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Chapter

Alternatives to CU Applications in Viticulture: How R&D Projects Can Provide Applied Solutions, Helping to Establish Legislation Limits

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Abstract

Copper (Cu) and its based preparations have been used for over 200 years to control fungi and bacterial diseases in cultivated plants. Downy mildew caused by the obligate biotrophic oomycete *Plasmopara viticola* is one of the most relevant and recurrent diseases of grapevines. Recently, the use of Cu is being limited by some regulations because of its high impact at different levels (health and environmental problems). Due to its accumulation in soil, this metal causes a little controversy with the principles of sustainable production. Therefore, international legislation and initiatives have recently been arisen to start limiting its use, with the main goal to replace it. In this framework, some alternatives have been tested and others are recently being developed to replace, at least partially, the use of Cu in viticulture. Many of them, are being developed and tested under the scope of research and development EU funded projects. To not compromise sustainability targets in viticulture, results from these R&D projects need to be considered to assess the present risks of using Cu in viticulture and to better support establishing limits for its applications, considering soils vulnerability, while no sustainable alternatives are available in the market.

Keywords: *Plamospara viticola*, Sustainability, Copper, Downey mildew, innovation

1. Introduction

Cu based preparations have been used for over 200 years to control fungi and bacterial diseases in cultivated plants. Downy mildew caused by *Plasmopara viticola*, which occurs throughout the world, is one of the most destructive of all grapevine diseases. Cu-based fungicides are used to control grapevine diseases even in organic vineyards. Their use had a worldwide development after the accidental

discovery of a Bordeaux mixture in the 1880s', when the winegrowers of this region, using a mixture of Cu, sulphate and lime to avoid people to pick up and eating these grapes. Due to this practice, a French scientist called Millardet noted these covered grapes did not present a downy mildew damage. By 1885, Millardet completed experiments, that confirmed the capability of this mixture to control this disease at a relatively low cost. Therefore, the Bordeaux mixture became the first fungicide to be used on a large scale, worldwide level [1].

Cu is an essential element for plant growth occurring naturally in soils in concentrations between 5 and 30 mg kg^{-1}, although exceptionally in soils developed on some type of basic parent material may reach values between 100 and 250 mg kg^{-1} [2, 3]. However, the historical use of Cu based-fungicides in vineyards leads to important increases of Cu concentrations in soils, because due to its low mobility it tends to accumulate in the upper soil layers, after rainfall removal from the vines, deposition of the senescent leaves or accidental spills [4]. Thus, in vineyard's soils in Europe is possible to find Cu concentrations higher than 100 or even 200 mg kg^{-1}, while in subtropical areas of Brazil values higher than 1000 mg kg^{-1} were already found [5].

In 2018, a new publication of JRC [6] that maps Cu concentration in European Union topsoils, finds that vineyards have almost three times the average soil Cu concentration (49.26 mg/kg compared to the overall average of 16.85 mg/kg), followed by olive groves (33.49 mg/kg) and orchards (27.32 mg/kg). However, Cu distribution in the soil is strongly influenced by climate and topsoil properties. The climate will affect the number of treatments and leaching of Cu into soils, whereas soil properties have a strong influence on its behavior in this matrix [3, 4]. Once in soils, Cu is strongly complexed or sorbed by OM, oxides of Fe and Mn and clay minerals, whereas low pH values tend to promote its mobilization [3, 5].

The continuous increase of Cu concentrations in soils devoted to vineyards cause an increasing concern because high concentrations of Cu in soils may cause negative impacts on soils-organism functions and diversity, and also on vineyards surrounding ecosystems. Indeed, environmental values of Cu commonly found in soils under inputs of Cu-based fungicides are shown to be toxic not only to non-target soil organisms like worms and microbial communities but also to aquatic organisms such as *Vibrio fischeri* and *Daphnia magna* [3]. Values ranging between 26.3 and 31.8 mg·Cu $kg^{-1}$of soil, which are lower than for example the mean Cu concentration found in European vineyard soils, has been proposed to guarantee the protection of terrestrial elements and ecosystems functioning [7]. Nevertheless, when assessing the toxicity of Cu and its impacts on the environment, not only total concentrations in soils should be considered, but also its bioavailability and mobility, which are both strongly affected by the soil properties and aging processes [3]. The toxicity of Cu is also dependent on the chemical species present in soil solution (i.e. free and complexed) [3, 5]. The mobility of Cu influences its ability to migrate through the soil profile up to other environmental compartments, for example, reaching water masses more easily [3].

Due to the environmental problems related to the accumulation of Cu in soils and potential contamination of the aquatic environment, since 2007[1], Cu use has been limited by European regulation, being a little controversial with principles of organic farming. Furthermore, the EU regulate by laws[2] the list of approved active substances and its potential risks for protection of water and non-target organisms concerning countries to realize e.g. buffer zones to these identified risks

[1] Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products.

[2] REGULATION (EU) No 540/2011. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0540&from=EN.

and risk mitigation measures where appropriate. In the past, regular inputs of Cu up to 30 kg·ha^{-1} (per every 5 years) were frequently attained and allowed. After each application, the residue is typically accumulated in the upper 15 cm of soil, given the high affinity of Cu with the soil organic matter (SOM), that contains several reactive groups, like carboxylic and phenolic groups, which can complex Cu cations, after deprotonation, reducing its mobility in soils [8].

Not only in Europe, in California, but there were also some studies which have shown that there was an increase in the use of Cu in vineyards, caused an accumulation in soils from 6 to 9 kg·ha^{-1} [9] during the last years of the 90s'.

