62	0.438

Bold numbers indicate values that were statistically significantly different

unsuitable stands. By contrast, no significant differences were observed in adult stands.

Another stepwise discriminant function analysis was carried out to develop a model from the foliar concentration data (Table 2, Fig. 1b). This model was able to discriminate the two groups of stands with different tree growth on the basis of the foliar concentrations of Fe (previously transformed by the Neperian logarithm to achieve the necessary assumptions in the discriminant analysis) and B (previously transformed by the Common logarithm) (Wilks' lambda = 0.296, approx. F (2.25) = 29.736, $P < 0.0000$). Stands were classified correctly in 96.43% of cases. The classification functions from foliar nutrients are shown in Table 2. The factor structure of the canonical variables (pooled-within-groups correlations) suggests the strong influence of both foliar Fe and B concentrations on growth of the plantations (−0.638 and −0.617, respectively). The model revealed that poorer tree growth was associated with lower concentrations of B and Fe (Fig. 1b).

Analyses of variance for the concentrations of Fe and B showed significant interactions between age and site quality (Table 3). Tukey's HSD tests (Fig. 3a, b) revealed a higher concentration of B in suitable stands, although in young stands only. By contrast, there was a significantly higher concentration of Fe in suitable stands, only in adult stands. While the ANOVAs and Tukey's HSD tests for the concentrations of N and P (Fig. 3c, d) revealed similar patterns to that observed for the concentration of B (only in young stands a higher concentration of both nutrients was observed in suitable stands), the concentrations of Mg were significantly different, according to site quality, only in adult stands (Fig. 3f). As regards the concentration of K and Cu, only a significant effect of site quality was found (Table 3, Fig. 3 e.g.).

Relating nutritional status and tree growth to visual crown conditions

Crown condition was evaluated as visual crown transparency (VCT) and visual crown discoloration (VCD). As often observed in many species, age had a strong effect on both variables (VCT and VCD). Thus, in all young stands (3–7 years old), the values of VCT and VCD were lower than 15%. By contrast, crown conditions in the mature stands (8–14 years old) with poor growth rates (unsuitable stands) were poorer than in stands with higher growth rates (suitable stands). In unsuitable sites, the mean values of VCT and VCD were about 40 and 18%, respectively, approximately four times higher than in the suitable sites. The values of crown conditions in young and mature plantations with different growth rates are shown in Fig. 4.

In order to evaluate the influence of soil properties and foliar nutrient concentrations on crown conditions, a three-step approach, based on PCA, Pearson's correlation matrices and regression procedures, was followed.

Principal component analysis (PCA) was used as a first approach in identifying the main soil properties affecting VCT and VCD. The approach accounted for 67% of the total variance in the three principal components. The first and third axes were strongly associated with high content of coarse fragments, low pH, soil organic matter and exchangeable Ca, and the poorest crown condition. The second axis was influenced by the texture, which determines the cationic exchange capacity (Table 4). The ordination diagram from axes 1 and 3 (correlated with crown conditions) revealed age, coarse fragment content and sand as the variables most closely related to crown condition variables (Fig. 5a).

The three principal components of PCA of foliar nutrients accounted for 79% of the total variance. The first axis

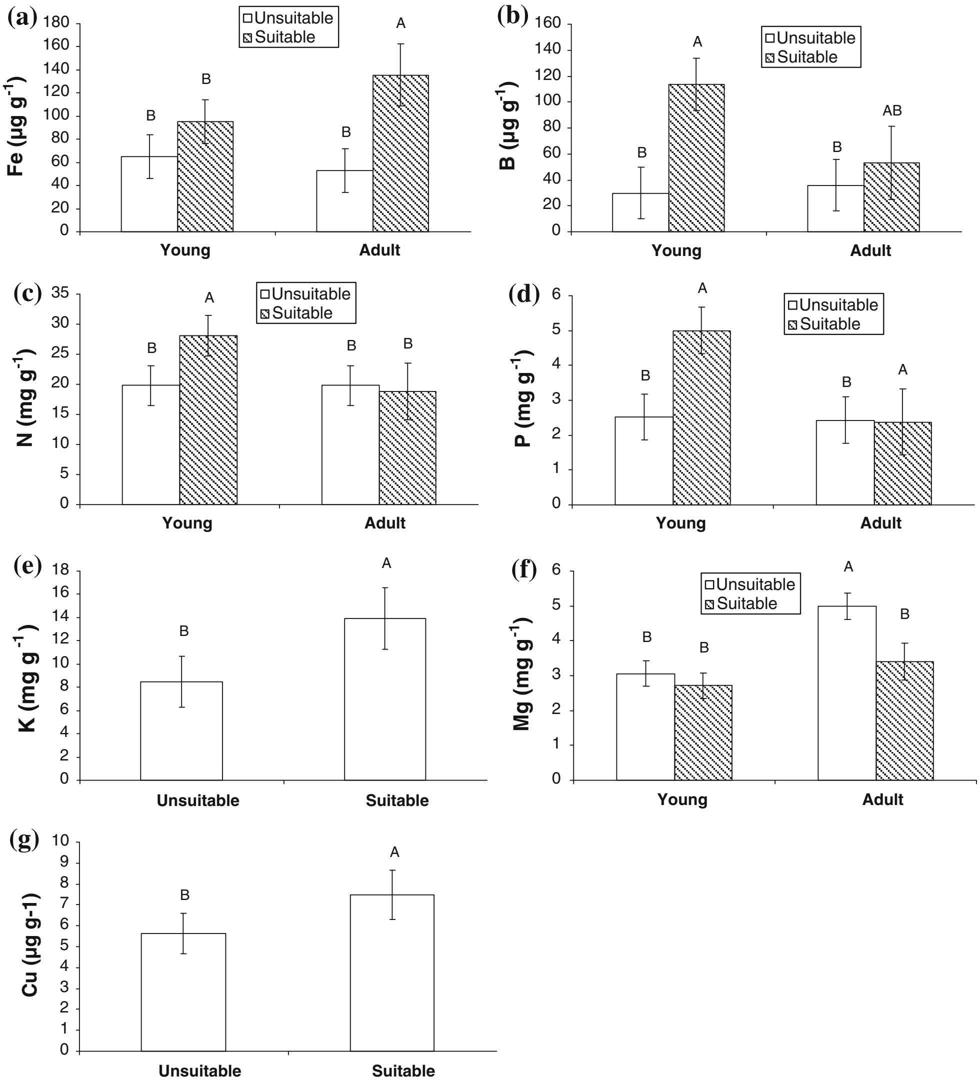

Fig. 3 Mean values (±95 confidence intervals) of foliar nutrient concentrations **a** Fe (µg g^{-1}), **b** B (µg g^{-1}), **c** N (mg g^{-1}), **d** P (mg g^{-1}), **e** K (mg g^{-1}), **f** Mg (mg g^{-1}) and **g** Cu (µg g^{-1}) according to age and site quality. *Bars* with *different letters* indicate significantly different means (Tukey's post hoc test, $\alpha = 0.05$)

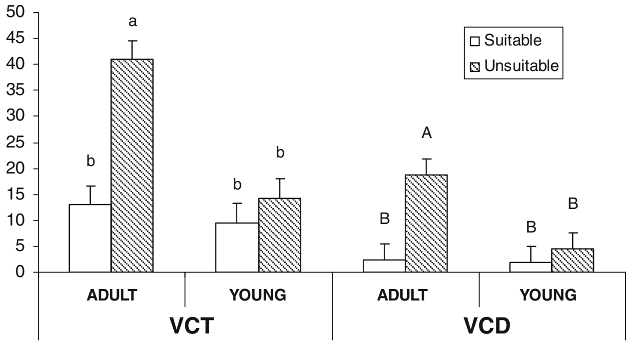

Fig. 4 Mean values (±95 confidence intervals) of visual crown transparency (VCT) and visual crown discoloration per poplar stand, for different site qualities. Within each crown condition variable, *bars* with *different letters* indicate significantly different means (Tukey's post hoc test, $\alpha = 0.05$)

was strongly influenced by age, some variables correlated with age and crown conditions, the second axis, by those variables not correlated with coarse fragments (foliar S, N and Ca), and the third axis, by Fe concentration and, to a lesser extent, crown condition (Table 4). The ordination diagram from axes 1 and 3 (correlated with crown conditions) showed that several variables (including age) influenced the first axis and that only the concentration of Fe influenced another axis (Fig. 5b).

The second approach in identifying the main soil properties and foliar nutrients affecting VCT and VCD was carried out on the basis of Pearson's correlation matrices. The coefficients of correlation between crown condition and nutritional status are shown in Table 5. As regards soil properties, the crown condition parameters (both VCT and VCD) were positively correlated with the coarse fragment

Table 4 Factorial coordinates of the three first axes obtained in principal components analyses of soil properties and foliar nutrient concentration

Soil properties	Axis 1	Axis 2	Axis 3	Foliar nutrients	Axis 1	Axis 2	Axis 3
Age	0.409	−0.564	−0.363	Age	0.852	−0.282	−0.107
VCT	0.612	−0.353	−0.627	VCT	0.901	−0.259	0.236
VCD	0.657	−0.295	−0.558	VCD	0.812	−0.265	0.409
Coarse fragment	0.570	−0.053	−0.526	S	0.205	−0.817	−0.144
Sand	0.548	0.692	−0.350	N	−0.517	−0.732	0.134
Silt	−0.686	−0.515	0.228	P	−0.694	−0.530	0.321
Clay	−0.025	−0.662	0.393	Ca	0.464	−0.669	−0.367
pH	−0.787	0.201	−0.370	Mg	0.859	−0.250	0.147
P	0.518	−0.162	0.572	K	−0.700	−0.071	−0.030
K	0.460	−0.273	0.183	Mn	0.656	−0.614	−0.090
Ca	−0.738	−0.231	−0.471	Fe	−0.407	−0.292	−0.817
Mg	0.246	−0.790	0.024	B	−0.679	−0.587	0.191
CEC	−0.474	−0.740	−0.058	Zn	−0.677	−0.231	0.326
N	−0.168	−0.493	−0.299	Cu	−0.609	−0.515	0.054
OM	−0.465	−0.160	−0.582				
C/*N*	−0.476	0.407	−0.384				

VCT visual crown transparency, *VCD* visual crown discoloration

content and exchangeable Mg. The crown condition parameters were negatively related to exchangeable K. Negative relationships between crown condition and foliar concentrations of K, Zn, Fe, B and Cu were also found. By contrast, positive relationships were observed between crown condition and foliar Ca and Mg.

Finally, the third step in identifying the relationships between crown conditions and soil properties or foliar nutrient concentrations was carried out by multiple stepwise regressions. Thus, two crown condition models, which showed a high percentage of explained variance, were identified. The largest amount of variance corresponded to crown transparency (Table 6). In one of the models, the variables entered were age and coarse fragment content. The second model included age and foliar Fe concentration.

Discussion

Influence of forest nutrition on tree growth

The results of the study revealed that the high content of coarse fragments in the soil had a negative influence on tree growth. Thus, a course fragment content higher than 30% was associated with unsuitable stands (Fig. 2a), possibly due to the small volume of soil available for the development of roots, the lower amount of available nutrients per unit of soil volume or a lower soil water-holding capacity.

Although several nutrients, such as N, P, K, Fe and B, appear to affect tree growth in these poplar plantations, Fe and B appear to be the most limiting elements. Thus, foliar concentrations of Fe lower than 70 µg g^{-1} were associated with unsuitable stands in adult plantations (Fig. 3a). This is consistent with the value reported in a study of a Fe deficient hybrid poplar (*P* × *euramericana*) (42.5 µg g^{-1}: Lombard et al. 2010). Others studies have reported higher concentrations in other non-nutrient-limited plantations of *Populus alba* (250 µg g^{-1}: Madejón et al. 2004) and *P* × *canadensis* (135–486 µg g^{-1}: Celik et al. 2010).

On the other hand, foliar B appears to be a limiting element in young stands (Fig. 3b). Thus, concentrations of B below 50 µg g^{-1} also resulted in unsuitable stands in young plantations. Deficient levels of B have been reported for *Populus tremuloides* (Wikner 1983, 1985) and other genera of the same family, such as *Betula* spp. (genus of the same family as *Populus* sp, Fam. *Salicaceae*) (Braekke 1983a, b).

Deficient concentrations of B and Fe have not been reported for forest species in the region; however, fertilization with B is common in cropland. Soils with high contents of coarse fragments were also deficient in Fe and B ($r = -0.39$, $P = 0.04$ and $r = -0.43$, $P = 0.02$, respectively), which implies lower availability of these nutrients as well as a lower moisture-holding capacity in superficial soil horizons, since moisture is required for uptake of the nutrients. Although soil acidity affects the availability of these elements (Brady and Weil 1999; Goldberg 1997), the foliar concentrations of B and Fe were not related to soil pH ($r = 0.01$, $P = 0.942$ and $r = -0.19$, $P = 0.317$, respectively). In addition, as the plantations

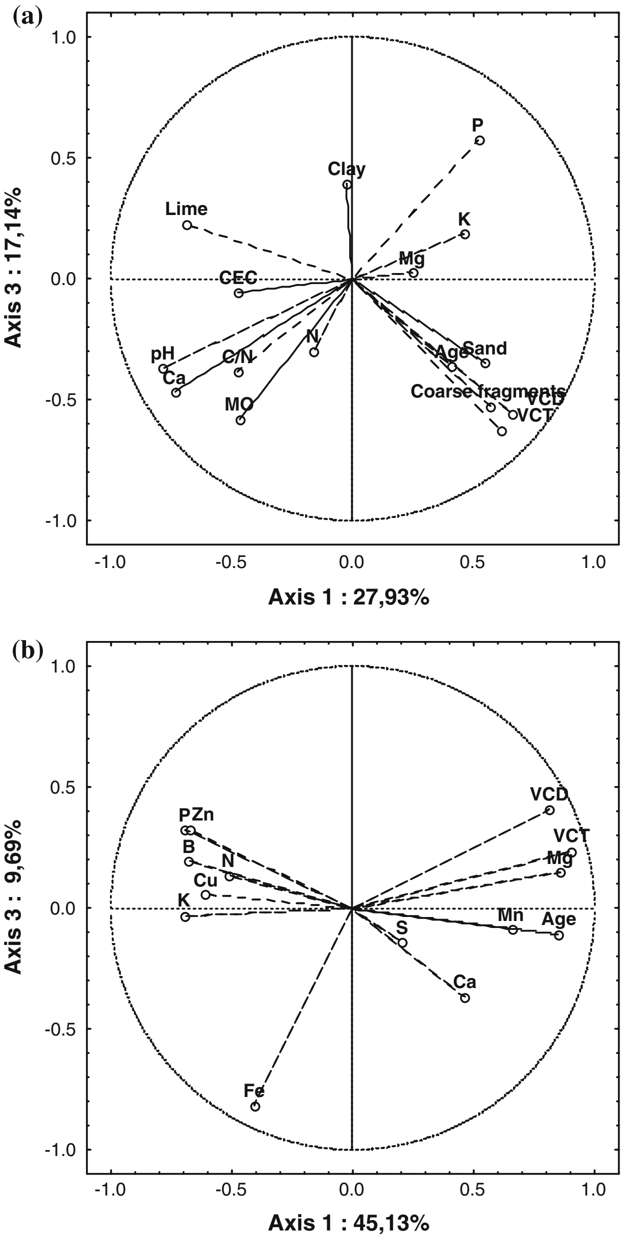

Fig. 5 Principal component analysis biplot for the stands under study, for **a** soil properties and **b** foliar nutrients

under study were not fertilized, it is unlikely that addition of macronutrients hindered the uptake of these elements, as previously reported (Carter and Brockley 1990; Stone 1990).

Low foliar N concentrations also had a negative effect on tree growth in young plantations. Thus, growth was poor in young stands with foliar N levels below 23 mg g^{-1} (Fig. 3c). This value is similar to those reported by other authors, who proposed a level of 24 mg N g^{-1} as critical for *Populus balsamifera* (McLennan 1996) and a level of 17 mg N g^{-1} for *Populus trichocarpa* and *P. tremula* × *P. tremuloides* (Jug et al. 1999). The data are also consistent with those of van den Driessche (1999), who reported that soil N and organic matter content could be used as indicators of site potential for *Populus trichocarpa* × *P.deltoides*. In the present study, foliar N levels were affected by the soil *C*/*N* ratio ($r = -0.39$, $P = 0.039$) and pH ($r = -0.48$, $P = 0.01$). Thus, deficient foliar N levels were found in young stands planted in soils with high *C*/*N* ratios and pH values. The soil *C*/*N* ratio appears to be a limiting factor in young plantations, and soil *C*/*N* ratios higher than 15 were associated with unsuitable stands in these plantations (Fig. 2b). The fact that high soil *C*/*N* ratios were only found in young stands may be due to an increase in soil N with age of the stand (Table 3, Fig. 3c) as a result of the return of some nutrients in soils through litterfall (mainly in adult plantations: Das and Chaturvedi (2005)), or enhancement of N mineralization as a result of a well-developed root system (Browaldh 1997). Furthermore, negative effects on soil microbial activity and therefore organic matter decomposition may be due to high soil pH (Yuan et al. 2007).

A similar pattern was observed for P, and low foliar P concentrations also had a negative effect on tree growth in young stands. Thus, P levels below 3.2 mg g^{-1} were associated with unsuitable stands in young plantations (Fig. 3d). This value is consistent with the optimum level established for the hybrid *Populus trichocarpa* × *P. deltoides*, that is, approximately 2.5 mg g^{-1} (Brown and van den Driessche 2005; van den Driessche 1999), although higher than those given for *Populus trichocarpa* and *P. tremula* × *P. tremuloides*, that is, 1.6 and 1.8 mg g^{-1}, respectively (Jug et al. 1999).

Foliar K did not appear to be influenced by the age of plantation, and the concentrations were only determined by site quality. Thus, growth was poor with foliar K levels below 11 mg g^{-1} (Fig. 3e). McLennan (1996) established a critical level of foliar K of 17.6 mg g^{-1} for *P. balsamifera*, whereas a critical value of approximately 7–8 mg g^{-1} was reported for *Populus trichocarpa* and *P. tremula* × *P. tremuloides* (Jug et al. 1999). The negative relationship between foliar K and Mg ($r = -0.80$; $P < 0.05$) suggests that the excess of Mg interferes with K uptake, probably because ionic and osmotic balance is maintained in leaf vacuoles (Diem and Godbold 1993).

According to the reference values provided by McLennan (1996), concentrations of Mg above 1.9 mg g^{-1} indicate a good supply of these elements for *Populus* hybrids. The concentrations Mg in the stands under study were higher than 2 mg g^{-1}. However, values higher than 4.5 mg g^{-1} appeared to reduce tree growth in the adult stands (Fig. 3f). The low growth in these plantations may be due to three types of indirect effects. The first two of these are the positive relationships between foliar Mg and content of coarse soil fragments ($r = 0.54$, $P = 0.002$),

Table 5 Coefficients of correlation between nutritional status and crown condition

Soil properties	VCT	VCD	Foliar nutrients	VCT	VCD
Age	0.669***	0.553**	Age	0.669***	0.553**
Coarse fragment	0.660***	0.654***	S	0.278	0.286
Sand	0.240	0.278	N	−0.254	−0.199
Silt	−0.315	−0.329	P	−0.395*	−0.285
Clay	0.015	−0.047	Ca	0.511**	0.404*
pH	−0.302	−0.316	Mg	0.811***	0.773***
P	−0.040	−0.035	K	−0.524**	−0.426*
Ln (K)	−0.352*	−0.381*	Mn	0.730***	0.643***
Ca	−0.069	−0.122	Fe	−0.450*	−0.537**
Mg	0.441*	0.427*	B	−0.413*	−0.332
CEC	−0.017	−0.064	Zn	−0.488**	−0.353
N	0.069	−0.016	Cu	−0.378*	−0.334
OM	0.029	−0.053			
C/N	−0.134	−0.163			

* Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$

Table 6 Multiple regressions between crown condition and soil properties and foliar nutrient concentrations

	Regression equation	R^2_{adj}	*P*
Soil properties	$VCT = -11.407 + 2.054Age + 11.431Lg(CF)$	0.656	**<0.0001**
	$VCD = -9.466 + 0.959Age + 7.036\ Lg(CF)$	0.527	**<0.0001**
Foliar nutrient concentrations	$VCT = -34.152 + 3.1421Age - 11.605Ln(Fe)$	0.886	**<0.0001**
	$VCD = -27.932 + 1.5054Age - 9.1212Ln(Fe)$	0.750	**<0.0001**

VCT visual crown transparency, *VCD* visual crown discoloration, *CF* coarse fragments

Bold numbers indicate values that were statistically significantly different

and between foliar Mg and pH ($r = -0.50$, $P = 0.007$), which had a negative effect on the availability of P, Fe and other micronutrients. The third type of indirect effect is the low foliar K levels found in stands with high foliar Mg, as described above.

With respect to Cu, unsuitable stands were associated with foliar concentrations below 6.5 µg g^{-1} (Fig. 3g). Such low levels occurred in trees growing in soils with high contents of coarse elements ($r = -0.39$, $P = 0.04$). Van den Driessche (2000) proposed 2.4 µg g^{-1} as the critical level for the hybrid *Populus trichocarpa* × *P. deltoides*. All the stands studied in the present study displayed higher concentrations than this reference value.

While deficient levels of Cu are sometimes associated with disorders induced by phosphorus, this did not appear to be a problem in the stands under study, since in all plantations the ratios of P/Cu were lower than 1,100, a value that has identified unhealthy *Populus* × *euramericana* individuals (Teng and Timmer 1990). On the contrary, P and Cu were positively correlated ($r = 0.6071$, $P < 0.001$), in contrast with results obtained by other authors (van den Driessche 2000; Teng and Timmer 1990). Therefore, the fact that the lowest concentrations of Cu were found in unsuitable stands may be due to an indirect effect caused by the coarse fragments, since these factors were negatively correlated ($r = -0.39$, $P = 0.04$).

Using visual crown conditions as an indicator of nutritional status and tree growth

The results of the study revealed that crown condition can be used as an early predictor of growth problems, which in the study area are mainly related to the poor quality of the soils. Thus, visual crown conditions reflected the poor nutritional status of the soils in some stands. Biotic damage in the crown was also recorded, but there were so few instances of such damage that it was not considered further (Martín-García et al. 2011).

Principal component analyses and Pearson's correlation matrices appear to indicate that age and coarse fragments are the most important factors explaining the crown conditions, but several correlations (although weak) between nutrients and crown conditions reflected the negative effect of the deficient concentrations (found in many plantations) on forest health. Positive correlations between crown conditions and other nutrients, such as foliar Ca and Mg, may reflect the abundance of these elements in the alkaline soils, in which levels of other nutrients are deficient.

Nevertheless, in the predictive models of crown condition developed in this study, a high percentage of variance was explained by only two variables. Multiple stepwise regressions avoid the use of correlated variables. Thus, variables such as P, K, B, Zn or Cu were not entered in the model, although deficiencies in these elements may also influence crown transparency. Models showed that about 60 and 80% of the variability in crown condition can be explained by age, coarse fragment content and foliar Fe concentration. These results are much better than those obtained by other authors (Hendriks et al. 2000; Ke and Skelly 1994), who developed models with at least 10 predictor variables, in which the variance percentages accounted for by R^2_{adj} was only 22–67%. In the present study, the use of monoclonal plantations may have provided a more accurate assessment because of the higher homogeneity of the tree response to nutritional status.

The models developed in the present study included age as a predictor of crown condition, reflecting the fact that the mature poplars displayed poorer health than the young stands. This finding, already pointed out in a previous paper (Martín-García et al. 2009), is probably a cumulative detrimental effect with age, due to the increased demand for some nutrients. Similar results have been found for other forest species, such as *Picea abies, Pinus sylvestris, Quercus robur, Quercus* ilex and *Fagus sylvatica*, in which an increase in crown transparency has also been observed in older trees (Ewald 2005; Hendriks et al. 2000; Klap et al. 2000).

Moreover, coarse fragment content and foliar Fe appear to be key factors in crown condition. A high content of coarse fragments reduces the uptake of many nutrients. Thus, Fe deficiency often causes defoliation and interveinal chlorosis (Bennett 1996), because this element is essential for chlorophyll synthesis and is involved in photosynthesis, respiratory enzyme systems and protein metabolism.

Development of useful indicators for early detection of forest decline has been a challenge for researchers and managers for many years. Although various studies (e.g., Ewald 2005; Musio et al. 2007) have attempted to relate foliar symptoms and nutritional status in different tree species, relationships involving crown conditions have been difficult to establish because of interactions with other environmental factors, such as air pollution and genetic variability. Unlike other forest systems, those in the present study were much more homogeneous with respect to environmental factors (similar altitude, lack of air pollution) and genetic variability (use of monoclonal plantations). The homogeneous conditions favored the development of the model, which was useful, in this case, for detecting nutrient deficiencies. However, although several weak correlations between nutrients and crown conditions were found with PCA and Pearson's correlation matrices, fertilization regimes cannot be recommended on the basis of these results, and further research is necessary to achieve this aim.

Conclusions

The present study identified the main nutritional factors limiting tree growth in monoclonal poplar plantations of *Populus* × *euramericana* in an area of Southern Europe. Most of the nutritional problems were due to the high contents of coarse fragments in many soils. Tree growth was also affected by several nutrients, the most clearly limiting were Fe and B, and to a lesser extent N, P and K.

Visual crown conditions of the stands reflected nutritional deficiencies and were associated with poor tree growth. Thus, crown condition assessment may be used in these plantations as a useful tool for early detection of nutrition-related problems in growth, in the absence of biotic damage.

Growers are encouraged to use the classification functions developed from soil properties to ensure that new plantations will be established on suitable land. Thus, growers could predict the suitability of their land (with a high probability of success) on the basis of the coarse fragment content and *C*/*N* ratio, which can be determined easily and cheaply in commercial laboratories. Furthermore, soils with more than 30% coarse fragments should be avoided during site selection to ensure optimum growth and crown condition of the plantations. Nevertheless, further research is required to analyze the effect of fertilization on tree growth and to monitor changes in crown conditions.

