WildFood Project

Eating the wild: Improving the value-chain of Mediterranean Wild Food Products (WFP)

Report on management and planning control systems for sustainability and quality in WFP value-chains: pilot experiences in Mediterranean countries

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Executive summary

The present report focuses on the innovation actions carried out in the WildFood project to address quality, safety and sustainability through all stages of the wild food products (WFP) value-chains by gathering existing information and implementing pilot projects and study cases at local level in different stages of the targeted products chains.

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1. INTRODUCTION

Forests and scrublands of the Mediterranean area countries have great natural resources (both in quality and quality) used as wild food: aromatic plants, berries, pine nuts, mushrooms, truffles, pine nuts/seeds, but their production varies a lot between years. Additionally, the provisioning quality system is not fully guaranteed, creating short circuits in the associated value-chains. Moreover, the context of climate change puts the supply of this forest resource on alert. In this sense, innovative methods and techniques are required to address quality, safety and sustainability through all stages of the wild food products (WFP) value-chains.

Under the WildFood project (2020-2023), several innovation actions were carried out in different Mediterranean countries by gathering existing information and implementing pilot projects and study cases at local level in different stages of the targeted products chains. For the development of the innovation actions the following key elements have been taken into account: Already existing information on traceability methods and control systems for quality, safety and sustainability of targeted WFPs; Large number of products, uses, and markets which leads to a long and complex supply chains – difficulties to trace product back to its source; Unique circumstances of ecology, habitat, and pressures on resource for each species; Providing a framework of principles and criteria that can be applied to management and planning systems of WFP species and their ecosystems to improve quality and sustainability.

The innovation actions include the design and implementation of a system for risks analysis and critical control points, including production control innovation, harvesting mechanization controls, improvements in manipulation process, maintenance of equipment and installations, pest control, storage, packaging and transport.

The pilot projects are the following ones:

Mushrooms and truffles

1) Establishing mycological parks (Tarragona, Spain), to assess and control mushrooms collection and guarantee a sustainable mycological use with appropriate mushrooms collection, while integrating social function in this activity (innovation in production and use) (coordinated by CTFC)
2) Participating in development of innovative biological agents in pest control on truffle sites / plantations (coordinated by SFI)
3) Preparing of laboratory protocols for certification and identification of selected WFP (coordinated by SFI)
4) Elaborating a production monitoring protocol for truffle (coordinated by UNIPD)

Pine nuts and acorns

5) Quantifying pinecone production, using sensors and drones in Catalonia, Spain (coordinated by CTFC)
6) Prediction systems for the annual supply of acorn and flour as raw material for human food products (coordinated by ISA)
7) Implementing some innovation systems for production, transformation and distribution of acorn related products (coordinated by ISA&HFM)

Aromatic plants

8) New production and transformation process for aromatic plants in Tunisia (INRGREF)

Besides the Innovation Actions, the WildFood project developed two reports linked to the pilot projects: Protocols and innovative modelling tools for the sustainability within the WFP value-chains; and guidelines, including techniques and know-how, aiming to ensure the quality, safety and sustainability of products provisioning.

The factsheets of the pilot project as well as other information and deliverables can be consulted on the project website: https://wildfood.ctfc.cat/.

In next section, the innovation actions (pilot projects) developed under the WildFood project are described in more detail, explaining the context, objectives of the pilot, methodology, implementation phases, results and outputs and lessons learnt.

2. INNOVATION ACTIONS

2.1. Mushrooms & truffles

Innovation Action 1
Establishing mycological parks to assess and control mushrooms collection and guarantee a sustainable mycological use with appropriate mushrooms collection, while integrating social function in this activity (innovation in production and use). Coordinated by CTFC

Authors: José Antonio Bonet & Juan Martínez de Aragón (CTFC)

Context and current state of the art

Mushrooms are probably the most popular non-wood forest product in Spain. In certain regions such as Catalonia, it is estimated that more than 15% of the population picks mushrooms at least once a year. This circumstance can create an overpressure to the forests.

For that reason, several Spanish regions (Figure 1) have stablished a specific normative including a mushroom permit system. These norms often standardize categories of picking activities and specifications for mushroom reserve signposting. The legal basis for establishing a mushroom picking regulation derives from the Spanish Civil Code and from the Forest Act which stipulates that mushroom ownership is a landholder's right. However, and in spite of the increasing perception of mushroom picking congestion in certain Catalonian counties (Figure 2), this activity has not been regulated by law.

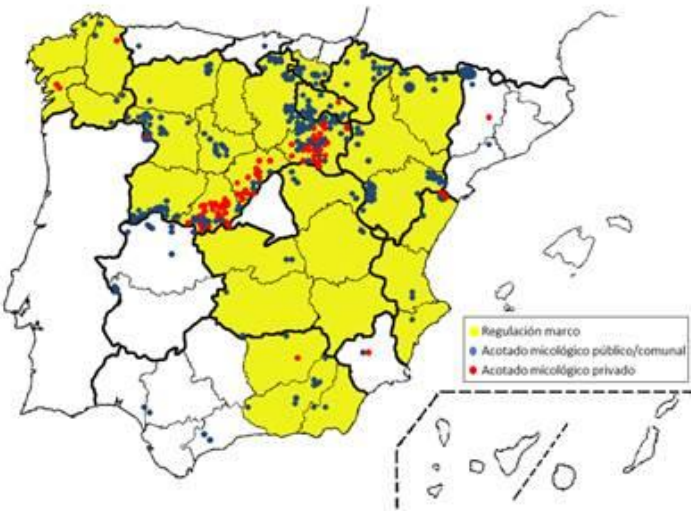

Figure 1: Mushroom picking regulation in Spanish regions – in yellow (Górriz-Mifsud & Bonet, 2017)

The motivation for mushroom pickers far exceeds the economic value of the collected edible fungi, increasingly seeking a recreational, close to nature activity. This activity has been named mushroom tourism or mycotourism and tries to connect the fungal discovery with gastronomy and fungal related tourism activities. Parallel to this, the figure of mycological parks has appeared. Mycological parks are areas in which the managers try to combine mushroom productivity, fungal conservation and the promotion of mycotourism. However, this figure is relatively new and there are still no specific agreed rules on how the mycological parks can be created, supported and monitored.

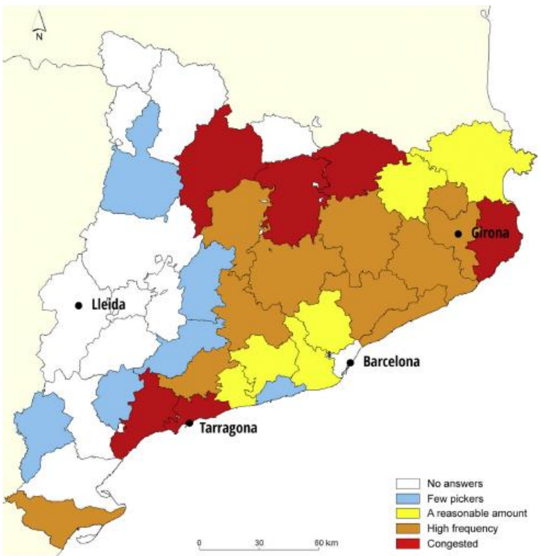

Figure 2: Perception of mushroom picking congestion per

county in Catalonia (Górriz-Mifsud et al. 2017)

Objective of the pilot

The objective of this pilot project was to establish the basis for the creation of a mycological park in Catalonia, aiming to make compatible mushroom picking with fungal conservation and the development of mycotourism. The pilot project has been held in the Protected Forest Area of Poblet (PNIN of Poblet) in which the combination of mycotourism facilities (i.e., restaurants, information panels, tour guides, etc..) with the mushroom species catalogue, optimize the different mushroom related services that can be offered in the mycological park (innovation by product). The pilot experience of PNIN of Poblet allowed us to generate a set of recommendations for the creation, improvement and monitoring of a mycological park. The obtained results in the pilot area can be transferred to the other areas in the Mediterranean.

Methodology

The evaluation of mushroom yields and diversity has been done through weekly inventories of the fungal fruitbodies in permanent mushroom plots established in the PNIN of Poblet (Tarragona) (Bonet et al. 2012). The inventory allowed us to have an approximation of the quantity and diversity of the fungal community. In parallel, we have analysed other mycological parks that has been established in other areas from the point of view of normative and procedures that can be applied to the Catalonian case study. On the other hand, we have listed the mycotourism facilities in the surrounding area that can be benefited by mushroom related activities.

Implementation of the pilot project

During the pilot project, we completed the analysis of other norms that includes the figure of Mycological Parks. At Spanish scale, we only found such reference in the mycological normative of the Castilla y León Region (Decree 31/2017 of 5th October that regulates the wild mycological resources in the region). This Decree states that mycological parks are areas of special interest for mycology, including the mycotourism. The mycological parks need to be managed by one authority that will represent the totality of all the landowners, establishing common rules for the mycological resources management. The minimum surface of a mycological park has been established in 10,000 ha. At international scale, the European Mycological Institute (EMI), an international organisation that promotes projects and cooperation actions related to the management and valorisation of mycological resources (www.eumi.eu) also promoted the figure of Mycological Parks. As an example, EMI has recently evaluated (October 2021) the Albarracín Community Mycological Park applying their own set of rules.

The pilot project also included the analysis of mycological resources in order to match the mushroom offer with the potential demand. Due to the huge variability observed in mushroom yields, we agreed in carrying out the fungal inventories through the establishment of permanent plots (100 m2 each) in the main forest typologies of the park during three consecutive productive seasons. The fungal inventory was carried out during the Autumn seasons of the years 2020, 2021 and 2022.

Finally, the third axis of the pilot project included the list of touristic facilities that can be potentially integrated in the process of constitution of a Mycological Park. The team in charge of the pilot project completed the list of facilities, that included hotels, restaurants and other touristic actors.

Results and outputs

The evaluation of the mushroom yields in Poblet Protected area during the autumn of 2020, 2021 and 2022 (See Table 1) shows a huge variability between forest typologies and specially between years. The analysis of normative in other areas that can be applied to the pilot project confirmed that the set of rules fixed by the Castilla y León region can be applied to the Catalonian region, but without fixing a minimum surface for the Mycological Park declaration. Finally, the list of mycotourism facilities already present in the surrounding area that will be benefited by mushroom related activities highlighted the need of establishing a common strategy of the touristic sector based on the mycological products. This can include the inclusion of mushrooms in the restaurant offer, the establishment of mycological fairs that can attract tourists and the need of highlighting the mycological values (fungal diversity) that is already present in the protected area.

Table 1: Total, edible and commercial mushroom yields identified by forest typology during the autumn mushroom seasons of 2020, 2021 & 2022.

Mushroom yield per forest typology	Total Yield 2020 kg/ha)	Edible Yield 2020 (kg/ha)	Commercial Yield 2020 (kg/ha)	Total Yield 2021 (kg/ha)	Edible Yield 2021 (kg/ha)	Commercial Yield 2021 (kg/ha)	Total Yield 2022 kg/ha)	Edible Yield 2022 (kg/ha)	Commercial Yield 2022 (kg/ha)
Pinus halepensis	18,84	3,78	5,54	6,08	0,69	0	0,20	0	0
Pinus pinaster	32,35	18,02	2,88	47,73	42,71	0,1	1,33	0,12	1,21
Pinus sylvestris	43,18	24,09	8,46	108,73	60,75	3,26	36,96	23,51	0
Quercus ilex	4,94	0,92	1,21	4,67	1,84	0,13	0,02	0	0

Lessons learnt

- The concept of Mycological Park is still relatively new in the Mediterranean area and thus, their application represents a challenge for both forestry and touristic actors.
- The Variability of mushroom yields, that are an intrinsic feature of the fungi, difficult to have a regular offer of mycotourism based activities.
- There is a need to design a mycotourism strategy adapted to every specific area, aligning all the actors that are involved in the mycological parks.

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Innovation Action 2
Elaborating a production monitoring protocol for truffle

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Context and current state of the art

Among the different types of wild food, truffles could give an important contribution to the rural economies of Southern-European countries (Buntgen et al. 2017). Nevertheless, a significant decline of the Southern-European production is predicted between 2071 and 2100 (78–100% less) (Thomas and Büntgen 2019) both as cultivated and wild. Moreover, in Spain, for example, a 29% reduction in the suitable area for the production is expected by 2040 (Fischer et al. 2017) as probably it will occur in south Italy. Nonetheless, new sensors and ecophysiological models are today new tools that allow to reduce the production loss, especially in plantation, where there is still a limited knowledge on the interaction between plants and fungi (Reyna and Garcia-Barreda 2014). The decrease in wild production is determined by several factors, among which climate change and land abandonment are the main ones (MiPAAF 2018). In the future, with a slight increase in temperature, it will probably be possible to grow truffles in different areas like, for example, in Central Europe (Čejka et al. 2020) or the inner part of the Alps. This shift through Northern areas is likely to happen if we consider that the current distribution of the truffle depends, besides the climatic characteristics, also on the distribution of the host plant species. It is possible that suitable climate zones will develop, where the host trees arrive and bring with them also the fungus (Mello et al. 2006). Understanding the needs of these fungi and the mechanism that regulate the symbiosis will make it possible to promptly adapt cultivation and management methods to climate change. In the management of a truffle orchard the most considered parameters are environmental and soil parameters. The actual literature like papers and handbook stress the importance of both pre-production and production operations, for example irrigation and soil tillage (Fischer et al. 2017, Salerni, Iotti, et al. 2014, (Raglione 2011)Figliuolo et al. 2013), but also mulching and deep soil processing has been considered important for production and productivity (Chevalier 2012). New methods are developed to improve truffle production, like the MRT method (rational method of truffle cultivation), which imposes operations like removing grass, deep operations to break roots, intense pruning (Chevalier and Pargney 2014).Soil analysis are considered of fundamental importance for a conscious management of production (Bragato et al. 2021). Analysed characteristics are, for example, soil texture, quantity and quality of the clay, pH, organic matter (Raglione 2011). Many studies also linked productions with atmospheric parameters, such as temperature and precipitations (Salerni, Perini, et al. 2014).

Different studies report different effects of the precipitations on the production. Rainfall during July and August seems to have a more important effect on *Tuber melanosporum* Vittad. than those in September (Garcia-Barreda and Camarero 2020), while those between October and November seem to have even a negative effect (Büntgen et al. 2019). However, the studies report a climatic condition of some area in Spain, and further studies would be need in different soil and climatic conditions. In fact, truffles could also benefit from a period of drought (Garcia-Barreda et al. 2019, Pacioni et al. 2014), and in cultivated truffle orchard an intermediate level of irrigation seems positively affecting the productivity (Olivera et al. 2014,Bonet et al. 2006), which let us intend that climatic and soil interaction are crucial, but only a clear understanding of the tree water uptake can disclose the dynamics that allow to increase production and productivity of truffle orchards and forests. On the contrary of Spanish authors, the importance of a moderate water supply was confirmed in natural environment during the fall and wintertime. For example, the whitish truffle (*Tuber borchii* Vitt.) needs a good humidity and high precipitation (Salerni, Perini, et al. 2014).

Few studies emphasize the importance of considering the point of view of those who feed the truffle: the plants. As seen above, the management operations are mainly focused on the fungus and environment (i.e., how truffle react to different precipitation, soil properties…). However, monitoring plant's physiological parameters would allow to unveil the mechanisms those regulate the processes through which the fungus feeds. In the cultivation of truffles, it is of fundamental importance to know the state of the plant. For example, Le Tacon et al. (2013) labelled a hazel plant with $^{13}CO_2$ and then measured the transfer of ^{13}C from the leaves to the fine roots and *Tuber melanosporum* Vitt. mycorrhizas: the study showed that "the carbon allocated to the fruiting bodies was only coming from the carbon assimilated by the tree during the growing season" (Le Tacon et al. 2013). This means that the life of truffle depends on how much carbon the plant is able to fix and allocate to the roots. In light of this, it is necessary to investigate how the physiological status of the plant influences the release of carbon that nourish the truffle.