Nowadays, and after recognizing the risks of copper accumulation in soils, the use of Cu in the European vineyard is limited to a maximum of 28 kg Cu·ha^{-1} and over 7 years[3]. This limit is usually applied to organic farming, whilst for conventional viticulture, there are alternative plant protection products available resulting in much lower Cu quantities. Some countries e.g. Germany and Austria had more strict limits (3 kg·yr.$^{-1}$·ha^{-1}) when necessary. Private organic organizations, like. Biodynamic growers with Demeter certification[4] and other biodynamic groups as ECOVIN, Bioland, Natruland, Bio-Austria, etc. can only use a maximum of 3 kg·yr.$^{-1}$·ha^{-1}. In France, the national legally allowed application rate of Cu is 6 kg·yr.$^{-1}$·ha^{-1} with flexible mechanisms (30 kg·yr.$^{-1}$·ha^{-1}) for organic agriculture. Furthermore, the France Minister of Agriculture and Food launched a national program "Ecophyto[5]" aimed at reducing the use of pesticides in agriculture.

Other standards like Slovenian or the Australian and New Zealand guidelines, focus on risk assessment of contaminated sites and give support decisions about remediation measures. In general, where total Cu concentrations in soil exceeding 60 mg·kg^{-1}, sites require environmental investigations [10, 11].

Despite the efforts for reducing the use of copper, the situation is challenging for organic agriculture for which synthetic active substances cannot be part of the solution.

2. Possible different alternatives and approaches to the use of Cu

2.1 Animal origins

Chitoplant©, Enzicur© and other extracts from animal origin (*Lumbricus humus*, propolis, milk protein and hydrolyzed proteins) have been proposed to reduce downy mildew symptoms [12], as they can form semipermeable films protecting plant tissues and stimulating plant's defense mechanisms.

Chitosan hydrochloride is a kind of resistance promoter that enhances plant protection against pathogenic infections. It has proven effects against bacteria and fungi (such as *P. viticola*), and it was approved for use in agriculture as a plant protection product by European Commission[6] [13].

However, their impacts on grapes and must quality have to be carefully assessed, as some studies point to negative effects. Garde-Cérdan et al. [14]. observed that both copper hydroxide and chitosan applications to the grapevines decreased the

[3] REGULATION (EU) 2018/1981 of 13 December 2018: total application of maximum 28 kg of Cu per hectare over a period of 7 years; Member States may in particular decide to set a maximum annual application rate not exceeding 4 kg/ha of Cu; expiration of approval: 31 December, 2025.

[4] https://www.demeter.net/wp-content/uploads/2021/04/20201204_bfdi_standard_for2021_final_sc.pdf

[5] https://agriculture.gouv.fr/le-plan-ecophyto-quest-ce-que-cest

[6] Regulation (EU) number 563/2014 of 23 May 2014, following Regulation (EC) number 1107/2009 of the European Parliament and Council. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R0563&from=EN

concentrations of all amino acids in must, except for Lys, and only when chitosan is applied alone. Romanazzi et al., [13], also recorded lower net photosynthesis, stomatal conductance, leaf area, and weight of leaves and pruned branches, as a consequence of chitosan treatments. The authors concluded that these side effects may be very risky for obtaining high berry quality.

Lactoperoxidase system (Enzicur©) is a natural anti-microbial system usually employed in the control of powdery mildew in various crops[7]. The product is based on naturally occurring salts (potassium iodide and potassium thiocyanate) and the lactoperoxidase system, active in different animals including in the bovine liver. Enzymes (lactoperoxidase) and substrates. The LP-system is a non-immune defense system, that promotes the formation of reactive oxygen species that inactivate microorganisms by protein's peroxidation.

2.2 Biocontrol agents (BCAs)

Bacillus subtilis (Serenade Max©) and *Trichoderma harzianum* (Trichodex©) has been found as promising candidates for replacing Cu as a biocontrol agent for protecting against downy mildew [12], and other fungi diseases.

Among some tested antagonists, the highest efficiency was observed for *Trichoderma harzianum*-based products. Its efficiency was significantly higher in the treated plot when compared with untreated one but decreased just before harvest. However, this *Trichoderma harzianum*-based product did not provide a level of *P. viticola* control similar to Cu in some trials [15]. Despite the positive results found in some experimental studies, it was realized that the ability of *Thricoderma* (T39) to induce resistance depends on grapevine cultivars. Thus, it is necessary to understand which are the molecular components and signaling pathways modulating the response to this resistance inducer to apply this biocontrol to the most responsive cultivars, enhancing the benefits of this biocontrol treatment [16].

Other results [17] showed the relevance of environmental conditions on BCAs activity (four-year trial). Prevention of fungal sporous germination at least in some years could means an interaction between the pathogen and the microorganism that can lead to a reduction of severity of primary foci.

On other hand, *Bacillus* and *Trichoderma* strains have a great ability to produce a wide range of active molecules with broad effects on the control of different grapevine diseases, by preventively inducing plants systemic resistance or inhibiting other fungi diseases development.

These works show that microorganisms could be a promising tool to reach a reduction of primary inoculum and thus contribute to a low impact and sustainable agriculture.

2.3 Cultural practices

In the case of an epidemic disease like the downy mildew, combat strategies relied only on chemical control and its optimization. Sanitation measures targeting to reduce the overwintering inoculum and therefore, to reduce early and linearly the primary infection, and regulation of the crop load are a good management strategy [18].

Another relevant and additional strategy is the strict regulation of Cu spray rates. In the field, rates between 200 and 400 g Cu·ha^{-1} (equivalent to 5 and 10 mg Cu·m-^{2}, respectively) was able to significantly reduce downy mildew (72–89% efficacy). These confirmed results (previously obtained from leaf disks assays in the lab), provided sufficient control, although it depends on the infection pressure [19].

[7] https://www.koppert.mx/enzicur/

Forecasting models linked to Cu applications could be an interesting approach. **Coptimizer**[8] is a model-driven decision support system designed to help growers to optimize and track the use of Cu-based fungicides against grapevine downy mildew in European organic viticulture. Results showed that by using Coptimizer (including historical data and several experiments under field conditions), growers could be able to maintain the same level of protection applying only half the amount of the fungicide [20].