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ARTICLE IV

Martín-García, J., Jactel, H. & Diez, J.J. (2011) Patterns and monitoring of *Sesia apiformis* infestations in poplar plantations at different spatial scales. *Journal of Applied Entomology* 135 (5): 382-392

RESUMEN

Patrón de comportamiento y seguimiento de *Sesia apiformis* en plantaciones de chopo a diferentes escalas espaciales

Aunque la superficie destinada a plantaciones de chopo está incrementando notablemente poco se conoce de la plaga *Sesia apiformis*. Treinta y dos plantaciones de chopo del clon I-214 fueron muestreadas en España, las cuales fueron seleccionadas según un diseño factorial con tres factores: edad, calidad de estación y selvicultura aplicada al sotobosque, para investigar los factores que condicionan la selección del hábitat tanto a nivel de árbol como de parcela de *S. apiformis*. En cada plantación, una trampa de feromonas fue utilizada para evaluar la relación entre capturas y porcentaje de árboles atacados. La proporción de otras plantaciones de chopo circundantes fue usada como una covariable en los modelos predictivos desarrollados. Un mayor porcentaje de árboles atacados fue encontrado en las parcelas adultas, mientras que en las parcelas jóvenes este porcentaje incrementó con la cobertura de sotobosque. No se observó un efecto de la calidad de estación en el grado de ataque, y tampoco influyeron la altura de los árboles, el diámetro o las condiciones de copa, lo cual sugiere que *S. apiformis* podría comportarse como un perforador primario. El presente trabajo establece la hipótesis que el estado más limitante en su ciclo biológico podría ser la instalación de la larva, la cual podría beneficiarse de la protección que una corteza gruesa con grandes grietas en chopos adultos y del sotobosque en las plantaciones jóvenes. Una correlación positiva fue encontrada entre capturas y porcentaje de árboles atacados en un radio circular de 100 m alrededor de la trampa. Dicha regresión fue mejorada cuando la superficie de otras choperas circundantes dentro de una distancia de 600 m fue incorporada en el modelo. Esto sugiere que las plantaciones circundantes podrían estar aportando capturas a las parcelas de estudio. Los resultados confirman que *S. apiformis* debería ser considerada como una potencial plaga de plantaciones de chopo y que un sistema basado en trampas de feromonas sería una herramienta idónea para el seguimiento de sus poblaciones.

Palabras clave: Chopera, *Populus*, paisaje, feromona, trampa.

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Patterns and monitoring of *Sesia apiformis* infestations in poplar plantations at different spatial scales

J. Martín-García[1,2], H. Jactel[3,4] & J. J. Diez[1,2]

1 Forest Pathology and Entomology Laboratory, Department of Plant Production and Forest Resources, University of Valladolid, Palencia, Spain
2 Sustainable Forestry Research Institute, INIA-University of Valladolid, Palencia, Spain
3 INRA, UMR 1202 BIOGECO, Laboratory of Forest Entomology & Biodiversity, Cestas Cedex, France
4 Université de Bordeaux, UMR 1202, Bordeaux, France

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Populus, hornet clearwing moth, landscape, management, pheromone, trap

Correspondence
Jorge Martín-García (corresponding author), Forest Pathology and Entomology Laboratory, Department of Plant Production and Forest Resources, University of Valladolid, Avenida de Madrid 44, 34071, Palencia, Spain.
E-mail: jorgemg@pvs.uva.es

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Abstract

Poplar plantations are expanding worldwide but little is known about the hornet clearwing moth, *Sesia apiformis*, one of their most severe pests. Thirty-two poplar plantations of I-214 clone were sampled in Spain, according to a factorial design combining stand age, site condition and understorey management to investigate the main drivers of *S. apiformis* habitat selection at both tree and stand level. In each plantation, one pheromone trap was activated during the flight season to test the correlation between captures and percentage of attacked trees. The proportion of other poplar forests in surrounding landscapes was calculated and used as a covariate in predictive models of trap catches. There were significantly more attacked trees in older stands. In young stands, the percentage of infested trees increased with the percentage cover of understorey vegetation. There was no significant effect of site quality on the rate of infestation and no difference in tree height, diameter or crown condition between attacked and un-attacked trees within each stand, suggesting that *S. apiformis* could behave as primary pest. We hypothesized that the critical stage in the life cycle of the moth was the first instar larvae, which may benefit from protection of deep bark cracks in older stands and understorey vegetation in younger stands. We observed a positive correlation between trap capture and percentage of attacked trees in a radius of 100 m around the trap. The regression of trap catches against percentage of attacked trees was improved when the area of other poplar plantations within a distance of 600 m was incorporated in the model. This suggests that surrounding poplar stands may act as sources of immigrating moths in monitored stands. Our findings confirm that *S. apiformis* should be considered as a potential threat to poplar plantations and that pheromone trapping provides a suitable monitoring tool.

Introduction

Poplar plantations like other short rotation forests are expanding worldwide due to an increasing demand for wood, fibre, and more recently for carbon sequestration and as a biofuel feedstock. For example, poplar forest area in China increased by 2.8 million hectare between 2004 and 2007 (10% per year) (FAO 2008). Recent research has focused on increasing the productivity of poplar plantations via genetic selection of new clones, improvement of silvicultural practices, and resistance to biotic and abiotic damage. Various pest species can cause severe damage to poplar plantations (de Tillesse et al. 2007) and considerable effort has been made to study defoliators such as *Chrysomela* spp. (Gruppe et al. 1999; Brückmann et al. 2002; Pauchet et al. 2009) and *Leucoma salicis* (Jakubowska et al. 2005). However, there is a lack of information concerning wood borers, particularly the hornet clearwing moth, *Sesia apiformis* (Clerck) (Lepidoptera, *Sesiidae*). *S. apiformis* is a relatively widespread pest of poplar in Europe. Many studies have shown that *S. apiformis* can cause moderate to severe damage in Europe (Chrystal 1937; Georgiev and Beshkov 2000), particularly in Spain (Sierra 2001; Pérez et al. 2002; Romanyk and Cadahia 2002). Recently, *S. apiformis* has been classified amongst the most aggressive European bark and wood boring insects in living trees (Grégoire and Evans 2004) and de Tillesse et al. (2007) considered *S. apiformis* an internationally important insect species damaging poplar. However, to date little is known about its patterns of infestation or its economic impact. This may be due to its cryptic behaviour, as its presence is only revealed by exit holes of emerging adults from the base of the trunk (Pérez et al. 2002; Romanyk and Cadahia 2002). Severe infestations can occur in both old and young plantations (Chrystal 1937; Speight 1986; Jobling 1990). Although damage may be more severe in young trees, because larval galleries can disturb the sap flow, Arundell and Straw (2001) did not find any relationship between infestation density and tree age or size. While *S. apiformis* is considered a secondary pest in the UK, where the moth has been described as attacking mainly weakened poplar trees (Arundell and Straw 2001; Straw et al. 2007), there is no empirical evidence to support this assumption in Spain (Romanyk and Cadahia 2002).

Sesia apiformis is found all over Europe, from Finland to the Mediterranean basin, the Middle East, Asia Minor, and China (Anon. 1977). It was introduced to the USA in the late nineteenth century and more recently to Canada (Morris 1986). In northern Spain adult moths emerge in May–June, mate and lay numerous eggs (between 1500 and 2500) at the base of poplar stems. Larvae hatch after approximately 20 days and penetrate the bark at the base of the trunk where they create galleries of 20–50 cm long towards the roots. After 2 years of feeding, larvae migrate from the roots to the bottom of tree trunk where they weave a cocoon of woody debris inside the tree. After approximately 3 weeks of pupation, young adults emerge through a round hole at the base of the main stem (Pérez et al. 2002; Muñoz et al. 2007; de Tillesse et al. 2007). The main flight period for this species in Spain is from the end of May to the end of July. *S. apiformis* larvae damage cambial layers and outer wood in the lower part of the trunk, which results in wood discoloration (Pérez et al. 2002; de Tillesse et al. 2007), a point of entry for fungal infections (Francke et al. 2004), decreased plant vigour, and in some cases death of trees (Chrystal 1937; Pérez et al. 2002; Francke et al. 2004). In addition infested trees are more prone to wind breakage at ground level (Pérez et al. 2002).

Two key issues currently hinder the sustainable management of *S. apiformis* in plantation grown poplar stands: (1) a lack of information on the effects of poplar stand management on hornet clearwing moth infestations, despite its clear importance as a moderator of biotic risk factors (Jactel et al. 2009) and (2) no quantitative data on the distribution and abundance of *S. apiformis* in poplar plantations. To date the only available technique to estimate population levels of *S. apiformis* is to count exit holes at the base of tree trunks. This method is both inefficient and inaccurate as holes are frequently overlooked because they are located very close to the ground and can be camouflaged by stones or soil. The identification and synthesis of the sex pheromone (Francke et al. 2004) for *S. apiformis* provides an opportunity to develop pheromone trapping methods to monitor populations. However, this approach relies on a positive relationship between trap capture and local population density (Howse et al. 1998), a relationship that has not been empirically tested.

In our study, we surveyed poplar stands using a factorial design that combined site quality, stand age and understorey management to evaluate the susceptibility of poplar plantations to the hornet clearwing moth. Our main hypothesis was that these three stand attributes that influence individual tree vigour can in turn confer resistance to *S. apiformis*.

In the same plantations we activated pheromone traps to quantify the relationship between trap capture and the number of attacked trees within the same stand and in the surrounding landscape. Our objectives were to test whether the relationship between pheromone trap capture and local infestation level may be obscured by trap interception of wandering moths emigrating from other nearby poplar stands and to lay the foundations of a pheromone monitoring system for *S. apiformis*.

Materials and Methods

Site description and experimental design

The present study was carried out in Castile and Leon (Spain), an area with approximately 45 000 ha of poplar plantations, mostly *Populus deltoides* × *Populus nigra* (*P.* × *euramericana* (Dode) Guinier). Several clones are grown but the clone I-214 constitutes about 70% of the total poplar plantation area (Fernández and Hernanz 2004). The density of trees in clonal poplar plantations is kept constant during the whole forestry cycle, either at 278 or 400 stems/ha, planted with a spacing of 6 × 6 or 5 × 5 m, respectively. Every year during the first 6 years, the understorey vegetation is controlled with disc harrowing and trees are pruned to a final height of 6–8 m above ground level. Poplar plantations are clear-cut at 14–15 years.

The experiment consisted of a complete factorial scheme with three factors and two treatments per factor: stand age (young stands of 3–7 years old or old stands of 8–14 years old), site quality (rich site (quality 1 and 2) or poor site (quality 3 and 4)), according to the site quality abacus developed for the *P.* × *euramericana* clone I-214 in the river Duero basin (Bravo et al. 1995), and understorey management (harrowed or not harrowed). Each combination of the factors was replicated four times, as four independent I-214 clonal plantations. A total of 32 poplar stands were therefore sampled in the north of the Palencia province, within the Carrion river basin (from 346.405 to 366.495 and 4.686.275 to 4.712.381, latitude and longitude, respectively, in UTM coordinates). The area of sampled stands varied from 1 to 38 ha (10 ha on average).

Sampling of *Sesia apiformis* attacks and populations

In each stand two circular subplots with a radius of 15 m were established 50 m apart from each other in 2005. Within each subplot we measured tree density (stems/ha), and the diameter at breast height (DBH, cm), total height (TH, m) and presence/absence of moth emergence holes at the base of the stem in all trees (a total of 1366 poplar trees). At the centre of each subplot, nine 2 × 2 m quadrats (36 m^2 in total) were also laid out in order to quantify the understorey vegetation. The percentage cover of all vascular plant species was estimated visually using the Braun-Blanquet (1964) scale during the spring of 2005. Crown condition of all trees was assessed during the summer of 2005 (within the first 2 weeks of July) as recommended in the ICP-Forest protocol. Crown transparency and discoloration were estimated visually and quantified using 20 classes of 5% width, according to Level I European network methodology (Eichhorn et al. 2006; see Martín-García et al. 2009 for details).

During spring and summer 2005, populations of hornet clearwing moth were monitored with pheromone traps in all 32 stands. We used funnel traps (Uni-Trap; PHEROBANK, Wageningen, The Netherlands) baited with commercial lures (PHEROBANK) of *S. apiformis* sex pheromone. Pheromone dispensers were loaded with a racaemic mixture of (*Z*,*Z*)-3,13-octadecadienyl acetate and (*E*,*Z*)-2,13-octadecadienal, with a release rate of 0.22 μg/h at 25°C. Traps were assessed every fortnight for a total of six times from May to August 2005, and lures were renewed once (after 42 days).

Spatial analyses

Habitat mapping within the study area was performed using colour aerial orthophotographs at the scale 1 : 1500, based on photo-interpretation and field cross-validation. Habitat type was assigned to each landscape patch according to the following classification: poplar plantation, hedgerows (because they may also contain hybrid poplars), riparian forests (because they can contain wild black poplars, *Populus nigra*) and others habitats. Circular buffered areas of 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 m were established around each of the 32 stands to calculate the percentage cover of each habitat (ArGis 9.1; ESRI Inc., Redlands, CA, USA).

Using this information we calculated the area of the sampled poplar stand encompassed within the 50–1000 m buffers. Because we found no significant difference in mean percentage of attacked trees between sub-plots (see below for method) we assumed a uniform, within stand distribution of *S. apiformis* infestations. We then estimated the number of trees attacked by *S. apiformis* in the sampled stand within a given distance (the buffer radius) from the pheromone trap according to the following equation (Eqn 1):

$$\text{NAT-d} = \text{PAT} \times \text{DENS} \times \text{AREA-d} \qquad (1)$$

where NAT-d is the number of attacked trees within distance *d* from the trap; PAT the % attacked trees in the stand; DENS the density of trees in the stand; and AREA-d is the area of the stand encompassed in a buffer zone of radius *d* around the trap location.

We also calculated the area of other poplar stands within each buffer zone.

Statistical analyses

Analyses of the variance (ANOVA) were carried out to test the effects of stand attributes (age, site quality and understorey management) on the percentage of trees attacked by *S. apiformis* (after angular transformation of the percentage value) and on the mean capture of *S. apiformis* male moths per stand and day (after log(x + 1) transformation). The effect of mean tree crown transparency (VCT) and discolouration (VCD) per stand on the mean tree size (DBH and total height) and on the percentage of attacked trees by *S. apiformis* were tested with the Pearson coefficient of correlation. We checked data homoscedasticity with a Bartley's test and normality of residuals with a Shapiro–Wilk's test. Multiple means comparisons were made with Tukey's tests.

We used a paired comparison test (Wilcoxon signed rank test, V statistic) of the percentage of attacked trees in the two sub-plots of each stand to check for within stand homogeneity of *S. apidormis* infestation.

To investigate the tree characteristics that influence *S. apiformis* host selection, we compared individual tree diameter (DBH), total height (TH), crown transparency (VCT) and discoloration (VCD) between attacked and un-attacked trees. Because we found a significant Age × Management interaction we tested the significance of these differences with Wilcoxon paired comparison tests in young and old stands, harrowed or not harrowed, separately.

To test the effect of stand harrowing on the risk of *S. apiformis* infestation we made simple linear regression between the percentage of attacked trees and the total cover of understorey vegetation (%) per stand, in young and old stands separately.

To interpret pheromone trap captures of *S. apiformis* we made two assumptions and then used a two-step approach to test these assumptions. Our first hypothesis was that the number of moths caught would be dependent on the attraction radius of the pheromone trap. To test this assumption we calculated the Pearson's correlation matrix between the total catch per stand per day and the estimated number of attacked trees in the same stand within buffers of increasing radius (i.e. increasing distance from the trap) using eqn 1. Then we identified the buffer radius associated with the highest correlation coefficient and calculated the corresponding simple linear regression model (step 1). Our second hypothesis was that some moths caught did not originate from the same stand but may have flown from surrounding poplar stands, depending on their flight capacity. To test this second assumption we calculated the residual of observed trap capture per pheromone trap (observed – predicted) using the best linear regression model of trap capture against the number of attacked trees within the same stand (as defined in step 1).

Then we calculated the Pearson's correlation matrix between the residuals and the percentage cover of other poplar stands, hedgerows or riparian forests (a proxy of the number of poplar trees) within buffer zones of increasing radius. We could then identify the buffer radius associated with the highest correlation coefficient (step 2). We used this residual regression approach to deal with the multi-collinearity problem in multiple regressions (Graham 2003) because variables of number of attacked trees in a stand within increasing distances from the trap were highly correlated between each other as well as variables of percentage cover of other poplar stands within concentric buffers. Finally we developed a multiple linear regression to relate trap captures to the number of attacked trees in the same stand within a given distance from the trap (best correlated variable in the first step) and the percentage cover of other poplar stands within a given distance (best correlated variable in the second step). The information on the age or management of other poplar stands, hedgerows or riparian forests was not available.

Statistical analyses were performed using XLSTAT software.

Results

Patterns of *Sesia apiformis* infestation at stand and tree level

The percentage of attacked trees by *S. apiformis* per stand varied from 0% to 71% with an average (±SE) of 25.5 ± 4.3%. The percentage of attacked trees did not differ significantly between the two sampled plots in each stand (V = 201.5, P = 0.33). Analysis of the variance indicated that poplar stand age was a key determinant of attack rate by *S. apiformis* (table 1). Old stands had almost five times the infestation rate compared to young stands (fig. 1a). However there was a significant interaction effect between stand age and understorey management on infestation (table 1). Young, not harrowed stands, showed significantly higher percentage of attacked

Table 1 Effect of poplar stand characteristics on the percentage of attacked trees by *Sesia apiformis* (after angular transformation) and on the mean capture of *Sesia apiformis* moths per stand and day (after log(x + 1) transformation). P values in bold indicate that the factor is significant at the 5% level

		Percentage attacked trees		Mean capture/ stand/day	
Independent variables	d.f.	F	P > F	F	P > F
Age	1	46.19	**<0.0001**	9.35	**0.018**
Site quality	1	0.25	0.621	2.95	0.279
Management	1	0.09	0.765	1.93	0.562
Age × site quality	1	2.28	0.144	1.24	0.869
Age × management	1	4.66	**0.041**	4.08	0.067
Site quality × management	1	0.62	0.440	2.27	0.203
Age × site quality × management	1	0.52	0.476	1.14	0.382

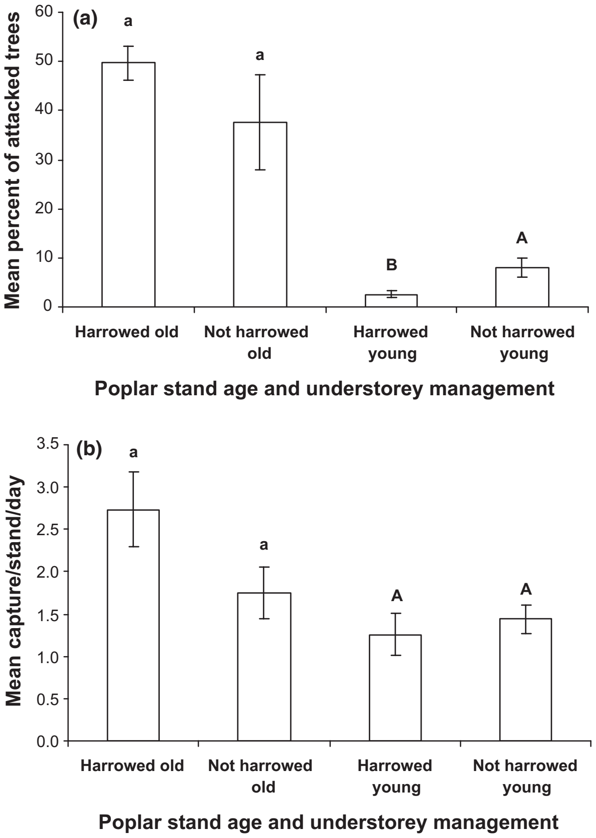

Fig. 1 Effects of poplar stand age and understorey management on the mean (±SE) percentage of attacked trees by *Sesia apiformis* (a) and on the mean (±SE) capture of *S. apiformis* male moths per stand (i.e. per trap) per day (b). For the mean percentage of attacked trees, bars with different letters – within the same class of stand age – indicate significant differences (Tukey's test, α = 5%).

trees than young harrowed stands, whereas no significant effect of understorey management was observed in old stands (fig. 1a). Site quality did not influence the percentage of attacked trees (table 1).

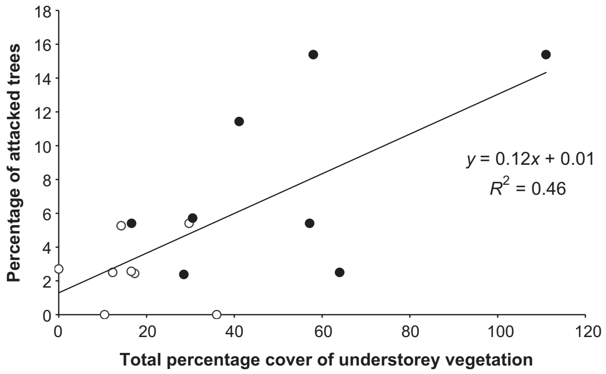

Fig. 2 Relationship between the percentage of attacked trees by *Sesia apiformis* and the total percentage cover of understorey vegetation in young poplar stands (white dots – harrowed stands, black dots – not harrowed stands). The percentage cover was calculated as the sum of percentage covers of annual, perennial, woody and bryophyte plants; it can be higher than 100% due to overlapping.

Harrowing significantly reduced the total cover of understorey vegetation in young stands (n = 16, F = 7.21, P = 0.01) and adult stands (n = 16, F = 29.82, P < 0.001). Furthermore, in young stands, we found a significant and positive relationship (n = 16, F = 10.96, P = 0.006, R^2 = 0.46) between the mean percentage of attacked trees per stand and the total cover (%) of understorey vegetation (fig. 2). There was no significant relationship between these variables in old stands (n = 16, F = 0.07, P = 0.80).

There were no significant differences in diameter, height and crown condition (VCT and VCD) between attacked and un-attacked trees within each stand. There was no significant correlation between mean tree crown transparency (VCT) and discolouration (VCD) per stand and the percentage of attacked trees per stand (n = 32, P = 0.07 and P = 0.18, respectively). Similarly there were no significant correlations between of tree crown condition (VCT and

VCD) and mean tree diameter ($n = 32$, $P = 0.06$ and $P = 0.16$, respectively) or height ($n = 32$, $P = 0.10$ and $P = 0.21$, respectively).

Monitoring hornet clearwing moth population with pheromone traps

On average, pheromone traps caught 1.8 ± 0.2 *S. apiformis* male moths per trap per day. Analysis of variance indicated that poplar stand age significantly influenced the mean capture per day (table 1) with higher capture in older stands (fig. 1b). The mean capture per day was significantly correlated with the total number of attacked trees per stand ($R = 0.65$, $P < 0.0001$) and with the estimated number of attacked trees within all tested distances (50–1 000 m) from the trap. The best correlation however, was observed with the number of attacked trees in the same stand within a distance of 100 m from the trap ($R = 0.78$, $R^2 = 0.61$, fig. 3). We could improve the prediction of pheromone trap capture by taking into account the amount of other poplar stands around the sampled stand. For that we calculated the residual of mean capture per trap and day (Eqn 2).

$$\text{Residual} = \text{MCS} - \left(1.1 + 3.5 \times 10^{-5} \times \text{NAT} - 100\text{m}\right) \quad (2)$$

where MCS is the mean capture of *Sesia* moths per stand per day; and NAT-100 m is the number of attacked trees in the sampled poplar stand within a circular area of 100 m radius around the trap.

The residual of mean capture per trap and day was significantly correlated with the area of other poplar stands within a distance of 200–1000 m from the trap (fig. 4) but the best correlation was observed for a distance of 600 m. On the other hand, area of surrounding hedgerows and riparian forests had no significant effect on number of captures with pheromone traps.

Then the best multiple linear model, including the number of attacked trees within a distance of 100 m and the area of other poplars stands within a distance of 600 m as predictive variables (Eqn 3), could explain ca. 70% of trap capture variability ($n = 32$, $F = 34.8$, $P < 0.0001$, $R^2_{\text{adj}} = 0.69$).

$$\begin{aligned}\text{MCS} = 0.74 + 3.5 \times 10^{-5} \times \text{NAT} - 100\text{m} \\ + 2.0 \times 10^{-2} \times \text{APS} - 600\text{m}\end{aligned} \quad (3)$$

where MCS is the mean capture of *Sesia* moth per stand per day; NAT-100 m the number of attacked trees in the sampled poplar stand within a circular area of 100 m radius around the trap; and APS-600 m is the area (ha) of other *Poplar* stands within a circular area of 600 m radius around the trap.

Discussion

This study demonstrated that poplar plantations of *P.* × *euramericana* in Spain can sustain high infestation rates of *S. apiformis*, up to 71% of attacked trees per stand. This is consistent with other studies that found *P.* × *euramericana* to be more susceptible to *S. apiformis* than other poplar species such as *P. nigra*, *P. trichocarpa* and *P. alba* (Arundell and Straw 2001; Straw et al. 2007).

The percentage of trees attacked by *S. apiformis* was mainly explained by stand age at stand level, since old stands (8–14 years) were much more susceptible than young stands (3–7 years). Higher infestation rates in old stands did not result from

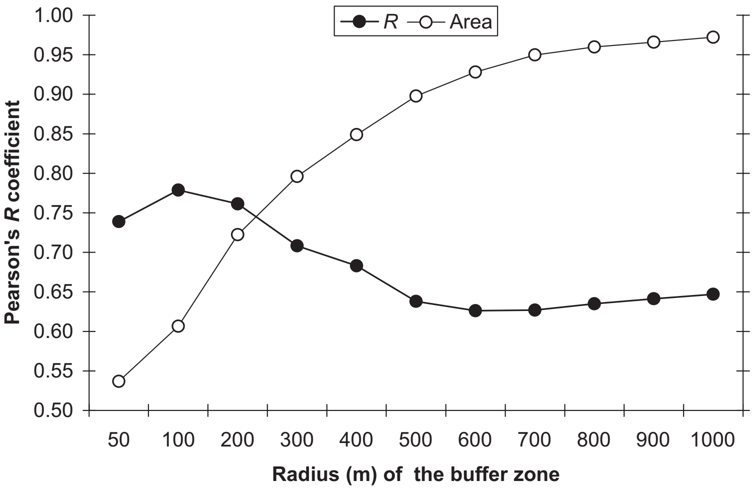

Fig. 3 Coefficient of correlation of the mean pheromone trap capture of *Sesia* moth per day vs. the number of attacked trees in the sampled poplar stand within circular areas (buffer zones) around the trap, at different spatial scales (black dots). Evolution of the area of sampled poplar stand intercepted by a circular area around the trap, at the same spatial scales (white dots).

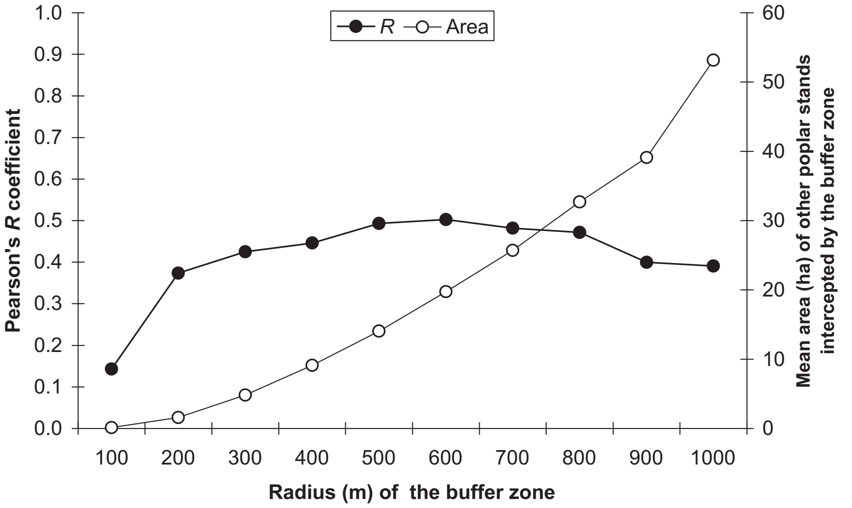

Fig. 4 Coefficient of correlation of residual mean pheromone trap capture of Sesia moth per day (observed capture – predicted capture according to the number of attacked trees within a circular area of 100 m radius around the trap) vs. the amount (area) of other poplar stands within circular areas (buffer zone) around the trap, at different spatial scales (black dots). Evolution of the area of other poplar stands intercepted by a circular area around the trap, at the same spatial scales (white dots).

recurrent attacks accumulated from previous years since most of exit holes were qualified as fresh. Furthermore, pheromone trap captures were also higher in old stands, which is an instantaneous measure of population level. It is then likely that older stands are more infested because older trees are preferred by *S. apiformis*. However, many driving factors of host selection by forest insects can be correlated with tree age such as tree size and tree vigour.

