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Testing the effect of native biodiversity and natural biotic resistance on grape losses

Dissertação de Mestrado em Ecologia, orientada pelo Professor Doutor Jaime Ramos (Universidade de Coimbra) e pelo Doutor Rúben Heleno (Universidade de Coimbra), apresentada ao Departamento de Ciências da Vida da Universidade de Coimbra

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UNIVERSIDADE DE COIMBRA

DEPARTAMENTO DE CIÊNCIAS DA VIDA

Faculdade de Ciências e Tecnologia da
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2017

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“*Man, unlike anything organic or inorganic in the universe, grows beyond his work, walks up the stairs of his concepts, emerges ahead of his accomplishments*.”- John Ernst Steinbeck (*As Vinhas da Ira*)

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Resumo

As práticas agrícolas modernas têm vindo a afetar a biodiversidade das zonas agrícolas e os serviços dos ecossistemas que estas áreas fornecem. Um dos serviços que tem vindo a ser mais afetado é o da resistência biológica a possíveis pragas.

A produção de vinho é um tipo de agricultura muito praticada no mundo e que em Portugal tem uma enorme importância histórica e económica. Mas este tipo de agricultura está sujeito a um grande número de pragas que afetam a produção, o que leva os produtores a usar uma grande quantidade de pesticidas para combater essas pragas, afetando assim a biodiversidade que habita e usa as vinhas para alimentação.

Neste estudo, que decorreu em seis vinhas na região demarcada da Bairrada, analisamos as perdas em quatro castas (Touriga, Baga, Arinto e Chardonnay) causadas por insetos, aves e fungos. Avaliamos se existe uma preferência por alguma casta ou cor (branca e tinta) das uvas. As comunidades de aves e insetos foram identificadas e divididas por grupo funcional (praga, neutra ou auxiliar), para comparar com as perdas e perceber se as comunidades locais por si só tem uma capacidade de controlo das pragas (Hipótese da resistência biótica). Pretendeu-se também estudar se o efeito de orla influencia as perdas de uvas.

No estudo foi registado um baixo número de perdas, mas verificámos que os insetos têm mais preferência pela casta Baga e Chardonnay. Observamos que a cor das castas não influenciou as perdas tanto por aves como por insetos. Em relação aos fungos registámos uma preferência pela casta Baga. A maioria das aves e insetos observados nas vinhas são considerados pragas, o que sugere um baixo potencial para efetuarem uma efetiva resistência biológica neste ecossistema agrícola. Apesar de a relação da orla com as perdas não ter sido significativa verificou-se um maior número de perdas nos primeiros 100 metros a contar da orla.

Futuros estudos serão necessários para avaliar melhor o papel da biodiversidade local nas vinhas. Para avaliar melhor a hipótese da resistência biótica seria importante efetuar amostragem numa vinha onde não sejam utilizados pesticidas – nomeadamente numa exploração de vinho biológico.

Palavras-chave: Resistência biótica; controlo biológico; biodiversidade natural; vinhas; pragas das uvas; perda de uvas;

Abstract

The agricultural practices strongly influence the biodiversity of agricultural areas, and the ecological services that these ecosystems provide. One of the services that have been most affected is the biological resistance to pests.

Wine production is a common type of agriculture throughout the world, and it has a strong historical and economic importance for certain countries such as Portugal. But this type of agriculture is subject a high number of pests that affect the production, and farmers use pesticides to combat these pests, thus affecting the biodiversity that inhabits and uses the vineyards for food.

This study took place in the Bairrada region in Central Portugal. We used six vineyards to assess the losses caused by fungi, birds and insects in four castes (Touriga, Baga, Arinto and Chardonnay), and evaluated whether pests have a preference for any caste or color (white and red). Bird and insect communities were studied and divided into functional guilds (pest, neutral or auxiliary), to compare with grape losses and assess if these natural communities hold a potential to naturally control wine pests (biotic resistance hypothesis). The edge effect in grape losses was also evaluated.

We recorded a small proportion of grape losses, but we verified that insects had a preference for the Baga and Chardonnay castes. We observed that color did not influence the birds and insect's losses. In relation to the fungi losses, we registered a preference for the caste Baga. Most insects and birds observed in vineyards were pests, which entails a low level of biological control in this agricultural ecosystem. Although the relation between the edge and grape losses was not significant, there were more losses in the first 100 meters from the edge.

Further studies will be necessaries to fully evaluate the role of local biodiversity in vineyards. To better evaluate the biotic resistance hypothesis it would be necessary to sample an organic vineyard, i.e. without the use of the pesticides.

Keywords: Biotic resistance; biological control; natural biodiversity; vineyards; grape pests; grape losses.

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1. Introduction

All organisms modify their environment, and humans are no exception. The current world population is around 7 billion (www.census.gov/population/international/). To satisfy human needs more and more agricultural areas are necessary, but the current levels of agricultural production are not environmentally sustainable. The area of land required to support our necessities, i.e. air, clean water, food, etc., would be at least five times the area of our planet if the total world population has the living standard of western developed countries (Gurr et al., 2012).

Natural ecosystems have been replaced by agricultural areas (crops, animal production), human settlements and commercial forests. About 50% of the World terrestrial area is now occupied by agricultural areas, 20% of commercial forests, 25% by human settlements, and only 5% are undamaged and uninhabited (Pimentel et al., 1992). Pimentel et al. in (1992) measured the biodiversity in different German ecosystems and found that 60 to 65% of species occur in areas used by humans (agriculture, urban areas, etc.).

