PRIMA SECTION 2

D.1.1 / TECHNOLOGICAL, REGULATORY AND STAKEHOLDERS DATABASE (TRSd)

(WP1 Project baseline and sustainable innovation framework design)

SUSTEMICROP PROJECT

Development of eco-sustainable systemic technologies and strategies in Mediterranean crop systems contributing to small farming socio-economic resilience

Coordinator:

UNIVERSITY OF LEON (ULE)

Project details and Deliverable information

✓ **Project Details**

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Project Type	Research and Innovation Actions (RIA). Topic 2.2.1. Up-scaling field practices based on agroecology principles to increase ecosystem services and biodiversity, to adapt the small farming systems to climate change and to increase farmers' incomes
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Type: R: Document, report (excluding the periodic and final reports); DEM: Demonstrator, pilot, prototype, plan designs; DEC: Websites, patents filing, press & media actions, videos, etc.; OTHER: Software, technical diagram, etc.	Report (R)

Document history and quality check

✓ **Document History**

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1.0	20/10/2022	Juan José Rubio Coque (ULE); Dominique This (INRAE); Lamia Hamrouni (INRGREF); Sebastjan Radisek (IHPS); Alfredo Vera AGBIO); Eric Chantelot (IFV); Mohammed Aziz EL HOUMAIZI (UMP)	Structure of the document and all contents
2.0	23/10/22	Juan José Rubio Coque (ULE); Irene Solé and Violeta Martín (Artica+i)	Refinement and corrections
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✓ **Quality Check Review**

Reviewer (s)	Main Changes
ARTICA+i	Overall review of the document
Rebeca Cobos (ULE)	Overall review of the document and quality check

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List of abbreviations and definitions

Abbreviation	Definition
AF	Antifungal
BCA	Biocontrol Agent
BF	Biofertilizer
cfu	Colony Forming Unit
D	Deliverable
Foa	*Fusarium oxysporum* f. sp. *albedinis*
GTD	Grapevine Trunk Diseases
PB	Project Baseline
SFP	SUSTEMICROP Full Proposal
TCS	Technological Case of Studies
TRSd	Technological, Regulatory and Stakeholder database
WP	Work Package
YGD	Young Grapevine Decline

Statement for open documents & Copyrights

1.- Executive Summary

The development of a Technological, Regulatory and Stakeholder database (TRSd) will serve as a multi-sectorial and multi-actor assistance network, to identify key aspects and current available agro-ecological solutions of interest for all the partners and stakeholder for the development of SUSTEMICROP Project.

Initially, the creation of the TRSd will aim to define the project baseline (PB) of SUSTEMICROP in order to provide all the partners a collection of data including the last scientific advances related to each particular approach on the *Technological Case of Studies* (TCS*)*, other solutions emerging in the market, or other R&D projects that address the same target crop pathologies and difficulties on management. Information on any advances in the regulation should also be included as well as a comprehensive list of relevant stakeholders.

Additionally, the elaboration of a TRSd will enable other objectives to be achieved, including:

➢ To create engagement and synergies with interested entities.

➢To represent a technological and legal surveillance tool, as well as a networking tool involving a group of representative stakeholders in the participants' countries

➢To include the selected stakeholders as the focus in the communication and dissemination plan of the Project, as well as in the exploitation plan.

2.- Introduction

The creation of a TRSd is one of the first deliverables of the SUSTEMICROP project (D1.1), to be delivered by Month 4. Creation of TRSd is part of the Project Baseline and sustainable innovation framework design (WP1).

At the SUSTEMICROP KIN Kick-Off Meeting (21-22 July, 2022, León, Spain) we discussed about how to address the engagement strategy for the elaboration of the TRSd. This database is a very important element in the realization of the project's aim and dissemination of the project's findings. It is therefore important to have a good discussion on which stakeholders are relevant to the project and how we can engage them.

This document will allow to define the project baseline (PB) of SUSTEMICROP in order to provide all the partners a collection of data including the last scientific advances related to each particular approach on the *Technological Case of Studies* (TCS*),* other solutions emerging in the market, or other R&D projects that address the same target crop pathologies and difficulties on management. Information on any advances in the regulation should also be included as well as a comprehensive list of relevant stakeholders interested in the SUSTEMICROP findings and developments.

3.- Objectives and overall structure of TRSd database.

The creation of a TRSd of SUSTEMICROP project aims to achieve two fundamental objectives:

➢Firstly, we will try to provide all the partners a general review of the state of the art related to each particular approach on the Technological Case of Studies (TCS) (**Table 1**). Trsd, and given its confidential nature, will be shared by all SUSTEMICROP partners in the shared cloud repository (i.e. shared Dropbox folder DELIVERABLES). Otherwise the document will also be accessible to partners through the private area that will be created in the next days on the project website.

➢ An additional objective is the elaboration of a list of relevant stakeholders interested in the SUSTEMICROP findings and developments as a way to include as the focus in the communication and dissemination plan of the Project, as well as in the exploitation plan.

As it is indicated in the SUSTEMICROP Full Proposal (SFP) our project is pursuing the valorisation of natural resources from Mediterranean crops (crops residues, essential oils, soils microbiome, bacterial metabolism, grapevine varieties more resistant to climate change conditions) their selection, purification and optimization, and the evaluation of their effect in controlled laboratory, or small-scale field conditions, against key pathogens and diseases in the target crops (grapevine, hops and date palm).

Accordingly, and in order to simplify the elaboration of the TRSd the document will be organised in different sections around the different problems SUSTEMICROP is facing: young grapevine decline, hop crop yield limited by soil borne fungal pathogens, Bayoud disease affecting date palm, development of new biopesticides to fight downy and powdery mildew in hops and grapevine, and the challenge of new grape varieties resistant to climate change.

In every section, we will deep into these challenges reviewing different aspects that will include the last scientific advances related to each particular challenge, other solutions emerging in the market, other R&D projects that address the same target crop pathologies, putative difficulties on management, as well as information on any advances in the regulation.

Table 1.- SUSTEMICROP Technological Cases of Study (TCS)

TCS nº	Leader	TCS Title	Country	Crop
1	ULE/UM 6P	Development of a BF (BCA-enriched compost) to reduce the incidence of the causal agents of YGD	Portugal	Grapevine
2	ULE	Development of a biofertilizer (BCA-enriched compost) able to reduce the incidence of the causal agents of Fusarium Canker	Spain	Hops
3	ULE	Development of a BF based on a hops compost and enriched with PSB to increase the Alpha acid content of hops	Spain	Hops
4	IHPS	Development of a BF (BCA-enriched compost) able to reduce the incidence of the causal agents of Verticillium wilt	Slovenia	Hops
5	INRGREF	New biopesticides from essential oils and extracts from aromatic plants to control *Botrytis* and *Alternaria*	Tunisia	Grapevine/ hop
6	INRGREF	New Biopesticides from essential oils and extracts from aromatic plants to control downy-powdery mildew	Slovenia	Hops
7	IFV	New Biopesticides from essential oils and extracts of aromatic plants addressing downy mildew	France	Grapevine
8	AGBIO	New Biopesticides based on bacterial secondary metabolites (priming-molecules) against phytopathogenic fungi	Spain	Grapevine
9	UM6P	Development of a BF (BCA-enriched compost) to control *Bayoud* disease affecting date palm	Morocco	Date palm
10	INRAE	Screening and monitoring of grapevine varieties (on current and new breeding programmes) for resilience and adaptation to climatic change with or without resistance genes against several fungal diseases, identifying key traits for breeding more resilient varieties	Lebanon/ Greece/ France Tunisia	Grapevine and table grape

4.- Section 1: Young Grapevine Decline (YGD).

4.1.- Young Grapevine Decline: introduction.

Grapevine trunk diseases (GTDs) are considered the most destructive diseases of grapevine and produce important economic losses all around the word. In young vineyards (<5 years old), decline symptoms or syndrome known as Young Grapevine Decline (YGD) have dramatically increased all over the world since the early 1990s (Gramaje and Armengol, 2011). The main fungal trunk diseases associated with YGD are **black-foot** and **Petri disease.** Affected plants exhibit external symptoms such as stunted growth, reduced vigor, retarded or absent sprouting, shortened internodes, sparse and chlorotic foliage with necrotic margins, wilting, dieback and finally death at a very high rate (Gramaje and Armengol, 2011) (**Figure 1**). A brief introduction of both diseases in shown next:

- **Petri disease.** Petri disease can be recognized by the presence of dark-colored phenolic compounds in xylem vessels of the trunks, which exude out of the vessels when cut in cross sections and dark streaks in longitudinal section (Rooney-Latham *et al*. 2005) (**Figure 1**).

Figure 1.- Vineyard affected by YGD with plants exhibiting deficiencies in growth and premature death (left) and internal symptoms of Petri disease detected in cross sections of plants (right). (Courtesy R. Cobos)

The fungal species associated with Petri disease include: *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., *Pleurostoma richardsiae,* and *Cadophora* spp. (Araújo da Silva *et al*. 2017; Gramaje and Armengol 2011; Gramaje *et al*. 2015; Halleen *et al*. 2007; Travadon *et al*. 2015). Among the different *Phaeoacremonium* spp. and *Cadophora* spp. occurring in Petri disease symptomatic vines, *Phaeoacremonium minimum* and *Cadophora luteo-olivacea* are the most prevalent (Gramaje *et al*. 2011; Mostert *et al*. 2006).

- **Black foot.** Black foot can be recognized by black, sunken, necrotic lesions on roots and reddish brown discoloration in the base of the trunk of affected vines. Bark removal reveals black discoloration and necrosis of wood tissue that develops from the base of the root- stock, causing death of young vines (Halleen *et al*. 2006). Up to 24 species in the genera *Campylocarpon, Cylindrocladiella, Dactylonectria, Ilyonectria, Neonectria,* and *Thelonectria* have been reported to cause black foot disease (Agustí-Brisach and Armengol 2013; Carlucci *et al*. 2017; Lombard *et al*. 2014). Once the pathogens enter and colonize plant tissues no effective treatments are available. For that reason most of the current efforts are focused in the development of methods able to efficiently control or reduce the population of fungal pathogens in soil. In this sense, SUSTEMICROP aims to contribute through the development of biofertilizers (BF) produced from compost obtained from crop residues and enriched with selected

Figure 2.- Typical symptoms of plants affected by Black foot disease (Courtesy R. Cobos)

biocontrol agents (BCAs) to control the development of these pathogens in soil and diminish their populations in soils to a level that are not able to infect plants, developing "suppressive soils".

4.2.- Young Grapevine Decline: brief review of studies for the development of control methods.

Since the banning of sodium arsenite because of its toxicity, a worrying progression of diseases in vineyards around the world is taking place. So far, no grape varieties are known to be immune to GTD (Bertsch *et al*., 2013). Given the great negative impact of GTDs in the wine sector, it should come as no surprise that, over the last 25 years, enormous efforts have been made to develop chemical control methods (both pesticides and natural products) or biocontrol agents (BCAs), as reviewed by Mondello *et al.* 2018, to fight these pathologies. However, in many developed countries, including those of the European Union, policies have been implemented in recent years to ban and/ or limit the use of pesticides for crop pest control (e.g., the European Green Deal initiative, one of whose objectives is to limit the use of synthetic chemical pesticides in the agrifood sector as much as possible by 2030). As a result, there are currently no chemicals available on the market with a proven efficacy against GTDs (Cobos *et al*. 2022).

However, we will next review some of the most relevant studies for the control of GTDs/YGDs using chemicals compounds or BCAs.

➢**Chemical compounds:** The most commonly tested chemical compounds include benzimidazoles, triazoles, and strobilurins (Surico *et al*. 2006).

The most efficient benzimidazoles were benomyl, carbendazim, and thiophanate-methyl, which consistently showed great efficacy for pruning wound protection and nurseries. Their effectiveness against the major GTD was mostly attributable to their broad-spectrum fungicidal action, persistence, and systemic activity. A disadvantage of benzimidazoles is that fungi can acquire resistance to them, as demonstrated by P. minimum's resistance towards carbendazim (Martín and Martín, 2013). In nurseries, benzimidazoles effectively reduced the presence of vascular pathogens. When applied during hydration or before grafting, benomyl and carbendazim, for example, decreased the amount of pathogen inoculum in grafted plants (Fourie and Halleen, 2006).

Triazoles are the most common group among synthetic chemicals. They are very effective at inhibiting conidial germination and reducing the mycelial growth of GTD pathogens (Gramaje *et al*. 2009).

Strobilurins are used to control downy and powdery mildew in vineyards. Recently, they have been examined to see if they could protect pruning wounds in particular. In both in vitro and wound protection trials, pyraclostrobin has shown to be the most effective against GTD pathogens (Amponsah *et al*. 2012).

Natural molecules as chitosan have shown *in vitro* and *in vivo* fungicidal effect against the most important grapevine wood fungi. The results showed that chitosan was effective in reducing mycelial growth of all fungi and significantly improved plant growth and decrease diseased incidence compared with untreated plants (Nascimento *et al*., 2007).

➢ **BCAs.** Since the 1990s, numerous studies have been conducted on the control of GTDs by using BCAs. More than 40 BCAs have been tested at different levels (*in vitro*, *in planta* and in the field), as reviewed by Mondello *et al*. (2018). Unfortunately, despite the fact that many different microorganisms have been tested, currently, only *Trichoderma* spp. have been shown to be the most suitable agents for biological control of GTDs, in both the field and nurseries, although with limited efficacy. This is why researchers have recently redoubled their efforts to isolate and characterize more efficient BCAs.

+ One promising tool for the control of GTDs caused by soil-borne fungal pathogens infecting plants through the root system is the use of **Actinobacteria-based BCAs.** A very interesting group of Actinobacteria is that of the Streptomycetales and, particularly, the genus *Streptomyces*, which are surprisingly diverse (around 600 species). They are responsible for the production of half of all known antibiotics (Labeda *et al*., 2012) and are well-known for their ability to control plant diseases, including soil-borne fungal pathogens (Coque *et al*., 2020; Vurukonda *et al*., 2018). Álvarez-Pérez and colleagues (2017) isolated many different strains from 1-year-old grafted plants. The field trial led to the identification of one endophytic strain, *Streptomyces* sp. VV/E1, and two rhizospheric isolates, *Streptomyces* sp. VV/R1 and Streptomyces sp. VV/R4, which significantly reduced the infection rate produced by the fungal pathogens *Dactylonectria* sp.–*Ilyonectria* sp. group, *P. chlamydospora* and *Phaeoacreomonium minimum* (77.8–88.9%) (Álvarez-Pérez *et al*., 2017). *Streptomyces* sp. VV/E1 and VV/R4 strains were also tested in a comparative study, together with other bacterial and fungal BCAs (Martínez-Diz *et al*., 2021). Both strains were applied jointly to 1-year-old hot-water-treated plants (cv. Tempranillo) by immersion of their root systems in a bacterial suspension. Both BCAs were highly effective in reducing the black-foot disease incidence of *Dactylonectria torresensis* and *D. macrodidyma* pathogens in plants of both ages. These strains also reduced the severity of infection in 2-year-old plants in the basal ends. However, the effect of these Actinobacteria against Petri disease pathogens after 2 years in the field was low.

+ BCAs based on other types of bacteria. Different studies have investigated other types of bacteria as BCAs. In planta trials have confirmed that *Bacillus*, *Paenibacillus* and *Pantoea agglomerans* strains are promising BCAS in the control of GTDs (Haidar *et al*., 2016; Haidar *et al*., 2021).

+ BCAS based on rhizosphere or endophytic fungi. One of the most promising fungal BCAs against GTDs is the oomycete *Pythium oligandrum*, which is a frequent inhabitant of the grapevine root system. In *in planta* trials the strain *P. oligandrum* Po37 was able to significantly reduce *P. chlamydospora* and *P. minimum* infections (Martínez-Diz *et al*., 2021).

Arbuscular mycorrhizal fungi have been shown to increase tolerance of grapevine rootstocks to black foot disease caused by *Ilyonectria* spp., and changes in the function of the rhizosphere microbial community (Jones *et al*., 2014). Petit and Gubler (2006) also indicated that grapevines inoculated with an arbuscular-mycorrhizal fungus, *Glomus intraradices,* were less susceptible to black-foot disease than non-mycorrhizal plants.

Unfortunately, and in spite all the research efforts to date there is no in the market a BCA with proven efficacy against all the pathogens involved in YGD. Thus the study of Martínez-Diz *et al*. (2021) have shown that Streptomyces sp. VV/E1 and VV/R4 strains are highly effective in controlling black foot, whereas their efficacy in the control of Petri disease is limited. On the contrary *P. oligandrum* was able to significantly reduce *P. chlamydospora* and *P. minimum* infections, causing Petri disease, whereas their effect on fungi causing Black foot was very limited. In a similar way the different *Trichoderma* sp. strains tested exhibited a better efficacy against fungi causing Petri disease, but also a reduced efficacy against Black foot-causing fungi.

4.3.- Products in the market with some efficacy to control YGD.

The following list of products with some effectiveness in the control of YGD corresponds to the products approved for use in Spain as of 22 September 2022. A major problem in the agricultural sector in the European Union is that the list of authorised products can vary from one country to another which creates comparative disadvantages for farmers. The list is as follows (**Table 2**):

Table 2.- List of products on the market with some efficacy against fungi involved in YGD

Product	Type product	Registration number	Owner company	Formulation
BLINDAR	BCA	25924	Gowan Crop Protection	*Trichoderma asperellum* (strain ICC012) + *Trichoderma gamsii* (strain ICC080). 2% (3 X 10^7 cfu/g. Sum of both microorganisms)
ESQUIVE WP	BCA	25961	Agrauxine S.A.	*Trichoderma atroviride* (strain I-1237) 5% (1 X 10^8 cfu/g)
VINTEC	BCA	ES-00468	BIPA N.V.	*Trichoderma atroviride* (strain SC1 1). 1 x 10^{10} cfu/g

4.4.-Later advances to fight YGD.

The resistance of the pathogen to chemicals is one of the major constraints of phytosanitary treatments, it is due to the often-prolonged misuse of these products. Alternatives are at the centre of farmers' major concerns. Several biocontrol agents have been tested to limit wood diseases, which have a battery of potentially usable attack mechanisms.

Several *Trichoderma* spp. have proven to be effective at diminishing natural infection levels of fungi involved in black foot and Petri disease in the grapevine plant root system. In addition to *T. atroviridae* mentioned above, other species have been used for control GTD, *T. asperellum, T. gamsii* and *T. harzianum* and *Phythium olygandrum* Po37 (Martínez-Diz *et al.*, 2021).

In addition to the aforementioned BCA-based treatments, other products are being studied, including molecules capable of transporting the pathogen through the plant.

