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Biomolecules from olive pruning waste in Sierra Mágina

Engaging the energy transition by multi-actor and multidisciplinary analyses

Marianne Cohen[1], Gilles Lepesant[2], Farida Lamari[3], Clelia Bilodeau[4], Petra Benyei[5], Stéphane Angles[4], Julien Bouillon[6], Kevin Bourrand[6], Ramla Landoulsi[1,6], Delphine Jaboeuf[1,6], Maria Alonso-Roldan[7], Isidro Espadas[7], Véronica Belandria[8,9], Philippe Silar[6], Moussa Dicko[3,*]

[1]Univ Paris Sorbonne, Sorbonne Universités, UMR 8185 ENeC, 75005 Paris, France

[2] CNRS, Géographie-Cités, Paris, 75005, France

[3]Univ Paris 13, Sorbonne Paris Cité, CNRS LSPM UPR 3407, 93430 Villetaneuse, France

[4] Univ Paris Diderot, Sorbonne Paris Cité, LADYSS, 75205 Paris CEDEX 13, France

[5]Universitat Autònoma de Barcelona, Institut de Ciència i Tecnología Ambientals (ICTA), Laboratori d'Anàlisi de Sistemes Socioecològics en la Globalització, 08193 Barcelona, Spain

[6] Univ Paris Diderot, Sorbonne Paris Cité, LIED, 75205 Paris CEDEX 13, France

[7] Non governemental organization PASOS (Participación y Sostenabilidad), Granada, Spain

[8]Institut de Combustion, Aérothermique, Réactivité, et Environnement (ICARE)-CNRS UPR3021, 1C avenue de la recherche scientifique 45071 Orléans Cedex 2, France

[9] Université d'Orléans, Institut Universitaire de Technologie, 16 rue d'Issoudun BP16724 45067 Orléans Cedex 2, France

* corresponding author

Moussa Dicko

E-mail: moussa.dicko@univ-paris13.fr, Tel: +33149403441, Fax: +33149403414

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Abstract

The volatility of fossil resources' prices, the uncertainty of their long-term availability and the environmental, climatic and societal problems posed by their operation, lead to the imperative of an energy transition enabling the development and utilization of other alternative and sustainable resources. Acknowledging that indirect land-use change can increase greenhouse gas emission, the European Union (EU) has reshaped its biofuel policy. It has set criteria for sustainability to ensure that the use of biofuels guarantees real carbon savings and protects biodiversity. From a sustainability perspective, biofuels and bioliquids offer indeed both advantages (*e.g.*, more secure energy supply, emissions reductions, reduced air pollution and production of high added-value molecules) as well as risks (monocultures, reduced biodiversity and even higher emissions through land use change). Approaching economic, environmental and social sustainability at the local level and in an integrated way should help to maximize benefits and minimize risks. This approach has been adopted and is described in the present work that combines chemical, biological, social and territorial studies for the management of pruning waste residues from olive trees in the Sierra Mágina olive-growing area in Spain. Biological and social analysis helped to orientate the research towards an attractive chemical process based on extraction and pyrolysis in which high added value molecules are recovered in the extracts and the residual biochar may be used as pathogen-free fertilizer. In this region where farmers face declining margins, the new intended method may both solve greenhouse gas emission problems and provide farmers with additional revenues and convenient fertilizer. Further research with a larger partnership will consolidate the results and tackle issues such as the logistics one, which stemmed from geographic analysis.

1.Introduction

1.1 General context and main issues

In Mediterranean Europe, olive-growing systems play an important role to ensure cohesion of rural areas and to prevent economic and social marginalisation, especially on sloping lands (Stroosnijder et al., 2008). While European countries account for almost 70% of the global production of olive oil (mean 2010-15, IOC, 2015), a production expected by the EU to continue growing significantly in the future, the olive-growing regions are currently facing serious problems, especially those triggered by the one crop systems. The fall of margins and operating incomes over the past fifteen years (EC, 2012b) is combined with increasing environmental threats (Beaufoy, 2001), such as water shortage (Garrote et al., 2015), soil erosion (CHG, 2010), biodiversity loss (Camarsa et al., 2010) and demands to lower air pollution as well as CO_2 emissions, both resulting in part from the burning of pruning waste (AAE 2013).

This environmental impact concerns an increasing amount of agricultural land in EU Member states, about 5 million hectares in 2007 (Camarsa et al., 2010). The carbon balance of olive-growing systems could be greatly improved by the valorisation of pruning wastes that amount to several tens of kilos per tree (La Cal Herrera, 2013; AAE, 2013). In Southern Spain, this waste is currently partly burnt on agricultural plots with significant greenhouse gases (GHGs) emissions, partly chipped and used for mulch (Calatrava and Franco, 2011). New income opportunities should be searched to face such challenges, for example a better valorization of agricultural residues. Moreover, the identified solutions should contribute to the mitigation of the carbon balance of rural Mediterranean regions, which are vulnerable to climate change (Gualdi et al., 2013), with important consequences on the decreased yields of olive trees (Tunalıoglu et al., 2012).

The human factor plays a crucial role in the success and failure of sustainable energy policies. Land-use change towards energy-oriented crops (*e.g. Miscanthus*) and associated large industrial plants face serious societal challenges (*e.g.* low social acceptation of rural landscape change, considered as a cultural heritage). However, the objectives of food and energy production, often considered as conflicting, may be conciliated through the valorization of agricultural biomass from existing agro-systems that are otherwise neglected (Söderberg and Eckerberg, 2013). Our project aims to develop innovative and locally-based initiatives, engaging farms and other types of micro-enterprises such as olive mills in research and demonstration activities. Enhancing the range of biomasses used in second generation small bio-refineries, integrated with olive mills, will help avoid food/fuel conflicts and support economic development of rural areas in the Mediterranean Europe (Gómez-Vázquez et al., 2009). Through a multi-actor approach, in partnership with local actors and responding to a social demand to improve the environmental impact of their agro-systems, we designed a biorefinery concept, which embraces the diagnosis of the local context, its congruence with emerging European policies, the evaluation of the risk due to the innovation, and finally its feasibility. The attention focused on the local level through this three-step method (diagnosis-risk-solution), has strengthened the collaboration between the disciplines involved in the project.

1.2 Case study at the local level

Sierra Mágina is a rural county, located in Jaén Province, Western Andalusia (Southern Spain). It is highly specialized in olive-growing, with 80% of arable lands occupied by olive groves, totalizing an area of 50,617ha (40 % of the total area of 15 municipalities, around 1390 km^2, geographical data basis SIGPAC, MAGRAMA 2006). Olive groves are located mainly on sloping lands from 294 meters up to 1466 meters altitude, surrounding a Natural Park and its highest peak (19,794 ha, 2167 m, average slope 23°), and along the course of important rivers, like Guadalquivir or Guadalbullón, which are used for irrigation for half of the olive-groves surface area (Sánchez-Martínez and Gallego-Simón, 2009). An important concern for local actors is the economic valorization of the olive oil, a great challenge in the context of decreasing prices on international markets. According to Sanz Cañada et al. (2013), only 20% of the production of olive oil is sold as protected designation of origin (PDO), while the majority is sold in bulk.