An innovative cultural practice has been recently tested consisting in the application of different cover crops mixtures to interfere with the dispersal of the soil-transient pathogen, such as *P. viticola* [21]. Fall sowing of cover crops allowed to have enough vegetation in spring, during the most relevant period of downy mildew primary infections, to delay the onset of first disease symptoms and reduce the final incidence of the epidemic. This cultural practice can result in a final saving in treatment numbers as well as a reduced amount of copper used during the first seasonal treatments.

In summary, when *P, viticola* pressure is low to intermediate, a reduction in the sprayed Cu quantity provides the same efficiency as standard strategies and allows to decrease two-fold to three-fold the sprayed Cu quantity [15].

2.4 Inorganic materials

Some inorganic salts have shown promising results under controlled conditions (greenhouse and potted plants) like potassium bicarbonates (Armicarb© and SaluKarb©); K-P product based on betaine, carbohydrates and amino acids (Gro-stim©); N-K products with oligosaccharide and glutathione (Kendal©) or Aluminum oxide and silicon oxide with S (Ulmasud©) showed to be as effective as Cu hydroxide treatment. However, in field trials, only the potassium bicarbonate (Armicarb©) provided control of infection on bunches greater than 60% [17].

2.5 Microbial and plant product extracts or derivates

Under controlled conditions (greenhouse and potted plants), some microbial extracts have shown a good efficacy to control downy mildew [17]. Extracts from inactivated *Pseudomonas aureofaciens* (Agat 25 K© and Diamant©) were an effective treatments at concentrations above 10%. This product was effective in field trials, providing control of infection on bunches greater than 60%.

Many plants' oils or water and alcohol extracts showed reduce downy mildew expression compared with the untreated control [12, 17], under controlled conditions (greenhouse and potted plants):

- Siva 50©, and Tecnobiol© (fatty acid-based products like gibberellic acid-GBA plant wash soap), significantly reduced downy mildew expression.

- Penergetic-p liquid© (cane sugar) and Phyto-Vital© (lignin derivate) were the only natural derivative treatments that showed the same effectiveness as Cu hydroxide.

Therefore, plant and other extract products isolated used without Cu can reduce their efficacy when *P. viticola* cause a high pressure in the vineyards.

Hedera helix (leaves in water), *Quercus spec*. (bark in alcohol), *Primula veris* (roots in water), *Rhamnus frangula* (roots in alcohol), *Solidago spec*. (leaves in

[8] https://www.haifaup.co.il/startup/coptimizer

alcohol), *Salix spec*. (bark in water) showed promising effects in the laboratory [22], and these effects increased with the concentration of plant material used to obtain the extract. Extracts from *Rhamnus* and *Primula* had significant effects, reducing disease severity by 30–35% if applied after infection.

In field trials, some of the extracts, such as those from *Chenopodium quinoa*; *Inula viscosa*; *Melaleuca alternifolia* (Timorex©); *Salix alba*; *Solidago virgaurea and Salvia officinalis* provided more than 60% of control of bunches infection [17].

In general, preventive effects were much better in lab conditions (70–90% reduction of disease severity) than the results in field experiments (34–40% disease reduction) for the species tested [22]. In particular, *Yucca schidigera* (Norponin BS© liquid and Saponin©) has been also found as some of the most promising candidates for replacing Cu, because it provided more than 60% control of leaves and bunches infection [17]. However, some variability in *Yucca* extract efficiency under a low *P. viticola* pressure was already observed in some studies [15].

Trials with potted plants showed that *Salix* extract is a promising alternative to Cu, with no risk for the development of *P. viticola* resistant strains. Salix extract was as efficient, being the 4th day between elicitation and inoculation the appropriate moment to control the disease. Nevertheless, its action is strictly preventive and *Salix* extract should be applied before rainfall splash dispersion of fungi, which are impossible to forecast and in case of strong pressure this protection could be insufficient [23].

Therefore, available results also showed that the use of plant extracts (alone or in combinations among them) can reduce the doses of Cu and should be tested in future as a real alternative.

2.6 Synthetic materials

Under high *P. viticola* pressure, Cu-based treatments and potassium phosphonate (PP) are the most efficient products to control downy mildew. Beta-aminobutyric acid (BABA), benzothiadiazole, and high levels of polyoxyethylene sorbitan monooleate (Tween 80©) were as effective as the Cu hydroxide treatments in indoor trials [17], but no relevant effects were recorded in field trials.

Clay-based treatments such as Mycosin© are promising alternatives, giving in some trials a level of protection higher than 60% in leaves and bunches [15], but it is important to understand the impact of Al cations provided by this product. However, under a high disease pressure, the efficiency of these clay-based products is low for commercial vineyard protection.

Some vineyards trials in Germany and Austria showed that PP has a direct effect on *P. viticola*, and in addition, it activates the plant's defense mechanism (EFSA 2012[9]) which is one of the basic principles of organic plant protection, as stated in the European Organic Regulation[10]. PP is absorbed by the plant and systemically distributed. Due to the distribution through the plant and the resistance-inducing effect, this substance particularly protects newly grown leaves and shoots. It also reaches the pathogens that have already penetrated the leaves. Apart from the protective effects, the substance also has a curative effect during the first days of infection and incubation (approx. 25% of elapsed incubation time).

PP was used in organic viticulture in a few countries as a plant strengthener until 2014. When used until the end of the flowering period, it showed great support of Cu products in protection against *P. viticola* under high infection pressure.

[9] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2012.2963

[10] EU No. 834/2007

Efficacy of PP, stone meal as well as new Cu formulations, has been recorded as good reference treatments (Folpan 80 WDG©, a.i. folpet© and "organic standard" mixture of Cu, sulfur and stone meal), when the *P. viticola*. The pressure was low, considering the low amount of total Cu applied (less than 2 kg/ha), the results were promising [24]. Moreover, the use of PP as a plant protection product in organic vineyards contributes to a Cu use reduction to levels <3 kg of pure $Cu{\cdot}ha^{-1}{\cdot}yr.^{-1}$, and it has been a practice adopted in Germany and Austria. Therefore, PP can be considered in Cu-reducing strategies.