Identify the innovation actions to be developed in the pilot

The main parameters considered in truffle management are soil characteristics, precipitation and temperatures. In some cases, handbooks suggest intervention on plants, such us pruning or deep soil operations to cut roots. However, it is not clearly explained why and how these activities influence the relationship between the plant and the mushroom. The innovative action of the pilot is to carry out a monitoring of the plant's physiological parameters, to understand the mechanisms of carbon release with root exudates, that could feed truffle. Sensors (in this case, sap flow sensors and dendrometers) would allow to know the physiological state of the plant: for example, if the plants are under water stress or if they're able to fix carbon. This innovation considers the fundamental role of the plant. Knowing the state of the host tree, it will be possible to choose the best practices to manage the plantation. Moreover, it would allow a more rational use of irrigation in cultivated truffle orchards. In fact, knowing the real needs of the plant, it will be possible to only bring water if it is necessary, thus allowing to save water resources. In natural forests, the information obtained from the plants can be used to carry out interventions that affect light and humidity (for example, selective thinning). Controlling light and humidity of the soil could induce particular response in plant physiology, influencing the release of carbon from roots. Consciously managing truffle orchards allows an improvement in productivity using resources more sparingly, and this can positively affect the economy of rural areas. Management models need to be developed to improve and stabilize the production.

The aim of the pilot project is to characterize the physiological predisposition of the plant in the establishment and maintenance of symbiotic relations with truffles. To do this, a monitoring of the parameters of productive and non-productive plants will be conducted. From the comparison of the parameters, it will emerge what is the physiological state of the plant that mainly favours the production of truffles. Using these new tools, it will be possible to elaborate a production monitoring protocol for truffle. The final objective is to use the identified indicators to facilitate the forest management oriented to truffle production. In particular, we expect significant differences between the productive and non-productive trees in the monitored physiological parameters, which thus could be used as indicators for forest interventions aimed at increasing the truffle productivity. In this context, a key objective is to estimate the overall cost of this monitoring system and of the associated data.

Methodology

We set up two experimental sites in Caltrano (Vicenza, Italy) and Carlino (Udine, Italy) to investigate the physiology of the target tree species in relation to the surrounding environmental conditions and to their truffle productivity. The site in Caltrano is a mixed forest plantation with predominance of *Ostrya carpinifolia* Scop. with stable production of *Tuber aestivum* Vittad*.* The site in Carlino is a relict lowland mixed forest, with predominance of *Carpinus betulus* L., associated to the truffle species *Tuber magnatum* Pico.

The physiology of the target trees will be investigated in relation to the surrounding environmental conditions in two different classes of high and low truffle productivity. At each site, we chose four target trees in a representative truffle-productive and a non-productive area. On each tree we installed a sap flow sensor and a stem dendrometer. The sap flow sensor (Granier type) allows the continuous measurement of the rate of the sap flowing along the stem at an interval of 15 minutes, during the whole growing season. The sap flow sensor is made of two probes inserted in the trunk, one above the other, at 10 cm distance (Figure 2).

Figure 1: Granier sensor on an *Ostrya carpinifolia* Scop. tree

Photo: Sartori (2021)

Figure 2: Granier sensor on a *Carpinus betulus* L. tree

Photo: Sartori (2021)

The stem dendrometer (Figure 3 and Figure 4) allows to measure the continuous variation of the stem diameter at an interval of 15 minutes, accounting for the actual growth, but also for the bark shrinking and swelling following the tissue dehydration and rehydration, respectively. We expect to observe daily and seasonal variations in the consumption of water (sap flow) and in the shrinkage/swelling of the bark tissue, as response of the monitored environmental parameters.

In each area we installed 1-2 TDR (Time-Domain Reflectometry) soil sensors for the measurement of the soil water content at an interval of 15 minutes, indicating the availability of soil water, but also its continuous variation due to plant uptake and evaporation to the atmosphere. Furthermore, we installed in each area a hygrometer (Figure 5 and Figure 6) providing measurements of air temperature and relative humidity.

Figure 3: Dendrometer sensor on *Ostrya carpinifolia* Scop. tree

Photo: Vidale (2021). Note: the plastic bag is used to prevent moisture damage

Figure 4: Dendrometer sensor on a *Carpinus betulus* L. tree

Photo: Sartori (2021). Note: the plastic bag is used to prevent moisture damage

Figure 5: Hygrometer in the sites of Caltrano

Photo: Sartori (2021)

Figure 6:Hygrometer in the sites of Carlino

Photo: Sartori (2021)

Implementation progress of the pilot project (first phase)

Between January 2021 and March 2021 all sensors were prepared and installed. At the time of installation, the maximum care must be used due to the fragility of some sensors. For instance, sap flow sensors may be damaged if not carefully handled and installed. Furthermore, it is important to create an adequate space when the probes are inserted in the tree, otherwise the trunk, growing, could compress the wires and break them. The interventions to repair possible damages to the sensors must be as timely as possible, so a remote connection is required to detect the problem.

The environmental data collected were soil humidity and temperature (Figure 7), but also air temperature and relative humidity.

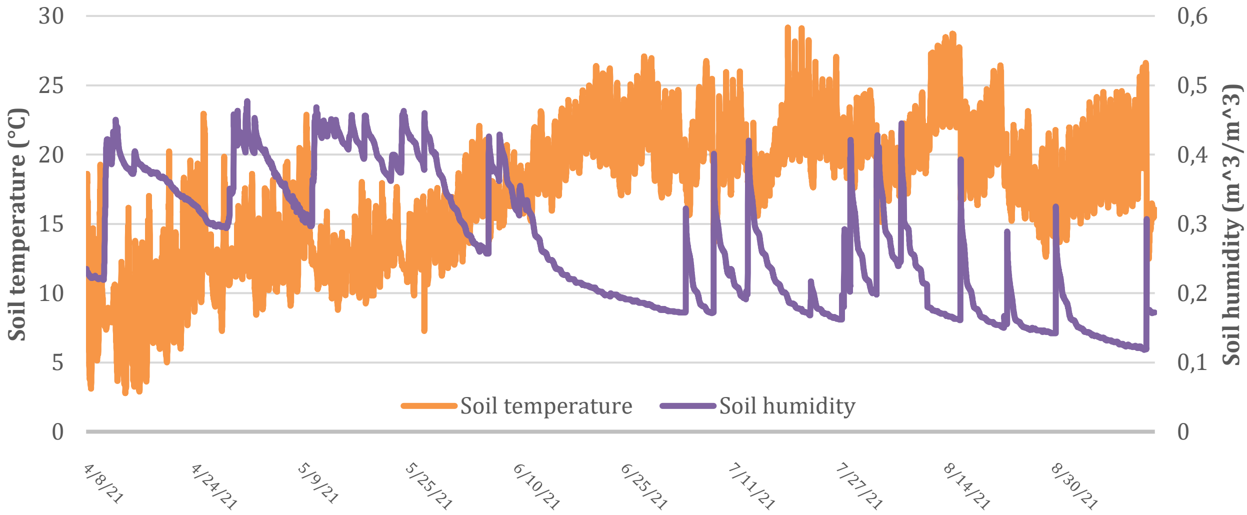

Figure 7: Soil temperature and humidity in Caltrano

Using the data of air temperature and relative humidity, it is possible to calculate the vapour pressure deficit (Figure 8), which influences the transpiration.

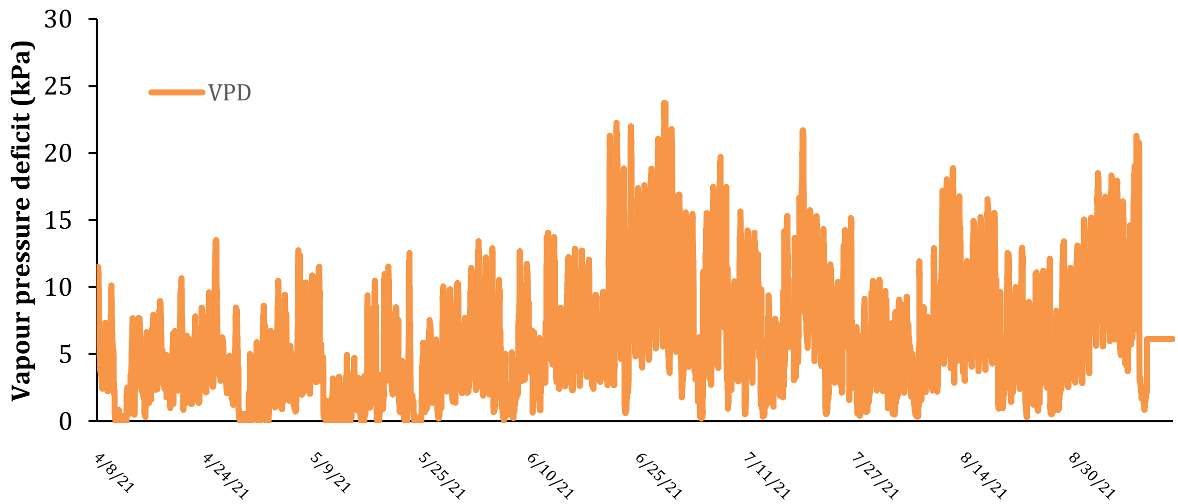

Figure 8: Vapour pressure deficit in Caltrano

Thanks to the Granier sensors and dendrometers, we collected stem diameter data and sap flow data (Figure 10).

For example, in Figure 9 are compared the periodical stem variation of a productive and a non-productive plant: it can be observed that the oscillations in the non-productive plant are greater than in the productive plant.

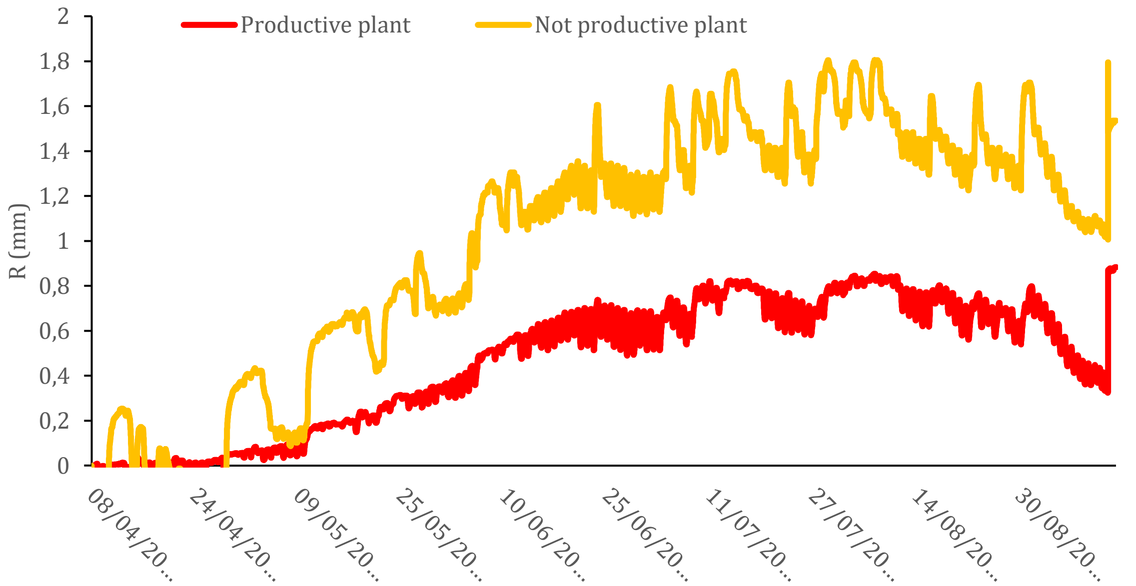

Figure 9: periodical variation in stem diameter, comparison between productive and non-productive plant

The sensors can measure also the daily variation in sapflow and diameter, as showed in Figure 10.

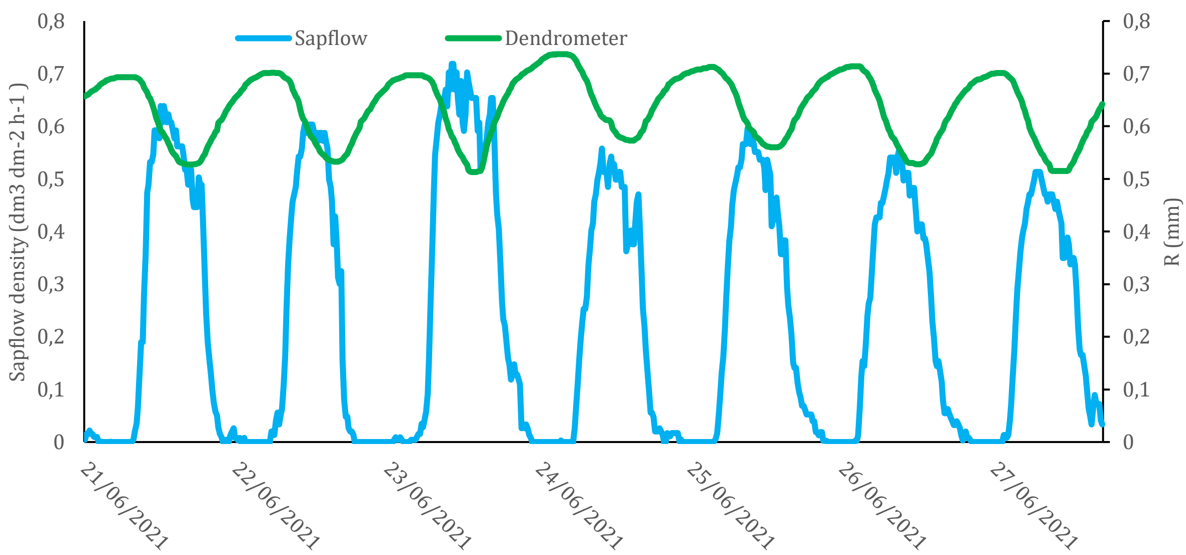

Figure 10: daily sapflow density and stem diameter variations in Caltrano

These data can provide useful information about the physiological status of the plant and carbon fixation.

One of the collected data is also the productivity of the truffle orchard (Figure 11), that has to be compared with the other measured parameters, in order to understand the relationship between.
For example, in Figure 11 it can be observed that the production peaks are preceded by a phase of low humidity and then by an increase. This may suggest the need for a cycle of water stress and subsequent recovery.

