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Abundance and diversity of the order Coleoptera in vineyards from different wine regions of Portugal

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Abstract

Insects of the order Coleoptera plays an essential role in managing and enhancing the sustainability of agricultural ecosystems by supporting relevant ecosystem services. Currently, they are the largest group of insects ever described and they are present in almost all agricultural environments. In vineyards, Coleoptera may occupy different ecological niches, highlighting the predatory action of some families against important vine pests. In this sense, the present study aimed to evaluate the abundance and diversity of Coleoptera's families and species of Coccinellidae in the vine canopy and vegetation cover of vineyards from different Portuguese Wine Regions, namely, Bairrada, Beira Interior, Douro, Península de Setúbal, Trás-os-Montes, and Vinhos Verdes. For this, in 2019, on three different periods (July, September, and October), the vegetation cover of 36 vineyards scattered in the different wine regions were sampled. For each plot, 20 samples were collected, ten in the canopy and ten on the vegetation cover, using the sweeping technique. In total 2954 Coleoptera individuals were collected belonging to 36 families, among these, 315 were Coccinellidae grouped in 22 species. The most abundant families were Chrysomelidae, Coccinellidae and Latridiidae. For Coccinellidae species, the most observed ones were *Scymnus apetzi*, *Scymnus interruptus* and *Coccinella septempunctata*. Results points toward a higher abundance of Coleoptera families and Coccinellidae species at the Douro Region. Most specimens were concentrated in July and at the vegetation cover, for all the Wine Regions. Vegetation cover was found to have a great importance in Coleoptera and Coccinellidae refugee and performance shelter as a microhabitat within the vineyards. The diversity index of Coleoptera indicates a higher diversity in July at Douro grapevines. Also, for Coccinellidae species, the higher diversity in canopy and in vegetation cover was observed in July and September, respectively in the same region. Further studies on pests and natural enemies' dynamics, the use of different variables and increase sampling dates should address non explained patterns in this study.

Keywords: Entomofauna; grapevine; vegetation cover; auxiliary fauna; ecosystem services.

Resumo

Os insetos da ordem Coleoptera desempenham um papel fundamental na manutenção e no incremento da sustentabilidade dos ecossistemas agrários, ao servir de suporte a relevantes serviços ecossistémicos. Atualmente, são o maior grupo de insetos já descritos e estão presentes em quase todos os ambientes agrícolas. Na vinha, os coleópteros podem ocupar diferentes nichos ecológicos, sendo de destacar a ação predadora de algumas famílias contra pragas importantes da videira. Neste sentido, com o presente trabalho pretendeu-se avaliar a abundância e diversidade funcional das famílias de coleópteros e especies de coccinelideos na copa e no coberto vegetal de vinhas de diferentes regiões vinícolas portuguesas nomeadamente região do Douro, Bairrada, Beira Interior, Península de Setúbal, Trás-os-Montes e Vinhos Verdes. Para tal, em 2019, em três períodos distintos (julho, setembro e outubro) procedeu-se à amostragem do coberto vegetal de 36 vinhas dispersas nas diferentes regiões vinícolas. Para cada parcela, foram colhidas 20 amostras, 10 na copa da cepa e 10 no coberto vegetal, utilizando a técnica de varredura. Foram recolhidos 2954 indivíduos de 36 famílias de Coleoptera, dos quais 315 eram Coccinellidae agrupados em 22 espécies. As famílias mais numerosas foram Chrysomelidae, Coccinellidae e Latridiidae. Para Coccinellidae, o maior número observado pertencia às espécies *Scymnus apetzi*, *Scymnus interruptus* e *Coccinella septempunctata*. Os resultados apontam uma maior abundância de Coleoptera e Coccinellidae na região do Douro. Em todas as regiões vinícolas, maior número de indivíduos foi observado no coberto vegetal em julho. A cobertura vegetal mostrou ter grande importância como refúgio de Coleoptera e Coccinellidae. Os índices de diversidade de Coleoptera indicam uma maior diversidade no Douro, em julho. Na mesma região, para as espécies de Coccinellidae, houve maior diversidade na copa em julho e na cobertura vegetal em setembro. Novos estudos sobre a dinâmica de pragas e inimigos naturais, o uso de diferentes variáveis e o aumento das datas de amostragem devem abordar padrões não explicados neste estudo.

Palavras-Chave: Entomofauna; videira; coberto vegetal; fauna auxiliar; serviços ecossistémicos.

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Chapter 1.

General Introduction

1.1. Vine’s historical and economic importance

In Portugal, vineyards are cultivated from the ancient times, before the foundation of the country. The country is one of the oldest with winery tradition. It is estimated that the vineyard was cultivated for the first time in the Iberian Peninsula (Tejo and Sado valley), about 2,000 years BC, by the Tartessians (Magalhães, 2008).

The importance of wine in the Portuguese agriculture, economy, and society is well known. Portugal is the 11th worldwide and 5th producer in the European Union. Vineyards currently occupy around 190,322 hectares in vines-growing areas destined for wine and total production of 6 061 243 hectoliters (hl) (IVV, 2018). Also the country is one of the greatest *per capita* wine consumers (Magalhães, 2008). After centuries of being one of the main product that shaped Portuguese national identity and culture, wine is historically the most important export-oriented product in the country’s economy (Panzone & Simões, 2009). In the national food culture, the wine remains a product of daily local consumption. At the same time, grape (*Vitis vinifera* L.) production and vine growing still plays an essential role in Portuguese rural economy, yet remaining a dominant element of the national agricultural landscape, with economic, environmental, technological and social impacts (Simões, 2003).

The viticulture’s economic dimension does not limit its importance to the country. It’s relation with the soil, climate, technique, and society requires versatility. In other words, it contributes to the local community, development of poor soil conditions areas, recreational activities, tourism, Iberian Peninsula art, and culture (Simões, 2003). Not only the wine culture can promote economic relations, but also social and political interests that have been leading governmental interventions. In order to optimize the industry and its production, it is essential to study all the dynamics of the winemaking process, from wine grapes growing to the wine distribution market (Panzone & Simões, 2009).

1.2. Characterization of the vineyard in Portugal

Globally, it is possible to grow vines in a wide range of edaphoclimatic conditions. At the broadest scale of global suitability for viticulture, it has been considered that producing zones are found between either the mean annual 10-20 °C (Figure 1.1.) (Böhm, 2020). The ideal range of temperature in summer is the maximum of 22 °C and in winter, a minimum of 3°C (Jones, 2007), although some vines are cultivated for wine much nearer the equator in the country such as Peru and Bolivia (Malheiro et al., 2010).

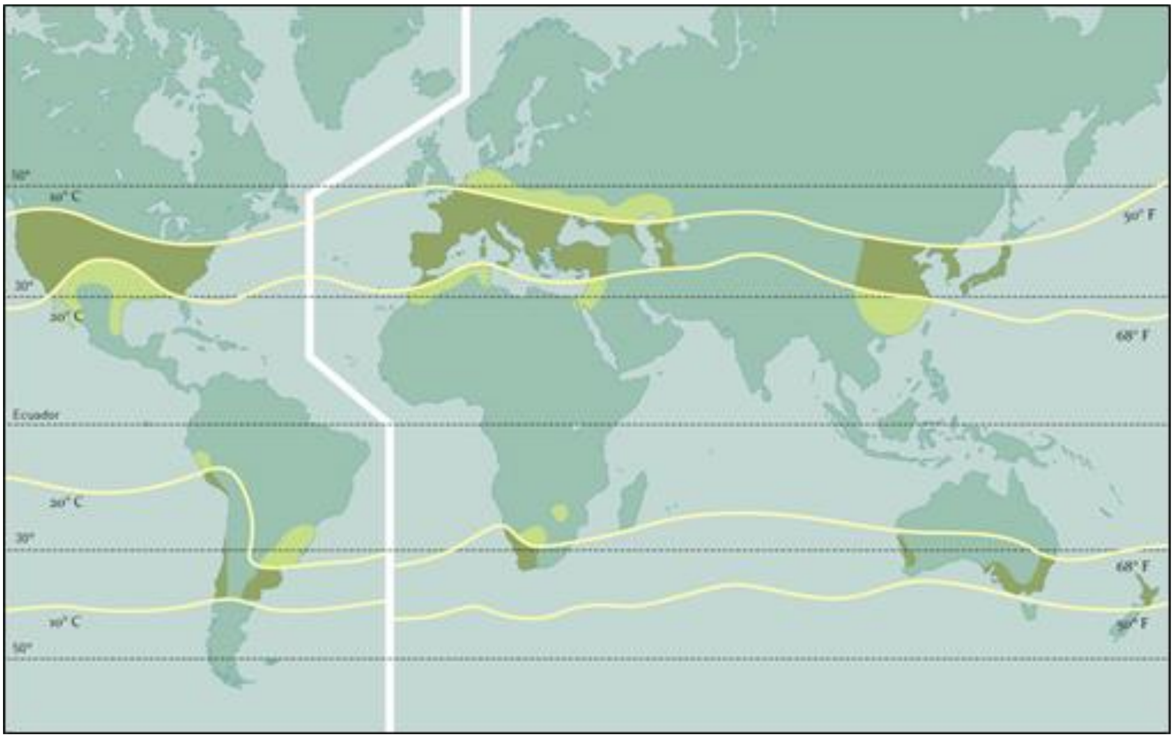

Figure 1.1. Global distribution of viticulture in the 10-20 °C isotherm according to Blij, 1983 (Source amended, after Gómez-Miguel, 2009) (Retrieved from: https://www.vinetowinecircle.com/terroir/factores-del-ambiente-vitivinicola-y-sus-limitaciones/).

In Portugal, the wine growing is distributed in a range of latitude from 37° to 42° North and longitude between 7° and 9.5° West, resulting in more than thirty wine regions. The popular viticultural regions are historically distinguishable by their predominant environmental characteristics, such as climate, soils, and varieties grown (Fraga et al., 2012). This complex and interactive system, including specific soil, topography, climate, landscape characteristics, and biodiversity features, is called of *Terroir* (Magalhães, 2008).

Vineyards are grown in all regions of the country, however, within each region, certain areas of production develop an identity with its local wine (Figure 1.2.). In order to protect this important agricultural and economic asset, policies have leaned towards the development of a reputation over the stronger wine production areas (Panzone & Simões, 2009). The stimulations of its reputation occurred after Portugal joined the former European Economic Community (EEC), which later became the European Union. The European directives oriented the regions to regulate geographical origins and identifying relevant wine density areas that had a strong link between production and locality. In terms of production standards, the quality of wines was improved by the accession to the EEC, furthermore, the wine became valued by its regional origin and by the reputation that a region has acquired over time (Panzone & Simões, 2009).

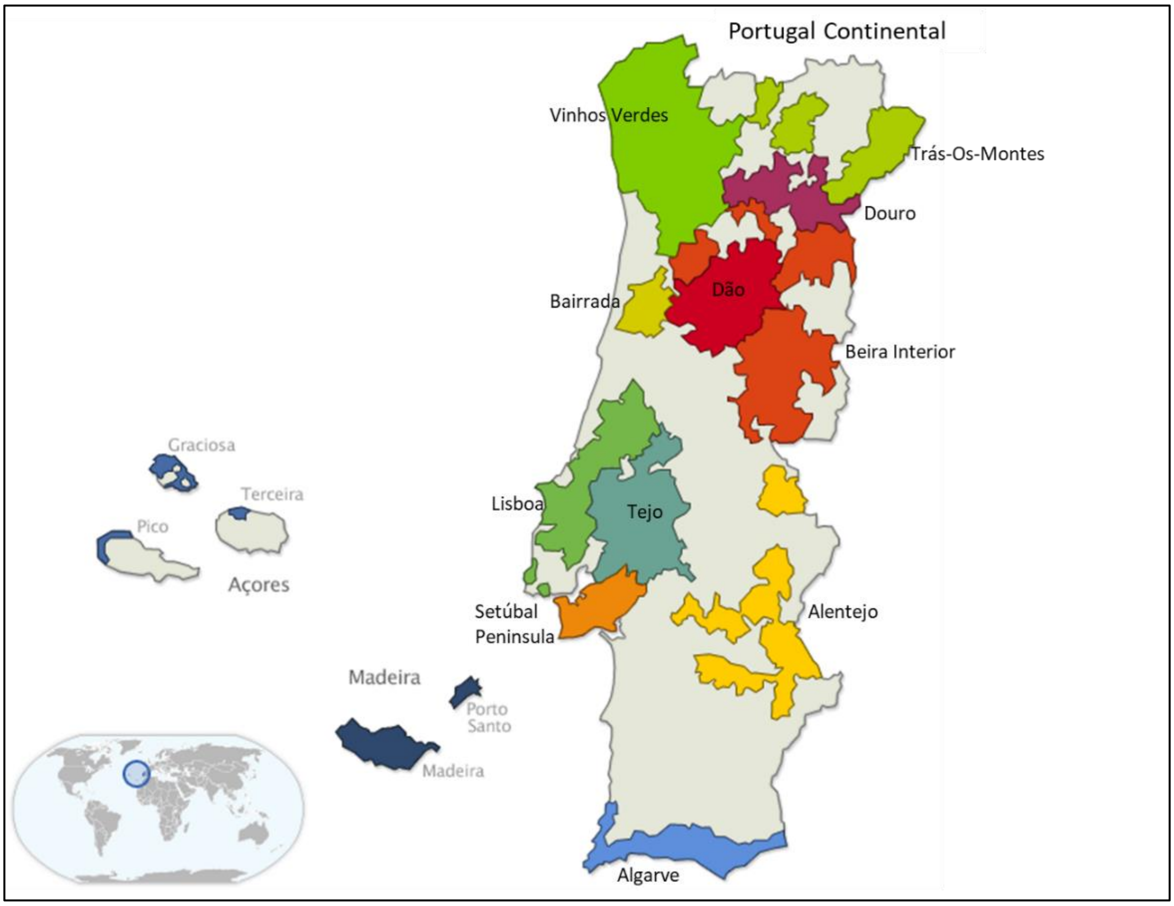

Figure 1.2. Map of the Portuguese Wine Regions. (Adapted from: http://www.infovini.com/pagina.php?codNode=18012).