The development of sophisticated biopolymer-based systems is the current trend in encapsulation application in agriculture, i.e., microcapsule formulations combining two active agents. Given the wide range of encapsulation procedures for encapsulated chemical agents, there are few investigations on simultaneous encapsulation and delivery of biological and chemical agents in the literature (Locatelli *et al.,* 2018). More recently, Peil *et al*. 2020 have developed a spore-compatible layer-by-layer assembly to encapsulate the spores of a new mycoparasite strain of *Trichoderma reesei* IBWF 034-05 in a bio-sourced and biodegradable lignin shell creating a surfactant-free, self-stabilizing spore dispersion.

Phytocompounds should be much more advantageous than synthetic pesticides since they are biodegradable, non-polluting, and do not have residual or phytotoxic properties. Ammad *et al*., 2018 have demonstrated that the essential oil of lemon *(Citrus limon L.)* to prevent fungal diseases of grapevine.

4.5.-Other projects addressing YGD.

+ Spanish national projects:

- COMBOBIOVID "*Implementation of different biocontrol strategies in the nursery to produce grafted grapevine plants with a high health guarantee that reduces premature death and enhances the longevity of the vineyard*". This project is currently under development by Viveros Villanueva Vides S.L (Larraga, Spain) financed by CDTI (Centre for the Development of Industrial Technology) and aims to optimise and analyse whether the simultaneous use of BCAs with different specificity can help control YGD.

+ European projects:

- e LIFE EU Life Green Grapes Project "*New approaches for protection in a modern sustainable viticulture: from nursery to harvesting*". The main objective of LIFE GREEN GRAPES is to improve the anti-parasitic response of vineyards through the use of innovative natural products and to increase the biodiversity associated with vineyards (https://webgate.ec.europa.eu/life/publicWebsite/index.cfm?fuseaction=search.dspPage&n_proj_id=6321)

➢ "*BIO-Based pESTicides production for sustainable agriculture management plan*". This project is developing an effective and cost-efficient biopesticide to fight GTDs based is based on the use of the oomycete *Pythium oligandrum* strain I-5180 as BCA (https://cordis.europa.eu/project/id/886776)

➢ NOVATERRA "*Integrated novel strategies for reducing the use and impact of pesticides, towards sustainable mediterranean vineyards and olive groves*". NOVATERRA project will bring together farmers, researchers and SMEs to investigate novel ways to reduce the use of contentious plant protection products (PPP) in grapevines and olives – the two main Mediterranean crops – and mitigate their negative impacts. Specifically, it will develop combinations of alternative, non-synthetic products and biological control techniques for plant protection (https://cordis.europa.eu/project/id/101000554)

4.6.-List of relevant stakeholders with interest in YGD.

➢ Potentially, any of the 2.4 million European winegrowing farms could be interested in the results. Particularly all the wineries included in one of the 70 "Denominaciones de Origen" existing in Spain, that group hundreds of cellar. Among them we can mention as the most representative DO Rioja, DO Ribera de Duero, DO La Mancha, DO Priorat, DO Rueda, among others. Also and more specifically the 2.0 Million European small and medium winegrowing farms in southern regions (Spain, Greece, Portugal, Roumania, south of France).

➢ Nursery companies are also the first potential users of the results, including the biggest grapevine nurseries in Spain such as Viveros Villanueva Vides, Viveros Enrique Bravo o Agromillora Iberia S.L.

➢ Plant protection companies interested in the development of natural methods to control fungal pathologies, including the company Agrogenia Biotech, which is partner of our projects.

➢ Companies specialized in the composting of agricultural residues

4.7.- Advances in regulation involving YGD.

We are not aware of any plans to modify regulatory aspects for the control of this pathology.

4.8.- YGD-related Bibliography

➢ Agustí-Brisach, C., and Armengol, J. 2013. Black-foot disease of grapevine: an update on taxonomy, epidemiology and management strategies. Phytopathol. Mediterr. 52:245-261

➢ Álvarez-Pérez, J.M.; González-García, S.; Cobos, R.; Olego, M.Á.; Ibañez, A.; Díez-Galán, A.; Garzón-Jimeno, E.; Coque, J.J.R. Use of endophytic and rhizosphere actinobacteria from grapevine plants to reduce nursery fungal graft infections that lead to young grapevine decline. Appl. Environ. Microbiol. 2017, 83, e01564-17

➢ Ammad, F.; Moumen, O.; Gasem, A.; Othmane, S.; Hisashi, K.N.; Zebib, B.; Merah, O. The potency of lemon (*Citrus limon* L.) essential oil to control some fungal diseases of grapevine wood. Comptes Rendus Biol. 2018, 341, 97–101.

➢ Amponsah, N.T.; Jones, E.; Ridgway, H.J.; Jaspers, M.V. Evaluation of fungicides for the management of Botryosphaeria dieback diseases of grapevines. Pest Manag. Sci. 2012, 68, 676–683.

➢ Araújo da Silva, M., Correia,K. C., Barbosa, M. A. G.,Câmara, M. P. S., Gramaje, D., and Michereff, S. J. 2017. Characterization of *Phaeoacremonium* isolates associated with Petri disease of table grape in Northeastern Brazil, with description of *Phaeoacremonium nordesticola* sp.nov.Eur.J.Plant Pathol.179:695-709

➢ Bertsch, C., Ramírez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., Spagnolo, A., Clément, C., & Fontaine, F. (2013). "Grapevine trunk diseases: complex and still poorly understood". Plant Pathology, 62(2), 243-265.

- Carlucci, A., Lops, F., Mostert, L., Halleen, F., and Raimondo, M. L. 2017. Occurrence fungi causing black foot on young grapevines and nursery rootstock plants in Italy. Phytopathol. Mediterr. 56:10-39
- Cobos, R.; lbañez, A.; Diez-Galán, A.; Calvo-Peña, C.; Ghoreshizadeh, S.; Coque, J.J.R. The Grapevine Microbiome to the Rescue: lmplications for the Biocontrol of Trunk Diseases. Plants 2022, 11, 840. https://doi.org/10.3390/ plantsl1070840
- Coque, J.J.R.; Álvarez-Pérez, J.M.; Cobos, R.; González-García, S.; Ibáñez, A.M.; Diez Galán, A.; Calvo-Peña, C. Advances in the control of phytopathogenic fungi that infect crops through their root system. In Advances in Applied Microbiology; Geoffrey Michael Gadd, S.S., Ed.; Academic Press: Cambridge, MA, USA, 2020; Volume 111, pp. 123–170, ISBN 9780128207055.
- Fourie, P.H.; Halleen, F. Chemical and biological protection of grapevine propagation material from trunk disease pathogens. Eur. J. Plant Pathol. 2006, 116, 255–265.
- Gramaje, D.; Aroca, Á.; Raposo, R.; García-Jiménez, J.; Armengol, J. Evaluation of fungicides to control Petri disease pathogens in the grapevine propagation process. Crop Prot. 2009, 28, 1091–1097.
- Gramaje, D., and Armengol, J. (2011). "Fungal trunk pathogens in the grapevine propagation process: potential inoculum sources, detection, identification, and management strategies". Plant Disease, 95(9), 1040-1055.
- Gramaje, D., Mostert, L., and Armengol, J. 2011. Characterization of *Cadophora luteo-olivacea* and *C. melinii* isolates obtained from grapevines and environmental samples from grapevine nurseries in Spain. Phytopathol. Mediterr. 50:S112-S126.
- Gramaje, D., Mostert, L., Groenewald, J. Z., and Crous, P.W. 2015. *Phaeoacremonium*: from esca disease to phaeohyphomycosis. Fungal Biol. 119:759-783.
- Haidar, R.; Roudet, J.; Bonnard, O.; Dufour, M.C.; Corio-Costet, M.F.; Fert, M.; Gautier, T.; Deschamps, A.; Fermaud, M. Screeningand modes of action of antagonistic bacteria to control the fungal pathogen *Phaeomoniella chlamydospora* involved in grapevine trunk diseases. Microbiol. Res. 2016, 192, 172–184
- Haidar, R.; Amira, Y.; Roudet, J.; Marc, F.; Patrice, R. Application methods and modes of action of *Pantoea agglomerans* and Paenibacillus sp. to control the grapevine trunk disease-pathogen, *Neofusicoccum parvum*. OENO One 2021, 55, 1–16
- Halleen, F., Fourie, P. H., and Crous, P. W. 2006. A review of black foot disease of grapevine. Phytopathol. Mediterr. 45:S55-S67
- Halleen, F., Mostert, L., and Crous, P. W. 2007. Pathogenicity testing of lesser-known vascular fungi of grapevines. Australas. Plant Pathol. 36:277-285
- Jones, E.E., Hammond, S., Blond, C., Brown, D.S., & Ridgway, H.J. (2014). "Interaction between arbuscular mycorrhizal fungi and rootstock cultivar on the susceptibility to infection by *Ilyonectria* species". Phytopathologia Mediterranea, 53(3), 582-583.
- Labeda, D.P.; Goodfellow, M.; Brown, R.;Ward, A.C.; Lanoot, B.; Vanncanneyt, M.; Swings, J.; Kim, S.-B.; Liu, Z.; Chun, J.; *et al*. Phylogenetic study of the species within the family Streptomycetaceae. Antonie Van Leeuwenhoek 2012, 101, 73–104
- Lombard, L., Van Der Merwe, N. A., Groenewald, J. Z., and Crous, P W. 2014. Lineages in Nectriaceae: re-evaluating the generic status of *Ilyonectria* and allied genera. Phytopathol. Mediterr. 53:515-532
- Locatelli, G.O.; Dos Santos, G.F.; Botelho, P.S.; Finkler, C.L.L.; Bueno, L.A. Development of *Trichoderma* sp. formulations in encapsulated granules (CG) and evaluation of conidia shelf-life. Biol. Control 2018, 117, 21–29.
- Martín, L.; Martín, M.T. Characterization of fungicide resistant isolates of *Phaeoacremonium aleophilum* infecting grapevines in Spain. Crop Prot. 2013, 52, 141–150
- Martínez-Diz, P, M.; Díaz-Losada, E.; Andrés-Sodupe, M.; Bujanda, R.; Maldonado-González, M.M.; Ojeda, S.; Yacoub, A.;Rey, P.; Gramaje, D. Field evaluation of biocontrol agents against black-foot and Petri diseases of grapevine. Pest Manag. Sci. 2021,77, 697–708
- Mondello, V.; Songy, A.; Battiston, E.; Pinto, C.; Coppin, C.; Trotel-Aziz, P.; Clément, C.; Mugnai, L.; Fontaine, F. Grapevine Trunk Diseases: A Review of Fifteen Years of Trials for Their Control with Chemicals and Biocontrol Agents. Plant Dis. 2018, 102, 1189-1217
- Mostert, L., Groenewald, J. Z., Summerbell, R. C., Gams, W., and Crous, P. W. 2006. Taxonomy and pathology of *Togninia* (Diaporthales)and its *Phaeoacremonium* anamorphs. Stud. Mycol. 54:1-113
- Nascimento T., Rego, C., & Oliveira, H. (2007). "Potential use of chitosan in the control of grapevine trunk diseases". Phytopathologia Mediterranea, 46, 218–224
- Peil, S.; Beckers, S.J.; Fischer, J.; Wurm, F.R. Biodegradable, lignin-based encapsulation enables delivery of *Trichoderma reesei* with programmed enzymatic release against grapevine trunk diseases. Mater. Today Bio 2020, 7, 100061.
- Petit, E., and Gubler, W.D. (2006). Influence of *Glomus intraradices* on black foot disease caused by *Cylindrocarpon macrodidymum* on *Vitis rupestris* under controlled conditions. Plant Disease, 90, 1481–1484.

- Rooney-Latham, S., Eskalen, A., and Gubler, W. D. 2005. Occurrence of *Togninia minima* perithecia in esca-affected vineyards in California. Plant Dis. 89: 867-871
- Surico, G.; Marchi, G.; Mugnai, L. Older and more recent observations on esca: A critical overview. Phytopathol. Mediterr. 2006, 1000–1019.
- Travadon, R., Lawrence, D. P., Rooney-Latham, S., Gubler, W. D., Wilcox, W. F., Rolshausen, P. E., and Baumgartner, K. 2015. *Cadophora* species associated with wood-decay of grapevine in North America. Fungal Biol. 119:53-66.
- Vurukonda, S.S.K.P.; Giovanardi, D.; Stefani, E. Plant growth promoting and biocontrol activity of *Streptomyces* spp. As endophytes. Int. J. Mol. Sci. 2018, 19, 952

5.- Section 2: Pathologies caused by soil-borne fungal pathogens on hop.

5.1.- Verticillium wilt on hop.

5.1.1.- Verticillium wilt on hop: Introduction.

Verticillium wilt of hops (*Humulus lupulus*) is caused by the soil borne fungi *Verticillium nonalfalfae* [*V. nonalfalfae* Inderb., HW Platt, RM Bostock, RM Davis & KV Subbarao, sp. nov. - before *V. albo-atrum sensu* lato Reinke & Berthold] and *V. dahliae* [*V. dahliae* Klebahn], which are pathogens of many other dicotyledonous plants. Hop present susceptible host which is frequently replanted on the same fields, therefore contamination of soil can lead to repeated infection and abandonment of production sites. *V. nonalfalfae* is most frequently isolated from hop and causes the majority of outbreaks, whereas *V. dahliae* infections are comparatively rare and less severe. The disease is particularly devastating when infections are caused by highly virulent (lethal) pathotypes of *V. nonalfalfae*. Since both fungi can be transmitted by planting material they are regulated in EU as non-quarantine pests for hop planting material (Commission Implementing Regulation (EU) 2019/2072).

- Verticillium wilt symptoms. The disease on hops appears in mild or lethal forms, depending on the pathogen virulence, the sensitivity of the cultivars and ecological factors. Disease symptoms in both forms include yellowing and wilting of the leaves, first at the base of the plant and then up the plant (**Figure 3**). Chlorotic and necrotic tissue appears on the affected leaves, especially on the margins and between the leaf veins. Leaf margins are characterised by upward curling of the leaf margins and leaf dropping when lightly touched. Another important sign of the disease is the brownish conductive tissue observed when the bine is cut at the lower part of the plant, up to a height of 1,5 m.

Figure 3.- Yellowing and wilting of leaves caused by Verticillium wilt on hop (Courtesy S. Radišek)

Mild form: The characteristic symptoms of the mild form of verticillium wilt occur when susceptible varieties are infected with *V. dahliae* or less virulent pathotypes of *V. nonalfalfae*. The first signs of the disease are observed in July and continue until harvest. In this form, the disease is limited to only one or two bines per plant, where the affected leaves appear mainly in the lower part of the plant and spread slowly towards the top until the end of the growing season. Lateral shoots emerging from the leaf axils of the affected leaves do not usually show signs of the disease. The most obvious sign of disease is the abnormal thickening of diseased bines at the base. A considerable thickening of the bines usually occurs, and the browning of the vascular tissue is limited to the centre (**Figure 4**). In the mild form of the disease, the plants rarely dies and grow normally the following year, but the re-emergence of the disease the following year is strongly dependent on factors such as high rainfall and fertilisation with nitrogen fertilisers.

Figure 4.- Mild form of Verticillium wilt on hop: (left) affected swollen and normal bine intertwined; (middle) transverse and (right) longitudinal sections of infected hop bine showing brown vascular tissue which is limited to the centre. (Courtesy S. Radišek)

Lethal form: The lethal form of the disease occurs only in susceptible varieties infected with the more virulent pathotypes of *V. nonalfalfae*. The first signs of the disease are observed in June and continue until harvest. The infection progresses rapidly and within 2 to 3 weeks also affects the lateral shoots, causing wilting of the plant and leaf drop. The disease affects all hop bines, which do not thicken as in the mild form, but in cross-section the entire conductive tissue is heavily browned (**Figure 4**). The lethal form causes rapid plant dieback and occurs in infected hop fields with less dependence on abiotic factors (**Figure 5**).

➢ Life cycle and spreading. Both fungi overwinter in soil in the form of resting organs, which in the case of *V. nonalfalfae* present resting melanized swollen mycelium, whereas *V. dahliae* form microsclerotia. Resting organs allow survival in the soil for up to 4 years in *V. nonalfalfae* and up to 10 years in *V. dahliae*, taking into account the absence of host plants. At the beginning of the growing season, germination of the resting organs begins, from which hyphae sprout, which then penetrate the root system of the plant. After colonization of the root cortex, infection leads to the invasion of the xylem tissue in which conidia are formed. The transpiration stream carries the conidia upwards, which are trapped in border pits or at vessel end walls. For colonization to continue, germ tubes must be produced, which penetrate adjacent vessel elements and begin a new infection cycle. The accumulation of fungal biomass in the xylem disturbs the conduction of water and nutrients. As the plant organ die or enter senescence, new resting organs begin to form. These are released into the soil with the decomposition of plant materials and thus provide infection potential for the next disease cycle.

The most important source of Verticillium wilt are the remains of infected bines and fallen leaves, and soil and weeds in the vicinity of infected plants. In hop gardens, the disease is transmitted by various agro-technological (ploughing, pruning, harvesting, spraying, etc.), most often in the direction of cultivation. Between hop fields, the disease can be transmitted by uncleaned machinery, hop waste and infected planting material.

Figure 4.- Lethal form of Verticillium wilt on hop: (left) extensive and rapid dieback of leaves and lateral shoots; (right) longitudinal section of hop bine show brown discoloration of whole vascular tissue (Courtesy S. Radišek)

Figure 5.- An outbreak of lethal Verticillium wilt on hop (Courtesy S. Radišek)

5.1.2.- Verticillium wilt on hop: disease control

The disease is difficult to control since there are no effective chemical treatments to cure infected plants or prevent the infection. Also, the two causal agents persist in the soil for several years, their infectious potential rising rapidly and declining slowly. Soil chemical fumigation treatments are effective however they are not used in the case of hop gardens and they are environmentally problematic. Thus, in the case of hop infections, phytosanitary measures, such are removing of infected plants, plant waste composting and decontamination of tools and machinery are important for prevention of further spreading. Since the soil contamination is the main infection source, crop rotation using non host plants present important measure to reduce soil infection potential before replanting of new hop garden. Nevertheless, fields with verticillosis history should be replanted by resistant cultivars which present the most successful means of controlling the disease. In the case of replanting of contaminated fields by susceptible varieties there is high probability that the disease will emerge again. Both fungi can be carried undetected by planting material therefore production of planting material should be obtained from *Verticillium* free (tested) propagation stock kept in greenhouses or alternatively from hop gardens that are *Verticillium* free.

5.1.3.- Advances in disease management.