However, PP were registered as plant protection agents in the EU and therefore, not listed or allowed to use in organic viticulture. This led to big problems in years with high infection pressure in different regions all over Europe (like in 2016).

2.7 Other or new Cu formulations

New Cu formulations available in the market showed efficacy similar to Cu hydroxide, however, are not efficient at low concentrations. Cu is a preventive fungicide allowed in organic agriculture that is active only in tissues where is applied (i.e. it is a non-systemic substance), so plant growth results in unprotected tissues. In areas where disease incidence is high, weekly Cu applications are made by growers increasing the risk of exceeding the fixed threshold.

Some low Cu formulations were able to control grape downy mildew in the field using a third (Glutex Cu 90©) or a sixth (Labicuper©) of the amount of Cu in comparison with the Cu hydroxide [25].

Cu gluconate (containing 8% of Cu^{2+}) showed efficacy comparable to Cu hydroxide (containing 35% of Cu^{2+}) in vineyard trials for managing downy mildew [26]. Acylbenzolar-s methyl (Bion 50 WG©) also confirmed its efficacy in vineyard trials.

Several new tested Cu formulations or mixtures provided effective disease control, but their efficacy levels decreased when lower rates of Cu^{2+} were used, and this pattern was similar for different formulations. Nevertheless, some general conclusions should be mentioned:

- The level of downy mildew control decreases negatively and logarithmically with to Cu levels.
- There is a threshold of Cu necessary for effective control of downy mildew.
- Higher concentrations of Cu (> 0.6 $g{\cdot}l^{-1}$) do not increase the efficiency of the treatment.

2.8 Technosoils or recovering soils. Measures to minimize the negative impacts of Cu in soils

The use of amendments is a promising strategy for recovering soils. The use of limestone is an effective strategy to reduce Cu availability and phytotoxicity that has been used for many years [27–29]. Limestone promotes the increase in soil pH, causing deprotonation of acidic functional groups of reactive soil particles. This increases cation exchange capacity (CEC) and Cu adsorption, decreasing bioavailability and potential uptake by plants. Grapevines grown in soil treated with limestone showed increased growth, dry matter yield and photosynthetic efficiency in young grapevines in parallel with a lowest Cu concentration in root tissues.

Also, compost and biochar could help in slightly moderate acidic soils, with some positive effects of Cu^{2+} reductions by liming. In general, organic soil

amendments could achieve similar effects of Cu^{2+} reduction than liming, but they might be more valuable because of their beneficial effects on physicochemical soil characteristics and decreased risk of soil erosion. Therefore, compost and biochar are promising solutions because usually are non-expensive treatments and, biochar go beyond a simple liming effect [30].

Nevertheless, depending on its characteristics, the addition of organic amendments can result in the opposite effect (mobilization of Cu due to its complexation with low molecular weight and soluble organic compounds) [27]. Thus, the use of this agronomic practice must be evaluated case by case to not deteriorate the already altered soil conditions. In its turn, biochar can overcome this problem due to its different mechanisms of Cu complexation. Also, the application of treated coal fly ash can be a solution, especially if mixing with compost, overcoming the potential problems of Cu leaching and availability that may arise from the application of the compost alone.

Pyoverdine (Pvd) is a bacterial siderophore produced by some *Pseudomonas* species that can bind Cu in addition to iron in the soil. Pvd is expected to alter the dynamics and the ecotoxicity of Cu in vineyard soils. Cu phytoavailability depends to a great extent on Cu complexation in soil pore water, the latter being highly sensitive to pH: vineyard topsoils with pH ranging from 5.9 to 8.6 can present Cu mobility differences of six times and, a Cu phytoavailability differing by a factor of 5000 among them. The Pvd action depends on Fe soil availability, the soil composition (e.g. carbonate soils more easily mobilized Cu) and other factors [28].

Besides, many several bacterial strains can hyper-accumulate and/or sequestrate Cu [27].

Another example is the mutualistic association between arbuscular mycorrhizal fungi (AMF) and plant roots that can minimize the toxic effects of Cu in plants, due to the complexation of this element with organic substance produced and released by them. Also, AMF can store Cu in cellular compartments such as vesicles and spores [27].

2.9 Modeling downy mildew

In the last decades, many epidemiological models have been elaborated to better manage fungicide application schedules. The correlations among environmental factors, host susceptibility and the pathogen have been well known for a long time: the so-called 3–10 rule (3 days under 10 mm or more effective precipitation) was the first attempt to predict primary infections of *P. viticola* [31]. Similar models have been developed in France [32, 33], Germany [34], USA [35, 36], and Australia [37, 38]. Unfortunately, they often fail to predict the real development of epidemics and their practical use is restricted [39]. Empirical models have shown some critical restrictions and limitations being too simple, due to the lack of robust cause-effect relationships in many model equations and therefore, requiring some corrections and calibrations to adapt to grape-growing areas or environmental conditions different from those used for the model development [40].

A mechanistic dynamic model was recently elaborated in Italy [41], which accounts for the biological effects of weather on the different stages of the primary infection chain, from the progressive breaking of dormancy in the overwintering oospore population to infection establishment during the grapevine-growing season. The model of Rossi et al. [42] was evaluated in more than 100 vineyards in Italy (from 1995 to 2007) as well as in the environmental conditions in the province of Quebec, Eastern Canada, by comparing the time of first lesion occurrence predicted by the model with field observations [42, 43]. This model always showed very high accuracy [44] and when used to schedule fungicide application against

downy mildew, allowed a reduction from 50 to 66% in fungicide applications, corresponding to an average saving of 174 and 224 €·ha^{-1}, respectively [42]. Finally, it was integrated into a DSS named vite.net® [45].

Moreover, Caffi et al. [46] developed a weather-driven model to predict *P. viticola* population dynamics on grape leaf surfaces during a discrete wet period. The authors positively correlated the post-inoculation efficacy of two cooper fungicides with the proportion of *P. viticola* sporangia on a leaf that had not yet caused the infection. Model simulations suggested that the efficacy of a copper treatment increased when the environmental conditions were less conducive for disease development. Therefore, this model can be used to predict whether a fungicide application during a discrete infection period will be effective [42].

