OIV COLLECTIVE EXPERTISE

FUNCTIONAL BIODIVERSITY IN THE VINEYARD

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SCOPE

This document will present an overview about functional biodiversity in vineyards and try to illustrate major aspects of functional biodiversity in the viticultural sector:

- Why enhancement of biodiversity and on what extent is beneficial for the vineyard?
- Identify and illustrate different ecological infrastructures existing in the vineyards;
- Identify and illustrate major mechanisms of interactions between species or group of species;
- Identify and illustrate how these interactions can be used for the benefit of the vineyard.

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INDEX

1. INTRODUCTION **7**

2. ECOLOGICAL CONCEPTS **9**

2.1 Definition of biodiversity 9
2.2 What is a Functional Biodiversity approach? 10
2.3 Ecological infrastructures: definition and general criteria 12

Definition 12
Which surface of the farmland should be dedicated to EI? 14

2.4 Ecological distances 14
2.5 Functional Units 14

Plant and Soil (micro scale; plot level). 14
Vineyard (meso scale; farm or vineyard level). 15
Landscape (macro or large scale; region level). 16

2.6 IOBC Guidelines for biodiversity and ecological infrastructures 17

3. IMPROVEMENT OF FUNCTIONAL BIODIVERSITY **21**

3.1 Implementation of EI. Conservation actions 21
3.1.1. Green cover crops 23
3.1.2 Hedges and woodlands remnants 26
3.1.3 Dry-stone walls 29
3.2 Sustainable use of PPP 31
3.2.1 Decision Support Systems 32
3.2.2 Selection of low toxicity PPP 32
3.2.3 Mating disruption 33
3.2.4 Microbial biocontrol agents 33
3.2.5 Active ingredients of natural origin 35

4. EVALUATING ECOLOGICAL QUALITY **37**

4.1. Evaluating ecological quality of EI 39
4.2. Evaluating ecological quality of soil 39

5. PLANNING, COSTS, PUBLIC INCENTIVES **41**

6. THREATS TO THE APPLICATION OF FUNCTIONAL BIODIVERSITY APPROACH **42**

REFERENCES **43**

WEBSITES AND INTERNATIONAL PROJECTS **47**

1. INTRODUCTION

For centuries traditional viticulture was part of a multifunctional agricultural system including low-input grasslands and fruit trees resulting in high functional biodiversity (FB). However, in the last decades the vineyard has suffered an intensive management leaded by a high mechanisation (including frequent tilling) and/or use of Plant Protection Products (PPP) in which several ecosystem services are been affected, causing high rates of soil erosion, degradation of soil structure and fertility, contamination of groundwater and high levels of agricultural inputs (Zaller *et al.*, 2015). Therefore, there is general agreement that agricultural intensification has a deep impact on biodiversity with possible cascade effects on ecosystem functions and service delivery (Trivellone *et al.*, 2014).

In fact, biodiversity in agro-ecosystems are under considerable pressure through both intensified farming and land abandonment. The simplification of cultivated landscapes is particularly acute in wine grape regions as the geographic branding of wine (e.g. premiums paid for wine produced in several regions) further encourages regional land use conversion from natural habitat to high-value wine grape production. The loss of both agrobiodiversity and natural habitats that surround agro-ecosystems can lead to the loss of multiple ecosystem services, including biological control (as reviewed by Miles *et al*. 2012).

By contrast, there is compelling evidence that wildlife-friendly farming practices, aimed at reducing the negative impacts of intensive agriculture by implementing conservation actions in farmed landscapes, can be effective in conserving and restoring biodiversity, as reviewed by Pywell *et al*. (2015).

In this context, it is well known how physical (such as edaphology or climate) and anthropological (such as viticulture and oenological techniques) factors work together to determine the identity of a vitivinicultural product from a particular region, establishing the concept of *terroir* (OIV VITI 333-2010 resolution). However, this complex concept also includes the biodiversity as a definitory part. Thus, conservation of biodiversity is crucial not only for maintaining or increasing the sustainability and stability of farming systems, either as for crop production or for nature conservation, but also to preserve the biological heritage of wine regions (Belda *et al*., 2017).

Abandonment of vineyards has a favorable short term effect on biodiversity, but it becomes unfavorable in the medium term due to competition mechanisms (Cohen *et al*., 2015) due to the post-farming succession on abandoned terraced plots is influenced by fires, the destruction of walls, and later land use patterns. Abandoning vineyards is frequently associated with a loss of the small sedentary fauna of plain (e.g. Beaujolais; Guittet *et al*., 2011).

In multifunctional agricultural systems, biodiversity provides important ecological services, such as the improvement of soil fertility, increasing organic matter, improvement of soil structure, storage of carbon, management of undesirable organisms (conservation biological control) and regulation of hydrological cycle and microclimate. Vegetative cover of a forest or grassland prevents soil erosion, replenishes ground water and controls flooding by enhancing infiltration and reducing water runoff (Perry, 1994). In agricultural ecosystems, biodiversity provides (beyond production of food, fiber, fuel, etc.) many different services to agricultural production, such as pollination, biological pest control, maintenance of soil structure and fertility, nutrient cycling and hydrological services.

2. ECOLOGICAL CONCEPTS

2.1. DEFINITION OF BIODIVERSITY

Biodiversity refers to all species of plants, animals and micro-organisms existing and interacting within an ecosystem (Vandermeer and Perfecto, 1995).

Biodiversity operates at three different levels (Boller et. al, 2004):

- Genetic diversity (ex. varieties),
- Taxonomic diversity (species),
- Diversity of communities of organisms, including the environment (diversity of ecosystems).

Biodiversity is an important regulator of agroecosystem functions, not only in the strictly biological sense of impact on production, but also in satisfying a variety of needs of the farmer and society at large. Agro-ecosystem managers, including farmers, can build upon, enhance and manage the essential ecosystem services provided by biodiversity, in order to work towards sustainable agricultural production. This can be achieved through **good farming practices** which follow ecosystem-based approaches designed to improve sustainability of production systems. They aim at meeting consumer needs for products that are of high quality, safe and produced in an environmentally and socially responsible way.