Comparison of tree diameter or total height between attacked and un-attacked trees within each stand did not show any significant differences, suggesting that *S. apiformis* may not use tree size as a cue to select host trees. Some studies have assessed the effect of diameter on attack of *S. apiformis* with contradictory results; Arundell and Straw (2001) did not observe any significant relationship between *S. apiformis* infestation and tree size (girth or height) whereas Straw et al. (2007) found a positive correlation in areas with high *S. apiformis* populations. Because of the long life-cycle of *S. apiformis* its infestation may be more related to past tree condition rather than present vigour. We tested this hypothesis using crown transparency or discoloration as indicators of past tree condition. We found no significant differences in crown condition between attacked and un-attacked trees by *S. apiformis*, which may indicate that stressed trees were not more prone to its infestation than vigorous trees.

Previous studies (e.g. Straw et al. 2007) have suggested that the relationships between *S. apiformis* infestation and tree condition are more evident at the site or plot scales than at tree scale. Site quality has been shown to reflect important differences in mean tree diameter, height and crown condition, with trees growing more slowly on poor quality sites (Martín-García et al. 2009), and yet we found no significant effect of site quality on the level of *S. apiformis* infestation per stand. We did not find either any significant correlation between mean tree condition per stand and the percentage of attacked trees, thus suggesting that low stand vigour may not be a predisposing factor of *S. apiformis* infestation at the stand scale.

Understorey management is a traditional technique in poplar plantations, aimed at increasing the growth of trees. In a previous study conducted in the same poplar stands we found that harrowing improved crown condition on poor sites, probably through reduction in competition for water between poplar trees and understorey vegetation (Martín-García et al. 2009). However, we did not observe any significant effect of site quality or site quality × understorey management interaction on *S. apiformis* infestation. Furthermore, the effect of understorey management on *S. apiformis* attacks was only significant in young stands, with higher rate of attacks in not harrowed stand where the competition between trees and understorey vegetation is likely to be greater.

The association between higher rates of infestation and the presence of understorey vegetation in young plantations could have arisen because trees in these situations were suffering a greater degree of water stress. However we found no significant correlation between the percentage cover of understorey vegetation and mean tree size per stand. So, our findings seem to suggest that low tree vigour is not a predisposing factor of poplar infestation either at tree level or at stand level, and that *S. apiformis* seems to behave as a primary pest able to attack healthy trees, at least in Spain. This contradicts the view that *S. apiformis* is a purely secondary pest (Arrundel and Straw, 2001; Straw et al. 2007). However our study was only based on a 1-year survey and no information on the number of exit holes per tree was available, which makes it difficult to judge exactly how damaging the moth was in poplar plantations. Further research on the population dynamics of *S. apiformis* is thus needed to clarify its status of primary vs. secondary pest.

Previous studies have indicated that the type of understorey may influence *S. apiformis* infestations (Coleman and Boyle 2000; Arundell and Straw 2001; Straw et al. 2007). These authors observed that attacks were more frequent where the understorey vegetation was dense, particularly where it consisted of shrub, tall weed or rough grass. We observed a similar positive response to understorey biomass in young stands, with more attacks in not harrowed stands and a significant correlation between the rate of attacks and the percentage cover of understorey vegetation. *S. apiformis* female moths can lay up to 1500–2500 eggs, on the surface or into crevices of the bark at the base of poplar trees. Eggs are totally dry without any sticky substance (Romanyk and Cadahia 2002). Mortality during the first instar, between eggs hatch and establishment under the bark, appears to be particularly high as only 5–6% of eggs result in established larvae (Srot 1969). These features lead us to propose a new theory of host colonization by *S. apiformis*. Once in a poplar stand, female moths would not select suitable host trees according to any physical or chemical cues but would just lay eggs on some trees at random. In old stands, trees have thick bark with deep cracks, providing eggs with protection from adverse climatic conditions or predation. As a result of higher first instar survival, trees would be then more likely to be successfully colonized by *S. apiformis* in old than in young stands. In young stands, where trees have only thin bark, most of the eggs would be laid unprotected by bark cracks and would be more susceptible to predators and adverse weather conditions. It has been already suggested that eggs need relatively high humidity to hatch (de Tillesse et al. 2007) and that more predation by birds might occur when vegetation is very low (Coleman and Boyle 2000). In these situations the understorey vegetation would provide eggs and egg-laying females with more protection, supporting the pattern of higher rate of attacked trees in young stands with higher cover of ground vegetation.

Another important result of our study is that pheromone trapping of *S. apiformis* provides relevant information on stand infestation. Surprisingly there has been no published study on the use of pheromone traps to monitor *S. apiformis* populations since the identification and synthesis of its sex pheromone (Francke et al. 2004). And yet pheromone trapping has proved to be a reliable tool to estimate abundance of other forest moth species, such as pine processionary moth (Jactel et al. 2006), eastern spruce budworm (Sanders 1988), western spruce budworm (Sweeney et al. 1990), gypsy moth (Ravlin 1991), nun moth (Morewood et al. 2000) and western hemlock looper (Evenden et al. 1995).

We observed that a single trap could provide reliable information to estimate *S. apiformis* attacks in poplar plantations, whereas a minimum of three traps were necessary to properly estimate pine processionary moth (*Thaumetopoea pityocampa*) populations in maritime pine plantations (Jactel et al. 2006). This discrepancy is likely to originate from differences of stand homogeneity, as poplar forestry is based on monoclonal stands with regular spacing of trees.

The best correlation between mean trap capture and number of attacked trees in circular areas of increasing radius around the trap was obtained for a radius of 100 m. In contrast the number of poplar trees in the same circular areas continuously increased with the radius, so the number of trap capture did not just correlate with possible source of emerging moths beyond this 100 m maximum. These results support the hypothesis that the active radius of attraction by pheromone traps, baited with *S. apiformis* sex pheromone at a release rate of 0.22 μg/h, is about 100 m. This outcome is consistent with the observation that most of the attraction radii of forest insect pheromone traps are in the order of tens to hundreds of meters (Schlyter 1992; Zhang et al. 1996).

The number of attacked trees in the vicinity of traps only accounted for ca. 60% of trap capture variability and in the three stands where no attacked trees were observed the pheromone trap still caught some *S. apiformis* moths (ca. 0.8 moths/stand/day). Some moths immigrating from other poplar stands may have been caught by pheromone traps. Accordingly, when we took into account the amount of other poplar stands in the surroundings of sampled stands we could improve the prediction of trap capture.

The best multiple linear model was obtained when the amount of other poplar stands was calculated within a buffer zone of 600 m. These results suggest that *S. apiformis* male moths could fly around 600 m and indicate that landscape composition, and particularly the percentage cover by forest insect habitat, should be considered when interpreting pheromone trap captures. For the same reason the abundance of suitable poplar host trees was probably too low in surrounding hedgerows and riparian forest to affect the local population level of the moth and then to be retained in multiple linear models.

We have therefore demonstrated that pheromone trapping offers a reliable monitoring tool for hornet clearwing moth population which can be easily applied on a larger scale than the usual sampling method based on exit hole counts. However further studies should be undertaken to optimize the trapping technique and evaluate the relevance of pheromone trapping for the prediction of *S. apiformis* abundance in the next generation in order to better develop control methods.

Conclusions

This study sheds new light on the process of *S. apiformis* infestation in poplar plantations.

- Plantations of *P.* × *euramericana* (clone I-214) can exhibit high infestation rates of *S. apiformis* (up to 70% of attacked trees) in Spain and low tree vigour does not seem to be a predisposing factor of infestation either at the stand level or at the tree level, thus indicating that the hornet clearwing moth may be a serious primary pest for some poplar plantations. This potential threat should be better surveyed.
- Pheromone trapping may offer a user friendly and reliable method of monitoring. One pheromone trap per stand seems enough to deliver relevant estimate of infestation rate at the stand scale and proposal has been made to improve the accuracy of local estimate through a correction based on the amount of other poplar stands in the surrounding landscape.
- Old poplar stands are more infested than young plantations and, according to trapping results, other poplars stands in the surrounding landscape (within an area of ca. 100 ha) would serve as sources of infesting insects. On the other hand, tree size and vigour are not drivers of host tree selection within each poplar plantation. This suggests that there might be an orientated selection of poplar habitat at the landscape scale by *S. apiformis*, but a random colonization of host trees at the stand scale.
- Deep bark cracks (in older stands) or understorey vegetation (in young stands) would provide eggs and first instar larvae with protection against predation and adverse condition at the base of host tree trunks. Meanwhile if serious damage is detected in young poplar plantations, it might be advised to reduce the cover of understorey vegetation in order to decrease the population level of the pest.

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ARTICLE V

Martín-García, J., Espiga, E., Pando, V. & Diez, J.J. (2011) Factors influencing endophytic communities in poplar plantations. *Silva Fennica* 45 (2): 169-180

RESUMEN

Factores que influyen en las comunidades de endófitos en las plantaciones de chopos

Los hongos endófitos asociados a hojas y ramillos de 12 plantaciones de chopo (*Populus x euramericana*, clon I-214) seleccionadas según un diseño factorial con dos factores (edad y calidad de estación) fueron estudiadas para evaluar su comportamiento frente a diferentes factores. Para lo cual, condiciones de copa, variables dendrométricas y concentración de nutrientes foliares fueron evaluadas en cada árbol muestreado. La riqueza de especies y la frecuencia relativa fueron mayores en las plantaciones jóvenes que en las adultas. Además, la calidad de estación influyó sobre la riqueza, observándose una menor riqueza de especies en las parcelas adultas establecidas en calidades de estación pobres. A nivel de parcela, las comunidades de endófitos variaron en función de la calidad de estación, y en menor medida de la edad. Por otro lado, decoloración de la copa, altura total del árbol y concentraciones foliares de Fe y Zn podrían ser indicadores de las comunidades de endófitos en plantaciones de chopo.

Palabras clave: Chopera, *Populus*, sanidad forestal, calidad de estación, nutrientes foliares.

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Factors Influencing Endophytic Communities in Poplar Plantations

Jorge Martín-García, Elba Espiga, Valentín Pando and Julio Javier Diez

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The fungal species associated with leaves and twigs from stands of *Populus × euramericana* in northern Spain were studied with the aim of evaluating the effects of several factors on endophytic communities in these plantations. Endophyte assemblages were analysed in 12 poplar plantations (clone I-214), chosen according to a factorial scheme with two factors: age and site quality. Crown condition, dendrometric variables and foliar nutrients were recorded in each sampled tree to evaluate their effects on endophytic communities. Fungal species richness and relative isolation frequency (RIF) were higher in young stands than in adult stands. Moreover, the age-related differences depended on site quality, with the lowest richness levels observed in adult stands located in poor sites. At stand level, endophyte assemblages varied among stands according to site quality and, to a lesser extent, stand age. On the other hand, crown discoloration, total height and foliar concentrations of iron and zinc may be key indicators of endophytic communities in poplar plantations, at tree level.

Keywords endophyte, poplar, management, site quality, foliar nutrients, forest health
Addresses *Martín-García*, Sustainable Forest Management Research Institute, University of Valladolid – INIA, Avenida de Madrid 57, 34004 Palencia, Spain, and Forestry Engineering, University of Extremadura, Plasencia, Spain; *Espiga* and *Diez*, Sustainable Forest Management Research Institute, University of Valladolid – INIA, Palencia, Spain; *Pando*, Statistics and Operations Research Department, University of Valladolid, Spain
E-mail jorgemg@pvs.uva.es, jorgemg@unex.es
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1 Introduction

Interest in hybrid poplar plantations is increasing in Spain because of the economic value of the trees. The profits associated with poplar plantations can reach between 1200 and 2400 €/ha/yr on optimum land (Díaz and Romero 2001). Therefore, although the area covered by this species in the region, estimated at about 45000 ha, is relatively low, the trees are a potentially important source of wood products (plywood), non-wood products (fuelwood) and services (shelter, shade and protection of soil, water and livestock). The environmental and economic applications of poplar plantations are therefore driving factors for sustainable forestry and rural development (Rueda et al. 1997, Ball et al. 2005).

Plantations of *Populus × euramericana* (Dode) Guinier (*P. deltoides* Marsh. ♀ × *P. nigra* L. ♂) are monoclonal; although several clones are used, clone I-214 is the most commonly planted in Spain, and covers about 70 % of the total area covered by poplar stands (Fernández and Hernanz 2004). Plantations are managed on short rotations (12–16 years), and intensive tillage practices are usually applied (Fernández 1998). Mechanical tillage, logging residue management, pruning and weed control are widely used techniques. The density of poplar plantations is maintained constant during the whole rotation, at about 278–400 stems/ha, depending on the planting distance, 6×6 or 5×5 meters, respectively. This species has a deep rooting system and requires large amounts of water; striplings are thus placed in direct contact with the water table, which is usually at a depth of between 1 and 2.5 meters (De Mier 2001, Fernández and Hernanz 2004).

However, despite the intensive management required, the profitability of poplar plantations varies greatly, as with other types of forest (Ke and Skelly 1994, Ouimet and Camiré 1995, Hallet et al. 2006), and depends, amongst other factors, on the health status of the stand (Camps 2001, Sierra 2001). The importance of forest health has been recognised in recent decades. A forest health monitoring programme has been carried out in Europe since the 1980s within the International Co-operative Programme, ICP Forest (Level I European network). More recently, sustainable forest management programmes have focused huge efforts on assessing forest health. Such programmes have assessed forest health by monitoring crown condition (crown transparency and discoloration), as well as fungal and insect pests. However, other important biotic agents, such as endophytes, have not yet been studied, although many authors have recognised the importance of endophytic communities in forest health (Bettucci and Alonso 1997, Bettucci et al. 1999, Gennaro et al. 2003, Ragazzi et al. 2003, Santamaría and Diez 2005, Zamora et al. 2008, Botella et al. 2010).

Many definitions of endophytes have been reported (Hyde and Soytong 2008); some researchers define endophytes as those fungi that are able to infect their hosts without causing visible disease symptoms (Petrini 1991, Wilson 1995, Schulz and Boyle 2005) and other authors established the term endophyte as synonymous with mutualism (Saikkonen et al. 2004, Backman and Sikora 2008). However, the distinction between pathogenic and endophytic organisms is not clear, and the same fungus or even the same isolate may behave as a saprophyte or pathogen according to the host vigour (Schulz et al. 1999). In the present study we considered those fungi isolated from surface-sterilized samples as endophytes.

Age and environmental conditions have important effects on endophytic communities (Petrini and Carroll 1981, Legault et al. 1989, Carroll 1994, Helander et al. 2006, Kauhanen et al. 2006). More recently, Botella et al. (2010) have demonstrated that several abiotic factors, including water availability, shade, light exposure, age, elevation and mean temperature, appear to influence endophytic communities and forest health in Allepo pine in Spain. However, so far no research has been carried out to determine the effect of these variables on endophytes of poplars. In addition, there is an obvious lack of research designed to clarify the effect of site quality and host nutrient status on endophytic communities.

Taking into account the great importance of endophytic communities and the lack of research on endophytic fungi in *Populus × euramericana*, the main goals of this study were: 1) to analyse whether factors such as age and site quality affect endophytic communities at stand level, and 2) to study whether soil nutrient status, dendrometric variables and crown condition could explain endophytic communities at tree level.

2 Materials and Methods

2.1 Site Description and Sampling Procedure

The present study was carried out in Castilla y León (NW Spain). The altitude of the study area ranges between 800 and 900 m. above sea level and in most stands the topography is almost flat. The average annual precipitation varies between 496 and 630 mm and the average annual temperature, between 9 and 11.4 °C (Ninyerola et al. 2005).

The experimental design consisted of a factorial scheme with two factors, stand age (young: 3–7 years old stands, or adult: 8–14 years old stands) and site quality. Stands were assigned a site quality, with rich sites (quality I and II) and poor quality sites (quality III and IV) differentiated on the basis of the site quality curves developed for *Populus × euramericana* clone I-214 in the river Duero basin (Bravo et al. 1995). These site indexes are related to a basal area (at the breast height of all trees planted in 1 ha) for stand age up to ten years. The specific values of the site indexes are 20.21, 16.77, 13.31 and 9.87 m^2 ha^{-1} for site qualities I, II, III and IV, respectively. Three I-214 clonal plantations were sampled, and two trees were chosen within each plantation for each combination of factors. A total of 12 poplar stands and 24 trees were finally selected for study.

The health status of each tree was evaluated during the summer (first two weeks of July) of 2005, on the basis of crown condition (crown transparency and crown discoloration). To avoid possible sources of error due to the subjectivity of human assessment of factors including weather conditions, crown appearance, tree species, tree age and social status (Innes et al. 1993, Ghosh et al. 1995, Solberg and Strand 1999, Wulff 2002, Redfern and Boswell 2004), crown transparency was determined by a more accurate variable, designated Digital Crown Transparency (DCT). This variable is estimated by means of digital photographs obtained by use of a semiautomatic image analysis system, known as CROCO (Mizoue 2002). An automatic thresholding algorithm is used in CROCO to obtain crown silhouette images, where foliage and branches are transformed to black pixels and background sky to white pixels (Mizoue and Inoue 2001). CROCO calculates two fractal dimensions to estimate the crown transparency of the tree silhouette (Ds) and outline (Do). The index of crown transparency, obtained by the CROCO method (DSO), was calculated as the difference between Ds and Do (Mizoue and Dobbertin 2003). DSO was subsequently converted into DCT by means of a calibration equation previously developed for *Populus × euramericana* (Martín-García et al. 2009).

Crown discoloration (VCD) was estimated visually and quantified by considering twenty 5%-interval classes, according to Level I of the European network methodology (Eichhorn et al. 2006). Before sampling, the operator took part in an intercalibration session with the Spanish field crew of the European Level I network. Parts of the crown directly influenced by crown interactions or competition were excluded; trees were assessed from a distance of about one tree length, with the observer taking care to avoid looking into the sun (Eichhorn et al. 2006). Biotic damage in the crown was also recorded but there were so few instances of such damage that it was not taken into further consideration.

Foliar sampling was carried out during the first two weeks of September 2005, the period when foliar nutrients are most stable in poplar trees (Bengoa and Rueda 2001). Between 12 and 15 green leaves were removed per tree, from two main branches of the upper third of the canopy (north and south sectors). The samples were transported to the laboratory, stored at 4 °C and processed within 24 hours. The oven-dried (60 °C) samples of leaves were milled (0.25 mm) and digested with HNO_3 in a microwave oven. Total C and N in milled foliar samples were analysed by combustion, with a Leco analyzer (LECO, St Joseph, Michigan, USA). The total concentrations of P, K, Ca, Mg, Fe, Mn, Zn, Cu, B, Ni S, Al, Cr, As, Mo, Cd, Co, Na and Pb in the digested foliar samples were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer, Wellesley, MA, USA).

Finally, diameter at breast height (DBH), total height (TH), pruned height (PH), crown diameter (CD) and crown volume (VOL) were also measured in all trees during autumn in 2005.

2.2 Fungal Isolation and Identification

Leaves and twigs from branches collected for foliar analyses were used for fungal isolation. Surface sterilization of the leaves and twigs was performed by a modified version of the procedure of Kaneko and Kaneko (2004). Samples (both leaves and twigs) were dipped in ethanol (70% v/v) for 60 s, then in sodium hypochlorite solution (2% v/v) for 2 min (leaves) or 3 min (twigs), in ethanol (70% v/v) for 30 s (leaves) or 60 s (twigs), and then washed three times in sterile distilled water.

Twelve pieces of leaves (0.5 × 0.5 cm) and twelve twig segments (0.5 cm diam., 0.5–1 cm thick) from each tree were placed in Petri dishes containing "potato dextrose agar" (PDA) medium. The plates were sealed with Parafilm® and incubated in the dark at 20 °C for one month. The outgrowing fungi were transferred to fresh PDA and grown in pure culture until sporulation. Fungal isolates were identified according to morphological characteristics, using a stereomicroscope and analysing the shape and colour of the colonies, and the main characteristics of fungal structures. Different taxonomic keys were used to identify the fungi (Lanier et al. 1978, Von Arx 1981, McGinnis et al. 1982, Barnet and Hunter 1987, Goidanich 1990, Watanabe 1994, Kiffer and Morelet 1997).

2.3 Statistical Analyses

Univariate statistics The effect of factors (age and site quality) and tissue sampled (leaves or twigs) on species richness of endophytic fungi and on the relative isolation frequencies (RIF) was evaluated by a Mixed Analysis of Variance Model. This model was carried out with three fixed factors in a complete 2^3 factorial design and using different error variances for each of the eight treatments in the model. The RIF were calculated as $\mathrm{RIF} = n_{ijk}/N_{ijk}$, where n_{ijk} is the number of isolates recorded for site quality i, age j and tissue k, and where N_{ijk} is the number of samples examined for site quality i, age j and tissue k (Santamaría and Diez 2005). Two linear mixed models (PROC MIXED) were therefore applied by use of SAS (version 9.1) software.

Multivariate statistics Two types of analyses were carried out. Firstly, correspondence Analyses (CA) were carried out at stand level, for the composition of fungal species isolated from leaves only, twigs only and leaves plus twigs, and with 'isolated fungal species composition' as the response variable, in order to assess the influence of both factors, age and site quality, on fungal occurrence. The response variable was transformed by means of log $(x+1)$ to comply with normality assumptions. Although fungi isolated from only one stand were excluded from these analyses, the downweighting option was also used to reduce the importance of rare species. For presentation in figures, plots were labelled by age and site quality (young/adult and rich/poor respectively).

The second analysis – Canonical Correspondence Analysis (CCA) – was carried out at tree level, to study the influence of the main explanatory variables (nutrient status, dendrometric variables and crown conditions) on the occurrence of fungi. A forward selection procedure with the Monte Carlo test was then applied to determine the significance of the results, with 499 permutations for exploratory analysis and 999 for the final results (Legendre and Legendre 1998). The constrained ordinations were performed with CANOCO software for Windows, version 4.5 (Ter Braak and Smiluaer 2002).

3 Results

The values of the dendrometric variables (diameter and height) were considerably higher in rich sites than in poor sites. The opposite was true for crown conditions, since DCT and VCD were higher in poor quality sites than in high quality sites, as expected (Table 1).

The fungal species (recovered from at least two poplar plantations) used in multivariate statistical analyses, as well as their relative isolation frequency (RIF), are shown in Table 2. A total of 43 species or morphological types were isolated from 576 plant fragments (288 plant fragments for each tissue), of which the most frequent were *Ulocladium* spp. and *Cladosporium herbarum* (Pers.) Link. ex S.F.Gray. On the other hand, *Glonium*

Table 1. Site characteristics associated with the stands.

Site quality	Age	Coordinate UTM	Diameter (cm)	Height (m)	DCT (%)	VCD (%)	N (mg g^{-1})	P (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	K (mg g^{-1})	Mn (µg g^{-1})	Fe (µg g^{-1})	B (µg g^{-1})	Zn (µg g^{-1})	Cu (µg g^{-1})	N/P
Rich	Young	357.945–4706.111	16.74	15.08	10.47	0.41	31.60	4.30	30.03	3.20	12.11	379.7	163.0	74.9	68.2	7.1	7.3
Rich	Young	364.479–4694.555	17.09	17.16	10.77	5.77	20.80	4.72	22.64	2.98	16.52	42.0	74.5	98.3	152.7	8.4	4.4
Rich	Young	353.909–4711.072	16.73	13.13	8.75	0	36.20	5.51	20.04	2.29	16.98	230.7	117.9	137.3	86.3	11.7	6.6
Rich	Adult	359.952–4701.961	32.20	24.15	15.97	5	20.70	2.58	46.54	4.13	7.78	376.1	155.6	56.7	101.1	8.0	8.0
Rich	Adult	365.346–4693.152	24.20	23.03	12.65	0	15.90	2.76	23.51	2.34	22.86	114.1	177.7	48.5	110.5	4.7	5.8
Rich	Adult	361.242–4699.292	27.54	22.97	12.09	0	19.20	2.08	39.00	3.33	8.87	67.8	110.4	48.3	28.3	6.5	9.2
Poor	Young	356.735–4706.840	11.04	11.03	9.32	0	16.60	2.50	20.34	2.55	8.50	48.5	75.3	14.5	82.4	4.5	6.6
Poor	Young	353.216–4706.840	13.04	10.61	31.93	20.5	28.40	3.48	27.01	3.47	8.44	354.2	62.6	42.3	89.5	6.2	8.2
Poor	Young	366.495–4692.036	9.71	10.34	13.20	0.13	17.50	1.82	15.23	3.14	3.82	41.3	59.1	10.0	70.2	3.6	9.6
Poor	Adult	355.360–4697.636	12.68	10.57	50.70	19.76	22.50	2.86	45.29	4.38	4.20	576.1	62.5	20.8	78.4	5.1	7.9
Poor	Adult	346.405–4696.185	19.59	16.39	44.62	19.02	17.60	1.89	42.21	4.92	3.04	589.0	52.6	29.5	32.9	4.9	9.3
Poor	Adult	359.336–4691.912	16.67	15.33	36.56	17.54	18.30	2.25	23.95	5.34	5.93	263.2	50.7	29.2	51.3	3.7	8.1

DCT: Digital crown transparency. VCD: Visual crown discoloration

spp., *Pestalotia* spp., *Trichotecium roseum* (Persoon) Link. Es S.F.Gray, and several unidentified Deuteromycetes and sterile mycelia occurred at lower frequencies.

The mixed linear model showed that fungal species richness did not differ significantly between site qualities or between tissues (Table 3), but did differ between ages (richness was higher in young stands than in adult stands). Moreover, the differences in richness between ages depend on the site quality, with the lowest richness values observed in old stands located on poor sites (Age × Site quality: $p = 0.03$; Fig. 1). The same pattern was found for RIF values (Fig. 1).

Correspondence Analysis (CA) performed on the relative frequencies of fungi isolated from leaves only, twigs only or leaves plus twigs revealed similar results, although the grouping of the stands according to age and site quality was clearer for leaves plus twigs. For this reason, in addition to the non significant differences found in the mixed lineal model for the variable 'tissue' (Table 3), the individual CA for leaves and twigs are not shown.

