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4

5 **Biomolecules from olive pruning waste in Sierra Mágina**

6 **Engaging the energy transition by multi-actor and multidisciplinary analyses**

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26 **Keywords:** Biorefinery, Pyrolysis-GCMS, Waste reduction, Low-carbon olive-growing systems,
27 Pathogenic strain, EU policy.

28

29

30 **Abstract**

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

51

52 **1.Introduction**

53

54 **1.1 General context and main issues**

55 In Mediterranean Europe, olive-growing systems play an important role to ensure cohesion of rural
56 areas and to prevent economic and social marginalisation, especially on sloping lands (Stroosnijder et
57 al., 2008). While European countries account for almost 70% of the global production of olive oil
58 (mean 2010-15, IOC, 2015), a production expected by the EU to continue growing significantly in the
59 future, the olive-growing regions are currently facing serious problems, especially those triggered by
60 the one crop systems. The fall of margins and operating incomes over the past fifteen years (EC,
61 2012b) is combined with increasing environmental threats (Beaufoy, 2001), such as water shortage
62 (Garrote et al., 2015), soil erosion (CHG, 2010), biodiversity loss (Camarsa et al., 2010) and demands
63 to lower air pollution as well as CO₂ emissions, both resulting in part from the burning of pruning
64 waste (AAE 2013).

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

75 The human factor plays a crucial role in the success and failure of sustainable energy policies. Land-
76 use change towards energy-oriented crops (*e.g. Miscanthus*) and associated large industrial plants
77 face serious societal challenges (*e.g.* low social acceptance of rural landscape change, considered as
78 a cultural heritage). However, the objectives of food and energy production, often considered as
79 conflicting, may be conciliated through the valorization of agricultural biomass from existing agro-
80 systems that are otherwise neglected (Söderberg and Eckerberg, 2013). Our project aims to develop
81 innovative and locally-based initiatives, engaging farms and other types of micro-enterprises such as
82 olive mills in research and demonstration activities. Enhancing the range of biomasses used in second
83 generation small bio-refineries, integrated with olive mills, will help avoid food/fuel conflicts and
84 support economic development of rural areas in the Mediterranean Europe (Gómez-Vázquez et al.,
85 2009). Through a multi-actor approach, in partnership with local actors and responding to a social
86 demand to improve the environmental impact of their agro-systems, we designed a biorefinery
87 concept, which embraces the diagnosis of the local context, its congruence with emerging European
88 policies, the evaluation of the risk due to the innovation, and finally its feasibility. The attention
89 focused on the local level through this three-step method (diagnosis-risk-solution), has strengthened
90 the collaboration between the disciplines involved in the project.

91

92 **1.2 Case study at the local level**

93 Sierra Mágina is a rural county, located in Jaén Province, Western Andalusia (Southern Spain). It is
94 highly specialized in olive-growing, with 80% of arable lands occupied by olive groves, totalizing an
95 area of 50,617ha (40 % of the total area of 15 municipalities, around 1390 km², geographical data
96 basis SIGPAC, MAGRAMA 2006). Olive groves are located mainly on sloping lands from 294 meters up
97 to 1466 meters altitude, surrounding a Natural Park and its highest peak (19,794 ha, 2167 m, average
98 slope 23°), and along the course of important rivers, like Guadalquivir or Guadalbullón, which are
99 used for irrigation for half of the olive-groves surface area (Sánchez-Martínez and Gallego-Simón,
100 2009). An important concern for local actors is the economic valorization of the olive oil, a great
101 challenge in the context of decreasing prices on international markets. According to Sanz Cañada et
102 al. (2013), only 20% of the production of olive oil is sold as protected designation of origin (PDO),
103 while the majority is sold in bulk.

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

112 Olive yields are stimulated by pruning practices, with annual frequency in young groves and biennial
113 in older ones. Due to the important areas covered with olive groves in the region, the growth habit of
114 the ultra-predominant variety (*Picual*), the width (up to 45m²) and height (up to 3m) of the trees, and
115 the local practices of drastic pruning, this agriculture produces a high quantity of ligneous pruning
116 waste. Until a few years ago, pruning waste was fully burnt in the agricultural plots, contributing to
117 greenhouse gas emissions. Olive mill managers were concerned about this matter, because of the
118 negative regional image it was giving to final consumers, contributing in their rationale to the low
119 valorization of olive oil. In the same way than in other Southern Spain regions, chopping and
120 spreading the residue on the ground, partly replaces the traditional waste management.

121

122 **1.3 OLIZERO Biorefinery concept**

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

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

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

154

155 **2. Materials and methods**

156

157 **2.1. General methodological framework**

158 Our research had two objectives:

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

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

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

168

169 **2.2 Inquiries at the European and local levels**

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

172 **2.2.1. European Union's policy:**

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

179 **2.2.2. Social perception of the OLIZERO biorefinery project at the local level**

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

184 **2.3. Diagnosis of the situation of pruning residues**

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

188 **2.3.1. Relation between tree crown size and pruning residue weight**

189 We weighted the pruning residue of georeferenced trees on field and tested the correlation with tree
190 crown sizes digitized on aerial photography using GIS. We obtained a highly significant correlation (r
191 $= 0,985$, $p=0.02$) despite the small size of our tree sample (5 trees).