2.10 Decision support system

To help growers optimize the scheduling and dosages of fungicides against downy mildew, decision support systems were developed based on weather data, disease risk, and plant growth [45, 47].

The DSS vite.net® is an Internet-based platform for sustainable vineyard management [41] that has two main components: (i) an integrated system for real-time monitoring of vineyard data, and (ii) a web-based tool that analyses data by using mechanistic and, dynamic models that can predict grapevine growth, risk of disease infection, and residual protection by the last fungicide application. Each of these models has been published and their accuracy validated [45–50].

The combination of site-specific weather data, monitoring reports and advice from a DSS enables growers to protect their vineyards by modulating the frequency and timing of copper applications, based on disease risk [51].

The DSS vite.net® was tested in 21 organic farms and allowed the reduction of copper applications by an average of 24%, and the total amount of copper applied by 37% compared to a calendar-scheduling of copper application that provided the same level of protection in organic vineyards, with an average saving of 195 €·ha^{-1}·year^{-1} compared to the common farm practice [52].

3. International legislation for PPPs application in vineyards

Regarding the international legislation, the aim of reducing pesticides in viticulture has been addressed by European and international bodies and organizations.

The International Organization of Vine and Wine (OIV) is an intergovernmental organization established under the Agreement of 3 of April 2001, which is directly related to a previous agreement (OIV Treaty, 1924) made for the creation in Paris of an International Wine Office.

OIV is an intergovernmental organization (47 countries), comprising scientific and technical knowledge in grapevines, wine and wine-based beverages, table grapes, dried grapes, and other vine-based products, with an international reputation and generally recognized competencies. OIV countries represent more than 80% of total world wine production, and, being present in main continents worldwide.

The principal objective of OIV is to contribute to the international harmonization of existing practices and standards and, if needed, to draft new international standards for grapevine and wine products. OIV is also cooperating strongly with international organizations intergovernmental or non-intergovernmental like *Codex Alimentarius* or World Health Organization (WHO) among others.

Under a proposal from one of its group of experts (Vine protection and viticulture techniques "PROTEC"), OIV wanted to suggest some recommendations or good practices for minimizing the impacts associated with the application of plant protection products (PPPs) in vineyards.

A questionnaire was launched between 2014 and 2015 to its Member States and, answers showed some relevant results. For example, all of them have an Official List for prohibited and allowed products for grapevine protection and almost all of them (90%), has an official methodology about applications limits [53].

This new resolution (VITI 592–2018[11]) includes some relevant points above described:

1. Methodology. Recommendations for the application of the PPP should be established based on the different factors that may help to determine the optimum volume of application (key factor, but not only) like Phenological stages of grapevines; Leaf area development; Varietal susceptibility to diseases suppressed; Climate and soil conditions; Training and trellising system; etc.

2. Products. Methods should define a specific limit for each product referring to the range among the treatments or doses used for it. It recommends undertaking (before its authorization) field trials and external audits given by official national departments or independent competent bodies. Pathogen resistance should be considered, and the product should be specific as possible for the intended target pest organism.

3. Doses. Quantities of PPP per hectare and treatment must be determined based on the volume or surface to be targeted or treated. Two models are strongly recommended: Tree Row Volume (TRV) or Leaf Wall Area (LWA) (Annex I).

4. Machinery for PPPs applications. General recommendations about the use of most efficient and environmentally friendly technologies for the vineyard treatments, like spraying or air-assisted sprayer techniques combined with injection nozzles or techniques which allow a homogenous application side by side and if possible, its recycling systems too (panels or other recovery systems). Calibrating procedures will be essential for the right dose rate adjustment. Drift Reduction Technology should be also encouraged.

5. Handling of plant protection products, training programs and national PPPs Plan should be drafted as guidelines for each member state.

The resolution was completed with five annexes with most used models, decision support systems (DSS), conversion factors and an official list from departments and websites related to PPPs national rules and recommendations.

Talking about the EU framework[12], some regulations should be considered, especially for organic production. As mentioned before, the rules for the implementation of organic production and labelling of organic products and control, describes quite well in article 5 and its Annex II. Pesticides — plant protection products, the use of Cu as fungicide up to 6 kg Cu per ha per year. For perennial crops, Member States may provide that the 6 kg Cu limit can be exceeded in a year provided that the average quantity actually used over 5 years consisting of that year and the four preceding years does not exceed 6 kg (it means 30 kg·ha^{-1} for 5 years

[11] https://www.oiv.int/public/medias/6450/oiv-viti-592-2018-en.pdf

[12] EC N° 834/2007

limitation). Cu can be applied under the form of Cu hydroxide, Cu oxychloride, (tribasic), Cu sulphate, cuprous oxide, Cu octanoate.

Recently, this limit was revised (based on some EFSA reports) and consequently, Cu compounds were designated as candidate substances for substitution and reduced applications, restricting the use of plant protection products containing Cu compounds to a maximum application rate of 28 kg/ha of Cu over 7 years (i.e. on average 4 $kg{\cdot}ha^{-1}{\cdot}year^{-1}$). This is described in clause 15 in the first statement (EC N° 1981/2018) and it has two annexes with the use and forms of Cu and their specific provisions. This regulation shall be applied until 2025 or previous revision.

It also is remarked that Cu sulphate was authorized in organic wine production until 31 July 2015 (EC N° 203/2012).

Therefore, within this framework, the research focused on real alternatives to reduce or substitute the Cu products, with other active principles or compounds for controlling the pest and diseases in grapevines are a key challenge for the sustainability of the wine sector.