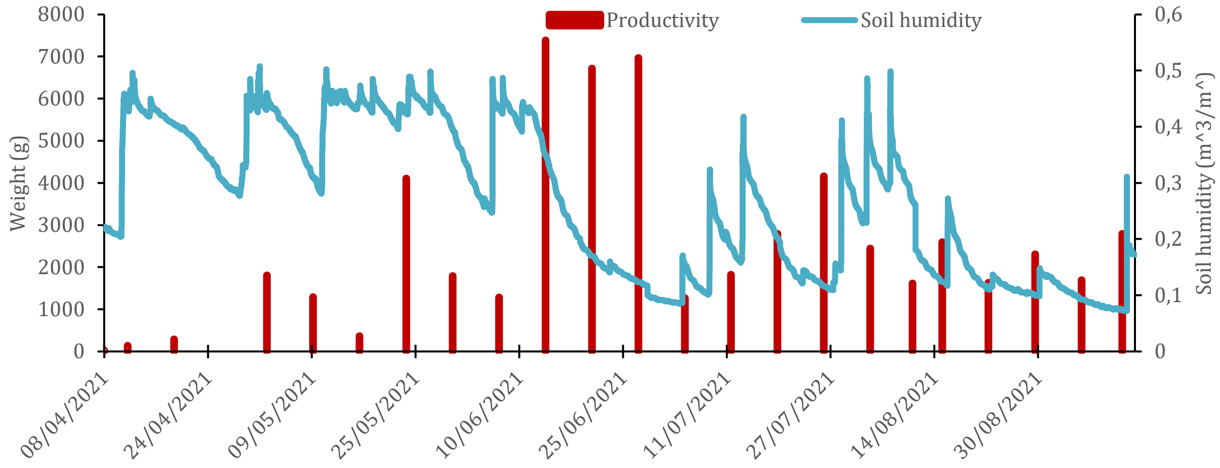

Figure 11: Productivity of the truffle orchard compared to soil humidity in Caltrano

However, the predation of the local fauna (wild boar and roe deer, among the others) prevented from getting the precise yield. In addition, illegal truffle harvesting activities have been detected, which represent an additional problem for obtaining precise yield data. Some solutions for the next season could be the placement of fences. Finally, wild animals have in some cases damaged the sensors, and this will make necessary to replace them.

With regard to the white truffle, it would be useful to study the parameters of a species such as poplar, one of the trees that are most involved in this symbiosis. In fact, in the site of Carlino (UD), the white poplar (*Populus alba* L.) was eliminated due to the interventions of previous management. The analysis of the parameters of this species would be very important to understand the development of the white truffle and enhance the production.

During this first year, data were also collected and analysed. Regarding the data collection, one of the problems was the absence of a remote connection. A remote connection is necessary for a quick intervention in case of damaged sensors. Without a remote connection, it is not possible to intervene immediately to repair or replace the sensors, thus causing loss of data series.

Implementation progress of the pilot project (second phase)

The monitoring of the two sites (Caltrano, in the province of Vicenza, and Carlino, in the province of Udine) has been continued through sensors which provided information about plant physiology and water status (dendrometers and sapflow sensors), but also about environmental features (sensors for soil and air temperature and humidity). The site in Caltrano is a mixed forest plantation with predominance of *Ostrya carpinifolia* Scop. with stable production of *Tuber aestivum* Vittad., while the site of Carlino is a relict lowland mixed forest, with predominance of *Carpinus betulus* L., that was one associated with *Tuber magnatum* Pico.

During this second period, a modem has been installed for the remote transmission of data, so it became possible to control the activity of the sensors in real time and intervene promptly in case of failures. In addition, a new datalogging system was developed using an Arduino UNO platform: the board was tested by connecting it to two dendrometers. First data collected have similar resolution to those collected with more expensive datalogger.

A spatial and dendrometrical data collection was carried out in the first months of 2022 in the truffle orchard of Caltrano, obtaining the precise position of the plants (using a submetric GPS technology) and measures of tree height, diameter at breast height and depth of the canopy, in order to determine whether there is a physiological difference between productive (mycorrhizal) and non-productive (non-mycorrhizal) plants.

In the forest of Carlino, currently non-productive, a selective thinning has been applied, to investigate the effects of the reduction of competition between plants, but also the bigger growth of canopy (fig.1). On the base of what observed in the site of Caltrano, this could influence photosynthetic activity.

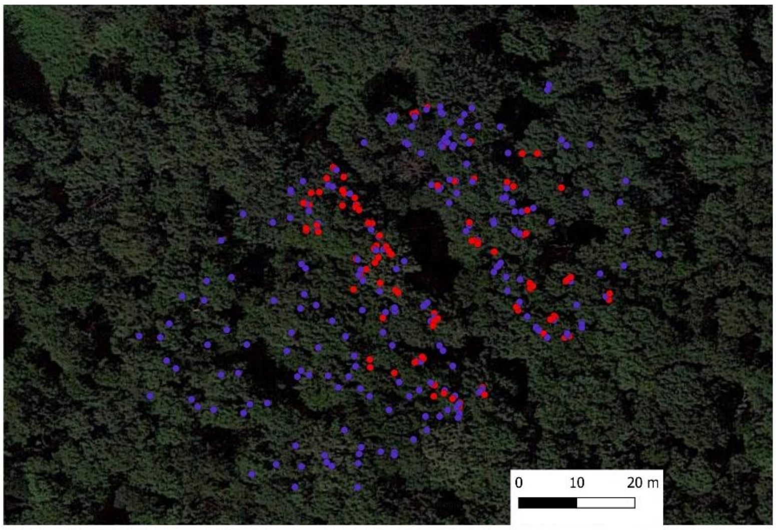

Figure 122: The relic forest in Carlino: the positions of all the trees in the survey area were detected using a submetric GPS technology (blue dots). Then in half of the survey area it was applied a selective thinning, cutting down part of the trees (red dots).

Among the main problems detected, there is the high energy consumption of the sap flow sensors, which could make it more difficult to build a low-cost monitoring station that also include this type of technology. Moreover, at the time of installation, it is important to remember the fragility of the sap flow sensors: if the space for the probes it's too small, the trunk, growing, could compress the wires and break them.

In the monitoring of the truffle orchards, it is necessary to consider the interference that other living organisms may have with the monitoring station. For example, the entry of insects into the structure containing the dataloggers has led to a failure of the equipment. Also, some animals can damage the power supply cables. Finally, truffle collection data are influenced by the activity of animals that feed on it (but also by human collection).

Results and outputs

The results obtained show a strong link between fluctuations of the dendrometers data (which highlight the water status of the plant) and soil moisture. Therefore, the data of the dendrometers provide precise and reliable indications on the water status of plants, that could be used to organize the management of truffle orchards. From the graphs in figure 2, it could be said that productive plants can control dehydration more than nonproductive plants: better hydration means a greater ability to perform photosynthesis and thus higher carbon fixation.

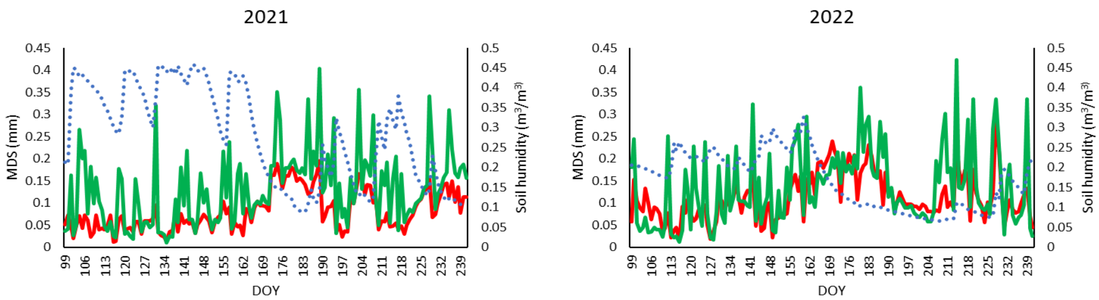

Figure 13: maximum daily shrinkage and soil humidity during seasons 2021 and 2022. In green, the fluctuations of non-productive plants, that are much wider than those of the productive plants (in red). The blue dotted line represents the soil humidity: when humidity is lower, fluctuations of non-productive plants are stronger. In 2022 the behaviour of the productive plants is more like that of the non-productive plant: this is probably due to the stress caused by the summer drought.

Georeferencing the plants of the truffle orchard in Caltrano made possible to distinguish between productive areas and non-productive areas (fig.3). For each plant (about 500) the position was recorded, the measurements of height, depth of crown and diameter at dbh were taken, and the status was indicated (productive or non-productive). This was possible through the collaboration with the manager of the plantation, who was able to point out which plants were productive, and which were not.

Figure 14: map of the plantation, that shows which plants are productive (red dots) and which are not (green dots). The georeferencing of plants and the analysis with GIS softwares could help in detecting patterns in production

Thanks to the dendrometrical measurements, it was possible to calculate the tree trunk volume and the tree canopy volume for each plant, to determine differences in the trunk/canopy ratio between productive and non-productive plants.

The analysis confirms that there are not substantial differences between the two groups, and this could suggest that the effect of mycorrhization does not have an obvious positive impact on plants in the production of new biomass (fig. 4).

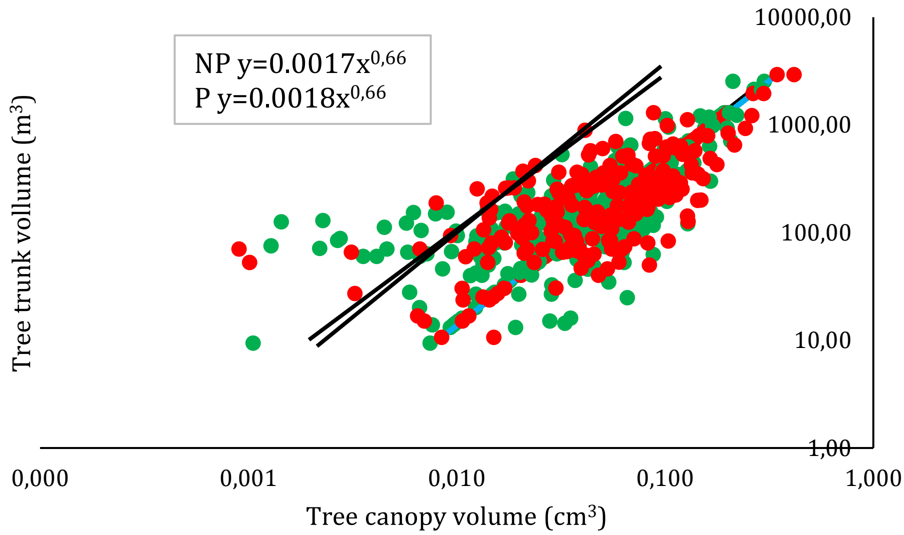

Figure 15: comparation of trunk/canopy ratio in productive (red dots) and non-productive (green dots) trees. In the two groups, with the same volume of crown, there are no differences in the volume of the stem. This means that productive plants, and therefore mycorrhized, do not grow more, although they can better control dehydration (as seen in the graphs reporting diametrical fluctuations).

A possible hypothesis is that the plant can produce NSCs (non-structural carbohydrates) useful for physiological mechanisms (for example, osmoregulation), but that it does not use them for its own growth: consumption by mushrooms could only be a secondary use of the NSCs. This could be a new hint in the management of truffle orchards, but also in the management of forests.

The production data first demonstrates how it is possible to extend the target plant data to the entire stand (fig.5 and 6). In fact, both in 2021 and 2022, the production data of a target plant (in this case, the number 7 was chosen as an example) have the same trend as the production data of the entire truffle farm. It is interesting to note that production peaks coincide with moments of recovery from water stress. Often, in fact, following periods of water abundance, production decreases (as occurred in May 2021).

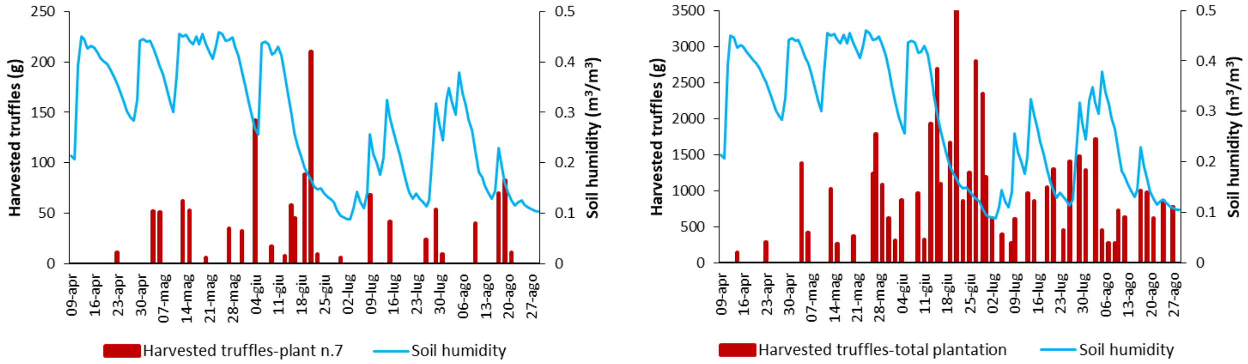

Figure 16: Truffle production compared to soil moisture. In the graph on the left is shown the production of the plant number 7, while in the graph on the right that of the entire truffle orchard for the year 2021. The production of truffles does not benefit from maximum moisture peaks, but rather from stress and recovery cycles.

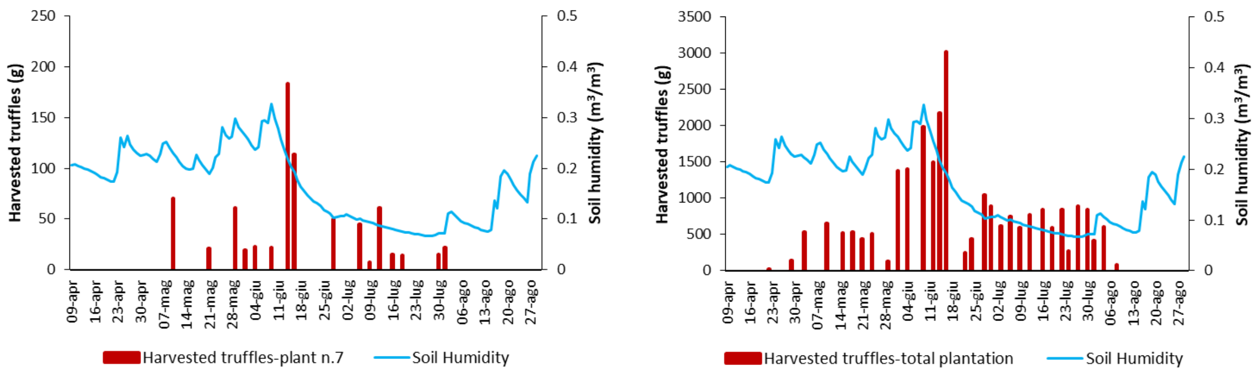

Figure 17: Truffle production compared to soil moisture. In the graph on the left is shown the production of the plant number 7, while in the graph on the right that of the entire truffle orchard for the year 2022. Compared to 2021, soil moisture has reached lower values and production has decreased.

The production peaks of both years do not fall at the moment of greater water availability, but at the moment of greater availability of daylight hours, in June. Increased availability of light means more photosynthesis and more carbohydrates that can also be transferred to the fungus in the form of radical exudates.

At the beginning of the project, we expected to observe significant differences between the productive and non-productive trees in the monitored physiological parameters, and this hypothesis has been confirmed.

The cost for data collection, carried out with the most used sensors and dataloggers for data collection in field, is more than €6000 (tab.1).

Table 1: costs of one monitoring station with the most commonly used sensors and datalogger

Costs for 1 station (Caltrano)			
Item	N°	Cost per unit (€)	Total cost (€)
Solar panel	1	130	130
Battery	1	77	77
Charge controller	1	37	37
Power supply circuit	1	160	160
Modem + antenna	1	300	300
Accessories (cables, etc..)	1	190	190
CR1000X datalogger	1	1600	1600
Dendrometers	8	82	656
TDR CS625 (humidity and soil temperature sensor)	2	209	418
T-RH sensor	1	227	227
Shield for T-RH sensor	1	72	72
Sap flow sensors (Granier type)	8	220	1760
Kit for installation	1	60	60
Field assistance	5	120	600
		TOT(€)=	6287

Using a programming card instead of the datalogger, and cheaper sensors (but still allowing the necessary data resolution) the cost drops to €1463 (tab.2). In this case the use of flow sensors has been omitted, since the dendrometers already provide precise information about the water status of the plant.