1.3. Agrobiodiversity of the vine

Biodiversity is one of the main components of the ecosystems' structures, which can be defined as the variability between living organisms, in addition to all the ecological processes of which such organisms are part of (Stout, 1972). The loss of biodiversity represents the greatest threat to ecosystems and their ability to sustain basic ecological processes that support life (Andrade & Romeiro, 2009). Cataloging the diversity of the vineyards and understanding how to preserve it is essential for the sustainable development of agricultural production and food security. Arthropods are part of the functional agrobiodiversity and provide numerous ecosystem services, including pollination, biological control of pests, soil aeration, waste decomposition and nutrient cycling (Saunders, 2018).

1.3.1. Trophic organization

The ecological function of the arthropods population in an ecosystem is the result of their modes of feeding and the food-acquisition system (Wallace & Webster, 1996). The concept of feeding guilds was originally proposed by Root (1967) and divides living organisms in trophic groups, which are based not only on the type of resources but also in the morphological and behavioral mechanisms to achieve these resources (Cummins & Klug, 1979). Functional Feeding Groups (FFG) are defined as a group of species, which explore the same class of food incomes in a similar way, rather than taxonomic affiliation (Shimano et al., 2012). The benefit of this method is that instead of multiple of different taxa to be studied, a small number of groups of organisms can be understood collectively based on the way they function and process energy in the stream ecosystem (Marinoni, 2001).

According to Blondel (2003), guilds are defined based on "similarity in resource sharing" and functional groups on "similarity in ecosystem function". The first exploits resources in a similar way and the second "processes the same resources or habitat features (e.g. soil) for and ecosystem service". The concepts of guilds / trophic groups and functional groups may be two facets of the same coin (structural vs. functional), however, a functional group can group more than one guild, while a guild cannot group more than one functional group (Blondel, 2003).

In order to classify trophic groups, Marinoni (2001) used the concept of trophic group as a natural unit, defined by the animal's eating habits. The guild, being an artificial unit, is defined by the research objectives, for comparisons between components of the ecosystem. Then, five trophic groups are recognized in which Coleoptera can be classified. Herbivores, and algivorous as primary consumers, fungivores, detritivores and carnivores as secondary or higher-level consumers (Marinoni, 2001).

Primary consumers, herbivores and algivorous, are organisms that use part or a whole of a producer organism as food, without any transfer of energy by another organism. Likewise, herbivores eat plants or parts of plants, such as leaves, bark, stem, fruit, etc. Another group of primary consumers is the species that feed on algae. This eating habit has greater significance in studies of aquatic invertebrate communities (Cummins & Klug, 1979). Secondary consumers, firstly, there are fungivores, which include all feeders on any type and part of fungi. In addition, detritivores eat particles (residuals, fragments, including liquids), or product of the decomposition of animal, or plant cells and tissues. The group of carnivores includes all organisms that feed on the tissues, cells or internal liquid of a living animal, such as parasites or parasitoids or recently killed by the action of the food ingested, such as predators (Marinoni, 2001).

1.3.2. Main pests of the vineyards

As a crop, the vine can be attacked by different enemies that can lead to a consequent reduction of the yield and in more severe cases to the death of the plant (Amaro, 2004). Mites, leafhoppers, mealybugs, lepidopterans, and some coleopterans are well kwon plagues that are responsible for serious damage in the vineyard (Neves, 2000). Secondly, in the vine, there is also the presence of insect vectors of pathogens (mostly Cicadomorpha) responsible for serious diseases such as Flavescence dorée (Chuche et al., 2017) and Pierce's disease (Sicard et al., 2018).

The Grape Phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Hemiptera: Phylloxeridae), is a worldwide pest of the grapevine, native from North America, and it is a microscope aphid that lives and feeds on roots (Downie, 2002). The leaf galls caused by grape phylloxera are unsightly harmful, however, attacks on the roots can be difficult to control and can lead to severe root pruning and decline of vines (Forneck & Huber, 2009). However, nowadays there is no more expression of this plague in Europe (Garnett et al., 2001).

Many species of mites are harmful to the vines. In Portugal, the red spider mite, *Panonychus ulmi* (Koch) (Acari: Tetranychidae) and the yellow spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae), are the most common (Costa, 2006). In ecological balance conditions, the spider mites are naturally controlled by its natural antagonists and are not considered dangerous for the crop, however, they have a high biological potential, being able to increase its population exponentially. Severe attacks can lead to the premature fall of the leaves, which affects the quality of the production and the vigor of the plant (Costa, 2006). Female adults lay the eggs during spring-summer on the bottom page of the leaves. From August, they go to the woody parts of the vine, where they do the winter postures. Usually, attacks are only detected in midsummer, sometimes when the populations have already reached very high levels (Rabbinge, 1976).

The leafhoppers *Empoasca vitis* and *Scaphoideus titanus* (Hemiptera: Cicadellidae) are a concern that have been increasing in the vine (Amaro, 2004). *Empoasca vitis* feed on the leaves with their piercing-sucking mouthparts, causing necrosis and the subsequent premature fall of the leaves. The main damage caused by this pest occurs mainly in the summer, which corresponds to the middle of the vegetative cycle of the vine (Raposo, 2003). The Cicadellidae *S. titanus* is a vector of the grapevine phytoplasma disease flavescence dorée. This disease is responsible for the death of young vines and for the reduction of the productivity of old vines (Chuche & Thiéry, 2014). Thus, another Cicadomorpha that has a consistent presence in the vines is the *Jacobiasca lybica* (Bergevin & Zanon) (Hemiptera: Cicadellidae) (Quartau & Rebelo, 1992). *Jacobiasca lybica* feed on the phloem, causing a typical symptomatology in the vine which consists of premature reddening (red strains) or yellowing (yellow strains) of the leaf edges, causing necrosis and the subsequent premature fall of the leaves (Quartau & Rebelo, 1992). In the vineyards, it is possible to find other individuals of the order Cicadomorpha that are considered potential vectors of *Xylella fastidiosa* (Wells et al.) (Xanthomonadales: Xanthomonadaceae), the causal agent of the Pierce's disease (Cornara et al., 2019). However, in Portugal, there are still no reports of this disease in vineyards.

The cotton mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) when present in the maturing branches, causes its blackening as a result of the honeydew excreted and the fumagine that develops on them, greatly depreciating the fruit (Neves, 2000). The most common species of grapevine moth is the *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae). It is responsible for "fake *Véraison* phase", which means blackening the berry before the it reaches the ideal size and development (Vennila & Agarwal, 2013). With the beginning of low temperatures, in the fall they return to hibernation. The direct damage caused by these caterpillars is the partial destruction of branches with a consequent decrease in production. Nevertheless, as indirect damage, they can cause several types of rot, in particular gray rot. In terms of life cycle, during the spring it settles on the branches and leaves, while in summer they attack happens on the bunches (Neves, 2000).

Among Coleoptera, *Xylotrechus arvicola* (Olivier) (Coleoptera: Cerambycidae) is an important pest in vineyards in the main wine-producing regions of Spain (Ocete et al., 2002). It's a xylophagous Cerambycidae, associated wood damage by the larvae habits of excavating galleries on the timber and degrading by facilitation to fungal diseases and/or inoculation by nematodes (Ocete et al., 2017).

1.3.3. Natural enemies

A complete management of the viticultural ecosystem can only be accomplished if considered the whole system based on a development that takes into account both ecological pest knowledge, their devastating potential, and complex adoption of the most appropriate measures for the natural control of the damaging species (Enoiu et al., 2013). Natural enemies of vine pests, also called "antagonists" or "beneficial arthropods" are organisms that act as regulatory factors for pest populations, often preventing them from causing damage. Beneficial arthropods are usually grouped into predators, parasitoids and parasites (Gonçalves et al., 2013). Predators and parasitoids naturally occurring in vineyards are the main natural biotic factor limiting pest populations (Portugal et al., 2017).

Beneficial arthropods can be a free and renewable natural limitation of pests present in all agrarian ecosystems (Nunes et al., 2015). Some cultural techniques used in vineyards, especially treatments against pests and diseases, can directly influence the diversity and abundance of natural beneficial arthropods, with direct implications in maintaining the natural balance within the vine's ecosystem (Enoiu et al., 2013). The most common and important predators belong to the class Araneae, and families Chrysopidae (Neuroptera), Syrphidae (Diptera), Myridae and Nabidae (Hemiptera), Coccinellidae, Malachiidae, Carabidae and others from the Order Coleoptera, further discussed.

Spiders (Araneae) are frequent predators of insects in the vineyard. Many species live in vegetation, while others live on the soil surface, preferring soils with plant residues. Harvestmen (Opiliones) feed on small insects, although some species may also consume decomposing material (animal or plant) and fungus (Coutinho, 2007; Gonçalves et al., 2013).

Phytoseiids are the main natural limiting agents of populations of phytophagous mites. They are often seen in the inflorescences of the vine and they feed on tetraniquid mites, thrips, cicadellids and aphids. Erythraeids (Trombidiformes) are parasites in the larvae phase and free predators, when adults; are often observed parasitizing leafhopper nymphs (Coutinho, 2007).

Lacewings (Neuroptera: Chrysopidae) are generalist predators, very abundant in the viticulture ecosystem, where they feed on enemies, like the grape moth, the leafhopper, mites and mealybugs (Carlos et al., 2005; Gonçalves et al., 2013). Syrphids (Diptera: Syrphidae) are predators only in the larval stage, feeding on aphids, mealybugs and other soft-bodied insects (Carlos et al., 2005). They are interesting predators from the point of view of the natural limitation of crop enemies, particularly as adults have great mobility, ensuring a rapid re-colonization of crops, both in the spring and after the completion of phytosanitary treatments (Carlos et al., 2005; Gonçalves et al., 2013).

On the Order Heteroptera, Myridae are known in the vineyard as predators of mites and cicadellids, while the Nabids, are seen as playing a complementary role to that of other predators in relation to caterpillars (Coutinho, 2007). Another family that is considered of greatest interest as potential predators, are the syrphids known fundamentally as predators of aphids, being able to feed on young caterpillars and psilas (Carlos et al., 2005).

And at last the Order Coleoptera, this group of arthropods play an important role in the natural limitation of the populations of enemies of the vine, mostly the families of the Coccinellidae, Cleridae, Cantharidae, Malachiidae, Carabidae, and Staphylinidae. Among these families, the best known are the Coccinellidae, widely used in biological control programs worldwide (Obrycki & Kring, 1998; Pervez, 2004). Coccinellidae are, either in the adult or immature state, mostly predominant predators of aphids, mealybugs and mites (Sloggett & Majerus, 2000). In the vineyard, the natural limitation that they exert on insects and mites is well known, particularly the species that feed on grape moths (Coutinho, 2007; Obrycki & Kring, 1998). Cantharidae are predators, both in adult and larva, feeding on other insects. Adults of some species can also feed on pollen, which is why they are often observed on flowers (Gonçalves et al., 2013). Malachiids' larvae stage are exclusively predators, however, adults feed mainly on pollen and flower nectar, although in some species adults also feed on insects (Gonçalves et al., 2013). Carabidae are both adult and larval predators (Rainio & Niemelä, 2003). They have essentially edaphic habits, where they feed on insects and other invertebrates. However, they can also go up to the vegetation to look for their prey (Coutinho, 2007; Lövei & Sunderland, 1996). Like Carabidae, Staphylinidae are predators in both adult and larvae stage (Balog et al., 2011). They live essentially in the soil, but are able to go up to the foliage to hunt (Frank & Kanamitsu, 1987).

In order to maintain beneficial arthropods in the agroecosystem in or near the crop, conditions must facilitate their presence, through selection of agricultural practices without negative impact on their populations and promote conservation practices and/or installation of ecological infrastructures, which provide shelter and alternative foods for the periods of prey scarcity (Nunes et al., 2015). In addition to the economic benefits achieved by reducing the use of pesticides, the promotion of obtaining higher quality products with lower risks to human health and the environment is promoted (Hoddle & Van Driesche, 2009). For example, the vegetation cover is one of the beneficial agricultural practices adopted in integrated production, which is a production method that encompasses integrated protection as a phytosanitary practice, and a set of other techniques that aims to balance culture and preserve natural resources, in order to improve the quality of the final product, in this case, the vine (Nunes et al., 2015). At the same time, it naturally limits pests' infection by fostering populations of auxiliary organisms, which reduces the need of use of phytosanitary products (Andrade & Romeiro, 2009).

1.3.4. Order Coleoptera

The Coleoptera order comprises about 350 thousand species, which represents 40% of all insects and 30% of animals, forming the largest group of organisms on earth (Borror & DeLong, 1969; Costa Lima, 1952; McHugh & Liebherr, 2009). Coleoptera is found in almost all environments. The vast number of beetle species is reflected by a bewildering array of anatomical and biological diversity in the order (McHugh & Liebherr, 2009).

Most of Coleoptera have four wings and the main characteristic of the members of this order is the first modified pair of wings, hard-shelled outer wings called the "elytra". The second set of wings is membranous or see-through and is folded under the elytra when not in use (Camargo et al., 2015; Costa Lima, 1952). The main function of elytra is usually protection, while in flight, the second pair of wings do most of the work (Borror & DeLong, 1969). The elytra may act as stabilizers or even as rudders, but even when they are artificially reduced (in case it has been trimmed) the ability to fly is not reduced. Also, there are many species of beetles without the second pair of wings (Borror & DeLong, 1969). This flightless animals have no need for wings as they usually live on the ground or within the litter (Hangay & Zborowski, 2010). The structure permits beetles to utilize diverse resources and engage in a broad range of activities that otherwise would be restricted to either winged or wingless insects (Crowson, 1960).

As characteristics of insects, we find the body divided into three parts (heteronomy): head, chest, and abdomen. It is a common characteristic to have bilateral symmetry with three pairs of legs (hexapods) and a pair of antennae (Audino, Nogueira, & Silva, 2007; McHugh & Liebherr, 2009; Silveira, 2009). The antennae are cephalic, and the legs, like the wings, are thoracic. Their mouthparts indicate the type of feeding and is an important element in characterization (Borror & DeLong, 1969).

Beetles undergo complete metamorphosis (holometabolic), comprising the phases of egg, larva, pupa and adult (Figure 1.3.). Larvae vary considerably in shape in different families. Borror and DeLong (1969) point out that the life cycle in that order varies from four generations per year to one generation in several years (Audino et al., 2007). Normally, species have only one generation per year. Their cycles are generally short, they can multiply quickly under favorable conditions, and winter can be crossed at any stage of the cycle, depending on the species (Borror & DeLong, 1969).