Correspondence Analysis revealed that the principal coordinate axes 1–2, which explained about 42% of the total inertia, separated two distinct clusters of stands according to site quality. Thus, rich stands corresponded to low and high values on axes 1 and 2 respectively, unlike poor quality sites (Fig. 2a). Such groupings associated with site quality are characterised by a clear gradient in the distribution of fungal species; from species exclusively (*Mste16*) or mainly (*Ccla, Mste18*) isolated from poor quality stands, to those exclusively (*Prsp*) or mainly (*Mste1, Mste6, Mste7*) isolated from rich sites (Table 2, Fig. 2a). Two distinct clusters were identified when plots were considered by age (Fig. 2b), although the grouping was not as clear as that observed for site quality. A weak gradient in the distribution of fungal species was also observed according to age; from species mainly isolated from adult stands (*Cher, Deu2, Mste3, Mste13*), to those exclusively (*Hacr, Mste17*) or mainly isolated from young stands (*Apull, Tvir, Mste2*) (Table 2, Fig. 2b). For other CA plots, such as with the first and third or second and third axes, no groupings were observed for the factors studied.

Five variables were retained in the CCA, three

Table 2. Distribution and isolation frequencies for fungi isolated from at least two poplar plantations. The isolation frequencies for each species are the percentages with respect to the total number of fragments collected in each sample tissue (leaves and twigs) and for each treatment (combinations of age (Y: young and A: adult) and site quality (R: rich and P: poor)). The column labelled "Total" refers to the percentages of isolates for each species with respect to the total number of the fragments cultured throughout the sampling.

Fungi	Code	Leaves				Twigs				Total
		YR	AR	YP	AP	YR	AR	YP	AP	
Alternaria alternata complex. Ness ex Fr.	*Acom*	4.2	-	12.5	4.2	11.1	2.8	5.6	2.8	5.4
Aspergillus niger van Tieghem	*Anig*	1.4	-	-	1.4	4.2	-	-	1.4	1.0
Aureobasidium pullulans Viala & Boyer	*Apull*	1.4	-	2.8	1.4	-	-	-	-	0.7
Chaetomium spp.	*Chsp*	-	-	-	-	1.4	1.4	-	-	0.3
Cladosporium cladosporioides Link. ex Fr.	*Ccla*	4.2	-	5.6	2.8	-	1.4	19.4	-	4.2
Cladosporium herbarum (Pers.) Link. ex S.F.Gray	*Cher*	11.1	16.7	16.7	20.8	6.9	-	4.2	11.1	10.9
Epicoccum nigrum Link.	*Enig*	5.6	2.8	2.8	1.4	12.5	4.2	1.4	6.9	4.7
Harcia acremonoides (Harz) Cost.	*Hacr*	4.2	-	2.8	-	4.2	-	-	-	1.4
Penicillium spp.	*Pssp.*	-	2.8	2.8	2.8	1.4	-	1.4	4.2	1.9
Preussia spp.	*Prsp*	1.4	-	-	-	-	1.4	-	-	0.3
Trichoderma viride Pers. Es S.F.Gray	*Tvir*	-	-	1.4	-	6.9	1.4	1.4	2.8	1.7
Ulocladium spp.	*Ussp*	18.1	12.5	12.5	9.7	30.6	26.4	16.7	6.9	16.7
Deuteromicete 1	Deu 1	4.2	1.4	22.2	1.4	4.2	5.6	22.2	8.3	8.7
Deuteromicete 2	Deu 2	-	1.4	1.4	1.4	-	12.5	2.8	6.9	3.3
Sterile mycelium 1	Mste1	11.1	19.4	1.4	-	2.8	9.7	4.2	5.6	6.8
Sterile mycelium 2	Mste2	-	1.4	4.2	1.4	4.2	1.4	2.8	2.8	2.3
Sterile mycelium 3	Mste3	4.2	15.3	6.9	5.6	2.8	1.4	9.7	4.2	6.3
Sterile mycelium 4	Mste4	4.2	6.9	-	4.2	1.4	4.2	1.4	1.4	3.0
Sterile mycelium 5	Mste5	-	1.4	2.8	-	-	-	2.8	-	0.9
Sterile mycelium 6	Mste6	-	4.2	-	-	-	-	1.4	-	0.7
Sterile mycelium 7	Mste7	1.4	1.4	1.4	-	1.4	1.4	-	-	0.9
Sterile mycelium 8	Mste8	4.2	1.4	8.3	1.4	5.6	8.3	-	1.4	3.8
Sterile mycelium 9	Mste9	-	-	1.4	-	-	1.4	-	-	0.3
Sterile mycelium 10	Mste10	-	-	1.4	1.4	-	1.4	1.4	-	0.7
Sterile mycelium 11	Mste11	6.9	6.9	1.4	-	6.9	1.4	1.4	4.2	3.6
Sterile mycelium 12	Mste12	-	2.8	2.8	-	2.8	1.4	-	-	1.2
Sterile mycelium 13	Mste13	2.8	4.2	2.8	4.2	5.6	5.6	6.9	2.8	4.3
Sterile mycelium 14	Mste14	1.4	-	-	-	4.2	1.4	1.4	-	1.0
Sterile mycelium 15	Mste15	1.4	2.8	1.4	-	1.4	2.8	-	-	1.2
Sterile mycelium 16	Mste16	-	-	5.6	2.8	-	-	1.4	-	1.2
Sterile mycelium 17	Mste17	-	-	1.4	-	2.8	-	-	-	0.5
Sterile mycelium 18	Mste18	1.4	-	1.4	-	1.4	-	1.4	-	0.7

associated with the nutrient status of the trees (concentrations of iron and zinc, and relation between concentrations of nitrogen and phosphorus), a dendrometric variable (Total height) and another crown condition variable (Visual Crown Discoloration) (Fig. 3). The eigenvalues (λ) for axes 1 and 2 were 0.208 and 0.123, respectively, and the model was significant according to the results of the Monte Carlo test ($F=1.452$, $p=0.008$, 499 permutations).

The first axis was positively correlated with VCD and concentration of Zn, and negatively with TH and concentration of Fe. Examination of the CCA plot shows several species associated with high VCD or low concentration of Fe and total height values (*Anig*, *Apull, Pssp, Mste16, Mste18*) and vice versa (*Prsp, Mste1, Mste6*). The second axis was positively and negatively correlated with the N/P ratio and concentration of Zn, respectively. The CCA plot revealed that

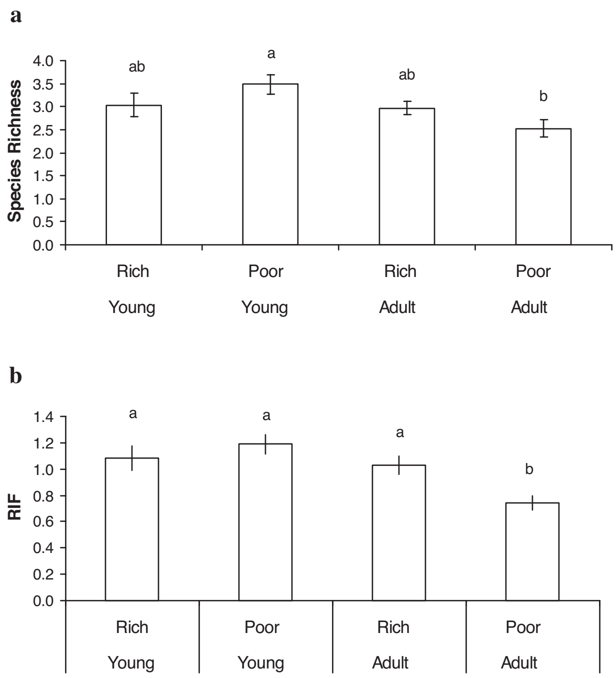

Fig. 1. Mean (± S.E.) (a) Species richness and (b) Relative isolation frequencies (RIF) values per poplar stand for each site quality according to stand age. Different letters above the bars indicate significantly different means (Two-tailed t-test with $\alpha=0.05$).

Table 3. Linear mixed models (PROC MIXED) for mean values of Species richness and Relative isolation frequencies (RIF) per poplar stand (N = 12), used to evaluate the effect of stand age, site quality and tissue.

N = 12		Species richness			RIF	
Source	df1	df2	F	Pr > F	F	Pr > F
Age	1	134	6.17	0.01	11.67	<0.01
Site quality	1	134	0.00	0.99	1.51	0.22
Tissue	1	134	0.43	0.51	0.14	0.71
Age × Site quality	1	134	4.70	0.03	7.14	<0.01
Age × Tissue	1	134	0.15	0.70	0.22	0.64
Site quality × Tissue	1	134	1.01	0.32	0.89	0.35
Age × Site quality × Tissue	1	134	1.01	0.32	2.59	0.11

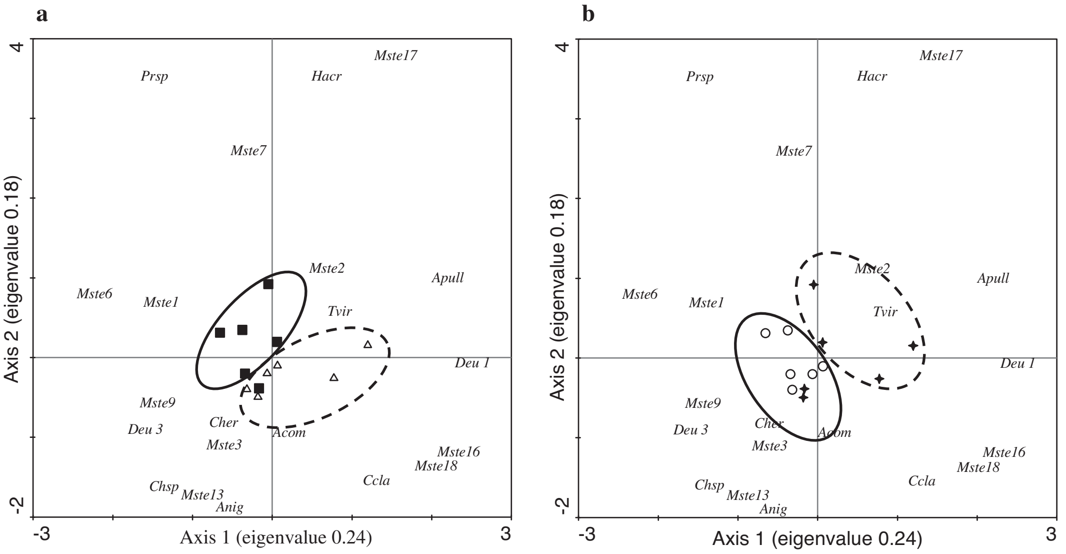

Fig. 2. Correspondence Analysis ordination of the 12 inventoried plots labelled by (a) site quality and (b) age (axes 1 and 2). Plot types: Rich sites (black squares), Poor quality sites (white triangles), Young stands (black stars) and Adult stands (white circles).

several species were associated with high N/P or low concentrations of Zn (*Cher, Hacr, Tvir, Mste15, Mste17*) or vice versa (*Mste5, Mste10, Mste9, Mste14*) (Fig. 3).

4 Discussion

The number of taxa recorded in the present study was similar to the numbers reported in previous surveys on fungal communities associated with other tree hosts under a temperate climate, such as *Populus tremula* (Santamaría and Diez 2005), *Betula pendula* (Green 2004), *Eucalyptus globulus* and *E. grandis* (Bettucci et al. 1999) or several species of pine and oak (Martín-Pinto et al. 2004, Zamora et al. 2008, Botella et al. 2010).

The most abundant species (RIF>3%) observed in the present study are ubiquitous taxa, such as *A. alternata complex*, *C. cladosporoides*, *E. nigrum* and *Ulocladium* sp. The same pattern was also found for *Populus tremula* (Santamaría and Diez 2005), *Salix fragilis* (Petrini and Fisher 1990), *Eucalyptus grandis* (Bettucci and Alonso 1997),

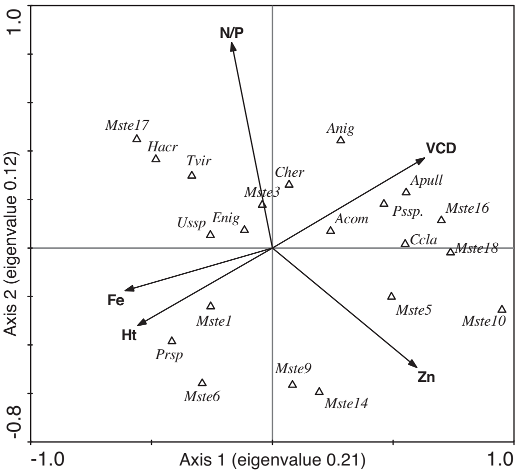

Fig. 3. Canonical Correspondence Analysis ordination biplot (axes 1 and 2), with nutrient status, dendrometric and forest health variables represented by *arrows* and fungal species by triangles. For explanation of abbreviations used for the fungal species, see Table 1.

pine plantations (Zamora et al. 2008) and pine and oak seedlings (Martín-Pinto et al. 2004).

The present findings show that young trees may acquire higher richness and frequency of fungal species than mature trees (especially those in poor sites), contrary to the findings of Kauhanen et al. (2006). There are several possible explanations for this. On one hand, the pruned height of young trees is lower, so that the first branch will be closer to the reservoir of inocula present in the previous year's litter, and therefore fungi may spread relatively rapidly towards the upper third of the canopy. However, multivariate analysis (CCA) showed that pruned height does not affect the endophytic community. On the other hand, several authors (Petrini and Carroll 1981, Helander et al. 1994, Müller and Hallaksela 1998, Collado et al. 1999) indicate that stand density and canopy cover are key factors related to relative humidity, and that these factors may therefore affect the frequency of endophytes in trees. Nevertheless, this does not appear to explain the findings as variables related to canopy cover, such as crown volume and crown transparency, did not have significant effects on endophytic communities, according to the results of the multivariate analysis (CCA). Another hypothesis is that pioneer fungi would quickly colonise young trees and then be replaced over time by more competitive species, as reported by Minter and Millar (1980), who found that *Lophodermium pinastri* replaced other fungi. This appears even more likely when it is taken into account that poplar plantations are subjected to clear cutting, which may eliminate the transmission of inocula of endophytic fungi, as noted by Kriel et al. (2000).

Although several authors have pointed out the importance of edaphoclimatic variables in the development of endophytic communities (Carroll 1994, Sieber et al. 1999, Botella et al. 2010), to our knowledge no specific research has been carried out to study the effect of site quality on fungal assemblages. Korkama et al. (2006) demonstrated that growth rate and size of the host affect the diversity and community structure of ectomycorrhizal species. However, these authors compared eight Norway spruce clones, and therefore could not differentiate between effect of the clone and site quality.

Separation of stands of different site quality according to the associated fungal assemblages has been demonstrated in the present study at clone level (removing the genetic effect of tree host). This may be due to a stress factor caused by nutrient or water deficits in poor quality sites, which appears to be supported by the results of multivariate analysis (CCA), since discoloration, total height and the concentrations of several nutrients were shown to be key variables affecting endophytic communities. It is possible that some endophyte species, such as *Periconiella* spp. (Collado et al. 1999) and *Cytospora* spp. (Bettucci and Alonso 1997, Callan 1998), require trees to be exposed to stress conditions before colonisation.

Although for culturable and sporulating mycelia, identification based on morphology may be of interest, because of the limited number of sequences reported (Kauhanen et al. 2006), the large number of sterile mycelia observed in the present study indicates that sequence-based identification would be advisable in future investigations involving identification of fungal endophytes in poplar plantations. However, taking into account that many fungi (possibly hundreds of thousands) have not yet been classified (Hawksworth and Rossman 1987, Sieber 2007), it would not be surprising if some new species were isolated from *P.* × *euramericana* in the present study.

In conclusion, the present results indicate that several endophytes colonise poplar plantations and that factors such as cutting cycle, selection of land according to site quality or possible fertilization regimes will affect endophytic fungi. These outcomes may be of great interest, not only because of the importance of endophytes as a source of ecological diversity, but also because of their enormous potential as indicators of forest health, owing to their role in acting against forest pests and diseases.

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ARTICLE VI

Martín-García, J., Müller, M. & Diez, J.J. (2012) ITS-based comparison of endophytic mycota in twigs of native *Populus nigra* and cultivated *P. x euramericana* (cv. I214) stands in Northern Spain. *Annals of Forest Science* 69: 49-57.

RESUMEN

Comparación de la comunidad endofítica en ramillos de un chopo autóctono (*Populus nigra*) y un clon híbrido (*P. x euramericana*) basado en la región ITS del rDNA

El álamo negro (*Populus nigra*) es una de los árboles autóctonos más amenazadas en Europa. Por el contrario, la superficie de plantaciones monoclonales de chopos híbridos está incrementándose notablemente debido a su alta rentabilidad económica. El objetivo de este estudio es describir y comparar las comunidades de hongos endófitos en ramillos de las choperas autóctonas (*P. nigra*) y las plantaciones monoclonales (*P. x euramericana*, clon I-214). Los hongos endófitos de ramillos de tres choperas autóctonas y de tres plantaciones monoclonales de Palencia (Norte de España) fueron aislados. Tres árboles fueron elegidos aleatoriamente dentro de cada parcela muestreada. Los aislamientos fueron identificados a partir de las secuencias de la región ITS de su rDNA. El estudio se centró en las especies dominantes. Un total de 13 especies fueron encontradas. Seis de las cuales fueron exclusivas de *P. nigra* (*Stemphylium vesicarium*, *Alternaria* spp., *Aureobasidium pullulans*, *Fimetariella rabenhorstii*, *Plagiostoma fraxini* y *Cryptodiaporthe salicella*) y cuatro de *P. x euramericana* (*Cytospora chrysosperma*, *Athelia bombacina*, *Biscogniauxia mediterranea* y una especie no identificada), mientras que tres fueron encontradas en los dos tipos de árboles (*Lewia infectoria*, *Alternaria alternata* y *Epicoccum nigrum*). Así, los resultados sugieren que la comunidad de hongos endófitos en ramillos varía entre el chopo autóctono y el híbrido.

Palabras clave: Chopera, autóctono, híbrido, endófito, plantación

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ORIGINAL PAPER

ITS-based comparison of endophytic mycota in twigs of native *Populus nigra* and cultivated *P. x euramericana* (cv. I-214) stands in Northern Spain

Jorge Martín-García · Michael M. Müller · Julio Javier Diez

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Abstract
· ***Context*** The European black poplar (*Populus nigra*) is considered one of the most seriously endangered indigenous tree species. However, the total area covered by monoclonal plantations of hybrid poplar is growing, because of the high profitability of these trees.
· ***Aim*** The aim of this study is to describe and compare the endophytic mycota in twigs from native poplar (*P. nigra*) and hybrid poplar plantations (*P. x euramericana* clone I-214).
· ***Methods*** Twig endophytes were isolated from three native and three hybrid poplar stands in Palencia (N. Spain). Three trees were chosen at random within each stand sampled. Isolates were identified according to sequences of the internal transcribed spacer region of their rDNA. The study focused on dominant species.
· ***Results*** A total of 13 species were found. Six species originated from *P. nigra* only (*Stemphylium vesicarium*, *Alternaria* spp., *Aureobasidium pullulans*, *Fimetariella rabenhorstii*, *Plagiostoma fraxini* and *Cryptodiaporthe salicella*) and four from *P. x euramericana* only (*Cytospora chrysosperma*, *Athelia bombacina*, *Biscogniauxia mediterranea* and Unidentified sp. 1), whereas three were found on both types of trees (*Lewia infectoria*, *Alternaria alternata* and *Epicoccum nigrum*).
· ***Conclusion*** The results show that the endophyte community on poplar twigs differs between native and hybrid poplars

Keywords Native · Hybrid · Poplar · Endophyte · Twig

1 Introduction

Populus nigra L., the European black poplar, is a dioecious, heliophilous, pioneer tree species in alluvial forests and considered a keystone species in floodplain forests in Europe. Although *P. nigra* is widely distributed throughout Europe, it is one of the most seriously endangered indigenous forest tree species and is threatened by three main factors. Firstly, hydraulic structures that regulate flood periods have been modified to favour hardwood species. Secondly, *P. nigra* has been replaced by hybrid poplars because of the higher profitability of the latter. Finally, gene introgression from cultivated poplars is a potential threat to *P. nigra*, as few clones are extensively cultivated, and they contribute to a large proportion of pollen and seed pools (Lefèvre et al. 1998; Smulders et al. 2008).

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Contribution of the co-authors Jorge Martín-García: designing the experiment, recollecting samples, working in laboratory (sample treatment and molecular identification of isolates), running the data analysis and writing the paper.
Michael M. Müller: designing the experiment, reviewing the paper and supervising the work.
Julio Javier Diez: designing the experiment, reviewing the paper and coordinating the research project

J. Martín-García · J. J. Diez
Sustainable Forest Management Research Institute,
University of Valladolid - INIA,
Avenida Madrid, 57,
34004 Palencia, Spain

J. J. Diez
e-mail: jdcasero@pvs.uva.es

J. Martín-García (✉)
Forestry Engineering, University of Extremadura,
Avenida Virgen del Puerto 2,
10600 Plasencia, Spain
e-mail: jorgemg@pvs.uva.es

M. M. Müller
The Finnish Forest Research Institute,
P.O. Box 18, 01301 Vantaa, Finland
e-mail: michael.mueller@metla.fi

Plantations of hybrid poplar are of great interest in Spain, because of their economic value as a source of plywood. The profits associated with cultivated poplar reach between 1,200 and 2,400 €/ha/year for highly favourable sites (Díaz and Romero 2001). Single plantations are monoclonal and although several hybrids are used in Spain, *Populus x euramericana* (Dode) Guinier clone I-214 (*P. deltoides* Marsh. ♀ × *P. nigra* L. ♂) is the most common. It covers about 70% of the total area covered by poplar plantations (Fernández and Hernanz 2004).

A current goal of national and regional forest policy is to increase the area occupied by poplar plantations. Castilla and León, the region with the largest area covered by poplar plantations in Spain (ca. two thirds of the total), has drawn up a first draft of a "strategy for poplar growing", the main objective of which is to double the area covered by poplar plantations in the next two decades.

In light of this, a trade-off between economic benefits and the maintenance of biological and functional diversity in poplars is necessary.

Decreased cover by *P. nigra* through replacement with *P x euramericana* or a possible drastic decrease in genetic variation through intensive introgression would probably also cause indirect effects on other organisms associated with *P. nigra*. However, while great importance is given to macroscopic life forms, little attention is paid to the potential consequences of the loss of microbial diversity (Strobel 2003). As plants are known to harbour host-specific microorganisms, disappearance of a plant species also results in the disappearance of these microorganisms. This is not desirable because e.g. the production of novel and biologically active secondary metabolites is common among fungal endophytes; therefore, this group of fungi is a potential source of new products for pharmaceutical and agrochemical industries. Transformation of native poplar stands into monoclonal plantations or hybrid zones appears to alter pre-existing fungal communities (Whitham et al. 1999). A trend towards decreased genetic variation in forestry may increase the phytosanitary risk because monoclonal forests are known to be more vulnerable to diseases than native forests (Pinon 1984).

Many definitions of the term endophyte have been used in the scientific literature. The term is often used to refer to fungi able to infect their hosts without causing visible disease symptoms (Schulz and Boyle 2005). In the present study, the term endophyte is used to refer to fungi isolated from healthy-looking surface-sterilized plant samples.

The aim of this study was to describe and compare the endophytic mycota on twigs of native poplar (*P. nigra*) and hybrid poplar trees (*P. x euramericana* clone I-214) in Spain.

2 Material and methods

2.1 Description of the study site and sampling

Three poplar plantations of clone I-214 and three native poplar stands (*P. nigra*) were chosen for study, in Palencia (N. Spain). The altitude, edaphic and climatic characteristics of all stands were similar (Table 1), and stands were situated at a maximum distance of 60 km from each other (Fig. 1). The poplar plantations (*P. x euramericana*, clone I-214) were 10-year-old monoclonal plantations established in 1995 by the Government of the Castile and Leon, whereas stands of native poplar (*P. nigra*) exist as remnants patches.

In October 2006, three trees were randomly chosen within each sampling stand. Native poplars of breast height diameter (10–20 cm) similar to those of the hybrid poplar sampling sites were selected for sampling. Four healthy branches from four horizontal sectors of the upper third of the canopy of each tree were collected. The branches were stored at 4°C and processed within 24 h.

2.2 Sample treatment

Four pieces of twig (1 year old, diam. 0.5 cm, length 5 cm) were cut from each branch and surface sterilized by: shaking in 70% ethanol (*v*/*v*) for 1 min, followed by shaking in 4% sodium hypochlorite (*v*/*v*) for 4 min, thereafter shaking in 70% ethanol (*v*/*v*) for 1 min and finally dipping for a few seconds in 70% ethanol (*v*/*v*). The samples were then dried for 1–5 min in a sterile laminar flow cabinet in open Petri dishes. Each twig piece was split into four longitudinal segments and placed in serial order on Petri dishes containing "potato dextrose agar" (PDA) medium (Scharlau product, Barcelona, Spain, prepared

Table 1 Edaphic and climatic characteristics of the stands

Stand	Altitude (m)	Annual precipitation[a] (mm)	Mean annual temperature[a] (°C)	Soil type[b]
Pn 1	800	429	11	Alluvial
Pn 2	800	467	11	Alluvial
Pn 3	996	637	10	Alluvial
Px 1	900	596	11	Alluvial
Px 2	1000	687	10	Alluvial
Px 3	859	535	11	Fluvial terraces

Px P. x euramericana, *Pn P. nigra*

[a] According to "Atlas Climático Digital de la Península Ibérica" (Ninyerola et al. 2005)

[b] According to "Mapa geológico y Minero de Castilla y León" (SIEMCALSA 1997)

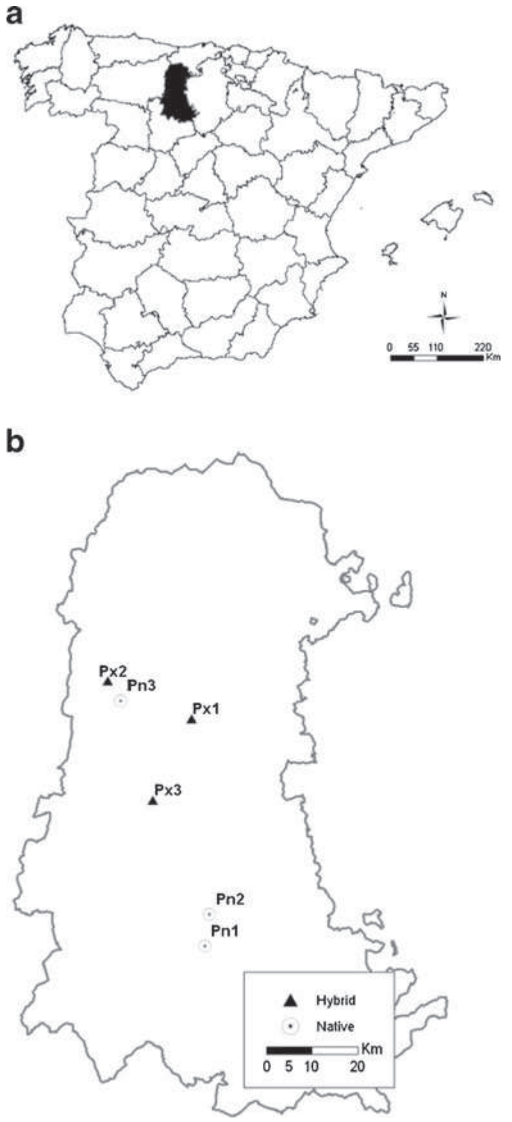


Fig. 1 Geographical location of the six sites surveyed. **a** Geographic location in Spain; **b** geographic location in the province of Palencia

according to the manufacturer's instructions). The plates were sealed with Parafilm® and incubated in darkness at 22°C for 3 weeks. One outgrowing colony from each branch was chosen according to the serial order of the Petri dish and transferred to a new Petri dish with PDA medium. Four isolates per sampled tree were obtained to provide 36 isolates per treatment/species (i.e. four isolates/tree × three trees/stand × three stands=36), with two types of poplar (i.e. a total of 72 isolates).