192 **2.3.2. Assessment of olive tree acreage and pruning residues**

193 We determine the olive tree acreage in the whole region and converted the estimated number into
194 pruning waste weight using the relation tree crownsize/waste weight. For this, we updated the GIS of
195 land use of the property plots, established by the Ministry of Agriculture and Environment for the
196 application of the Common Agricultural Policy (SIGPAC, MAGRAMA, 2006) using aerial photography
197 of 2010 and 2011 (Source: Junta de Andalucía, Jaboeuf 2015, unpublished), that were automatically
198 downloaded with the MEMOTF program from Bourrand et al. (2015). Between 2006 and 2010-2011,
199 1485 ha of new olive groves have been gained mainly upon grain lands, as observed in prior inquiries
200 (Alonso, 2010, Cohen et al., 2014). We applied an iso-cluster analysis on a spatial selection of the
201 aerial photography occupied by olive groves, after enhancing the contrast of the 3 colored bands.
202 This automatic procedure was the most efficient, showing the lowest difference with manual
203 digitalization of 11,177 trees. Despite this, a visual assessment carried on a sample of 522 parcels
204 showed that the olive tree acreage was correctly handled only in 193 plots. To improve this result,
205 we computed the NDVI index (Normalized Vegetation Index), from Landsat Images from the same
206 dates. There was a significant correlation between NDVI and the olive trees acreage in the 193 plots
207 ($r=0.7$, $p<0.0001$), as shown by Peña-Barragán et al. (2004). The NDVI improved significantly the
208 calculation of the density of olive-trees (Mann Whitney test, $p<0.0001$). The whole process was
209 performed using GIS (Arcgis10.2[®] software), in a simpler way than by remote sensing analysis (tree
210 counting analysis: Masson et al., 2004, Ke and Quackenbush, 2011; Mulla, 2013; CLUAS software:
211 García Torres et al., 2008).

212 **2.3.3. Determining the potential of processed pruning waste**

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

225

226 **2.4 Olive tree fungal endophyte sampling and identification.**

227 **2.4.1 Sampling procedure**

228 57 samples of olive branches were collected in 19 plots (3 trees per plot), selected to represent the
229 different altitudes (from 534 to 1057 meters) and exposure of olive groves. They were scattered in a
230 large area within a buffer of 250 meters along roads passable to traffic in order to accelerate the
231 sample collection. Samples were kept cool from the field collection until the laboratory. Among these
232 19 plots, 14, well-conserved, were selected to be processed in the laboratory, representing a range
233 of altitude from 573 to 1057 meters. We tested the influence of sampling parameters, *i.e.*, altitude
234 and exposure on the genus of fungi with a Chi square analysis. We also mapped the proportion of
235 potential pathogenic fungal in the samples, using their geographic coordinates (in UTM ED50).

236 **2.4.2 Identification**

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

246

247 **2.5. Chemical analysis**

248 **2.5.1. Samples:** Leaves for Soxhlet extraction were obtained from branches brought after the field
249 survey. For Py-GCMS, leaves sampled in different olive groves were used. Finally, a single location has
250 been selected to assess the effect of temperature since location had no impact on the results.

251 **2.5.2. Reagents:** Acetone and diethyl ether were purchased from Sigma Aldrich with 99% and 99.5%
252 purity respectively.

253 **2.5.3. Soxhlet extraction** : The samples (3 g) were extracted with 250 ml of each solvent (acetone and
254 diethyl ether) during 16 and 22 h (96 and 132 cycles respectively), refluxing in a Soxhlet apparatus.
255 The extract was then concentrated and chemically analyzed by GCMS analysis device. The solvents
256 were totally evaporated under reduced pressure in a rotary evaporator. The mass of extractives was
257 then measured. The GC methods were as follows. For acetone, it began at 303.15K (for 1 min)
258 followed by a heating rate of 5K/min during 12 minutes up to 363.15 K then a heating rate of 8K/min
259 during 23,7 minutes up to 553.15 K and stayed at 553.15K for 1 min. For diethyl ether, the method
260 began at 323.15K (for 1 min) followed by a heating rate of 10K/min during 5 minutes up to 373.15 K
261 then a heating rate of 8K/min during 22,5 minutes up to 553.15 K and stayed at 553.15K for 1 min.