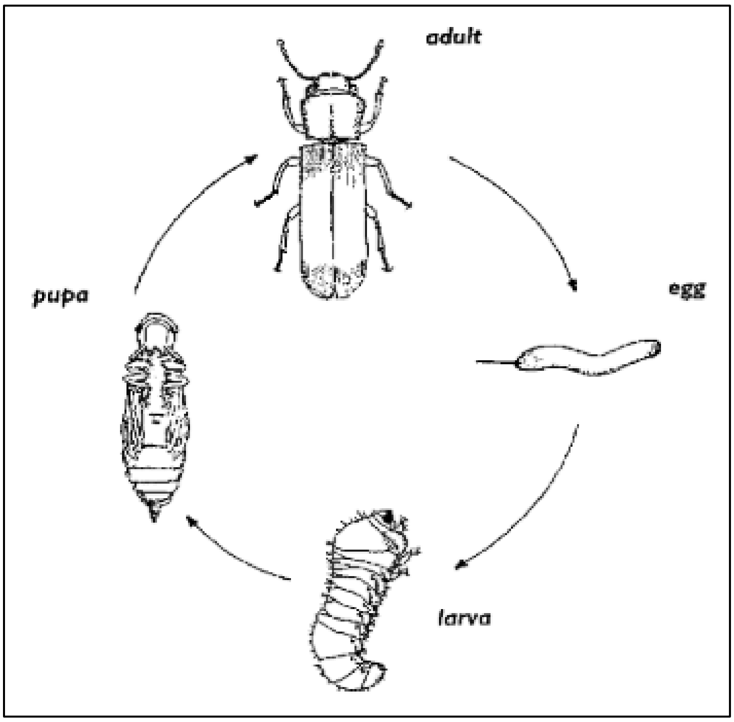

Figure 1.3. Stages of development of powderpost beetles, representing the phases of the egg, larva, pupa and adult (holometabolous). Adapted from Peters, Creffield & Eldridge (2002).

The terrestrial species are, the majority, phytophagous (feed on all parts of the plant – roots, leaves, flowers, fruits and pollen), necrophagous (carrion), coprophagous (excrement), predators, parasites or can infest stored products of animal or vegetable origin. In the aquatic environment, they can be predators or phytophagous (Camargo et al., 2015; Costa Lima, 1952). The greatest numbers are plant feeders in one form or another, such as nectar feeders (e.g. Buprestidae), foliage eaters (e.g. Chrysomelidae), seed-eaters (e.g. Curculionidae) or timber (e.g. Cerambycidae) or stem borers (e.g. Scolytidae). Others may feed on rotting wood (e.g. Lucanidae), carrion (e.g. Silphidae), manure (e.g.

Scarabaeidae), fungi or leaf litter (Borror & DeLong, 1969; Marinoni, 2001; Silveira, 2009). Some species are also predators (Carabidae) of other invertebrates (Marinoni, 2001; Silveira, 2009) . The feeding habits between larvae and adults may be the same or can vary. For example, some beetle species are predators when in the larval stage and plant-feeders when adults (Borror & DeLong, 1969; Crowson, 1960). Most Coleoptera have a feeding habit similar to the family taxonomic level, thus minimizing, in most cases, efforts in identifications (Silva & Silva, 2011). Marinoni (2001) divided the Order Coleoptera in five trophic groups (Table 1.1.): herbivores and algivores as primary consumers; fungivores, detritivores and carnivores as consumers secondary or higher level.

1.4. Objectives

The present work has as general objectives, to obtain data about the abundance and diversity Order Coleoptera associated to vineyards in different Wine Protected Designation of Origin (PDO) regions of Portugal, namely Bairrada, Beira Interior, Douro, Península de Setúbal, Trás-os-Montes and Vinhos Verdes. The general objectives were accomplished through the achievement of the following specific objectives, namely:

- Identification of Coleoptera families present in different strata, the soil vegetation cover and the canopy of vineyards of the different regions;

- Study of the patterns of abundance and richness and distribution of Coleoptera families;

- Identification at the species' level of the individuals of the Coccinellidae family and study of diversity and abundance of the family.

- Compare the different strata and regions.

1.5. Supplementary material

Table 1.1. Coleoptera trophic groups and subgroups organization. Adapted from Marinoni (2001).

Trophic group	Trophic subgroup	Food substrate
Herbivore	Philophagous	Leaf
	Xylophagous	Wood
	Stemborer	Stem
	Phleophagous	Phloem
	Antophagous	Flower
	Nectariphagous	Nectar
	Polyniphagous	Pollen
	Carpophagous	Fruit
	Spermophagous	Seed
	Rhizophagous	Root
Algivore		Algae
Fungivore	Myxomycophagous	Myxomycetes
	Ascomycophagous	Ascomycetes
	Basidiomycophagous	Basidiomycetes
	Deuteromycophagous	Deuteromycetes
Detritivore	Coprophagous	Excrement
	Phytoprophagous	Vegetable remains
	Saprophagous	Animal remains
Carnivore	Predator	Kills and feeds on other animals
	Parasite	Organism that lives on the surface or inside of another animal from which it obtains food, shelter or other advantages
	Parasitoid	Larvae lives in the host, internally or externally, killing it slowly close the end of their larval development

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Chapter 2.

Material and methods

2.1. Characterization of the Wine Regions

The present study was developed in six different Portuguese Wine Regions (Figure 2.1.), including: Vinhos Verdes, Douro, Bairrada, Beira Interior, Península de Setúbal, and Trás-os-Montes. According to Koppen-Geiger's climate classification, the study's areas is divided in two climate regions: one with temperate climate with rainy winter and dry and hot summer (Csa) and the other with temperate climate with rainy winter and dry and not very hot summer (Csb), commonly observed in Mediterranic areas (Andrade & Contente, 2020; Peel et al., 2007).

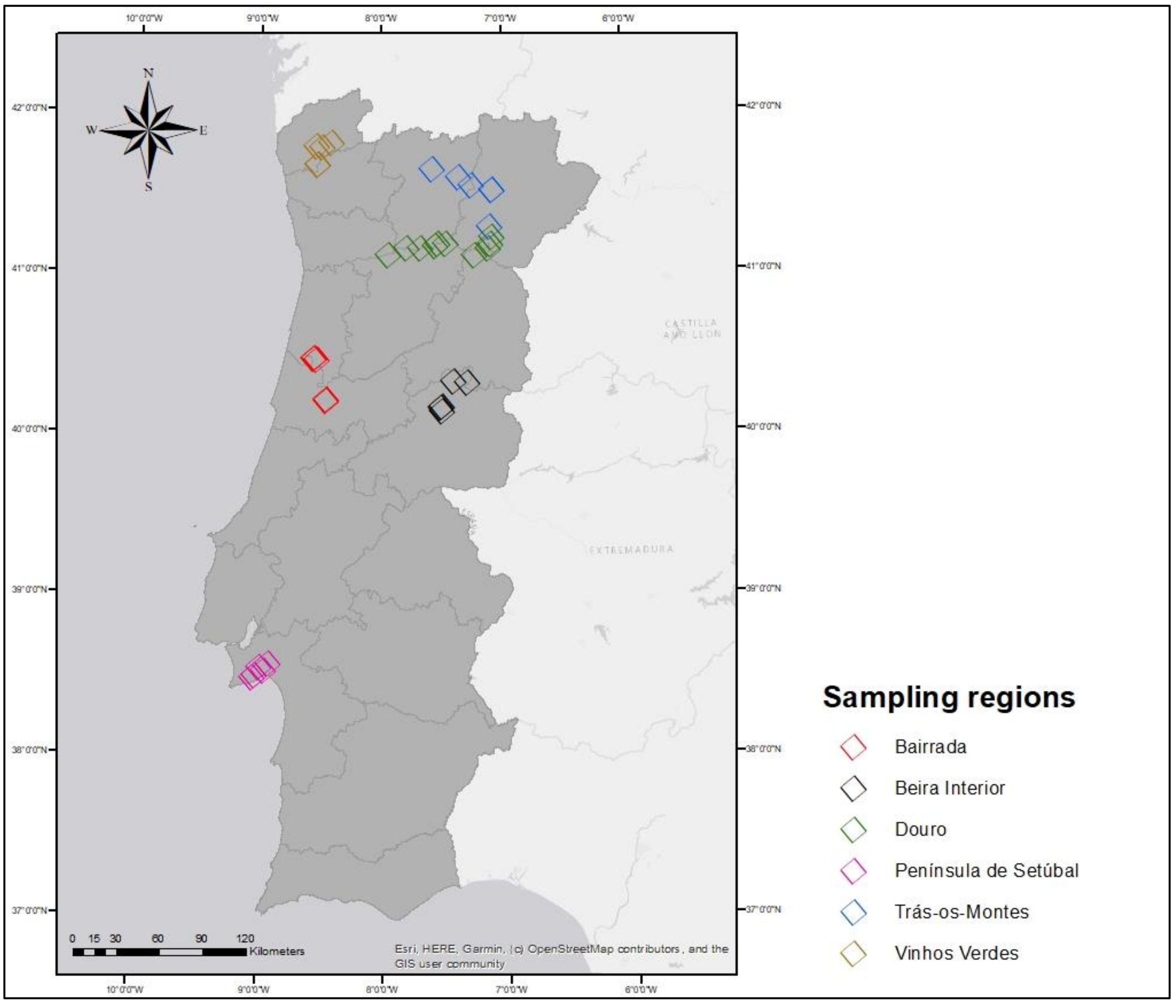

Figure 2.1. Map of Portugal and the location of the study area. Sampled plots indicated by regions.

2.1.1. Vinhos Verdes Region

The Vinhos Verdes region (Figure 2.2.) is located in the northwest of continental Portugal, extending throughout the northwest of the country, in the area traditionally known as Entre-Douro-e-Minho, corresponding to the largest Portuguese Demarcated Region (Figure 2.1.) (IVV, 2018). The vineyards extend across hills and valleys intersected by the hydrographic basins of the Minho, Lima, Cávado, Ave and Douro rivers and their affluent, where ocean winds easily penetrate up to 700-800m in mountain areas (Magalhães, 2008). Due to the ocean influence, the climate is temperate, with mild and rainy winters, with no significant thermal variation throughout the year (Carvalho, 2019). The annual precipitation generally exceeds 1,200 mm, with some regularity over the months and the soils are mostly granitic, ranging from fertile to very fertile and of high acidity (IVV, 2018).

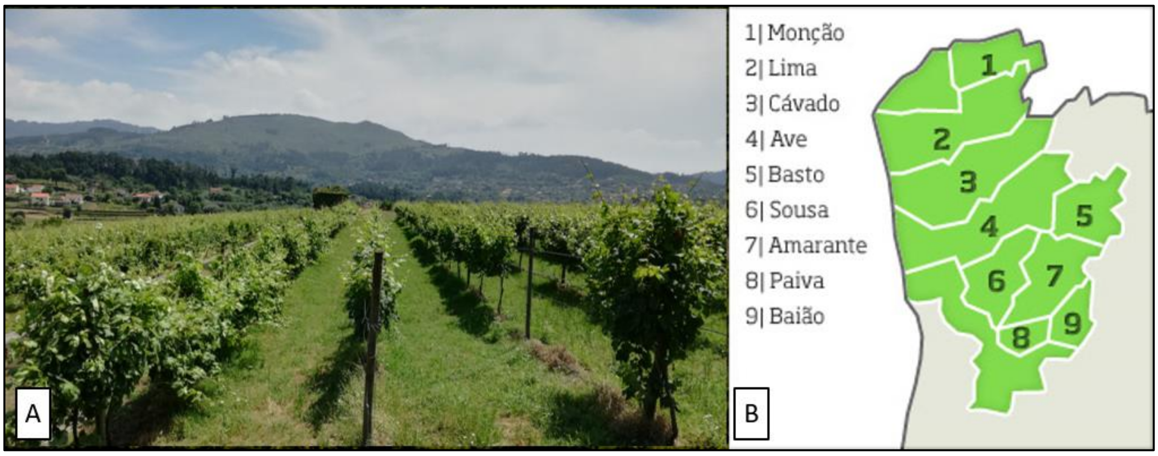

Figure 2.2. A - Vinhos Verdes Region's landscape; B - Map of the Region and its sub-regions. (Adapted from: Wines of Portugal, 2010).

2.1.2. Douro Region

The Douro region is located in the Northeast of the country, is heavily mountainous and is protected by the Serra do Marão and its climate is usually dry, with cold winters and very hot summers (Magalhães, 2008). Protected by the mountains that surround it, the Douro Demarcated Region, with its slopes of schist, it is benefited from the diversity of microclimates and is divided into three sub-regions: Baixo Corgo, Alto Corgo and Douro Superior (Figure 2.3.) (IVV, 2018).

In this region, climatic differences have produced different *terroirs* that are distinguished in wines: advancing upstream, the humidity decreases and the temperature increases, creating a drier climate and with greater thermal amplitudes (Magalhães, 2008). There are 250 thousand hectares of total area, which 44 thousand are for main production of two types of wines: Porto and Douro. The diversity that distinguishes the Douro Demarcated Region is its great wealth, being one of the few wine regions in the world with two Denomination of Origin (IVV, 2018).