Reducing the pathogen soil infection potential below the threshold of harmfulness is key to preventing soil pathogens. There are several methods of soil disinfection that are alternatives to chemical fumigants. Physical methods include steam treatments, which can be highly effective but on the other hand also highly energy consumptive. Soil solarisation, which is based on covering the soil with plastic foil and using solar irradiation to heat the soil, has been known effective for a long time. The main limitation is the dependence on weather and climatic factors and the inability to grow plants for several weeks. Well known is biofumigation, which is based on the incorporation of cruciferous residues into the soil and the release of isothiocyanates and other substances, which have a negative effect on certain soil pathogenic fungi. The use of biological soil disinfestation is also well known, which works by exposing the soil to anaerobic conditions and excreting volatile components that are toxic to soil organisms. Most of today's research is focused on the study and maintenance of soil microbiological activity, which is the basis for ensuring adequate soil health and soil fertility. One of the most promising methods is the use of enriched composts using biological control agents (BCA), which provide shelter and food for beneficial BCA and, when incorporated into the soil, make a significant contribution to soil suppressiveness against soil-borne pathogens.

5.1.4.- Other projects addressing Verticillium wilt on hop.

- Slovene national project “Biofumigation as alternative for chemical control of soil borne pathogens« (V4-1068, 1.10-30.9.2012)

5.1.5.- List of relevant stakeholders.

- Hop farmers and other farmers in crop production
- Hop growers cooperatives and organizations
- International Hop Growers’ Convention (IHGC) http://www.hmelj-giz.si/ihgc/obj.htm
- The Hop Growers Association of Slovenia http://hmeljarji.si/en/homepage/
- Chamber of Agriculture and Forestry of Slovenia https://www.kgzs.si/

- Administration for Foot Safety, Veterinary Sector and Plant Protection of Republic Slovenia https://www.gov.si/en/state-authorities/bodies-within-ministries/administration-for-food-safety-veterinary-sector-and-plant-protection/
- Agricultural advisory services
- Slovenian Research agency http://www.arrs.si/en/index.asp
- Researches and students on institutes and universities
- Plant protection companies

5.1.6.- Advances in regulation for controlling Verticillium wilt on hop.

There are no known future developments concerning the control of Verticillium wilt in hops.

5.1.7.- Hop Verticillium wilt-related Bibliography

- Berlanger I., Powelson M.L. (2000). Verticillium wilt. The Plant Health Instructor. DOI: 10.1094/PHI-I-PHI-I-2000-0801-01
- Inderbitzin P, Bostock RM, Davis RM, Usami T, Platt HW & Subbarao KV (2011) Phylogenetics and taxonomy of the fungal vascular wilt pathogen *Verticillium*, with the descriptions of five new species. *PLoS ONE* **6**: e28341. doi:10.1371/journal.pone.0028341.
- Inderbitzin P, Davis RM, Bostock RM, Subbarao KV (2013) Identification and differentiation of *Verticillium* species and *V. longisporum* lineages by simplex and multiplex PCR Assays. PLoS ONE **8**: e65990. doi:10.1371/journal.pone.0065990.
- Munda A, Radišek S., Seigner E., Jakše J., Štajner N., (2020), EPPO PM 7/78 (2) *Verticillium nonalfalfae* and *V. dahliae*. *Bulletin OEPP*. 2020, vol. 50, iss. 3
- Pegg G. F., Brady B. L. (2002). Verticillium Wilts. Wallingford, CABI Publishing
- Radišek S, Jakše J & Javornik B (2004) Development of pathotype specific SCAR markers for the detection of *Verticillium albo-atrum* isolates from hop. *Plant Disease* **88**, 1115-1122.
- Radišek S, Jakše J & Javornik B (2006) Genetic variability and virulence among *Verticillium albo-atrum* isolates from hop. *European Journal of Plant Pathology* **116**, 301-314.
- Radišek, S. Verticillium Wilt. (2009) In: MAHAFFEE, Walter F. (ed.), PETHYBRIDGE, Sarah J. (ed.), GENT, David H. (ed.). Compendium of hop diseases and pests. St. Paul (Minnesota): The American Phytopathological Society, 2009
- Sewell GWF & Wilson JF (1984) The nature and distribution of *Verticillium albo-atrum* strains highly pathogenic to the hop. *Plant pathology* **33,** 39-51.

5.2.- Fusarium canker on hop.

5.2.1.- Fusarium canker on hop: Introduction.

Fusarium canker is a destructive hop disease characterized by erratic outbreaks more frequents in wet seasons and frequently associated with the coinfection of Hop Stunt Viroid. Unfortunately, under favorable conditions severe outbreaks can occur and produce bine die-off or plant death (Ocamb and Gent, 2015). The disease is produced by the soil borne fungi *Fusarium* sp. Although the most frequently species associated to Fusarium canker are *F. sambucinum* (Cerruti *et al*., 2010; Thomas *et al*., 2022) and *F. meridionale* (Ferreiro Pinto *et al*., 2022)

- Symptoms. The most typical symptom of this pathology is swelling of the stem near the point of attachment on the crown, due to the inhibition of carbohydrate movement that accumulates in that area. The affected bines wilts rapidly and suddenly; often at flowering or during hot weather in such a way that they can be easily detached from the crown with a gentle tug. This means that any mechanical agitation (wind, tractors, sprayers, etc.) will cause them to break. If the bine remains connected until late in the season, it possibly will collapse in hot weather. Older leaves on the lower part of the bine may become yellow and then turn brown as they die. Leaves on wilted bines remain attached. Severely affected plants, particularly young plants, may die out during winter or under periods of high soil moisture.

5.2.2.- Fusarium canker on hop: disease control

The disease is difficult to control since there are no effective chemical or biological treatments currently available in the market. Control methods are reduced to agronomic measures to try to limit the spread of the pathology and include sanitary measures to propagate affected Hop Stunt Viroid plants, which are more sensitive to the disease, avoid excess moisture around the rhizome, control soil pH to avoid excessive acidification, avoid the use of ammonium fertilizers, mound soil around the base of bines to promote growth of new healthy roots or try to minimize injury to plants during tillage.

Figure 6.- Typical symptom of Fusarium canker consisting in swollen basal portions. Picture from Gent and Cerruti (Ocamb and Gent, 2015)

5.2.3.- Other projects addressing Fusarium canker on hop.

We are not aware of the existence of other projects underway in the European Union to address these pathologies.

5.2.4.- List of relevant stakeholders.

- Hop farmers and other farmers in crop production
- Hop growers cooperatives and organizations like Lúpulos de León, SAT (Hop Growers from Spain) which brings together the majority of Spanish producers (https://www.lupulosdeleon.es/)
- International Hop Growers' Convention (IHGC) http://www.hmelj-giz.si/ihgc/obj.htm
- Researches and students on institutes and universities
- Plant protection companies

5.2.5.- Bibiography related to Fusarium canker on hop

- Cerruti, NR. (2010). Examinations of *Fusarium sambucinum* on *Humulus lupulus* and Co-infection with Hop stunt viroid in Commercial Hop Fields. Doctoral Thesis, Oregon State University, OR, USA.

- Moretti Ferreira Pinto, F., Araujo, L., de Andrade, C.C.L. *et al*. First report *of Fusarium meridionale* causing canker in hop plants. Australasian Plant Dis. Notes 17, 13 (2022)

- Ocamb CM., Gent, DH. Fusarium canker. In: *Field Guide for Integrated Pest Management in Hops.* Third Edition. 2015. Walsh DB., Gent DH., Barbour JD., Boydston RA., George AE., James DG., Sirrine JR (editors). Published by Washington State University, Oregon State University, University of Idaho, and U.S. Department of Agriculture in cooperation with Michigan State University and Cornell University.

- Thomas WJ, Borland TG, Bergl DD, Claassen BJ, Flodquist TA, Montgomery AS, Rivedal HM, Woodhall J, Ocamb CM, Gent DH. A Quantitative PCR Assay for Detection and Quantification of *Fusarium sambucinum*. Plant Dis. 2022 Oct;106(10):2601-2606.

6.- Section 3: Downy Mildew and Powdery Mildew in hop.

6.1.- Downy Mildew in hop.

6.1.1.- Hop Downy Mildew: Introduction.

Hop downy mildew is caused by obligate biotrophic oomycete *Pseudoperonospora humuli* Miyabe et. Takahashi) G.W. Wilson. It is one of the most devastating diseases of cultivated hop that affects hop production in all regions of cultivation in the Northern Hemisphere and Argentina. The first report of this disease comes from Japan in 1905, in 1920 it first appeared in Europe in England, from where it spread rapidly throughout all European countries.

Downy mildew is economically important disease due to the potential for reduction in crop quality and quantity. Damage caused by downy mildew can be extensive, causing complete crop loss due to infection of flowers and cones. Furthermore, downy mildew can overwinter in hop rootstock, what can lead to progressive loss of vigour and plant death in subsequent production seasons.

➢ Symptoms. Hop downy mildew can develop on all organs of the hop plant, causing systemic and local infections. The characteristic systemic infection that allows this pathogen to overwinter can be seen on a section of the hop rhizome and underground stem. The diseased tissue is violet red in colour, spreading concentrically and covering the xylem tissue (**Figure 7**).

Figure 7.- Cross-section of a hop rhizome infected with systemic hop downy mildew infection. The affected tissue is observed as purple-red coloured tissue (Courtesy S. Radišek).

Affected rhizomes can produce up to 30 % less yield, but in the case of sensitive varieties, the entire plant may decay and die. From systemically infected rhizomes and buds, infected shoots (basal spikes) emerge in the spring, which can be recognized by shortened and thickened internodes and yellowish downward leaves (**Figure 8**). Under favourable conditions, a purple-greyish coating develops on the abaxial side of the leaves. In the case of secondary infections of the apical meristem of the shoots, secondary spikes are also formed, which are called lateral or terminal spikes depending of the shoot type.

Secondary infections occur as a result of local infections of leaves, flowers and cones. On infected leaves, pale yellow spots appear on the upper side of the leaf surface, which later turn into necrosis, limited by leaf veins (**Figure 9**). On the underside of the leaves, a dark greyish coating develops on the spots, which consists of sporangiophores and sporangia.

Diseased flowers turn brown and harden and later fall off. Cone infection develops on the bracts and bracteoles, which also have a brown discoloration (**Figure 10**). Severe infection can cause malformation and discolouration on the entire cone depending on the timing of infection. Cone infection results in crop damage through reduction of yield and levels of alfa acids.

Figure 8.- Hop downy mildew: Systemically infected hop shoots (spikes). Top left- lateral infected hop shoot, lower left- terminal infected shoot, right- basal infected hop shoot (Courtesy S. Radišek)

Figure 9.- Hop leaves infected with hop downy mildew (Courtesy S. Radišek)

Figure 10.- Hop downy mildew: left- infected cones; upper right- infection of cones and leaves; lower right- severe infection during cone formation, which turned brown and harden (Courtesy S. Radišek)

➢ Life cycle and spreading. *Pseudoperonospora humuli* overwinters as an intercellular mycelium in infected hop crowns. In the spring, the basal spikes are develop from infected buds. In wet and rainy weather, masses of sporangia on the underside of the leaves create a moldy coating. The sporangia are carried by wind and water in which they germinate and release 5-15 zoospores. The zoospores have two flagella that allow them to move to the stomata, where they encyst by forming cell walls. A germination tube then penetrates the plant cell wall. Once a host cell wall is penetrated, *P. humuli* colonise the plants with intercellular mycelium and forming haustoria within the host cells which allow the absorption of nutrients. New sporangiophores emerge from stomata with sporangia which cause new secondary infections (**Figure 11**). The role of sexual spores (oospores), which develop on disease tissue in the autumn, and the life cycle has not yet been fully understood, as researchers have not yet been able to prove their germination. Oospores are found readily in infected hop tissue and are particularly abundant in diseased cones. The presence of rain water on the leaves is essential for the germination of the sporangia, which release zoospores when the leaf is wet for more than 1 hour at temperatures from 5-28 °C, with an optimal temperature between 20-22 °C.

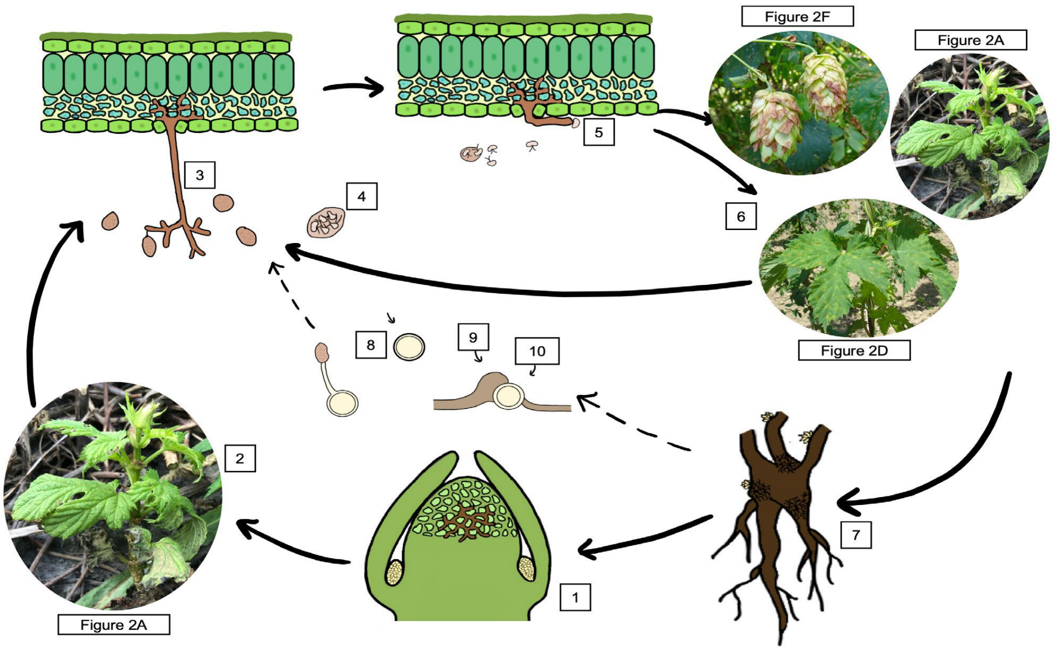


Figure 11.- : Life cycle of *Pseudoperonospora humuli*. (1) Mycelia overwinter in hop crowns and give rise to diseased shoots (2) in spring. Sporangia borne on sporangiophores (3) are present on infected tissue and are released in favorable conditions. Sporangia contain biflagellate zoospores (4) that when released settle on open stomata, penetrate the cell wall through a germ tube, and continue to grow. Sporangiophores appear on the newly infected tissue and the infection cycle continues with zoospores infecting leaves, buds, and cones (6). Infection travels down from aerial parts to hop crowns (7) where the mycelia overwinter. Sexual oospores (8) are formed throughout the infection cycle through fusion of antheridia (9) and oogonia (10) (Purayannur *et al.*, 2021).

6.1.2.- Hop Downy Mildew: disease control.

The protection of hop against hop powdery mildew is based on integrated approaches, which includes the breeding of resistant varieties, phytosanitary measures, monitoring and predicting the optimal spraying time, and taking into account cultivation technologies that do not stimulate the development of the disease. The prevention of hop downy mildew begins in the spring with the suppression of primary infections (basal spikes) which are the source of further infections in hop garden. In addition to the mechanical removal of infected buds at the time of cutting and removing of spikes during training of sprouts, the suppression of the primary infection is based on the use of systemic fungicides. Among those, the most commonly used fungicides are based in the active substance fosetyl-Al and metalaxyl-M. The use of systemic fungicides to manage primary infection is recommended if the proportion of plants with spikes in the hop garden exceeds 3 %. Contact fungicides tend to be used later in the season to suppress secondary infections. Various forecasting systems have been established based on weather conditions and spore trapping (**Figure 12**) to predict risk for infection and help with timing of fungicide application.

Figure 12.- Burkard spore trap for monitor downy mildew infection potential in hop gardens (Courtesy S. Radišek)

6.1.3.- Hop Downy Mildew: advances in disease management.

Due to the systemic nature of *P. humuli* it is important to control and produce healthy planting material. Breeding and planting resistant cultivars is a desirable strategy, however sources of resistance are rare and complete resistance has not been identified.

In order to develop more sustainable hop production many alternatives to chemical fungicides can be used and tested like are bio-fungicides, plant extracts, essential oils, disease resistance inducers and basic substances.

6.1.4.- Other projects addressing similar problems.

Some Slovene national projects addressing hop Downy Mildew are:

- Use of low risk plant protection methods in vegetable production (V4-1602)
- Assessment of the state of pesticide resistance in Slovenia (V4-1601)

6.1.5.- List of relevant stakeholders.

- Hop farmers and other farmers in crop production
- Hop growers cooperatives and organizations
- International Hop Growers' Convention (IHGC) http://www.hmelj-giz.si/ihgc/obj.htm
- The Hop Growers Association of Slovenia http://hmeljarji.si/en/homepage/
- Chamber of Agriculture and Forestry of Slovenia https://www.kgzs.si/
- Administration for Foot Safety, Veterinary Sector and Plant Protection of Republic Slovenia https://www.gov.si/en/state-authorities/bodies-within-ministries/administration-for-food-safety-veterinary-sector-and-plant-protection/
- Agricultural advisory services

- Slovenian Research agency http://www.arrs.si/en/index.asp
- Researches and students on institutes and universities
- Plant protection companies

6.1.6.- Bibiography related to hop Downy Mildew.

- Gent D.H., Ocamb C.M., Farnsworth J.L. (2010). Forecasting and management of hop downy mildew. Plant Disease, 94, 425-431.
- Index Fungorum (2011) http://www.indexfungorum.org/names/NamesRecord.asp?RecordID=119090
- Johnson D.A., (1991) Two degree day models for predicting initial mergence of hop shoots systemically infected with *Pseudoperonospora humuli*. Plant Disease 75, 285-287.
- Johnson, D. A., Engelhard, B., Gent, D. H. (2009). Downy Mildew. Str. 18-22 V (ed): Mahaffee, W. M., Pethybridge, S. J., and Gent, D. H., Compendium of Hop Diseases and Pests, APS Press, St. Paul, MN.
- Purayannur, S., Miles, T.D., Gent, D.H., Pigg, S., Quesada-Ocampo, L.M. (2020) Hop DOwny Mildew Caused by *Pseudoperonospora humuli:* A Diagnostic Guide. Plant Helath Progress. 21: 173-179.
- Purayannur, S., Gent, D.H., Miles, T.D., Radisek, S., Quesada-Ocampo, L.M. (2021) The hop downy mildew pathogen *Pseudoperonospora humuli.* Mol. Plant Pathol. 22: 755-768.

6.2.- Powdery Mildew in hop.

6.2.1.- Hop Powdery Mildew: Introduction.

Hop powdery mildew is one of the important diseases of hops, which without the use of fungicides can cause significant yield drop. The disease was first mentioned in the 18th century in the area of the country Kent in England, but later it spread to most hop-growing countries, with the exception of South Africa and Australia, where strict quarantine measures have so far managed to prevent its spread. At the beginning of the 20th century, the fungus *Sphaerotheca humuli* was identified as the causative agent of hop powdery mildew, which was renamed *Podosphaera macularis* (Wallr.) U. Braun & S. Takam in a recent taxonomic revision. Until now majority of hop growing coutries has been reported about the presence and outberaks of *P. macularis*. The disease severity is dependent on cultivar, environmental conditions and management programs. The polycyclic nature of powdery mildew can result in destructive epidemics when populations of highly susceptible hosts are cultivated in climates favorable to the disease.