262 **2.5.4. Py-GCMS method**: Samples of olive pruning waste (leaves), prepared with reproducible
263 weights of 0.5 +/- 0.1 mg, were analyzed using analytical pyrolysis with a Pyrolyzer PY3030 (Frontier
264 Lab) coupled to a gas chromatograph and mass spectrometer (GCMS-QP2010Ultra SHIMADZU) under
265 continuous Helium flow (1.24 ml/min). The gas chromatograph (GC) was equipped with a polar
266 capillary column ZB-1701 (30mx0.25µmx0.25µm) with a (14 %-cyanopropyl-phenyl-86 %-
267 dimethylpolysiloxane) phase. As a preliminary step, 2 samples were analyzed for 3 different locations
268 at 773.15K. Then, two pyrograms were produced from the same sample location at four different
269 temperatures (673.15K, 773.15, 873.15K, and 973.15K). In order to provide selective compound
270 separation, the GC heating method began at 303.15K (for 1 min) followed by a heating rate of 3K/min
271 during 13,3 minutes up to 343.15 K then a heating rate of 8K/min during 26,2 minutes up to 553.15K
272 and stayed at 553.15K for 1 min. For Py-GCMS and GCMS analysis, the temperatures of the injector
273 and detector were set at 553.15K and 473.15K respectively. The ionization mode on the MS was
274 electron impact. The mass range from m/z equal to 25 up to 600 was scanned and the identification
275 of the compounds relied on NIST Mass Spectral Library 2011. The results were relative and
276 qualitative. They were expressed in area percentages of the Total Ion Chromatogram (TIC).

277 **2.5.5. Thermogravimetric Analysis (TGA)**: The TGA of the samples was performed with a NETZSCH
278 STA 449 thermogravimetric analyzer under dynamic nitrogen atmosphere (30 mL/min). The olive
279 pruning residue was analyzed without treatment. A weighted sample of approximately 35 mg was
280 placed on an alumina crucible inside the furnace and vacuum was applied to create a minimum
281 oxygen environment. The sample was heated up to 1173.15K with a heating rate of 10K/min. The
282 sample temperature was measured with a type S (Pt–Rh10/Pt) thermocouple which was placed
283 under the sample holder and mass changes were recorded as a function of temperature. The overall
284 mass loss measurement uncertainty is expected to be within ± 0.5 %.

285

286 **3. Results**

287

288 **3.1. From UE policies to local level: socio-political feasibility of the biorefinery project**

289 **3.1.1. Congruence between OLIZERO biorefinery project and EU policy**

290 The OLIZERO project takes place against a background characterized both by an increasing share of
291 renewable energies in the European energy mix and by controversies related mainly to social
292 acceptance of these energies (especially in the case of wind energy) and to the carbon footprint of
293 bioenergy. Against this background, a better mobilization of cellulosic wastes and residues in EU

294 countries, estimated by Searle and Malins (2013) as the quantity of each feedstock left over after
295 environmental concerns and existing uses are taken into account, would be relevant. The amount of
296 material left on site under sustainable harvesting practices to protect against soil erosion and soil
297 carbon loss varies among EU Member States but is significant in some areas (Searle and Malins
298 2013). Bio-refineries supplied from lignocellulosic biomass and implementing emerging technologies
299 such as pyrolysis process in an olive-growing area would in this context be relevant as it would
300 address at the same time sustainability issues linked to bioenergy and uncertainties of the olive oil
301 sector in Europe.

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

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

328 **3.1.2. Socio-economic feasibility of OLIZERO biorefinery project at the local level**

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

334 A sociological analysis has shown that this change was not only triggered by economic or ecological
335 reasons, but by a complex farmers' rationale. Chipping and composting pruning waste is easier and

336 less dangerous than burning it in dense olive orchards, the more modern and intensive ones.
337 Moreover, it does not need a prior authorization, nowadays mandatory for burning pruning residue
338 due to the forest fire prevention policy. Another advantage of chipping and composting, according to
339 the farmers, is an expected improvement of the chemical and physical properties of the soil, the
340 latter being currently degraded (Ballais et al, 2013). Finally, the small number of chipping machines is
341 not an obstacle, due to lending practices between farmers and to the existence of pruning service
342 providers. According to a limited number of survey responses, one third of the farmers is currently
343 chipping, one third is burning, and the last third mix both practices (Benyei, 2015).

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

356

357 **3.2. The situation of pruning residue**

358 The map of biennial pruning waste, in tons per hectare, shows a wide range of values, from nearly
359 5 tons/hectare to almost 20 tons/hectare. The biennial pruning waste weight per surface is
360 significantly and negatively correlated with the distance to the Nature Park ($r=-0.313$, $p<0.0001$) and
361 more weakly with the size of the plots ($r=-0.278$, $p<0.0001$); the altitude and the slope of the plots
362 are very weakly but significantly correlated with the waste weight ($r=-0.023$, $p<0.0001$; $r=0.178$
363 $p<0.0001$; respectively). Large plots are generally newly planted olive groves, with smaller trees,
364 although with a higher density than traditional groves, and located in lands previously occupied by
365 crops, at the periphery of the Sierra Mágina district.