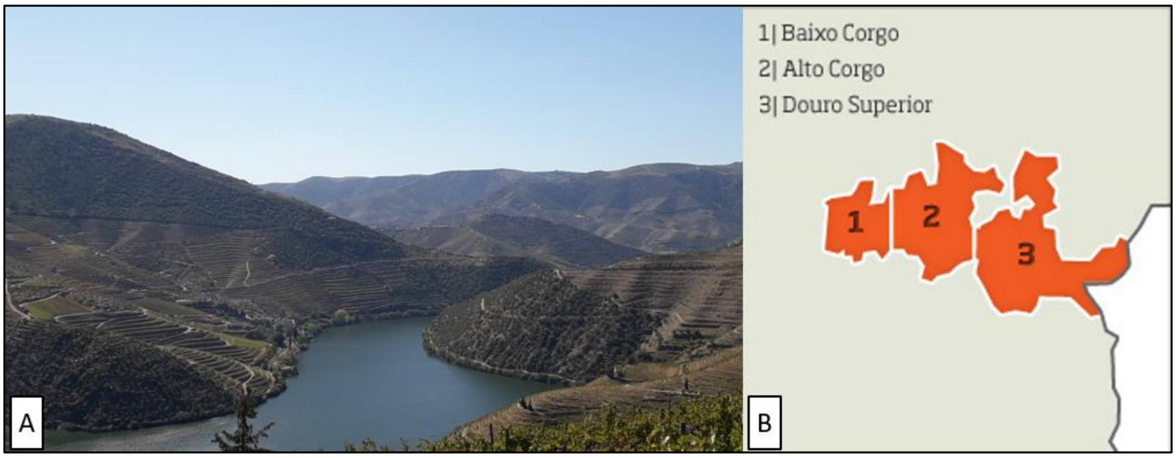

Figure 2.3. A - Douro Region's landscape; B - Map of the Region and its sub-regions. (Adapted from: Wines of Portugal, 2010).

2.1.3. Trás-os-Montes Region

Located in the northeast of continental Portugal, the Region of Trás-os-Montes is known for its scenario between hills and valleys in a large area. It is characterized by its high altitude, being conditioned by a rigorous continental climate, with long and hot summers, followed by long and cold winters (Magalhães, 2008). The soils are granitic, very poor and not very productive, with some schists stains (IVV, 2018). In the region of Trás-os-Montes, there are several microclimates, which, together with the differences in the soil's constitution, allowed the definition of three sub-regions (Figure 2.4.) for the production of quality wines entitled to DO Trás- os-Montes (Wines of Portugal, 2010).

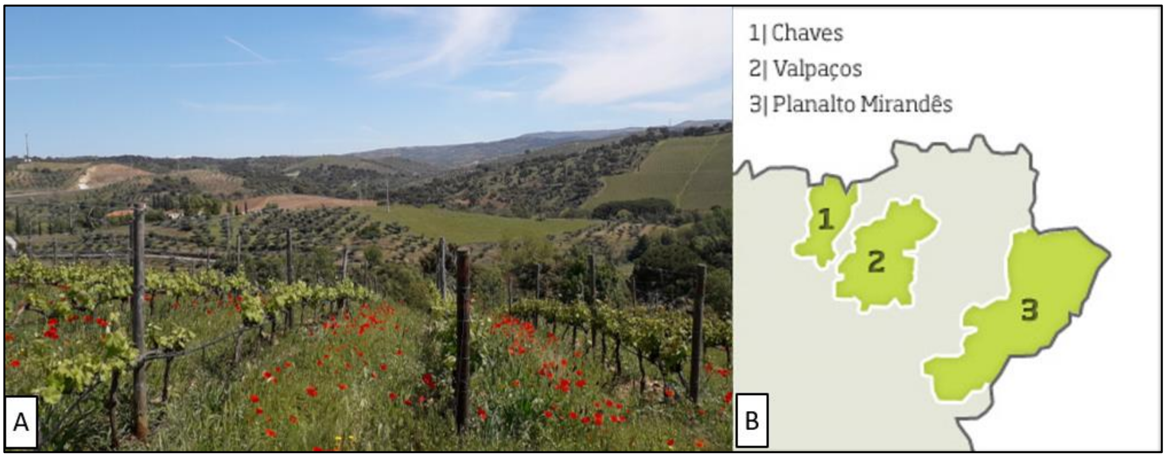

Figure 2.4. A – Trás-os-Montes Region's landscape; B - Map of the Region and its sub-regions. (Adapted from: Wines of Portugal, 2010).

2.1.4. Bairrada Region

Bairrada is a main plain region located in the center-coast of mainland Portugal (Figure 2.5.), between Minho and Alta Estremadura (IVV, 2018). The vines rarely exceed 120 meters in altitude and the orography is mainly plain, these characteristics combined with the proximity to the ocean has a climate temperate by a strong maritime influence with abundant rains and medium temperatures (Magalhães, 2008). The Bairradinos' soils, the clay-limestone soils and the sandy strips and soils are of different geological periods, with great diversity, sometimes to a small extent (Wines of Portugal, 2010).

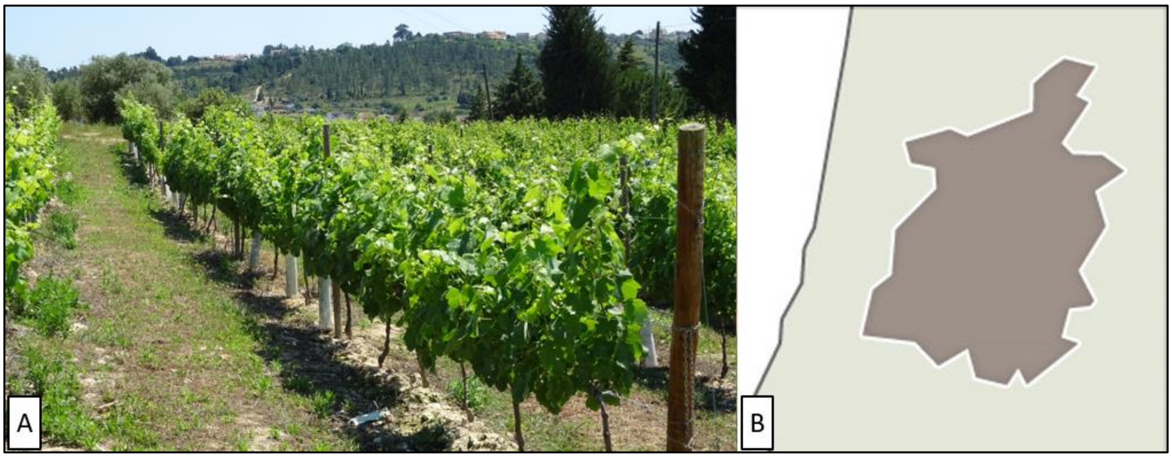

Figure 2.5. A - Bairrada Region's landscape; B - Map of the Region. (Adapted from: Wines of Portugal, 2010).

2.1.5. Beira Interior Region

The Beira Interior Region is located in the central part of continental Portugal and includes the districts of Castelo Branco and Guarda. It is characterized by an arduous and mountainous region (Simões et al., 2008). Beira Interior covers three wine-producing sub-regions: Castelo Rodrigo, Cova da Beira and Pinhel (Figure 2.6.). Influenced by the mountain air, the vines are cultivated at an altitude ranging between 400 and 700 meters. The climate has an extreme continental influence with impressive and day to day variations of temperature, short, hot and dry summers and prolonged and iced winters. The soils are in majority, of a granitic nature, with some presence of schist and sometimes, although less common, some sandy ascendency (IVV, 2018).

Figure 2.6. A – Beira Interior Region's landscape; B - Map of the Region and its sub-regions. (Adapted from Wines of Portugal, 2010).

2.1.6. Península de Setúbal Region

Located on the west coast of south Lisbon, it is in this wine region that the famous and much appreciated Moscatel de Setúbal and Moscatel Roxo de Setúbal are produced (Magalhães, 2008). This region can be divided into two completely different orographic zones: Palmela and Setúbal (Figure 2.7.). The first one in the South and Southwest, mountainous, these cut by valleys and hills, with altitudes between 100 and 500 m. The other, on the contrary, is flat, extending over an extensive plain by the river Sado (IVV, 2018). The climate is mixed, subtropical and Mediterranean (Magalhães, 2008). Influenced by the proximity of the sea, by the Tagus and Sado hydrographic basins, and by the mountains and hills that are located in the region, it has small thermal amplitudes and a rainfall stands between 400 to 500 mm (IVV, 2018). The soils vary among the region, alternating between the fine and deep sands of the flat land and the limestone and clay-limestone soils of the Serra da Arrábida (Wines of Portugal, 2010).

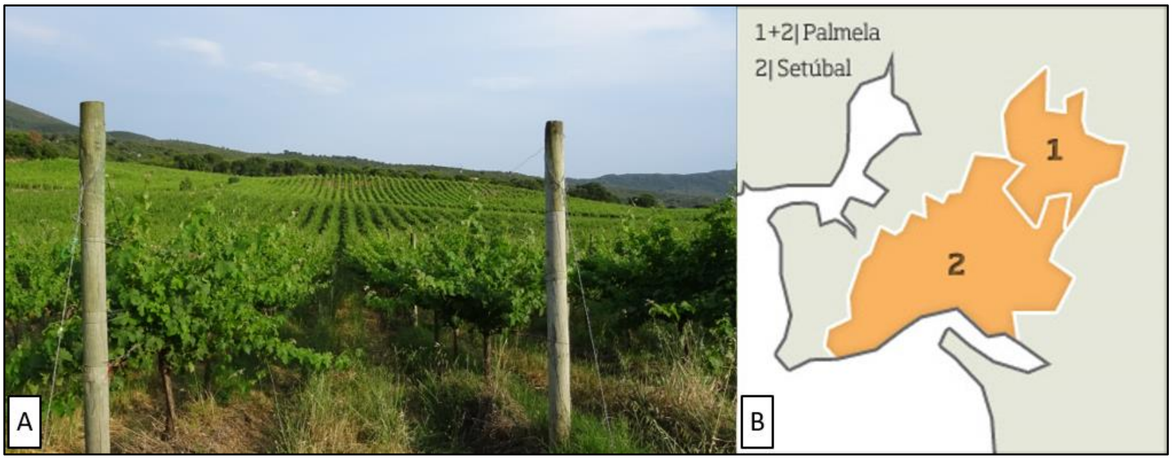

Figure 2.7. A – Península de Setúbal Region's landscape; B - Map of the Region and its sub-regions. (Adapted from: Wines of Portugal, 2010).

2.2. Sampling method

For each wine region, six vineyards (Figure 2.8.) considered representative of the region were selected, representing a total of 36 vineyards. The samplings occurred in 2019, in three different periods of the year. The first sampling date occurred in early July, the second in mid-September and the third on mid-October. For each vineyard two different strata were sampled: the herbaceous vegetation cover and the vine canopy.

For each vineyard and sampling date, randomly distributed over 1 ha, 10 samples of 10 consecutive sweeps were collected in the ground vegetation. Each sweep was performed by moving the entomological sweep 180 degrees. In the canopy of the vineyards 10 samples of 50 sweepings were collected. The content of the sweepings was emptied into a plastic bag, added ether and properly labelled and sealed. All samples were frozen at −20°C. Insects were separated under a stereoscopic microscope and conserved in ethanol 96% until further identification. All the adults of the order Coleoptera were identified up to the family taxonomic level. The individuals of the family Coccinellidae were the subject of a more detailed study in-depth identification to the species, this information having been dealt with in a chapter part (Chapter 4.). The taxonomic classification was based on appropriate keys and illustrations from Harde & Severa (1984), Raimundo & Alves (1986).

Figure 2.8. Sweeping techniques using entomologic net on the two strata levels: vegetation cover (A) and canopy (B).

2.3. Data analysis

The structure of the Coleoptera community was assessed in terms of abundance, richness, and diversity. It was decided to present the number of individuals by region, collection date, and strata, according to the purpose of the studied. The relative abundance was determined for the different families by the regions, calculating the frequency corresponding to each group, in relation to the total of observed specimens presented in the next section (Table 3.2.).

The Permutational Multivariate Analysis of Variance (PERMANOVA) was used to analyze the differences among coleopteran communities by strata (canopy and vegetation cover), collection dates (July, September, and October), and regions (Bairrada, Beira Interior, Douro, Setubal, Trás-os-Montes, and Vinhos Verdes) using the function adonis2 from the package "vegan" (Oksanen et al., 2019) in R software (R core Team, 2020). The square root of the abundance matrix was used for minimizing the influence of the most abundant groups. Then, the Bray-Curtis dissimilarity matrix was used as response and the strata, the dates, and the regions as explanatory variables. The main effects and three-way interactions were analyzed, and permutations were constrained by plot, being in total 999 permutations. In order to analyze which levels of the significant explanatory variables and/or interactions were significantly different, a pairwise comparison for each group level with Bonferroni correction for multiple testing was performed using pairwise permutational MANOVA with the function pairwise.perm.manova from the "RVAideMemoire" package (Hervé, 2020). Following the 'marginality principle' when non-null-interactions stood out, the main effects were not analyzed (Nelder, 1977).

The variance among communities grouped by strata-date and by strata-region were tested using *betadisper* function in the "vegan" package followed by a permutation test for homogeneity of multivariate dispersions using the *permutes* function in the same package (Xia et al., 2018). When differences were found, pairwise differences between groups were checked using a Tuckey's HSD test using the *TukeyHSD* function. Coleopteran communities by strata and date were visualized using nonmetric multidimensional scaling (NMDS) (999 permutations) plots, after grouping data by plot (metaMDS function from "vegan" package in R). Two plots were generated using the NMDS analysis for Coleopteran abundance by the interaction of strata and dates and another one for the interaction of regions and dates.

To visually represent the total diversity in strata collected, family accumulation curves were constructed for both canopy and vegetation cover, based on the number of individuals captured using the vegan package algorithm (Oksanen et al., 2019). To measure the diversity of species in the area of study, were used three diversity indexes: Shannon-Wiener Diversity Index (H'), Simpson Diversity Index (C) and Pielou Equability Index (J').

The most used index is the Shannon-Wiener index (H') and it gives greater importance to rare species (Magurran, 1988). The higher the Shannon-Wiener index is the greater is the species diversity of the study plot. The index is obtained by the equation:

$$H' = \frac{\left[N \ln(N) - \sum_{i=1}^{S} n_i \ln(n_i) \right]}{N}$$

Where:

S = total number of species;

ni = number of individuals of species i;

N = total number of individuals sampled;

ln = Napierian logarithm.

The Simpson index (C) refers to the probability of two individuals randomly picked from a community belonging to the same species (Brower & Zarr, 1984). The estimated value of C ranges from zero to one, and for values close to one, diversity is considered greater (Barros, 2007). It is given by the formula:

$$C = 1 - \frac{\sum_{i=1}^{S} n_i(n_i - 1)}{N(N-1)}$$

Where:

ni = number of individuals of species i;

N = total number of individuals sampled;

S = number of species.