The conservation and enhancement of biodiversity in cropping systems both above and below ground (e.g. soil biodiversity) are part of the foundation of **sustainable farming practices**. Such measures also lead to improved biodiversity in other parts of the environment which are adjacent to but not directly part of the cultivated area – such as water bodies and the broader agricultural landscape. The composition and diversity of the crop system strongly influences the nature of the associated diversity - plant, animal and microbial. A challenge is to integrate, through ecosystem approach strategies, the desirable biodiversity that is maintained with the associated diversity (for example, wild pollinators). It is also necessary to define sensitive biological indicators that could be useful to monitor the effect of farming practices on the functional biodiversity of vineyards. In this regard, the microbiota inhabiting soils and vine tissues, composed by several hundreds of microbial species, can be considered as a sensitive and high-resolution indicator of the evolution (increase, decrease or modification) of biodiversity (Wagg *et al*., 2014).

Development of **agroecological approaches**, which emphasise the conservation-regeneration of biodiversity, soil, water and other resources, is urgently needed to meet the growing array of socioeconomic and environmental challenges. These approaches, **fully integrating the functionalities of the biodiversity into the agricultural practices**, are the key ecological strategy to bring sustainability to productionproduction (Altieri, 1999).

2.2 WHAT IS A FUNCTIONAL BIODIVERSITY APPROACH?

According to Böller (2004), **Functional Biodiversity** (FB) can be defined as the utilitarian part of biodiversity that can be of direct use to the farmer (e.g. conservation biological control of pests). Functional biodiversity approach seeks to integrate **ecological infrastructures** (hedgerows, woodlands, dry-stone walls, ground covers, etc...) supporting and enhancing biodiversity into the vineyard and to improve their management increasing, simultaneously, the quality of the production, while maintaining the quality of the landscapes.

Functional biodiversity can provide many "hidden services" such as water retention, purification of water and air, maintenance of soil fertility, increase of nutrient bioavailability, improved nutritional quality of fodder, etc. (Böller *et al*., 2004). Another important aspect is the preventive and the sustainable regulation of pests by their natural enemies (biological control). In conservation biological control (CBC), humans actively manipulate the ecological infrastructures (EI) to increase the density of natural enemies and enhance their impact on pests. The success of a CBC strategy is strongly linked to the availability and quality of EI inside and outside the farm limits (within a radius of the order of 100-200 m).


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2.3 ECOLOGICAL INFRASTRUCTURES: DEFINITION AND GENERAL CRITERIA

Definition

According to Böller *et al*. (2004), **ecological Infrastructures** (EI) are any infrastructure at the farm or within a radius of the order of 150 m that has an ecological value to the farm, which judicious use increase the functional biodiversity of the farm, such as hedges, grassland, wildflower strips, ruderal area, conservation headlands, stone heap, etc.
In different words, ecological Infrastructures on the farm are the ecological compensation areas, which act as the most important tools to utilise to the fullest extent services of functional biodiversity.

The network of EI is composed of three basic elements according to their different functions (Böller *et al*., 2004):

- **Large permanent habitats** of the fauna.
- **Stepping stones** are habitats of smaller size allowing the build-up of temporary animal populations.
- **Corridor structures** assist animal species in moving between large habitats and small stepping stones.

Among EI, hedges, natural and seminatural grassland, high-stem orchards, forest borders, as well as wildflower strips, wildflower fallow, grassy beetle banks, field margins or conservation headlands are included (Terres, 2006). Also, rotational fallows, litter meadows, high-stem fruit trees or stone heaps could be useful in this sense.

Although ground cover of vineyards are usually not considered as part of the EI surface (cultivated area), a plant species rich green cover and its appropriate management is the pre-requesite for a diversified beneficial fauna in the vineyards, as it also causes considerable modifications in the microbiota inhabiting soils (Burns *et al*., 2016). A high potential for a species rich and natural green cover has been found in slopping vineyards with small-scale terraces, as reviewed by Böller *et al*. (2004). In fact, by concentrating fertilizer input and mechanical impact (mowing, spraying, harvesting) in the horizontal alley, the steep banks can remain largely undisturbed and flora can be converted into a plant community similar to that in meadows with low management intensity. This plant community contains several perennial plant species of value in fostering beneficial parasitoids (Picture 1).

Picture.1. - Example of a vineyard installed in one row terrace with high diversity found on banks. In this case, the banks serve as an internal EI of the vineyard and face the grapevines at very short distance. (Douro, Portugal. Credits: C. Carlos/ADVID).

Which surface of the farmland should be dedicated to EI?

The optimum surface of EI (including all structures of interest) to maintain an adequate diversity of species is estimated to be **close to 15%**. According to the International Organization for Biological and Integrated Control (IOBC), a minimum of **5% of farmland** is required to be designated as EI. The ideal size of EI for the vineyard will depend, however, more on the ecological quality, distribution, interactions and connectivity between the EI already existing with other EI existing outside the vineyard (Böller *et al*. 2004).

2.4 ECOLOGICAL DISTANCES

The distances between the crop area and important EI are important dimensions in FB, but not fully investigated. The **"functional units" (see box)** (1-field / plot; 2-farm and 3-landscape) of farm and landscape apparently go hand in hand with the ecological distances. Certain fractions of the field (ex. vineyard, orchard, groves) are more important than others in affecting biodiversity and quality of expected ecological services. They can have the function of EI inside the crop area.

In general sense, a minimum of 10m (most intensive herbivore pest, critical range of PPP drift, etc.) and maximum of 100m is usually recommended. Optimal distances are 10-50 m (Böller *et al*., 2004).

2.5 FUNCTIONAL UNITS

Plant and Soil (micro scale; plot level)

Plot level means a group of vines or a small local unit under the same variables (e.g. variety, rootstock, training system, cover crop, etc.). In order to endorse specific characteristics to plots, they should correspond to homogeneous terroir units (González-San José *et al*., 2010), considering all the variables included in the definition of terroir (OIV, 2010). At local level, plant biodiversity could be influenced by several factors: directly by disturbances engendered by farming practices, according to their intensity (e.g. tillage or mowing); indirectly by cultural variables, weakly or indirectly by environmental variables (Cohen *et al*., 2015). All these factors also influence the micro-biodiversity of soils and plants, being the former (soil microbiota) the most biodiverse place in vineyard, acting, indeed, as the reservoir of the vineyard microbiota during the absence of vine's aerial parts (leaves, flowers and grapes) (Zarraonaindia *et al*., 2015). Apart from that, due to the generation time of microbial species, their response to environmental or anthropological changes is more sensitive than the response of plant and animal communities, so they are precise early indicators of the influence of external factors in the biodiversity of the vineyard.