Table 2: costs of a monitoring station, using an Arduino board with some shields (e.g., for clock and data transmission) and cheaper sensors, that still allow a good resolution of the data

Costs for 1 "low-cost" station (Caltrano)			
Item	N°	Cost per unit (€)	Total cost (€)
Solar panel	1	130	130
Battery	1	77	77
Charge controller	1	37	37
Power supply circuit	1	160	160
Shield MikroElektronika GSM2 Click + Antenna	1	80	80
Accessories (cables, etc..)	1	170	170
Scheda arduino UNO	1	30	30
Clock and memory shield for arduino (clock and sd card)	1	10	10
SD card	1	16	16
Dendrometers	3	3.75	11.25
RS485 Soil Moisture & Temperature Sensor (S-Soil MT-02A)	1	82	82
T-RH sensor	1	227	227
Shield for T-RH sensor	1	72	72
Field assistance	3	120	360
		TOT(€)=	1463

Lessons learnt

The application of sensors in plantation, as reforestation or to produce truffles, shows a remarkable effectiveness in monitoring the abiotic stresses generated by intense climatic events such as drought, heavy rainfall or heat bubbles. The pilot project made it possible to relate environmental variables to water stress induced on the trees, as well as to monitor how environmental and tree's variables affect truffle production. The application for the first two and half years indicates how the use of low-cost sensors can be very useful for forecasting summer truffle production within 5-8 days, as well as the sensors can be employed to record carbon storage in real time in each stand. Further research would be needed to design a low-cost kit to be applied in small European forest holdings.

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Innovation Action 3

Innovative biological agents in pest control on truffle sites/plantations

Author: Tine Grebenc (SFI)

Context and current state of the art

In areas with higher average annual precipitation, oaks, in particularly *Quercus pubescens* and *Q. robur* tend to get regularly and abundantly infected by the powdery mildew. Powdery mildew of oak is caused by the fungus *Erysiphe alphitoides* (also known as *Microsphaera alphitoides*) and it is a common foliar pathogen of oak throughout Europe (Marçais and Desprez-Loustau, 2014). As powdery mildew grows well in high humidity and moderate temperatures conditions, it can appear and spread easily thus causing significant reduction of oaks leaf area and consequently photosynthesis (Copolovici et al. 2014). There are various ways known for preventing infestations of powdery mildew, based on various (non-specific) chemical methods, genetic resistance approaches, and use of biocontrol agents such as microbiological fungicide based on the antagonist fungus *Ampelomyces quisqualis* AQ-10 (Kiss, 2003). In particularly the effects of chemical fungicides and fungi-targeting biocontrol agents on truffle mycelium in truffle plantations remain unknown, thus we are trying to find other ways of preventing powdery mildew infestations without (or with a minimum) negative effects on truffle mycelium growth in soils. In addition, using of local strains of potential antagonists remain understudied.

Innovation actions to be developed in the pilot

The objective was to test the effects of a locally isolated strain of a harmless and potentially antagonistic phyllosphere fungus isolated directly from leaves where the development powdery mildew on leaves of oaks in selected young truffle plantations was observed as being reduced. The strain of the harmless phyllosphere fungus was isolated from healthy oak leaves and after a preliminary small-scale testing successfully reduced the appearance of a typical powdery mildew manifestation on oaks leaves. Besides confirmation of the preliminary results, this pilot study also aimed to quantify the ability of this fungus to prevent powdery mildew in young oak-truffle plantations, and to master the application method that would result in best prevention level. The proposed pilot study was done on several small-scale truffle plantations distributed in the SW area of Slovenia, with typical warm to hot climate, high dew occurrence and an average annual precipitation over 1400-1600 mm.

Methodology

Prior to the experimental setup a range of phyllosphere fungi were isolated from leaf surfaces of healthy oak leave on otherwise infected trees from a designated truffle plantation. Isolations and strain maintenance were provided by Biotechnical Faculty University of Ljubljana (dr. Janja Zajc and dr. Cene Gostinčar), where strains are also deposited and were borrowed for the purpose of this experiment. Among isolated strains one was selected based on its taxonomic position as potentially well adapted strain to extreme conditions and without known harmful effects on the plant (leaf) tissue. To test the potential effect of this locally isolated strain of a phyllosphere fungus against powdery mildew, we applied (sprayed) leaf surface of oaks in young truffle plantations with phyllosphere fungus strain and compared to the negative control (no spraying). We also implemented spraying with fermented milk that is to have similar effect on powdery mildew, and a control, where plain water was used. We applied those treatments on a sub-sample of 10 plants for each treatment.

Implementation progress of the pilot project (first phase)

In the first phase of the implementation the isolations of phyllosphere fungi were performed by a partner institution and subsequently deposited in their strain collection. The first phase also implemented a survey over three young truffle plantations executed in late summer 2020: plantation 1 (mainly *Q. pubescens*, 4 years after planting, about 200 seedlings from two seedling producers), plantation 2 (mainly C. avellana with individual *Q. pubescens* forming two lines, 3 years after planting, about 50 oak seedlings in otherwise 1.5 ha plantation), plantation 3 (Q. Pubescens, 2 years after planting, about 80 oak seedlings). Plantations were surveyed for the presence of powdery mildew on oak leaves from July till October, once per month and the most infected trees were selected and subsequently the plantation with highest number of heavily infected (> 90% leaves infected) was selected as an experimental site.

Implementation progress of the pilot project (second phase)

The second phase included spraying with a life culture of isolated strain of harmless and potentially antagonistic phyllosphere fungus. Culture was cultivated in weak Potatoe dextrose medium until late logarithmic phase and at that point kept refrigerated until application. No concentration was measured, and as the culture was in late log phase, we expected all easily accessible nutrients to be already used, thus culture was not filtered or washed. In addition, water and fermented milk was used in same volumes applied on different but comparably infected trees. The total volume of each solution was prepared in a way that overall, 0.1 L of the log stage culture/milk/water was being sprayed over one seedling (at time of application seedling were about 1.2m tall, well developed and 4 years old). Application was made in late May before the first visible infections of Powderly mildew appeared. The infection rate was assessed as a number of infected leaves among first 100 fully developed leaves counted on 3-5 randomly selected side branches.

Results and outputs

The main result of the pilot project is an improved application method for prevention of powdery mildew in young oak-truffle plantations using a *phyllosphere* fungal isolate of a local origin, and an approach with no predicted negative effects on the truffle mycelium in soils. The efficiency of the phyllosphere fungal isolate was significant. The treated with water only water showed 84,7% (±9,9%) infected leaves, and treated with fermented milk 76,2% (± 12.8%). On the other hand, trees treated with harmless and potentially antagonistic phyllosphere fungus showed a significant reduction of the percentage of infected leaves at 31,7% (± 8.3%) (for each treatment n=10). Given the results we concluded that fermented milk had no significant effect on mildew, and so was the case for water, while the application of the phyllosphere fungus significantly reduced the infection rate.

(a) (b)

Figure 1: Results of applying phyllosphere fungal isolate: (a) no application, (b) application done

Lessons learnt

Isolate of phyllosphere fungus regarded as harmless and potentially antagonistic proved to be an effective way to control and mitigate the effect of powdery mildew in oak plantations, and while there are no known negative environmental effects it might be considered as environmentally friendly as well.

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Innovation Action 4

Laboratory protocols for certification and identification of selected wild food products

Author: Tine Grebenc (SFI)

Context and current state of the art

Truffles are among the most precious wild food products collected in the Mediterranean area and sold globally. The main speciality of truffles are their irresistible aromas. Only fresh truffles (live fruiting bodies) produce their original aroma that gives them the adequate price and the real gastronomic value. Truffle aromas are generally species specific with detectable local/regional differences in the aroma components profile (Strojnik et al. 2020, Šiškovič et al. 2021). Also, molecular markers can distinguish between geographically distinct truffles among and within taxonomic species (Marozzi et al. 2020). Buyers of truffles should be informed and aware about these differences and well equipped with affordable and easy-to-perform methodologies for detecting truffles differences through specific tests that can prove the identity of the species (and with it the general aroma profiles) and its specific origin (with their local/ regional specific variants of aromas).

Innovation actions to be developed in the pilot

The objective is to gather alternative way to identify truffle species and their origin, truffle aromas and other potential characteristics that could help end users in identifying truffles as end products that they are buying. For each approach/methodology, a laboratory protocol for identification (and potential certification methodology) was collected and/or referred to.

Methodology

We have applied a gas chromatography approach in determining aroma components of all commercial truffles available in Slovenia (native or marketed at least 20 truffles distinct sporocarps were analysed per species). Results represented different aroma components (like alcohols, aldehydes, fatty acids etc.), Different for each species. This enabled us to identify truffle solely by its aroma composition – their relative shares, which are distinctive for each species (Figure 1). Basically, we read the chemical trails that each individual truffle was releasing in time of the analysis. The approach is costly, and it requires a specialized laboratory equipment, but.is on the other hand very sensitive.

Implementation progress of the pilot project (first phase)

In the first phase we have collected roughly 1500 samples of truffles from different species (mainly European species) and from a range of countries. These samples were being collected from 2017 until 2020, used for a molecular barcoding using rDNA ITS marker following the protocol in Sulzbacher et al. (2020) (results not part of this report). Analysed collections are kept in herbarium collection at LJF and are available upon request. In addition to reference storage a selection of ascocarps showing no obvious damage or rotting were separated promptly after collection for aromas analysis (after the methodology published in Strojnik et al. (2020). In the first phase the data from past studies and additional collections from 2020 and 2021 were reevaluated for this study.

Implementation progress of the pilot project (second phase)

In the second phase the data from previous phase were published.

Results and outputs

While the molecular barcoding confirmed its reliability and correspondence of marker variability among species and detectable differences among various geographic regions, a detailed aroma analysis confirmed the observed molecular identity / variability at the species level (Figure 1).

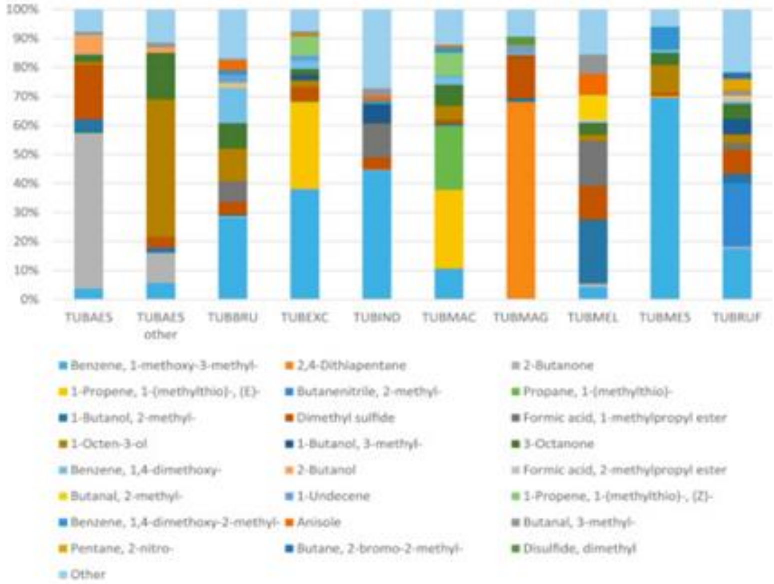

Figure 1: Results of gas chromatography analysis of 20 native truffle distinct sporocarps (from Strojnik et al. 2020)

Additional and more in-depth analysis of aroma composition aroma profiles of only the most frequently collected and analysed species showd that truffle aroma does not only discriminate well at the species level but can at a high enough number of samples from same location (in our case for *Tuber aestivum* – the summer truffle), also successfully distinguish among each other also sampling areas (Figure 2). However, we did not attempt to analyse specific reasons for a geographic variability in % of different chemicals (volatile compounds) among analysed individual truffles, yet a geographic distance seems to highly correlate with observed (small) differences.

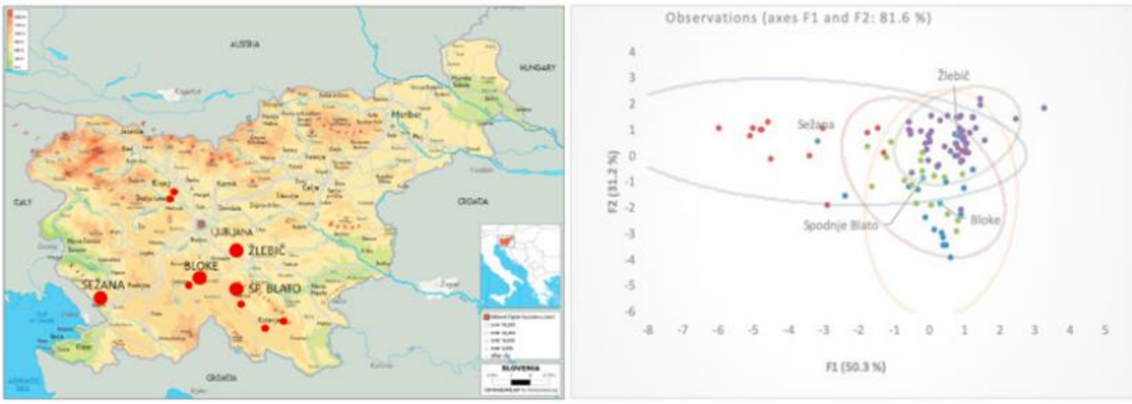

Figure 2: Intraspecific variation in *Tuber aestivum* – mainly in quantity of individual aroma components enabled to group truffles from one location (from Strojnik et al. 2020)

Lessons learnt

Truffles were and remain among most mysterious fungi and however well studied, there is still space for scientific and technical improvements. While the molecular analysis, applied on a large number of samples confirmed the previous information about species diversity and haplotype distribution, a wide-range aroma analysis of several truffle species, never till date performed on such a large number of samples, confirmed the methodology to be suitable for identification as well. Unfortunately, like molecular tools, also aroma analysis requires an expensive equipment, highly skilled personnel and for getting reproducible and accurate data also fast handling of collections. While there are only few molecular markers published for disclosing of truffle geographic origin (e.g. Murat et al. 2013), an aroma analysis based on a sufficient number of samples and a quality reference base with high geographic and ecological resolution can serve as a repeatable and relevant way of tracing the origin of an unknown truffle ascocarps.

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2.2. Pine nuts and acorns

Innovation Action 5
Quantifying pinecone production, with sensors and drones

Author: Míriam Piqué (CTFC) and Shawn C. Kefauver (UAB)

Context and current state of the art

The edible pine nut of the stone pine (*Pinus pinea* L.) constitutes, due to its high nutritional value, excellent flavor and connection to the Mediterranean diet, one of the most emblematic WFP's of the Mediterranean forests, with an important impact on the world market (Calama et al., 2020). However, cone production is highly variable between trees and years, making it difficult to predict annual production and consequently to organize the harvesting of the pinecones, from which the pine nuts will be obtained. In this sense, it is very common that forest owners (private or public) do not know the production of their forests or plantations and are not able to organize the correct collection of cones in time and space, with proper harvesting techniques, avoiding collection outside the harvest season or pinecones robberies.