The Pielou Equability Index (J') is derived from the Shannon diversity index and allows to represent the uniform distribution of individuals among existing species (Pielou, 1966). It ranges from 0 to 1, where 1 represents the maximum diversity, that is, all species are equally abundant, given by the formula:

$$J = \frac{H'}{H\max.}$$

Where:

H' = Shannon-Weaver diversity index;

Hmáx= ln(S)

S = total number of species sampled.

Biodiversity indices, accumulation curves and differences among Coccinellidae communities were analyzed using the same procedure used for Coleopteran families.

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Chapter 3.

Abundance and diversity of the order Coleoptera families in the canopy and vegetation cover of vineyards from different wine regions of Portugal

Abundance and diversity of the order Coleoptera families in the canopy and vegetation cover of vineyards from different wine regions of Portugal

Abstract

Insects of the order Coleoptera play an essential role in managing and enhancing the sustainability of agrarian ecosystems by supporting relevant ecosystem services. Currently, they are the largest group of insects ever described and they are present in almost all agricultural environments. In the vineyard, Coleoptera may occupy different ecological niches, highlighting the predatory action of some families against important vine pests. In this sense the present study aimed to evaluate the abundance and diversity of Coleoptera's families in the canopy and vegetation cover of vineyards from different Portuguese Wine Regions, namely, Bairrada, Beira Interior, Douro, Península de Setúbal, Trás-os-Montes and Vinhos Verdes. For this, in 2019, on three different dates (July, September and October), the vegetation cover of 36 vineyards scattered in the different wine regions, six per region, were sampled. For each plot, 20 samples were collected, ten in the canopy and ten on the vegetation cover, using the sweeping technique. A total of 2954 individuals from 36 families were collected. The most numerous Coleoptera families in order of importance were Chrysomelidae, Coccinellidae and Latridiidae. Results points toward a higher abundance of Coleoptera families at the Douro Region. Mostly were concentrated in July and at the vegetation cover, for all the Wine Regions. Vegetation cover was found to have a great importance in Coleoptera refugee and performance shelter as a microhabitat within the vineyards. Diversity index of Coleoptera indicates a higher diversity at Douro, in July. Further studies on pests and natural enemies' dynamics, the use of different variables and increase sampling dates should address non explained patterns in this study.

Keywords: entomofauna; auxiliary fauna; grapevine; vegetation cover; ecosystem services.

3.1. Introduction

The implication of the vines on the Portuguese agriculture, economy and society is widely known. Portugal is one of the oldest and strongest nations in the winery culture and this tradition performs an important role in the Portuguese rural economy and still nowadays a dominant element of the countrywide agricultural landscape, with economic, environmental, technological and, social effects (Simões, 2003). Through time, the intensification of vineyards changes the diversity and microclimate of the ecosystem, creating new edaphic conditions that may favor some pests (Altieri & Nicholls, 2002). It is crucial to comprehend the whole management of the viticultural environment, and it can only be accomplished if considered the whole development that takes into consideration both ecological pest knowledge, their devastation potential, and adoption of the appropriate measures for maintaining the natural balance within the vineyard's ecosystem (Enoiu, 2013).

The vine can be a target of different kinds of pests, that may affect its production depending on the situation of the vineyard, region and edaphoclimatic conditions (Amaro, 2004). The antagonists, also referred to as natural enemies or beneficial, are the organisms that act as regulatory agents for pest populations, often preventing them from developing and inflicting more damage (Coutinho, 2007). Predators and parasitoids naturally occurring in vineyards are the main natural biotic agents limiting pest populations harmful to cultivation (Portugal et al., 2017). In these beneficial arthropods, the order Coleoptera stands out for its high taxonomic and functional variety (Bouchard, 2014), with an international description of about 350000 species (McHugh & Liebherr, 2009).

Along with basic morphological characters, the presence of elytra and the position of the abdominal spiracles which, opening in an air chamber formed between the elytra and the abdomen, protect them against desiccation and allow the presence of several species in extremely dry places. The beetles have a chewing-type mouthpiece (McHugh & Liebherr, 2009). This type of device not only allows individuals of this order to break and grind food but also presents adaptive modifications that turn them into a skilled group in the use of all food resources available in nature (Marinoni, 2001).

Coleoptera occupies various ecological niches (Brown et al., 2015; Marinoni, 2001) with diverse feeding habits, eating plant and animal resources, including herbivores and predators, as well as fungi, detritivores, and some parasites (Brown et al., 2015; Marinoni, 2001; McHugh & Liebherr, 2009). This range of trophic groups makes Coleoptera an effective performer of ecological processes that are central to the functioning of these ecosystems, including the decomposition of organic matter, seed dispersal, conservation of soil structure, nutrient cycling, and regulation of pests populations of the vineyard (Balog et al., 2011; Marinoni, 2001; McHugh & Liebherr, 2009; Obrycki & Kring, 1998).

The taxonomic level of Coleoptera usually follows the same feeding habits within a family, minimizing sometimes efforts in identification (Silva & Silva, 2011). Marinoni (2001) suggests five trophic groups for Coleoptera: herbivores and algivores as primary consumers; fungivores, detritivores and carnivores as consumers secondary or higher level, described more precisely in section 1.7.

The objectives of the present study were to evaluate patterns of diversity of the Coleoptera families in Portugal for different dates (July, September and October) and strata (canopy and vegetation cover) in several important wine regions.

3.2. Material and methods

The study area, sampling method and data analysis are detailed described in Chapter 2. The individuals were identified with a stereoscopic microscope, acknowledged its taxonomic family level and grouped into guilds according to Marinoni (2001) classification. The taxonomic classification was based on appropriate keys and illustrations from Harde & Severa (1984).

3.3. Results and discussion

In total, 2954 individuals of the order Coleoptera were collected, belonging to 36 families (Table 3.1.). The most frequent families were Chrysomelidae with 753 individuals, that represents 25.49% of the total individual, Coccinellidae with 325 individuals, representing 11%, and Latriididae with 301 individuals (10.19%) (Figure 3.1). The least frequent families were Throscidae with two individuals and Catopidae and Scarabaeidae with only one individual. Regarding to Coleoptera trophic groups, a great representation of herbivorous was observed with the most representative families being Bruchidae and Phalacridae. Trophic groups of each family are specified at table 3.3. in the supplementary material.

Figure 3.1. Specimens of the most abundant families of Coleoptera. A – Chrysomelidae; B – Coccinellidae; C – Latridiidae.

The members of the family Chrysomelidae are phytophagous and are the largest Coleoptera family in the world (Arnett et al., 2002; Audino et al., 2007). The adults feed on living plant material, usually consuming aerial parts, sometimes leaves or various flower parts, including pollen (Arnett et al., 2002). As many species regularly generate large populations have become a serious horticultural or agricultural pest, however, many species have been used successfully in biological weed control (Audino et al., 2007). In a study that occurred in Canada, Chrysomelidae adults were found to feed on the vine's foliage but not in the berries. They were not associated with the vine itself, but rather with weeds growing within the vineyards (Lesage et al., 2008), which agrees with the results found in this study.

The second most abundant family was the Coccinellidae, which are well known for its predaceous nature and its importance on the natural limitation of crop enemies' populations (Audino et al., 2007; Gonçalves et al., 2013; Enoiu et al., 2013). The occurrence of predaceous Coccinellidae may be associated not only with the availability of food, but also with quality of the food (Hodek, 1967). Their life cycle can be synchronized to the prey's and Coccinellidae can change their habitat while searching for food. This is particularly true for the temporary incidence of the aphidophagous Coccinellidae (Takahashi & Naito, 1984; Hodek, 1967) and they may be influenced by the suitability of their diet (Bouvet et al., 2019). Dixon (2000) believes that the number of species largely depends on the number of preys.

Table 3.1. Total number of individuals (N) and frequency (%) of Coleoptera families collected on the canopy and vegetation cover by region: Bairrada (B), Beira Interior (BI), Douro (D), Península de Setúbal (S), Trás-os-Montes (TM) and Vinhos Verdes (VV).

Families	Canopy												Vegetation cover											
	B		BI		D		S		TM		VV		B		BI		D		S		TM		VV	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Anobiidae															2	0.40			1	0.20	2	0.40		
Anthicidae					1	0.50	1	0.50					1	0.04	3	0.13	7	0.29	8	0.33	4	0.17	1	0.04
Bruchidae	7	0.22	7	0.22	4	0.13	13	0.41	1	0.03			27	0.14	19	0.10	46	0.23	36	0.18	62	0.31	9	0.05
Buprestidae			5	0.63					3	0.38			1	0.05	3	0.14	6	0.27			12	0.55		
Byrrhidae																	4	1.00						
Cantaridae	1	0.13			4	0.50			1	0.13	2	0.25					2	0.20					8	0.80
Carabidae					3	1.00							2	0.03	6	0.08	42	0.56	3	0.04	20	0.27	2	0.03
Catopidae											1	1												
Cerambycidae			1	1											2	0.22	6	0.67					1	0.11
Chrysomelidae	48	0.33	21	0.14	40	0.28	15	0.10	8	0.06	13	0.09	64	0.11	101	0.17	220	0.36	52	0.09	121	0.20	50	0.08
Clambidae	1	0.50	1	0.50									2	0.40	1	0.20					1	0.20	1	0.20
Coccinellidae	7	0.18	5	0.13	19	0.50	2	0.05	3	0.08	2	0.05	30	0.10	19	0.07	135	0.47	38	0.13	62	0.22	3	0.01
Cryptophagidae					4	1.00									4	0.29	10	0.71						
Cucujidae			1	0.11	7	0.78	1	0.11							11	0.85	1	0.08			1	0.08		
Curculionidae	9	0.29	3	0.10	12	0.39	3	0.10	3	0.10	1	0.03	12	0.08	46	0.29	62	0.39	5	0.03	12	0.08	22	0.14
Cybocephalidae			1	1											2	0.50	2	0.50						
Dermestidae	1	1																			1	1.00		
Elateridae					1	0.50					1	0.50					1	0.33					2	0.67

Histeridae			1	1													1	1.00						
Lagriinae			1	0.10	9	0.90											8	0.80					2	0.20
Latridiidae	48	0.34	4	0.03	12	0.08	54	0.38	3	0.02	21	0.15	57	0.36	9	0.06	19	0.12	32	0.20	7	0.04	35	0.22
Malachiidae					10	0.77			2	0.15	1	0.08			20	0.37	32	0.59			1	0.02	1	0.02
Melyridae					3	0.60	2	0.40					1	0.01	34	0.24	97	0.69	6	0.04	2	0.01		
Mordellidae					12	0.63	6	0.32	1	0.05			11	0.13	4	0.05	19	0.22	38	0.44	14	0.16		
Nitidulidae	1	0.11			3	0.33	2	0.22			3	0.33	4	0.17	10	0.43	5	0.22	2	0.09			2	0.09
Oedemeridae					2	1.00									28	0.56	14	0.28			8	0.16		
Orthoperidae	2	1.00													5	0.42	1	0.08	3	0.25			3	0.25
Phalacridae	4	0.16	5	0.20	8	0.32	4	0.16			4	0.16	22	0.08	111	0.42	55	0.21	10	0.04	51	0.19	13	0.05
Scarabaeidae	1	1.00																						
Scirtidae													3	1										
Scolytidae					3	1.00							2	1										
Scraptiidae	2	0.22	1	0.11			4	0.44	2	0.22									1	1.00				
Serropalpidae	2	0.10	7	0.35			9	0.45			2	0.10											2	1.00
Staphylinidae	2	0.06	1	0.03	17	0.55	7	0.23			4	0.13	9	0.07	3	0.02	68	0.56	10	0.08	7	0.06	25	0.20
Tenebrionidae															2	0.67	1	0.33						
Throscidae			2	1																				
Total	136		67		174		123		27		55		248		445		864		245		388		182	

Adults and larvae of Latridiidae are fungivorous and feed on mycelia and spores. Member of this family can be found on the leaf litter and are most widespread in the wetter periods (Arnett et al., 2002). They can usually be found in areas where there is moisture and rot material (Harde & Severa, 1984).

For every sampled wine region there were high abundance of Coleoptera in the vegetation cover, representing 80.33% of the total recovered individual in the whole study, in comparison to the vine canopy. Vineyards inter-rows with cover crops are an important biodiversity hotspot within an agricultural ecosystem (Fiera et al., 2020). Overall, it is argued that vegetation adjacent to vineyards and inter-row cover crops frequently increases the abundance of natural enemies (Thomson & Hoffmann, 2009; Lundgren & Fergen, 2010). Fiera et al. (2020) refers that species richness and activity density of Coleoptera community is increased with vegetation cover. In addition, Lundgren & Fergen (2010) emphasize a greater availability of alternative resources, greater niche differentiation in complex habitats and favorable microclimates for the presence of these arthropods. More presence of insects in the vegetation cover can be explained since these insects benefit from additional sources of food, moisture, and shelter (Carmona & Landis, 1999).

The highest abundance of Coleoptera was observed in the Douro region, both in the canopy (174 individuals) and in the vegetation cover (864 individuals), when compared to the other regions. On contrary the less abundance was obtained in Vinhos Verdes region with 237 individuals, approximately 8% of the total number of Coleoptera (Table 3.1.). The Douro region itself was responsible for approximately 35.14% of the individuals collected. The Shannon (H') and Simpson (C) indexes also point towards higher values of diversity, especially in July for this region, both in the canopy and in the vegetation cover (Table 3.2.).

The analysis on diversity rates (Table 3.2.) of the canopy for Coleoptera families shows the lowest values for the Península de Setúbal, in September. Both diversity indexes show the same pattern. Within the vegetation cover, the lowest diversity indexes indicate October in Trás-os-Montes as the less diverse site. Temperature is instrumental in activating many insects and in determining development rates and adult reproductive activity (Wolda, 1988). July represents the end of spring and the beginning of summer, where the mild temperatures are more favorable to insect's reproduction, dispersion and metabolic rates are higher (Beck, 1983). Also, diapause is found to be mediated by photoperiod and humidity (Wolda, 1988; Danks, 1987), which suggests that active periods too. In this season, the adults of the first generation of arthropods begin to appear from the middle of June to the middle of July, and of the second generation from the middle of July until September (Hagen, 1962).

Table 3.2. Diversity indexes of Coleoptera for each sampling date and region. Shannon-Wiener Diversity Index (H'), Simpson Diversity Index (C) and Pielou Equability Index (J').