Vineyard (meso scale; farm or vineyard level)

Studies of farmed landscape biodiversity most frequently are undertaken on a local scale in a specific region and involve a single crop type. They generally focus on functional groups, which may improve crop production (beneficial) and are sensitive to intensification (Cohen *et al*., 2015). Intensification is linked to agricultural practices, depending on their type, frequency and intensity. Among these practices, weeding and ploughing usually harm biodiversity more than mowing, sowing or grazing. Fertilisation and irrigation have a direct positive effect on herbaceous biomass and plant competition, but the influence of the agriculture type, is hard to isolate from other factors. In fact, according to data reported by Burns *et al*. (2016), the structure of soil-borne microbial communities is influenced by soil properties, typically affected by crop management practices.


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Furthermore, the microbial community seems to be more responsive to a given management practice or factor depending on the inherent characteristics associated with a soil type. Thus, the characterisation of homogeneous *terroir* units in a vineyard and its use to design specific viticulture practices for each, is of great importance for applying precision viticulture approaches to increase crop sustainability.

On the plot (including the surrounding edges) and landscape scales, some studies suggest that a high proportion of natural landscapes around groves and vineyards favors agricultural plant biodiversity (Cohen *et al*., 2015). Neighbouring landscape with potentially significant impact on the farmed vineyards lies within a belt of approx. 100-200 m (Böller *et al*., 2004).

Landscape (macro or large scale; region level)

At the landscape level, biodiversity can be supported by maintaining the diversity of habitats, both cropped and uncropped, at a diversity of spatial and temporal.

On large geographical scales, land use, economic specialisation and development projects of a *terroir* have a direct and/or indirect influence on plant biodiversity and the environmental filter effect increases with proximity to large urban areas (Cohen *et al*., 2015). Landscape and urbanisation have an effect on biodiversity, and it is more noticeable at regional scale than on small scales (Cohen *et al*., 2015). At this scale the percentage of surface for each EI, lineal meters for hedges or minimum distance from one EI to another, etc. are relevant index in order to evaluate and measure the biodiversity (Guenser and Van Helden, 2010). One example of landscape or at large scale study was the LIFE + BioDiVine project (http://www.biodivine.eu/).

2.6 IOBC GUIDELINES FOR BIODIVERSITY AND ECOLOGICAL INFRASTRUCTURES

According to the IOBC (International Organisation for Biological Control) guidelines for integrated production of grapes (Malavolta and Boller, 2009), existing EI on the farms must be preserved. Adequate EI are essential for the development and efficiency of important benefits (antagonists of pests). They provide refuges, hibernation sites, alternative host animals, prey for the juveniles stages of predators, nectar, pollen and honeydew for the maturation and reproduction of all parasitoids, many predators and pollinators (e.g. Bees) (Boller *et al*. 2004). Headland attractants (flowering field margins) should be established as reservoirs of pest antagonists. Regional organizations must establish lists of plants to be avoided (e.g. sources of infestations of major diseases, viruses etc.). Areas of linear elements (e.g. flowering border strips, hedges, ditches, stone walls) and non-linear elements (e.g. groups of trees, ponds, etc.) present on the farm or planned should be combined in a manner to obtain spatial and temporal continuity as a pre-requisite for the enhancement of faunistic diversity and for the maintenance of a diverse landscape. (Practical examples on the evaluation of the ecological quality of the infrastructures, their functions, establishment and maintenance are given in the IOBC Toolbox on internet http://www.iobc-wprs.org/ip_ipm/index.html). Important elements of ecological infrastructures in vineyards are e.g. border areas and slopes of terraced plots rich in plant species, stone walls and ruderal areas. Particular attention must be devoted to headlands and hedges. High diversity of their composition and structure should be the aim, using or encouraging where possible native species. A ground cover during winter (or the rainy season) is mandatory.

National/regional guidelines have to provide a list of possible options for the active enhancement of biological diversity. At least two of these ecological options have to be chosen and implemented (Malavolta and Böller, 2009).

Ecological options valid for vineyards can be the followings:

- Edges and banks of terraced plots are rich in plant species;
- Hedges and headlands of high diversity (composition and structure) whereby native plant species are preferentially encouraged;
- In alleyways alternating mowing regime of green cover with permanent supply of flowering plants;
- Stone walls and ruderal areas (wasteland, old fields, waysides or highly disturbed sites – Böller, 2004) are maintained.

According to Böller *et al*. (2004) three elements must be incorporated that are instrumental for functional biodiversity:

- A regional list of key pests, diseases and weeds, which need a regular attention and plant protection measures;
- A list of 2 key antagonists (Böller *et al*., 2004)
- The designation of maintenance of at least 5% total surface of the farm as EI.

Natural pest control services are determined by processes acting at multiple spatial scales and depending on species characteristics. There are some mechanisms between landscape characteristics, several major vine pests (e.g. grapevine moths), and their biological control by different natural enemies (Sage *et al*., 2015).

In the case of *Lobesia botrana/Eudemis*, one of the major vineyards key-pest, natural enemies include several parasitoids, according to Loni *et al*., (2014) mainly belonging to hymenoptera and diptera, and predators including arthropods (e.g. spiders, lacewings, syrphids), birds, reptiles, mammals (bats), etc.

Potential key pests in vineyards

Among arthropod pests of grapevines in Europe, grape berry moths (*Lobesia botrana* and *Eupoecilia ambiguella/ Cochylis*) are of economic importance in most areas. Additionally, *Sparganothis pilleriana(pyrale)* is currently limited to specific areas. The first species is invasive and recently colonised Californian vineyards (Gilligan *et al*., 2011), causing major damage to Napa valley production. A number of leafhoppers (e.g. *Empoasca vitis* and *Scaphoideus titanus*), scales (e.g. *Parthenolecanium corni* and *Planococcus ficus*) and spider mites (e.g. P. *ulmi* and *Eotetranychus carpini*) are locally important (Pertot *et al*. 2016) and can be vectors of important diseases.