Sierra Mágina benefits from a Mediterranean climate, with rainfall varying from 350 mm to 800 mm. As for many other Mediterranean regions, the climate is expected to change in the future, with a decrease of 9% in rainfall by 2050 and consequently of olive yields (7% in rain-fed and 3.5% in irrigated olive groves, Ronchail et al., 2014). Adaptation to these changes is a priority issue, but it must be addressed taking into consideration the concerns about the carbon balance of the olive-growing system and the mitigation of climate change. The carbon footprint of olive grove monoculture is currently unsatisfactory for two reasons: the importance of hydric erosion despite a dedicated policy (Ballais et al., 2013), and the low valorization of the pruning residues (Benyei, 2015).

Olive yields are stimulated by pruning practices, with annual frequency in young groves and biennial in older ones. Due to the important areas covered with olive groves in the region, the growth habit of the ultra-predominant variety (*Picual*), the width (up to 45m^2) and height (up to 3m) of the trees, and the local practices of drastic pruning, this agriculture produces a high quantity of ligneous pruning waste. Until a few years ago, pruning waste was fully burnt in the agricultural plots, contributing to greenhouse gas emissions. Olive mill managers were concerned about this matter, because of the negative regional image it was giving to final consumers, contributing in their rationale to the low valorization of olive oil. In the same way than in other Southern Spain regions, chopping and spreading the residue on the ground, partly replaces the traditional waste management.

1.3 OLIZERO Biorefinery concept

The proposed OLIZERO biorefinery concept is part of an interdisciplinary thinking that aims to optimize the use of pruning waste available in a specific territory. It defines local and integrated production of intermediate and/or finished products generated by wastes, including high-added-value molecules (from solvents to aroma, or flavors and products of medical interest) and biofertilizer. The final objective is thus to develop innovative methods for recovering valuable chemicals and products from the most difficult fractions produced by olive trees, the lignocellulosic ones. It proposes to recover first high value molecules with chemical extraction, followed by pyrolysis. The final residue, the biochar is then reintroduced in soils as fertilizer. Within this biorefinery concept, we also assessed the potentialities and constraints due to the endophytic fungi contained in the pruning residues. A pre-fungal attack may lower the cost of the biorefinery process, as long as these fungi do not constitute a disease risk for olive-trees.

Pyrolysis is an advanced technology able to produce biofuels and biomolecules from biomass

(Czernik and Bridgwater, 2004). Pyrolysis is an attractive alternative to a simple combustion which would cause GHG emissions and low performance of the process due to high ash contents (Garcia et al., 2012). Different extraction methods have previously been performed on olive tree cuttings. However, most published studies focused on phenolic compounds in olive leaves (Altiok et al., 2004; Le Floch et al., 1998; Talhaoui et al., 2014). Indeed, these polyphenols have been demonstrated to exhibit anti-carcinogenic, anti-inflammatory and antimicrobial proprieties (El and Karakaya, 2009; Talhaoui et al. 2014). In the present biorefinery investigation, Soxhlet extraction and pyrolysis coupled with Gas Chromatography Mass Spectroscopy (Py-GCMS) were performed on olive leaves from pruning waste. This second technique is a powerful tool to determine the composition of evolved gases from biomass (Akalina and Karagöz, 2014). It provides a picture of what kind of chemicals may be obtained using a pyrolysis process. The pyrolysis of agricultural residues, including olive mill wastes, has been widely reported in the literature (Zanzi et al., 2002; Encinar et al., 1998; Oasmaa et al., 2010). However, pruning waste has received less attention than olive mill waste and has been essentially dealt with by the Biomass group of Pr. Zabaniotou (Pütün et al., 2005; Zabaniotou et al. 2000, 2015a, 2015b; Valenzuela Calahorro et al., 1992). Importantly, their work is systematically focused on the energetic valorization of the residues. Overall, a different model of waste management in agricultural territories is proposed in the present work. It explores the opportunity to produce simultaneously biomolecules of interest (specialty chemicals, with cosmetic applications…), along with biofuels and fertilizers.

2. Materials and methods

2.1. General methodological framework

Our research had two objectives:

1) Assessing the capability of olive-growing territories to move towards a low energy model. Our integrated method combines remote sensing and GIS with social and economic inquiries and the analysis of the congruence of Common Agricultural Policy (CAP) and energy European policies.

2) Demonstrating and developing a technology able to generate new bio-energy and bio-chemicals, thanks to collaborations with chemical engineers and biologists. This biorefinery concept could then be implemented on test-territories with private partners (small enterprises, olive-mills, cooperatives and an engineering company).

We present our methodology through a three steps process: diagnosis, risk analysis and proposed solution to generate innovative bio-energy and further bio-chemicals.

2.2 Inquiries at the European and local levels

We analyzed the socio-political feasibility of our project at both the European and local levels, using different methods.

2.2.1. European Union's policy:

The analysis of the EU's role in setting an overall framework for biofuels development across Europe is based on a review of the literature produced by the EU institutions, mainly the European Commission and the European Parliament. Additional details and up-dated information have been collected thanks to interviews carried-out at DG ENER, DG REGIO, DG AGRI, DG GROW, DG ENV, which are in charge of Energy, Regional policy, Agriculture, Internal market and Environment, respectively, in 2015 and 2016.

2.2.2. Social perception of the OLIZERO biorefinery project at the local level

The social perception of the OLIZERO project has been evaluated along with the changes in pruning practices by a sociological survey. The evaluation relied on field enquiries, with 20 in-depth interviews, 40 questionnaires randomly sampled in 8 municipalities and 2 participatory workshops (Benyei, 2015).

2.3. Diagnosis of the situation of pruning residues

We determined the tonnage of pruning waste by means of a crossed analysis between field data, remote sensing and Geographical Information System (GIS) analysis of the olive tree cover all over the Sierra Mágina region.

2.3.1. Relation between tree crown size and pruning residue weight

We weighted the pruning residue of georeferenced trees on field and tested the correlation with tree crown sizes digitized on aerial photography using GIS. We obtained a highly significant correlation ($r = 0,985$, $p=0.02$) despite the small size of our tree sample (5 trees).

2.3.2. Assessment of olive tree acreage and pruning residues

We determine the olive tree acreage in the whole region and converted the estimated number into pruning waste weight using the relation tree crownsize/waste weight. For this, we updated the GIS of land use of the property plots, established by the Ministry of Agriculture and Environment for the application of the Common Agricultural Policy (SIGPAC, MAGRAMA, 2006) using aerial photography of 2010 and 2011 (Source: Junta de Andalucía, Jaboeuf 2015, unpublished), that were automatically downloaded with the MEMOTF program from Bourrand et al. (2015). Between 2006 and 2010-2011, 1485 ha of new olive groves have been gained mainly upon grain lands, as observed in prior inquiries (Alonso, 2010, Cohen et al., 2014). We applied an iso-cluster analysis on a spatial selection of the aerial photography occupied by olive groves, after enhancing the contrast of the 3 colored bands. This automatic procedure was the most efficient, showing the lowest difference with manual digitalization of 11,177 trees. Despite this, a visual assessment carried on a sample of 522 parcels showed that the olive tree acreage was correctly handled only in 193 plots. To improve this result, we computed the NDVI index (Normalized Vegetation Index), from Landsat Images from the same dates. There was a significant correlation between NDVI and the olive trees acreage in the 193 plots ($r=0.7$, $p<0.0001$), as shown by Peña-Barragán et al. (2004). The NDVI improved significantly the calculation of the density of olive-trees (Mann Whitney test, $p<0.0001$). The whole process was performed using GIS (Arcgis10.2© software), in a simpler way than by remote sensing analysis (tree counting analysis: Masson et al., 2004, Ke and Quackenbush, 2011; Mulla, 2013; CLUAS software: García Torres et al., 2008).