- Hop Powdery Mildew: symptoms. Powdery mildew fungi are obligatory biotrophic plant pathogens that can live on the surface of all green plant organs and can infect a wide range of agriculturally and horticulturally important angiosperms. This group has a wide host range, but individual species of powdery mildew fungi tend to be highly specialized, and each species often infects only a single host species. Hop powdery mildew is caused by the ascomycete fungus *P. macularis*. The host range of the organism was thought to be limited to plants of the genus *Humulus* until recently, when it was expanded to include plants of genus *Cannabis*. Typical symptoms of the disease on the leaves are seen in the form of white dust colonies, which can also unite and cover the entire surface of the leaf in the case of young leaves (**Figure 13**).

Figure 13.- Symptoms of hop powdery mildew on leaves and cones (Courtesy S. Radišek)

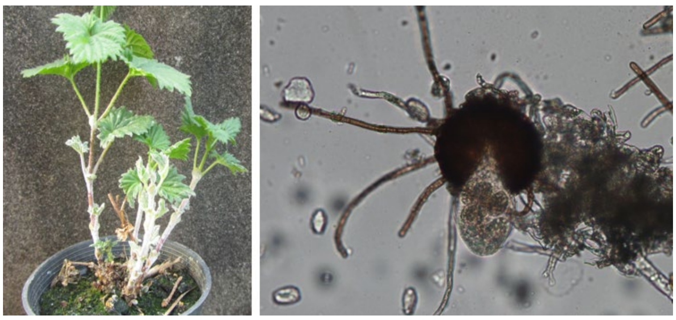
As hyphal colonies expand on above-ground green hop tissue, they produce hundreds of thousands of infectious, wind-dispersed conidia. These conidia spread to adjacent leaves as the disease increases throughout the first half of the growing season. When lateral hop shoots develop and produce flowers, is the period of greatest risk for the disease to transition from foliar infection to pronounced deformations in cone development (**Figure 14**). Burrs and young cones are very susceptible to infection, which can lead to cone distortion, substantial yield reduction, diminished alpha-acids content, color defects, premature ripening, low-aromas and complete crop loss. Heavily infected flowers wither completely and fall off. Infections of older, already developed cones are mild and mostly limited to only part of the cone, but they have a significant impact on hop cone quality and yield. Cones become less susceptible to powdery mildew with maturity, although they never become fully immune to the disease. Infection during the later stages of cone development can lead to browning and hastened maturity. Powdery mildew resulting from bud infection appears in the spring whitestunted shoots called "flag shoots." Late-season powdery mildew can be easily confused with other diseases such as Alternaria cone disorder, gray mold, other cone diseases or spider mite damage.

Figure 14.- Hop powdery mildew: deformation and flower stunting (right); infected cones turn brown and gray during harvesting, and cleistothecia develop on them (Courtesy S. Radišek)

➢ <u>Hop Powdery Mildew: life cycle and spreading</u>

The fungus *P. macularis* overwinters as chasmothecia (Figure 3) or as mycelia in infected buds. The chasmothecia can ripen already in November, but by April of the following year at the latest. They contain ascus with 4-8 spores that are released in late spring (end of April) and infect young leaves. Moisture and a temperature above 10°C are crucial for the release of ascospores. If the fungus overwinters in the buds, infections are visible immediately when the shoots are emerged (**Figure 15**).

Figure 15.- Hop powdery mildew: (left) Infected emerged hop shoots; (right) chasmothecia with ascus (Courtesy S. Radišek)



Ascospores are discharged and land on newly emerged shoots or leaves where they germinate, infect and eventually produce a new spore mass of asexual spores (conidia). Young shoots are fully colonized and turn white due to the presence of mycelium and then become stunted. Conidia arising from secondary infection are produced in large numbers over multiple cycles within the season as long as conditions are favorable and are dispersed via wind and rain splash, and do not need water to germinate (**Figure 16**).

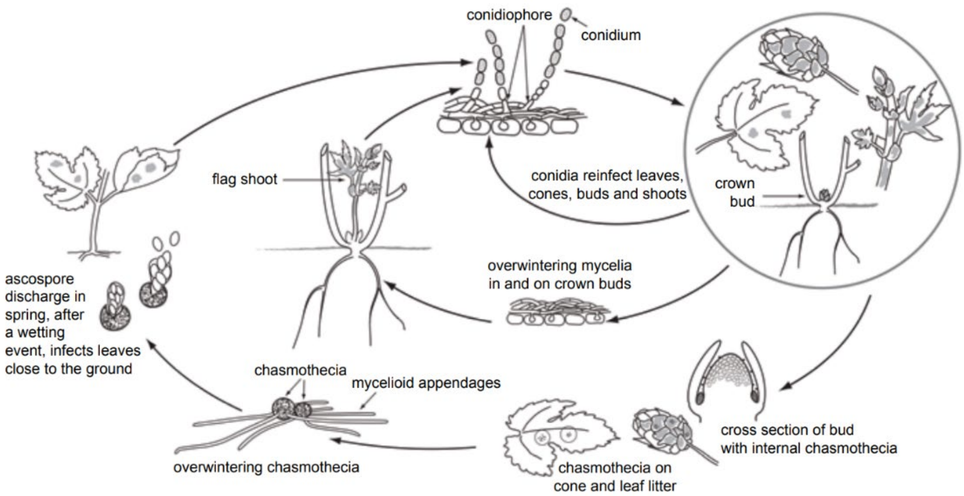


Figure 16.- The lifecycle of *Podosphaera macularis*, the causal agent of hop powdery mildew (Prepared by V. Brewster, Hop IPM Field Guide, 3rd edition)

Conditions that favor powdery mildew are reported to include low light levels resulting from cloud cover, canopy density, excessive fertility and high soil moisture. Leaf wetness from dew or rain does not directly impact powdery mildew infection, but results from high humidity and cloud cover, which favors disease. Infections occur at a temperature of 8-28 °C, disease risk decreases when temperatures consistently exceed 30 °C for six hours or more. On sensitive varieties, under optimal conditions, the conidia sprout an appressorium after 6 hours, pierce the epidermis and develop haustoria in the epidermis cells after 12- 15 hours. After 5 days, a colony develops at the site of infection, which already forms mature conidia and consequently spreads the infection. Young tissue is most sensitive, while infections of older leaves and cones are less aggressive.

6.2.2.- Hop Powdery Mildew: Disease control.

Disease management of hop powdery mildew is based on preventive fungicide applications, planting resistant varieties and appropriate cultivation technology. Mechanical control is carried out at the time of hop pruning, when the basal parts of the last year's bines with buds are removed with a cutter. The first wave of infections in Europe usually occurs in the months of June and July and then continue up to middle of August. In that time preventive spraying is necessary especially on susceptible varieties using fungicides based on different active substances like are myclobutanil, metrafenone, sulphur, trifloxstrobin, quinoxyfen, fluopyram, tebuconazole and potassium bicarbonate. Appropriated fungicide resistance management is important by practicing rotation of different fungicides or using different class of fungicides in the same spraying. Fungicide applications made during the early stages of hop cone development have the strongest effect on suppressing powdery mildew on cones. Mechanical or chemical removal of basal foliage after flowering phase can help in the reduction of infection potential. Important is also avoiding to excessive amount of nitrogen fertilisers and too dense canopy which crates shadowing of plant foliage.

6.2.3.- Hop Powdery Mildew: Advances in disease management.

Implementation and development of hop powdery mildew forecasting models can be used to achieve target fungicide application. Resistance breeding and planting resistant cultivars is one of the most efficient way for disease control but it can be dependent from market demands. In recent years there has been growing interest in using alternatives to fungicides in order to obtain more sustainable crop production. These alternatives includes using different BCAs like beneficial bacteria and fungal antagonists, essential oils, disease resistance inducers and basic substances.

6.2.4.- Other projects addressing Hops Powdery Mildew.

Some Slovene national projects dealing with this subject are:

- Use of low risk plant protection methods in vegetable production (V4-1602)
- Assessment of the state of pesticide resistance in Slovenia (V4-1601)

6.2.5.- List of relevant stakeholders.

- Hop farmers and other farmers in crop production
- Hop growers cooperatives and organizations
- International Hop Growers' Convention (IHGC) http://www.hmelj-giz.si/ihgc/obj.htm
- The Hop Growers Association of Slovenia http://hmeljarji.si/en/homepage/
- Chamber of Agriculture and Forestry of Slovenia https://www.kgzs.si/
- Administration for Foot Safety, Veterinary Sector and Plant Protection of Republic Slovenia https://www.gov.si/en/state-authorities/bodies-within-ministries/administration-for-food-safety-veterinary-sector-and-plant-protection/
- Agricultural advisory services
- Slovenian Research agency http://www.arrs.si/en/index.asp
- Researches and students on institutes and universities
- Plant protection companies

6.2.6.- Advances in regulation for controlling hop powdery mildew.

We are not aware of any plans to modify regulatory aspects for the control of this pathology.

6.2.7.- Hop powdery mildew -related Bibliography

- Gent, D. H., Nelson, M. E., George, A. E., Grove, G. G., Mahaffee, W. F., Ocamb, C. M., Barbour, J. D., Peetz, A., and Turechek, W. W. 2008. A decade of hop powdery mildew in the pacific northwest. Online. Plant Health Progress doi:10.1094/PHP-2008-0314-01-RV.
- Gent, D. H., Nelson, M. E., Grove, G. G., Mahaffee, W. F., Turechek, W. W., and Woods, J. L. 2012. Association of spring pruning practices with severity of powdery mildew and downy mildew on hop. Plant Dis. 96: 1343-1351
- Neve, R. 1991. Hops. Chapman and Hall, London.
- Radisek, S, Čerenak, A., Javornik, B. Žlahnjenje hmelja na odpornost na bolezni: postopki in tehnike pri selekciji križancev Hmeljarski bilten, ISSN 0350-0756, 2007, letn. 14, str. 5-10.
- Weldon, W.A., Gent, D.H., Gadoury, D.M., 2021. Management of Hop Powdery Mildew in the Context of Recent Advances in pathogen Ecology and Population Genetics. Plant Helth Progress. 22: 450-458.
- Wolfenbarger, S. N., Massie, S. T., Ocamb, C., Eck, E. B., Grove, G. G., Nelson, M. E., Probst, C., Twomey, M. C., and Gent, D. H. 2016. Distribution and characterization of *Podosphaera macularis* virulent on hop cultivars possessingR6-based resistance to powdery mildew. Plant Dis. 100:1212-1221.

7.- Section 4: Downy Mildew and Powdery Mildew in vines.

7.1.- Downy and powdery mildew in vines: introduction.

Plasmopara viticola or more commonly known as **grapevine downy mildew** is a fungus belonging to the Peronosporaceae family that develops on all herbaceous organs of the grapevine, more particularly during growth when the organs are rich in water. Its biological cycle is complex and implies alternation of both asexual and sexual phases (**Fig. 17**)

This fungus causes important damages on the vine (deformation of shoots, tendrils and bunches, premature fall of bunches leading to a delay in the ripening of fruits, weakening of the vines), sometimes leading to important losses of harvests but also to problems related to the quality of wines. This fungus has the particularity of being able to cause a total loss of harvest after 2 or 3 successive contaminations at flowering.

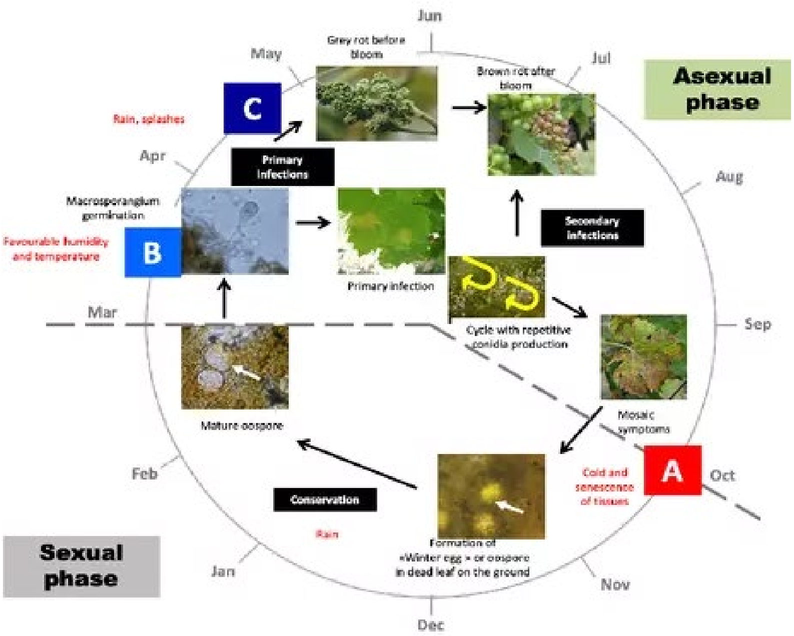


Figure 17.- *Plasmopara viticola* (downy mildew) biologique cycle

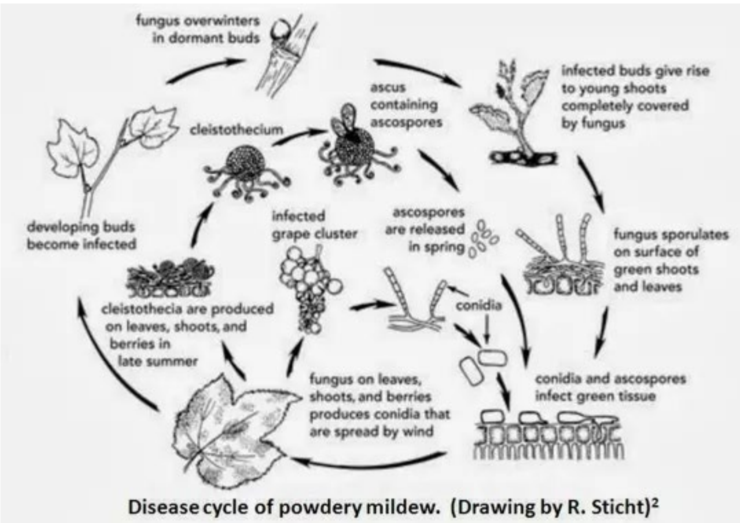


Figure 18.- *Erysiphe necátor (powdery mildew)* biological cycle

- **Powdery mildew** or *Erysiphe necator* is a filamentous fungus of the Erysiphaceae family. In the vineyard, this fungus is the cause of important grape losses during the harvest. When it occupies a large surface on the berries and bunches, in addition to giving off a musty odor, it can also cause berry bursts and increase the acidity of the latter, leading to a drop in wine quality. Its biological cycle can be observed in **Figure 18**.

7.2.- Downy and powdery mildew in vines: current control strategies.

First of all, we must mention the existence of a series of **prophylactic measures** that must be taken into account.

- On powdery mildew, the objectives are to control the vigor and to create a microclimate not very favourable to the development of the fungus. Two techniques have been identified (i) Disbudding and leaf removal which favours sunlight and aeration of the bunches (Oidium is sensitive to UV) and, (ii) the control of nitrogen fertilization and grassing to reduce vigour.
- On downy mildew, the objectives are to limit the formation of primary foci by (i) Drainage of the soil to limit water pools; (ii) Grass management, tillage and pruning to reduce the development of green organs; (iii) Tillage to destroy seedlings from seedlings; (iv) Disbudding and leaf removal to limit the accumulation of vegetation in order to reduce the duration of wetting of the bunches.

The fight against mildew and powdery mildew is basically carried out with **chemical fungicides**. A distinction must be made between preventive fungicides, which prevent the development of spores on the surface of the plant, and curative fungicides, which put an end to the development of a fungus already present in the plant. Nevertheless, both types of fungicides must be applied before the firs visible symptoms. None product have an eradicating effect (destruction of the mycelium on the surface of organs). On vineyards fungicides to control downy and powdery mildew represent 80% of the overall treatments. In average, 8 applications are realised during season.

The societal demand to reduce the use of synthetic inputs leads to the development of **biosolutions.** However, currently these products have a lower efficiency. Most of this solution are under evaluation to determine the best way to use them in a global strategy. Biosolution are classified in four main groups: macroorganisms, microorganisms, chemical mediators (acting mainly as elicitors or biostimulants), and natural substances.

SUSTEMICROP Project has the objective to evaluate some new natural substances, mainly essential oils, plant extracts and priming molecules.

7.3.- Natural antifungals in the market to control Downy and Powdery Mildew in vines.

Currently on French market one orange extract oil is authorized. This product is more positioned with a curative effect than a preventive one. This orange oil extract is commercialized by VIVAGRO and ORAO Agri Company in France under the commercial names Essen'ciel®, Limocide® and Orocide®. In the **Figure 19** some basic information about their application mode is resumed.

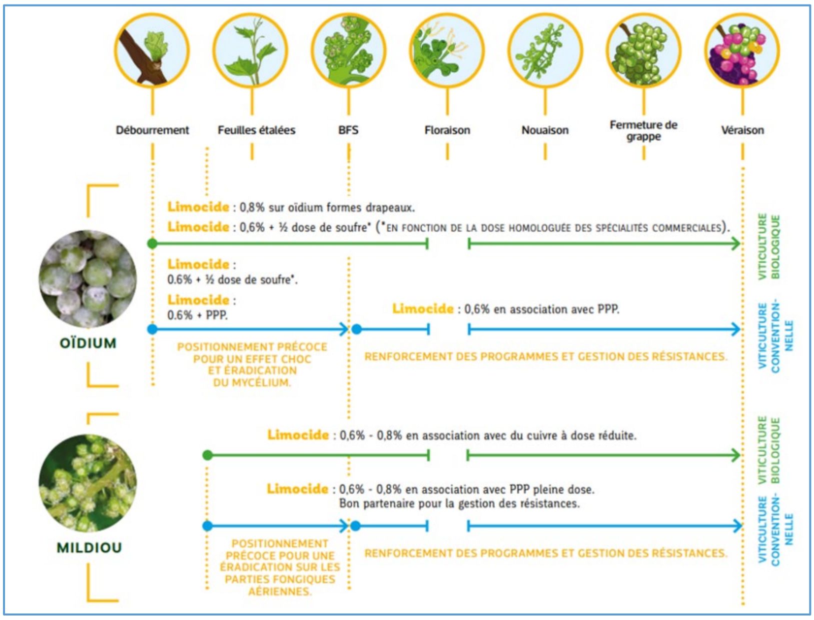


Figure 19.- Extract of technical positioning recommend by VIVAGRO for Limocide® (obtained from https://vivagro.fr/produit/limocide/?lang=en)

7.4.- Brief review of other projects addressing same problems

IFV is involved in two other European projects dealing with the development of biosolutions:

- COPPEREPLACE "*Development and integral implementation of new technologies, products and strategies to reduce the application of copper in vineyards and remedy of contaminated soils in the SUDOE region*" (https://eurecat.org/es/portfolio-items/coppereplace/)
- NOVATERRA "*Integrated novel strategies for reducing the use and impact of pesticides, towards sustainable mediterranean vineyards and olive groves*". NOVATERRA project will bring together farmers, researchers and SMEs to investigate novel ways to reduce the use of contentious plant protection products (PPP) in grapevines and olives – the two main Mediterranean crops – and mitigate their negative impacts. Specifically, it will develop combinations of alternative, non-synthetic products and biological control techniques for plant protection (https://cordis.europa.eu/project/id/101000554)

Nevertheless, these projects do not include an evaluation of essential oil as putative biosolutions. Some evaluation on essential oil have been carried out some years ago. The results were interesting in lab evaluation but we faced some issue to identify an effect in field trial. The hypothesis posed at the end of the project dealt with the formulation. Essential oil should need some coformulant to be more stable.