Currently, pinecone production (Figure 1) is quantified visually before the start of the collection with a subjective estimate of the number of pinecones in a selection of trees within the forest and, after that, the mean tree production is extrapolated to an estimation of the full productivity of the forest. Visual evaluation of cone production is complex, both in plantations and in forest stands, where in addition mobility and observation conditions are complicated. In this context, the use of cone production models with remote sensing has incalculable potential. Studies on cone production's estimations in stone pine stands with sensors are scarce (Schneider et al., 2020). Thus, it is necessary to examine innovative techniques and to implement new experiences to analyse the capabilities and drawbacks of using remote sensing techniques to evaluate cone production in stone pine stands at tree level.

Figure 1. Pinecone production and edible pine nuts from stone pine (Pinus pinea L.)

Innovation actions to be developed in the pilot

The objective is to evaluate pinecone production in sufficient time to organize the correct collection of cones in time and space, thus increasing the efficiency of a key phase of the pinecones harvesting as it is the evaluation of annual production.

The innovation presented in this pilot project is related to the establishment and provision of new technologies and protocols for directly quantifying pinecone production, using drones and multispectral sensors, to develop more efficient and precise evaluation of pinecones than the current visual pinecone estimation. The idea for improvement is the use of remote sensors to estimate pine productivity for a sufficient number of trees in a systematic way for the same extrapolation or even to partially count the pinecones directly and estimate productivity for every tree in a whole forest. The development and evaluation of the methodologies by using remote sensors are carried out on pilot plots.

The innovation is important and useful for different sectors of the value chain, as producers, pickers, harvesters and processors. For exemple:

- pine forests owners (private or public) can know the potential value of the annual harvesting, which allow them to organize the correct collection of cones in time and space, with proper harvesting techniques, avoiding collection outside the harvest season or pinecones robberies, also help them to negotiate the conditions for the pinecones sale or collection subcontracting
- harvesting companies can know more precisely the total production and proximately value for an specific forest area and to plan the most profitable method of harvesting (estimate the needs for mobilizing machinery and human resources for a campaign).
- processing companies will be able to plan the processing, commercialization and marketing campaigns with less uncertainty.

Methodology

The general objective is to establish a protocol and/or tool for direct quantification of pinecone production with remote sensors that allows a quicker and more objective evaluation than the current visual procedure. Methodology for that purpose has three main tasks:
1. Definition of the best differential response and the sensor needed to capture it.
2. Definition of the sample of pines to evaluate the production of the stand.
3. Image processing and obtaining results.

1. *Definition of the best differential responses and sensors:*

Regarding the differential response, it is necessary to find the way in which cones are most different from their environment (branches and leaves). It is necessary to define in which bands of the spectrum pinecones are most different, exploring different options and combinations, and by modulating the date and time of image capture. The first necessary tests can be carried out from the ground or even in the laboratory, testing visible bands, near and middle IF, ultraviolet, microwaves, radio waves, etc.

Different sensors of different EM bands are tested to find what makes the higher differences. A test is set up in the laboratory or in the field with a sample of pine crowns with pinecones, and images are captured with different bands. These images are combined to find the optimum where the mature pinecones are most different from their surroundings. As a partial result, a selection of EM bands to be tested in a real environment is obtained. The sensor required for the selected bands is mounted on a UAV and images of real trees are taken. The images are processed to establish whether the pattern observed in the initial test is maintained or whether the sensor and/or image processing needs to be slightly varied.

Once the necessary sensor is available, field flights are carried out in field plots (two periods, spring and autumn, see point 3.2).

Study site, sample and field data collection:

Study sites are two *Pinus pinea* plantations:

- Study site 1: **Garrigàs-Alt Empordà** (Girona, Catalonia), Coordinates UTM: 31N/ETRS89; x: 496289,5; y: 4669449,5.
- Study site 2: **Torre Marimón-Vallès Oriental** (Barcelona, Catalonia), Coordinates UTM: 31N/ETRS89; x: 431105; y: 4606621,5.

Figure 2. *Pinus pinea* plantation (study site 1)

Figure 3. *Pinus pinea* plantation (study site 2)

2. Sample selection:

The criteria for <u>plots selection</u> were as follows:

- Presence of pinecones.
- Accessibility with a normal vehicle.
- Accessibility for forestry machinery, gentle slopes.
- Protection against pinecone theft.
- Silvodasometric monitoring to obtain context information.
- Cone harvesting carried out in a controlled and monitored manner, ideally at individual tree scale.
- Location preferably outside areas restricted to the use of unmanned aerial vehicles (UAVs; Figure 4).

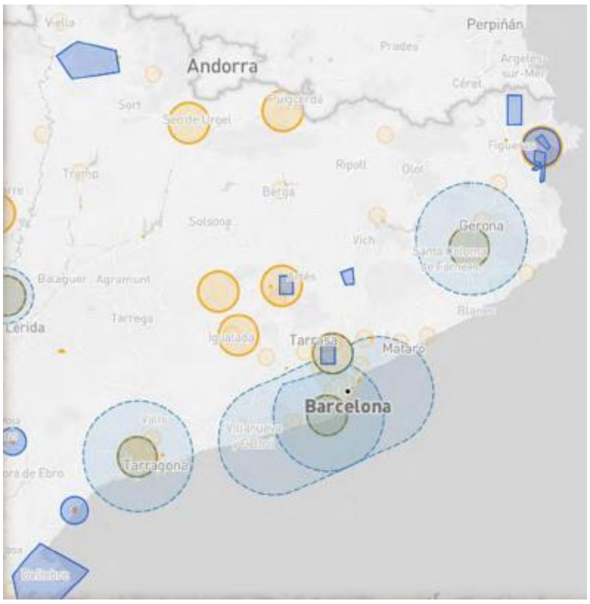

Figure 4. Areas restricted to unmanned aerial vehicles in Catalonia.

Data collection:

In each plot, both variables to (i) allow a silvicultural description of the plantation and variables to (ii) evaluate pinecone production are measured.

As for the dasometric variables, the following are measured:

- the diameter at 50 cm height, because many of the trees are grafted at 70-120 cm.
- crown diameter, based on the measurement of two crossed diameters, one in the direction of the centre of the plot and the other in a perpendicular direction.
- total height and height of the first live branch of the tree.
- sociological stratum.

The following estimates and measurements are made for the production variables:
- Visual estimation of cone production, by visually counting the number of 3rd year, 2n year and 1st year pinecones.

All trees are identified with a code.

3. Data processing

It includes the processing of the images obtained by the sensors and the processing of the data obtained in the field, both in terms of dasometric variables and cone production.

Once the differential response has been defined and the images have been obtained, it is necessary to define the automatic processing, based on pattern recognition with manual training.

Finally, it is necessary to contrast the experimental estimates with reference data, which will be those established as real production, in order to evaluate the degree of accuracy and to find out possible systematic biases.

Implementation progress of the pilot project (first phase)

During the first project period (June 2020 to December 2021) we have been working in collaboration with the University of Barcelona in:
- Definition of the best differential responses and sensors
- Field data collection (forest measurements and dron flights).
- Preliminariy processing of data (Figure 5).

During 2021, some survey flights and reflectance studies were carried out in collaboration with the University of Barcelona, in order to obtain initial results on the data collection methodology. The flights and data collection were carried out on the plots:
- Garrigàs-Alt Empordà plot (Girona, Catalonia).
- Torre Marimón-Vallès Oriental Plot (Barcelona, Catalonia)

Flights are carried out with multispectral sensors transported on unmanned aerial vehicles (UAV). The UAV Mikrokopter OktoXL 6S12 is used to capture images with RGB camera and multispectral camera.

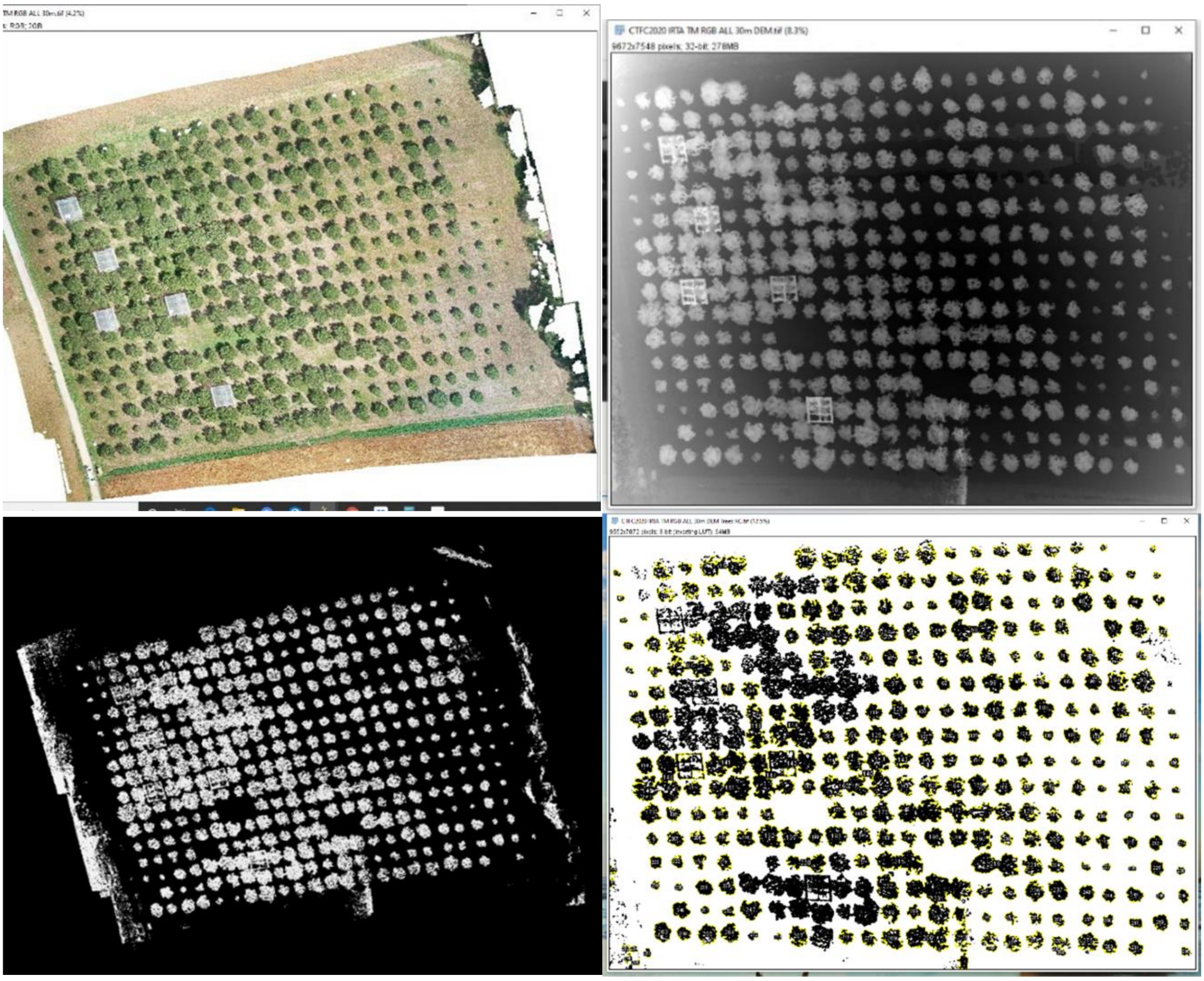

Figure 5. Example of images and 3D models obtained

Implementation progress of the pilot project (Second phase)

During the second period (January 2022 – November 2022) we have continued working in collaboration with the University of Barcelona on the following tasks:

- Have continued carrying out flights and field data collection.
- Processing images and data for cones estimation by means of Machine Learning.
- Preliminary elaboration of results.

New flights and data collection were implemented on the following areas:

- Garrigàs-Alt Empordà plot (Girona, Catalonia).
- Mixed pine forest plot (Hostalric, Girona).

Figure 1 shows aerial images of the areas where new flights were done.

Figure 1a. Garrigàs-Alt Empordà plot (Girona, Catalonia).

Figure 1b. Mixed pine forest plot (Hostalric, Girona).

In Garrigàs-Alt Empordà plot images were also taken laterally and from below with Lumix GX7 and Pixel 4XL with the aim of capturing images of mature cones. In the mixed pine forests images were taken with drone DJI Mavic 2 Pro and RGB camera. Together with the images, plots measurements were done to be used for validating sensors and images estimations. The variables measured were related to trees characteristics (size of trees) and pinecones production (pinecones of 1 and 3 years).

After field measurements, machine learning techniques have been used for processing images and data and estimates cones production (pinecones of 1 and 3 years).

Results and outputs

The new data collected were processed and used for the construction of artificial intelligence models for counting 1st year pinecones (flowers) from drones (UAV). Work has been carried out on the processing of the images and orthomosaics, both in the case of the plantation plots and in the case of the images taken from the forest (in 2021 and 2022). At the same time, new images processing techniques have been explored, using the latest machine learning techniques to count pinecones in the 1st and 3rd year, and to estimate the production of *Pinus pinea* cones using UAV and proximal sensors.

We have obtained good results (80% precision) for counting pinecones of the 1st year (flowers), by using high-resolution RGB images acquired from an unmanned aerial vehicle (UAV) at early stages of the crop (around may). Flowers are often overlapping in the same sector of the image. Previous works have found that there is a relation between number of flowers and number of pine cones in this species. VGG Image Annotator (VIA) was used to manually segment and quantify the flowers contained in the images and Coco Annotator to rescale the labels. The Mask R-CNN network was implemented to perform segmentation by instances, as a backbone (backbone/feature extractor) a ResNet50 was used. As a result, the model delimits and locates in the image the pixels corresponding to each flower obtaining an Average Precision (AP) of 79.89 %. In addition, the system performs the total count of detected flowers. The model was implemented using Python 3, Keras and TensorFlow.

Figures 2 and 3 show an example of the data set used to develop the models for pinecones estimation.

Figure 2. Original images of the data set.

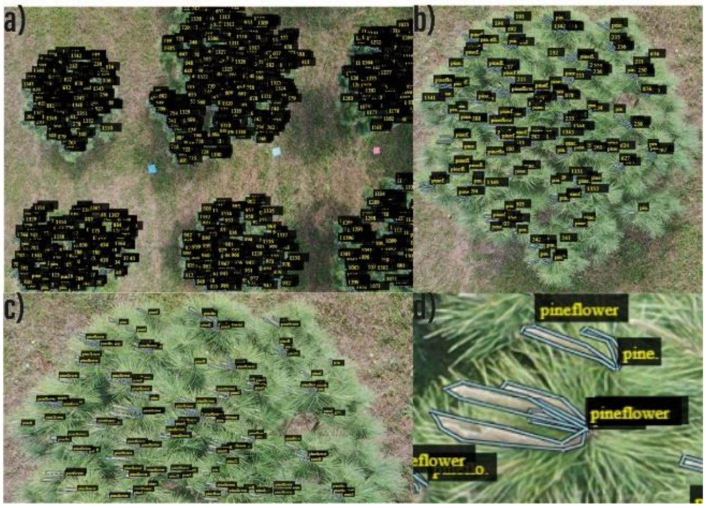

Figure 3. Image with the labels inserted by means of VGG Image Annotator (VIA). a) Full image with labels, b) Zoom of 2.5x, c) Zoom of 5x, d) Zoom cropping of 5x.

In the coming months efforts will be focus on the estimation of pinecones of 3rd year (Figure 4) and finalizing the tools and/or protocols for direct quantification of pinecone production with remote sensors that will allow a quicker and more objective evaluation of production than the current visual procedure.