2.3.3. Determining the potential of processed pruning waste

To determine the proportion of pruning waste easily removable by truck for processing, we used GIS analysis crossing georeferenced survey responses, road network and the map of pruning residues. From the 40 survey responses randomly performed in 8 municipalities (§ 2.2.2), the 55 plots that farmers had drawn on a topographical map were georeferenced using GIS and crossed with the digitized plots (source: SIGPAC, 2006; Landoulsi, 2015, unpublished). We estimated the spatial criteria of pruning waste management (burning, chipping or combined management) and considered that the type of waste management done by the farmers is a good proxy of the feasibility of our biorefinery concept. Both are relying on the accessibility of the parcel by chipping engines or by trucks for the removal of the pruning waste. We selected in the subsequent steps the roads allowing traffic (2239 km out of 5585 km). We computed the geographical features of the 55 olive groves with GIS: slope, altitude, surface area and distance to roads. We performed statistical tests and a classification tree to determine the driving factors of pruning waste management.

2.4 Olive tree fungal endophyte sampling and identification.

2.4.1 Sampling procedure

57 samples of olive branches were collected in 19 plots (3 trees per plot), selected to represent the different altitudes (from 534 to 1057 meters) and exposure of olive groves. They were scattered in a large area within a buffer of 250 meters along roads passable to traffic in order to accelerate the sample collection. Samples were kept cool from the field collection until the laboratory. Among these 19 plots, 14, well-conserved, were selected to be processed in the laboratory, representing a range of altitude from 573 to 1057 meters. We tested the influence of sampling parameters*, i.e.,* altitude and exposure on the genus of fungi with a Chi square analysis. We also mapped the proportion of potential pathogenic fungal in the samples, using their geographic coordinates (in UTM ED50).

2.4.2 Identification

Samples of olive pruning wastes were surface sterilized by incubating one minute in ethanol 96%, two minutes in NaOCl 10%, then 30 seconds in ethanol 96% and finally rinsed in sterile water. Sterilized leaves and small twigs were deposited onto potato dextrose agar (PDA) Petri plates and incubated for five days at 27°C. Fungal mycelia originating from the samples were collected and inoculated onto fresh PDA plates. Any one sample yielded from one up to six different fungal strains. DNA from the samples was then extracted using the protocol of Lecellier and Silar (1994). The ITS regions were amplified using the ITS1 and ITS4 primers (White et al. 1990) and send for sequencing. Identification was made by comparing the sequences with the GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and UNITE (https://unite.ut.ee/) databases.

2.5. Chemical analysis

2.5.1. Samples: Leaves for Soxhlet extraction were obtained from branches brought after the field survey. For Py-GCMS, leaves sampled in different olive groves were used. Finally, a single location has been selected to assess the effect of temperature since location had no impact on the results.

2.5.2. Reagents: Acetone and diethyl ether were purchased from Sigma Aldrich with 99% and 99.5% purity respectively.

2.5.3.Soxhlet extraction : The samples (3 g) were extracted with 250 ml of each solvent (acetone and diethyl ether) during 16 and 22 h (96 and 132 cycles respectively), refluxing in a Soxhlet apparatus. The extract was then concentrated and chemically analyzed by GCMS analysis device. The solvents were totally evaporated under reduced pressure in a rotary evaporator. The mass of extractives was then measured. The GC methods were as follows. For acetone, it began at 303.15K (for 1 min) followed by a heating rate of 5K/min during 12 minutes up to 363.15 K then a heating rate of 8K/min during 23,7 minutes up to 553.15 K and stayed at 553.15K for 1 min. For diethyl ether, the method began at 323.15K (for 1 min) followed by a heating rate of 10K/min during 5 minutes up to 373.15 K then a heating rate of 8K/min during 22,5 minutes up to 553.15 K and stayed at 553.15K for 1 min.

2.5.4. Py-GCMS method: Samples of olive pruning waste (leaves), prepared with reproducible weights of 0.5 +/- 0.1 mg, were analyzed using analytical pyrolysis with a Pyrolyzer PY3030 (Frontier Lab) coupled to a gas chromatograph and mass spectrometer (GCMS-QP2010Ultra SHIMADZU) under continuous Helium flow (1.24 ml/min). The gas chromatograph (GC) was equipped with a polar capillary column ZB-1701 (30mx0.25µmx0.25µm) with a (14 %-cyanopropyl-phenyl-86 %-dimethylpolysiloxane) phase. As a preliminary step, 2 samples were analyzed for 3 different locations at 773.15K. Then, two pyrograms were produced from the same sample location at four different temperatures (673.15K, 773.15, 873.15K, and 973.15K). In order to provide selective compound separation, the GC heating method began at 303.15K (for 1 min) followed by a heating rate of 3K/min during 13,3 minutes up to 343.15 K then a heating rate of 8K/min during 26,2 minutes up to 553.15K and stayed at 553.15K for 1 min. For Py-GCMS and GCMS analysis, the temperatures of the injector and detector were set at553.15K and 473.15K respectively. The ionization mode on the MS was electron impact. The mass range from m/z equal to 25 up to 600 was scanned and the identification of the compounds relied on NIST Mass Spectral Library 2011. The results were relative and qualitative. They were expressed in area percentages of the Total Ion Chromatogram (TIC).

2.5.5. Thermogravimetric Analysis (TGA): The TGA of the samples was performed with a NETZSCH STA 449 thermogravimetric analyzer under dynamic nitrogen atmosphere (30 mL/min). The olive pruning residue was analyzed without treatment. A weighted sample of approximately 35 mg was placed on an alumina crucible inside the furnace and vacuum was applied to create a minimum oxygen environment. The sample was heated up to 1173.15K with a heating rate of 10K/min. The sample temperature was measured with a type S (Pt–Rh10/Pt) thermocouple which was placed under the sample holder and mass changes were recorded as a function of temperature. The overall mass loss measurement uncertainty is expected to be within ± 0.5 %.

3. Results

3.1. From UE policies to local level: socio-political feasibility of the biorefinery project

3.1.1. Congruence between OLIZERO biorefinery project and EU policy

The OLIZERO project takes place against a background characterized both by an increasing share of renewable energies in the European energy mix and by controversies related mainly to social acceptance of these energies (especially in the case of wind energy) and to the carbon footprint of bioenergy. Against this background, a better mobilization of cellulosic wastes and residues in EU countries, estimated by Searle and Malins (2013) as the quantity of each feedstock left over after environmental concerns and existing uses are taken into account, would be relevant. The amount of material left on site under sustainable harvesting practices to protect against soil erosion and soil carbon loss varies among EU Member States but is significant in some areas (Searle and Malins 2013). Bio-refineries supplied from lignocellulosic biomass and implementing emerging technologies such as pyrolysis process in an olive-growing area would in this context be relevant as it would address at the same time sustainability issues linked to bioenergy and uncertainties of the olive oil sector in Europe.