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

373 In Figure 1, the quantity of biennial waste is colored in different tons of red, when they are located
374 less than 256 meter from a road and in different shades of green when they are located further. We
375 observe that most removable pruning waste is located less than 10 kilometers from an olive mill,
376 except at the extreme east of the county. These industrial scale olive mills could be used to shelter
377 and process the pruning residue. This moderate distance is important to reduce the cost of

378 transportation, an important issue notified by farmers during the engaging workshops. In conclusion,
379 41% of biennial pruning waste is removable by trucks, representing an amount of 340,734 tons. Every
380 year, around 170,367 tons are thus potentially available for biomass processing.

381

382 **3.3. Endophytic fungi analysis**

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

398 The type of fungi (potentially pathogenic or not) was significantly linked with the altitude category,
399 according to the chi square analysis (observed value 14.36, critical value 5.99, $p=0.0008$). Potentially
400 pathogenic fungi are significantly over-represented in a range of altitude from 573 to 736 meters and
401 under-represented in a range of altitude from 797 to 944 meters of altitude. At an altitude of over
402 1000 meters, pathogenic fungi are under-represented, without this being significant. The type of
403 fungal genus is not significantly linked with sun exposure. The map of the proportion of pathogenic
404 fungal varies does not show a clear spatial pattern (Figure 2). This suggests that pruning residues are
405 much more expected to host fungal parasites in olive-groves located at lower altitude.

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

410

411 **3.4. Biomolecules production**

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

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

429 Py-GCMS experimental device enabled to identify the decomposition products of the three major
430 polymers in lignocelluloses, lignin, cellulose and hemicelluloses (Chen, 2014). Additional Py-GCMS
431 data of olive leaves have not been found in the literature. The effect of the localization of the trees
432 has been assessed by testing different samples (E1=Pt=45², E2=Pt=25², E3=Pt=24¹) at the same
433 temperature (773.15K). As can be seen in Figure 3, no effect was observed on the pyrograms.

434 In order to have an overview of the main biomolecules that could be produced via pyrolysis, the
435 samples have been injected in a preheated oven at four different temperatures (673.15, 773.15,
436 873.15 and 973.15 K). Results are presented in Table 2 and a typical pyrogram in Figure 4. As
437 expected from the thermogravimetric (TGA) experiment (Figure 5), a broad range of pyrolysis
438 temperature can be selected to favor the emission of volatiles. Indeed, most of the compounds are
439 volatilized over the 585-949K range (e.g. ~69% mass change). Therefore, pyrolysis temperature has
440 been set 20 degrees after the second peak at 673.15K where the majority of the volatiles were
441 emitted and subsequently at three higher temperatures. The results evidenced the molecules
442 expected from lignocelluloses degradation with high intensity peaks. Indeed, typical phenolics,
443 aldehydes, ketone and acetic acid were recovered. The applications of these molecules are well
444 known in fields such as resins, solvents, chemicals, aroma, *etc.* (de Wild, 2011). It is noticeable that
445 several compounds seemed to originate from the extractive part (musk ambrette, linoleic acid,
446 tetramethyl-2-hexadecen-1-ol...). The increase in temperature favored the formation of low
447 molecular weight molecules and less-functionalized molecules (toluene, xylene). The proportion of
448 linear fractionation products increased while the one-cyclized compounds decreased. The
449 complementary analysis of TGA and pyrolysis extractions, coupled with GCMS analysis, helped to
450 identify the biomolecules production potential. These molecules of interest have various possible
451 applications ranging from cosmetics, chemicals, materials to antioxidant precursors synthesis.

452

453 **4. Discussion**

454 Our interdisciplinary approach highlighted the challenges that are faced while implementing the new
455 process. The technical and economic feasibility must be demonstrated in accordance with the
456 collection of resources and plants positioning issues. Involvement of local stakeholders is crucial in
457 order to understand their awareness about the risk of trees infection, their positions towards rural
458 development policies and their opinion about different technologies such as pyrolysis. The following
459 discussion encompasses the main steps of our analysis. First we question the congruence between
460 the EU policy and the local situation. Secondly, we discuss about the feasibility of the innovation

461 according to our geographical and socio-economical diagnosis. Thirdly we assessed the risks linked to
462 the current practices, and finally the feasibility of the OLIZERO concept.

463 **4.1. Discussion from the European policy analysis**

464 Solid and gaseous biomass is by far the biggest source of renewable energy in the EU and is expected
465 to make a key contribution to the 20% EU renewable energy target by 2020. Back to 2003, the EU
466 adopted a biofuels support policy aiming at lowering CO₂ emissions in the transport sector. In order
467 to comply with this policy, Member States introduced market support price mechanisms and excise
468 duty exemptions. This policy has however triggered critic as it might have an adverse impact on food
469 prices, which levels may increase due to induced indirect land use change (ILUC). Although estimates
470 vary regarding the exact impact of biofuels production on global grain prices, a related concern has
471 emerged on the carbon footprint of biofuels. Agricultural land expansion induces ILUC at the expense
472 of forests and of grassland and cultivation increases CO₂ emissions. Again, studies vary on this
473 specific issue but the European Commission acknowledged as soon as in 2010 that ILUC can increase
474 CO₂ emissions and supported a precautionary approach (European Parliament, 2015).