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Potential antagonists

Predators- Among arthropods, the most relevant in conservation biological control of vineyard pests are: spiders, lacewings, predatory mites (*Phytoseiidae*), predadory bugs (anthocorids, mirids and nabids), syrphids and ladybugs; however, several other groups could play a significant role, depending on the agro-ecosystem (birds, bats, reptiles, etc).

For instance, the introduction of the New Zealand Falcon (*Falco novaeseelandiae*) into vineyards in New Zealand saw a 95% reduction in the number of grapes removed by introduced pest birds relative to vineyards without the falcon (Kross *et al*. 2012).

Parasitoids- egg, larval or pupal parasitoids of grapevine moths, leafhoppers, etc.

Other biocontrol agents – fungus (e.g. *Beauveria bassiana*, *Ampelomyces sp*., *Trichoderma sp.*) and bacteria (e.g. *Bacillus thurigiensis*) are used as biocontrol agents. *Beauveria bassiana* (Bals.) is an ascomycete that can live as endophyte in grapevine, *Vitis vinifera (L.)* plants and still maintain its antagonistic potential against insect pests or even other microbial pathogens such as Plasmospara viticola (Rondot and Reineke, 2013).

The surface of ecological infrastructure should eventually increase to 10%. The 5% rule needs not to be applied to each individual farm in areas with predominantly small farms, with highly scattered properties, and where a surface of 5% or more of a comparable and homogeneous agro-climatic unit (e.g. same municipal district), has been set aside as ecological infrastructure by official and well documented regional programs. In this case, it has to be shown that the ecological infrastructure areas are well distributed in time and space in the municipal area, thus providing a guaranteed continuity (Baur *et al*. 2011).

3. IMPROVEMENT OF FUNCTIONAL BIODIVERSITY

3.1. IMPLEMENTATION OF EI. CONSERVATION ACTIONS

Appropriate farming practices and agro-ecosystem planning may play a crucial role in functional biodiversity enhancement (Terres, J.M., 2006).

1.

Planting shrubs at the ends of each row, in places where they do not interfere with work. Criteria for the selection of shrubs include their attraction for butterflies and other insects, the provision of nesting opportunities, root symbiosis, and the use of any fruit. Native species are to be preferred.

2.

Interspersing hedges with the vines. Dependent on local circumstances, there should be at least 2 20-metre hedges per hectare. Hedges constitute biological hotspots, acting as corridors linking up ecological areas. Moreover they constitute a natural barrier preventing the spread of harmful fungi.

3.

Planting fruit trees as a way of improving vertical diversity. The presence of trees in the middle of a low-growing and little-structured field/vineyard is a great way of attracting birds, insects and other groups of animals. They are also a way of promoting the long-term colonisation of an ecosystem. At least one tree per hectare should be planted amidst the vines, and no point of the vineyard should be further than 50 meters away from a tree.

4.

The provision of compensatory areas (at least 50 m2 per hectare) as diversity hotspots both within and on the perimeter of a vineyard. These areas become the home of aromatic herbs and wild flowers.

5.

The provision of structural elements, such as piles of stones or wood. These provide a habitat for reptiles and insects. The provision of nesting aids for bees, insects and birds. These can be integrated into trellis posts. Perches for birds of prey, with the latter helping to keep the rodent population in check.

6.

Favor the regular supplantations, to avoid the total uprooting of the old vines. The young vines are taken from the vineyard using massal selection and grafted onto existing root structures onsite. In doing so, selection perfectly adapted to the terroir takes place over generations. The achieved genetic diversity reduces the likelihood of infections through pests, boosts wine quality and also improves vine resilience to prevailing conditions.

3.1.1. GREEN COVER CROPS

Green or ground cover (GC) based on natural vegetation is diverse and valuable functionally, it is advisable to improve its presence, since its adaptation to the local conditions (ex. soil, climate). Sometimes its presence can be enhanced through the simple correction of pH. In some specific cases (herbicide accumulation, weed domination, soil compaction, soil pH, vigor control, etc.), the GC can be installed or changed, taking into account some requirements, related to, for example: nitrogen balance, organic matter production, height, active/blooming season and water demand.

Regarding their management, some points should be taken into account:

• If possible, preference is given to natural ground covers or mixtures instead of seeded or new species. Local plants are more adapted to local conditions than species not adapted to the vineyard ecosystem;

• Water availability and enough soil holding capacity during the growth season.

• Some parameters should be taken into account before mowing or doing a soil management: mowing level (avoiding the excessive and early destruction of GC), soil status (organic matter, nutritients and propierties), type of machine, period and frequency, time of the day, etc.

• Alternating mowing should be preferred, providing constant flower supply. Whereas traditional mowing eliminates instantly all flowering units from the entire surface, alternating mowing of every second bank allows maintenance of a flower supply of varying intensity and quality throughout the growth season. Important for the survival and reproduction of adult beneficial is the avoidance of gaps in the nutritional food sources for more than 10 days.

Improvement of functional biodiversity

Advantages

■ **Pest control** - The presence of a diversified GC increases the abundance of natural enemies of pests (predators and parasitoids), since it provide them natural resources (pollen, nectar, alternative preys, shelter and water).

■ **Reduce the risk of erosion and water runoff** - The presence of GC reduces the velocity of raindrops before they hit the soil surface, preventing soil from splashing and running off of nutrients.

■ **Improvement of soil fertility** - Besides increasing soil nitrogen and organic matter, decomposed cover crops increase the soil cation exchange capacity.

■ **Improvement of soil structure and water holding capacity** – GC roots help aggregate soils as fine roots penetrate the soil profile (especially grasses). Large tap roots help to create macropores when the plants die, which greatly assist the movement of air and water into the soil profile. Also organic matter is a food source for macro and micro-organisms. Many of them assist in recycling cover crops into the soil, while improving soil physical qualities in the process. Particularly the increasing of earthworm populations is a good indicator of soil health and improved physical conditions.