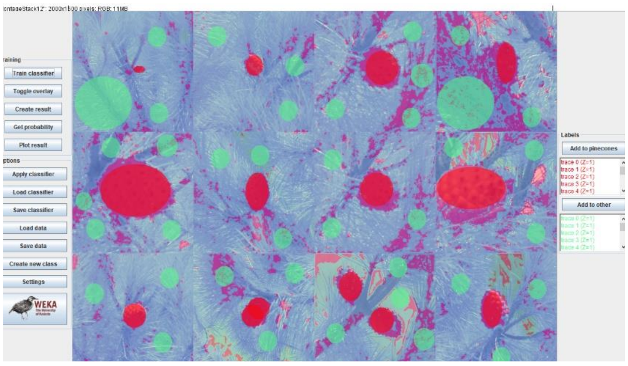

Figure 4: Lateral images with pinecones of 3rd year

Lessons learnt

We have attempted a systematic approach to the challenge of automatic quantification of 1st and 3rd year *Pinus pinea* cones by using sensors and drones (unmanned aerial vehicle, UAV). The results have been successful, but we have detected some limitations in estimating current year harvest (3rd year cones) when using images from sensors transported by drones.

We have obtained good results for counting pinecones by using high-resolution RGB images. The models developed for the image-based automatic pinecone counts in both, from drones and field level, with a normal

RGB camera are good, with a high correlation with the image-based manual pinecone counts.

We have got good results for counting pinecones of the 1st year (80% precision), by using high-resolution RGB images acquired from an unmanned aerial vehicle (UAV), but it is very complicated the visibility of 3rd year pinecones with the aerial perspective of the drones, since 3rd year pinecones are not in the top of the pine crown, they are hidden, covered by the tree branches.

So then, for counting 3rd year pinecones would be more promising to take the images from the field and therefore there may be some potential to quantify current year pinecones harvest from a profile perspective, depending on the tree canopy.

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Innovation Action 6

Prediction systems for the annual supply of acorn and flour as raw material for human food products

Author: Joana Amaral Paulo and Inês Conceição (ISA)

Context and current state of the art

Oak acorns have been part of human diet for thousands of years in many countries around the world. However, oak acorns, like many other wild food products, were gradually displaced over time by increasingly more refined products and have received little attention as resources for improving nutrition and supporting livelihoods. Like many wild forest products, oak acorns are not only an important source of nutrition and sustenance. In fact, they are also growing in relevance for the food industry as "nutraceuticals" and functional foods (FAO, 2021).

Within the Mediterranean Basin, the Quercus spp. is widely distributed across the Iberian Peninsula landscapes, setting up silvopastoral systems with high ecological and socioeconomic value. In Portugal, the Montado areas are typical landscapes in the Southern and Central areas, with the prevalence of *Quercus suber* and *Quercus rotundifolia*. In these systems acorn production is known to be highly variable between years, geographic locations and even between nearby trees. This variability is a source of uncertainty regarding acorn production to an emerging sector, and a challenge for modelling the production of acorns (Crous-Duran, 2016).

Despite an increase in interest regarding acorns from many oak species, there is a limited number of studies and data regarding acorn yield in Portugal, and those existing are mainly focused on *Quercus rotundifolia* in the Alentejo region. Data collection, research and the development of prediction tools for acorn yield is crucial for developing a sustainable acorn economic row.

Having in mind the context of circular economy, the importance of utilizing by-products is a concern nowadays. Regarding acorns, the usage of cupules and fruit wall (pericarp) for bioactive compounds might be of interest (Mébarki *et al*., 2019).

Innovation actions to be developed in the pilot

This pilot project aims at fulfilling the following innovation actions:

1) Separately account for the yield and percentages of acorn and cupules.
2) Develop predictive models for estimating acorn and cupule productions of *Quercus suber* and *Quercus rotundifolia* for Alentejo and Ribatejo regions.
3) Develop specific drying curves for acorns and cupules of cork oak and holm oak species.

Methodology

The main task carried out in the period included in this report was the collection of data regarding cork oak and holm oak acorn production, at tree level. Gathering this data was challenging, since it implies to find available and suitable plots that allow to get the precise yield, and that are fenced to prevent predation from wild animals and livestock during the months of acorn production (ideally from September to January, approximately). We were able to collect data from two sites: Herdade do Freixo do Meio in Alentejo region and Quinta Grande, Coruche in Ribatejo region. On the first site, sixteen trees were marked, half being cork oak trees and half being holm oak trees. On the second site, we were able to collect data from three trees, all cork oak trees of large dimensions considering diameter at breast height and crown projection area.

Cork oak trees produce acorns earlier in the season, while most acorns from holm oak trees are still falling in December and January. Since acorns were picked up from the ground (Figure 1) after they fall, periodic tree monitoring was carried out along this period (every one- or two-weeks interval).

Figure 1. Collecting acorns from the ground in the Montado, Alentejo (Photo: Inês Bento).

All acorns and cupules were picked and weighted in the field, using a weighing scale with a precision of 10 g. Regarding acorns, they were picked despite the development stage they presented. This included stages f, g, and h, as proposed by Gómez-Casero *et al.* (2007).

The collected material was brought to the laboratory where it was kept under refrigerated conditions until the drying procedure was initiated for the determination of humidity content and for the development of acorn and cupule drying curves. The drying procedure was carried out separately for cupules, acorns under stage f+g, and acorns under stage h. In addition, a sub sample was taken for the determination of "acorn bark" percentage.

All sampled trees were measured considering dendrometric variables: diameter at breast height (using diameter tape), crown radius (using Vertex), and cork thickness (using a cork thickness gauge) for the case of cork oak (Figure 2).

Figure 2. Cork oak trees in the Montado, Alentejo (Photo: Inês Conceição).

Implementation progress of the pilot project (first phase)

In the first period of the pilot project, we collected data by periodic tree monitoring in both regions. We went to the field approximately once a week, since October 2021 (Figure 4). We intended to have 30 trees, but this was not possible due to the encountered constraints. The sample size was lower to what was previously planned, in particular due to difficulties in controlling the access of wild boars to the sampling areas of Herdade do Freixo do Meio.

Figure 4. Grass covering the ground in the month of January (Photo: Inês Conceição).

Drying and other laboratory procedures were developed. Preliminary relations between acorn yield, cupule yield and tree dendrometric variables were also analysed.

Implementation progress of the pilot project (second phase)

During the acorn production season that took place between October 2021 and January 2022, data was collected from two sites according to the procedures described in the first phase. As mentioned before, the sample size was lower to what was previously planned. Nevertheless, it was possible to arrive at production values at tree level and account for the acorn yield, also considering the percentages of each component, namely nuts and cupules. This is an important factor, as such components may be valued by different industries.

An issue to be considered by producers is that it's not possible to harvest the entire acorn production, as one must include animal feed and natural regeneration or nursery material, essential for the maintenance of the ecosystem.

After the harvest and quantification of acorn production yield, the moisture content of acorn samples was determined and the development of acorn and cupule drying curves was carried out.

Results and outputs

I. Separately account for the yield and percentages of acorn and cupules

The following figures show acorn production by tree basal area (Figure 1 and 2) and by tree canopy area (Figure 3 and 4). In our sample, it is possible to see that holm oaks have a higher correlation between production and basal area (r=0,96), compared to cork oaks (r=0,56). The same phenomenon is observed in relation to the canopy area, though not as strong (with r=0,45 for holm oaks and r=0,02 for cork oaks).

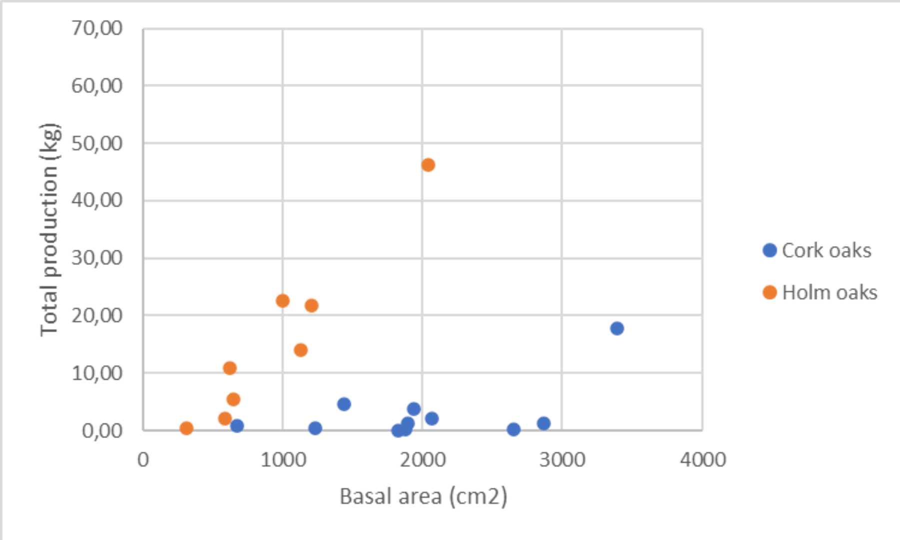

Figure 1. Total production (acorns + cupules) in dry weight by basal area

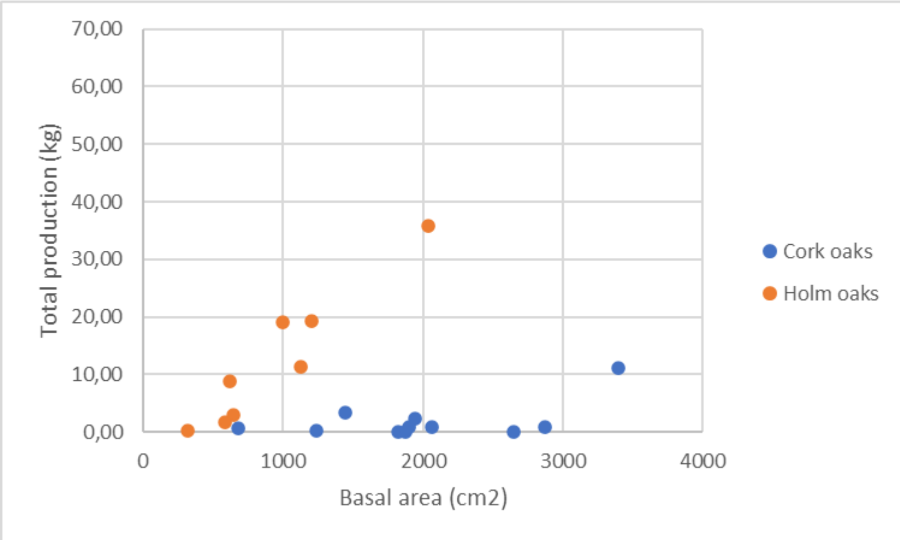

Figure 2. Total production (acorns with nutshells) in dry weight by basal area

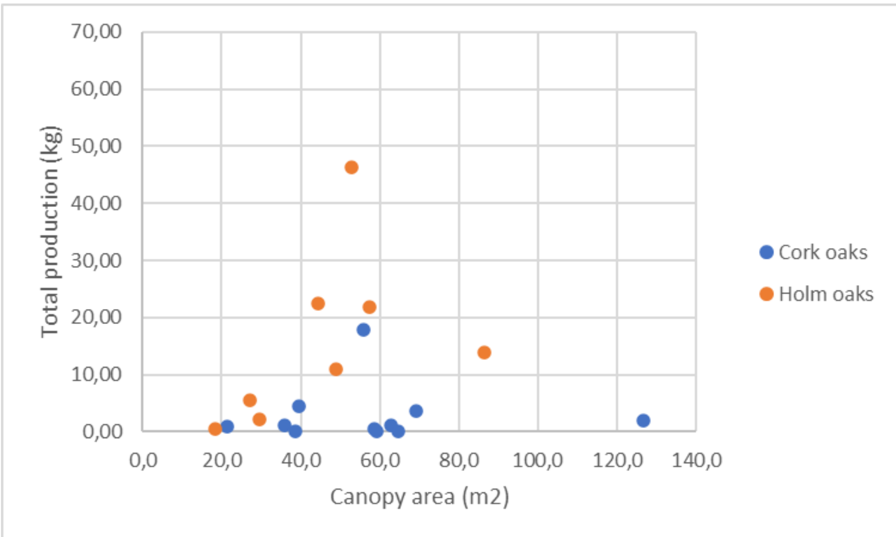

Figure 3. Total production (acorns + cupules) in dry weight by canopy area

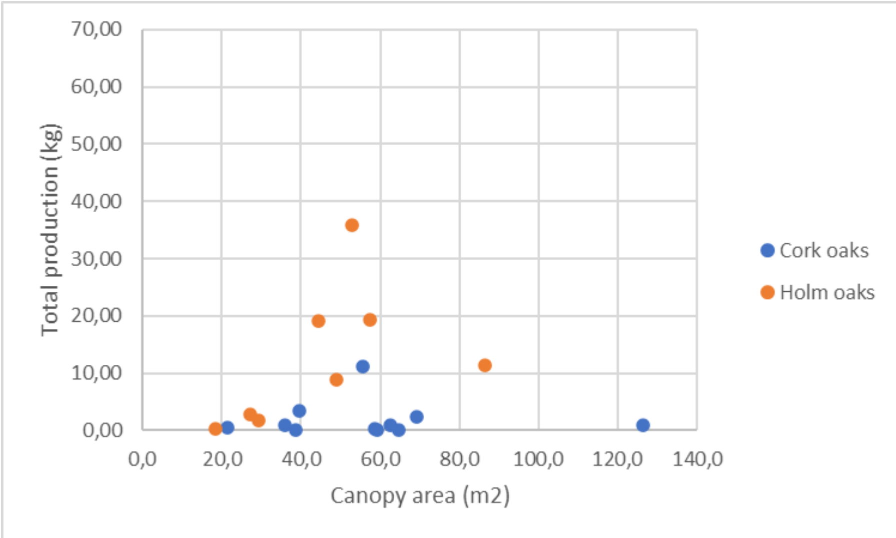

Figure 4. Total production (acorns with nutshells) in dry weight by canopy area

The drying process is essential to obtain a high-quality acorn flour. Based on the interest of using acorns of various species, we carried out a test with a different species of oak, the Portuguese oak, in comparison with cork oak acorns. The results are shown in Figure 5.

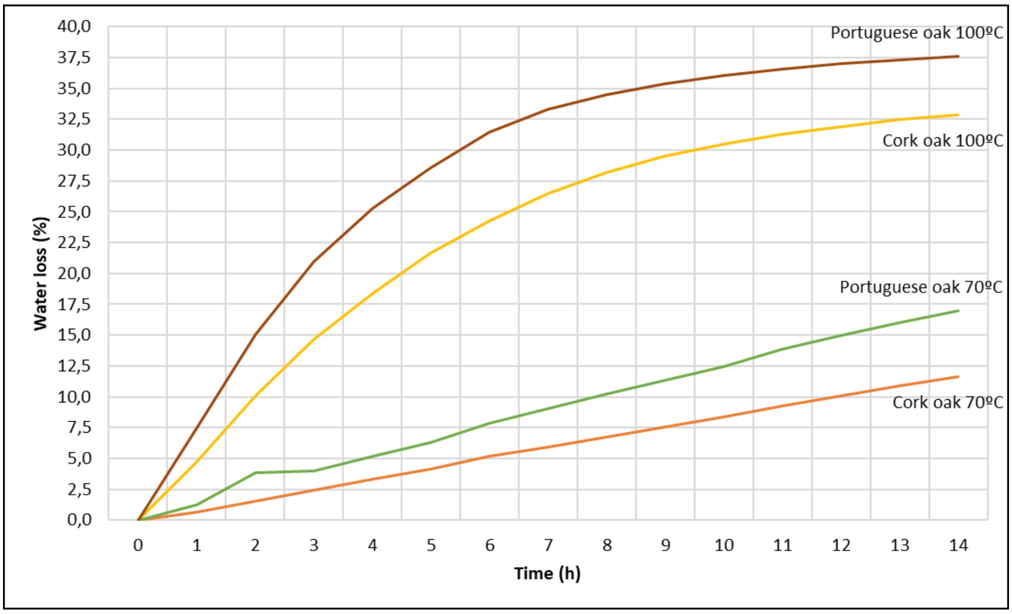

Figure 5. *Q. suber* and *Q. faginea* acorns drying curves at 70 ºC and 100 ºC

The following figures show acorns drying curves – we used consumable acorns with shell, as shown in Figure 6 – at two temperatures, 70ºC and 100ºC (Figure 7 and 8).