475 In this context, the Commission decided in 2015 to amend its biofuels quality Directive and its
476 renewable energy directive (EP, 2015/1513, Council 9/9/2015) and proposed a 5% threshold for first
477 generation biofuels in the energy mix of transport by 2020. Finally, a compromise was found with the
478 European Parliament and the Council on a 7% cap on conventional biofuels, including biofuels
479 produced from energy crops. Furthermore, transition towards advanced biofuels (defined as biofuels
480 produced from feedstock that do not compete directly with food and feed crops, such as wastes and
481 agricultural residues as listed in the Annex IX of the 2015 Directive) is to be encouraged. The
482 compromise found at the EU level stated that Member states will be required to adopt a target
483 above a reference of 0.5 percentage points of the 10% target for renewable energy in transport. This
484 renewed EU framework opens opportunities to local actors that are willing to engage in energy
485 production without being exposed to the risk of increasing ILUC and CO₂ emissions.

486 However, one of our findings is that implementing a cross-cutting approach to foster sustainable
487 bottom-up energy policies is very much required from local and regional actors. EU territorial
488 development policies are indeed part of the toolbox to address opportunities and challenges related
489 to biomass. In this respect, the Cohesion policy as well as the Rural Development Policy, also known
490 as the second pillar of the Common Agricultural Policy, offer both funding and instruments to
491 implement innovative policies, linking agricultural development, energy production and reduction of
492 CO₂ emissions. Beneficiary countries have introduced into their programming documents for the
493 2014-2020 period specific actions in this respect, but local and regional actors may find it difficult to
494 make full use of all the possibilities available across different EU policies.

495 **4.2. Discussion from pruning waste and socio-economic diagnosis**

496 The quantity of pruning waste is higher and more variable than assessed in previous studies (15,400
497 m³/ha/yr in irrigated olive-orchard, with 100 trees/ha, La Cal Herrera, 2013, 3 ton/ha, AAE 2013). This
498 quantity is potentially available for biomass process. But potential is not real. We have to consider
499 several obstacles. First of all, the pruning waste is currently processed by farmers in their private
500 lands. They consider the chipping cost affordable because of the advantages of composting residue
501 on soils, including increasing organic matter content and avoiding erosion and excessive evaporation.
502 Yet, chipping is more expensive (70 €) than burning (53 €, La Cal Herrera, 2013). Chipping is less

503 widespread than in other regions (amounting to half the groves in the whole Jaén Province, La Cal
504 Herrera, 2013; and in the Granada Province, Calatrava and Franco, 2011). Except the role of social
505 interactions between farmers and of the low quality of soils, the drivers of chipping diffusion are
506 different from those observed in 2005 by Calatrava and Franco (2011).

507 Farmers are unwilling to give up the supposed benefit for soils without compensation. As the
508 economic balance of our biorefinery concept is still under study, it is risky to include a payment for
509 pruning residue. This is one of the reasons why we redesigned the project by integrating a return as
510 fertilizer of biochar, the ultimate residue of the pyrolysis (Steiner et al., 2007; Zabaniotou et al.,
511 2015a). Note that farmers expressed certain unwillingness regarding one of our initial proposals to
512 perform a fungal attack on waste residues before pyrolysis, as they feared the spreading diseases.
513 This fungal pretreatment was aimed at improving yield of recovery of high-value molecules. It is thus
514 for now absent from the process. However, we will further discuss this point in section 4.2, in the
515 light of the results obtained on fungal endophytes. Another obstacle is the economic feasibility of our
516 biorefinery concept. To evaluate it, we should consider the costs including transport by trucks in
517 sloping land of voluminous pruning waste (modelling is in process) and quantities of available
518 biochar, as well as the benefits such as the concentration of high-value molecules, biofuel
519 production, soil improvement by biochar, reduction of greenhouse emission and a better image of
520 the region leading to better valorization of olive oil.

521 **4.3. Discussion from risk analysis**

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

538 In the context of climatic change, the threat of pathogenic fungal should increase. In low altitude
539 olive-groves, generally located near rivers, drip irrigation is largely used (Cohen et al., 2014). The
540 future increase in maximum temperature (from 1 to 3°C, in 2030-2050, Ronchail et al., 2014), jointly
541 with the moisture maintained by drip irrigation on the root system, should enable potential
542 pathogenic fungi to turn into pathogenic fungi, increasing the vulnerability of olive-growing. These
543 low altitude olive-groves are generally composed with younger trees which means that they
544 represent the future of olive-growing in the region. For this reason, our prospect of a more

545 sustainable waste management is part of the solution for the adaptation to climate change, and it is
546 also a way to mitigate the ecological footprint of olive-growing and thus to contribute to the
547 reduction of climate change itself.