■ **Improvement of beneficial microbial communities**. Arbuscular mycorrhizal fungi are important to grapevine nutrition, particularly in challenging soils. Cover crop strategies can increase the likelihood of fungal colonisation of grapevine roots, facilitating the transfer and uptake of nutrients from cover crops to grapevines. Site characteristics and vineyard management strategies that foster root growth, such as planting vines in soil with adequate texture and structure and irrigating vines during periods of rapid root growth, benefit grapevine roots and mycorrhizal fungi, will likely have greater effects on grapevine nutrition than practices that focus solely on enhancing populations of mycorrhizal fungi, such as the application of fungal inoculants to vineyard soil (Baumgartner, 2003).

Negative agronomic aspects

■ **Competition for water resources**- Cover crops for semi-arid areas show a favourable effect, but careful management is needed to avoid excessive water consumption by the cover crop (Medrano *et al*., 2015). In order to maximise the potential benefits of specific cover crops and to avoid the undesirable ones, the accurate selection of species and varieties are key points in the decision-making process. As reviewed by Medrano *et al*. (2015), the results about cover crops competition for water resources with vineyards are ambiguous. Several studies showed that cover crop interfere with grapevine water use by decreasing water resources and thus increasing grapevine water stress (mainly early during the spring). Whereas, in other studies, it has been shown that cover-cropped vineyards do not always exhibit higher water stress, compared to those with bare soil. Even in warm climate (Linares *et al*., 2014; Steenwerth *et al*., 2016), it has been demonstrated that changes in weed community composition in response to tillage and cover cropping were not always associated with increased water competition with grapevines. Therefore, it is evident that the timing for sowing the GC is a key point for managing the vine water stress, and can produce some effects on juice characteristics, nutrition parameters, or vine vegetative growth, yield and fruit load (Ravaz index).

■ **Fertilisers**. The establishment of green cover could requires an additional quantity of nitrogen or other inputs. Increasing costs and labours and also, their environmental impact should be taken into account.

3.1.2 HEDGES AND WOODLANDS REMNANTS

Hedgerows are defined as lines or groups of trees, shrubs, perennial forbs, and grasses that grow naturally or are planted along road ways, fences, field edges or other non-cropped areas.

Hedges are important sources of predatory mites immigrating from outside into the vineyards. Predatory mites (*Phytoseiidae*) are one of the most important and efficient beneficial in viticulture worldwide, being economically relevant in vineyard management if they are situated in proximity to vineyards (Böller *et al*., 2004). The presence of hedges, garrigue and forest around vineyards allows floral richness and traits diversity to be increased on the plot and edges scale (Cohen *et al*., 2015).

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Each geographic region has many animal species, especially the immature stages of insects, which are highly adapted to the local hedge flora. Local and typical shrubs and trees are valuable components of hedges since every type of regional landscape has its characteristic types of hedges. Local climate, soil characteristics (e.g. soil base saturation/pH and soil moisture) and altitude strongly influence botanical composition and structures of the hedges.

Hedges also represent a biodiversity reservoir, being a shelter and an ideal place for numerous animal species. A huge number of invertebrates develops there as auxiliaries to the vines (spiders, hymenopterans, etc.). The diversity inside them offers some places for nesting and feeding to certain birds (thrushes, partridge) and the structure of the hedge is used as biological corridor to mammals (rabbit, fox).

Regarding the maintenance of hedges, some points should be taken into account:

• In new planted hedges, weed control is key for a well managing.

• Renew the old hedges. Cutting to ground level in interval of some years (3-10 depending of the space and height).

• Selected plants with strong growth should be frequently trimmed to ground level.

• Long hedges require a "sector-wise" trimming technique (alternative trimming for each area).

• Maintenance of lateral grass strips.

Improvement of functional biodiversity

Advantages

■ Serve as **habitat** for beneficial insects, pollinators and another wildlife.

■ **Providing** corridors for migrant animals.

■ **Protection** against **rain and erosion**, wind and sun.

■ **Weed control**

■ **Stabilise waterways**. Increasing surface water infiltration, reducing non-point source water pollution and groundwater pollution. Regulating soil moisture content.

■ **Buffer**, reducing pesticides drift.

■ Act as **living fences** and boundary lines.

■ **Provide** an aesthetic resource (oenotourism, image of the vineyard for the consumer), or eventually serve to hide dissonant elements.

Negative agronomic aspects

■ **Risk of introducing invasive organisms** (plants/pathogens/pests) (e.g. potential host plants of quarantine organisms (e.g. phytoplams, bacteria). The species of plants and their origins must be carefully chosen in order to avoid such negative potential effect;

■ **Microclimate and competition in root zones**;

■ **Land resources** (obstacles to optimal operation);

■ **Need of labour for maintenance** (irrigation in the first years, pruning, etc.).

■ **Host plants for plant diseases or vertebrate, insects pests**.

3.1.3 DRY-STONE WALLS

Dry-stone walls are constructions without connecting elements, built with local stone with dry joint (schists, granite or limestone). They are traditional handmade elements placed with different purposes: defining boundaries, preserving the biodiversity, adding value to the vineyard landscape or providing a flat surface on which vines could be planted (high slope or mountain's vineyards).

There are some requirements for a wall habitat: hibernation sites, food sources, proximity to ecological infrastructures, etc. The mean number of protected species is usually lower on edges of vineyards than on the plot. However, among all the studied species, a 20% were only found on edges, which thus contribute to increasing plant biodiversity on the plot and surrounding edges scale (Cohen *et al*., 2015).

Some recommendations are given for obtaining a durable dry-stone wall:

• Select stones of different sizes and place them in a correct order (e.g. as flatter and bigger stones should be placed at a lower levels).

• Preserving autochthonous flora inside the wall and on it.

• Oldstones from local rocks found near the vineyard are preferred to bare, alien or new stones .

• Maintenance of one area without treatments for enhancing the biodiversity of the species.

• Conservation actions (repairing or rebuilding) like splattering the face with manure, soil and local seeds are highly recommended.

Advantages

■ allow the implantation of vineyards on the hillside,

■ turning work easier and increasing the arable land,

■ best system to mitigate erosion, hold the soil, slow down runoff of water, allow their penetration into the soil and replenish of sources,

■ play an important role on preservation of biodiversity, acting as a reservoir for several species of flora and fauna, being some of them, natural enemies of pests. The numerous cavities and crevices provide favorable conditions for many species of reptiles (wall lizards, snakes), mammals (hedgehogs and shrews), birds and insects, including wild bees, beetles and ants and also for spiders.