4. Cutting edge lines from R&D ongoing projects developed by the wine sector

The Framework Programmes for Research and Technological Development, also called Framework Programmes (FPs), are funding programmes established by European Commission to support and promote research in the European Research Area (ERA). Since 1984, European Community research and technological development activities have been defined, implemented and founded by a series of multi-annual FPs (**Figure 1**)[13], getting close to €100 billion for the new Horizon Europe (2021–2027) and the Euratom Research and Training Programme.

Soil degradation is a global problem, often caused by several factors: unsustainable management and agricultural overexploitation practices, climate change, pollution, and deforestation. Soil degradation may intensify the impacts of natural disasters and contributes to social issues, (e.g. depopulation or migrations). The EU suffers from different levels of land degradation, and thirteen EU Member States have declared themselves as affected Parties under the United Nations Convention to Combat Desertification (UNCCD). The EU itself is one of the signatory members since 1998. Unfortunately, recently published studies and expert's opinions, released by the European Environment Agency's 2020 State of the Environment Report, the Special IPCC report on Climate Change and Land and the IPBES Assessment Report on Land Degradation and Restoration demonstrated that during the last years soils have been degraded dramatically at European and global level. In response, in May 2021 the EU announced a new Biodiversity Strategy for 2030. It adopts a comprehensive, ambitious, long-term plan for protecting nature and reversing the degradation of ecosystems, including a whole section dedicated to the soil.

It is expected that this new strategy will deliver a powerful tool to raise awareness on the importance of soils, engage citizens, create knowledge, and develop solutions for restoring soil's health and functions. Research and innovation are crucial to better understand, monitor and measure the specific effects of agricultural and forestry activities on soils and ecosystems functions. Transfer of knowledge and know-how are required to improve soil biological, chemical, and physical properties. Outstanding and breakthrough ideas are essential for achieving the objectives

[13] https://ec.europa.eu/info/sites/default/files/budget-may2018-research-innovation_en.pdf

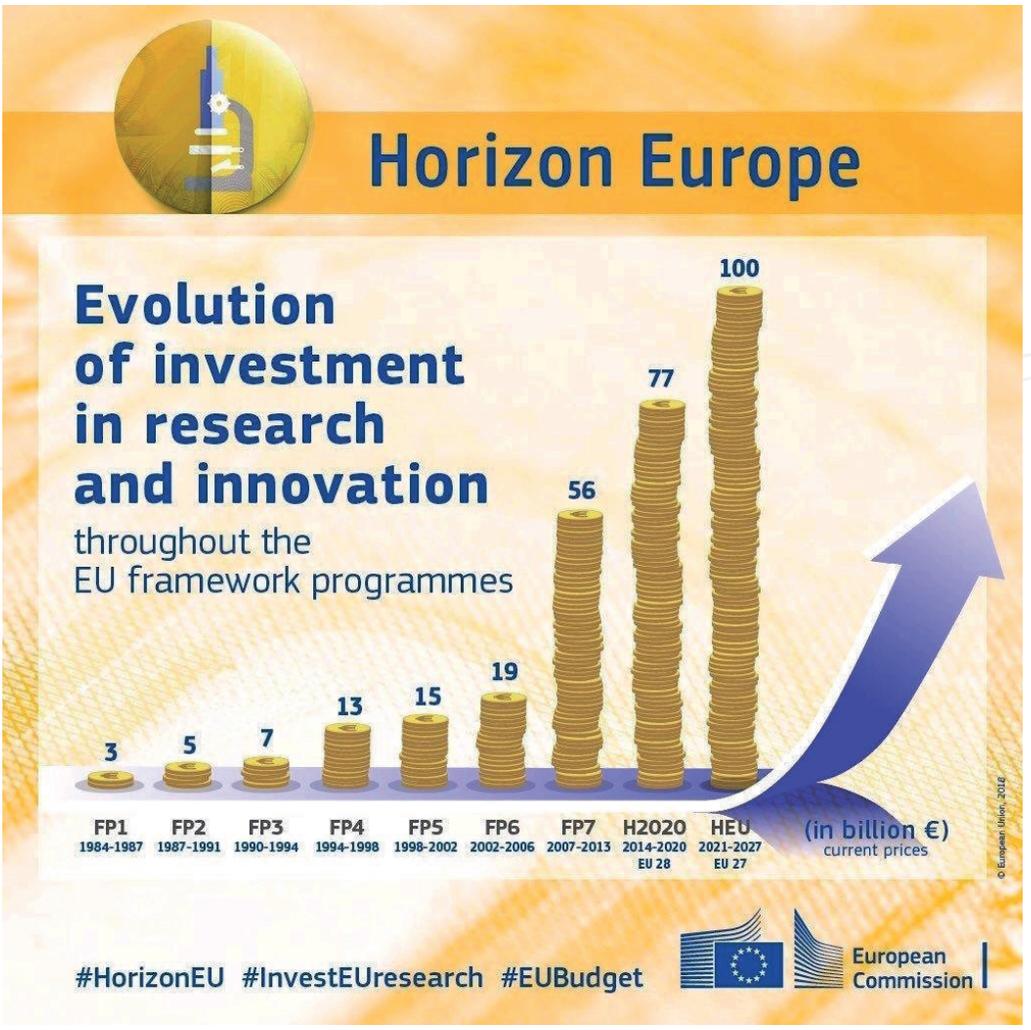


Figure 1.
EU framework programmes budget evolution.

of the European Green Deal, which is a set of policy initiatives of the European Commission with the overarching aim of making Europe climate neutral by 2050.

Horizon Europe is presently, the European Union's flagship Research and Innovation programme, part of the EU-long-term Multiannual Financial Framework with a budget of €95,5bn to spend over seven years (2021–2027). Previously, technological development and innovation in ERA have been carried out under the scope of project calls launched during the period 2014–2020 in the frame of Horizon 2020 (H2020). Indeed, one of the identified challenges of this H2020 program was named: "Food Security, Sustainable Agriculture and Forestry, Marine, Maritime and Inland Water Research and Bioeconomy". To achieve the objectives highlighted in this challenge, the European Commission, provided a budget of around 3.7 billion euros, out of which at least 1.5 billion euros were dedicated to carrying out research projects in agriculture and forestry.