The EU accounts for almost three quarters of the global production of olive oil, the bulk of which being concentrated in four countries (Spain, Italy, Greece and Portugal). Spain, and especially Andalusia, depends heavily on big holdings, whereas the sector is more fragmented in other producing countries (EC, 2012a). The European Commission expects the production to grow significantly in the years to come and the area of irrigated olive grove could expand accordingly between 2011 (681,000 ha) and 2020 (771,000 ha). Olive oil production could reach 1.68 million tons by 2020, the exact figure being influenced by climatic conditions. This trend is however disconnected from margins and income indicators that have shown a clear downward trend since 2000. From 2000 to 2009, income in Spanish olive farms has experienced a one-third drop in nominal terms (-38% in family income per work unit) in a context of lower market prices (EC, 2012b). Thus, harnessing the full value of their production through additional by-products makes sense for farmers, especially in areas such as Andalusia where additional income sources are scarce.

In its Action Plan adopted in 2012 (EC, 2012c) to support the olive oil sector, the European Commission did acknowledge the overproduction crisis of the sector in Spain but didn't mention the role the sector could play in energy supply. The stress was instead put on improving quality standards and on better structuration of the supply chain in order to strengthen the bargaining power of producers. Agri-environmental measures have also been advocated in the framework of the second pillar of Common Agricultural Policy (CAP). The CAP has indeed been reshaped for the 2014-2020 programming period and substantial changes have been introduced concerning environmental protection. 30% of direct payments to farmers are now asked to comply with environmental "greening" measures. Furthermore, environmental protection, including climate change aspects and the production of renewable energy has been strengthened in the Rural Development Policy. In each Member State, 30% of rural development funds have to be spent on measures linked to the environment policy or to climate change mitigation. Hence, funding for innovative solutions aimed at optimizing the use of available residues might be found in the Cohesion and in the Rural Development policies rather than in the framework of the Energy policy.

3.1.2. Socio-economic feasibility of OLIZERO biorefinery project at the local level

According to our inquiries, part of the farmers, incentivized by olive mills and local authorities, has recently changed practices. The burning of pruning waste is currently being replaced in part by chipping and composting the chips on the soils, a practice with less emission of GHG, but which generates a potential risk of pest contamination (Koski and Jacobi, 2004), mostly unknown by farmers.

A sociological analysis has shown that this change was not only triggered by economic or ecological reasons, but by a complex farmers' rationale. Chipping and composting pruning waste is easier and less dangerous than burning it in dense olive orchards, the more modern and intensive ones. Moreover, it does not need a prior authorization, nowadays mandatory for burning pruning residue due to the forest fire prevention policy. Another advantage of chipping and composting, according to the farmers, is an expected improvement of the chemical and physical properties of the soil, the latter being currently degraded (Ballais et al, 2013). Finally, the small number of chipping machines is not an obstacle, due to lending practices between farmers and to the existence of pruning service providers. According to a limited number of survey responses, one third of the farmers is currently chipping, one third is burning, and the last third mix both practices (Benyei, 2015).

Our purpose of implementing a new process of valorization of pruning waste has been largely debated in participatory workshops with farmers and institutional actors, leading us to improve our proposals, as exposed in the discussion section. Local stakeholders showed their interest for the OLIZERO concept, but made suggestions that the team took into consideration in a multi-actor approach. The first suggestion was to fully integrate the carbon balance of the innovation (for example the cost of transporting the pruning waste), the second concern was the economic viability of the innovation, and the possible return for the farmers who already invested in machines and energy for pruning waste chipping. Moreover, according to farmers, chipping pruning waste and composting on the ground bring benefits for the soil, avoiding erosion and fertilizing it. This is one of the reasons, why the process includes the return of biochar to compensate the “loss” of the compost due to the project (chips will be used in the pyrolysis process), as this residue is a recognized fertilizer (Steiner et al., 2007).

3.2. The situation of pruning residue

The map of biennal pruning waste, in tons per hectare, shows a wide range of values, from nearly 5 tons/hectare to almost 20 tons/hectare. The biennal pruning waste weight per surface is significantly and negatively correlated with the distance to the Nature Park ($r=-0.313$, $p<0.0001$) and more weakly with the size of the plots ($r=-0.278$, $p<0.0001$); the altitude and the slope of the plots are very weakly but significantly correlated with the waste weight ($r=-0.023$, $p<0.0001$; $r=0.178$ $p<0.0001$; respectively). Large plots are generally newly planted olive groves, with smaller trees, although with a higher density than traditional groves, and located in lands previously occupied by crops, at the periphery of the Sierra Mágina district.

Among the geographical features computed in the 55 selected surveyed olive-groves: altitude, slope, surface of plots and minimum distance to wide asphalt roads, the last one is significantly linked with the type of waste management (Kruskal-Wallis test, p=0,017). The average distance to roads is higher in plots where pruning waste is burnt (343 m), than in parcels with mixed (213 m) or chipping practices (106 m). The threshold of 256 meters has been found using a classification tree. Above this distance, 17 out of 19 olive groves are totally or partially burnt, and only 2 are not burnt. We further built a buffer area of 512 meters along the road network.

In Figure 1, the quantity of biennal waste is colored in different tons of red, when they are located less than 256 meter from a road and in different shades of green when they are located further. We observe that most removable pruning waste is located less than 10 kilometers from an olive mill, except at the extreme east of the county. These industrial scale olive mills could be used to shelter and process the pruning residue. This moderate distance is important to reduce the cost of transportation, an important issue notified by farmers during the engaging workshops. In conclusion, 41% of biennal pruning waste is removable by trucks, representing an amount of 340,734 tons. Every year, around 170,367 tons are thus potentially available for biomass processing.

3.3. Endophytic fungi analysis

Because unattended pruning residues may nurture parasites, we analysed the fungi they may contain, as fungi usually represent 85% of the diseases of plants. To this end, fresh leaves and twigs sampled from several pruned trees were surface-sterilized and endophytic fungi were isolated on PDA. Identification was made by sequencing the barcode, *i.e.*, the Intergenic Transcribed Spacers (ITS) of the rRDNA cluster. Seventy five strains were successfully cultivated and analysed for their barcode (Table 1). Analysis showed that the most frequent species (24 out of 75) belonged to the genus *Alternaria* and its relative *Pleospora*, as previously found in Olive trees from the Baleares (Fisher et al., 1992). Many strains belonging to both genus are plant pathogens, including some pathogenic to Spanish olive trees (Moral et al., 2008). Other frequent endophytes belonged to genus *Biscogniauxia* and *Preussia*. The *Biscogniauxia* species were related to *Biscogniauxia mediterranea*, a pathogen of oaks (Henriques et al., 2014). Additional species were found to belong to *Pezizomycetes*, *Dothideomycetes* and *Sordariomycetes*, some of which were related to strains potentially pathogenic to the olive trees, such as *Cladosporium sp.* and *Aureobasidium pullulans* (Chliyeh et al., 2014). Olive tree pruning residues of the Sierra Mágina harbour thus fungi frequently recovered in surveys of endophytes, but also that are potential plant pathogens, including some known to attack olive trees.

The type of fungi (potentially pathogenic or not) was significantly linked with the altitude category, according to the chi square analysis (observed value 14.36, critical value 5.99, p=0.0008). Potentially pathogenic fungi are significantly over-represented in a range of altitude from 573 to 736 meters and under-represented in a range of altitude from 797 to 944 meters of altitude. At an altitude of over 1000 meters, pathogenic fungi are under-represented, without this being significant. The type of fungal genus is not significantly linked with sun exposure. The map of the proportion of pathogenic fungal varies does not show a clear spatial pattern (Figure 2). This suggests that pruning residues are much more expected to host fungal parasites in olive-groves located at lower altitude.