Region	Date	Canopy			Vegetation cover		
		H'	C	J'	H'	C	J'
Bairrada	July	1.79	0.77	0.78	2.03	0.84	0.82
	September	1.41	0.69	0.79	1.94	0.83	0.88
	October	1.38	0.67	0.66	1.72	0.72	0.69
Beira Interior	July	2.12	0.83	0.83	2.39	0.88	0.83
	September	1.59	0.75	0.82	1.72	0.72	0.72
	October	1.28	0.70	0.92	1.20	0.54	0.62
Douro	July	2.40	0.89	0.87	2.42	0.88	0.79
	September	1.89	0.83	0.91	1.70	0.74	0.74
	October	2.07	0.83	0.83	1.61	0.70	0.61
Setúbal	July	2.00	0.84	0.87	1.91	0.80	0.80
	September	0.81	0.32	0.39	1.61	0.74	0.78
	October	1.72	0.79	0.88	1.54	0.67	0.70
Trás-os-Montes	July	1.99	0.85	0.96	2.12	0.84	0.78
	September	1.21	0.66	0.88	1.48	0.63	0.62
	October	1.56	0.78	0.97	1.05	0.46	0.48
Vinhos Verdes	July	1.99	0.85	0.96	1.97	0.83	0.85
	September	1.09	0.60	0.78	1.89	0.81	0.86
	October	1.66	0.77	0.86	1.73	0.77	0.69

The PERMANOVA analysis showed that interaction among strata, date and region and the interaction between strata and region did not significantly influence the coleopteran community, however the interactions between date and region and between strata and date did it (Table 3.3.) After the Bonferroni correction, any of the pairwise comparisons between date and region was significant. The interaction between strata and date was responsible for the 2.7% of the differences (Table 3.3.).

Table 3.3. Differences among coleopteran communities by strata, collection dates, and regions according to the PERMANOVA analysis of Coleoptera families. Df – degrees of freedom; SS – sum of squares.

Variable	Df	SS	R^2	F	P value
Strata	1	2.144	0.039	9.111	0.001
Date	2	3.752	0.068	7.974	0.001
Region	5	4.537	0.083	3.857	0.001
Strata:Date	2	1.483	0.027	3.152	0.001
Strata:Region	5	1.648	0.030	1.401	0.051
Date:Region	10	4.475	0.082	1.902	0.001
Strata:Date:Region	10	1.986	0.036	0.844	0.852
Residual	148	34.820	0.635		
Total	183	54.845	1		

Further research should address the effect of variables not included in the analysis, such as precipitation, temperature, the management, or landscape structure, which could be potentially explain the patterns of the Coleoptera community. Erwin & Scott (1980) argue that Coleoptera's richness is influenced by humidity and temperature. Also, temperature limits biological activity in arthropods, such that low and high temperature and optimal temperature can be estimated for all major life processes (Roy et al., 2002). The variables not addressed in this study could have been the key for explaining such patterns.

The pairwise comparison for the levels of the strata and date interaction showed that the coleopteran community did not vary for the canopy in any date (all $p > 0.075$) and in September and October when compared with the herbaceous layer in September ($p = 0.57$ and 0.78 respectively). The coleopteran community in all the other comparisons were significantly different, i.e., (i) the coleopterans found in the herbaceous layer in the three dates, (ii) the found in the herbaceous layer in July and October and the three dates in the canopy and (iii) the found in the herbaceous layer in September and the canopy in July (p = 0.015 in all cases). This was partially corroborated by the NMDS (Figure 3.2.)

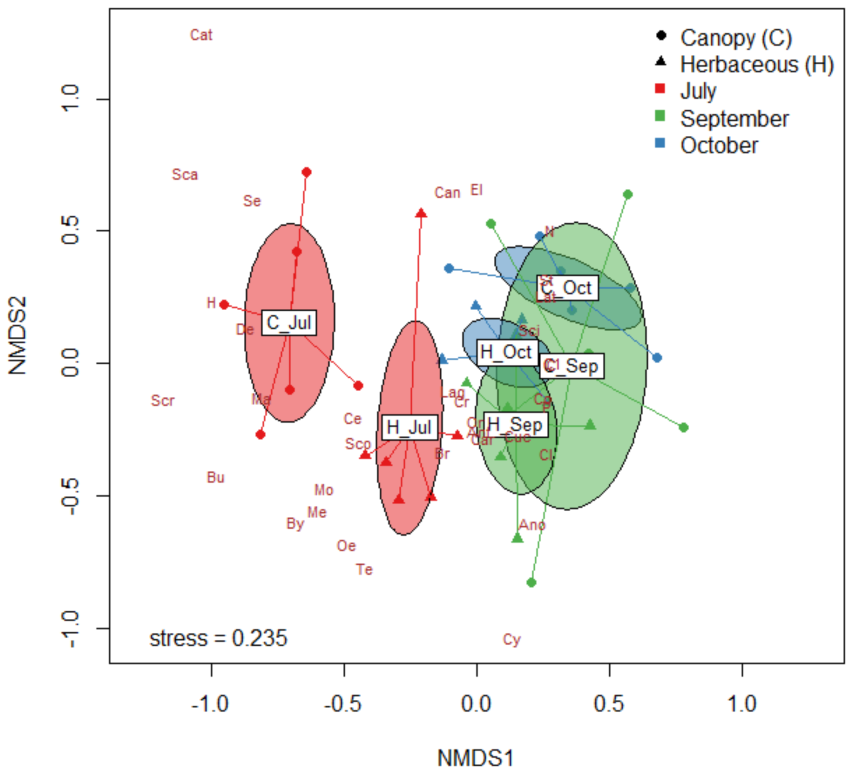

Figure 3.2. Non-metric multidimensional scaling (NMDS) analysis for Coleopteran abundance by the interaction of strata [canopy (C) and vegetation cover (H)], and date [July (Jul), September (Sep) and October (Oct)]. Sites are connected to the centroid in each group, and the 95% confident interval of each centroid is shown by shaded ellipses. Families: Anobiidae (Ano), Anthicidae (Ant), Bruchidae (Bru), Buprestidae (Bup), Byrrhidae (Byr), Cantaridae (Can), Carabidae (Car), Catopidae (Cat), Cerambycidae (Cer), Chrysomelidae (Chry), Clambidae (Clam), Coccinellidae (Coc), Cryptophagidae (Cryp), Cucujidae (Cuc), Curculionidae (Cur), Cybocephalidae (Cyb), Dermestidae (Der), Elateridae (Ela), Histeridae (His), Lagriinae (Lag), Latridiidae (Lat), Malachiidae (Mal), Melyridae (Mel), Mordellidae (Mor), Nitidulidae (Nit), Oedemeridae (Oed), Orthoperidae (Ort), Phalacridae (Pha), Scarabaeidae (Sca), Scirtidae (Sci), Scolytidae (Sco), Scraptiidae (Scra), Serropalpidae (Ser), Staphylinidae (Sta), Tenebrionidae (Ten) and Throscidae (Thr).

According to the permutation test for homogeneity, the variance of Coleoptera communities by strata and date was heterogeneous (F = 8.621, df = 5, p = 0.001), which could explain why the NMDS results did not completely corroborate the PERMANOVA results. The Tuckey HSD test showed that those differences were due to a lower distance from the centroid in the coleopterans from vegetation cover collected in October (Figure 3.3.). According to Carlos et al. (2019), species richness and abundance were observed to be higher in the minor rainy season (in this case of study, July) and less in major rainy season samples (October).

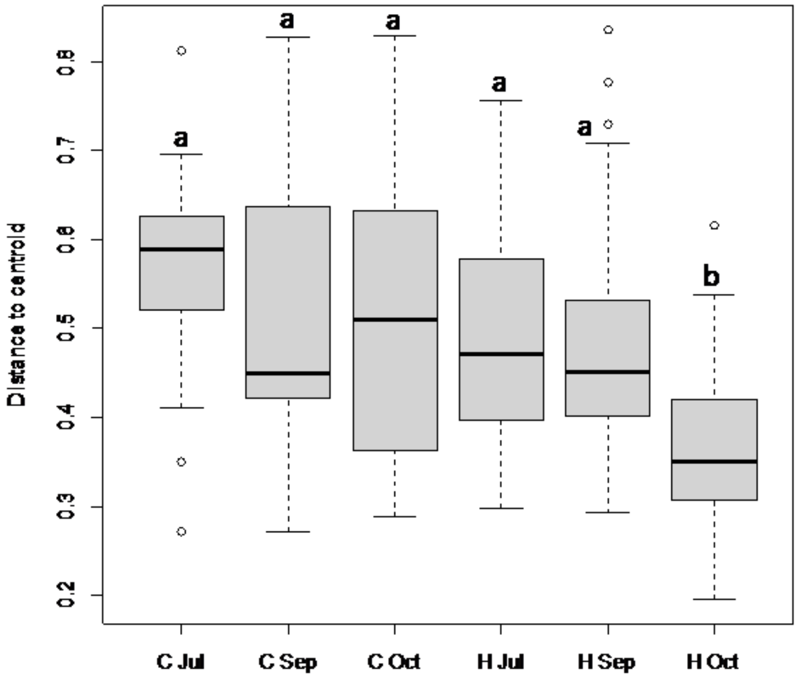

Figure 3.3. Variance of the coleopteran communities by strata (H- vegetation cover; C – canopy) and data (Jul – July, Sep –September, Oct – October) as the distance to the centroid of the Bray-Curtis distance matrix. Different letters indicate significant differences using a Tukey HSD test. Upper and lower hinges in the boxplot represent the first and third quartiles, error bar indicate 95% confidence intervals, and bar represents the median.

Regarding total diversity, the family's accumulation curves indicate a higher diversity in terms of richness for July, both in the canopy and in the vegetation cover (Figure 3.4.), for the same number of individuals collected. The stabilization of the curve at the expected mean family richness indicates that the sampling effort was enough to predict the richness. In July, a greater richness of the sampled families was observed. The role of temperature is associated in the growing rates, seasonal development, and ecological adaptations of insects (Beck, 1983) and has been discussed before in the present study.

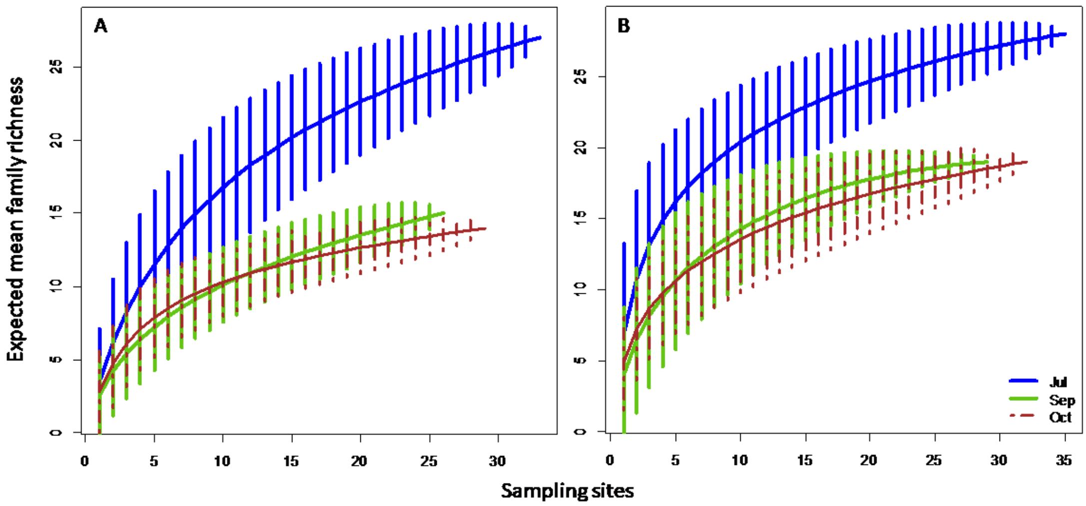

Figure 3.4. Coleoptera family's accumulation curves in the vineyard canopy (A) and in the vegetation cover (B) in July, September and October. Vertical lines indicated the standard errors.

3.4 Conclusion

In this study, among every Wine Region, Douro had a higher Coleoptera abundance. When compared to the vine's canopy, the vegetation cover showed a higher diversity. This result indicated that vegetation cover was found to be an important biodiversity hotspot in vineyards ecosystem. This finding was probably related with the existence of alternative resources, such foods and shelters. Niche differentiation and additional sources of food, moisture, and shelter at the vegetation interrow crops can perform more favorable conditions for the presence of these arthropods. Among the sampling dates, July had the maximum diversity rates, which can represent the end of spring and the beginning of summer, where the mild temperatures are more favorable to insect's reproduction, dispersion, and metabolic rates can be higher. However, it still necessary further research to address diversity patterns not explained by this study.

3.5. Supplementary material

Table 3.4. Total families of Coleoptera identified in all samples collected at the 36 sites studied in 2019 and its respectively feeding guilds and food preferences. Trophic groups: C – Carnivorous; D – Detritivores; F – Fungivorous; H – Herbivorous.