548

549 **4.4. Discussion from chemical analysis**

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

572

573 **5. Conclusion**

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

582 The collaboration between social, biological and chemical engineering sciences brought many
583 advantages: first, we chose a territory with a social demand to improve the waste management,
584 along with a capacity of innovation and a high waste production. This encouraged us to improve the
585 agronomical and economic benefits of the proposed innovation. Secondly, the analysis of endophytic

586 fungi showed the potential risks of the changing practices along with the potential benefit of our
587 innovation. Finally, the chemical and Py-GCMS analyses highlighted the opportunity of a two-step
588 process making possible to extract high added-value molecules, produce energy and fertilizers.
589 Further investigations, such as on-going laboratory and pilot scale pyrolysis, are needed to address
590 the technical challenges in the production processes and in the design of appropriate separation
591 technologies. However, knowing that potential molecules of interest can be recovered is encouraging
592 for the future developments of the proposed OLIZERO concept. It will be necessary to refine the
593 geographic data (workflow, localization, mapping, etc.) to assess the availability of the biomass
594 according to harvest periods as well as the physicochemical characteristics of the residues to assess
595 the nature of bio-molecules that can be extracted (volumes and material flow). In the prospect of this
596 global study that involved several partners with complementary tools and skills, both Spanish and
597 French, future work will be dedicated to the consolidation of this innovative methodology at UE level
598 by enlarging the partnership to other academics and private companies interested in waste
599 management through alternative biomolecules and fuels production.

600

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610

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617 the range of 300–1173 K and at a constant heating rate of 10K/min in a flow of nitrogen.

618

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

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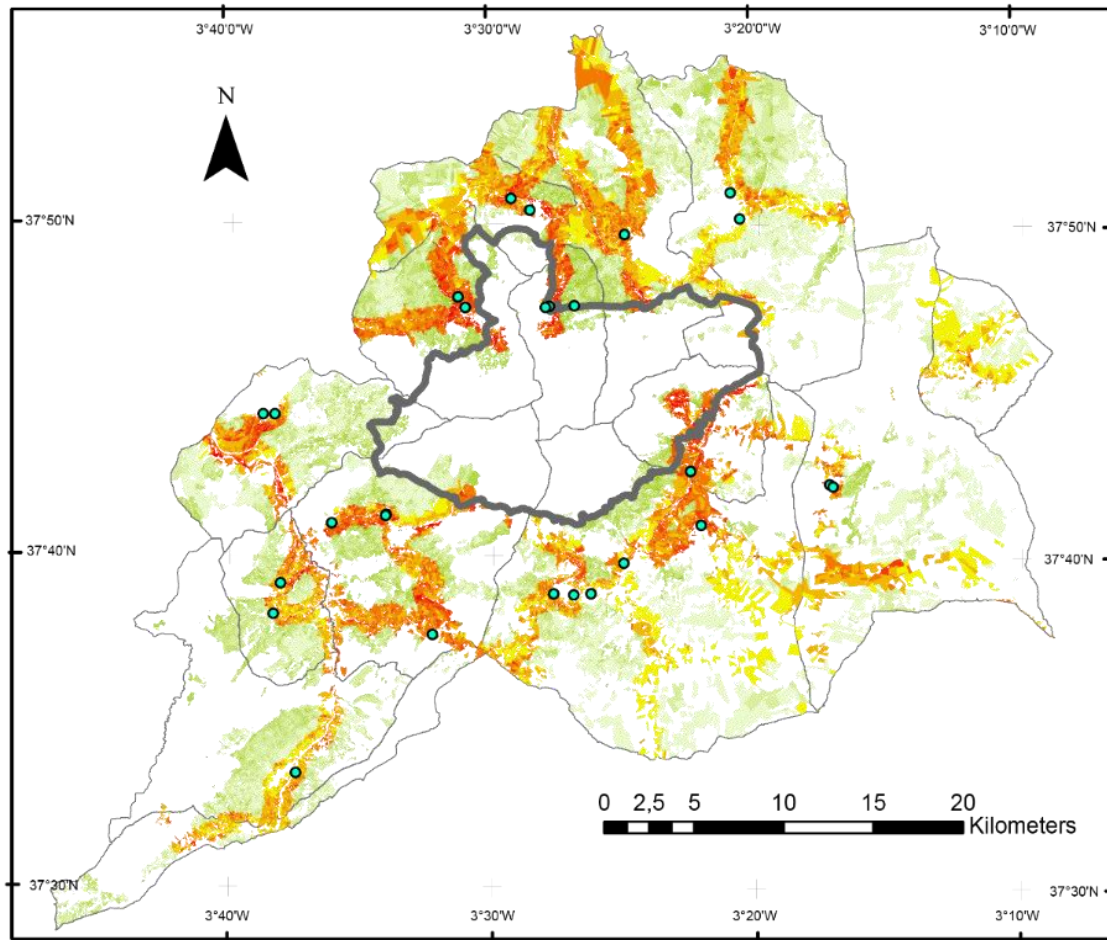
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632