Overall, the data suggest that unattended pruning residues may indeed host and nurture fungal parasites. It would therefore be recommended to dispose of the waste in a way that limit potential threat from fungi*, i.e.,* that would sterilize the pruning residues. Biochar is well-suited for such purpose.

3.4. Biomolecules production

Olive leaf extracts have already gained particular attention for their healing properties (El and Karakaya, 2009). These properties are attributed to polyphenols, such as oleuropein and tyrosol, because of their antioxidant activity. Numerous publications are dedicated to the extraction and the analysis of these compounds. HPLC is the preferred technique for such analysis since the polyphenols are mostly non-volatile (Herrero et al., 2011; Pereira et al., 2011; Abaza et al., 2015).In this work, extractives were recovered with acetone or diethyl ether and analyzed by GCMS. Despite the medium quality of the obtained chromatograms, it was sufficient to observe various families of molecules, such as acids, fatty acids / alcohols, condensed polyphenols. Some may also be found by

Py-GCMS analysis (see below). Precise identification of all the molecules would require the injection in the GC of standard compounds. Indeed, the similarity level with NIST database varied from high values (97% for acetic acid, dodecene, phenol, 2.4-bis (1.1-dimethylethyl)-) to intermediate (88% for eicosanol) and low (75% for benzoin). From a quantitative perspective, extractives are often present in small amounts (approximately 5%wt in lignocellulosic materials). Here, 10%wt extractives were recovered using acetone as extraction solvent and 3.3%wt with diethyl ether. More products were detected in acetone extracts than in diethyl ether. If future work confirms the presence in sufficient amount of extractives then useful applications in various fields such as lubricants, solvents, plastics, surface agents, cosmetics, polymers, resins, soap, detergents and fragrance could be considered.

Py-GCMS experimental device enabled to identify the decomposition products of the three major polymers in lignocelluloses, lignin, cellulose and hemicelluloses (Chen, 2014). Additional Py-GCMS data of olive leaves have not been found in the literature. The effect of the localization of the trees has been assessed by testing different samples (E1=Pt=45[2], E2=Pt=25[2], E3=Pt=24[1]) at the same temperature (773.15K). As can be seen in Figure 3, no effect was observed on the pyrograms.

In order to have an overview of the main biomolecules that could be produced via pyrolysis, the samples have been injected in a preheated oven at four different temperatures (673.15, 773.15, 873.15 and 973.15 K). Results are presented in Table 2 and a typical pyrogram in Figure 4. As expected from the thermogravimetric (TGA) experiment (Figure 5), a broad range of pyrolysis temperature can be selected to favor the emission of volatiles. Indeed, most of the compounds are volatilized over the 585-949K range (e.g. ~69% mass change). Therefore, pyrolysis temperature has been set 20 degrees after the second peak at 673.15K where the majority of the volatiles were emitted and subsequently at three higher temperatures. The results evidenced the molecules expected from lignocelluloses degradation with high intensity peaks. Indeed, typical phenolics, aldehydes, ketone and acetic acid were recovered. The applications of these molecules are well known in fields such as resins, solvents, chemicals, aroma*, etc.* (de Wild, 2011). It is noticeable that several compounds seemed to originate from the extractive part (musk ambrette, linoleic acid, tetramethyl-2-hexadecen-1-ol…). The increase in temperature favored the formation of low molecular weight molecules and less-functionalized molecules (toluene, xylene). The proportion of linear fractionation products increased while the one-cyclized compounds decreased. The complementary analysis of TGA and pyrolysis extractions, coupled with GCMS analysis, helped to identify the biomolecules production potential. These molecules of interest have various possible applications ranging from cosmetics, chemicals, materials to antioxidant precursors synthesis.

4. Discussion

Our interdisciplinary approach highlighted the challenges that are faced while implementing the new process. The technical and economic feasibility must be demonstrated in accordance with the collection of resources and plants positioning issues. Involvement of local stakeholders is crucial in order to understand their awareness about the risk of trees infection, their positions towards rural development policies and their opinion about different technologies such as pyrolysis. The following discussion encompasses the main steps of our analysis. First we question the congruence between the EU policy and the local situation. Secondly, we discuss about the feasibility of the innovation according to our geographical and socio-economical diagnosis. Thirdly we assessed the risks linked to the current practices, and finally the feasibility of the OLIZERO concept.

4.1. Discussion from the European policy analysis

Solid and gaseous biomass is by far the biggest source of renewable energy in the EU and is expected to make a key contribution to the 20% EU renewable energy target by 2020. Back to 2003, the EU adopted a biofuels support policy aiming at lowering CO_2 emissions in the transport sector. In order to comply with this policy, Member States introduced market support price mechanisms and excise duty exemptions. This policy has however triggered critic as it might have an adverse impact on food prices, which levels may increase due to induced indirect land use change (ILUC). Although estimates vary regarding the exact impact of biofuels production on global grain prices, a related concern has emerged on the carbon footprint of biofuels. Agricultural land expansion induces ILUC at the expense of forests and of grassland and cultivation increases CO_2 emissions. Again, studies vary on this specific issue but the European Commission acknowledged as soon as in 2010 that ILUC can increase CO_2 emissions and supported a precautionary approach (European Parliament, 2015).

In this context, the Commission decided in 2015 to amend its biofuels quality Directive and its renewable energy directive (EP, 2015/1513, Council 9/9/2015) and proposed a 5% threshold for first generation biofuels in the energy mix of transport by 2020. Finally, a compromise was found with the European Parliament and the Council on a 7% cap on conventional biofuels, including biofuels produced from energy crops. Furthermore, transition towards advanced biofuels (defined as biofuels produced from feedstock that do not compete directly with food and feed crops, such as wastes and agricultural residues as listed in the Annex IX of the 2015 Directive) is to be encouraged. The compromise found at the EU level stated that Member states will be required to adopt a target above a reference of 0.5 percentage points of the 10% target for renewable energy in transport. This renewed EU framework opens opportunities to local actors that are willing to engage in energy production without being exposed to the risk of increasing ILUC and CO_2 emissions.

However, one of our findings is that implementing a cross-cutting approach to foster sustainable bottom-up energy policies is very much required from local and regional actors. EU territorial development policies are indeed part of the toolbox to address opportunities and challenges related to biomass. In this respect, the Cohesion policy as well as the Rural Development Policy, also known as the second pillar of the Common Agricultural Policy, offer both funding and instruments to implement innovative policies, linking agricultural development, energy production and reduction of CO_2 emissions. Beneficiary countries have introduced into their programming documents for the 2014-2020 period specific actions in this respect, but local and regional actors may find it difficult to make full use of all the possibilities available across different EU policies.

4.2. Discussion from pruning waste and socio-economic diagnosis

The quantity of pruning waste is higher and more variable than assessed in previous studies (15,400 m^3/ha/yr in irrigated olive-orchard, with 100 trees/ha, La Cal Herrera, 2013, 3 ton/ha, AAE 2013).This quantity is potentially available for biomass process. But potential is not real. We have to consider several obstacles. First of all, the pruning waste is currently processed by farmers in their private lands. They consider the chipping cost affordable because of the advantages of composting residue on soils, including increasing organic matter content and avoiding erosion and excessive evaporation. Yet, chipping is more expensive (70 €) than burning (53 €, La Cal Herrera, 2013). Chipping is less widespread than in other regions (amounting to half the groves in the whole Jaén Province, La Cal Herrera, 2013; and in the Granada Province, Calatrava and Franco, 2011). Except the role of social interactions between farmers and of the low quality of soils, the drivers of chipping diffusion are different from those observed in 2005 by Calatrava and Franco (2011).