7.5.- List of relevant stakeholders.

- SUDVINBIO : https://www.sudvinbio.com/. It is a French company that has been promoting organic viticulture since 1991. The interprofessional association Sudvinbio was created in 1991 on the initiative of winegrowers who practise this form of agriculture, in order to collectively promote their wines.

- SVBNA : http://www.vigneronsbionouvelleaquitaine.fr/. Vignerons Bio Nouvelle-Aquitaine brings together more than 200 organic and biodynamic winemakers from the Nouvelle Aquitaine region. What binds all their partners is the desire to sustain an Organic Agriculture that is certified, plural and economically viable.

- Cave HERACLES : https://caveau-heracles.com/ . The Héraclès cooperative winery brings together 80 winegrowers and has been producing organic wines for more than 25 years on land near the Perrier spring.

- The results could also been of Potential interest for any of the 2.4 millions European winegrowing farms including large private companies like Moët & Hennessy, Mumm or AdVini in France or Torres in Spain, but more specifically the 2.0 Million European winegrowing farms in southern regions (Spain, Greece, Portugal, Roumania, south of France).

7.6.- Information of any advances regarding regulation

To our knowledge the development of essential oils to control different fungal pathologies could be authorized in Europe in two ways:

1.- The essential would be recognized as a basic substance: http://itab.asso.fr/downloads/substances%20de%20base/reglement_dexecution_354_2014_ue.pdf. However we should point out that, for the timebeing, most of the essential oils in Europe are not recognized as basic substance because of potential toxicity (as to be confirmed).

2.- The extract oil is authorized as a phytosanitary products.

8.- Section 5: Bayoud disease in date palm

8.1.- Brief introduction to Bayoud disease

Date palm (*Phoenix dactylifera* L.) is one of the oldest fruit trees grown in arid regions of the Arabian Peninsula, North Africa, and the Middle East (Munier, 1973; Chao and Krueger, 2007). It has been cultivated for ecological, economic, and social purposes.

According to the Food and Agriculture Organization (FAO), the global production volume of dates amounted to about 9.45 million metric tons in 2020 (FAOSTAT, 2020). Date fruit are considered a good source of sugars (fructose, glucose, and sucrose), fibers, and essential vitamins and minerals (Amanat *et al*., 2012). The date palm production area in Morocco is estimated to be close to 60,000 ha (2020) (MAPMDREF, 2021), concentrated mainly in the oases of Draâ (42%), Ziz (28.3%), Tata (20%), Tiznit (3.73%), Guelmim (3.32%) and Figuig (2.3%) (Larbi, 1990; Haddouch, 1993). Total date production from Morocco was reported to be 140.000 tons 2020 (MAPMDREF, 2021), which comprise of over 220 varieties including a high proportion of Khalts (45%) (mainly originated from seed) and other high-value varieties such as Boufeggous, Mejhoul, Bouskri, and Aziza Bouzid (Hasnaoui *et al*., 2011). Morocco continues to import 80,000 tons of dates annually, mainly from Tunisia, especially Deglet Nour, which is the most marketed variety (about 90%) in Europe (CBI, 2020).

"Bayoud": (the name Bayoud comes from the Arabic word "abiadh", meaning white, which refers to the whitening of the fonds of diseased trees (Djerbi, 1983). Bayoud disease, caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa), appeared for the first time in 1870 in the Draa valley in the south of Morocco. It was described as the most important disease of date palm. The pathogen invades the plant through the roots producing foliar withering, and leading to the death of the date palm tree. Foa produces typical micro- and macroconidia, as well as chlamydospores, allowing the pathogen to survive under adverse environmental conditions

Since its presence, the problem has developed as an epidemic. In one century, the whole area of date palm plantations in Morocco was affected and only a fraction of the initial number of trees is still productive. The spread of the disease in Morocco can be explained by the advancement from one oasis to another along the river valleys. From Morocco, Bayoud entered Algeria via oases close to the border. From there the fungus travelled via the caravan- routes in infested wood and offshoots to the oases of central Algeria Bayoud causes not only reduction in the production of dates, the principal food of humans and animals in the desert, but also an imbalance of the oasis ecosystem (desertification, disappearance of the subjacent cultures: cereal, fodder and vegetables cultures and fruit trees). Consequently, the dense Moroccan palm plantations were transformed in one century into clearings. Morocco which was previously a date exporter has now to import dates to satisfy its own internal consumption (El modafar 2010)

Growing date palm is becoming a serious challenge due to the increasing incidence of fungal diseases that attack and limit the sin grown areas and even sometimes kill the plant. One such disease is Bayoud of palm date known as Bayoud, caused by the ascomycete fungus *Fusarium oxysporum* f. sp. *Albedinis.*

I- Disease of date palm: case study of Bayoud

Bayoud disease has been first reported in Morocco in 1870, in the Daraa valley north of Zagora before spreading to neighbor countries such as Algeria (Djerbi, 1982) and Mauritania (Sedra, 2003). The dangerousness of this disease can be related by its destructive impact evaluated over 12 million trees in Morocco (Djerbi, 1983) and 3 million in Algeria (Kada, 1974). Bayoud has now been detected in many palm-growing areas across Africa, Asia and America (Corte, 1973; Feather, 1979; Sedra, 2013; Benzohra, 2015; Samo, 2020), with North Africa being the most affected. Its spread causes huge damage to the production of dates, therefore also threatening food security because the date is increasingly consumed and is an essential component in the diet of Arab populations. An estimated 15 million palm trees in oasis habitats of Morocco and Algeria have now been completely wiped out by the Bayoud disease, which continues to progress at an alarming rate despite prophylactic measures to contain pathogen spread (Samo, 2020). Moreover, the exacerbation of Bayoud disease caused the desertification and disappearance of the underlying crops, leading to an imbalance of the oasis ecosystem

Foa is a soilborne fungus deuteromycete that penetrates through the roots, reaches the vessels, then spreads throughout the plant to the terminal bud thus causing the death of the plant (Kettout and Rahmania, 2010).

Foa produces microconidia, macroconidia, and chlamydospores which correspond to different forms of spores that play an important role in the survival of fungi, especially those reproducing asexually. These spore types represent different forms of resistance and survival of the fungus when conditions are unfavorable. When the plant is affected, it often shows symptoms, synonymous with the presence of the pathogen. But the presence of the pathogen in the environment does not necessarily mean the presence of symptoms. Under favourable conditions chlamydospores germinate and infect date palm trees through their roots. It starts its life cycle with a biotrophic phase that later on turns necrotrophic. During the biotrophic phase, the mycelia penetrate the plant via the roots and develop intracellularly into the root cortex to reach the xylem. The development of the pathogen in the xylem causes a blockage of the vessels that result in severe water stress and nutrient uptake causing the plant to wilt. Still from the xylem, the pathogen produces microconidia that migrate through the sap to reach the upper part of the plant. From this stage, the microconidia germinate and the pathogen turns necrotrophic causing foliar necrosis, drying and eventually plant death.

The disease symptoms are first visible on the leaves of the middle crown. The infected leaves are discolored and they brownish on one side of the leave from the base to the top before infecting the other side of the leave from the top to the base. The disease continues spreading and infect adjacent leaves the same manner. This phenomenon takes about 6 months to 2 years depending on the cultivar and when the terminal bud of the plant is reached the plant dies.

8.1.1.- External symptoms.

The first external symptom of the disease appears on one or more leaves of the middle crown. Affected leaves show a leaden color, then wilting is observed from base to top. Pinnae or stunted thorns on one side of the leaf turns white, then disease progression is observed from the base to the apex. After one side of the leaf is reached, he will do the same for the other side of the same leaf but from the top to the base. The observation of a brown spot along the length of the dorsal side of the leaf corresponds to the passage of mycelium in the vessels of the spine and then the leaf appears arched. Similar symptoms then begin to appear on the other leaves of the palm tree and can cause its death when the terminal bud is affected. Palm death can take place 6 weeks to 2 years after the first symptoms appear but depends on the cultivar and the planting conditions. The final stage of the disease is observed when the branches at the base are reached (Dar *et al*., 1982) (Figure 20).

Figure 20.- External symptoms of Bayoud of date palm in a natural oasis in Morocco (Courtesy Prof. Med Aziz Elhoumaizi)

8.1.2.- Internal symptoms.

Reddish color of many roots is observed when the affected plant is uprooted. Towards the base of the stipe, the colored areas are large and numerous. Higher up, the colored vascular bundles separate from healthy tissue. Palm leaves showing externally the symptoms present a reddish-brown color when cut, showing highly colored vascular bundles. Consequently, a persistence of vascular symptoms exists from the roots of the palm tree to the tips of the fronds (**Figure 21**) (Tran & Bastianelli, 2015).

Figure 21.- Internal symptoms of Bayoud of date palm in a natural oasis in Morocco (Courtesy Prof. Med Aziz Elhoumaizi)

The ability of these fungi to persist in the environment largely contribute to its pathogenicity. In fact, Foa produce chlamydospores that can survive in dead tissues of infected plants and in the soil for several years and, thereby, re-infect new plants when conditions become favorable (Benzohra *et al.*, 2015).

Chlamydospores can also be transported by irrigation water, within the grove, or by contaminated materials, outside the grove, and increase the occurrence of the phytopathogen. Foa has also been reported to have the capability of colonizing roots of other crops grown in intercropping system with date palm. These healthy carriers contribute to the presence and persistence of the fungal pathogen in palm cultivated lands. Foa is transmitted through the soil and is specific to the host, although the henna bush when paired with date palm, has been reported to serve as an asymptomatic carrier of the fungus (Freeman and Maymon 2000).

8.2.- Bayoud disease management.

Faced with this disease which is threatening the oasis with extinction, good management must be put in place to prevent its spread. So many strategies have been developed by agricultural structures to control Bayoud disease as we will review next.

Disease management for a wide range of crops is being a big challenge in the agri-food sector. To address this issue, disease management technics such as crop rotation or pesticide use have been integrated in good farming practices. However, these management technics have been very limited in giving efficient results within time. Pest management relying exclusively on commonly used chemical pesticides are neither eco-friendly nor cost-effective. One of their most debatable concerns is the deleterious impact they have on the ecosystem and human health. When pesticides are applied in a farming ecosystem to fight or prevent disease occurrence, chemical residues remain in the soil and contaminate the underground water (Kristoforović-Ilić, 2004; Aktar *et al.*, 2009). Contaminated water, in turn, negatively impacts the environment and human health (Forget *et al*. 1993; Igbedioh 1991). Another downside of the heavy use of pesticides is that they don't only suppress targeted harmful pathogenic species. Instead, quite often they affect large classes of organisms that are not posing any threat to crops, such is the case of beneficial organisms (Sarwar, 2015) resulting in a loss of biodiversity. This has led to a strict regulation in the use of pesticides in many areas around the world.

Alternative solutions need to be considered and investigated for a safer and more sustainable agriculture. Today, resorting to plant-associated microbes is a promising solution to fight plant bioaggressors whilst also conserving ecosystem health. Therefore, the agricultural research sector has been, for several decades, focusing on the use of microorganisms, either as antagonist or competitors to plant pathogens as an alternative to the use of common chemicals or other management technics.

The destructiveness of Foa has made of the Bayoud disease an alerting problem that need to be addressed urgently. Therefore, several methods are or have been applied to manage the disease.

8.2.1.- Prophylactic control.

The fight against Bayoud's disease has long been considered a challenge that has long preoccupied farmers in oasis areas. Several traditional practices have been tried, including burning infected trees. Prophylactic measures are based on the protection of disease-free fields by prohibiting the introduction of plant material and soil from contaminated areas (Louvet *et al.*, 1970). These measures are not a viable mitigation option in Morocco where all productive palm plantations are already affected (Louvet *et al.*, 1970). This measure has given satisfactory results in Tunisian palm groves, where the Deglat-Noor variety sensitive to Bayoud has been protected so far (Rhouma, 1996). However, this method cannot be a long-term solution and it becomes ineffective once the field is infected, as is the case with most Moroccan palm groves and some Algerian palm groves. Cultivation techniques and in particular planting alternating sensitive and resistant cultivars are likely to limit the spread of contamination but they cannot stop it.

8.2.2.- Chemical control.

The use of chemicals (fumigants such as methyl bromide or pesticides such as benomyl) to control Foa in the fields appears restrictive and inefficient. Ineffective because the fungus can reach great depths in the soil in the form of chlamydospores (Djerbi, 1982) thus escaping chemical treatment (Nash, 1978). In addition, these chemicals constitute a potential source of pollution for the ecosystem of fragile oases and can exert selective pressure on Foa populations leading to the possible selection of pathogen populations resistant to fungicides. The only case where it can be considered would be for the eradication of a new outbreak in a healthy area (Bounaga and Djerbi, 1990), the effects of soil solarization and metham sodium, alone or in combination, on the survival of total fungi and Fusarium spp. were studied in the field and they showed an inhibition efficiency, especially in depths ranging from 0 to 40cm (Essarioui and Sedra, 2010).

8.2.3.- Selection and breeding for resistance and high quality.

The method that seems effective and promising is genetic control consisting of selecting varieties resistant to Bayoud. This procedure is unfortunately accompanied by genetic factors unfavorable to quality (Louvet and Toutain, 1973). The search for genotypes that are both resistant and of good fruit quality from natural hybrids (Khalts) or controlled crosses between resistant males and good-quality females has been the subject of numerous selection programs (Djerbi *et al*., 1985; Louvet and Toutain, 1973). However, this method is long and its result is not always sure.

In addition, this method is likely to induce a selection of parasite populations capable of circumventing this resistance. The rehabilitation of palm groves, devastated by the Bayoud, by planting vitro-plants is not a sustainable solution because it does not allow to offer protection against the pathogen and the disease remains a real threat for these vitro-plants. It must be taken into consideration that these are long-term programs and that there is a possibility of the appearance of new physiological races of the pathogen. Thus, the search for new ways to control this fusarium wilt is essential.

8.2.4.- Natural control methods adopted to manage Bayoud disease including BCAs.

Biological control refers to the use of living organisms that are naturally antagonistic to other living organisms particularly the ones that cause damages to plants. These damage-causing organisms can be divided into two groups:

- Pests (insects, nematodes, weeds)
- Pathogens (fungi, bacteria and virus).

Pests and pathogens cause serious damages to plants that result in the reduction of their production. Thus, ecosystem services provided by plants such as human nutrition, animal feeding, medicines, constructions, fuel production etc. are being less sustainable. It is estimated that 20 to 40 percent of the global food production is destroyed by pests and pathogens (FAO 2018). This destroyed food adversely impact on efforts that are being made to provide sufficient food to the world population. The global food security is, then, threatened in a situation of fast population growth, predicted to reach 9.7 billion in 2050, and 10.9 billion in 2100 (ONU, 2019). Compared to pesticides, biological control plays a more important role in the Sustainable Development Goals. Furthermore, its development process is less time-consuming and more money-saving. When the R&D process for a new pesticide requires $286 million and about nine years (Mcdougall, 2016), biopesticides development involves less than $10 million and about 2-4 years

(Olson, 2015). Presently, the word is more and more tending to biological farming concept. Biological farming aims to reduce the use of pesticides in cultivated lands. This approach is a strong driving factor in the rapid adoption of biocontrol, coupled with the increasing demand of organic food.

The discovery of new effective **antifungal (AF) agents of a biological nature** without negative influence on the environment is one of the goals that encourage researchers to discover, develop and synthesize new molecules that are effective, active, and less toxic. toxic for systemic activities.

The potentiation of the defense mechanisms of the date palm by the use of molecules of different natures is one of the strategies adopted in this direction. These molecules can be phytohormones for example jasmonic acid which has shown an increase in the activity of defense enzymes in date palm (Jaiti *et al*., 2009), also the exogenous application of signaling molecules for example salicylic acid led to a significant reduction in the mortality rates of date palm inoculated with Foa (Dihazi *et al*., 2003). Natural products have also been used, such as the product Stifénia (fenugreek seed extract) (Abouraïcha *et al*., 2008) and chitosan (partially deacetylated derivative obtained from the chitin of shellfish waste from crustaceans) which led to an accumulation of phenolic compounds involved in palm resistance to Bayoud (El Hassni *et al*., 2004). Recently, Bouissil *et al* (2022) found that the use of alginate is a promising prospect in agriculture as an inducer that triggers immunity of plants against telluric pathogens in general and of date palm against Foa.

The **ability to potentiate defense reactions** was also assessed for mycorrhizal fungi based on their ability to colonize root systems. Thus, the mycorrhization of date palms by arbuscular mycorrhizal fungi, in particular by species of *Glomus* and *Sclerocystis*, reduced the severity of the disease (Oihabi, 1991; Jaiti *et al*., 2007, 2008). By checking the receptivity of palm plantation soils to mycorrhizal fungi, this approach could be an effective alternative not only to protect the palm against Bayoud disease but also to improve mineral nutrition and plant resistance to water stress and thus the significant improvement of plant growth. Research on soils suppressing Bayoud's disease is an alternative that has been the subject of many studies. The work of Sedra and Roxel (1989), reported the existence of certain soils resistant to Bayoud, such as the example of the soil of the palm grove of Marrakech which remained unscathed. The resistance of these soils is linked to a phenomenon of competition exerted on Foa by the *Fusarium* flora and to the antagonism of several bacteria, mainly actinomycetes, and fungi (Maslouhy, 1989). As a result, research on biological control has been directed toward the use of microbial antagonism against Foa by bacteria, in particular various species of *Bacillus* spp. (Dihazi *et al*., 2012a), *Rhizobium* spp. and *Pseudomonas* spp. (El Hassni *et al*., 2007), and fungal species including hyperaggressive isolates of *Fusarium* (El Hassni *et al*., 2005). Thus, the modification of palm plantation soils by these Foa antagonistic microorganisms has been suggested as a biological control. However, these control approaches by antagonistic microorganisms and mycorrhizal fungi remain far from being applied in this particular ecosystem, where the introduction of these microorganisms could lead to their ecological rejection. The **use of aromatic and medicinal plant extracts** represents a new approach to biological control against Foa initiated by a few researchers.