633 Figure 1 : Pruning waste biennial production in Sierra Mágina district



Legend

- Olive Mills
- Natural Park
- Municipalities

**Waste near roads
in T/ha/2 years**

- [4,75 - 8,35[
- [8,35 - 9,35[
- [9,35 - 10,45[
- [10,45 - 12,09[
- [12,09 - 18,09[

**Waste
in T/ha/2 years**

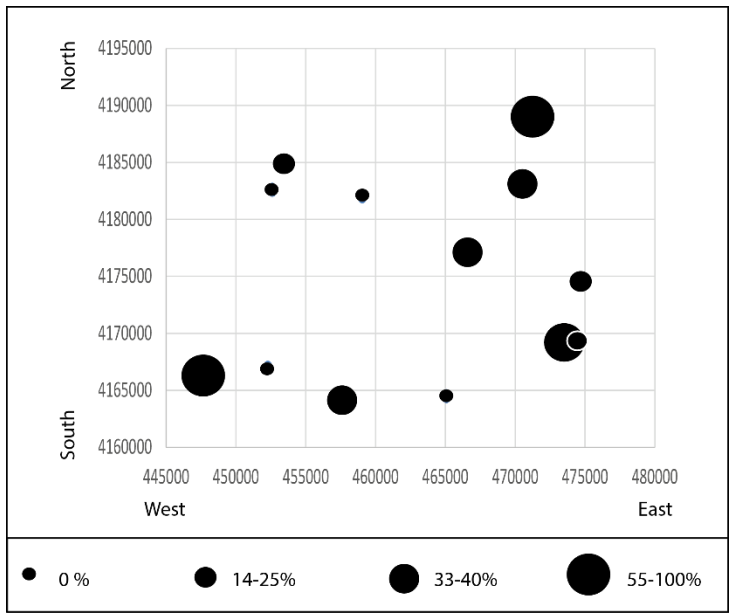
- [4,75 - 8,64[
- [8,64 - 9,54[
- [9,54 - 10,56[
- [10,56 - 12,18[
- [12,18 - 19,15[

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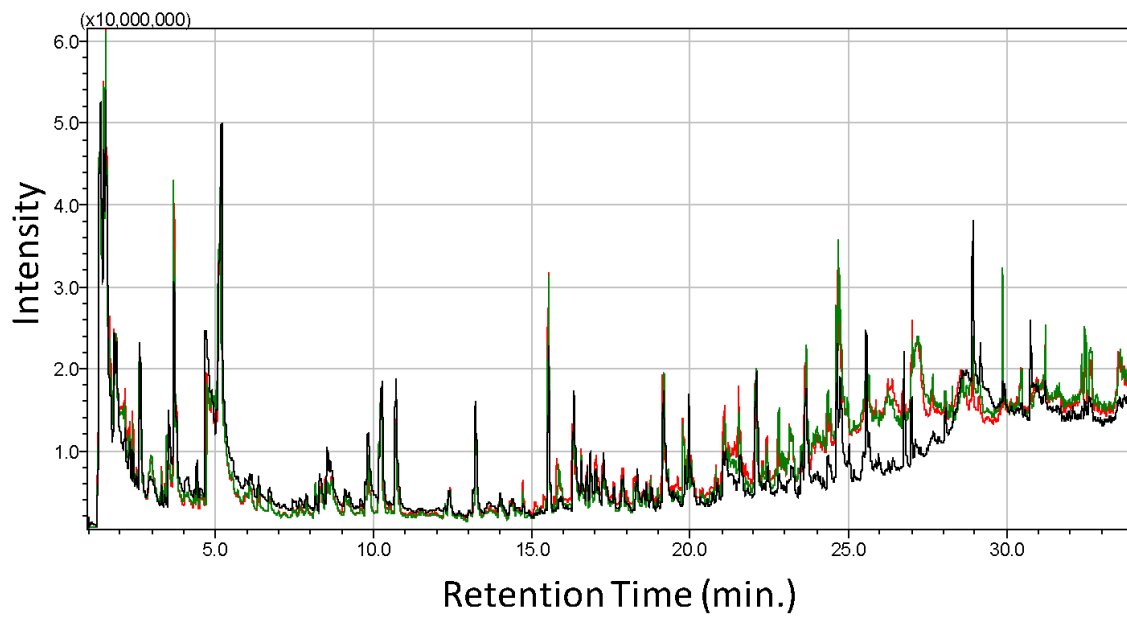
637 Figure 2: Geographic localization of samples and proportion of potential pathogenic fungal