Besides, during the H2020 8FP soils were the target of increasing political attention at European and global levels. The United Nations declared 2015 as the International Year of Soils, while the International Union of Soil Sciences at the Vienna Soil Declaration on Dec. 7th of 2015 proclaimed that 2015–2024 would be the International Decade of Soils.

In this context, and due to the serious environmental problems caused by the continuous use of Cu-derived phytosanitary products for decades, several projects to decrease/substitute Cu use in agriculture, have been granted within 7FP or 8FT (H2020).

Project acronym	Project title	Project duration	Project budget
COPPEREPLACE	Development and integral implementation of new technologies, products, and strategies to reduce the application of Cu in vineyards and remedy of contaminated soils in the SUDOE region	2020–2023	€ 1.638.340,72
NOVATERRA	Integrated novel strategies for reducing the use and impact of pesticides, towards sustainable Mediterranean vineyards and olive groves	2020–2024	€ 5.507.110,20
RELACS	REpLAcement of Contentious inputs in organic farming Systems	2018–2022	€ 3.999.675
BioAvenger	Biofungicide saves plants from fungal attacks.	2019–2019	€ 71.429
ProEcoWine	Development of a process to generate a novel plant protection product enriched with micronutrients to replace Cu in organic viticulture	2012–2014	€ 1.579.149,71
MicroWine	Microbial metagenomics and the modern wine industry	2015–2018	€ 3.945.597,12
DROPSA	Strategies to develop effective, innovative, and practical approaches to protect major European fruit crops from pests and pathogens	2014–2018	€ 8.602.632,24
WILDWINE	Multi-strain indigenous Yeast and Bacterial starters for 'Wild-ferment' Wine production	2012–2015	€ 1.592.302,40
CO-FREE	Innovative strategies for Cu-free low input and organic farming systems	2012–2016	€ 3.994.513,60
INNOVINE	Combining innovation in vineyard management and genetic diversity for a sustainable European viticulture	2013–2016	€ 8.489.665

Table 1.
Projects funded by European Commission aimed at promoting organic agriculture.

Table 1 highlights some of them, as well as their executions, which started in 2012. Besides the Framework Programmes, European Union (EU) has other instruments to fund projects with high impact on Regional development like Interreg programme, which supports cooperation across borders through project funding. The main aim of Interreg is to jointly tackle common challenges and find shared solutions in fields such as health, environment, research, education, transport, sustainable energy and more.

COPPEREPLACE [54] project, co-founded by the Interreg SUDOE programme, aims to develop and validate a series of integrated, innovative, and viable solutions to reduce the use of Cu and its environmental impact in vineyards. The solutions promoted within the project will be transferable and durable to allow the wine sector complies with the new European legislation and to promote environmentally sustainable production. **COPPEREPLACE** is led by the Wine Technology Platform (PTV) and has an international consortium comprised of Spanish, French and Portuguese entities: the Associaçao para o Desenvolvimento da Viticultura Duriense (ADVID), Institut Français de la Vigne et du Vin (IFV), Sogrape Vinhos, Centro de Valorización Ambiental del Norte (CVAN), Vignerons Bio

Nouvelle-Aquitaine (SVBNA), Eurecat, Família Torres, University of Porto and its Sustainable Agrifood Research Centre-GreenUPorto (Portugal), University of Vigo and Polytechnic University of Catalonia (Spain), LBS (Gérard Bertrand) and Jean Leon. In addition, the consortium has the support of Artica Ingeniería y Innovación (artica+i) consultancy. **COPPEREPLACE** will create a network of stakeholders that includes wine growers and other representatives of the international grape and wine-growing sector.

NOVATERRA project, funded by EC H2020 [55] with the main objective of reduction of the use and impact of pesticides used in Mediterranean vineyards and olive groves, while maintaining sustainable yields and quality of final products. Three are the pillars to achieve the goals: new natural plant protection products, smart farming techniques, (which include optimized spray applications, early detection of symptoms, decision support systems, and robotics) and soil management practices, enhancing functional biodiversity. These three pillars are being tested and analyzed in case of studies through Greece, Italy, France, Spain, and Portugal, under different conditions. Results will be analyzed by cost–benefit and impact analysis, final users' acceptance and adoption, consumers' willingness to pay, and validated by multidisciplinary stakeholders. Finally, new Integrated Pest Management strategies will be designed and disseminated aiming to reduce the environmental and health-related damages of food production.

The general objective of the H2020 **RELACS** project [56] is to foster development and facilitate the adoption of cost-efficient and environmentally safe tools and technologies, to phase out the dependency on and use of contentious inputs in organic farming systems. It is expected that the know-how generated under **RELACS** project will reduce the use of Cu and mineral oil, manure from conventional farms. As part of project deliverables, reports/technical descriptions defining alternatives to excessive use of anthelmintics in small ruminants, to reduce antibiotic use in dairy cattle, and moderate reliance on synthetic vitamins in cattle and poultry production were planned.

As it was mentioned in the previous sections, agricultural and horticultural industries need a way of dealing with fungal infections in non-chemical ways. The EU-funded **BioAvenger** [57] project begun the development of such an alternative. The project's prototype of the same name is a bio-fungicide for soil treatment. According to the project consortium, **BioAvenger** combated fungal infection in plants in a natural way. The product could be applied as either a cure or a preventative treatment. Obtained results demonstrated that in case the crop plants were sick, use of the treatment improved health within a month. Usual dosing over several months resulted in the eradication of over 90% of the invading fungi and up to 50% more plant growth. Unfortunately, the product is not yet developed or available in the market.