8.2.5.- Pathogen detection and rapid identification.

Nowadays the molecular method seems to have a lot of scope in the field of research. The molecular method, used, remains an effective tool because it allows to identify early detection of Foa (its genome) in an environment whether in the soil or plants. To develop a diagnostic tool for the detection of Foa, insertion sites of two DNA bands were cloned and sequenced to determine the sequences as PCR primers. Thus, two pairs of primer pairs were defined: BIO3-FOA1 and FOA28-TL3 (Fernandez *et al*., 1998). The pairs of primers BIO3-FOA1 and FOA28-TL3 respectively lead to the identification of 95% and 99% of the isolates of Foa. However, no amplification was obtained by the two sets of primers in isolates of other pathogenic and non-pathogenic *Fusarium* species (Fernandez *et al*., 1998). It should also be noted that this is an effective means for better management of Bayoud to prevent its propagation.

8.2.6.- Eradication of infected foci.

The biocide-antagonism tandem, guaranteed upstream by soil disinfection by a localized treatment based on the combination of solarization with fumigation and downstream by rigorous management of palm orchards, would have its justification in a control strategy. integrated against the Bayoud of the date palm (Essarioui and Sedra, 2017). In the interest of protecting the extension areas against contamination from active Bayoud outbreaks, this approach also focuses on 3 main axes:

(i) detection and mapping of the disease in the oasis regions;

(ii) demarcation of priority palm grove areas for rehabilitation;

(iii) deployment of new resistant varieties for the rehabilitation of areas devastated by the Bayoud.

8.3.- Recent advances in Bayoud management.

➢ The **selection of varieties** has been used by INRA for Bayoud disease management. In fact, several resistant varieties and clones have been selected (Sedra, 2015), such as the varieties Mabrouk (INRA_1394), Najda (INRA_3014), Khair (INRA_3300), Tanourte (INRA_3414), Ayour (INRA_3415), Tafoukte (INRA_3416), Al Baraka (INRA_3417) and Hiba (INRA_3419). This varietal selection has been multiplied on a large scale by tissue culture techniques and used for the rehabilitation of areas devastated by this disease. Genetic control effectively manages Bayoud in areas that have been infested for several decades (Essarioui, *et al* 2018). The use of the Najda variety (INRA_ 3014) has generated selection pressure on foa populations which risks evolving towards new, more resistant races (Essarioui, *et al* 2018).

Knowledge of the geographical distribution of the disease is a crucial element that can provide more visibility on its dynamic evolution. A mapping study of infected soils (Aoufous, Errachidia, etc.) was carried out at INRA Morocco. Field surveys of laboratory studies of samples have confirmed the presence of Foa in the prospected areas (Essarioui *et al*., 2018). Thus the early detection of infected areas is an effective way to limit the rapid spread of this pathogen which threatens all natural oases.

Despite their inapplicability in large-scale infested areas, the results of their study show that in the event of the introduction of Bayoud disease into new plantations, a timely spot treatment, based on the combination of solarization with fumigation, could eradicate the disease and prevent its progression in these areas. The technical management of palm groves in the extension areas, in particular the practice of localized irrigation which does not allow the spread of the pathogen by abundant irrigation water, and the rigorous measures of prevention and exclusion practiced by investors, are also factors that complement this strategy of struggle. This could help protect plantations against the devastating effects of the disease and thus secure long-term investments (Essarioui and Sedra, 2017).

➢ **Phytosanitary measures**. Morocco have implemented internal quarantine on all contaminated oases to prevent the movement of offshoots from diseased areas to healthy ones. For the purpose of development in Morocco, this country put in place the law 01/06 in 2007 for the sustainable development of palm groves and the protection of the date palm and Order No. 2027-15 of June 20, 2015 (modified in 2018 by decree 1812-18) fixing the conditions of production, circulation, transfer and planting of date palms in certain zones protected against Bayoud disease. Morocco has also demarcated zones for planting disease-free date palms and has adopted phytosanitary measures to be implemented in these zones.

Date-producing countries are advised to prohibit the importation of the following from countries where Bayoud disease is present (EPPO, 2022):

- all date-palm material (offshoots, leaves, handicrafts, etc., but not fruits);
- soil and plants for planting (with roots, cuttings) accompanied by soil;
- plants for planting of *Lawsonia inermis* (except seeds)

8.4.- Conclusions.

The date palm Bayoud disease found in most of Morocco is caused by *Fusarium oxysporum* f. sp. *albedinis*. Several studies have focused on the management of Bayoud of date palm through the morphological identification of the pathogen, the use of chemicals, including methyl bromide and other soil fumigants such as chloropicrin (Freeman & Maymon, 2000) and sometimes the burning of affected palms. However, success of these methods has been very limited. Many date palms remain infected, although symptomless and the pathogen could be recovered in many of the treated areas (Rafiqi *et al*., 2021). Besides, these chemical treatments are also harmful to the environment due, which combined with burning of infected palm trees, contributes to soil degradation in oases. Morphological criteria are insufficient for the detection of Foa (Freeman & Maymon, 2000). For rapid and effective detection of the pathogen, molecular methods, such as PCR amplification of genomic DNA specific to the pathogen, are largely reliable. However, in many cases, combination macroscopic morphological observations and molecular methods can be useful.

8.5.- Brief review of other projects addressing Bayoud disease.

➢ Bayoud disease control project A "*Bayoud Disease Control*" project was implemented in North Africa in two phases between 1988 and 1991. /RAB/84/018. The project was funded by UNDP and implemented by FAO. The project served as a regional example. Its aim was to limit the spread of Bayoud disease on date palms that have a significant economic importance in Algeria, Morocco and Tunisia. Its immediate objectives included: developing an effective system to reduce the spread of Bayoud disease, performing research on a regional level of a selection of Bayoud-resistant date palm; and establishing a mechanism for regional propagation of new date palms

➢ Project "*Control of Bayoud Disease in date Palm*" (RAF/5/035). FAO/IAEA inter-regional project RAF/5/035 was started in 1995 (completed in 2001) with a major objective to isolate Bayoud disease resistant date palm mutants by using *in vitro* propagation and selection, and gamma irradiation. This project continued as RAF/5/049 (active since 2001) with the main objective field evaluation of Bayoud disease resistance date palm mutants. The major components of these projects are: in vitro culture of Deglet Nour, Tegaza, and Mejhool varieties, radiation induced mutations, screening of mutants and their field evaluation, and molecular marker analysis. Already, *in vitro* propagation methods, somatic embryogenesis and organogenesis, is developed and plants are ready for hardening under the controlled conditions before transferring to the field. Somatic embryogenesis technology seems to be promising for the isolation of mutants and their clonal propagation in large numbers by using bioreactor. Bayoud disease toxin can be isolated from the causal fungus FOA, and it can be used for in vitro selection against this disease.

8.6.- List of relevant stakeholders.

We selected two types of farmers interested in the control of Bayoud disease:

➢ Ten farmers with less than 5 ha:

Farmer	Region	Surface
Ahmed Agramin	Boudnib	0,25 ha
Hassan Bouhafsi	Ksar Sahli	0,5 ha
Abdelwafi Fatah	Ksar Sahli	0,5a
Omar Benomar	Boudnib center	1 ha
Giat Samhamed	Boudnib center	1,5 ha
Sabar mahomet	Boudnib center	1 ha
Rashid Benikin	KSar Bani Wazim	0,5 ha
Rabeh Mimouni	Ksar Walad Ali	3 ha
RAhou jamal	KsarTazokart	5 ha
Adel Mansour	Ksar Walad Ali	3 ha

- 8 farmers with more the 50 ha:

Farmer	Region	Surface
Domaine Oumadliss	Bouanane	80 ha
Domaine Hachimi Agri	Boudnib	50 ha
Domaine Agroplam	Boudnib	85 ha
Domaine Lina	Boubernous-Boudnib	85 ha
Agrounivers Domaine Elhmouti	Lahmada Boudnib	120 ha
La Belle Datte	Assanr-Oued Naan-Tazgart	120 ha
Domaine Snousia	Boudnib	150 ha
Domaine Jannat Annakhil	Boudnib	105 ha

8.7.- Bayoud disease related bibliography.

- Abouraïcha EL. F., Jay-Allemand C., Coumans M., Martinez C. et El Hadrami I., 2008. Développement de nouvelles stratégies de lutte contre le bayoud : la potentialisation des réactions de défense du palmier dattier au moyen de stifénia. Actes du 3è séminaire international du Réseau Palmier dattier, 18-20 novembre 2008, IRD-Montpellier-France.
- Aktar, Md Wasim, Dwaipayan Sengupta, and Ashim Chowdhury. 2009. “Impact of Pesticides Use in Agriculture: Their Benefits and Hazards.” Interdisciplinary Toxicology 2 (1): 1–12. https://doi.org/10.2478/v10102-009-0001-7.
- Amanat, A., Waly, M., Essa, M., Sankar, D., 2012. Nutritional and medicinal value of date Fruit. In: Manickavasagan, A. (Ed.), Dates: Production, Processing, Food, and Medicinal Values. CRC Press, pp. 361–371.
- Bounaga N. et Djerbi M., 1990. Pathologie du palmier dattier. Systèmes agricoles oasiens Options Méditerranéennes, Sér. A I n 11, 127-132 p.
- CBI, 2020. Centre for the Promotion of Imports from developing countries (CBI). The European market potential for dates. Available online: https://www.cbi. eu/market-information/processed-fruit-vegetables-edible-nuts/dates/marketpotential
- Chao, C.T., Krueger, R.R., 2007. The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation. HortScience 42 (5), 1077–1082
- Corte A. La traceomicosi da *Fusarium oxysporum* f. sp. *albedinis* della *Phoenix canariensis*. Notiz Malattie Piante. 1973; 88–89:107–17.
- Dar, T. J. A., Haidari, H. S. E. L., & Shabana, H. R. (1982). The date palm. Date Palm Journal, May 1982, 153–316.
- Dihazi A., Jaiti F., Taktak W., kilani-Feki O., Jaoua S., Driouich A., Baaziz M., Daayf F. et Serghini M. A., 2012 a. Use of two bacteria for biological control of Bayoud disease caused by *Fusarium oxysporum* f. sp. *albedinis* in date palm (Phoenix dactylifera L.) seedlings. Plant Physiology and Biochemistry. 55:7-15p.
- Dihazi A., Jaiti F., Zouine J., EL Hassni M. et EL Hadrami I., 2003. Effect of salicylic acid on phenolic compounds related to date palm resistance to *Fusarium oxysporum* f. sp. *albedinis*. Phytopathol. Mediterr. 42:9–16 p.

- Djerbi M, Aouad L, Filali H, Saaidi M, Chtioui A, Sedra MH, Allaoui M, Hamdaoui T, Oubrich M. (1986) Preliminary results of selection of high-quality Bayoud-resistant clones among natural date palm population in Morocco. In: Proceedings of the Second Symposium on the Date Palm, Saudi Arabia, pp. 383-399.
- Djerbi M. (1982) Bayoud disease in North Africa: history distribution, diagnosis and control. DatePalm Journal, 1: 153-197.
- El Hassni M., 2005. Interaction Palmier dattier-*Fusarium oxysporum albedinis*: Elicitation desréactions de défense et développement de nouvelles stratégies pour le biocontrôle de la maladie du Bayoud. Thèse de doctorat. Université Cadi Ayyad, Faculté des Sciences Semlalia, Marrakech, Maroc.
- El Hassni M., El Hadrami A., Daayf F., Chérif M., Ait Barka E. et El Hadrami I., 2007. Biological control of Bayoud disease in date palm: selection of microorganisms inhibiting the causal agent and inducing defense reactions. Environmental and Experimental Botany. 59(2): 224-234 p.
- EPPO (2022) *Fusarium oxysporum* f. sp. *albedinis*. EPPO datasheets on pests recommended for regulation. Available online. https://gd.eppo.int
- Essarioui A. et Sedra M. Lutte contre la maladie du bayoud par solarisation et fumigation du sol. Une expérimentation dans les palmeraies du Maroc. Cah. Agric. 2017, 26, 45010
- Essarioui A. et Sedra My. H. 2010. Biocides, Soil Solarization and Fumigation to Control *Fusarium oxysporum* f. sp. *albedinis* Inciting Bayoud Disease on Date Palm. Proc. 4th Int. Date Palm Conference. Acta Hort. 882.
- Essarioui A., Ben-amar H., Khoulassa S., Meziani r., Amamou a., Mokrini f. Gestion du Bayoud du palmier dattier dans les oasis marocaines 537-543/ 2018-12-20
- FAOSTAT, 2020. FAOSTAT Database. website: WWW.fao.org
- Feather TVaM, H.D. Wilt and dieback of Canary Island palm in California. Calif Agric. 1979; 33:19–20.
- Fernandez, D. (1993). Conaparaisorn entre *Pusurim oxysporum* f. sp. *ulbedhis* et *Fusarium oxysporum* des sols de paimeraies par l'étode du polymorphisme de longueur des fragments de restriction (RFLP). Phytopathologia Mediterranea, 235–244.
- Fernandez, Diana, Ouinten, M., Tantaoui, A., Geiger, J., Daboussi, E., Langin, T., & Icrobiol, A. P. P. L. E. N. M. (1998). Genome Provide Diagnostic PCR Targets for Detection of the Date Palm Pathogen. Appl Environ Microbiol, 64(2), 633–636.
- Forget, Gilles, Tracey Goodman, and A De Villiers. 1993. Impact of Pesticide Use on Health in Developing Countries: Proceedings of a Symposium Held in Ottawa, Canada, 17-20 Sept. 1990. IDRC, Ottawa, ON, CA.
- Freeman, S., & Maymon, M. (2000). Reliable detection of the fungal pathogen *Fusarium oxysporum* f.sp. *albedinis*, causal agent of Bayoud disease of date palm, using molecular techniques. Phytoparasitica, 28(4), 341–348.
- Haddouch, M., 1993. Situation actuelle et perspectives de développement du palmier dattier au Maroc importance du secteur phoenicicole importance socioéconomique oasis. In: Ferry, M., Greiner, D. (Eds.), Le palmier dattier dans l'agriculture d'oasis des pays méditerranéens. Zaragoza: CIHEAM, pp. 63–79.
- Hasnaoui, A., Elhoumaizi, M.A., Hakkou, A., Wathelet, B., Sindic, M., 2011. Physicochemical characterization, classification and quality evaluation of date palm fruits of some Moroccan cultivars. J. Sci. Res. 3 (1), 139–149.
- Hussein, Adil A ElAlhasan, R. E. M., & Abdelwahab, S. A. (2016). Isolation and Identification of Streptomyces rochei Strain Active against Isolation and Identification of *Streptomyces rochei* Strain Active against Phytopathogenic Fungi. British Microbiology Research Journal, 4(10) (January 2014), 1057–1068.
- Ibrahim Elkhalil Benzohra MMaRB. Bayoud disease of date palm in Algeria: History, epidemiology and integrated disease management. African Journal of Biotechnology. 2015; 14(7):542–50. https://doi.org/ 10.5897/AJBX2014.14292
- Ibrahim, E. B., Mohamed, M., & Rafik, B. (2015). Bayoud disease of date palm in Algeria: History, epidemiology and integrated disease management. African Journal of Biotechnology, 14(7), 542–550.
- Igbedioh, S O. 1991. "Effects of Agricultural Pesticides on Humans, Animals, and Higher Plants in Developing Countries." Archives of Environmental Health 46 (4): 218–24. https://doi.org/10.1080/00039896.1991.9937452.
- Jaiti F, Verdeil JL, El Hadrami I. (2009) Effect of jasmonic acid on the induction of polyphenoloxidase and peroxidase activities in relation to date palm resistance against *Fusarium oxysporum* f. sp. *albedinis*. Physiol. Mol. Plant Pathol, 74: 84–90.
- Jaiti F., Meddich A. et El Hadrami I., 2007. Effectivess of arbuscular mycorrhizal fungi in the protection of date palm against bayoud disease, Physiological and Molecular Plant Pathology, 71: 166-173 p.
- Kettout, T. A., & Rahmania, F. (2010). Identification par CG-SM de l'acide phénylacétique produit par *Fusarium oxysporum* f. sp. *albedinis*, agent causal du bayoud. Comptes Rendus - Biologies, 333(11–12), 808–813.
- Kristoforović-Ilić, Miroslava. 2004. "Pesticides and the Environment." Medicinski Pregled 57 (11–12): 523–35. https://doi.org/10.2298/MPNS0412523K.

- Louvet J, Toutain G. (1973) Recherches sur les fusarioses. VIII-Nouvelles observations sur la fusariose du palmier dattier et précisions concernant la lutte. Ann Phytopathol, 5:35-52.
- Louvet J., Bulit J., Touîain G. et Ribuf P., 1970. Le Bayoud, fusariose vasculaire du palmier dattier symptômes et nature de la maladie moyens de lutte, Al Awamia. 35:16l-162 p.
- Maslouhy My A., 1989. Contribution à l'étude in vitro et in situ des antagonistes de *Fusarium oxysporum* f. sp. *albedinis*, agent causal du Bayoud. Thèse de 3éme cycle. Faculté des sciences, Marrakech.
- Mcdougall, Phillips. 2016. "The Cost of New Agrochemical Product Discovery, Development and Registration." R&D Study, no. March 2016: 3.
- Mirza Hussain Samo NJI. solation and characterization of *Fusarium oxysporum* f. sp. *albedinis* causing bayoud disease of date palm tree from Gambat, district Khairpur, Sindh, Pakistan. 2020; 04 (1):43–7.
- Munier, P., 1973. Le palmier dattier. Techniques Agricoles et Productions Tropicales. Paris 5ème, Maisonneuve et Larose, 217.
- Nash SS. (1978) Comparison of germination of pathogen *Fusarium oxysporum* f. sp. *Albedinis* chlamydospores in host rhizosphere soils conductive and suppressive to wilts. Phytopathol, 67: 502-10.
- Oihabi A., 1991. Etude de l'influence des mycorhizes à vésicules et arbuscules sur le Bayoud et la nutrition du palmier dattier. Doctorat d'Etat, Université Cadi Ayyad, Faculté des SciencesSemlalia-Marrakech,Maroc. 110 p.
- Olson, Sara. 2015. "An Analysis of the Biopesticide Market Now and Where It Is Going." Outlooks on Pest Management 26 (5). https://doi.org/10.1564/v26_oct_04.
- ONU. 2019. World Population Prospects 2019. Department of Economic and Social Affairs. World Population Prospects 2019.
- Rafiqi M, Jelonek L, Diouf AM, Mbaye A, Rep M, Diarra A (2022) Profile of the *in silico* secretome of the palm dieback pathogen, *Fusarium oxysporum* f. sp. *albedinis*, a fungus that puts natural oases at risk. PLoS ONE 17(5): e0260830. https://doi.org/10.1371/journal.pone.0260830
- Raho, Ouissame & Boutasknit, Abderrahim & Anli, Mohamed & Ben-Laouane, Raja & Ait Rahou, Youssef & Ouhaddou, Redouane & Duponnois, Robin & Douira, Allal & El Modafar, C. & Abdelilah, Meddich. (2022). Impact of Native Biostimulants/ Biofertilizers and Their Synergistic Interactions On the Agro-physiological and Biochemical Responses of Date Palm SeedlingsAuswirkungen von Biostimulanzien/Biodünger und ihrer synergistischen Wechselwirkungen auf die agrophysiologischen und biochemischen Reaktionen von Dattelpalmen-Setzlingen. Gesunde Pflanzen. 10.1007/s10343-022-00668-5.
- Rhouma A. (1996) Le palmier dattier en Tunisie: un secteur en plein expansion. Option Méditerranéens, Série A, Séminaires Méditerranéens, 28:85-104.
- Sedra MH. The Bayoud (Vascular Wilt) of Date Palm in North Africa: Situation, Research Achievements and Applications ActaHorticulturae. 2013;(994) : 59–75. https://doi.org/10.17660/ActaHortic.2013.994.4
- Sedra My. H. et Roxel F., 1989. Résistance des sols aux maladies. Mise en évidence de la résistance d'un sol de la palmeraie de Marrakech aux fusarioses vasculaires. Al Awamia 66: 35-54 p.
- Tran, G., & Bastianelli, D. (2015). Date palm seeds Date palm seed in animal nutrition A datasheet available freely online Datasheet citation Heuzé V ., Tran G ., Delagarde R ., Bastianelli D ., Lebas F ., 2015 . Date palm seeds. Feedipedia, a programme by INRA , CIRAD , AFZ and FAO . Ext. Feedipedia, May.