Obviously, the total diversity of endophytes cannot be measured and quantified comprehensively so that we decided to focus on dominant species to achieve a trade-off between ecological rigour and economic efficiency. A sample size of 36 isolates per population allows detection (at $p>0.95$) of a species occurring with a frequency of at least 0.08 [$p=1-(1-f)^n$, where $f=$ frequency and $n=$sample size].

2.3 Molecular identification of isolates

To obtain agar-free mycelial samples for DNA isolation, the isolates were cultivated on agar plates and covered with Cellophane (Surface Specialities, Wigton Cumbria, UK). MOS agar was used to allow fast growth (Müller et al. 1994). Total DNA was isolated according to Vainio et al. (1998). The protocols included cell disruption with quartz sand and a FastPrep® cell disrupter (Qbiogene, Inc., Cedex, France) for 2×20 s at 4 $\mathrm{m s^{-1}}$, two phenol/chloroform/isoamyl alcohol (50:49:1) and one chloroform/isoamyl alcohol (24:1) extraction, precipitation with polyethylene glycol and drying. DNA was resuspended in 50 μl of TE-buffer (pH 8.0), containing 10 mM Tris–HCl and 1 mM ethylenediaminetetraacetic acid. This procedure was adequate for most isolates, although for some isolates, up to four extractions in phenol/chloroform/isoamyl alcohol (50:49:1) were required to remove visible pigment from the extract.

The internal transcribed spacer (ITS) region of the rDNA was amplified with primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). All amplifications were performed according to White et al. (1990) and Vainio and Hantula (2000).

PCR products were purified with the High Pure PCR Product Purification Kit (Roche, Mannheim, Germany), according to the manufacturer's instructions. The sequences were determined in an automated sequencing apparatus (Li-Cor Global Edition IR^2 system; Li-Cor Inc., Lincoln, NE, USA), by following the manufacturer's instructions. DNA fragments were sequenced in both directions at the same time. Finally, the sequences were aligned with LI-COR software (ALIGN IR ver. 2.0). Consensus sequences were identified by comparing them with sequences deposited in GenBank (NCBI) database, by means of the BLAST algorithm. The isolates were named according to the best GenBank match if at least 97% matched at least 401 bp.

Sequences generated in this study have been submitted to GenBank under accession number FR648327–FR648398.

2.4 Statistical analyses

Differences in species richness and diversity between native (*P. nigra*) and hybrid poplars (*P. x euramericana*) were evaluated by Mann–Whitney *U* tests (nonparametric tests). Diversity was estimated by use of the Shannon index:

$$H=-\sum_{i=1}^{S} p_i \log p_i$$

where H is the number of species and p_i is proportion of the ith species in a sample.

Canonical correspondence analyses (CCA) were carried out to test whether endophyte assemblages were different between native and hybrid poplars. For this, type of poplar was considered as environmental data (nominal variables), and frequency of species was transformed by log (x+1). The statistical significance was tested by means of a Monte Carlo permutation test with 499 permutations of total inertia.

Univariate analyses were performed with the Statistical 6.0 package (StatSoft 2001) and multivariate analyses with CANOCO 4 (Ter Braak and Šmilauer 2002).

Richness and diversity accumulation curves were developed with EstimateS software, version 8.2 (Colwell 2005).

Phylogenetic and molecular evolutionary analyses were conducted with MEGA, version 4 (Molecular Evolutionary Genetics Analysis) (Tamura et al. 2007).

3 Results

At least one isolate was obtained from all twig samples. The ITS sequences obtained varied from 401 to 548 bp in length, including the complete or partial ITS1, complete 5.8 S ribosomal RNA gene and complete or partial ITS2. Two of the 72 sequences did not provide an accepted match with any sequence in the GenBank (min. 97% similarity of at least 401 bp sequence length). A total of 13 species were found. Nine species originated from *P. nigra*, seven from *P. x euramericana* and three [(*Lewia infectoria* (Fuckel) M.E. Barr & E.G. Simmons (anamorph: *Alternaria infectoria* E. G. Simmons), *Alternaria alternata* (Fr.) Keissl. and *Epicoccum nigrum* Link)] were found on both types of trees (Table 2). Although *A. alternata* was more frequent on *P. nigra* ($p<0.03$; Fisher's exact test), it was also found on one hybrid tree.

Species found only on samples of *P. nigra* were: *Alternaria* spp., *Aureobasidium pullulans* (de Bary) G. Arnaud, *Cryptodiaporthe salicella* (Fr.) Petr., *Fimetariella rabenhorstii* (Niessl) N. Lundq., *Plagiostoma fraxini* (Redlin & Stack) Sogonov and *Stemphylium vesicarium* (Wallr.) E.G. Simmons. On the other hand, *Athelia bombacina* Pers., *Biscogniauxia mediterranea* (De Not.) Kuntze, *Cytospora chrysosperma* (Pers.: Fr.) Fr. (teleomorph: *Valsa sordida* Nitschke) and an unidentified species were only found on *P x euramericana*. *C. chrysosperma* was isolated from seven out of nine hybrid poplar trees sampled; this frequency was significantly higher than the complete absence on *P. nigra* ($p<0.01$, Table 2).

A phylogenetic analysis, based on the ITS sequences (Fig. 2), showed that all clusters including isolates from only one of the two types of poplar stand (e.g. *C. chrysosperma, A. pullulans* and *F. rabenhorstii*) differed greatly from the next nearby cluster with isolates from the other poplar type. For instance, the mismatch between isolates Pn3_1_2 (*C. chrysosperma* from hybrid poplar) and Px3_2_1 (*C. salicella* from *P. nigra*) of two nearby clusters was 67 bp of a 367-bp long sequence in common (i.e. 18%).

Species richness and diversity, as expressed by the Shannon index of isolates from native poplar, were higher than those for isolates obtained from hybrid poplar (Table 2), although the differences were not significant ($p=0.10$ and $p=0.07$, respectively).

The logarithmic equation provided a good explanation for the richness and diversity accumulation curves (Fig. 3). The logarithmic equations (Fig. 3) predict a low increase in number of species with increasing number of trees sampled. For instance, for native and hybrid poplars, 16 and 12 species, respectively, would have been isolated from a sample of 100 trees, whereas we found nine and seven species from nine trees, respectively.

A CCA showed that fungal assemblages differed according to type of poplar (Fig. 4). The result of the Monte Carlo test, with 499 permutations, was significant ($p<0.01$), and CCA showed that 17.4% of the total variation in species data could be explained by type of poplar. An ordination diagram displayed species exclusively isolated from native (*Alternaria* spp., *A. pullulans*, *C. salicella*, *F. rabenhorstii*, *P. fraxini* and *S. vesicarium*) or hybrid poplar (*A. bombacina*, *B. mediterranea*, *C. chrysosperma* and Unidentified *1*). Furthermore, *A. alternata*, which was isolated from native and hybrid poplar, was more closely associated with native poplars.

4 Discussion

The results of the study show that the endophyte community of poplar twigs differs between native *P. nigra* and clone I-214, as only three out of 13 observed species were found on both poplars and the frequency of two species differed significantly (Table 2). Identification of the isolates in this study is based solely on DNA sequence blast results in GenBank and therefore must be regarded as tentative.

The sampling intensity used in the study is too low to reveal specific endophyte species for *P. nigra* from among species occurring at low frequency. As already mentioned, the present study therefore concentrates on species occurring at high frequencies, i.e. dominant species.

The cumulative curves for species richness and diversity (Fig. 3) show that the number of trees sampled in this study provided a reasonably good indication of twig endophyte richness and diversity of the two types of poplar in the stands under study. The diversity indices would not have

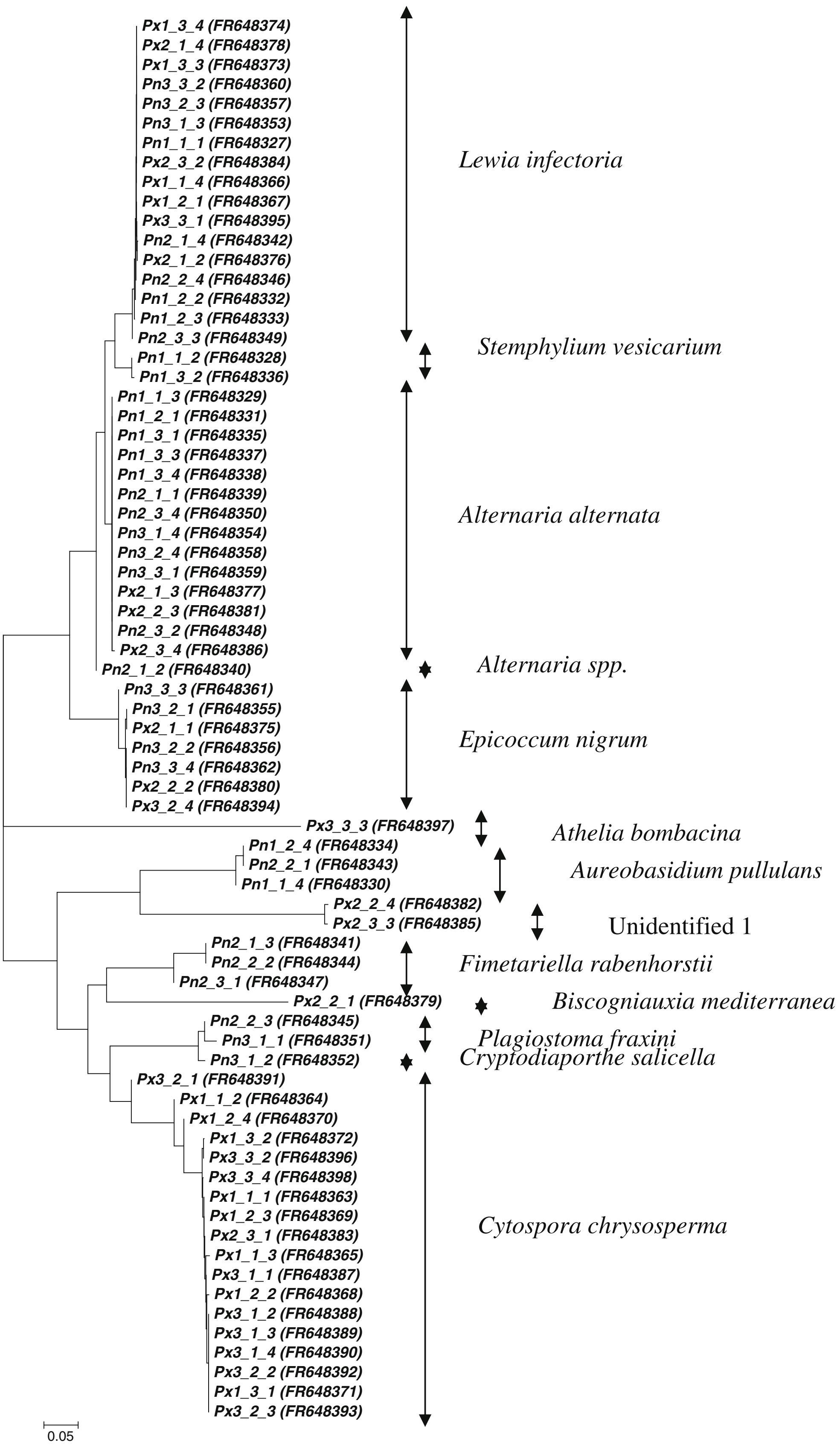
Table 2 Distribution and relative abundance of the fungal species isolated in the study

Accession no.	Closest GenBank taxa	Code	Similarity (%)	Relative abundance (%)[a]							
				Populus nigra				*P. x euramericana*			
				Pn1	Pn2	Pn3	Mean	Px1	Px2	Px3	Mean
FR648329, FR648331, FR648335, FR648337, FR648338, FR648339, FR648348, FR648350, FR648354, FR648358, FR648359, FR648377, FR648381, FR648386	*Alternaria alternata*	Aalt	99–100	33.3	25	25	27.8±28a	0	25	0	8.3±8.3b
FR648340	Alternaria spp.	Aspp	100	0	8.3	0	2.8±2.8	0	0	0	0
FR648397	*Athelia bombacina*	Abom	99	0	0	0	0	0	0	8.3	2.8±2.8
FR648330, FR648334, FR648343	*Aureobasidium pullulans*	Apul	100	16.7	8.3	0	8.3±4.8	0	0	0	0
FR648379	*Biscogniauxia mediterranea*	Bmed	100	0	0	0	0	0	8.3	0	2.8±2.8
FR648352	*Cryptodiaporthe salicella*	Csal	99	0	0	8.3	2.8±2.8	0	0	0	0
FR648363, FR648364, FR648365, FR648368, FR648369, FR648370, FR648371, FR648372, FR648383, FR648387, FR648388, FR648389, FR648390, FR648391, FR648392, FR648393, FR648396, FR648398	*Cytospora chrysosperma*	Cchr	99–100	0	0	0	0a	66.7	8.3	75	50±21b
FR648355, FR648356, FR648361, FR648362, FR648375, FR648380, FR648394	*Epicoccum nigrum*	Enig	99–100	0	0	33.3	11.1±11.1	0	16.7	8.3	8.6±4.8
FR648341, FR648344, FR648347	*Fimetariella rabenhorstii*	Frab	98-99	0	25	0	8.3±8.3	0	0	0	0
FR648345, FR648351	*Plagiostoma fraxini*	Pfra	97	0	8.3	8.3	5.6±2.8	0	0	0	0
FR648327, FR648332, FR648333, FR648342, FR648346, FR648349, FR648353, FR648357, FR648360, FR648366, FR648367, FR648373, FR648374, FR648376, FR648378, FR648384, FR648395	*Lewia infectoria*	Linf	99 - 100	25	25	25	25±0	33.3	25	8.3	22.2±7.3
FR648328, FR648336	*Stemphylium vesicarium*	Sver	99	16.7	0	0	5.6±5.6	0	0	0	0
FR648382, FR648385	Unidentified sp. 1	U1		0	0	0	0	0	16.7	0	5.6±5.6
Species richness				3.00	3.67	3.33	3.33±0.19	2.00	3.67	2.00	2.56±0.56
Shannon–Weaver index				0.996	1.271	1.271	1.179±0.09	0.606	1.271	0.534	0.804±0.23

Mean values with different superscript letter—within the same endophyte—differ significantly at $p<0.05$ (Fishers exact test)

[a] 100% corresponds to isolation of a species from all 12 branches sampled from a stand

Fig. 2 Neighbour-joining tree generated from ITS sequences of 72 isolates. Codes at the ends of branches correspond to host (*Px P. x euramericana* or *Pn P. nigra*), number of stand, number of tree and number of isolate. Accession numbers are shown in *brackets*



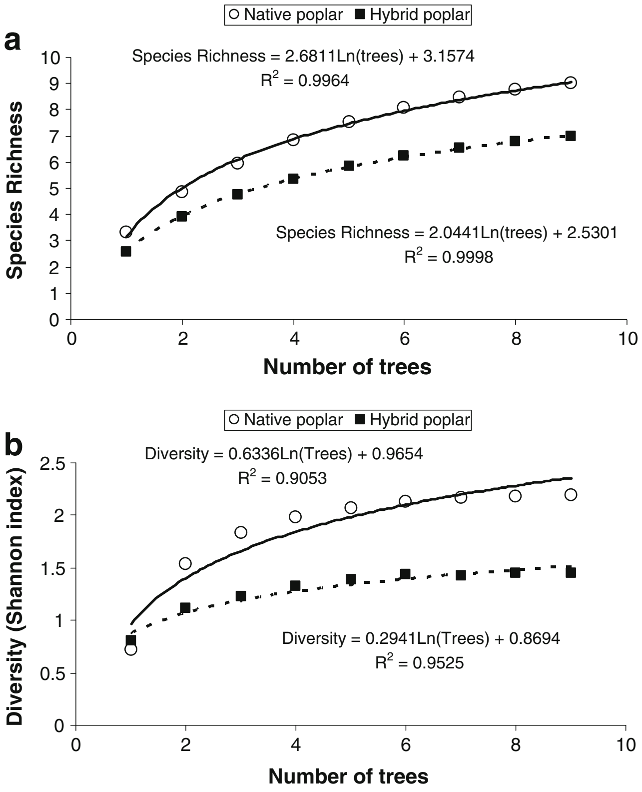


Fig. 3 **a** Richness and **b** diversity accumulation curves for native and hybrid poplars

changed greatly even if many more samples had been processed. A greater number of sample trees would probably have revealed more occasional and rare species but not the predominant specific endophyte species on poplar.

Several most common species found on *P. nigra* (*A. alternata, E. nigrum* and *L. infectoria*) were also found on hybrid clone I-214 (Table 2 and Fig. 2), which all can be classified as generalist species (Schulz and Boyle 2005). However, *C. chrysosperma*, the most common endophyte of clone I-214 was absent from *P. nigra*.

The community structure of twig endophytes of the native *P. nigra* and clone I-214 differed greatly according to a Monte-Carlo test and are clearly visualized by the canonical correspondence analysis (Fig. 4). This general difference is noteworthy as clone I-214 is a 50% descendant of *P. nigra*. In previous studies, distinct differences between endophytic communities were found on comparison of different tree species (Fisher and Petrini 1990). Significant differences in the community structure of ectomycorrhizal fungi in various clones of Norway spruce have also been reported (Korkama et al. 2006).

A. pullulans was only isolated from *P. nigra*, even though it is also considered as a generalist species. This endophyte has previously been isolated from *P. x euramericana* (clone I-214) (Martín-García et al. 2011), so that it is likely that it would have been found on *P. x euramericana* in this study if a larger number of samples had been examined.

Two of the species found on native poplars (*F. rabenhorstii* and *S. vesicarium*) have not previously been reported from *P. x euramericana* (Callan 1998; Ginns 1986; Martín-García et al. 2011), *Populus tremula* (Santamaría and Diez 2005) or genera that share the same forest habitat such as *Salix* sp. (Petrini and Fisher 1990) and *Alnus* sp. (Fisher and Petrini 1990). Furthermore, *C. salicella* appears to be a common species in riparian habitats, since it has been isolated from *Salix* sp. (Ginns 1986), *Alnus* sp. (Ginns 1986), *Populus tremuloides* (Callan 1998; Ginns 1986), *P. trichocarpa* (Callan 1998; Ginns 1986) and *P. canadensis* (*P. deltoides x nigra x eugenei*) (Ginns 1986). *P. fraxini* is apparently not specific to the genera *Fraxinus* sp. and *Chionanthus* sp., as earlier suggested (Sogonov et al. 2008).

Species isolated from hybrid poplars, except *A. bombacina*, have previously been isolated from other tree species. Thus,

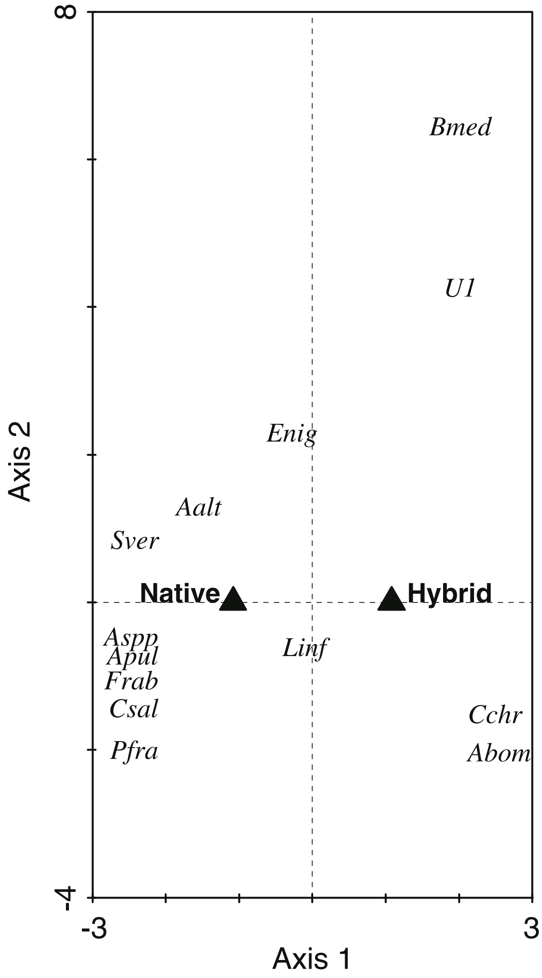


Fig. 4 Canonical correspondence analysis ordination biplot (axes 1 and 2) of fungal species and type of poplar. See Table 1 for species abbreviations

C. chrysosperma was isolated from *Populus tremuloides* (Callan 1998), *P. tremula* (Santamaría and Diez 2005), *P. balsamifera* (Callan 1998), *P. trichocarpa* (Callan 1998), *Populus* hybrid (Callan 1998) and *Eucalyptus grandis* (Bettucci and Alonso 1997). Furthermore, Petrini and Fisher (1990) found *Cytospora* sp. colonising *Salix fragilis*. Moreover, *B. mediterranea* is regarded as a ubiquitous species as it has been isolated from numerous other tree genera (Nungent et al. 2005).

Most of the species detected are probably "true endophytes", as they have not been reported from plant tissues with visible disease symptoms. However, three species (*S. vesicarium*, *C. chrysosperma* and *B. mediterranea*) can also be regarded as weak pathogens detected during latency. *S. vesicarium* is known as a pathogen able to cause severe damage to pear (Rossi and Pattori 2009) and onion (Aveling and Snyman 1993) amongst other species but has not previously been reported from poplar. *B. mediterranea* is generally known to occur in healthy living trees as an endophyte, occasionally becoming invasive under water stress conditions (Nungent et al. 2005). *C. chrysosperma* is known to be a typical weak pathogen of poplars and is widely distributed throughout the northern hemisphere and Australia, although it is able to cause severe damage under certain conditions (Worrall et al. 2008).

The occurrence of *C. chrysosperma* on only hybrid poplars can be explained by the origin of the hybrid poplar seedlings or different susceptibility of the poplar types to this weak pathogen or endophyte. The hybrid poplars in each sampled stand probably originated from cuttings of a single *ortet*, which may have been infested by *Cytospora*, and it is therefore possible that all cuttings used in the plantation already carried this fungus as a latent infection. On the other hand, hybrid poplar may be more susceptible than native poplar, because of the hybrid susceptibility pattern (Fritz 1999). It is well known that poplar species and even clones differ in susceptibility to attacks of various fungal species. For instance *P. trichocarpa* is more susceptible to *C. chrysosperma* than *P. x canadensis* (syn. *P. x euramericana*) (Bloomberg 1962a,b), and Alonso et al. (2000) noted that the susceptibility of *P. x euramericana* to *C. chrysosperma* varies according to the clone.

P. x euramericana can be considered as a partly exotic species because *P. deltoides*, the other parent species, originates from North America. The hybrid poplar may therefore carry resistance factors (genes), which are new to the fungal flora in Spain. This may explain some of the observed differences in the twig endophyte assemblages between native and hybrid poplars. The hybrid poplar is known to grow considerably faster than the native poplar, which is the main reason for its popularity among foresters. The speed of growth may affect the mycobiota associated with the tree, as shown by Korkama et al. (2006) in studies on the mycorrhizal diversity of various spruce clones. The results of the latter study showed significant differences between the ectomycorrhizal diversity of slow and fast growing Norway spruce clones.

Edaphic, climatic and other site-specific factors are not likely causes of differences in the observed structure of endophyte species on the two poplar types, as the characteristics of all stands were similar.

The present results show that the structure of endophytic species found on *P. nigra* twigs differed from that found on twigs the hybrid poplar *P x euramericana*, but that all of the three most common species observed on *P. nigra* were also found on the hybrid clone. These results should be considered as preliminary findings, and further studies on leaf, wood and root endophytes as well as on mycorrhizal species should be carried out to evaluate the risk of loss of area covered by *P. nigra* through replacement with *P x euramericana* or a possible drastic decrease in genetic variation through intensive introgression.

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ARTICLE VII

Martín-García, Barbaro, L., Diez, J.J. & Jactel, H. (2012) Contribution of poplar plantations to bird conservation in riparian landscapes. (In prep. to be submitted to *Biodiversity and Conservation*).

RESUMEN

Contribución de las plantaciones de chopo a la conservación de las aves en los paisajes riparios

Los bosques de ribera son un ecotono especialmente importante para la biodiversidad, especialmente en los ambientes mediterráneos donde el marcado gradiente de humedad determina los procesos ecológicos y provee a la fauna de corredores. Sin embargo, la vegetación autóctona de las zonas riparias ha sido tradicionalmente modificada o eliminada por las actividades humanas a una velocidad alarmante durante las últimas cinco décadas. El objetivo de este estudio fue investigar la influencia de las plantaciones de chopo sobre la diversidad de aves en los paisajes riparios, tanto a una escala local como a nivel de paisaje, para estimar las implicaciones ecológicas de un incremento de la superficie destinada a plantaciones de chopo. La avifauna fue censada en veinticuatro plantaciones de chopo (*Populus x euramericana*, clon I-214), seleccionadas según un diseño factorial combinando edad y selvicultura aplicada al sotobosque. Además, las únicas tres parcelas de bosques de ribera encontradas en el área de estudio fueron también muestreadas para comparar las comunidad de aves de bosques naturales de ribera y plantaciones de chopo. Las variables explicativas fueron (1) dendrométricas, (2) de sotobosque y (3) de paisaje en seis diferentes radios circulares (100, 200, 300, 400, 500 y 1000 m). Los bosques de ribera presentaron una mayor riqueza de especies y a una mayor frecuencia que las plantaciones de chopo. Además, mientras que los bosques de ribera albergaron una avifauna típica de riberas bien preservadas, las plantaciones de chopo hospedaron principalmente especies de aves generalistas. Un efecto notable del paisaje fue encontrado en las plantaciones de chopo, en particular el porcentaje de otras plantaciones de chopo circundantes (radio circular de 400 m) fue la variable más influyente en la diversidad de aves. Por otro lado, a nivel de parcela, la cobertura de sotobosque fue también un factor clave para las comunidades de aves. Por lo tanto, las plantaciones de chopo no deberían ser utilizadas como sustitutas de los bosques de ribera. Si bien, una adecuada gestión selvícola a nivel de parcela (minimizando el número de gradeos) y sobre el paisaje (manteniendo un alto porcentaje de plantaciones de chopo con una distancia entre ellas menor de 400 m) sería deseable desde un punto de vista de la diversidad de aves.

Palabras clave: *Populus x euramericana*, clon I-214, híbrido, autóctono, híbrido, gestión selvícola.