Family	Trophic Group	Observations	Source
Anobiidae	H	Agricultural pests;	Lima et al. (2010), Erwin & Scott (1980)
Anthicidae	C, D	Predators and scavengers;	Audino et al. (2007), Erwin & Scott (1980)
Byrrhidae	H	Mostly moss feeders and rarely feed on bryophytes;	Arnett et al. (2002)
Bruchidae	H	Spermophagous larvae, adults philophagous and agricultural pests;	Lima et al. (2010); Arnett et al. (2002)
Buprestidae	H	Xylophagous;	Lima et al. (2010), Audino et al. (2007)
Cantaridae	H, C	Polyniphagous, nectariphagous and predaceous larvae;	Audino et al. (2007), Erwin & Scott (1980)
Carabidae	C, H	Most predadors, but can be philophagous, or parasites;	Arnett et al. (2002); Audino et al. (2007)
Catopidae	D	Animal and vegetable remain;	Peck & Cook (2002)
Cerambycidae	H	Polyniphagous, carpophagous and stemborer larvae;	Fagundes et al. (2011); Audino et al. (2007)
Chrysomelidae	H	Mostly philophagous, but some polyniphagous and anthophagous;	Arnett et al. (2002); Audino et al. (2007); Marinoni et al. (2001)
Clambidae	F	Mycetophagous;	Lawrence et al. (2000)
Coccinellidae	C, H	Mostly predators of other invertebrates, also can be herbivorous;	Arnett et al. (2002); Audino et al. (2007); Marinoni et al. (2001)
Cryptophagidae	F, D	Mostly fungal hyphae, spores, and conidia while some are saprophagous;	Arnett et al. (2002)
Cucujidae	C	Adults and larvae are reported to be predacious;	Fagundes et al. (2011); Arnett et. al. (2002)
Curculionidae	H	Philophagous, carpophagous, anthophagous, polyniphagous;	Fagundes et al. (2011); Audino et al. (2007)
Cybocephalidae	H, F	Xylophagous, stemborers and fungivores;	Arnett et al. (2002)

Dermestidae	H, F, D	Pollen and mold feeders, decaying organic matter and larvae feed on rotten wood;	Arnett et al. (2002)
Elateridae	C, H	Predators, also rhizophagous and considered agricultural plagues;	Lima et al. (2010); Audino et al. (2007)
Histeridae	C	Predators of Diptera and Coleoptera larvae;	Fagundes et al. (2011); Audino et al. (2007)
Lagriinae	H	Rotten wood, decaying vegetation;	Arnett et al. (2002)
Latridiidae	F	Spores, molds and considered pests beacuse of products stored in humid locations;	Audino et al. (2007), Erwin & Scott (1980)
Malachiidae	C, D	Scavengers and predators, feeding primarily on small arthropods, pollen and nectar;	Marinoni et al. (2001); Arnett et al. (2002)
Melyridae	C, H	Feed on both plant and animal material. Many feeds on pollen or flowering plants;	Marinoni et al. (2001); Arnett et al. (2002)
Mordellidae	H, D	Rotten wood, decaying vegetation and herbaceous plants;	Fagundes et al. (2011); Audino et al. (2007)
Nitidulidae	C, F, H, D	Animal and vegetable remain, fungivore, predators and leafy decomposing material;	Fagundes et al. (2011); Audino et al. (2007)
Oedemeridae	H, D	Carpophagous, phylophagous and decaying wood;	Arnett et al. (2002)
Orthoperidae	D, F	Decomposing plant material and mycophagus (spores and mycelium);	Watson & Dallwitz (2003)
Phalacridae	H, F	Mostly herbivorous (Antophagous) and fungivorous;	Lima et al. (2010); Audino et al. (2007)
Scarabaeidae	H, D	Coprophagous, animal and vegetable remain and fruits;	Fagundes et al. (2011); Audino et al. (2007)
Scirtidae	D	Vegetable remains;	Epler (2010)
Scolytidae	H, F	Most species are strictly phytophagous;	Fagundes et al. (2011)
Scraptiidae	H	Feeds and reproduce on flowers;	Arnett et al. (2002)
Serropalpidae	H, F	Mostly fungivorous, but also xylophagous;	Arnett et al. (2002)
Staphylinidae	C, D, F	Predator, algivorous, fungivorous and Diptera pupa's parasites;	Fagundes et al. (2011); Audino et al. (2007)
Tenebrionidae	C, H, D, F	Vegetable remains, rotten wood, fungivores and some algivore. Only few predators;	Fagundes et al. (2011); Audino et al. (2007)
Throscidae	H, F	Generalist pollen and mold feeders;	Arnett et al. (2002)

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Chapter 4.

Abundance and diversity of the Coccinellidae (Coleoptera) species in the canopy and vegetation cover of vineyards from different wine regions of Portugal

Abundance and diversity of the Coccinellidae (Coleoptera) species in the canopy and vegetation cover of vineyards from different wine regions of Portugal

Abstract

The family Coccinellidae (Coleoptera) plays an essential role in managing and enhancing the sustainability of agrarian ecosystems by supporting relevant ecosystem services. In the vineyard, Coccinellidae may occupy different ecological niches, highlighting the predatory action of some species against important vine pests. In this sense the present study aimed to evaluate the abundance and diversity of Coccinellidae species in the canopy and vegetation cover of vineyards from different Portuguese Wine Regions, namely, Bairrada, Beira Interior, Douro, Península de Setúbal, Trás-os-Montes and Vinhos Verdes. To this end, in 2019, on three different dates (July, September and October), the vegetation cover of 36 vineyards, 6 per wine region, were sampled. For each plot, 20 samples were collected, ten in the canopy and ten on the vegetation cover, using the sweeping technique. A total of 315 Coccinellidae distributed in 22 species were collected. The most numerous Coccinellidae species in order of abundance were *Scymnus apetzi*, *Scymnus interruptus* and *Coccinella septempunctata*. Results pointed toward a higher abundance of Coccinellidae species at the Douro Region. Most specimens were concentrated in July and at the vegetation cover, for all the Wine Regions. Vegetation cover was found to have a great importance in Coccinellidae dispersion and performance shelter for species as a microhabitat within the vineyards. Diversity indexes of Coccinellidae species, at the Douro, were higher in the canopy at July and in the vegetation cover at September. Further studies on pests and natural enemies' dynamics, the use of different variables and increase sampling dates should address non explained patterns in this study.

Keywords: natural enemies; entomofauna; grapevines; biological control.

4.1. Introduction

The family Coccinellidae are beetles from the Order Coleoptera, commonly known as ladybugs, that consists of over 5000 described species. They are well-known group of small, oval, convex insects and their elytra are strong and often brightly colored (Majerus, 2009). The majority of species have great economic importance due to their predaceous nature and the role they play in the natural limitation of the populations of enemies of agricultural crops (Audino et. al, 2007; Gonçalves et al., 2013; Enoiu et al., 2013; Borror & DeLong, 1969; Hagen, 1962). Coccinellidae are, either in the adult or immature state, mostly predators of aphids, mealybugs and mites (Majerus, 2009; Obrycki & Kring, 1998). However, while most Coccinellidae are predaceous, some are phytophagous and can be injurious to the crops (Giorgi et al., 2009).

Coccinellidae present wide diversity in habitat and dietary preferences and specificity (Sloggett & Majerus, 2000). The abundance, dispersion, and pest management of this family are influenced by their set of natural enemies (Hodek, 1996; Hodek, 1967).

In general, different species of Coccinellidae can be divided into generalists and specialists based on their diet and variety of habitats (Majerus, 2009). For example, to aphids, most species feed on a variety of aphid species and move from one host plant to another as aphid colonies increase and decrease (Majerus, 2009). However, some species have a specialized diet and therefore, they are restricted to specific habitats where their food occurs (Hodek, 1996). Besides the aphid feeders, the specialization occurs for many of the species with different diet targets, such as coccids, mildew, plant foliage or pollen and flower nectar as the main food (Giorgi et al., 2009; Majerus, 2009; Weber & Lundgren, 2009). Unlikely other insects, the two feeding stages (larvae and adults) frequently have the same feeding habits (Majerus, 2009).

Perhaps equally important are intraguild predation (including other Coccinellidae) that regularly consume Coccinellidae eggs and larvae (Weber & Lundgren, 2009). Pressure from intraguild competitors and other natural enemies influence on Coccinellidae's predation capacity on many scales (Riddick et al., 2009). Intraguild predation is not found to be a negative force within the community, on the other hand, they respond to and affect their communities in beneficial ways, generally to reduction of herbivore populations (Snyder, 2009). This is explained perhaps because of the niche spatial and temporal differences that reduce the encounters among species. The best explanation would be that Coccinellidae may be an essential complement to others from the same community, contributing to improving biological control with greater predator's diversity (Snyder, 2009; Weber & Lundgren, 2009).

In terms of biology and life cycle, Coccinellidae hibernate as adults, almost always in groups, in different shelters, protected from humidity (Coutinho, 2007). In regions with winter and summer seasons, some species spend the winter as dormant adults (Majerus, 2009). Others, such as *Coccinella septempunctata* (Linnaeus), can migrate to distant mountain areas, where they spend the winter in diapause, and from where they return in the spring to the cultivated areas. This migration, among other objectives, will escape the natural predators that ladybugs also have (Coutinho, 2007). In case of wet and dry season climates, like tropical regions, many Coccinellidae can be dormant during the dry season and, consequently, starting to reproduce on the wet season, when the food became more available (Majerus, 2009). The same pattern occurs in place with hot summers, with some species being dormant in the hottest months and sometimes having a second reproduction period in the fall (Majerus, 2009).

The present study aims to evaluate the abundance and diversity of Coccinellidae species on the canopy and vegetation cover of six different Portuguese Wine Regions in July, September and October.

4.2. Materials and methods

The study area, sampling method and data analysis are detailed described in Chapter 2.

The individuals were identified with a stereoscopic microscope. Acknowledged its taxonomic species level based on appropriate keys and illustrations from Harde & Severa (1984), Raimundo & Alves (1986).

4.3. Results and discussion

In total, 315 individuals of the family Coccinellidae were collected, belonging to 22 different species. Table 4.1. shows the list of Coccinellidae species captured in the sampled regions. The maximum species richness (15 species) was observed in Douro Region, followed by Bairrada, with 9 species identified. As far as concerns the species abundance, *Scymnus apetzi* (Mulsant) had maximum abundance with 96 individuals (30.48% of the total), the second was *Scymnus interruptus* (Goeze) with 61 individuals (19,37%), in third place *Coccinella septempunctata* (Linnaeus) with 43 individuals (13,65%) and *Scymnus subvillosus* (Goeze) with 41 individuals (13%) (Figure 4.1.). The least abundant species were *Adalia bipunctata* (Linnaeus)*, Adalia decempunctata* (Linnaeus)*, Nephus helgae* (Frürsch), *Oenopia conglobata* (Linnaeus) and others with only one individual (Table 4.1.).

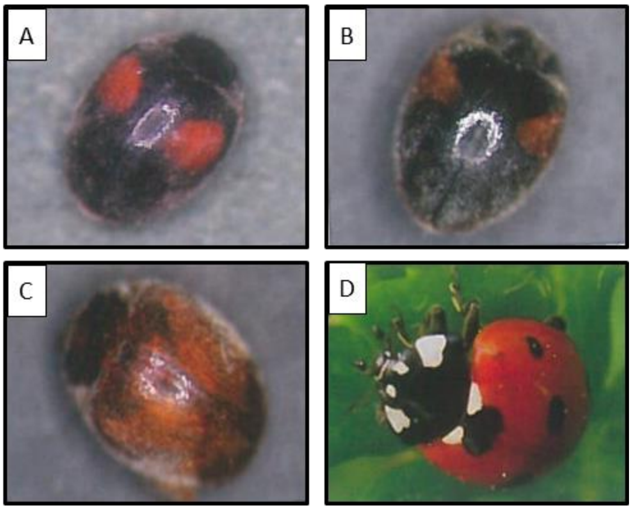

Figure 4.1. Most numerous species of Coccinellidae found in the study. A - *Scymnus apetzi* (Mulsant); B - *Scymnus interruptus* (Goeze); C - *Scymnus subvillosus* (Goeze); D - *Coccinella septempunctata* (Linnaeus). Adapted from Gonçalves et al., (2010).

Among the four most abundant Coccinellidae, three species belonging to the genus *Scymninae*: *S. apetzi*, *S. interruptus* and *S. subvillosus*. *Scymninae* are voracious predators of scales and aphids (Giorgi et al., 2009), and leafhopper, although of reduced potential efficacy (Carlos et al., 2005). Other species like *C. septempunctata* are found to be mainly aphidophagous (Iperti, 1999).

Table 4.1. Species of Coccinellidae and total number of individuals collected on the canopy and vegetation cover by region: Bairrada (B), Beira Interior (BI), Douro (D), Península de Setúbal (S), Trás-os-Montes (TM) and Vinhos Verdes (VV).

Species	Canopy						Vegetation cover					
	B	BI	D	S	TM	VV	B	BI	D	S	TM	VV
Adalia bipunctata											1	
Adalia decempunctata								1				
Anisosticta novemdecimpunctata									2			
Coccidula rufa							2		2			
Coccinella novemnotata									3			
Coccinella septempunctata	1	5	3		2	1		13	13		5	
Hippodamia variegata								1	9		4	1
Nephus binotatus									3	1		
Nephus helgae												1
Nephus redtenbacheri									5			
Oenopia conglobata				1								
Propylaea quatuordecimpunctata	1											
Rhizobius chrysomeloides										1		
Rhizobius forestieri							2					
Rhizobius litura									1			
Scymnus abietis									3			
Scymnus apetzi			8				2	2	33	12	39	
Scymnus interruptus	2		2		1		4		42	4	5	1
Scymnus mediterraneus							4		1	1	2	
Scymnus rufipes									2			
Scymnus subvillosus			4				3	1	14	18	1	
Stethorus punctillum	3		2	1		1	10			1	1	
Total	7	5	19	2	3	2	27	18	133	38	58	3

The present study shows a larger number of Coccinellidae in the vegetation cover than on the canopy in all the Wine Regions. This result agrees with what described by other authors about the importance of herbaceous cover crops for predators (Canovai & Loni, 2019; Thomson & Hoffman, 2013). The presence of Coccinellidae in vineyards is observed to be higher when there is adjacent woody vegetation and/or vegetation in between the lines (Thomson & Hoffmann, 2013). It can be explained since the inter-rows vegetation cover provides additional sources of food, moisture and shelter (Carmona & Landis, 1999; Lundgren & Fergen, 2010). Further research about the landscape structure would be interesting to evaluate these patterns.

At the Douro Region, both in the canopy (19 individuals) and in the vegetation cover (133 individuals) (Table 4.1.) had maximum abundance. The number of individuals recovered in the Douro region represents approximately 48.25% of the total specimens collected. In this region, both Shannon (H') and Simpson (C) indexes points towards higher values of diversity, in the canopy at July and in the vegetation cover at September (Table 4.2.). These results agree with Carlos et al. (2005), which study at the Douro indicates the months of July and August, as the time of greatest activity of the genus *Scymnus*, the genus that dominated in the present study.

July also represents the suitable temperatures and dry season in temperate regions, where the favorable climate leads to insects reproduction, dispersion and metabolic rates are found to be higher (Beck, 1983). Besides temperature, photoperiod and humidity can influence on insects ecological dynamics (Wolda, 1988; Danks, 1987), which are also responsible for more active periods too. At temperate climates, at this period of the year the adults of the first generation of insects begin to appear from the middle of June to the middle of July, and of the second generation from the middle of July until September (Hagen, 1962).