9.- Section 6: Essential oils for the control of fungal pathologies

9.1.- Brief introduction to essential oils

Global food supply is highly dependent on industrial agriculture, which in turn would not be possible without the intensive use of pesticides against fungal diseases and other pests. Responding to consumers' increasing demands for a sustainable food production implies developing alternatives to conventional synthetic plant protection products. Long-term fungicide applications have consequently led to increased resistances of pathogens and detrimental impacts on ecosystems and humans, followed by a decreasing acceptance by consumers. This is particularly true for grapevine (*Vitis vinifera* L.), which is highly sensitive to fungal diseases such as downy mildew. This organism is one of the most devastating diseases of viticulture worldwide, which explains that the application of relatively large amounts of pesticides in viticulture, when compared to other crops, is necessary to guarantee yield and quality of grape production.

To reduce synthetic pesticides, organic production is one alternative to conventional farming but still highly dependent on copper (Cu), the oldest and still a very efficient treatment against downy mildew. It remains, however, a heavy metal accumulating in vineyard soils. Cultivation of disease resistant varieties is certainly one of the most ecological solutions to reduce pesticides and, due to tremendous efforts of international public breeding program, a large choice of disease-resistant grape cultivars is nowadays available to growers. However, the organoleptic quality is still often inferior to the one of traditional cultivars, making them less attractive to consumers and thus producers. For these last reasons, access of organic wines to the wine market is still difficult. Furthermore, the durability of resistance factors is often not stable, in particular in monogenetic cultivars. Genetically modified organisms are not a solution either, since they are so far neither premised nor authorised in most producing countries. Alternative plant protection strategies are thus utterly needed to guarantee a sustainable viticulture that is both environment- and consumer-friendly. Also, the natural substance proposed for pest management in agriculture should have the following properties: efficacy against the target organism, safety and biological selectivity, standardized composition and formulation and ready availability. Natural compounds generally have lower persistence and toxicity than synthetic compounds, reducing their potential environmental impact. The **essential oils** are complex mixtures of several chemical compounds including terpenes, alcohols, aldehydes and phenols, and these materials exhibit potential herbicidal and fungicidal properties. Some essential oils have antimicrobial properties, are antiviral, or antimycotic, and can have roles in weed control. In recent years, there has been an increased interest in the use of essential oil combinations to improve their natural antimicrobial and antifungal activities. Many researchers observed increased antifungal effects caused by combinations of essential oils from *Syzygium. aromaticum* and *Rosmarinus officinalis*, among others.

In this context our objectives for SUSTEMICROP project are:

- To obtain novel essential oils by hydrodistillation from a wide variety of aromatic and medicinal plants
- To analyze and characterize the physico-chemical properties of the obtained essential oils.
- To detect AF and antioxidant activities of essential oils and identify the molecules responsible.
- To formulate novel biopesticides based on natural AFs, from aromatic plants and bacterial priming-molecules
- To test the new biopesticides effectiveness on plant assays (greenhouse and small-scale field assays), addressing key pathogens which threaten several cultures in the Mediterranean area in the project crops (hops, grapevine and date palm)

This project also aims to study the antifungal activity of natural extracts and essential oils obtained from many aromatic plants, like *Eucalyptus gomphocephala*, *Thymus capitatus*, *Rosmarinus officinalis*, *Pinus halepensis*, *Cupressus sempervirens*, and many others against phytopathogenic fungi, like *Fusarium oxysporum*, *Rhizoctonia solani* and *Alternaria solani*.

These extracts will also be tested for their effectiveness against mildew and odium. Finally, the chemical constituents of the extracts/HE will be identified by GC/MS analysis, as mentioned earlier in section 4 of this document, where some example of the application of essential oils for the control of downy and powdery mildew on vineyards is discussed in more detail.

9.2.- List of relevant stakeholders.

- SUDVINBIO : https://www.sudvinbio.com/. It is a French company that has been promoting organic viticulture since 1991. The interprofessional association Sudvinbio was created in 1991 on the initiative of winegrowers who practise this form of agriculture, in order to collectively promote their wines.

- SVBNA : http://www.vigneronsbionouvelleaquitaine.fr/. Vignerons Bio Nouvelle-Aquitaine brings together more than 200 organic and biodynamic winemakers from the Nouvelle Aquitaine region. What binds all their partners is the desire to sustain an Organic Agriculture that is certified, plural and economically viable.

- Cave HERACLES : https://caveau-heracles.com/ . The Héraclès cooperative winery brings together 80 winegrowers and has been producing organic wines for more than 25 years on land near the Perrier spring.

- The results could also been of Potential interest for any of the 2.4 million European winegrowing farms including large private companies like Moët & Hennessy, Mumm or AdVini in France or Torres in Spain, but more specifically the 2.0 Million European winegrowing farms in southern regions (Spain, Greece, Portugal, Roumania, south of France).

9.3.- Bibliography related to essential oils.

- Abd Rashed, A.; Rathi, D.-N.G.; Ahmad Nasir, N.A.H.; Abd Rahman, A.Z. (2021). Antifungal Properties of Essential Oils and Their Compounds for Application in Skin Fungal Infections: Conventional and Nonconventional Approaches. Molecules 26, 1093. https://doi.org/10.3390/ molecules26041093

- Nazzaro, F., Fratianni, F., Coppola, R., and De Feo, V. (2017). Essential Oils and Antifungal Activity. Pharmaceuticals 2017, 10, 86; doi:10.3390/ph10040086

- Singh, G and Maurya, S. (2005). Antimicrobial, antifungal and insecticidal investigations on essential oils. An Overview. Natural Product Radiance, 4: 179-192.

10.- Section 7: Priming molecules for the control of fungal pathologies

10.1.- Priming molecules for the control of fungal pathologies: an introduction.

Agrogenia Biotec,S.L. participates in SUSTEMICROP Project in the development of "*Agricultural Biostimulants*", specifically of "Active Biomolecules" derived from metabolic extracts of bacteria that produce a "priming effect". These biomolecules should be oriented as tools to help in the biocontrol of pathogen infections to be studied in the project, specifically "Downy and Powdery mildew, *Botritys*, and *Alternaria* fungal phytopathogens in hops and grapevines.

A bibliographic study of bacterial active biomolecules that develop priming effects against these diseases has been carried out although few references have been found. They are mainly related to molecules of the lipopeptide type with priming function and polysaccharides of bacterial origin, especially wall lipopolysaccharides for controlling diseases in grapevines, while except for an article from 2022 and related to the company's advisory team, no studies have yet been carried out for bacterial signalling and communication molecules (see list of references below in section 9.2).

The molecules that produce the "priming effect" are actually activators of the plant's immune system, activating the induced resistance of plants against possible pathogens. The priming effect consists of stimulating the passive defence capacity of the plants so that they are in a permanent state of alert without having to have their defences constantly activated and so that the plants are able to detect possible damage and react more quickly and effectively.

When a priming biomolecule comes into contact with a plant, it establishes a state of alertness, increasing the ability to recognise the harmful effect of unfavourable biotic stress earlier and better and facilitating the transcription of these defence proteins when they are actually needed, i.e. in the presence of aggression, in many cases even preventing the harmful effect of the biotic stress from occurring.

The use of induced resistance will become a realistic technique for sustainable agriculture 2.0. By stimulating the immune system of plants, they are "primed" to respond quickly and effectively to different stress factors. They are another tool at the service of the farmer to achieve healthier and therefore more productive crops.

Most of the biomolecules used for priming are of inorganic or organic origin derived from plants or fungi and very few molecules are derived from bacteria. Molecules derived from plants, oomycetes, fungi and bacteria have been reported. The priming molecules to be developed at Agrobenia Biotech, S.L. are derived from bacterial extracts of rhizobacteria. Among these molecules, there is only one molecule bibliographically referred to that is being investigated by the University of Seville in collaboration with the EMBRAPA research institute in Brazil, which has recently published an article that relates the priming effect of these bacteria-plant signalling molecules with the biocontrol of *Xhantomonas campestris* pv campestris (Xcc) in *Brassica oleracea* plants, reaching the conclusion that these active biomolecules have an important biotechnological use as a molecule that activates the defence of the plants (Reis Santos *et al*., 2022).

10.2.- List of relevant stakeholders.

- Potentially, any of the 2.0 Million European small and medium winegrowing farms in southern regions (Spain, Greece, Portugal, Roumania, south of France) could be interested in the results. Particularly all the wineries included in one of the 70 “Denominaciones de Origen” existing in Spain, that group hundreds of cellar.
- Also the results could be of interest for European Hop Growers and more specifically for the Spanish hope growers cooperatives and organizations like Lúpulos de León, SAT (Hop Growers from Spain) which brings together the majority of Spanish producers (https://www.lupulosdeleon.es/)
- Plant protection companies interested in the development of natural methods to control fungal pathologies, including the company Agrogenia Biotech, which is partner of our project.

10.3.- References related to priming molecules.

- Banani, H., Roatti, B., Ezzahi, B., Giovannini, O., Gessler, G., Pertot, I., et al. (2014). Characterization of resistance mechanisms activated by *Trichoderma harzianum* T39 and benzothiadiazole to downy mildew in different grapevine cultivars. Plant Pathol. 63, 334–343. doi: 10.1111/ppa.12089
- Bakker, P., Doornbos, R.F., Zamioudis, C., Berendsen, R.L. and Pieterse, C.M.J. (2013) Induced Systemic Resistance and the Rhizosphere Microbiome. The Plant Pathology Journal, 29, 136-143. https://doi.org/10.5423/PPJ.SI.07.2012.0111
- Brulé, D., Villano, C., Davies, L. J., Trdá, L., Claverie, J., Héloir, M. C., et al. (2019). The grapevine (*Vitis vinifera*) LysM receptor kinases VvLYK1-1 and VvLYK1-2 mediate chitooligosaccharide-triggered immunity. Plant Biotechnol. J. 17, 812–825. doi: 10.1111/pbi.13017
- Conrath, U. (2011). Molecular aspects of defence priming. Trends Plant Sci. 16, 524–531. doi: 10.1016/j.tplants.2011.06.004
- Delaunois, B., Farace, G., Jeandet, P., Clément, C., Baillieul, F., Dorey, S., et al. (2014). Elicitors as alternative strategy to pesticides in grapevine? Current knowledge on their mode of action from controlled conditions to vineyard. Environ. Sci. Pollut. Res. Int. 21, 4837–4846. doi: 10.1007/s11356-013-1841-4

- Desaki, Y., Kouzai, Y., Ninomiya, Y., Iwase, R., Shimizu, Y., Seko, K., et al. (2018). OsCERK1 plays a crucial role in the lipopolysaccharide-induced immune response of rice. New Phytol. 217, 1042–1049. doi: 10.1111/nph.14941
- Desaki, Y., Miya, A., Venkatesh, B., Tsuyumu, S., Yamane, H., Kaku, H., et al. (2006). Bacterial lipopolysaccharides induce defense responses associated with programmed cell death in rice cells. Plant Cell Physiol. 47, 1530–1540. doi: 10.1093/pcp/pcl019
- Farace, G., Fernandez, O., Jacquens, L., Coutte, F., Krier, F., Jacques, P., et al. (2015). Cyclic lipopeptides from *Bacillus subtilis* activate distinct patterns of defence responses in grapevine.Mol. Plant Pathol.16, 177–187. doi: 10.1111/mpp.12170
- Helóir, MC, Adrian M., Brulé D., Claverie J., Cordelier S., Daire X., Dorey S., Gauthier A., Lemaître-Guillier C., Negrel J., Trdá L., Trouvelor S., Vandelle E . and Poinssot B. 2019. Recognition of Elicitors in Grapevine: From MAMP and DAMP Perception to Induced Resistance. Front.Plant Sci., 18: https://doi.org/10.3389/fpls.2019.01117
- Henry, G., Deleu, M., Jourdan, E., Thonart, P., Ongena, M. (2011). The bacterial lipopeptide surfactin targets the lipid fraction of the plant plasma membrane to trigger immune-related defence responses. Cell Microbiol. 13, 1824–1837. doi: 10.1111/j.1462-5822.2011.01664.x
- Jourdan, E., Henry, G., Duby, F., Dommes, J., Barthelemy, J., Thonart, P., et al. (2009). insights into the defense-related events occurring in plant cells following perception of surfactin-type lipopeptide from *Bacillus subtilis*. Mol. Plant-Microbe Interact. 22, 456–468. doi: 10.1094/MPMI-22-4-0456
- Lemaître-Guillier, C., Hovasse, A., Schaeffer-Reiss, C., Recorbet, G., Poinssot, B., Trouvelot, S., et al. (2017). Proteomics towards the understanding of elicitor induced resistance of grapevine against downy mildew. J. Proteomics 156, 113–125. doi: 10.1016/j.jprot.2017.01.016
- Li, Y., Héloir, M. C., Zhang, X., Geissler, M., Trouvelot, S., Jacquens, L., et al. (2019). Surfactin and fengycin contribute to the protection of a *Bacillus subtilis* strain against grape downy mildew by both direct effect and defense stimulation. Mol. Plant Pathol. 20(8), 1037–1050 doi: 10.1111/mpp.12809
- Monnier, N., Furlan, A., Botcazon, C., Dahi, A., Mongelard, G., Cordelier, S., et al. (2018). Rhamnolipids from *Pseudomonas aeruginosa* are elicitors triggering Brassica napus protection against *Botrytis cinerea* without physiological disorders. Front. Plant Sci. 9, 1170. doi: 10.3389/fpls.2018.01170
- Paris, F., Krzyżaniak, Y., Gauvrit, C., Jamois, F., Domergue, F., Joubès, J., et al. (2016). An ethoxylated surfactant enhances the penetration of the sulfated laminarin through leaf cuticle and stomata, leading to increased induced resistance against grapevine downy mildew. Physiol. Plant 156, 338–350. doi: 10.1111/ppl.12394
- Ranf, S., Scheel, D., Lee, J. (2016). Challenges in the identification of microbe-associated molecular patterns in plant and animal innate immunity: a case study with bacterial lipopolysaccharide. Mol. Plant Pathol. 17, 1165–1169. doi: 10.1111/mpp.12452
- Silipo, A., Molinaro, A., Sturiale, L., Dow, J. M., Erbs, G., Lanzetta, R., et al. (2005). The elicitation of plant innate immunity by lipooligosaccharide of *Xanthomonas campestris*. J. Biol. Chem. 280, 33660–33668. doi: 10.1074/jbc.M506254200
- Trotel-Aziz, P., Eliane Abou-Mansour, Barbara Courteaux Fanja Rabenoelina Christophe Clément Florence Fontaine and Aziz A. 2019. *Bacillus subtilis* PTA-271 Counteracts Botryosphaeria Dieback in Grapevine, Triggering Immune Responses and Detoxification of Fungal Phytotoxins. Front. Plant Sci., 24: https://doi.org/10.3389/fpls.2019.00025.
- Trouvelot, S., Héloir, M. C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., et al. (2014). Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. Front. Plant Sci. 5, 592. doi: 10.3389/fpls.2014.00592
- Trouvelot, S., Varnier, A. L., Allègre, M., Mercier, L., Baillieul, F., Arnould, C., et al. (2008). A beta-1,3 glucan sulfate induces resistance in grapevine against *Plasmopara viticola* through priming of defense responses, including HR-like cell death. Mol. Plant Microbe Interact. 21, 232–243. doi: 10.1094/MPMI-21-2-0232
- van Aubel, G., Buonatesta, R., Van Cutsem, P. (2014). COS-OGA: a novel oligosaccharidic elicitor that protects grapes and cucumbers against powdery mildew. Crop Prot. 65, 129–137. doi: 10.1016/j.cropro.2014.07.015
- Varnier, A. L., Sanchez, L., Vatsa, P., Boudesocque, L., Garcia-Brugger, A., Rabenoelina, F., et al. (2009). Bacterial rhamnolipids are novel MAMPs conferring resistance to *Botrytis cinerea* in grapevine. Plant Cell Environ.32, 178–193. doi: 10.1111/j.1365-3040.2008.01911.x
- Wiesel, L., Newton, A. C., Elliott, I., Booty, D., Gilroy, E. M., Birch, P. R., et al. (2014). Molecular effects of resistance elicitors from biological origin and their potential for crop protection. Front. Plant Sci. 5, 655. doi: 10.3389/fpls.2014.00655
- Zhang, X., Zhou, Y., Li, Y., Fu, X., Wang, Q. (2017). Screening and characterization of endophytic Bacillus for biocontrol of grapevine downy mildew. Crop Prot. 96, 173–179. doi: 10.1016/j.cropro.2017.02.018

11.- Section 8: Report on Climate change and new selected grapevine varieties more adapted to dryer and warmer conditions.

11.1.- Brief introduction to the problem.

The change of the climate is now very well documented and its consequences on plants relatively clear. The predicted climate change will concern two main components, temperature and precipitation.

Regarding the mean air temperature, it will increase at a variable rate depending on the greenhouse gas emission scenarios (**Figure 22**)

Similarly, climate change will have an effect on the rains, even if it is still difficult to estimate finely the evolution of rains in the future: probably decrease everywhere but more severely in the south, but regional variations will be important.