CONTRIBUTION OF POPLAR PLANTATIONS TO BIRD CONSERVATION IN RIPARIAN LANDSCAPES

Jorge MARTÍN-GARCÍA[1], Luc BARBARO[2], Julio J. DIEZ[1], Hervé JACTEL[2]

[1] Sustainable Forest Management Research Institute, University of Valladolid – INIA. Palencia. Spain. Avenida Madrid, 57. 34004 Palencia, Spain.

[2] INRA, UMR 1202 BIOGECO, Laboratory of Forest Entomology & Biodiversity, 69 Route d'Arcachon, F-33612 Cestas cedex, France.

Abstract

- **Context:** Riparian zones are the interfaces between terrestrial and aquatic environments. In Mediterranean area this ecotone is particularly important to biodiversity, due to sharp gradients of moisture that determine ecological processes but also because they provide wildlife corridors. Nevertheless, virgin vegetation of riparian zones have been traditionally modified or lost by human activities at an alarming rate during the past five decades.
- **Aim:** The main objective of this study was to investigate the influence of poplar plantations on bird diversity in riparian zones at both local and landscape scales in order to estimate the ecological implications of a substantial increase in the cover of poplar plantations.
- **Methods:** Breeding birds were sampled by the point-count method in twenty-four poplar plantations of I-214 clone, according to a factorial design combining stand age and understory management. Furthermore, the only three native riparian forests found in the study area were also surveyed to compare bird assemblages between poplar plantations and native forests. Explanatory variables consisted of (1) dendrometric variables, (2) understory variables and (3) landscape variables at six different radii of circular buffers (100, 200, 300, 400, 500 and 1000 m).
- **Results:** Riparian forests showed higher number of species and total abundance than poplar plantations. Moreover, while riparian forests were associated with specific typical bird species of well preserved riparian forest areas, poplar plantations hosted mainly generalist species. A prominent influence of landscape variables on bird diversity was found in poplar plantations. In particular, percentage of poplar plantations in the surrounding landscape (buffer radius of 400 m) was the variable most important to explain bird diversity. Furthermore, local scale, and particularly understory cover, was also a key factor influences for bird assemblages.
- **Conclusion:** Poplar plantations should not be used as surrogates of native forests and a suitable management at local (minimizing the number of harrowings) and landscape levels (holding a high percentage of poplar plantations with distance between them lower than 400 m) would be desirable in poplar plantations from a point of view of bird diversity.

Keywords: *Populus x euramericana*, clone I-214, hybrid, native, management.

1. INTRODUCTION

Riparian zones are the interfaces between terrestrial and aquatic environments. In arid regions, such as Mediterranean area, this ecotone is particularly important to biodiversity, due to sharp gradients of moisture that determine ecological processes (Schnitzler, 1994) but also because they provide wildlife corridors (Gregory *et al.*, 1991; Naiman *et al.*, 1993; Machtans *et al.*, 1996; Naiman & Décamps, 1997).

Nevertheless, riparian zones have been disturbed or lost at an alarming rate during the past five decades in many European countries, including in the river Duero basin (Spain). Virgin vegetation of riparian zones was almost entirely lost when stream flow was regulated by storage reservoir and canalizations in the middle of the 20th century (Schnitzler, 1994; González & García, 2007). Such a regulation was followed by a major change from native vegetation (riparian forests) to crops or planted forests such as poplar plantations.

A current goal of national and regional forest policies is to increase the area occupied by plantation forests, since establishing plantations on degraded lands or agricultural crops may have multiple benefits, such as wood and biomass production but also biodiversity restoration (Hartley, 2002; Carnus *et al.*, 2006; Loyn *et al.*, 2007; Brockerhoff *et al.*, 2008). However, this statement has seldom been tested in Mediterranean landscapes consisting of riparian forests, poplar plantations and agricultural crops.

Obviously, the overall biodiversity in forest landscapes cannot be measured and quantified comprehensively; hence indicators may be a relevant option (Noss, 1999). Biodiversity indicators can be based on species richness, indicator species or functional diversity of one or several taxonomic groups. Among them birds are often considered as efficient indicators by playing an essential functional role in ecosystems at, or near, the top of the food chain (Ormerod & Watkinson, 2000; Gregory *et al.*, 2005; Gil-Tena *et al.*, 2007). Moreover, it is well known that bird diversity can rapidly respond to forest management, such as timber harvest (Hanowski *et al.*, 2007; Vanderwel *et al.*, 2007; Chizinski *et al.*, 2011) or site preparation (Lane *et al.*, 2011). On the other hand, birds are also responsive to signals that accumulate across local and landscape scales since bird communities typically select habitat features at multiple scales (MacFaden & Capen, 2002; Warren *et al.*, 2005; Mitchell *et al.*, 2006; Barbaro *et al.*, 2007). Nevertheless, at which spatial scale birds select their habitat remains a matter of debate. While several studies pointed out that landscape is the most relevant scale to be accounted for turnover in bird communities (Christian *et al.*, 1998; Saab, 1999; Bennett *et al.*, 2004; Barbaro *et al.*, 2007), other found that bird communities were mainly influenced by habitat patch features (MacFaden & Capen, 2002; Loyn *et al.*, 2007; Styring *et al.*, 2011), or that local and landscape variables are equally influential (Herrando & Brotons, 2002; Moreira *et al.*, 2005; Coreau & Martin, 2007).

The main objective of this study was therefore to investigate the influence of poplar plantations on biodiversity in riparian zones at both local and landscape scales in order to estimate the ecological implications of a substantial increase in the cover of poplar plantations. To achieve this objective the following two questions were addressed:

1. Can poplar plantations be surrogates of native riparian forests for native riparian bird communities?
2. What are the respective influences of local and landscape-scale forest features on bird diversity?

2. MATERIAL AND METHODS

2.1. Description of the study site and sampling

The present study was carried out in the Duero river basin, exactly in middle reach of the Carrión river (Castilla y León, NW Spain). The altitude in the study area ranges between 800 and 900 meters and, in most stands, the slope is almost flat. The average annual precipitation varies between 496 and 630 mm, and the average annual temperature, between 9 and 11.4 ºC (Ninyerola *et al*., 2005).

Formerly this riparian zone was characterized by several vegetation strips from the river to the external zone. A first vegetation strip had direct contact with watercourse, which consisted of species, mainly a shrub stratum, with high water requirement and ability to put up with floods. A second strip was located in alluvial meadow soils, which consisted of *Salix*, *Alnus*, *Populus*, *Ulmus*, or *Fraxinus* species, that just required a temporal water table with accessible deep (Lara *et al*., 2004). Nevertheless, nowadays the first strip of vegetation in the study area is very narrow (ca. 5-7 meters), and consists of a mixed tree and shrub stratum (mainly species of genus *Salix* sp. and *Alnus glutinosa*, and to a lower extent species of genus *Populus* sp), because of a lack of drastic and periodic floods. On the other hand, wetland forests of the second strip (mainly stands of alders, ashes or poplars) have been replaced with agricultural crops or poplar plantations, except the occasional remnant patches.

Poplar plantations were initially located next to the first strip of vegetation, where high edaphic moisture made lands impossible to cultivate, whereas other adjacent alluvial meadow soils were cultivated because of rich soils and irrigation. Nevertheless, poplar plantations are greatly increasing in the study area because of the high profitability of these trees (up to 2400 € ha^{-1} $year^{-1}$; Díaz & Romero, 2001). Poplar plantations are monoclonal and although several hybrids are used in Spain, *Populus x euramericana* (Dode) Guinier clone I-214 (*P. deltoides* Marsh. ♀ x *P. nigra* L. ♂) is the most common. It represents about 70 % of the total area covered by poplar plantations (Fernández & Hernanz, 2004). Poplar plantations are managed intensively in short rotations (12-16 years), and weed control techniques (mainly surface ploughing) are used regularly during the first six years. The density of poplar plantations, which is kept constant during the whole rotation, is approximately 278-400 stems/ha (De Mier, 2001; Fernández & Hernanz, 2004).

2.2. Sampling design

Twenty four *Populus x euramericana* (clone I-214) stands were selected for the study, which were chosen by use of a factorial scheme with two factors: (i) stand stage with two categories: young stands of 3-7 years old and adult stands of 8-14 years old, (ii) understory management with two categories: harrowed or not harrowed. While harrowed stands were ploughed each year, not harrowed stands were not harrowed at least the last two years. Eight clonal plantations for the young stands and four plantations for the old stands were therefore selected as replicates for each combination of the previous two factors (young-harrowed stands, young-not harrowed stands, adult-harrowed stands and adult-not harrowed stands).

Furthermore, the three native riparian forests found in our study area were sampled to compare bird assemblages between poplar plantations and native forests. These stands consisted of an upper storey of alders (*Alnus glutinosa*), a scattered lower storey of elders (*Sambucus nigra*), common hawthorn (*Crataegus monogyna*) and common dogwood (*Cornus sanguinea*), and a forb stratum.

All sampled forests were located within the same landscape of ca 3,500 ha (Fig. 1).

2.3. Bird sampling

Breeding birds were sampled by the point-count method with one visit in spring 2006 (Bibby *et al*., 1989). Unlimited distance was used within the stand, but all birds recorded were located within the limits of the sampled stands. All birds heard and seen were recorded and a semi-quantitative abundance index, with a score of 1 for singing male heard or pair or 0.5 for each non-singing bird heard or seen, was calculated (Norton *et al*., 2000). Point counts were performed within 3 hours after sunrise and rainy days were excluded.

2.4. Habitat and landscape description

In each poplar plantations, four circular subplots of 15 m radius were established for taking tree measurements. The subplots were located 50 metres apart from each other, at the ends of a cross located in the middle of the stand. All trees within each subplot were marked and sampled. Diameter at breast height (dbh), crown height, crown diameter and total height were measured in an average of 84 trees per stand (ranging from 68 to 112 trees per stand).

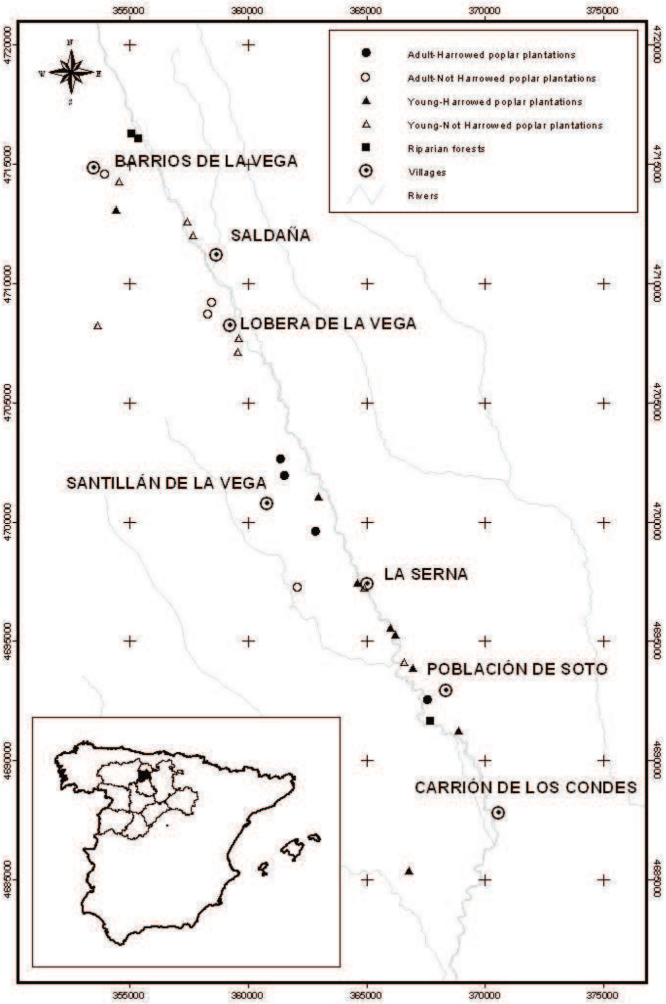

Figure 1. Geographical location of the sites surveyed

At the centre of each stand, nine 2 × 2 m quadrats (36 m^2 in total) were also laid out in order to quantify the understory vegetation. The species richness and percentage cover of all vascular plant species were estimated visually using the Braun-Blanquet (1964) scale during the spring of 2005.

Landscape mapping within the study area was performed in a GIS (ArcGis 9.3, ESRI) using colour aerial orthophotographs dated from 2004 at the scale 1: 1500, based on photo-interpretation and field cross-validation. Eight land cover types were assigned to each landscape patch according to the following classification: young poplar plantations (not closed canopy), adult poplar plantations (closed canopy), riparian forests, pine forests, oak forests, hedgerows, agricultural crops and roads. Landscape metrics were calculated within six different radii of circular buffers (100, 200, 300, 400, 500 and 1000 m) around each sampled stand to capture landscape features at different spatial scales, using Fragstat 3.3 in raster version and a cell size of 2.5 m (McGarigal *et al*., 2002). Within each buffer, we calculated the following metrics characterizing both composition and configuration of the landscape: the percentage cover of the eight land cover types, distance to river, edge density (in m ha^{-1}) and Shannon index of habitat diversity. Procedures and metrics are fully described in McGarigal *et al.* (2002).

2.5. Data analysis

Correspondence analysis (CA) was used to ordinate bird communities along a gradient of forest composition and structure from riparian forests to poplar plantations. For these analyses, as we only found three riparian forests, which were mature and were not ploughed, we used three adult-not harrowed poplar plantations for the purpose of comparison. Bird species abundances were transformed by means of log (x+1) to comply with normality assumptions. Analyses of variance (ANOVAs) and Tukey's HSD post-hoc test were carried out to test for the effect of habitat type on species richness and total abundance in poplar plantations. In addition, Mann-Witney tests were carried out to detect whether species richness and total abundance differ between riparian forests and adult-not harrowed poplar plantations.

To evaluate the relative influence of habitat and landscape variables on bird communities in poplar plantations, we used a two-step approach. First, a principal component analysis (PCAs) was carried out with dendrometric, understory and landscape variables calculated for each of the six different radii of circular buffers. Six multiple regressions (with forward selection) were made with bird species richness or total bird abundance as dependent variables and coordinates of sampled poplar plantations on the three first axes of each PCA as predictor variables in order to select the buffer radius for which bird variables were best explained.

Second, another multiple regressions were carried out to test the effects on bird species richness or total bird abundance of the single dendrometric, understory and landscape variables that were best correlated to the three first axes of the PCA calculated at the selected buffer radius.

Canonical correspondence analysis (CCA) was carried out to study the influence of the habitat and landscape variables (buffer radius selected by two-step approach) on the bird assemblages in poplar plantations. A forward selection procedure with Monte Carlo tests was then applied to determine the significance of the results, with 499 permutations. ANOVAs, Tukey's HSD post-hoc tests, Pearson's correlation matrix, PCA and multiple stepwise regressions were calculated using XLSTAT software and the constrained ordinations were performed with CANOCO software for Windows (Ter Braak & Smilauer, 2002).

3. RESULTS

Comparison between riparian forests versus poplar plantations (adult-not harrowed) reflected differences in bird species richness and abundance ($N = 6$, $Z = 1.99$, $p = 0.046$

and N = 6, Z = 1.96, *p* = 0.049, respectively), particularly riparian forests showed higher number of species and total abundance (18.3 ± 1.5 and 39.0 ± 0.3, respectively) than poplar plantations (11.7 ± 0.7 and 22.2 ± 2.9, respectively). In the same vein, correspondence analysis (CA) performed on the abundance of bird species revealed a complete turnover of bird assemblages from poplar plantations to riparian forests (Fig. 2). While riparian forests were characterized by several specific bird species, such as *Aegithalos caudatus*, *Cettia cetti*, *Columba palumbus*, *Cuculus canorus*, *Garrulus glandarius*, *Parus major*, *Phylloscopus collybita*, *Regulus ignicapillus* and *Serinus serinus*, poplar plantations did not show any specific species (Table 1, Fig. 1).

Table 1. List of bird species occurring in riparian forests and the three adult-not harrowed stands chose. Aerial, game and urban species were excluded. A six-letter abbreviation (first three letters of genus and species) was used for species names.

		Riparian		**Poplar**	
Bird species	Abbreviations	Nº stands	Total abundance	Nº stands	Total abundance
Fringilla coelebs	*FRICOE*	3	17	3	17
Phylloscopus collybita	*PHYCOL*	3	11	2	3
Cuculus canorus	*CUCCAN*	3	8,5	1	2
Columba palumbus	*COLPAL*	3	8	1	2
Aegithalos caudatus	*AEGCAU*	2	7,5	0	0
Serinus serinus	*SERSER*	3	7	1	1
Turdus merula	*TURMER*	3	6	3	7
Regulus ignicapa	*REGIGN*	2	5	0	0
Cettia cetti	*CETCET*	2	4	1	1
Corvus corone	*CORCOR*	2	4	2	5
Parus caeruleus	*PARCAE*	2	4	1	1
Parus major	*PARMAJ*	2	4	1	1
Sylvia atricapila	*SYLATR*	3	4	2	2
Carduelis chloris	*CARCHL*	3	3	3	4
Garrulus glandarius	*GARGLA*	2	3	0	0
Picus viridis	*PICVIR*	2	2	1	3
Troglodytes troglodytes	*TROTRO*	1	2	0	0
Anthus trivialis	*ANTTRI*	1	1	1	0,5
Carduelis carduelis	*CARCAR*	1	1	1	1
Certhia brachydactyla	*CERBRA*	1	1	0	0
Coccothraustes coccothraustes	*COCCOC*	1	1	1	1
Emberiza cirlus	*EMBCIR*	1	1	1	1
Emberiza citrinella	*EMBCIT*	1	1	0	0
Erithacus rubecula	*ERIRUB*	1	1	1	1
Hippolais polyglotta	*HIPPOL*	1	1	0	0
Jynx torquilla	*JYNTOR*	1	1	1	1
Luscinia megarhynchos	*LUSMEG*	1	1	0	0
Sylvia borin	*SYLBOR*	1	1	0	0
Sylvia melanocephala	*SYLMEL*	1	1	0	0
Turdus viscivorus	*TURVIS*	1	1	0	0
Carduelis cannabina	*CARCAN*	0	0	1	1
Dendrocopus major	*DENMAJ*	0	0	2	2,5
Parus ater	*PARATE*	0	0	1	2
Phylloscopus trochilus	*PHYTRO*	0	0	1	1
Sylvia communis	*SYLCOM*	0	0	1	1

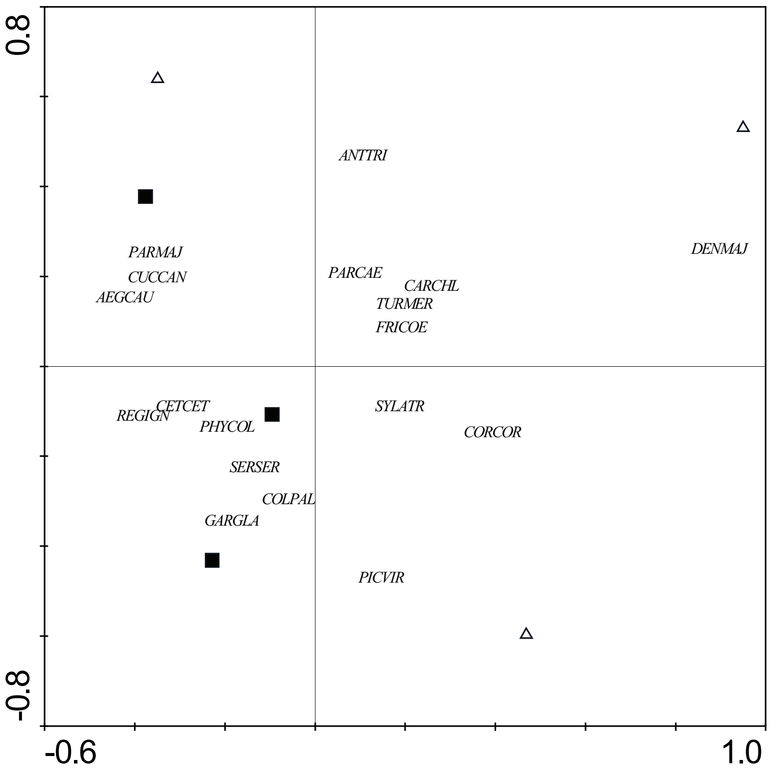

Figure 2. Ordination of bird species and stand. Type of forest: Riparian forests were represented by black square and poplar plantations (adult-not harrowed stands) by white triangles. See table 1for abbreviations of species name.

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When focusing on the dataset of 24 poplar plantations, a total of 45 species were recorded, of which 16 occurred in only one stand. The most abundant species were in decreasing order *Fringilla coelebs*, *Corvus corone*, *Turdus merula*, *Anthus trivialis*, *Phylloscopus collybita*, *Carduelis chloris* and *Columba palumbus*. Analyses of variance for mean species richness per stand (N=24) showed a significant interaction between age and management (N=24, F = 5.35, p = 0.03). Tukey's HSD test revealed that adult and harrowed stands held the lowest species richness and young harrowed stands the higher. By contrast total bird abundance did not differ according to the type of poplar plantations (Fig. 3).

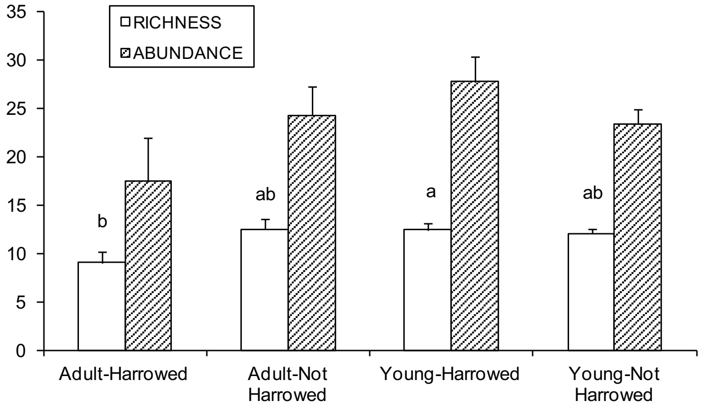

Figure 3. Mean (± S.E.) species richness and total abundance. Bars with different letters indicate significantly different means (Tukey's post hoc test, α = 0.05).

Principal component analyses (PCAs) and multiple regressions were performed to test the first assumption of the two-step approach, i.e. whether the effect of landscape may vary according to the spatial scale. The first three axes of PCAs separated clearly the effect of landscape, dendrometric and understory variables respectively (Table 2). Multiple regressions only retained the axis related to landscape variables across all the buffers, and the best correlation between bird diversity and explanatory variables was obtained with a buffer size of 400 meters (Fig. 4). Moreover, the multiple regressions carried out with variables calculated for a buffer size of 400m showed that bird species richness and total abundance were only related to the percentage of poplar plantations (excluding the area of the sampled stand) in the surrounding landscape (N = 24, F = 15.45, $p < 0.001$, $R^2 = 0.413$ and N = 24, F = 19.92, $p < 0.001$, $R^2 = 0.475$, respectively) (Fig. 5).

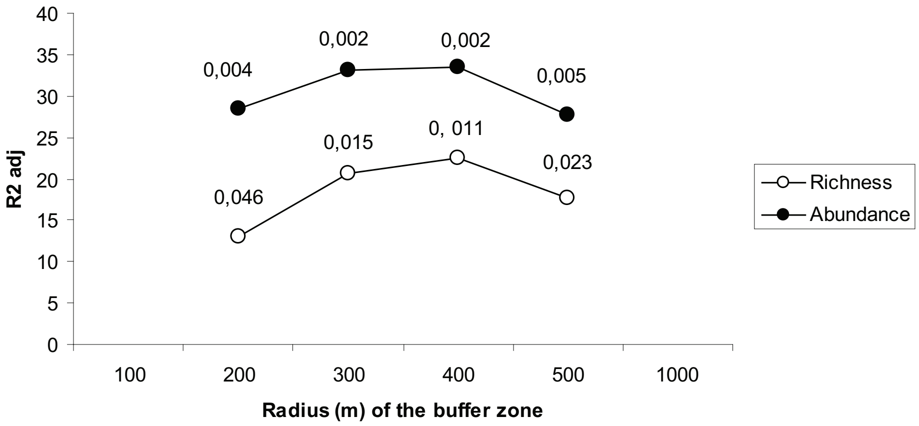

Figure 4. Percentage of explained variance of species richness and total abundance by the multiple linear model using as explanatory variables the coordinates of sampled poplar stands along the three first axes from PCAs. Above each line by each buffer size are showed the *p*-values per radius buffer. No values were represented for buffers of radius 100 and 1000 m because significant models were not found.

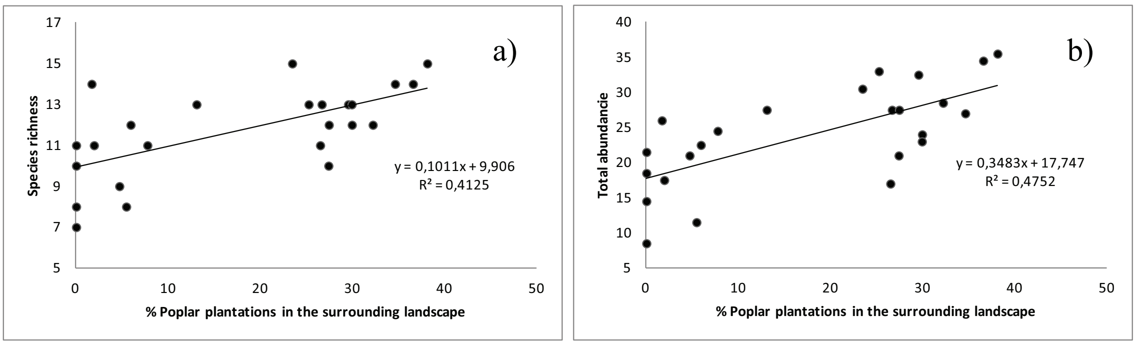

Figure 5. Relationship between (a) species richness and (b) total abundance and the percentage of poplar plantations (excluding area of sampled stand) within a circular buffer of 400 m radius.

Table 2. Coefficients of correlation (the highest in bold) between dendrometric, understory and landscape variables and the first three axes of principal component analysis (PCA) carried out at six different buffer radii.