Separately, cupules were dried as well, obtaining drying curves at the same temperatures (Figure 9 and 10). Note that in all graphs, the solid black line represents the mean.

Figure 6. Holm oak acorns and cupules samples

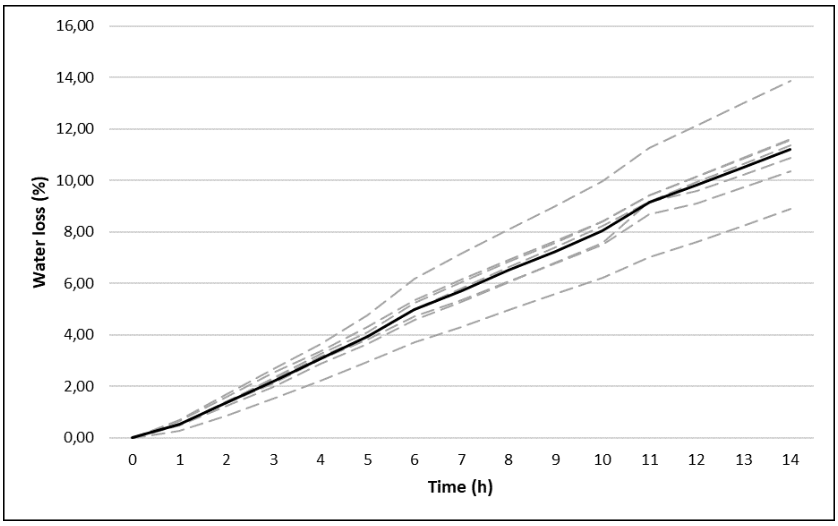

Figure 7. Holm oak acorns drying curves at 70 ºC.

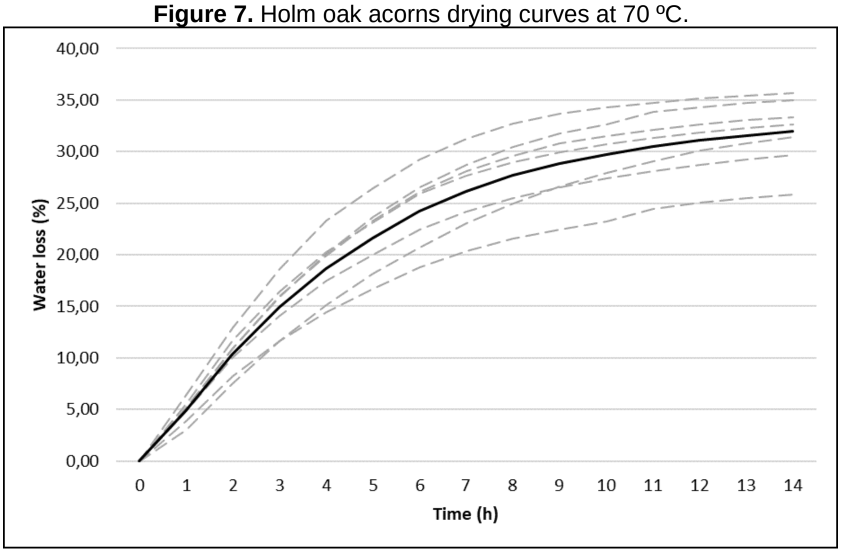

Figure 8. Holm oak acorns drying curves at 100 ºC

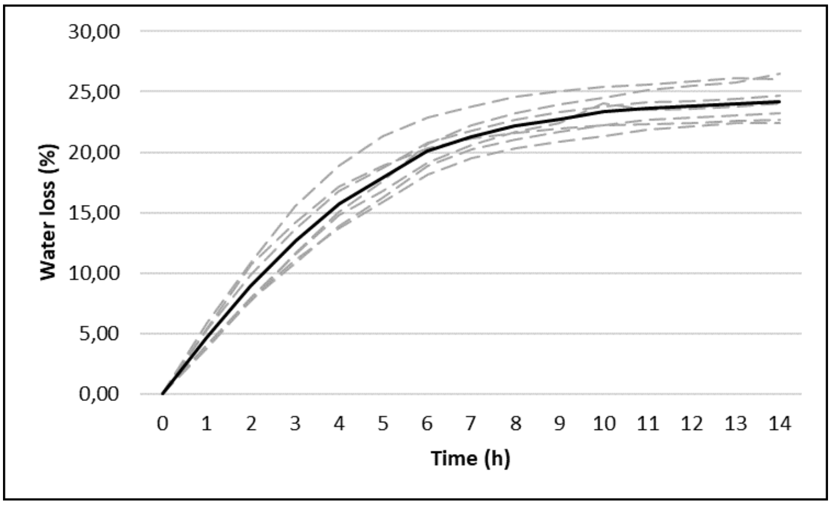

Figure 9. Holm oak cupules drying at 70 ºC

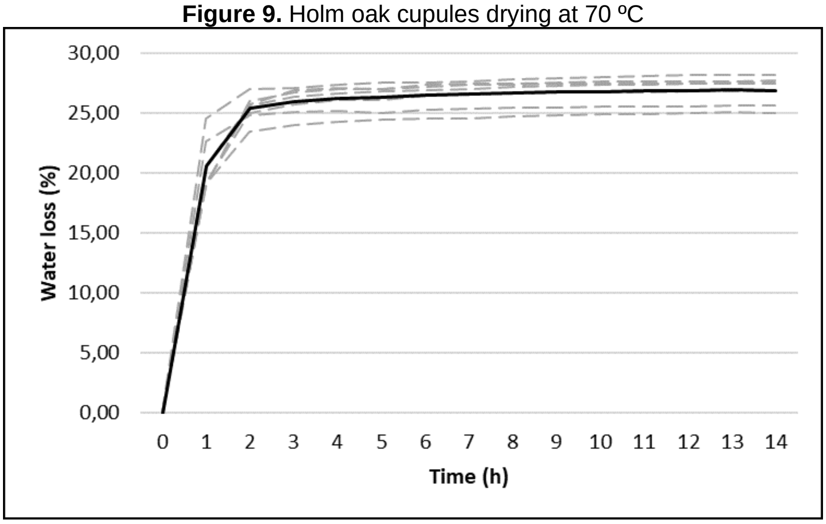

Figure 10. Holm oak cupules drying at 100 ºC

Lessons learnt

- Prediction models developed for holm oak are not suitable for predicting cork oak acorn production.
- The tree basal area had a significant correlation to acorn production in the holm oaks sampled.
- Acorn production in cork oak did not correlate significantly to any of the tree variables considered.
- The number of cork oak trees not producing, or producing quite low amounts of acorns, is high. Specific modelling techniques should be considered for this data.
- Acorn cupules drying process is quite fast (2 hours to 8 hours depending on the drying temperature).
- Acorn drying at 70ºC for 14h does not allow reaching dry weight at 0% humidity. To reach this requirement, acorns need to dry at 100ºC for at least 10 hours.

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Innovation Action 7

Innovation for quality, safety and sustainability in the wild food products (WFP) value chains: reformulating artisanal stages of acorn processing

Author: Ana Fonseca (HFM)

Context and current state of the art

The use of acorn for human consumption, dates back several centuries and is well documented in the literature and archaeological findings in the Iberian Peninsula. However, nowadays, it is mainly used to feed livestock. In Montado Alentejano, where the oaks that produce them abound, acorns are potentially one of the most important resources for human consumption in various forms: dried and peeled, in flours or in processed foods such as breads, soups, hamburgers, crackers and cookies or pâtés. This has been one of the main goals of Cooperativa de Usuários Freixo do Meio (CUFM), which seeks to produce food in a sustainable way. At CUFM, the acorn processing is carried out in a micro factory whose unsophisticated equipment is suitable for small-scale manual work. This way, the flow of processed raw materials is slow, expensive, and inconsistent.

The main challenges/issues faced are: i) maintaining the quality of the product, especially in the grinding phase with great variations both in granulometry and in the presence of bark traces, ii) the slow, expensive, and artisanal process (example the drying process), which results in overvalued products, which might otherwise be more accessible, iii) the inter-annual variability of acorn production, with consequences on the unpredictability of the production, and iv) acorn harvesting process.

The expected results are: i) anticipating the production of the raw material, allowing for better planning of its destination (internal flows for production on the farm or sale abroad), ii) greater quantity of processed product available in reduced time; iii) decrease in the need for labor and consequent cost reduction; iv) homogeneity and consistency to obtain a finished product with higher quality and better value.

Identify the innovation actions to be developed in the pilot

Regarding the **Production** phase we intend to innovate by developing a protocol that defines the harvesting method (when to start and how to do it so that the process is as sustainable and as efficient as possible maintaining the quality of the acorns):

- ACTION 1: Developing a Harvesting Protocol

Regarding the **Processing** phase we intend to have three different innovation actions, one to do with the drying, another with the peeling and a third one with the griding.

- ACTION 2: Improving the Drying Process
- ACTION 3: Improving Peeling Process
- ACTION 4: Improving the Grinding Process

Methodology

ACTION 1: Developing a Harvesting Protocol
Tools to be used are a map of the property with the different units (fences) it is divided and make a survey of the the age of the trees that bare acorns in each unit. Coordinating the different actors involved in this process is crucial (there are people employed by the Coop and people outsourced to perform this task). After experiencing the state of the art in 2021/2022 we intend to fully implement innovation in 2022/2023.

ACTION 2: Improving the Drying Process
The tools and equipment used are the two storerooms called "Caniço" where we dry the acorns with a source of heat in the bottom room, the cloister where the acorns are spread and wait for the turn to go to the Caniço. Experiments are to test different sources of heat the Caniço (that is less needy in inputs and labour and creating a method to dry the acorns faster) and record temperature and humidity. Also, we intend to develop distinct drying curves and predict quantity of final product.

ACTION 3: Improving Peeling Process
We have a universal peeler, and we will test how we can lower the need in labour and the quality of the process (less pieces of shells).

ACTION 4: Improving the Gridding Process
With the different mills we have we can test efficiency and quality of the final product. Finding the optimal humidity content to help the griding process. Considering trying to outsource this process and compare costs/benefits.

Implementation progress of the pilot project (first phase)

- ACTION 1: Developing a Harvesting Protocol

This action is undergoing but not finished. The different parts of the property with trees baring acorns are identified and the actors involved as well. It has not been possible to implemented fully this season but next season it will be fully experimented.

- ACTION 2: Improving the Drying Process

This action is undergoing but not finished. We are now not even halfway through the drying phase. We have bought a new source of heat for the dryer and are now make the first conclusions on the efficiency of this equipment. We have bought an equipment as well to measure humidity. We have tried different quantities of acorn inside the dryer and starting to make first conclusions. We are recording weight, temperature and humidity 2 times a week.

- ACTION 3: Improving Peeling Process

This action has not started yet.

- ACTION 4: Improving the Gridding Process

This action has not started yet.

Implementation progress of the pilot project (second phase)

In the year 2022, we had a very low production of acorns, so our work consisted more in finalizing the collection and processing of the data that were being collected when the last report was written. To this data treatment we added other analyses that were very interesting for our process.

ACTION 1: Developing a Harvesting Protocol
The acorn collection protocol has been completed and the collection period and areas selected. Due to big price variation of acorns sold by our partners (acorns collected in our farm) we decided to perform ourselves the collection of the acorns used to process. For now, we cannot process more than 4 Ton of acorns per year so it will represent the limit to collect until we improve sales, processing and storing capacity. The collection starts on 15 October and finishes on 15 November and is made by a team of 4 workers with the use of sticks, cloths, and of a lifting system to reach the highest canopies. The best trees are identified as the best collection areas. Beside this identification, an orchard of sweet acorn grafted trees was installed to increase the collection of high quality, big, sweet acorns.

ACTION 2: Improving the Drying Process
We finished the evaluation of the drying process and estimated an average moisture content for our acorns of 37%. We take a month to get acorns with 17% of moisture content, after pass 15 days in the cloister where they dry in a passive way and other 15 days in a forced air system. This is the average moisture content of dried acorns that we use to get a high-quality flour.

ACTION 3: Improving Peeling Process
We tested several peeling processes. The first method we tested was the traditional one that consists of drying the acorns, putting them in bags, and then beating them with a mallet to open them and remove the shell.
Then we tested an industrial device that breaks the acorns in two or three pieces and shell them using a centrifugation system. This machine is fast and efficient doing this work, so it was adopted as our device to shell the acorns. After this work of shelling the acorns, we use a blower that allows us to separate the kernel from the bark. The bark has economic value being sold to pharmaceutical companies, but in general is used as pig feed.

ACTION 4: Improving the Gridding Process
We tested four types of mills, a smaller wood mill, two industrial inox mills and an old-fashioned mill with stone grindstone. The smaller wood mill it is a mill for home use and makes very good, fine flour, but it is very slow, so we stopped using it and now it is only used to make chickpea flour when we need this ingredient in acorn burgers or acorn pâté.
The stainless-steel industrial mills have different powers and different vocations. This way we realize that the mill that makes crumb doesn't have enough power to make flour, so we use the less powerful mill to make crumb and the more powerful one to make flour. The crumb is needed for different recipes such as acorn

bread, “broa”, acorn burgers, among other recipes. The mill with a stone millstone cannot make flour since the acorn becomes paste-like because of the oil present in the acorn. This way we gave up its use for acorn milling and used it only for grains such as corn or wheat.

Results and outputs

After the implementation of the different actions related to the different phases of the acorn processing chain and the identification of the right equipment and protocols to implement them, we expect to turn all the process faster and more efficient. The last harvesting season was a lost season once we almost had no acorns, so we did not have the opportunity to experience the application of the established protocols in actions 1, 2 and 3. However, after estimating the moisture curves and the moisture content of our acorns, before and after the drying phases, we made use of our Task Sheets (that we fill when we produce the different products) to have a closer idea about the production costs. It helped a lot to define the right prices for these acorn products. We believe that the next production season will be better than the last one and we intent to apply the protocols and processes defined previously to improve our harvesting and drying phases. The work carried on in the action 4, was applied since them because we are still processing the acorns that we harvested in 2021, with the expected good results. Our work partners, like the owner of the Landroal bakery, and other partners who buy acorn flour from us, are the best attesters of the quality improvement of our flour.

Lessons learnt

The focus on the technological aspects of the acorn transformation was important for the improvement of the quality of the final products and for a greater profitability of the whole process. The quantification of the quantities of raw material in each phase of the value chain allowed a more accurate definition of prices of the different products. We intend to continue in this process of greater control of the different phases of the acorn value chain by permanently applying and adapting the established protocols. We also intend to implement the acorn oil production process in order to better monetize the final products obtained. With this work we realize that it is more advantageous for us to position ourselves as minimal processors of acorn products producing dehydrated acorn, flour, acorn infusion and other basic products, instead of focusing on a wide variety of products with a high level of processing. Thus, we intend to offer quality raw material to other operators located in the region and who have a greater vocation for the development of products with a high level of processing.