Negative agronomic aspects

■ **Land resources**. Stone walls could difficult the soil management (due to split the plots in many small parts, (obstacles to optimal operation).

■ **Costs**. Total costs can reach more than 250€/m3 (values of Douro Valley, Biodivine project), depending on several factors (stone availability, total perimeter, etc.).

3.2 SUSTAINABLE USE OF PPP

Problems associated with older generation of synthetic chemical PPP and consumer demand for residue free products have stimulated research into new tools for pest management. Chemical companies are developing new active substances with a favourable profile for human health and the environment, and new mechanism of action with lower risk of developing resistant pest populations. Alternatives to synthetic chemical PPP are represented by a number of microbial and botanical active ingredients and pheromone based tactics in the case of insecticides alone. Inoculate and inundate biocontrol techniques (e.g. release of predators or parasitoids commercially produced by biofactories) against insect pests have been less investigated in vineyards than in other agricultural systems such as greenhouse vegetables and ornamental plants. In contrast, conservation biocontrol strategies have attracted the interest of researchers in order to successfully manage various pests, mainly grape berry moths and leafhoppers (Pertot *et al*. 2016).

Growers should combine several different tools in order to reduce the input of synthetic chemical PPP on crop. Agronomic practices, i.e. reduction of the inoculum or improvement of the microclimate of the plant in order to avoid conditions favorable to pests and diseases, are commonly implemented in most of the grape growing areas. Resistant/tolerant varieties may represent a solution to reduce fungicide treatments, however their implementation is widely limited by the market, especially for wines produced in typical areas (e.g. AOC in France, DOC and DOCG in Italy). Biocontrol products based on microorganisms or natural molecules may represent an alternative to synthetic chemicals, however several of existing solutions have drawbacks or limiting factors, which prevent a fast uptake by the farmers (Lamichhane *et al*., 2016). On the contrary, beneficial arthropods and the use of semi chemicals may offer interesting and sustainable alternatives to synthetic chemical PPP in certain contexts. Due to all these reasons, the correct timing of the synthetic chemical application is still a crucial step to achieve a sustainable use of PPP (Pertot *et al*. 2016).

3.2.1 DECISION SUPPORT SYSTEMS

These are basic tools for integrated and biological plant protection models (e.g. VitiMeteo, Vite.net). Coupling these predictive algorithms with information about the base doses of pathogen's inoculum in the vineyard could be a winning strategy to control diseases development. In addition, as occurs in human health, some microbial-derived plant diseases break out after losing the normal balance in the normal microbiota. Thus, strategies focused on maintaining a diverse, stable and health-promoting microbiota in the vineyard are also recommended and are in the basis of the concept of 'disease-suppressive soils' (Berendsen *et al*., 2012).

At this point, it is still unclear the optimal level and structure of micro-biodiversity to keep vineyards in a resilient status, but the evidence says that the higher the biodiversity is, the better the resistance to disturbances (by covering a higher range of ecological nitches).

3.2.2 SELECTION OF LOW TOXICITY PPP

Finally, with a proper selection of PPP (with low toxicity) could be beneficial. The use of chemicals to control crop pests can cause a wide range of unintentional effects on beneficial parasitoids and predators (Thomson and Hoffmann, 2006). Therefore, as shown by Thomson *et al*. (2006), parasitism can be high in vineyards with low chemical use and particularly low sulphur inputs (Thomson *et al*., 2000) as it was shown to be highly toxic to parasitoids (Jepsen *et al*., 2007). Hence, the choice of chemicals with low toxicity to beneficial is a critical point, and should be carefully considered, in order to contribute to the preservation and maintenance of natural enemies in the vineyard.

3.2.3 MATING DISRUPTION

The use of mating disruption (MD), an environmentally friendly method to control pests, is widely recommended in several production systems (e.g. integrated production, organic) in order to control pests, reducing the use of conventional PPP.

The principles of this method usually relies on the use/releasing of a defined amount of synthetic pheromones of female pest on vineyards, interfering with males communication (males are not able to find females on a saturated environment of pheromone), with the goal of avoiding mating process (Carlos *et al*., 2013). It was shown to be effective for controlling *L. botrana* (Den. and Schiff.) after consecutive seasons with the application, when large areas were treated, and in years of low pest population density. Mating disruption for *Lobesia botrana* started in mideighties in Europe and took a long time to develop however now is applied on more than 200,000 ha of vineyards worldwide.

Another examples about the use of pheromones is the success in monitoring the vine mealybug (*Planococcus ficus*) and its relationship with its main parasitoids *Anagyrus pseudococci* in vineyard (Gonçalves *et al*., 2013) and of the use of MD using vibrational signals to disturb the behavior of *S. titanus*, the vector of Flavescence Dorée Phytoplasm.

The key of this technique realies on preventing *S. titanus* mating interrupting the sound vibrational sexual communication by transmitting (imitating) suitable disrupting signals. For more information http://www.winetwork-data.eu/intranet/libretti/0/libretto16489-01-1.pdf .

3.2.4 MICROBIAL BIOCONTROL AGENTS

Preventive actions for vineyard pathogens are linked to reduction of the inoculum and the planting of healthy nursery material. In relation to the plant, growers can use resistant or less susceptible varieties, clones and rootstock, while actions related to the environment mainly concern plant architecture (trellis and training systems) and a reduction in plant vigour, which can partially modify the microclimate, making it less favorable for the development of the pathogen. However, although theoretically these actions can substantially reduce the risk of disease, in practice quite often technical, economic and commercial barriers strongly reduce their feasibility.

The approach used to combat most of the plant pathogens is commonly different from that used against insect and mites, where treatments are often applied when an economic threshold is reached.

Indeed, for reaching a satisfactory pest control level, natural enemies are in most cases sufficient (Duso and Vettorazzo, 1999). In contrast, keeping the level of the inoculum of plant pathogens low is crucial. This is why in the last few years researchers have focused on techniques that can minimise the overwintering inoculum or on optimal control of primary infections and/or the initial stages of an epidemic (Caffi *et al*., 2013a,b).