		Buffer 100 m			**Buffer 200 m**			**Buffer 300 m**			**Buffer 400 m**			**Buffer 500 m**			**Buffer 1000 m**		
	Variables	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**
Dendrometric	**Total height**	0,899	-0,099	0,073	-0,055	-0,049	0,956	-0,135	-0,040	**0,959**	-0,155	-0,050	**0,958**	-0,168	-0,057	**0,959**	-0,075	-0,029	0,975
	Basal area	**0,928**	-0,099	0,063	-0,108	-0,062	**0,966**	-0,193	-0,047	0,956	-0,222	-0,053	0,950	-0,248	-0,057	0,943	-0,147	-0,039	**0,979**
	Canopy closure	0,914	-0,182	-0,011	-0,164	-0,116	0,962	-0,253	-0,100	0,951	-0,275	-0,112	0,942	-0,289	-0,117	0,938	-0,156	-0,108	0,963
Understory	**Total understory cover (%)**	0,119	0,564	-0,352	-0,324	0,695	-0,009	-0,225	0,763	-0,010	-0,188	0,768	0,015	-0,195	0,754	0,003	-0,171	0,601	0,018
	Total richness understory	-0,198	0,891	-0,106	-0,031	0,877	-0,247	0,095	0,852	-0,248	0,129	0,855	-0,246	0,146	0,857	-0,243	0,199	0,830	-0,233
	Shannon understory	-0,071	**0,953**	0,130	0,161	**0,923**	-0,099	0,228	**0,891**	-0,084	0,219	**0,899**	-0,088	0,209	**0,906**	-0,081	0,184	**0,934**	-0,110
	Eveness understory	0,062	0,619	0,421	0,408	0,594	0,108	0,374	0,554	0,139	0,312	0,562	0,132	0,273	0,569	0,141	0,145	0,648	0,058
Landscape	**Stand area**	-0,481	0,355	0,109	0,264	0,241	-0,295	0,345	0,182	-0,268	0,409	0,185	-0,258	0,481	0,189	-0,216	0,278	0,265	-0,320
	Distance river	0,273	-0,165	-0,515	-0,675	-0,122	0,074	-0,783	-0,020	-0,016	-0,803	-0,025	-0,042	-0,818	-0,037	-0,053	-0,869	-0,073	0,062
	% Total poplar (Surrounding)	0,072	0,064	**0,928**	**0,913**	-0,126	-0,133	**0,898**	-0,092	-0,308	**0,916**	-0,009	-0,290	**0,918**	0,005	-0,284	**0,936**	-0,070	-0,102
	% Adult poplar (Surrounding)	0,013	-0,130	0,756	0,753	0,023	-0,076	0,790	0,038	-0,162	0,837	0,014	-0,120	0,859	-0,029	-0,142	0,873	0,066	-0,011
	% Young poplar (Surrounding)	0,114	0,304	0,633	0,658	-0,220	-0,192	0,626	-0,187	-0,328	0,656	-0,029	-0,366	0,695	0,043	-0,357	0,699	-0,196	-0,167
	% Crop	0,657	0,132	-0,542	-0,825	-0,130	0,375	-0,882	-0,201	0,311	-0,890	-0,219	0,285	-0,895	-0,227	0,278	-0,867	-0,308	0,163
	% Urban	-0,166	0,539	0,098	-0,034	0,428	0,329	-0,070	0,486	0,320	-0,092	0,444	0,360	-0,122	0,405	0,350	-0,167	0,694	0,125
	% Riparian	-0,524	-0,403	0,278	0,532	-0,055	-0,502	0,645	0,041	-0,468	0,738	-0,044	-0,474	0,745	-0,067	-0,488	0,766	-0,075	-0,440
	% Hedgerow	0,740	0,068	0,016	-0,195	-0,071	0,712	-0,426	-0,144	0,572	-0,594	-0,114	0,508	-0,687	-0,150	0,349	-0,628	-0,202	0,407
	SHDI	0,501	0,071	0,230	0,777	0,354	-0,012	0,849	0,302	-0,163	0,847	0,282	-0,221	0,826	0,311	-0,260	0,823	0,347	-0,125

Only two variables were retained in the CCA, understory cover (local level) and percentage of adult poplar plantations (excluding the area of the sampled stand) in the surrounding landscape (landscape level) (Fig. 6). The eigenvalues (λ) for axes 1 and 2 were 0.106 and 0.079, respectively, and the model was significant according to the results of the Monte Carlo test (F = 1.834, *p* = 0.008, 499 permutations). The CCA biplot showed a clear gradient according to forest cover, since percentage of adult poplar plantations was positively correlated with axis 1, and to a lesser extent, negatively correlated with axis 2. While high percentages of adult poplar plantations were associated with *Regulus ignicapillus*, *Aegithalos caudatus, Cettia cetti*, *Carduelis cannabina*, *Carduelis carduelis*, and to a lesser extent *Corvus corone, Miliaria calandra* occurred at low plantation covers. On the other hand, understory cover also determined the bird community, with a high understory cover associated to a typical species assemblage including *Cettia cetti*, *Parus major, Carduelis carduelis*, and to lesser extent, *Aegithalos caudatus, Erithacus rubecula, Lullula arborea*, *Phylloscopus collybita* and *Sylvia atricapilla*.

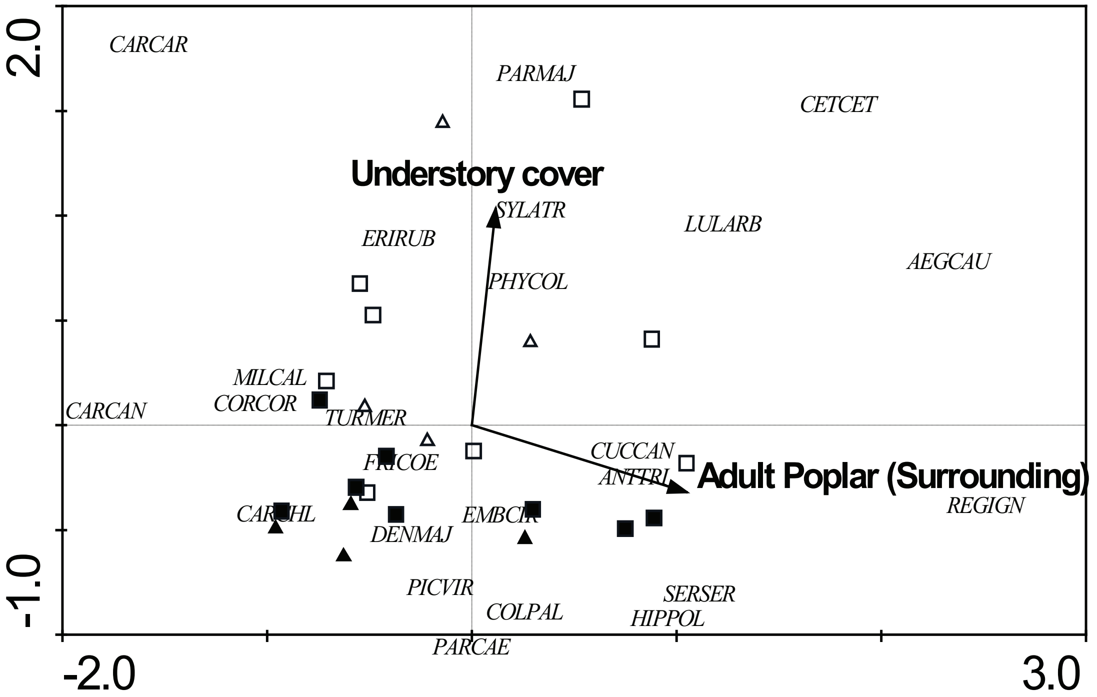

Figure 6. Ordination of bird species, stands, and the environmental variables (dendrometric, understory and landscape variables with a 400 m radius buffer) that were retained in CCA analysis. Type of forest: Adult-Harrowed stands were represented by black triangles, Young-Harrowed stands by black squares, Adult-not harrowed stands by white triangles and Young-Not Harrowed stands by white squares. See table 1 for abbreviations of species name

4. DISCUSSION

Our study confirms that both the type and the characteristics of forest habitats can influence bird diversity in riparian landscapes. Although the number of riparian forest stands available in the study area was very low (only three stands), it still hosted typical bird communities of natural riparian forests that were clearly distinct from those occurring in poplar plantations. Species richness and total abundance in adult poplar plantations were significantly lower than in riparian forests, which is consistent with findings in other riparian landscapes of North America, Australia or Europe (Hanowski *et al*., 1997; Christian *et al*., 1998; Twedt *et al*., 1999; Palmer & Bennett, 2006; Archaux & Martin, 2009). Bird assemblages also differed between riparian forest and poplar plantation. While poplar plantations hosted mainly generalist species, such as *Fringilla coelebs, Turdus merula* or *Corvus corone,* riparian forests were associated with specific typical bird species of well preserved riparian forest areas with a dense and unmanaged understory, such as *Cettia cetti*, *Phylloscopus collybita*, *Aegithalos caudatus*, *Columba palumbus*, *Cuculus canorus*, *Garrulus glandarius*, *Parus major*, *Regulus ignicapillus* and *Serinus serinus* (Jubete, 1997). Furthermore, when focusing on the dataset of 24 poplar plantations, significant differences were found among poplar plantation types, with adult and harrowed stands showing the lowest species richness and young harrowed the highest, suggesting an effect of stand age. This result is contrary to other studies conducted in tree plantations worldwide, which found an increase in species richness and abundance with increasing stand age (Lance *et al*., 1996; Hanowski *et al*., 1997; Vanhinsbergh *et al*., 2002; Barbaro *et al*., 2005; Styring *et al*., 2011). But in our study even the oldest poplar plantations were "young" (14 y.o.) from a natural forest perspective. Trees may have not been old enough for example to provide birds with suitable micro-habitats such as nesting cavities. Moreover, our results showed that understory vegetation determined bird assemblages in poplar plantations. Indeed, only poplar stands with high understory cover (not harrowed stands) were associated to bird species typical of natural riparian forests, such as *Cettia cetti*, *Aegithalos caudatus*, *Parus major*, or *Regulus ignicapillus* (Jubete, 1997).

On the other hand, we found a prominent influence of landscape variables on species richness, total abundance and bird assemblages in poplar plantations. This could be due to a lack of suitable habitats at local scale as a result of the small size of poplar plantations (average 6.37 ha, ranging between 0.97-19.71 ha) and a high degree of fragmentation (Andrén, 1994; Warren *et al*., 2005).

Most studies select a single landscape scale based on assumptions or previous researches, but the a priori selection of the most influential scale in a specific landscape matrix is still a controversial point. This study shows that the scale at which landscape matrix is more influential on bird diversity is about 50 ha (buffer radius of 400 m). This size is rather lower than the one found in other studies carried out in forest areas (see e.g., 1 x 1 km cells by Gil-Tena *et al*., 2007 or 10 x 10 km cells by Oja *et al*., 2005), but is consistent with findings in mixed farmland where landscape composition effect was stronger at smaller scales (200 - 400 m) than at larger radius (2 - 3 km) (Deconchat *et al*., 2009). This buffer range is also similar to dispersal distances of several birds, such as *Turdus migratorius* and *Toxostoma rufum* (ca. 200 m. within a breeding season,) in agricultural landscape with wooded patches (Haas, 1995), or other territorial passerines that have territories about 3 – 30 ha in size in riparian areas (Pearson & Manuwal, 2001). Mean distance in bird dispersal vary according to landscape composition and structure, and a complex landscape matrix is more likely to supply several types of habitat for foraging, nesting and sheltering in multi-habitat species through the mechanism of habitat complementation (Dunning *et al*., 1992). Furthermore, a landscape matrix with open areas is more sensitive to predation by corvids or raptors, and birds may therefore limit their movements outside poplar plantations (see e.g., Desrochers & Fortin, 2000).

The variable most important to explain species richness, abundance and bird assemblages was the percentage of poplar plantations in the surrounding landscape (within a 400m range). This probably involves a mechanism of landscape supplementation (Dunning *et al*., 1992; Brockerhoff *et al*., 2008), since the small size of poplar plantations in our study area may not allow to support species-rich bird communities. Furthermore, other poplar plantations in the surrounding could be used by birds as corridors or stepping stones for dispersing across complex landscape matrix with habitats as diverse as riparian forest and agricultural crops, thus acting like hedgerow networks in other areas (Parish *et al*., 1994; Baudry *et al*., 2000; Hinsley & Bellamy, 2000; Fuller *et al*., 2001). Indeed, only poplar stands with high cover of surrounding plantations were visited by bird species typical of natural riparian forests, such as *Cettia cetti*, *Aegithalos caudatus*, *Parus major*, or *Regulus ignicapillus* (Jubete, 1997), whereas poplar plantations within predominantly agricultural landscapes (i.e. with lower percentage of surrounding poplar plantations) were mainly visited by bird species associated to open areas, such as *Carduelis cannabina, Carduelis carduelis* or *Miliaria calandra* (Jubete, 1997).

CONCLUSIONS

Our results indicated that poplar plantations should not be used as surrogates of native riparian forests from the point of view of bird species diversity and that native riparian forests should be preserved or restored as far as possible. Nevertheless, bird communities occurring in poplar plantations can still accommodate rich communities of forest bird species, providing that suitable managements at local and landscape levels are applied. The landscape matrix should hold a high percentage of poplar plantations, with distance between them lower than 400 m, to provide forest bird species with well connected supplementary forest habitats. Moreover, poplar plantations should keep a high understory cover as long as it does not affect tree growth through competition and vitality through pest attacks (Martín-García *et al.*, 2011), in order to favour forest bird species.

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ARTICLE VIII

Martín-García, J., Jactel, H. & Diez, J.J. (2012) The role of poplar plantations on vascular plant diversity in riparian landscapes. (In prep. to be submitted to *Biodiversity and Conservation*),

RESUMEN

El papel de las plantaciones de chopo en la diversidad de las plantas vasculares en los paisajes riparios

Las zonas riparias desempeñan funciones ecológicas claves, entre otras proveer un microclima con una alta biodiversidad. Sin embargo, la vegetación autóctona de las zonas riparias ha sido deteriorada por las actividades humanas a una velocidad alarmante durante las últimas cinco décadas. El objetivo de este estudio fue investigar el papel de las plantaciones de chopo sobre la diversidad de plantas vasculares en los paisajes riparios para estimar las implicaciones ecológicas de un incremento de la superficie destinada a plantaciones de chopo. Treinta y dos plantaciones de chopo (*Populus x euramericana*, clon I-214), fueron seleccionadas, según un diseño factorial combinando edad, calidad de estación y selvicultura aplicada al sotobosque. La influencia de los factores de estrés y perturbación sobre la diversidad de plantas vasculares fue estudiada. Además, las únicas tres parcelas de bosques de ribera encontradas en el área de estudio fueron también muestreadas para evaluar si las plantaciones de chopo podrían ser utilizadas como sustitutas de las bosques naturales de ribera. Para esto, la diversidad fue estimada como riqueza de especies, especies indicadoras y diversidad funcional basada en la estrategia C-S-R. Las plantaciones de chopo mostraron una riqueza de especies similar a los bosques de ribera, sin embargo diferencias en las especies indicadoras y en la diversidad funcional fueron observadas según el tipo de masa. Perturbaciones, y principalmente el número de gradeos, generó un descenso en la riqueza de especies y modificó las especies indicadoras y la diversidad funcional, favoreciendo a las especies ruderales en detrimento de las especies con una estrategia intermedia entre tolerante a la sombra y competidores, las cuales son típicas de los bosques naturales de ribera. Las plantaciones de chopo no deberían ser usadas como sustitutas de los bosques naturales de ribera, si bien minimizar el número de gradeos en las plantaciones de chopo sería deseable desde un punto de vista de la diversidad de plantas vasculares.

Palabras clave: *Populus x euramericana*, clon I-214, híbrido, autóctono, híbrido, teoría C-S-R.

THE ROLE OF POPLAR PLANTATIONS ON VASCULAR PLANT DIVERSITY IN RIPARIAN LANDSCAPES

Jorge MARTÍN-GARCÍA[1], Hervé JACTEL[2], Julio J. DIEZ[1]

[1] Sustainable Forest Management Research Institute, University of Valladolid – INIA. Palencia. Spain. Avenida Madrid, 57. 34004 Palencia, Spain.

[2] INRA, UMR 1202 BIOGECO, Laboratory of Forest Entomology & Biodiversity, 69 Route d'Arcachon, F-33612 Cestas cedex, France.

Abstract

- **Context:** Riparian zones perform many key ecological functions, including the ability to provide a microclimate with high biodiversity. Nevertheless, virgin vegetation of riparian zones have been traditionally modified or lost by human activities at an alarming rate during the past century.
- **Aim:** The aims of this study were to investigate the role of poplar plantations on plant diversity in riparian zones and to estimate the ecological implications of a substantial increase in the cover of poplar plantations.
- **Methods:** Thirty-two poplar plantations of I-214 clone were surveyed, according to a factorial design combining stand age, site condition and understory management to examine the respective influences of stress and disturbance factors on vascular plant diversity. Furthermore, the only three native riparian forests found in the study area were also sampled to investigate whether poplar plantations could be surrogates of native riparian forests. For that, diversity was referred to species richness, indicator species and functional diversity based on C-S-R strategy.
- **Results:** Poplar plantations showed similar species richness than riparian forest, however very sharp differences in the indicator species and functional diversity were found according to type of habitat. Disturbance, and mainly the number of harrowings, led to a decline in the species richness, and modified the indicator species and functional diversity by facilitating ruderal species in detriment to Stress-Tolerant Competitors species that are better suited to riparian forests.
- **Conclusion:** Poplar plantations should not be used as surrogates of native forests and minimizing the use of harrowing in poplar plantations would be desirable from the point of view of vascular plant diversity.

Keywords: *Populus x euramericana*, clone I-214, hybrid, native, C-S-R theory.

1. INTRODUCTION

Riparian zones are the most diverse, dynamic and complex habitats, which play a key role as interfaces between terrestrial and aquatic environments (Gregory et al., 1991; Naiman et al., 1993). It is well known that riparian zones perform many key functions, such as the ability to reduce the eutrophication processes, to trap pesticides, to stabilize river banks and to provide a microclimate with high biodiversity (Kauffman et al., 1997; Naiman & Décamps, 1997; Stutter et al., 2012). Furthermore, this type of habitat is particularly important in arid regions, such as the Mediterranean area, due to high sharp gradients of moisture that determine ecological processes (Schnitzler, 1994).

Despite its numerous benefits, riparian zones have been disturbed by human activities over the past century (Kauffman et al., 1997; Naiman & Décamp, 1997). Virgin vegetation of riparian zones was almost totally lost when stream flow was regulated by storage reservoir and canalizations in the middle of the 20th century (González and García, 2007; Schnitzler, 1994). This allowed the natural vegetation to be replaced with crops or forest plantations, such as poplar, which are more profitable.

A current purpose of national and regional forest policies is to enlarge the area occupied by plantation forests, justifying the plantations established on degraded lands or agricultural crops, which may have numerous benefits, including restoration of biodiversity values (Brockerhoff et al., 2008; Carnus et al., 2006; Hartley, 2002). However, this statement has hardly ever been tested in Mediterranean landscapes consisting of riparian forests, poplar plantations and agricultural crops

Biodiversity can be inclusive everything (Wilson, 1996), but obviously, the overall biodiversity in forest ecosystems cannot be estimated and therefore indicators may be a relevant option (Noss, 1999). In this regard, vascular plants are often used as a general indicator of the broad state of wildlife (Gray & Azuma, 2005; Lindenmayer et al., 2000), because this taxonomic group is a faithful reflection of the situation of the forests. Vegetation is directly affected by any change whether by natural disturbance or by human management.

Diversity has been often referred to species richness, and to a lesser extent to indicator species. However, functional diversity has been traditionally ignored by researchers, despite being an aspect of crucial importance in determining ecosystem processes. It is well documented that ecosystem processes are more consistently associated with functional composition (defined as presence of certain plant functional types, i.e. set of plant species with the same collection of attributes) and functional richness (defined as number of different plant functional types) than with species richness (Díaz & Cabido, 2001; Fleishman et al., 2006). Nevertheless, these three components (species richness, indicator species, and functional diversity) don't contribute the same type of information, and should therefore be analysed as complementary variables.

In this vein, Grime (1973, 1974, 1977, 2001) reached a plant strategy theory, based on the concept of functional types to reduce the complexity of vascular plant communities and to understand vegetation processes. The Grime's theory establishes that two external factors (stress and disturbance) determine the plant communities.

Stress is defined as "the external constraints which limit the rate of dry matter production of all or part of the vegetation", e.g. shortages of light, water or mineral nutrients and suboptimal temperatures. On the other hand, disturbance is defined as "the mechanisms which limit the plant biomass by causing its partial or total destruction" e.g. activities of herbivores, pathogens, man and abiotic phenomena such as wind damage, frosts or fire. Grime's theory suggests that vascular plants can be classified in three primary strategies according to the local levels of stress and disturbance, and secondary strategies in those habitats with intermediate intensities of both factors. Thus, while low stress with low disturbance would give rise to Competitors species (C), high stress with low disturbance to Stress-tolerators species (S) and low stress with high disturbance to Ruderal species (R). In highly disturbed habitat, the effect of continuous and severe stress prevents recovery or reestablishment of the vegetation (Grime, 2001).

The general hypothesis was that vascular plant diversity in poplar plantations may differ from riparian forests, and that stress and disturbance factors may explain the differences in plant communities among the different type of habitats. The main objectives of this study were to investigate the role of poplar plantations on plant diversity in riparian zones and to estimate the ecological implications of a substantial increase in the cover of poplar plantations. To achieve these objectives the following questions were addressed:

1. Can poplar plantations be surrogates of native riparian forests for native riparian plant communities?
2. What are the respective influences of stress and disturbance stresses on vascular plant diversity in riparian landscapes?

2. MATERIAL AND METHODS

2.1. Description of the study site

The present study was carried out in the Duero river basin, exactly in middle reach of the Carrión river (Castilla y León, NW Spain). The altitude in the study area ranges between 800 and 900 meters and, in most stands, the slope is almost flat. The average annual precipitation varies between 496 and 630 mm, and the average annual temperature, between 9 and 11.4 ºC (Ninyerola et al. 2005).

Formerly this riparian zone showed several strips of vegetation that were characterized by a first strip coming into direct contact with watercourse, which consisted of species, mainly a shrub stratum, with high water requirement and ability to put up with floods. A second strip, located in alluvial meadow soils consisted of tree species that just required a temporal water table with accessible deep (Lara et al., 2004)

Nevertheless, because of a lack of drastic and periodic floods the first strip of vegetation in the study area is very narrow ca. 5-7 meters, and consists of a mixed tree and shrub stratum (mainly species of genus *Salix* sp. and *Alnus glutinosa*, and to a lesser extent species of genus *Populus* sp). On the other hand, wetland forests of the second strip (mainly stands of alders, ashes or poplars) have been replaced with agricultural crops or poplar plantations, except the occasional remnant patches.

Poplar plantations were initially located next to first strip of vegetation, where high edaphic moisture made lands impossible to cultivate. Whereas other adjacent alluvial meadow soils were cultivated, exploiting rich soils and irrigation. Nevertheless, poplar plantations are increasing greatly in these zones because of the high profitability of these trees (up to 2400 € ha^{-1} $year^{-1}$; Díaz and Romero, 2001).

Poplar plantations are monoclonal and although several hybrids are used in Spain, *Populus x euramericana* (Dode) Guinier clone I-214 (*P. deltoides* Marsh. ♀ x *P. nigra* L. ♂) is the most common. It covers about 70 % of the total area covered by poplar plantations (Fernández and Hernanz 2004). Poplar plantations are managed intensively in short rotations (12-16 years), and weed control techniques (mainly harrowing) are used regularly during the first six years. The density of poplar plantations, which is kept constant during the whole rotation, is approximately 278-400 stems/ha (De Mier 2001; Fernández and Hernanz 2004).

2.2. Sampling design

Thirty-two *Populus x euramericana* (clone I-214) stands were selected for study. These were chosen by use of a factorial scheme with three factors: (1) stand stage: young stands of 3-7 years old and adult stands of 8-14 years old, (2) understorey management: harrowed or not harrowed. Whereas harrowed stands were ploughed each year from their plantations, not harrowed stands were not ploughed for at least the last two years, (3) site quality: rich stands with optimum growth rates of poplar trees (site qualities I and II), and poor land, unsuitable for growth (site qualities III and IV). Site quality was determined by the model based on curves developed for *Populus x euramericana* clone I-214 in the Duero river basin (Bravo et al. 1995).

Four clonal plantations were therefore selected as replicates of each combination of the previous three factors. Furthermore, the only three native riparian forests found in our study area were sampled to compare bird assemblages between poplar plantations and native forests. These stands consisted of an upper storey of alders (*Alnus glutinosa*), a scattered lower storey of elders (*Sambucus nigra*), common hawthorn (*Crataegus monogyna*) and common dogwood (*Cornus sanguinea*), and a forb stratum.

2.3. Vascular plants sampling

At the centre of each stand, nine 2-m × 2-m quadrants (36 square meters) were laid out in order to quantify the understorey vegetation (i.e. a total of 315 quadrants were assessed). Each vascular plant was identified (field or laboratory) and its percentage cover and characteristics were collected during the spring of 2005.

The conversion of floristic data into a C-S-R signature was carried out automatically by the calculator (first part of the spreadsheet tool) developed by Hunt et al. (2004). This tool, once pastes-in data matrix containing quantitative records from one stand, calculates the percent abundance of each functional type and plots this net position onto a triangular representation of C-S-R space. Consequently, a C-S-R signature was obtained by each stand (see Hunt et al. 2004 for details).

2.4. Sampling of quantity of light transmitted to the understory

Several dendrometric variables can be related to the quantity of light transmitted to the understory, such as basal diameter, total height and canopy closure. Thus, in each of the poplar plantations, four circular subplots of 15 m radius were established for taking tree measurements. The subplots were located 50 metres apart from each other, at the ends of a cross located in the middle of the stand. All trees within each subplot were marked and sampled. Diameter at breast height (dbh), total height and canopy width (two perpendicular measures) were measured in an average of 84 trees per stand (ranging from 68 to 112 trees per stand).

Furthermore, taking into account that canopy closure depends not only on canopy area but is also influenced by the density of foliage, crown transparency of all trees each subplot was estimated visually during the summer of 2005 (within the first two weeks of July) as recommended in the ICP-Forest protocol (Eichhorn et al. 2006, see Martín-García et al. 2009 for details). Thus, canopy closure was determined by multiplying canopy area by mean crown transparency in each stand.

2.5. Sampling and analysis of soils

Mineral soil samples were collected from the upper 30 cm soil layer in each subplot (i.e. 4 samples per plot). The samples were mixed, homogenized and considered as a composite sample per plot. The pH was determined potentiometrically with a pH meter, in a soil solution (1:2.5, soil:water). Organic matter was determined by the $K_2Cr_2O_7$ method. Total N was determined by Kjeldahl digestion. Soil available P was extracted by the Olsen procedure and determined photometrically by the molybdenum-blue method. Soil exchangeable cations (K^+, Na^{+2}, Ca^{2+} and Mg^{2+}) were extracted with ammonium acetate and determined by atomic absorption/emission spectrometry. Particle-size distribution was determined by the Bouyoucos method (hydrometer method), and the ISSS (International Society of Soil Science) classification was applied. The Cationic Exchange Capacity (CEC) was determined by Bascomb's method (i.e. the exchange cations were displaced by Ba ions, which were then displaced by Mg ions and the remaining concentration of Mg was determined by titration against EDTA).

2.6. Statistical analyses

To evaluate the possible differences in the vascular plant diversity between riparian forests and poplar plantations, we used a two-step approach. Firstly, Mann-Witney test was carried out to detect whether species richness differs between riparian forests and poplar plantations. Secondly, Non-metric multidimensional scaling (NMDS), using (1) species frequency and (2) C-S-R signatures, were performed to detect the possible differences in the vascular plant communities between riparian forests and poplar plantations. NMDS is a nonparametric procedure, recommended for data that is non-normal because it uses ranked distances and does not depend on assumptions of linear relationships among variables (McCune and Grace, 2002). NMDS were conducted using Bray-Curtis as the distance metric, and the multivariate ordination was created using the metaMDS results. For these analyses, as we only found three riparian forests, which were mature and were not ploughed, we used three adult-not harrowed poplar plantations for the purpose of comparison.