2.3. Aromatic plants

Innovation Action 8

New production and transformation process for aromatic plants

Author: Issam Touhami, Ibtissem Taghouti and Mariem Khalfaoui (INRGREF)

Context and current state of the art

In north and north-western Tunisia, approximately 830,737 hectares of forest lands and 4,706,029 hectares of rangelands in southern Tunisia are suitable to produce wild growth of Aromatic and Medicinal Plants-AMP (Ghoudi, 2002). Most spontaneous and cultivated plants are highly dependent on environmental conditions. In Tunisia, AMP's production is advantageous compared to other countries due to its profitability (low collect and distillation costs) (Govindasamy et al. 2014).

AMP secretes complex chemicals that once extracted, could be incorporated into various consumer products. They can be found in food flavorings, aromatherapy/personal care products, pharmaceuticals, perfumes, and a variety of other products (insecticides, dyes, and coloring). There are two types of plant extracts: essential oils and fixed oils. Essential oils (King, 2006; Turek and Stintzing, 2013) are volatile essential oils and melt at higher temperatures. Several market research companies have evaluated the current and projected global essential oils markets (Moyler, 1991; GVR, 2022).

Innovation actions to be developed in the pilot

The main objective of the pilot project is to generate income for rural women by the exploitation of the AMP products for different uses. The innovation actions to be developed in the pilot project are:

1. Better productivity: Improving oil extraction yield and quality
    - Adoption of a technical improvement of the distiller which consists in adding a water amendment to control the water level in the compartment n°1 (see figure 4) of the distiller to obtain more oil quantities.
    - The yield of aromatic and medicinal plants in essential oil and active principles is very influenced by the type and duration of drying. For this reason, we will study the effect of the type and duration of drying by developing a drying table for the main species used at the GDA. This will allow us to have good yields of essential oils with better organoleptic and biochemical properties. It will also minimize energy costs by knowing the optimum duration of extraction.

2. Valorizing by-products

3. Increasing visibility by joining WIKI PAM cluster: Facility to make our products known, new opportunity (e.g., cluster with innovative visibility strategies. New type of association.)
    - The cluster is divided into 6 working groups to facilitate the work of all members. Each group addresses a theme identified as requiring work for the cluster: marketing, logistics, training, certification, research and development, and technical and economic collaboration. All groups contain multiple stakeholders, so that all interests are represented at every stage. The groups meet regularly to work together and establish development strategies.
    - The advantage for artisans of working in a cluster is the sharing of resources and knowledge. For example, a cluster researcher who studies AMP and discovers a new therapeutic property can inform the artisan, who can then use it in the best way. Or, if the cluster's marketing group does market research and discovers that a new packaging is preferred by consumers, this knowledge is shared with the producers, who adapt to the need.

Methodology

Essential oils are extracted from plant material by extraction methods adapted to the specific part of the plant that contains the oils. The most common extraction methods are: Steam distillation, solvent extraction, water distillation, etc. The extraction method affects the quality of the essential oil by the pressure and temperatures applied. Some extraction methods are better suited to particular types of plants and parts.

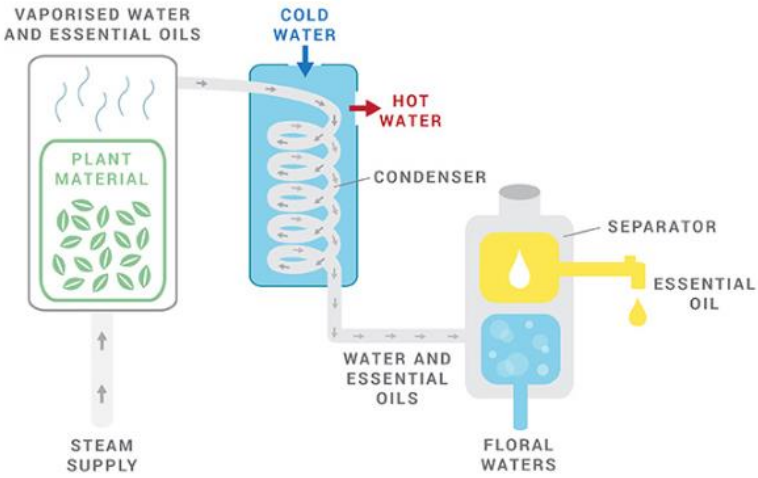

Figure 1. Steam distillation method used to extract and isolate essential oils from plants.

How can the extraction process be improved? There are several ways to increase extract efficiency (Figure 2).

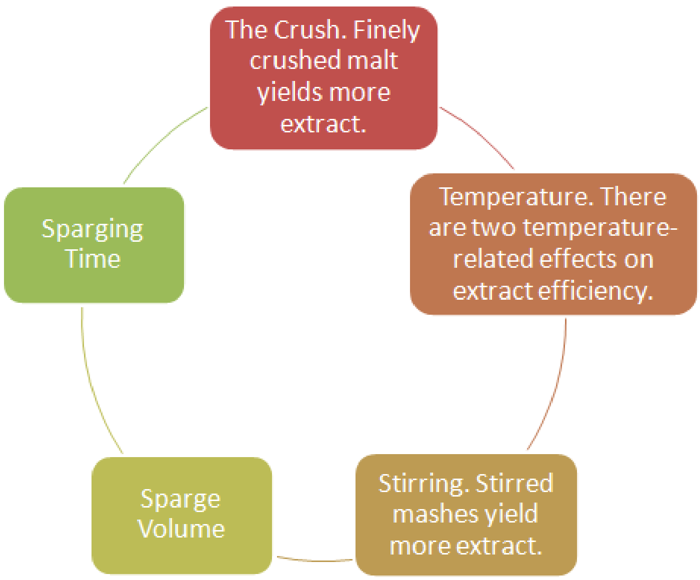

Figure 2. Several parameters need to be considered to improve oil extraction yield and quality.

Implementation progress of the pilot project (first phase)

<u>Action 3: Improving the skills and capacity building members by training sessions</u>
This action has started and will continue in Trimester 3 and 4 from the 2022 years. Some of the training sessions are illustrated in Figure 3 and 4.

Figure 3. First training session to improve oil extraction yield for the rural women in the GDA Borj Essougu. Date 20/01/2021

Figure 4. Second training session to improve oil extraction yield for the rural women in the GDA Borj Essougui. Date 6/05/20222.

Improving our extract efficiency has several benefits. Firstly, and most obviously, improving oil extraction yield and quality of the final products.

Action 4: Join the cluster WIKI PAM.

This action consists of helping the pilot case (GDA Borj Essougui) to join the WIKI PAM cluster. This action has several benefits. The cluster WIKI PAM is a collaborative network that unites all willing stakeholders of a value chain, with the common goal of developing said value chain. The wiki PAM network is made-up of many cooperatives who make essential oils from MAPs. It also includes small and medium-sized companies that export products, and representatives of the public entities that support the artisans.

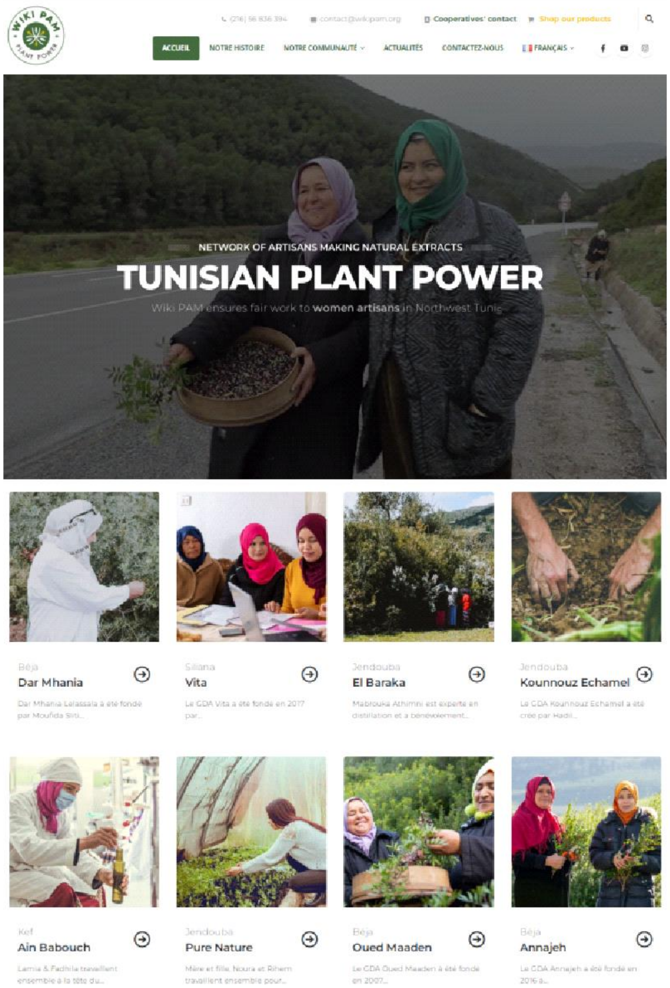

Figure 5. WIKI PAM cluster. https://wikipam.org/fr/acceuil/

Implementation progress of the pilot project (second phase)

The exploitation of aromatic and medicinal plants is a traditional activity in Tunisia. It currently represents a turnover of about 30 MTD and is an important source of income for the local population. Rosemary is one of the main species exploited on a large scale in Tunisia. It is used, either in natural form as culinary or medicinal plants, or in the form of essential oils or extracts. Rosemary essential oils are extracted from plant material by extraction methods adapted to the specific part of the plant that contains the oils. The most common extraction methods are: Steam distillation, solvent extraction, water distillation, etc. The extraction method affects the quality of the essential oil by the pressure and temperatures applied. Some extraction methods are better suited to particular types of plants and parts.

The methodology consists of monitoring the quality of rosemary oils extracted by controlling the distillation time and techniques. Decreasing the distillation time for essential oils, the most relevant in economic terms, allows producers to increase their production, improve the composition of essential oils and decrease energy costs.

Results and outputs

How can the extraction process be improved? There are several ways to increase extract efficiency. Experiments were conducted to determine the evolution of yield and composition of rosemary essential oils as a function of steam distillation and drying time (**Figure 1**).

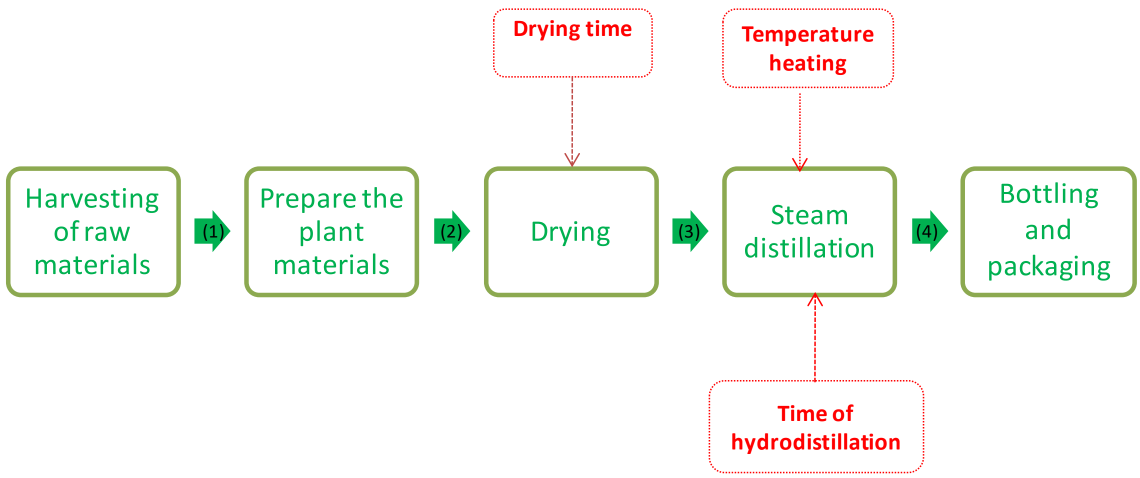

Figure 1. Process of rosemary essential oils extraction by controlling the following parameters: Drying time, temperature heating and time of hydro-distillation.

Extraction was performed under different drying conditions and extraction times by varying the temperature. The pressure was set at atmospheric pressure. However, 1 kilogram of rosemary leaves was tested for each experiment (6). Three drying times were tested, 0 days, 7days, and 14 days with three different distillation times of 150 min, 180 min, and 210 min, to compare their efficiency in terms of oil yield and oil composition. The results are presented in Table 1.

Table 1: Experimental design of the optimization of the hydro-distillation process of *Rosmarinus officinalis* for each experiment

Number of experiment	Time of hydrodistillation (min)	Drying time (Days)	Temperature heating	Yield (%)
1	150	0	250	1.22
2	210	0	250	1,79
3	150	7	300	1;54
4	**210**	**7**	**250**	**2,26**
5	210	14	250	2,10
6	180	7	300	1,74

For the rosemary EO, a hydro-distillation time of 210 min, a heating temperature of 250°C, and a drying time of 7 days are the optimal experimental conditions for a maximization of the yield towards a value of 2.26%. These results can be a basis for the extrapolation of the optimization of the experimental conditions for the extraction of essential oils by pilot or industrial process. Therefore, they can represent a great economic interest, especially after the confirmation of the effect of the hydro-distillation time and the duration of the drying on the maximization of the yield.

Chromatographic analyses of the different samples allowed us to determine the chemical composition of the essential oils of rosemary. About 11 components were detected and identified during these analyses (Table 2).

Table 2: Chemical composition of the studied essential oils extracted by the two types of distillation. Mean value of 3 trials ± Standard deviation.

Pic number	Composites	Industrial Hydrodistillation (%)	Hydrodistillation by Clevenger (%)
1	**Alpha-pinène**	**11,27 ±0.76**	**9,84 ±0.57**
2	**Camphène**	**4,53 ±0.28**	**4,27 ±0.39**
3	**Beta-pinène**	**8,11 ±0.17**	0,10 ±0.00
4	Beta-myrcène	1,25 ±0.01	1,94 ±0.03
5	**1,8-cineole**	**41,28 ±2.52**	**51,77 ±1.97**
6	p-cymène	2,23 ±0.25	0,51 ±0.09
7	**Camphre**	**22,82 ±1.96**	**22,31 ±1.03**
8	Acetate de Bornyle	**5,53 ±0.11**	1,02 ±0.05
9	Alpha-terpinéole	1,72 ±0.04	**5,04 ±0.07**
10	Bornéole	0,96 ±0.02	2,80 ±0.01
11	Verbenone	0,06 ±0.01	0,11 ±0.01
	Total	**99,75**	**99,70**

The analysis of essential oils by chromatography shows that the chemical compositions and majority compounds are different and variable depending on the extraction method used. This variation can be very interesting during the industrial research of a compound in quality and quantity and the method of distillation used.

The essential oil of rosemary obtained by hydro-distillation has a significant content of one of the 3 compounds: α-pinene (9.84-11.27%), 1,8-cineole (41.28-51.77%), camphor (22.31-22.82%).

Lessons learnt

The principal lessons learned from the transformation process of Medicinal and aromatic plants (MAP) in our pilot project, GDA Borj Essougui are the following:

- Transformation process of aromatic plants (e.g., rosemary, myrtle,) was performed under different drying conditions and extraction times by varying the temperature. This allows us to have good yields with better organoleptic and biochemical properties which allows them to improve their incomes and increase the added value of local products.
- The quality of plant extracts depends on quality of raw materials and the applied drying technique
- Improving the skills and capacity building of GDA members need to be supported by training sessions.

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