Farmers are unwilling to give up the supposed benefit for soils without compensation. As the economic balance of our biorefinery concept is still under study, it is risky to include a payment for pruning residue. This is one of the reasons why we redesigned the project by integrating a return as fertilizer of biochar, the ultimate residue of the pyrolysis (Steiner et al., 2007; Zabaniotou et al., 2015a). Note that farmers expressed certain unwillingness regarding one of our initial proposals to perform a fungal attack on waste residues before pyrolysis, as they feared the spreading diseases. This fungal pretreatment was aimed at improving yield of recovery of high-value molecules. It is thus for now absent from the process. However, we will further discuss this point in section 4.2, in the light of the results obtained on fungal endophytes. Another obstacle is the economic feasibility of our biorefinery concept. To evaluate it, we should consider the costs including transport by trucks in sloping land of voluminous pruning waste (modelling is in process) and quantities of available biochar, as well as the benefits such as the concentration of high-value molecules, biofuel production, soil improvement by biochar, reduction of greenhouse emission and a better image of the region leading to better valorization of olive oil.

4.3. Discussion from risk analysis

The potential risk due to the presence of endophytic fungi, potentially pathogen, is serious in a mono crop agricultural system and in the context of climatic change. This result leads us to the revision of our initial work hypothesis of using fungal attack in the OLIZERO concept. Moreover, the new practices of chipping and composting pruning waste may represent a danger for insect pests and fungal pathogen propagation (Koski and Jacobi, 2004). The recommended precautions about waste management are particularly addressed to olive-groves located at lower altitude, which are expected to host pathogenic fungi. In these olive-groves, the biorefinery concept that we propose should limit the potential threat from fungi and is feasible when the distance between olive-groves and roads remains below 256 meters. In olive-groves located at higher altitude, the risk due to potentially pathogenic fungi is not important. Nearly half of the pruning waste obtained in olive groves near the roads is located at an altitude higher than 797 meters, in the southern part of the district (161,439 out of 340,734 tons per hectare). Another part is located further away from the main roads, and for this reason pruning waste is not easily removable by trucks (Figure 1). Harmless endophytic fungi already present in the pruning residues may thus be used for a first attack of ligneous waste in these remote olive groves. The fungal attack should be an interesting alternative to reduce the volume of pruning waste in order to bring it to the road network for further processing (instead of burning it).

In the context of climatic change, the threat of pathogenic fungal should increase. In low altitude olive-groves, generally located near rivers, drip irrigation is largely used (Cohen et al., 2014). The future increase in maximum temperature (from 1 to 3°C, in 2030-2050, Ronchail et al., 2014), jointly with the moisture maintained by drip irrigation on the root system, should enable potential pathogenic fungi to turn into pathogenic fungi, increasing the vulnerability of olive-growing. These low altitude olive-groves are generally composed with younger trees which means that they represent the future of olive-growing in the region. For this reason, our prospect of a more sustainable waste management is part of the solution for the adaptation to climate change, and it is also a way to mitigate the ecological footprint of olive-growing and thus to contribute to the reduction of climate change itself.

4.4. Discussion from chemical analysis

Zabaniotou et al. (2015a) demonstrated that a pyrolysis process combined with olive mills and receiving pruning waste, kernels and pomace as feedstock can create a complete stand-alone decentralized bio-energy system. In their study, bio-oil is used for electricity generation. Not only it covers the energy requirements of the olive mill but also it produces a surplus. Pyrolysis is mainly developed for energetic purpose but it is also a way to produce chemicals (Scott et al., 1997; Czernik and Bridgwater, 2004; Yaman, 2004; de Wild, 2011; Zhang et al., 2013).The production of chemicals would require higher investments but could result in a more profitable plant if high value products are obtained. The previous results and the data published about extractions of olive leaves suggest the possibility to develop a two-step process. The first step would be an extraction process of high added-value molecules selected upon evaluation of the quantity and quality of the recovered extractives. Secondly, the residual lignocellulosic biomass could be dried and pyrolyzed in order to produce new molecules of interest, energy and finally biochar. The process temperature might be set at a lower level than usual (673K instead of 773K for wood) as suggested by TGA. Globally, this biorefinery would be able to produce at the same time olive oil, energy, high added-value molecules, biofuels and biochar for the soils. Sterile biochar would solve the issue of the potential risk of pathogens diffusion while improving soil fertility. The geographic analysis showed strong spatial heterogeneities emphasizing the importance of logistics and the need for an optimization of the waste collection. Different scenarios for a biorefinery implantation could be considered such as a mobile pyrolysis devices or a plant next to existing olive mills. Size, number and positioning of such devices must also be optimized. Further work will be necessary to produce actual bio-oil from pyrolysis gases, then to target specific molecules and optimize their recovery in extractives and condensed bio-oil.

5. Conclusion

In this work, we proposed a strategy for pruning waste valorization involving local stakeholders. The field study indicated that a favorable solution should emerge from a multi-actor approach integrating the concerns and perceptions of local actors. Farmers' belief that chipping pruning waste and composting on the ground would bring benefits for the soil has been seriously questioned by endophytic fungi analysis. These practices seemed to be risky since the detected strains might induce trees infection. In coordination with local stakeholders, our group performed preliminary studies, which indicate the path of an innovative biorefinery solution circumventing this problem, while coping with the need to maintain soil fertility.

The collaboration between social, biological and chemical engineering sciences brought many advantages: first, we chose a territory with a social demand to improve the waste management, along with a capacity of innovation and a high waste production. This encouraged us to improve the agronomical and economic benefits of the proposed innovation. Secondly, the analysis of endophytic fungi showed the potential risks of the changing practices along with the potential benefit of our innovation. Finally, the chemical and Py-GCMS analyses highlighted the opportunity of a two-step process making possible to extract high added-value molecules, produce energy and fertilizers. Further investigations, such as on-going laboratory and pilot scale pyrolysis, are needed to address the technical challenges in the production processes and in the design of appropriate separation technologies. However, knowing that potential molecules of interest can be recovered is encouraging for the future developments of the proposed OLIZERO concept. It will be necessary to refine the geographic data (workflow, localization, mapping, etc.) to assess the availability of the biomass according to harvest periods as well as the physicochemical characteristics of the residues to assess the nature of bio-molecules that can be extracted (volumes and material flow). In the prospect of this global study that involved several partners with complementary tools and skills, both Spanish and French, future work will be dedicated to the consolidation of this innovative methodology at UE level by enlarging the partnership to other academics and private companies interested in waste management through alternative biomolecules and fuels production.