Bacteria have many important beneficial roles in the metabolism and physiology of the host plant. They can:

i. stimulate the growth of plants by synthesizing plant growth hormones or enzymes;

ii. promote resistance of plants by inducing host defense mechanisms;

iii. control diseases by suppressing pathogens and/or

iv. solubilising phosphates and fixing nitrogen, making them available to their hosts (Rezgui et al., 2015).

Not only bacteria, but also some fungal species are also involved in plant-growth promoting functions such as phosphorus mobilisation and/or mycorrhiza formation.

Concerning pests, *Bacillus thuringiensis* (Bt) is a well-known microbial biocontrol agent of berry moths. The efficacy of Bt depends on the strain, formulation (e.g. wettable powder or dust), timing and frequency of application, spray volume, pest population density and cultivar features.

Improvement of functional biodiversity

In experiments carried out in northern Italy the use of *Beauveria bassiana* combined with *B. thuringiensis* slightly increased the efficacy of the latter in berry moth control. *B. bassiana* showed a significant effect against spider mites and trips, suggesting possible applications against grapevine pests (Vega *et al*., 2009). Recently a commercial formulate based on *B. bassiana* (ATCC 74040 strain) was authorised for the control of trips and spider mites in Italian vineyards. Among the PPP based on microbial metabolites, spinosad proved to be effective in controlling grape berry moths and trips (Vassiliou, 2011). However, spinosad has been reported to be nonselective towards predatory mites (Duso *et al*., 2014; Ahmad *et al*., 2015).

Different biological control approaches, such as conservation, augmentation or dissemination of natural enemies, have been identified as potential levers in controlling vineyard pests and vectors in the next 50 years (Thiery, 2011). Biological control against insect pests or vectors can be achieved either by natural populations of predators or parasitoids (Thiery *et al*., 2001; Rusch *et al*., 2015), but also by releasing natural enemies.

3.2.5 ACTIVE INGREDIENTS OF NATURAL ORIGIN

The effect of botanical PPP on grape berry moths has not been widely investigated, despite increasing interest in these compounds in organic viticulture. Pyrethrins have been used for a long time against berry moths and other pests, but their efficacy is questionable and the impact on beneficial organisms is a major concern. Field applications of Azadirachtin, originated from the neem tree, Azadirachta indica against berry moths reduced damage when compared to the untreated control. At the same time, azadirachtin proved to be relatively harmless towards beneficial organisms (e.g. predatory mites).

Among the remaining nonsynthetic chemical products, recent investigations have suggested that kaolin has an effect on *L. botrana* eggs and larvae. At the same time, this mineral product proved to be substantially selective towards *L. botrana* egg parasitoids (Pease *et al*., 2016).

4. EVALUATING ECOLOGICAL QUALITY

In 2010, declared by United Nations "International Year of Biodiversity", the World Biodiversity Association proposed "Biodiversity Friend", an innovative initiative to evaluate and score the biodiversity conservation in agriculture. The protocol evaluates 10 actions, considered by the WBA as the "Decalogue of Biodiversity" in agriculture: agricultural model, soil fertility conservation, sustainable water management, hedges and woods, agrobiodiversity, soil, water and air quality, renewable energy and environmental responsibility.

The environmental quality is evaluated by means of soil, water and air biodiversity indices, based on biomonitoring data, developed by the WBA International Scientific Committee (Caoduro *et al*., 2014).

The Soil Biodiversity Index (SBI-bf) is based on soil macroinvertebrates used as bioindicators. To each group of pedofauna (Annelids, Molluscs, Diplopoda, Chilopoda, Isopoda, Acarina, Opilionida, Pseudoscorpionida, Aranea and many orders of Insects) a score related to their sensitivity to environmental alterations is assigned.

Lichens are extremely sensitive to atmospheric pollution, therefore they are frequently used in biomonitoring of air quality, both in urban and rural environments. The Lichen Biodiversity Index (LBI-bf)

evaluates the state of lichen diversity in standard conditions, related to the air pollution of the vineyard. The calculation of the index is based on the epiphytic lichen communities on the tree barks.

Many groups of freshwater organisms (Plecoptera, Ephemeroptera, Trichoptera, Crustaceans, Molluscs, etc.) can be used as bioindicators to determine the Freshwater Biodiversity Index (FBI-bf). The survey considers the hydromorphology of the water course, the taxonomic diversity of the aquatic community and the tolerance of each group to water pollution; the principal physical-chemical parameters of the water are also measured: pH, temperature, electric conductivity and dissolved oxygen.

4.1. EVALUATING ECOLOGICAL QUALITY OF EI

The ecological quality of the agro-ecosystem vineyard is determined by the quality of the green cover abd that of neighbouring ecological infraestructures (Böller *et al*., 2014). The ecological infraestructures (EI) can be evaluated with record sheets, ilustrations or photos taken by diferent sample units or replicates from the ecological infraestructures in diferent seasons. A detailed questionnaire and a list of 13 plants indicators was described in Böller *et al*., 2014 for doing this kind of evaluation on neighbourhood and the current biodiversity. Number and types for fauna and flora individuals (taxonomy approach) and also, indicator plants, insects or the shipes and sizes of the trees (fruit trees, woodlans, etc.) and another arbustive species are parameters used in this evaluation system.

On the other hand, an evaluation system called RISE (Response Inducing Sustainability Evaluation) has been developed by the University of Applied Sciences (Zollikofen, Switzerland). It is an holistic approach which measures 12 indicators about the economical, ecological and social performance of the farm. It based on the evaluation of sustainabilityof the farm and the ecological quality of its ecological infraectructures (Böller *et al*, 2014).

4.2. EVALUATING ECOLOGICAL QUALITY OF SOIL

Soil is the habitat of a diverse array of organisms: archaea, bacteria, fungi, protozoans, algae and invertebrate animals, the activities of which contribute to the maintenance and productivity of agroecosystems by their influence on soil fertility (Swift and Bignell, 2001).

Taxonomic diversity in soil biota is high, but principal groups could be stablished in the following: Earthworms; Termites and ants; Other macrofauna (woodlice, millipedes and some types of insect larvae, centipedes, larger arachnids, some other types of insect); Nematodes; Mycorrhizas; Rhizobia and Microbial biomass (fungi, protists and bacteria).