ProEcoWine project [58] set out to develop a novel, nutrient-enriched bio fungicide to combat common grapevine fungal diseases. Project partners successfully cultivated several microalgae species against downy mildew and Botrytis under different conditions. They screened the strains for antifungal activity and identified the two most capable microalgae strains with over 90% fungicide efficiency. The two strains and their antifungal activity were validated in a series of greenhouse and field experiments. The project team developed effective and economically viable methods for high microalgae density growth. They scaled up the production, processing, and storage of microalgae formulations for application as a fungicide. Researchers evaluated downstream methods required to activate microalgae antifungal activity to determine the most cost-effective process for product manufacturing. They established the ideal formulation of microalgae concentrate, resulting in products with enhanced

shelf life. It is forecasted that thanks to **ProEcoWine**, the innovative microalgae plant protection product will increase vineyard productivity by up to 30%, and decrease production costs per unit by up to 20%. This in turn will increase the competitiveness of EU wines and support the development of organic markets. The antifungal activity of the developed products was monitored and showed that the **ProEcoWine** products fully inhibited the presence of pathogens and had no adverse effect on plants (phytotoxicity).

The **MicroWine** [59] network was created to train a new generation of researchers with the aim to develop tools and gather knowledge for a modern DNA-based approach to European winemaking. It is expected that specialized scientists will transfer their knowledge to decrease the amount of Cu used in agriculture.

Investigations carried out under this project allowed uncovering microbial contributions to several phases of winemaking, from microbial influence on plant health to the microbial role in fermentation processes and influence on wine aroma and sensory perception and, seasonal microbial dynamics on grapevine leaves under biocontrol and Cu fungicide treatments [60].

The aims of the **DROPSA** [61] project was to developing reliable, robust, and cost-effective approaches to protect the major European fruit crops from *Drosophila suzukii*, and quarantine pathogens *Pseudomonas syringae pv. actinidiae* (Psa), *Xanthomonas fragariae* (Xf) and *Xanthomona arboricola pv. pruni* (Xap). They are identified as major phytosanitary risks and pose significant challenges to fruit production. The project consortium reported that pests and pathogens cause losses to the EU fruit industry of €10 billion and 3 million tons of produce. **DROPSA** addressed Cu problems advancing options beyond those currently available in the market according to secure food production lines in the EU.

From Greece and Spain to Germany and Romania, Europe already enjoys a strong winemaking tradition with a remarkable variety of flavors and bouquets. Nevertheless, modern winemakers generally use commercially available yeast and lactic acid bacteria (LAB) starter kits, leading to more homogenous European wines. One way to return to regionally distinct wines is by using locally occurring yeast and LAB species to create 'wild-ferment' terroir wines. With this in mind, the **WILDWINE** [62] project investigated regional microbial diversity to develop original starter cultures that can be used to make such unique wines. During the project, scientists analyzed several dozens or hundreds of *Saccharomyces* and *non-Saccharomyces* strains and a few tens of *Oenococcus* and non-*Oenococcus* bacteria. One of the project objectives was to investigate how the presence of Cu could influence fermentation processes.

The **CO-FREE** [63] project aimed to develop innovative methods, tools, and concepts for the replacement of Cu in European organic agriculture (grapevine, potato and tomato) production systems. The project promotes alternative compounds and, 'smart' application tools for integrating them into traditional and novel Cu-free crop production systems. Some strategies were identified to develop 'smart' breeding goals through crop ideotypes and by, fostering the acceptance of novel disease-resistant cultivars by consumers and retailers. The innovations and production systems were evaluated in a multi-criteria assessment concerning agronomic, ecological, and economic performance. In **CO-FREE** a total of 17 alternative compounds were studied for which modes of action, formulations, and application strategies were explored in the lab and field. As a major success of this project, one active substance was approved and included in the EC regulation 1107/2009, with other five dossiers submitted or being studied due to the efficacy of three additional alternative compounds, but additional R&D is still necessary. Most **CO-FREE** candidates exhibited safe ecotoxicological profiles in detailed studies on non-target organisms (beneficial arthropods, aquatic and soil indicator organisms).

Costs for registration, however, are high and require a substantial initial investment by small or medium enterprises (SMEs). This means that considering that (i) Cu has broad-spectrum activity, (ii) it is unlikely that only one compound isolated will have the potential to completely replace Cu in all crops, (iii) the alternative compounds, at the best, will have similar efficacy as Cu, and (iv) the new compounds have to remain effective over time, several different candidate compounds are likely necessary to further reduce/replace Cu. **CO-FREE** has thus, contributed strongly with several candidate compounds with a technology readiness level of 8, which provided the foundation for the development of new products for the market.

INNOVINE [64] project globally led to a better understanding of the impact of vineyard practices and various abiotic stresses on grapevine physiology and berry composition in the context of climate change. The development of two grapevine models allowed us to simulate and predict those impacts in various climatic scenarios. Further models' implementation had to be addressed, taking into account differential impacts on different genotypes. Methods for screening germplasm for plasticity or for identifying key molecular pathways of adaptation to stress were proposed. Several non-destructive phenotyping tools based on fluorescence, reflectance, thermal imaging and or, hyperspectral imaging were experimented and validated in several work packages of **INNOVINE** to monitor the physiological status of the canopy, as well as the berry content or the onset of downy mildew attacks. Researchers from different scientific areas developed a foreground that allowed them to carry out strategies for sustainable control of diseases in the vineyards. The most important level for the diminution of pesticides was found to be the use of resistant varieties. A very important effort was carried out for the screening of yet uncharacterized germplasm collections for resistance to diseases and was made available through publication in papers and the European Vitis Database. However, it was also shown that the populations of downy and powdery mildews could slowly adapt to resistant varieties and overcome these resistances. The current disease models were improved to consider grapevine physiology and genetic diversity. Finally, **INNOVINE** showed that canopy management practices impact the berry size and therefore, the *Botrytis* incidence.

5. Conclusions

Even if several (R&D) projects have been developed in recent years, replacing or giving alternatives to the use of Cu in viticulture, this problem is still currently unsolved, being one of the most relevant challenge for the wine sustainable production. Before providing or modifying some new standards or rules, results from these projects should be considered to not compromise sustainability targets in viticulture, assess the present risks of using Cu in viticulture and to better support establishing limits for its applications.

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