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Figure 1 : Pruning waste biennal production in Sierra Mágina district

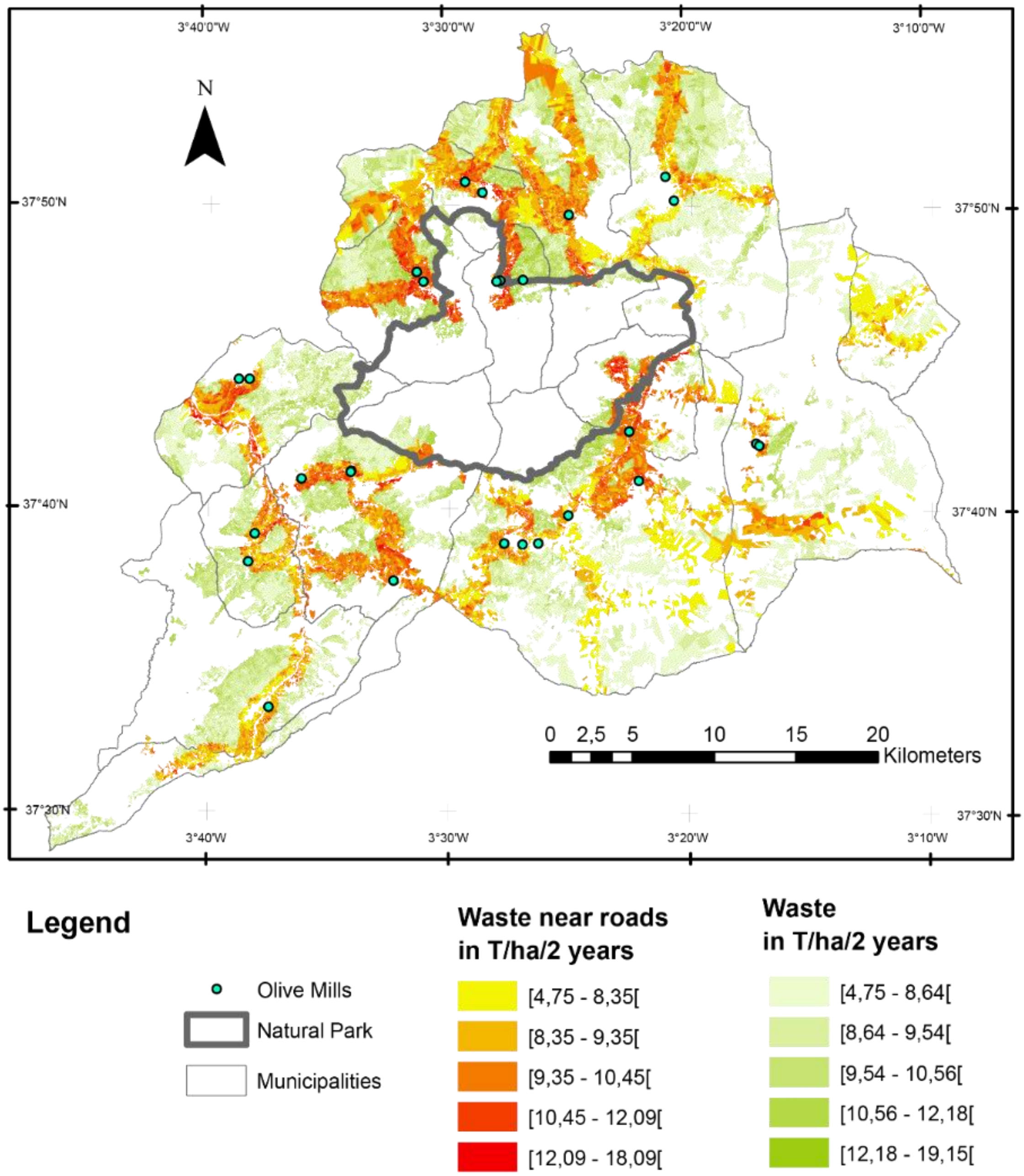


Figure 2: Geographic localization of samples and proportion of potential pathogenic fungal

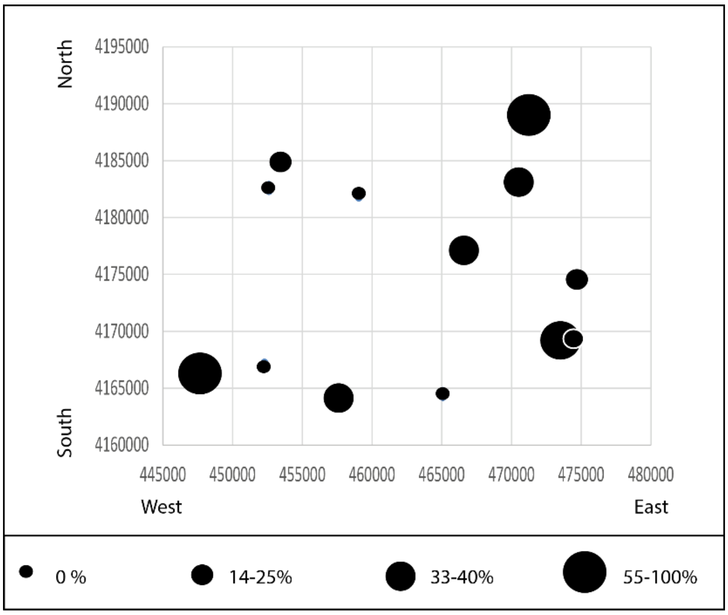


Figure 3: Pyrograms obtained from flash pyrolysis-GCMS at 773.15 K for different tree locations (different colors for each analyzed tree).

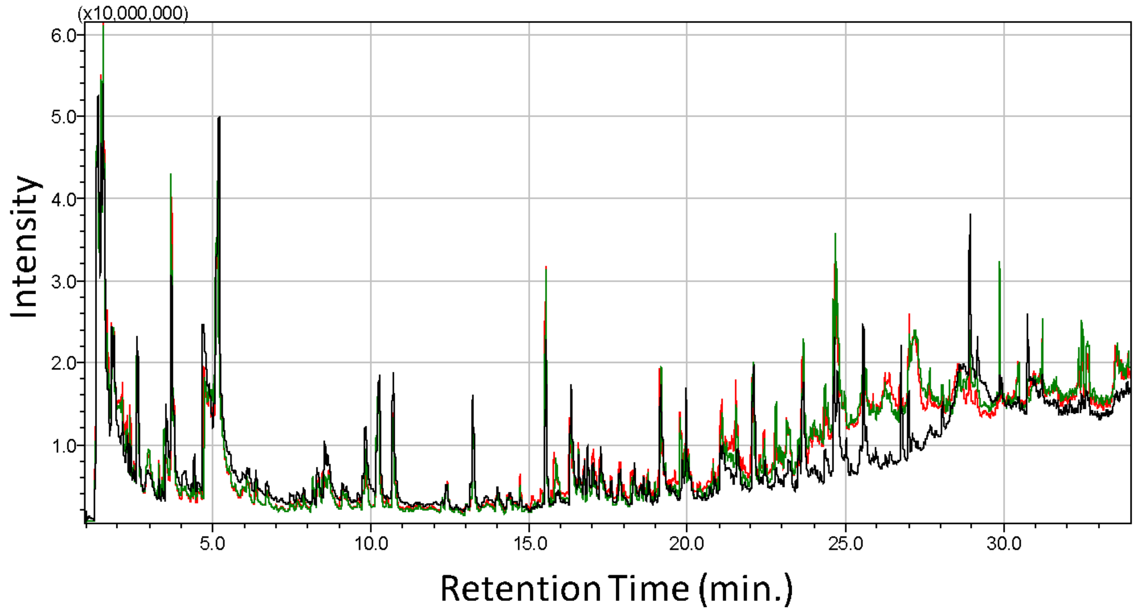
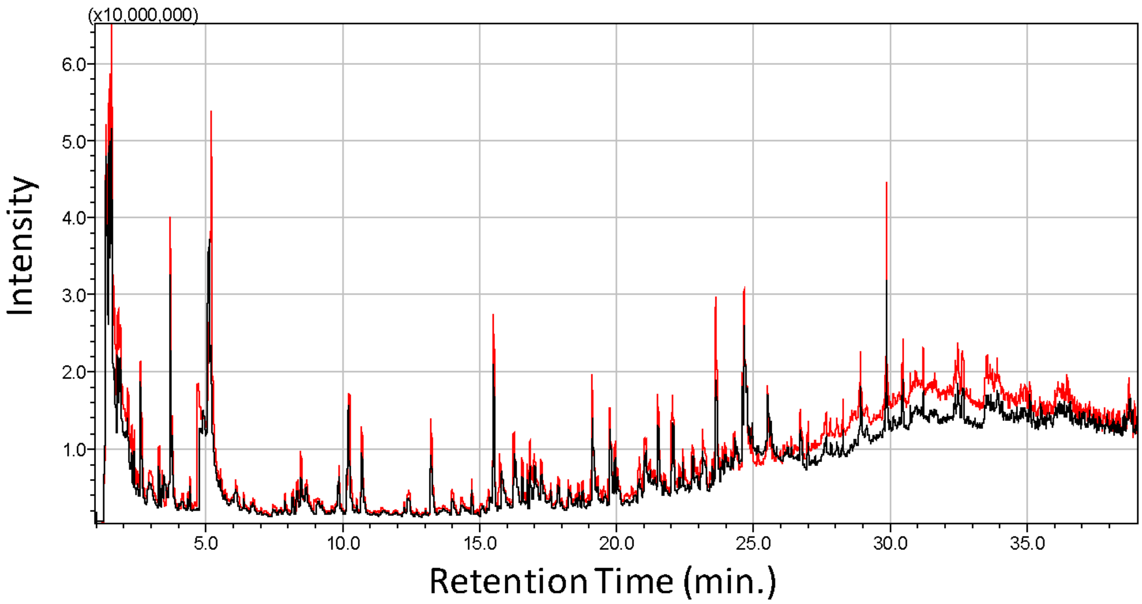