Bacteria have many important beneficial roles in the metabolism and physiology of the host plant. They can:

i. stimulate the growth of plants by synthesising plant growth hormones or enzymes;

ii. promote resistance of plants by inducing host defense mechanisms;

iii. control diseases by suppressing pathogens and/or

iv. solubilising phosphates and fixing nitrogen, making them available to their hosts (Rezgui et al., 2015).

Not only bacteria, but also some fungal species are also involved in plant-growth promoting functions such as phosphorus mobilisation and/or mycorrhize formation.

For evaluating the ecological quality, some methodology was described such as the “Standard methods for assessment of soil biodiversity and land use practice” (Swift and Bignell, 2001). This compendium includes methods for

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evaluating macroorganisms such as macrofauna or nematodes, and also specified methodologies to evaluate root-microsymbionts such as nitrogen fixing bacteria (also known as Rhizobia) or mycorrhizal fungus.

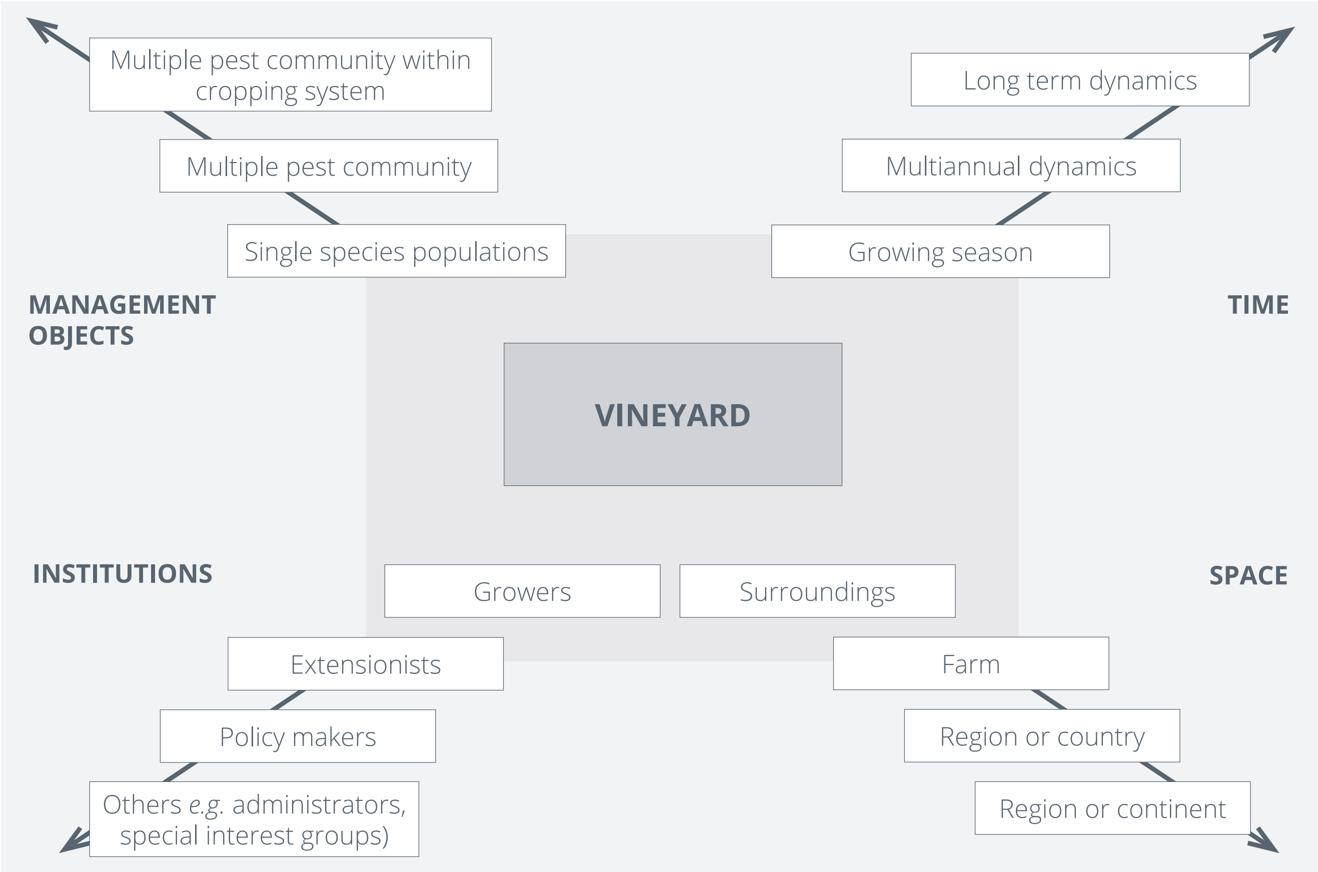
Finally, there are other methodologies which include also, the soil microbiota. Metagenomics (Next Generation DNA Sequencing) is the cutting edgeline for evaluating the whole micro-biodiversity. This technique improves previous one such as DGGE (Denaturing Gradient Gel Electrophoresis) or T-RFLP (Terminal restriction fragment length polymorphism) that provide partial information about microbial community structures (Rastogi and Sani, 2011; Rincon-Florez et al., 2013).

5. PLANNING, COSTS, PUBLIC INCENTIVES

Biodiversity at the vineyard level should be considered at a holistic approach. It should be planned in accordance with a proper space and time scale, such as the hierarchy theory (Graphic 1; Baumgärtner, 2013) used in integrated pest management.

In some countries, ecological direct payments (subsidies) to promote a high level of biodiversity are only granted to vine-growers that satisfy a number of ecological requirements (e.g. swiss vineyards), using a scored list of non-productive plants belonging to the species of particular interest (Trivellone *et al.*, 2014).

In addition, taking the example given by previous projects (e.g. BioDiVine), the viticulturist could choose some conservation actions for preserving the biodiversity, according to their own objectives and the expected results (planting hedges, promoting ground cover, applying mating disruption to avoid applying conventional insecticides, restoring dry stone walls, conservation of headlands).



Graphic.1.- Example of a hierarchy theory (Baumgärtner, 2013).

6. THREATS TO THE APPLICATION OF FUNCTIONAL BIODIVERSITY APPROACH

- Necessary human resources
- Necessary interdisciplinary resources
- Climate change
- Mandatory pest management and use of plant protection products
(e.g. flavescence dorée)

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