To evaluate the possible differences in the vascular plant diversity according to type of poplar plantation, a three-step approach was used. Firstly, analysis of variance (ANOVAs) and Tukey's HSD post-hoc test were carried out to test the effect of the type of habitat (combination of factors; age, site quality and understory management) on species richness in poplar plantations. Secondly, the detection of characteristic species for each habitat type was carried out with the Indicator Value (IndVal) method (Dufrêne and Legendre 1997). The value of $IndVal_{ij}$ is the product of two terms, the first referring to the performance of species *i* in terms of abundance over all groups and the other referring to the performance of the same species in terms of presence/absence within site group *j*. This index ranges from 0 to 100 for each species, corresponding to the largest IndVal value observed over the different habitats. A maximum IndVal value (100 %) is reached when all individuals of a species are found in one single habitat type and in all sites of that habitat. The IndVal of a species can be calculated for each level of a hierarchical site typology. This procedure differentiates between generalist (maximum IndVal at a higher cluster level) and stenotopic species (maximum at lower levels) (Dufrêne and Legendre 1997). A hierarchical site typology based on the habitat types, which were established a priori, was used. For that, the first level grouped all poplar plantations, and in the next steps the poplar plantations were separated in harrowed and not harrowed, then in rich and poor stand, and finally in young and adult stands. The statistical significance of the index was estimated at each level of the hierarchy by a random reallocation procedure of plots among plot groups based on 999 permutations (Dufrêne and Legendre 1997).

Thirdly, Non-metric multidimensional scaling (NMDS) and Multiple response permutation procedures (MRPP), using C-S-R signatures, were performed to explore the characteristics of the different types of poplar plantations and the associated stress and disturbance factors to the plant community. NMDS were also conducted using Bray-Curtis as the distance metric and environmental variables (stress variables: canopy closure and dendrometric variables as indicators of shortages of light, and soil properties as indicators of nutrient status; and disturbance factors: number of harrowings and years since the last harrowing) that were significantly correlated with one or more axes were overlaid on the ordination as vectors. In parallel, MRPP was used to test the null hypothesis of no vascular plants differences among poplar plantations according to the age (young vs. adult stands), site quality (poor vs. rich) and understorey management (harrowed vs. not harrowed). The MRPP compares the observed intra-group average distance with the average distance that would have resulted from all the other possible combinations of the data under the null hypothesis. For this, MRPP calculates a variable A (chance-corrected within-group agreement) and a p-value to assess significance of A. MRPP is also a nonparametric procedure that does not depend on assumptions such as normally distributed data or homogeneous variances. MRPP was also performed using Bray-Curtis dissimilarity and running 1000 permutations.

Mann-Witney test, ANOVAs and Tukey's HSD post-hoc tests have been performed with the software Statistica 7.0 for Windows (StatSoft Inc., 2004) and Indicator Value Indexes were calculated with software IndVal 2.1. Non-Metric Multidimensional Scaling (NMDS) and Multiple Response Permutation Procedure (MRPP) were carried out using the VEGAN package (Oksanen *et al*., 2011) implemented in the R software environment (R-Development-Core-Team, 2008). Rare species occurring in fewer than two stands were excluded from IndVal, NMDS and MRPP analyses

3. RESULTS

3.1. Comparison between riparian forests versus poplar plantations

Comparison between riparian forests versus poplar plantations did not reflect differences in species richness (N = 6, Z = 0.44, p = 0.66). Nevertheless, Non-metric multidimensional scaling (NMDS) using species frequency revealed a complete turnover of vascular plant assemblages from poplar plantations to riparian forests (Fig. 1). Particularly, twenty species were associated with riparian forests (*Alliaria petiolata*

[*Apet*], *Brachypodium sylvaticum* [*Bsyl*], *Carex cuprina* [*Ccup*], *Crataegus monogyna* [*Cmon*], *Epilobium parviflorum* [*Epar*], *Euphorbia amygdaloides* [*Eamy*], *Filipendula ulmaria* [*Fulm*], *Gallium palustre* [*Gpal*], *Geranium robertianum* [*Grob*], *Geum urbanum* [*Gurb*], *Humulus lupulus* [*Hlup*], *Ligustrum vulgare* [*Lvul*], *Loniera peryclimenum* [*Lper*], *Ribes rubrum* [*Rrub*], *Rosa canina* [*Rcan*], *Rubus caesius* [*Rcae*], *Rubus ulmifolius* [*Rulm*], *Sambucus nigra* [*Snig*], *Stachys sylvatica* [*Ssyl*] and *Urtica dioica* [*Udio*]). Whereas, *Agrostis stolonifera* [*Asto*], *Bromus sterilis* [*Bste*], *Chenopodium album* [*Calb*], *Cirsium arvense* [*Carv*], *Dactylis glomerata* [*Dglo*], *Daucus carota* [*Dcar*], *Holcus lanatus* [*Hlan*], *Hordeum murinum* [*Hmur*], *Lactuca serriola* [*Lser*], *Rumex crispus* [*Rcris*], *Taraxacum* gr. *officinale* [*Toff*], *Torilis nodosa* [*Tnod*] and *Trifolium repens* [*Trep*] were associated with poplar plantations.

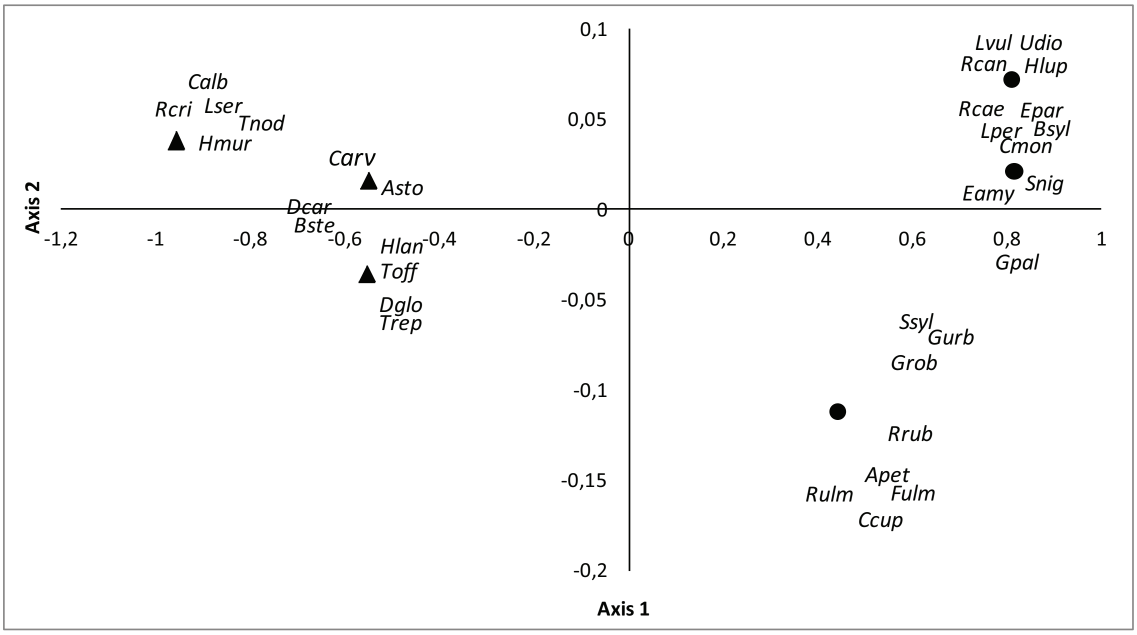


Figure 1. Ordination of vascular plants and stands. Type of forest: riparian forests were represented by circles and poplar plantations (adult-not harrowed stands) by triangles. Vascular plant species: Apet *Alliaria petiolata*, Asto *Agrostis stolonifera,* Bste *Bromus sterilis*, Bsyl *Brachypodium sylvaticum*, Calb *Chenopodium album*, Carv *Cirsium arvense*, Ccup *Carex cuprina*, Cmon *Crataegus monogyna*, Dcar *Daucus carota*, Dglo *Dactylis glomerata*, Epar *Epilobium parviflorum*, Eamy *Euphorbia amygdaloides*, Fulm *Filipendula ulmaria*, Gpal *Gallium palustre*, Grob *Geranium robertianum*, Gurb *Geum urbanum*, Hlan *Holcus lanatus*, Hlup *Humulus lupulus*, Hmur *Hordeum murinum*, Lser *Lactuca serriola*, Lvul *Ligustrum vulgare*, Lper *Loniera peryclimenum*, Rrub *Ribes rubrum*, Rcan *Rosa canina*, Rcae *Rubus caesius*, Rcris *Rumex crispus* Rulm *Rubus ulmifolius*, Snig *Sambucus nigra*, Ssyl *Stachys sylvatica*, Tnod *Torilis nodosa*, Toff *Taraxacum* gr. *Officinale*, Trep *Trifolium repens* and Udio *Urtica dioica*

In the same vein, NMDS ordination test reflected a clear differentiation between riparian forests and poplar plantations according to CSR strategists. Particularly, NMDS ordination showed that plant communities in riparian forests mainly consisted of Stress-Tolerant Competitors species (SC). On the other hand, a clear gradient towards Ruderal species (R) was found in poplar plantations (Fig. 2).

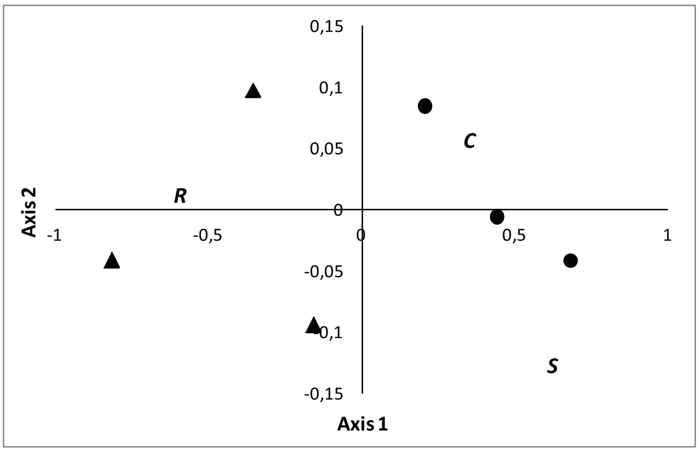


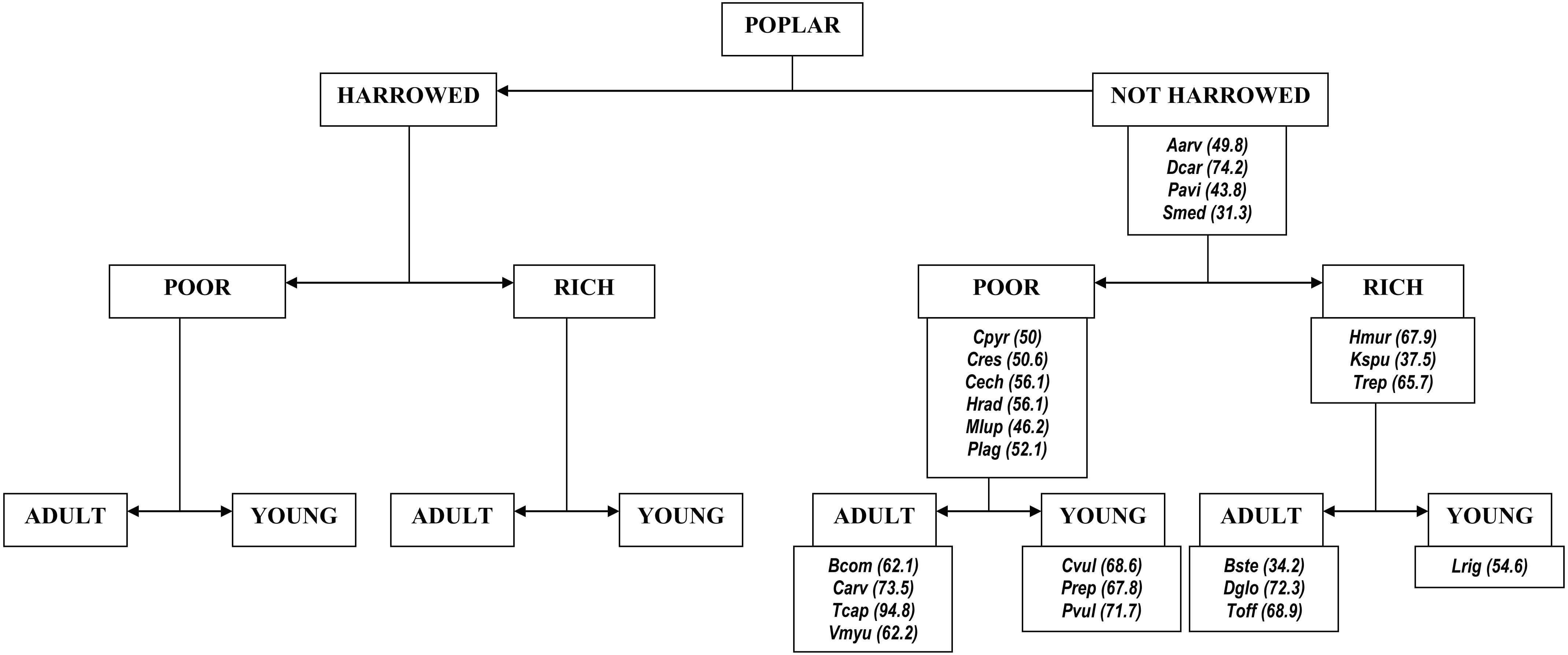
Figure 2. Ordination of C-S-R signatures and stands. Type of forest: riparian forests were represented by circles and poplar plantations (adult-not harrowed stands) by triangles. C-R-S signatures: C competitors species, R ruderal species and S stress-tolerators species.

3.2. Comparison between types of poplar plantations

A total of 133 species were recorded from the thirty-two poplar plantations, of which 44 occurred in only one stand. Analysis of variance for species richness mean values per stand showed significant differences according to the age (N = 32, F = 10.11, $p = 0.004$) and management (N = 32, F = 34.85, $p < 0.001$), but no significant effect of site quality or interaction was found. In particular, mean species richness in young stands was higher than adult stands (ca. 23 and 16, respectively), and harrowed stands showed a lower species richness than non harrowed stands (ca. 13 and 26, respectively).

IndVal analysis was performed with 89 species, of which 25 had a significant IndVal index at one or several levels of the typology (Fig. 3). Nevertheless, no indicator species were found in harrowed stands. In particular, five species had their maximum value at the first level according to management and other nine species were found at the second level, of which six species belonged to poor stands. Lastly, the third level detected eleven indicator species according to age (Fig. 3).

Figure 3. Indicator species for the different levels of the hierarchic site typology (indicator value in brackets). Aarv *Anthemis arvensis*, Bcom *Bromus commutatus*, Bste *Bromus sterilis*, Carv *Convolvulus arvensis*, Cech *Cynosurus echinatus*, Cpyr *Cirsium pyrenaicum*, Cres *Crespis* sp., Cvul *Cirsium vulgare*, Dcar *Daucus carota*, Dglo *Dactylis glomerata*, Hmur *Hordeum murinum*, Hrad *Hypochoeris radicata*, Kspu *Kickxia spuria*, Lrig *Lolium rigidum,* Mlup *Medicago lupulina*, Pavi *Polygonum aviculare*, Plag *Plantago lagopus*, Prep *Potentilla reptans*, Pvul *Prunella vulgaris*, Smed *Stellaria media*, Tcap *Taeniatherum caput-medusae*, Toff *Taraxacum* gr. *Officinale*, Trep *Trifolium repens*, Vmyu *Vulpia myuros*.



NMDS ordination and MRPP tests reflected a differentiation in the CSR strategies in poplar plantation according to age (A = 0.06, *p* = 0.02) and site quality (A = 0.03, *p* = 0.04). Particularly, NMDS ordination showed that plant communities in adult stands mainly consisted of Ruderal species (R), whereas young plantations were closer to Stress-Tolerant Competitors strategy (SC) (Fig. 4). On the other hand, site quality also seems to influence the strategy of plant community according to a gradient "Competitors to Stress-Tolerant". In particular, poplar plantations in poor sites held plant communities with a Stress-Tolerant strategy (S), whereas in rich sites understorey species showed a strategy closer to Competitors (C) (Fig. 4).

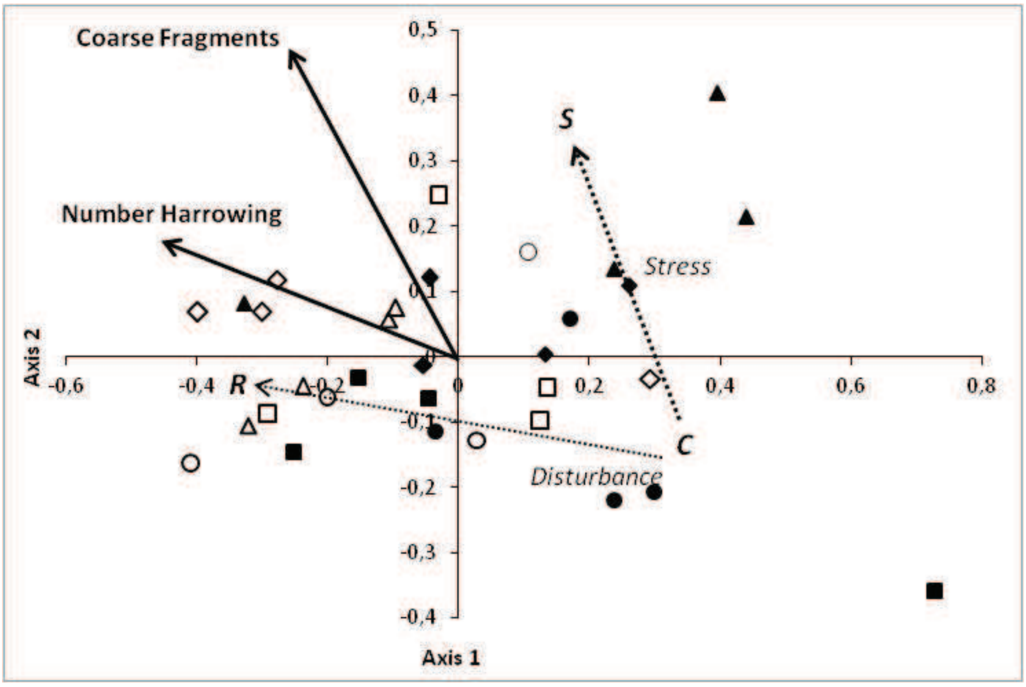


Figure 4. Ordination of vascular plants, stands and the environmental variables (represented by continuous arrows) that were retained in NMDS analysis. Type of forest: Adult-Poor-Harrowed were represented by black triangles, Adult-Poor-Not harrowed by black diamonds, Adult-Rich-Harrowed by black squares, Adult-Rich-Not harrowed by black circles, Young-Poor-Harrowed by white triangles, Young-Poor-Not harrowed by white diamonds, Young-Rich-Harrowed by white squares and Young-Rich-Not harrowed by white circles. C-R-S signatures: C competitors species, R ruderal species and S stress-tolerators species. Dotted arrows indicate the hypothetical axes (stress and disturbance) in terms of Grime's theory.

Furthermore, NMDS retained two significant environmental variables (number of harrowings [*p* = 0.04] and percentage of coarse fragments [*p* = 0.04]). While, the latter seems to indicate a gradient from Competitors (C) to Stress-Tolerant (S) strategies, the number of harrowings does the same with the pattern Competitors (C) to Ruderal species (R).

4. DISCUSSION

4.1. Comparison between riparian forests versus poplar plantations

It is widely thought that plantation forests have lower biodiversity than natural forests. Nevertheless several reviews have confirmed that it is not a universal truth (Brockerhoff et al., 2008; Carnus et al., 2006; Stephens et al., 2007). No differences in species richness between native forests and poplar plantations were found in this study, which seems to point out that the gain of disturbed habitat species could compensate for the loss of forest species (Roberts, 2002; Schoonmaker & McKee, 1988).

Nevertheless, NMDS analysis detected sharp differences according to the vascular plant species present at riparian forest and plantations. Most of the indicators species identified from natural forests (*Apet, Bsyl, Ccup, Eamy, Epar, Fulm, Gpal, Grob, Hlup, Lper, Lvul, Rcae, Rulm, Snig* and *Ssyl*) have been previously identified as species with high requirements in shade and/or edaphic moisture, even temporal flooding in some cases, which is just typical of riparian zones (Aizpuru et al., 1999; Alejandre et al., 2006).

Moreover, NMDS ordination reflected a clear differentiation between riparian forests and poplar plantations according to CSR strategies. Whereas plant communities in riparian forests mainly consisted of Stress-Tolerant Competitors species (SC), poplar plantations held mostly Ruderal species (R). NMDS ordination showed differences between both types of habitats mainly along the horizontal axis, which in terms of Grime's theory would be due to disturbance factor. On the other hand, no differences were found between types of habitats along the vertical axis, which would be influenced by stress factors in accordance with Grime's triangle (Grime, 1974, 1977, 2001). Thus, it appears logical to believe that disturbances (mechanicall site preparation for plantation and harrowing) carried out in poplar plantations are the key factor to modify plant communities and ecological processes, whereas stress seems to be less important. These results are consistent with findings in other areas, where continued physical disturbance has played a key role in primary succession (Caccianiga et al. 2006; Grime, 2001).

4.2. Comparison among types of poplar plantations

As noted previously, species richness differed according to age and management of the plantation. In particular, a declining trend of species richness as it matured was found, which seems to be due to a fall in the number of annual species or short-lived perennial species. This type of plants sets aside a considerable effort to produce a high number of seeds, which are able to survive in the soil (seed bank) for long periods and, if a disturbance, such as plantation or harrowing, exposes the seeds to light and the climate is favorable, they can quickly establish and grow (Decocq et al., 2004a; Grime, 1977).

Subsequently, a progressive canopy closure would reduce the available shortages of light for plants and a loss of generalist species should occur (Berthelot et al., 2005; Nagaike et al., 1996, Qian & Klinka, 1997; Small & McCarthy, 2005). Similar results were found by Archaux et al. (2010), who pointed out that a decline in species richness is expected until plantations are older than 12-13 years, at the point in time when some forest plant species could start to become established. This might explain the fact that no differences between young and mature stands in hardwood forests were found by Gilliam et al. (1995), since their young stands were around 20 yr-old and a recovery of forest plant species could have already been hold, which seems to be confirmed by the fact that species composition was quite similar regardless of stand age.

On the other hand, management (i.e. harrowing carried out the last year) decreased in half the species richness in poplar plantations, which was because of removal of species as a direct result of harrowing. While a half of species were able to be established in less than a year, the remaining could require a longer time, emphasizing the importance of life strategies to determine plant assemblages. However surprisingly, Laquerbe (1999, 2000) and Berthelot (2001) reported the opposite pattern in French poplar plantations. Lastly, differences in species richness according to site quality were not found in our study, contrasting with Chen et al. (2004) who observed differences in *Populus tremuloides* stands of northern British Columbia, Canada.

IndVal analysis seems to point out that disturbance carried out on poplars (mechanicall site preparation for plantation and harrowing) determine the vascular plant assemblages, since all indicator species found were, to a certain extent, ruderal plants (Aizpuru et al., 1999; Alejandre et al., 2006). Although species indicators were found according to site quality and age, clear groupings cannot be determined and probably these clusters could be due to a random effect as a result of the low number of replicates.

On the other hand, although poplar plantations hosted mainly ruderal species, NMDS ordination showed clearly the triangle of the Grime's theory (Grime, 2001), in which two factors (number of harrowings and percentage of coarse fragments) seem to determine the type of strategy CSR reached into each stand. This finding is very consistent with this theory, since the axes C-R and C-S, which in terms of Grime's CSR model should be determined by disturbance and stress factors respectively, are parallel to the number of harrowings and percentage of coarse fragments, correspondingly (Fig. 4). As regards disturbance, plant communities seem to be determined by the frequency of disturbance (number of harrowings [p = 0.04]) rather by the time since the last disturbance (years since the last harrowing [p = 0.91]). In the same vein, Decocq et al. (2004b) reported that some vascular plant species were mainly limited by the frequency of disturbance and not by its severity.

Our outcomes seem to indicate that soil properties, which determined productivity of habitat (i.e. conversely, stress levels), have an influence on vascular plant communities, whereas quantity of light transmitted to the understory was not a key factor. Thus, NMDS ordination retained the variable "percentage of coarse fragment", which was previously recorded as the best indicator of a gradient of productivity in these poplar plantations, since this variable determines the volume of soil available for the development of roots, the amount of available nutrients per unit of soil volume and the soil water holding capacity (Martín-García et al., 2012).

Moreover, variables related to the quantity of light, such as canopy closure or dendrometric variables, were not retained by the model. This could be due to the fact that the structure of poplar plantations (a low density of plantation around 278-400 stems/ha and that all trees are pruned up to 6-8 meters above ground level) allowing enough light to be transmitted to the understory. Similar results were reported by Chen et al. (2004) in *Populus tremuloides* stands of northern British Columbia and by Klinka et al. (1996) in several tree species on West of Vancouver Island (Canada).

MRPP tests confirmed differences in CSR strategies according to site quality, although this trend was observed to a larger extent in young stands (Fig. 4). This fact may be due to an increase in soil nutrients with age of the stand as a result of the return of some nutrients in soils through litterfall (Das & Chaturvedi, 2005) or enhancement of mineralization of some nutrients because of a well-developed root system (Browaldh, 1997). In turn, deficiencies of several nutrients, such as nitrogen, phosphorus and boron, were already noted mainly in these young stands (Martín-García et al., 2012).

On the other hand, MRPP tests reflected a clear differentiation of CSR strategies between poplar plantations according to age, but not with respect to management (harrowed or not harrowed during the last year). This fact could be due to the effect of harrowing during the last year, which is not as important as the historical disturbances, as was previously noted. Thus, the significant effect found by MRPP test according to age is probably an indirect effect because of a higher number of harrowings in adult stands (ANOVA, $F = 4.63$, $p < 0.04$). A direct effect of age would be expected with older poplar plantations, which is consistent with findings in other areas (Archaux et al., 2010; Laquerbe, 1999, 2000), but in practice this is unlikely because of the economic aspects.

5. CONCLUSIONS

Our outcomes indicated that poplar plantations did not hold specific riparian species and therefore poplar plantations should not be used as surrogates of native forests from the point of view of vascular plant diversity. Thus, native riparian forests should be preserved or restored as far as possible.

Disturbance, and mainly harrowing, seems to be the key factor to determine the vascular plants in poplar plantations, affecting not only plant assemblages, but also plant strategies. To determine the vascular plant communities, the number of harrowings is the most influential variable, even greater than the time, which has elapsed since the last harrowing. Furthermore, despite the fact that poplar plantations cannot accommodate typical riparian plant species, it was observed that a high number of harrowings provided numerous Ruderal species (R) in detriment to Stress-Tolerant Competitors species (SC). Thus, minimising the use of harrowing would be desirable, as long as it does not affect tree growth and vitality through competition, in order to favour Stress-Tolerant Competitors species, which is a plant strategy closer to native riparian forests.

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