The region with minimum abundance of Coccinellidae were Vinhos Verdes, with 5 individuals (1,60%). These results agree with what was referred by Batish et al. (2007), which was that major changes induced by economic policy occurred in the Vinho Verde Wine Industry during the 1990’s. For easy mechanization farmers were encouraged to plant "cordão" monoculture system. A study carried out between 1997 and 1999 showed that the number of insects collected from vineyards in agroforestry systems (arjoado) were larger than in monoculture vineyards. In arjoado systems, insect biodiversity was higher, probably due to the spatial heterogeneity and greater complexity of agroforestry (Batish et al, 2007). This agrees with Dixon (2000), which believes that the behavior of a predator species are largely depends on the number of preys. If there is low biodiversity of prey, it is likely to have low incidence of predators.

The vegetation cover plants sustained a higher population of Coccinellidae than the same plots’ canopy. Although the Vinhos Verde’s Region had the lowest number of individuals gathered (only two), the Equitability index (J’) shows the highest value of diversity in July. This is due to the specimens collected belonged to different species, which indicates a high level of diversity. At the vegetation cover, the highest Equitability Index points toward Bairrada (October and September), Vinhos Verdes (September) and Beira Interior (September). The same pattern is observed in there (Table 4.2.).

Table 4.2. Diversity indexes of Coccinellidae for each sampling date and region. Shannon-Wiener Diversity Index (H'), Simpson Diversity Index (C) and Pielou Equability Index (J').

Region	Date	Canopy			Vegetation cover		
		H'	C	J'	H'	C	J'
Bairrada	July	1.01	0.61	0.92	1.38	0.71	0.86
	September	0	0	0	1.10	0.67	1.00
	October	0	0	0	0.69	0.50	1.00
Beira Interior	July	0	0	0	0.51	0.26	0.46
	September	0	0	0	1.10	0.67	1.00
	October	0	0	0	0	0	0
Douro	July	1.52	0.76	0.94	1.83	0.79	0.83
	September	0	0	0	2.00	0.84	0.91
	October	0.50	0.32	0.72	1.09	0.59	0.79
Setúbal	July	0	0	0	0.27	0.14	0.39
	September	0	0	0	1.15	0.61	0.83
	October	0	0	0	0	0	0
Trás-os-Montes	July	0	0	0	0.86	0.41	0.53
	September	0	0	0	1.04	0.63	0.95
	October	0.00	0.00	0.00	0.95	0.56	0.86
Vinhos Verdes	July	0.69	0.50	1.00	0	0	0
	September	0	0	0	0.69	0.50	1.00
	October	0	0	0	0	0	0

PERMANOVA analysis showed that the Coccinellidae community was significantly influenced by the interaction between strata and date and date and region but not by the interaction among strata, date and region neither the interaction between strata and region (Table 4.3.). Regarding the interaction strata:date after the Bonferroni correction the pairwise comparison showed that the Coccinellidae community was significantly different between the canopy in July and October and the canopy in October and the herbaceous layer in all the dataes ($p < 0.03$ in all cases) and did not differ for the other comparisons between strata and date ($p > 0.165$ in all cases). Regarding the interaction region:date 66% of the 153 comparisons were significant ($p < 0.048$ in all cases). Possible patterns need to be analyze in further studies.

Table 4.3. Differences among Coccinellidae communities by strata, collection dates, and regions according to the PERMANOVA analysis of Coccinellidae species. Df – degrees of freedom; SS – sum of squares.

Permanova	Df	SS	R^2	F	Pr(>F)
Strata	1	0.746	0.025	2.295	0.028
Date	2	2.013	0.068	3.096	0.001
Region	5	3.343	0.113	2.056	0.003
Strata:Date	2	1.239	0.042	1.905	0.026
Strata:Region	5	1.340	0.045	0.824	0.799
Date:Region	9	4.801	0.163	1.641	0.003
Strata:Date:Region	4	0.754	0.026	0.580	0.974
Residual	47	15.281	0.518	NA	NA
Total	75	29.518	1	NA	NA

According to the permutation test for homogeneity, the variance of Coccinellidae species communities by strata and date were heterogeneous ($F = 9.593$, $df = 5$, $p < 0.001$). The Tuckey HSD test (Figure. 4.2.) showed that the heterogeneity was due to a lower distance from the centroid in the Coccinellidae collected in the canopy in October. This was explained because only one species was found in the canopy in October.

The permutation test for homogeneity showed that the variance of Coccinellidae species communities by region and date was homogeneous ($F = 1.605$, $df = 17$, $p = 0.115$). Factors such as rainy and dry seasons, climate, the use of pesticides and/or landscape structure, could explain the observed patterns on the community of Coccinellidae (Carlos et al., 2019; Iperti, 1999; Erwin & Scott, 1980; Roy, Brodeur & Cloutier, 2002). Thereby, the variables not considered in the present work could have addressed the observed patterns.

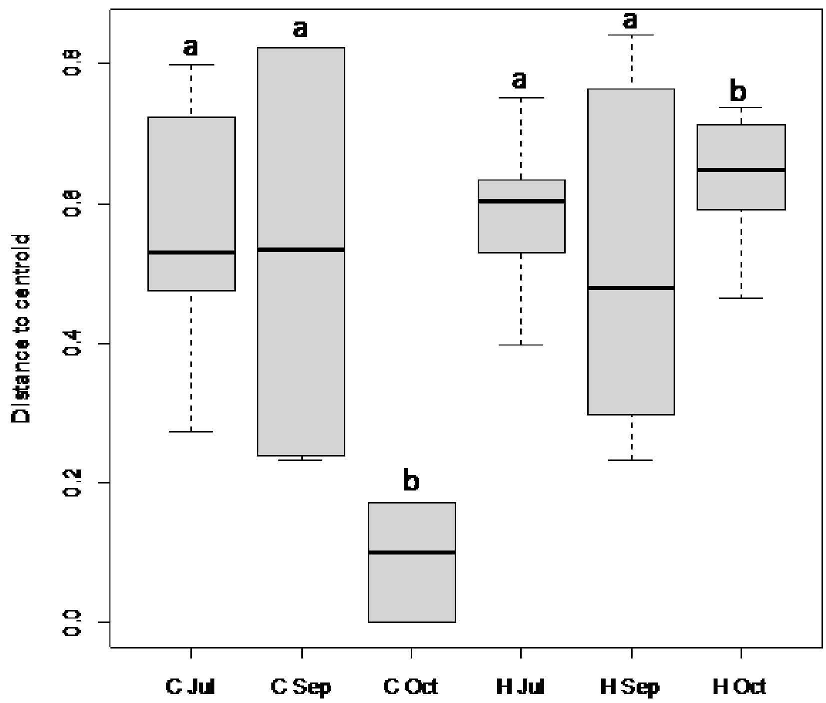

Figure 4.2. Variance of the Coccinellidae species by strata (H- herbaceous layer; C – canopy) and data (Jul – July, Sep –September, Oct – October) as the distance to centroid of the Bray-Curtis distance matrix. Different letters indicate significant differences using a Tukey HSD test. Upper and lower hinges in the boxplot represent the first and third quartiles, error bar indicate 95% confidence intervals, and bar represents the median.

The calculation of the species accumulation curve (Figure 4.3.) leads to the statement that, for the different sampling dates in the canopy, the month of July is the one that registers the higher number of species, in agreement with the previously commented about the diversity index for that same date and strata. In these accumulation curves for the canopy samples, only July and September are represented because in October there were no individuals of Coccinellidae. The accumulation curve of the vegetation cover shows that in October there was a greater number of species collected, exceeding the month of July. None of the months' curves were stabilized, so the estimated sampling effort required points towards the necessity to increase the number of samples at all dates.

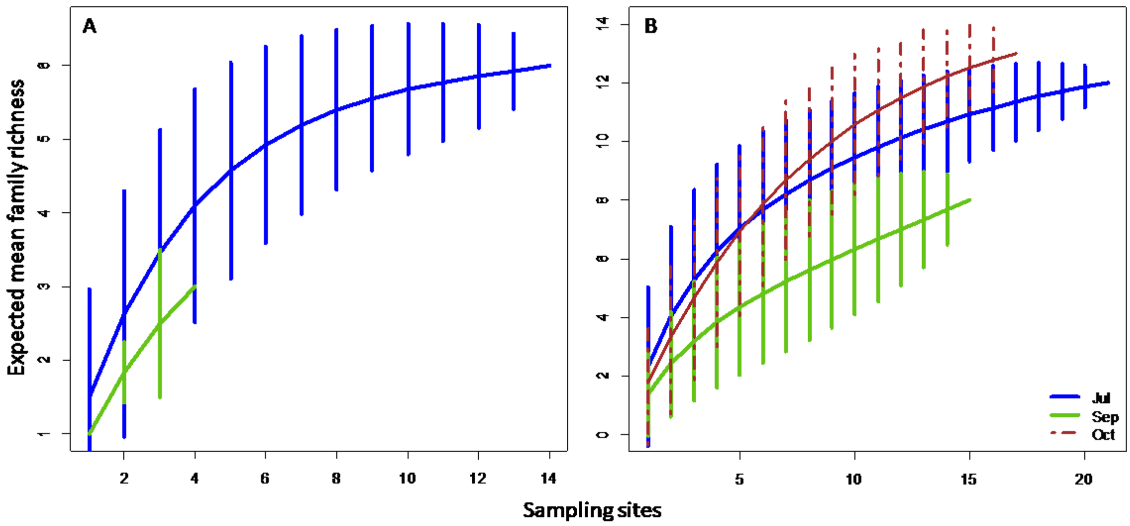

Figure 4.3. Coccinellidae species accumulation curves in the vineyard canopy (A) and in the vegetation cover (B) in July, September and October. Vertical lines indicated the standard errors.

Both accumulation patterns show September as less abundant dates. In Mediterranean areas, the summer is the start of the dry season, where the water is scarce, and the vegetation interrow-crops begin to compete with the vine for water (Afonso et al., 2003; Mercenaro et al., 2014) and is observed to decrease its vigor. September in this case is in the peak of the dry season. The vegetation cover crops, where multiple insects found its habitats, are beneficial for its additional sources of food, humidity and shelter (Carmona & Landis, 1999). Without these cover crops, diminished because of the dryness, many Coccinellidae can migrate when the climatic conditions, usually temperature and humidity, are not favorable (Iperti, 1999).

4.4. Conclusion

Among the most abundant species of Coccinellidae, most of them play an important role as a natural limitation of the vineyard's enemies. The larger genus found in this study, *Scymnus* and *Coccinella*, are predators of scale insects, aphids, and mites. The Douro Region was found to have the maximum species richness, both in the canopy and vegetation coverage. Among the sampling dates, July has had most individuals gathered, proving to be the most active period of Coccinellidae. It can be argued that the vegetation cover plants sustained a significantly higher population of Coccinellidae than the canopy. These results agree with what is described by other authors about the importance of herbaceous cover crops as an additional food source and its role as a biodiversity hotspot, shelter, and moisture. Also, the use of other environmental variables such as rainy and dry seasons, temperature, photoperiod, the use of pesticides, and/or landscape structure, could enricher the discussion of this study. The results could be richer if the sample were more detailed.

4.5. References

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Chapter 5.

General conclusions, conceptual overview and future perspective

5.1. General conclusions, conceptual overview and future perspective

The vineyards provided multiple habitats that supports a diverse community of Coleoptera. In total, 36 families of Coleoptera encompassing at least 2954 individuals, were found to inhabit the studied Wine Regions of Portugal. The most frequent families were Chrysomelidae, Coccinellidae and Latridiidae. The members of the family Chrysomelidae are phytophagous and some authors agree that Chrysomelidae adults are more likely to feed on the vine's foliage rather than in the berries, which agrees with the results found in this study. The second most abundant family was the Coccinellidae, which is well known for its predaceous nature and its importance on the natural limitation of vines pests' populations. Next, the family Latridiidae has been reported to feed on fungi and is usually associated with moisture and stored products.

For Coccinellidae, 315 individuals were collected, from 22 species. The most abundant species were *Scymnus apetzi* (Mulsant), *Scymnus interruptus* (Goeze) and *Coccinella septempunctata* (Linnaeus). The members of the genus *Scymnus* prey on scale insects and aphids and *Coccinella* are mainly found to be aphidophagous. Some authors agree that behavior and ecology of predaceous Coccinellidae are associated with their prey's habits.

Among all the Wine Regions, Douro had the greater Coleoptera and Coccinellidae abundance. When compared to the vine's canopy, the vegetation cover shelters a higher number of Coleoptera and Coccinellidae and were found to be an important biodiversity hotspot within a vineyard ecosystem, because of its alternative resources. Niche differentiation and additional sources of food, moisture and shelter at the vegetation interrow crops can perform more favorable conditions for the presence of these arthropods.

Species richness and abundance were observed to be higher in July, in both chapters, which represents the minor rainy season and less abundant in October, major rainy season. The results agree with other articles about July having more suitable temperatures and dryness in temperate regions, where the favorable climate increases insects reproduction, dispersion and metabolic rates. Also, photoperiod can influence on insects ecological dynamic. In terms of strata, there were more abundance of Coleoptera and Coccinellidae in the vegetation cover, rather than in the canopy. Herbaceous cover crops are an important biodiversity hotspot and many authors say it increases activity density and species richness. Niche differentiation, favorable microclimates, additional food resources, moisture and shelter are the main variables that explain such improvement.

For the groups of Coleoptera and Coccinellidae comparison between sample dates, diversity indexes indicates that July had the maximum diversity rates in both groups, which can represent the end of spring and beginning of summer, where the mild temperatures are more favorable to insects reproduction, dispersion and metabolic rates can be higher. Among Wine Regions, diversity indexes also point that Douro had higher diversity, both in the canopy and vegetation cover.

Further studies are required for revealing the factors driving the differences found in Coleoptera and Coccinellidae communities. More sampling dates and sites, as well as additional collection methods also would enrich the discussion about Coleoptera community. The consideration in future investigations of other variables not addressed in the current study, such as temperature, precipitation, use of pesticides, management and landscape structure, will increase the knowledge about Coleoptera and Coccinellidae communities in Portuguese vineyards . Also, more detailed study on the ecology of pests and predators need to be accomplished to improve the knowledge about Coccinellidae and other Coleoptera families.