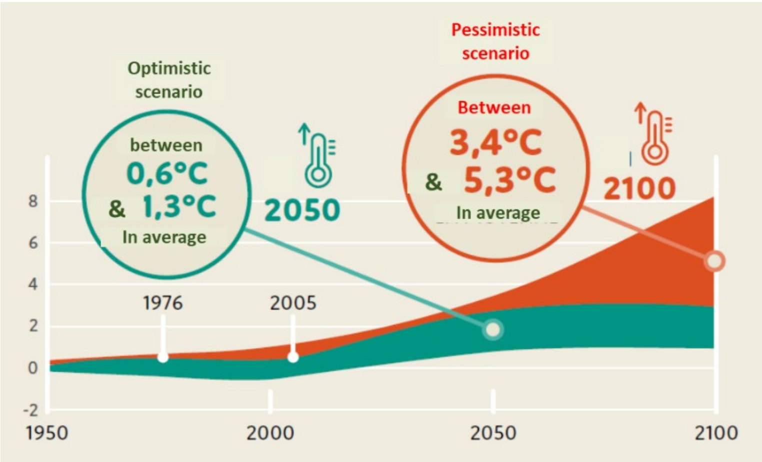


Figure 22.- Increase in average air temperatures under different climate scenarios. Increases are expressed in relation to the reference period (1976-2005). Uncertainties on the magnitude depending on emissions, regions, seasons and climate models (Sources : Jouzel *et al*, 2014, Directorate-General for Energy and Climate, Nathalie Ollat, Jean-Marc Touzard. La vigne, le vin, et le changement climatique en France - Projet LACCAVE - Horizon 2050. 2020)

The consequences on grape development, yield and quality are already visible and will probably intensify. The development of grape has been modified, with earlier harvest in most regions (**Figure 23**) when temperatures are higher

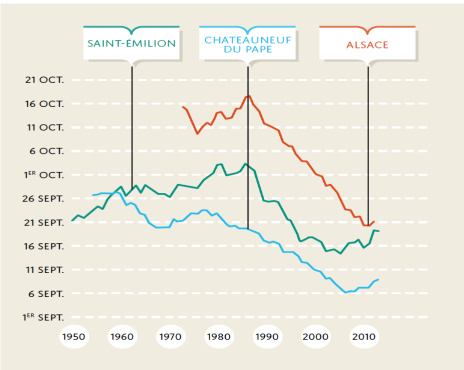


Figure 23.- Evolution of harvesting time in 3 regions of France. The advance is 15 days in 26 years in St-Emilion and in the Côtes du Rhône, and 26 days in Alsace (Sources : Nathalie Ollat, Jean-Marc Touzard. La vigne, le vin, et le changement climatique en France - Projet LACCAVE - Horizon 2050. 2020 (https://www6.inrae.fr/laccave/Actualites2/Plaquette-La-vigne-le-vin-et-le-changement-climatique).

Harvesting when temperatures are still summery can lead to problems in winemaking requiring adaptations, such as harvesting at night. But the climate change can also have direct effect on yield and wine quality, with increased sugar and reduced acidity (**Figure 24**).

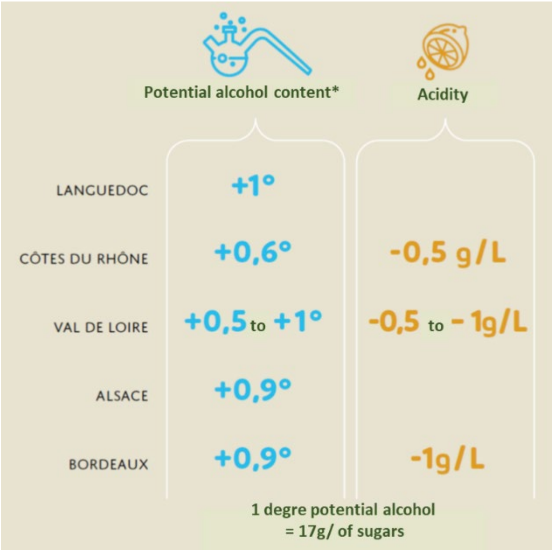


Figure 24.- Evolution of potential alcohol content and acidity in wines from 5 French regions (per decade). The potential alcohol content increased and acidity decreased everywhere [Sources : Nathalie Ollat, Jean-Marc Touzard. La vigne, le vin, et le changement climatique en France - Projet LACCAVE - Horizon 2050. 2020 (https://www6.inrae.fr/laccave/Actualites2/Plaquette-La-vigne-le-vin-et-le-changement-climatique]

11.2.- Brief review of current treatments / solutions already in the market

Figure 25 outlines different types of levers to reduce the effects of climate change. At the moment, some of them have been tested or are tested including

- innovative viticultural practices such as for example the use of water from wastewater treatment plants; https://www.veolia.fr/irri-alteau-solution-sauver-viticulture), green cover or grape shading device (project VitiSad ; https://www.vitisad.eu/) , irrigation regimes, canopy management, yield management, plant density and pruning types (Project INNOVINE ; https://cordis.europa.eu/project/id/311775/reporting/fr).
- Oenological process (such as the selection of yeast strain producing less alcohol and replacing it by glycerol, Tilloy *et al*, 2014).

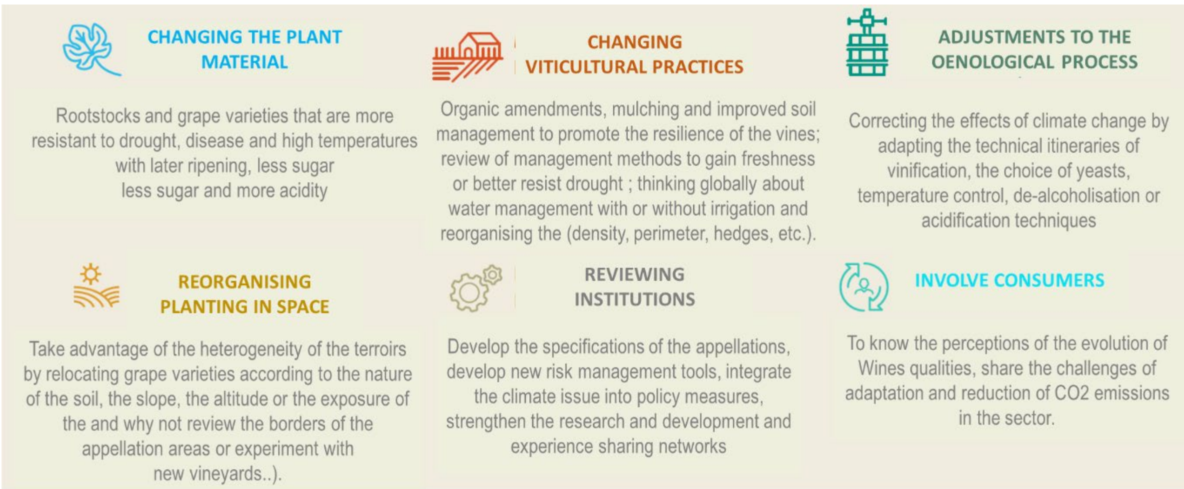


Figure 25.- Different technical, organisational and geographical levers to fight climate changes [Sources : Nathalie Ollat, Jean-Marc Touzard. La vigne, le vin, et le changement climatique en France - Projet LACCAVE - Horizon 2050. 2020 (https://www6.inrae.fr/laccave/Actualites2/Plaquette-La-vigne-le-vin-et-le-changement-climatique)]

- The idea of reorganising the planting area is currently only at the conceptual stage, and may be tested within the appellation areas, considering the relocation of some vineyards in areas that have been rather disadvantaged until now.
- Considering the plant material, much effort has been made in Europe and elsewhere on the development of new cultivars resistant toward the main fungal diseases (powdery and downy mildew), but until now, few efforts has been made to the development of grape cultivars more adapted to changing climate and with durable resistance to mildews. Few studies also concerned the selection of clones of classical varieties better adapted to Climate Change (Tempranillo and Graciano, VitiSAD project).

11.3.- Brief review of later advances to fight the problem.

Our project Sustemicop choose to concentrate one of its Work Package and one of its Technological Cases of Study on "Screening and monitoring of grapevine varieties (on current and new breeding programmes) for resilience and adaptation to climatic change with or without resistance genes against several fungal diseases, identifying key traits for breeding more resilient varieties.

In order to select better adapted grape varieties, considering the effects of climate changes on grape and wine production, one need to consider many traits in order to propose new innovative cultivars for the future of viticulture:

Phenology is an important trait to consider, and one need to take into account the different stages of development: bud bursting, flowering time, veraison time and of course harvest time, in order to move the harvesting time into more favourable climate and avoid late freezing time in some regions. Sugar and acidity need to be taken into account as well, with the selection of cultivars maintaining acidity and with lower sugar accumulation. Finally, drought tolerance, and plasticity are also traits of great importance. Since evolution of climate may be coupled with CO_2 concentration increase, it will also be quite relevant to test the behaviour of cultivars in different concentration of CO_2.

In addition, since the characterization of grape in controlled condition may present some limitations, it is also important to test the same cultivars in different regions with very different climate, some of them simulating the possible evolution of the others.

11.4.- Brief review of other projects addressing same problems

Many countries in Europe and other wine growing countries outside Europe (USA, South Africa, Chili, …) have initiated breeding project in order to create new innovative material better adapted to climate change, financed either by public or private money. .

Two recent projects financed by EU with the aim of selecting new plant material can be cited here:

➢ The project Vitisad, Sustainable winegrowing strategies and practices for adaptation to climate change (2020-2022), considered the selection of clones from cultivars Tempranillo and Graciano, with different phenology and berry composition, as well as the reintroduction of ancient cultivars, thus the characterization of ancient cultivars from the border territory of Spain, France and Andorra.

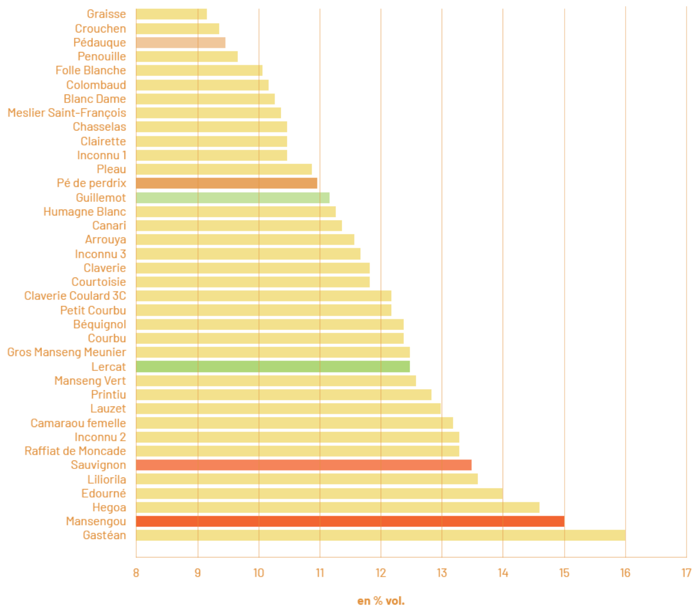


Figure 26.- Comparison of potential alcohol content of 38 cultivars at the same date in Pyrénées-Atlantique department in France. Alcohol expressed as percentage in volume. At this date, potential alcohol content varies up to 6 degrees.

Source: Guide Pratiques viticoles & adaptation au changement climatique dans la zone POCTEFA, projet Vitisad https://www.vitisad.eu/wp-content/uploads/2022/07/guide-vitisad-fr-FINAL.pdf

- The project INNOVINE, Combining innovation in vineyard management and genetic diversity for a sustainable European viticulture (2013-2016). Within this project WP3, aimed at exploiting the genetic diversity in grapevine defined several objectives in regards to innovative plant material:
  - Screening for new resistances to diseases and pests (mildews, black rot and Phylloxera) and adaptation to abiotic stresses in the grapevine germplasm
  - Study the genotypic diversity and plasticity in response to abiotic stresses: for example white and red cultivars of Tempranillo were used to study the effects of different abiotic stresses in greenhouse conditions (combining elevated CO2, elevated temperature and drought acting simultaneously or in interaction) and the partners looked for cultivars and clones with different behaviour under heat and drought conditions.
  - Provide a basis for a European strategy for the pyramiding of resistance genes in elite material

Main results of Innovine project were:

- Screening several collections of Tempranillo (syn. Aragonez) clones revealed variation for surface leaf temperature and ripening time under heat and drought stress. A short list of selected clones with extreme phenotypes was developed. Genetic variation for plasticity of the berry composition under drought has also been studied in a core collection of *V. vinifera* cultivars (Pinasseau *et al*, 2017).

In addition to these two project, it is worth to mention the LACCAVE project a systemic and multidisciplinary program to analyze the impacts from the vine to the region, to define adaptation strategies combining technical, spatial and organizational options and to evaluate the perception by the actors and consumers of climate change issues. Thermal variability was studied at local scale to develop high resolution atmospheric models which better simulate future climate trends. Impacts on growth/developmental conditions and vine responses were estimated from the calculation of eco-climatic indices and a combination of functional models. Genetic and physiological bases of grapevine adaptation to high temperature and drought were analyzed. Improving oenological and cultural practices as well as plant material innovation have been investigated as major technical adaptations. How these options could be implemented at the plot level was examined to elaborate decision tools. Multi-agent modelling was developed for this purpose. Surveys were performed to evaluate the perception of the main actors regarding climate change and their level of acceptability towards technical changes. Consumer acceptability of new types of wines was also investigated with an experimental economy approach. Finally, a foresight exercise was conducted to design four potential adaptation strategies: conservative, innovative, nomad and liberal. Outcomes of this exercise are now used as a tool for the French wine industry members to develop their own strategic plan for adaptation

11.5.- List of relevant stakeholders.

- Potentially, any of the 2.4 million European winegrowing farms could be interested in the results, including large private companies like Moët & Hennessy, Mumm or AdVini in France or Torres in Spain, but more specifically the 2.0 Million European winegrowing farms in southern regions (Spain, Greece, Italy?, Portugal, Roumania, south of France).

- Nursery companies are also the first potential users of the results, including big nurseries such as Mercier in France or VCR in Italy or the nursery Bakasieta from Greece, partner of the project (subcontractor).

11.6.- Information of any advances regarding regulation

The new Common Agricultural Policy of the European Union allows the production of "designation of origin" wines from vine varieties resulting from a cross between *Vitis vinifera* and other species of the genus *Vitis*. In Regulation EU 2021/2117 of the European Parliament and of the Council published on 6 December 2021, article 93 of regulation (EU) n°1308/2013 which only authorised the production of "designation of origin" wines “from vine varieties of the *Vitis vinifera* species” was modified as “which is obtained from vine varieties belonging to *Vitis vinifera* or a cross between the *Vitis vinifera* species and other species of the genus *Vitis*”.

Since the new cultivars resistant against fungal diseases and more adapted to climate change will be of interspecific hybrid origin, this new regulation will indeed allow the use of such varieties in appellation region. The modalities of deployment of the measure in the different regions are not yet known.

In France, in addition, the National Committee for Winegrowing AOCs (CNAOV) wanted the ODGs (Organisme de Défense et de Gestion - Defence and Management Organisation) to be able to evaluate new or old varieties that would present a potential for adaptation to a well-identified problem, while retaining the benefits of the SIQO.

For "varieties of interest for adaptation (VIFA)" which can be either resistant varieties or varieties adapted to a changing climate, operators who so wish to take part in the evaluation work in relation to their ODG and the INAO services during an observation period set at a minimum of 10 years under the following conditions:

- A limit of 5% of the vine stock of the holding
- Incorporation into blends of wines marketed under PDO limited to 10% in order to limit substantial changes in the characteristics of the wines
- The limitation of VIFAs to 10 varieties per AOP and per colour

- compliance with an agreement between each operator, the ODG and the INAO specifying that the ODG is to be provided with all the information needed to complete the cultural behaviour of these VIFAs in the various plots planted and that wine samples are to be provided, in particular samples of wines from VIFAs vinified separately

- in the case of a collective structure, the products of several holdings but of the same VIFA may be vinified together.

Similar system may also be available in other European countries.

The objective of this procedure is to allow operators to plant these varieties as of now and for the ODGs to benefit from a maximum of information necessary to make a final decision. At the end of the 10-year observation period during which the agreement on the future of the VIFA applies, the ODG will have three options, depending on the results of the cultivation and tasting observations:

- to propose the definitive integration of the VIFA in the specifications
- propose the withdrawal of the VIFA from the specification
- requesting the extension of the observation period for a period of 5 years

Several ODGs have already asked to benefit from the implementation of this procedure, such as the ODGs of the AOCs Languedoc, Corbières, Saint-Mont, Bordeaux and Bordeaux supérieur, Savoie, Côtes du Jura, Crémant de Die and Côtes de Provence, and Champagne.

11.7.- Bibliography

- Laccave project : https://www6.inrae.fr/laccave/Actualites2/Plaquette-La-vigne-le-vin-et-le-changement-climatique
- Ollat, Nathalie, Hervé Quénol, Gérard Barbeau, Cornelis van Leeuwen, Philippe Darriet, Inaki Garcia de Cortazar Atauri, Benjamin Bois, Hernan Ojeda, Eric Duchêne, Eric Lebon, Philippe Vivin, Laurent Torregrosa, Jean-Marie Sablayrolles, Geneviève Teil, Philippe Lagacherie, Eric Giraud-Héraud, Patrick Aigrain, and Jean-Marc Touzard. 2018. 'Adaptation to climate change of the French wine industry: a systemic approach – Main outcomes of the project LACCAVE', E3S Web Conf., 50: 01020. https://doi.org/10.1051/e3sconf/20185001020
- Pinasseau L, Vallverdu´ -Queralt A, Verbaere A, Roques M, Meudec E, Le Cunff L, P_eros J-P, Ageorges A, Sommerer N, Boulet J-C, *et al*. 2017 Cultivar diversity of grape skin polyphenol composition and changes in response to drought investigated by LC-MS based metabolomics.Front Plant Sci. 8:1826. doi:10.3389/fpls.2017.01826.
- Tilloy, V., A. Ortiz-Julien, and S. Dequin. 2014. 'Reduction of ethanol yield and improvement of glycerol formation by adaptive evolution of the wine yeast *Saccharomyces cerevisiae* under hyperosmotic conditions', Appl Environ Microbiol, 80: 2623-32. doi: 10.1128/AEM.03710-13

12.- Conclusions and next steps.

- Although the elaboration of the TRSd is an initial starting point for a review of the state of the art of the different TCSs addressed by the project, a more in-depth and specific review can be carried out in the future as the work of the different WPs progresses.
- In a similar way, each partner will be responsible for updating and expanding the list of stakeholders as the project progresses, especially incorporating all those entities or farmers that may be involved in the field trials.
- All these changes can be reflected in the successive reports to be produced during the course of the project.

List of figures

List of Tables