Figure 4: Pyrograms obtained from flash pyrolysis-GCMS at 773.15 K for one location and two different samples (in red and black).



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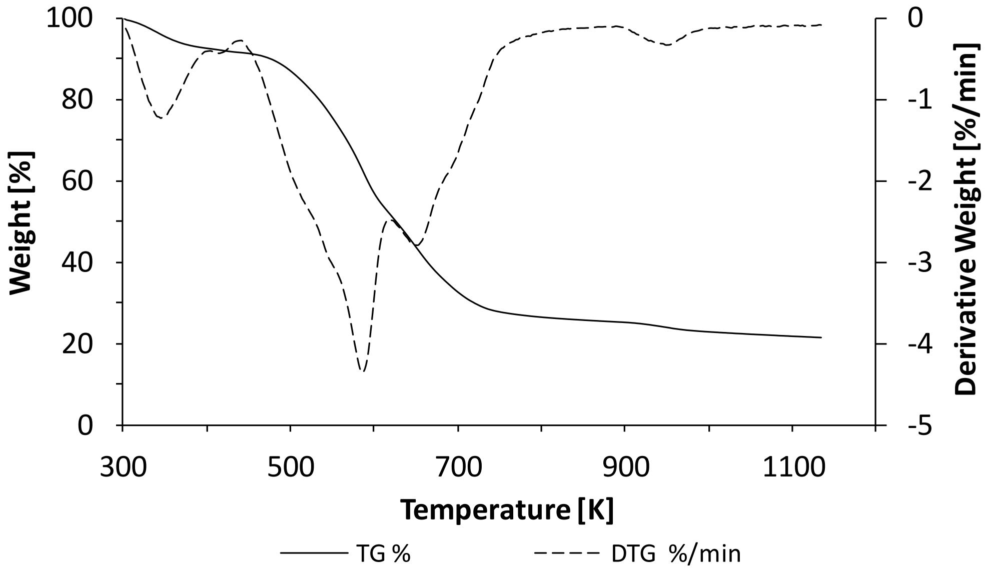

Tables

Table 1: Summary of endophyte analysis

Class	Genus	Number of isolated strains
Dothideomycetes	***Alternaria-Pleospora***	24
	Preussia	10
	Aureobasidium	2
	Peyronellaea glomerata	1
	Cladosporium sp.	1
	Phaeosphaeria sp.	1
Sordariomycetes	*Biscogniauxia*	10
	Chaetomium	5
	Coniocheta	5
	Nigrospora	4
	Sordaria	2
	Sordariales sp.	1
	Thielavia sp.	1
Pezizomycetes	*Ascorhizoctonia*	5
	Pseudotrichina	2
	Pezizomycetes sp.	1

Table 2: Major products obtained by Pyrolysis-GCMS analysis of olive tree leaves from cuttings at four temperatures

673,15 K	**773,15 K**	**873,15 K**	**973,15 K**
Formic acid	Acetic acid, oxo-	Butanal, 3-hydroxy-	Butanal, 3-hydroxy-
Acetaldehyde	Acetic formic anhydride	Acetic formic anhydride	Acetic formic anhydride
Methyl Alcohol	Methyl Alcohol	Methyl Alcohol	1-Pentanol
2,3-Butanedione	1,3-Butadiene, 2-methyl-	Cyclopropane, ethylidene-	1,3-Butadiene, 2-methyl-
2-Butenal	2-Propenal	2-Propenal	1,4-Pentadien-3-ol
Acetic acid	Acetone	1-Hexene	1-Hexene
Glycidol	2,3-Butanedione	Propanal, 2-methyl-	2-Pentene, 4-methyl-
Propanoic acid, 2-oxo-, methyl ester	2-Butenal	Furan, 2-methyl-	2-Hexen-1-ol, acetate, (Z)-
Furfural	Acetic acid	.alpha.-Acetobutyrolactone	1,3,5-Hexatriene, (Z)-
2-Propanone, 1-(acetyloxy)-	Propanoic acid, 2-oxo-, methyl ester	2,4-Hexadien-1-ol	3-Cyclohexen-1-ol, acetate
Benzaldehyde	2-Propanone, 1-(acetyloxy)-	2-Butenal	2,4-Hexadien-1-ol
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	Benzaldehyde	Toluene	1,5-Hexadien-3-yne
Phenol	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	Propanoic acid, 2-oxo-, methyl ester	2-Butenal
p-Cresol	Phenol	2-Propanone, 1-(acetyloxy)-	(*)3-Undecene, (E)-
Cyclopropyl carbinol	p-Cresol	Benzaldehyde	Toluene
Isosorbide	Cyclopropyl carbinol	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	Ethylbenzene
Benzofuran, 2,3-dihydro-	Isosorbide	Phenol	p-Xylene
(*)Tetramethyl-2-hexadecen-1-ol	Benzofuran, 2,3-dihydro-	p-Cresol	Benzaldehyde
(*)Oxacycloheptadec-8-en-2-one, (8Z)	(*)Tetramethyl-2-hexadecen-1-ol	Isosorbide	Benzofuran, 2,3-dihydro-

*: accurate identifications of molecules with large carbon numbers are difficult via MS

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