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639

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

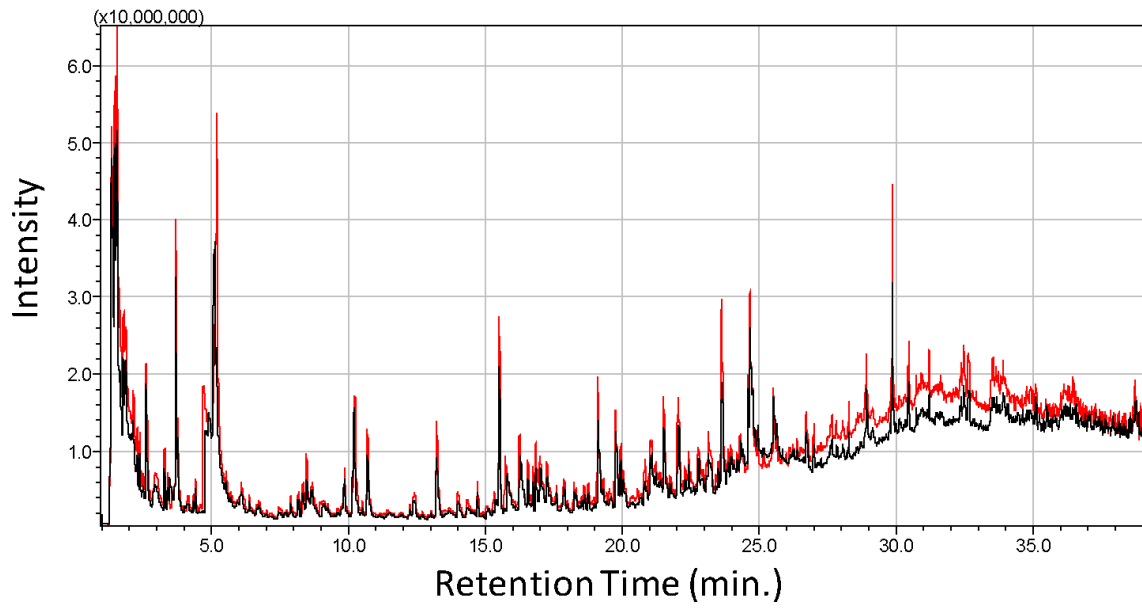


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644 **Figure 4:** Pyrograms obtained from flash pyrolysis-GCMS at 773.15 K for one location and
645 two different samples (in red and black).

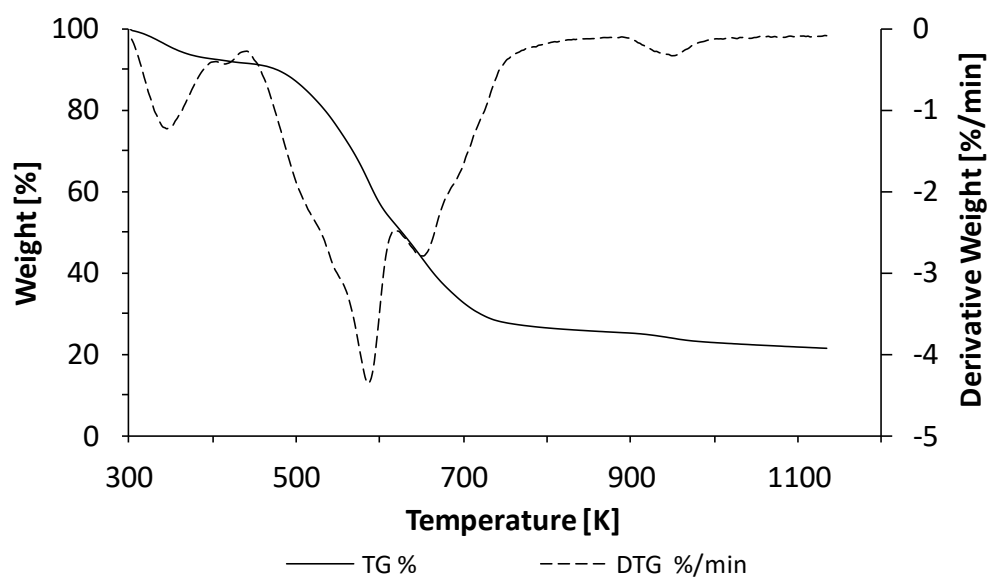
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649 **Figure 5:** Thermogravimetric (TG) and derivative thermogravimetric (DTG) curves for olive
650 leaves, at the range of 300–1173 K and at a constant heating rate of 10K/min in a flow of
651 nitrogen.



652 **Tables**

653

654

655 Table 1: Summary of endophyte analysis

656

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

657

658

659 Table 2: Major products obtained by Pyrolysis-GCMS analysis of olive tree leaves from cuttings at
 660 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- Acetic formic
Acetaldehyde	Acetic formic anhydride	Acetic formic anhydride	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- .alpha.-	2-Hexen-1-ol, acetate, (Z)-
Furfural	Acetic acid	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-

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

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