Modern agricultural systems, characterized by the use of pesticides, monoculture practices, and intensive use of heavy machinery, have been shown to negatively affect the biodiversity, as well as the resistance and resilience of agro-forestry systems (Altieri, 1999). The modern agriculture leads, directly and indirectly, to a reduction on the available niches, reflected for example in the lack of shelter, reproduction sites, and food sources for arthropods and vertebrates (Corbett & Rosenheim, 1996; Landis et al., 2000; Heimpel & Jervis, 2005). In turn, a high biodiversity is critical for ecosystems resilience, including in agricultural systems, and is responsible for many ecosystem services such as pollination, nutrient cycle, and seed dispersal. Biodiversity loss now represents one of the most critical environmental issues, threatening ecosystem services. One of such services is natural biological control. With a reduction in biodiversity there is a reduction in predation rate (Cardinale et al., 2006), and the number of predator and parasitic species capable of controlling potential pests rapidly declines (Thies & Tscharntke, 1999).

The economic value of natural biological control was estimated at 2$, 23$ and 24$ per hectare in forests, grasslands and croplands, respectively (Costanza et al., 1998).

This study focuses on the interplay between the pests and natural biodiversity occurring on vineyards, a very important type of agricultural in economic, cultural and ecological terms.

1.1 Biodiversity and pest control

For agriculture to be profitable it is critical to control the abundance of pests. The agricultural areas are not stable systems, they are characterized by extreme fluctuations in densities of organisms, and frequent invasions by new species (Gurr et al., 2012). In recent years the potential role of predators, parasites and pathogens to control pests has been explored - i.e. biocontrol (Gurr et al., 2000). Biocontrol, or biological control, is defined as the utilization of biological agents (predators, parasites and pathogens) to maintain the pest population below an economically significant threshold (De Bach, 1964).

There are four common approaches to biological control: the classical, the conservation, the inoculation and the inundation (Eilenberg, Hajek, & Lomer, 2001). The classical biological control focuses on the importation of pest enemies to target particular pest species. This type of biological control has been used since 1880, but it has several disadvantages: 1) Low suppression of target species (around 10%); 2) low success of the biocontrol agent’s establishment; 3) Biocontrol agents might attack other, non-target, species (Howarth, 1991) and; 4) the introduced agent may become a pest (Gurr et al., 2000). One example of this problem is the cane toad (*Rhinella marina*), initially introduced to control gray-backed cane beetles *(Dermolepida albohirtum*) in sugar- cane plantations in Australia, where this species has devoured and poisoned non-target native species and causing other adverse ecological effects in Australia (Shine, 2010). Despite such problems the classical biological control is considered important by many professionals (Thomas & Willis, 1998). This type of biological control had a database with many species that have a capacity of control pests and invasive species, so the cost of control was minimal because the development work had already been done. Other advantage is that the classical biological control has been used where alternative controls were not feasible. So the classical biological control is considered is many cases because it is an economical and sometimes the only practicable control method (Greathead, 2003).

The conservation biological control consists in the conservation and augmentation of natural enemies (Orr, 2009). This type of biocontrol is distinguished from other strategies in that natural enemies are not released. Instead, wild biological control agents are protected and provided with adequate resources so that they can naturally control introduced species (Eilenberg et al., 2001). Whenever possible, this type of biological control should receive priority principally if the release of other agents is not economically viable or if it poses serious threats. One example is the control of the pine processionary moth (*Thaumetopoea pityocampa;* Lepidoptera: Notodontidae) by insectivorous birds such as Cuckoos, hoopoes, tits and nightjars that can reduce 20% to 100% of this moth species in well preserved forests. Therefore, if avian natural biodiversity is not reduced by human activities it can naturally control the *T. pityocampa* outbreaks (Barbaro & Battisti, 2011).

Inoculation biocontrol is when an organism controls a target pest after multiplication. One example of this type of biocontrol is the release, in glasshouses, of *Encarsia formosa* (Hymenoptera: Aphelinidae) and other predators: the number of individuals released is not enough to control de pest, and the success of this strategy depends on the ability of *E. formosa* to multiply. In the end of the season, the glasshouse is emptied, and the biocontrol agent is not permanently established. When the next generation of plants grow the biocontrol agents are released again (Eilenberg et al., 2001).

Inundation biological control is when the biological control agents kill a sufficiently high proportion of the pest population, reducing the damage level. The success of this type of biocontrol depends exclusively on the released population and not on their progeny. One example is the use of living *Bacillus thuringiensis* spores for insect control. The *B. thuringiensis* is released in high numbers with the aim of killing quickly the target insects. The spores will decrease overtime and there is no expectation of long term pest control (Eilenberg et al., 2001).

1.1.1 Biotic resistance theory

Biotic resistance is the capacity of resident species in a certain community to reduce the success of invader species and pests (Levine, Adler, & Yelenik, 2004; Flower et al., 2014). The biotic resistance has been well studied in the context of herbivory by vertebrates and invertebrates and predation in marine environments, but studies in pests and their natural enemies are less common (Bürgi, Roltsch, & Mills, 2015).

Two studies have analyzed the biotic resistance capacity of natural biodiversity in two different ecosystems: a) Michaud (1999) investigated the control of *Toxoptera citricida*, a pest of citrus, by their natural enemies. The local's whit Coccinellids and Syrphids had a higher percentage of *Toxoptera citricida* colonies eliminated and these two families were responsible for a major pest elimination. b) Flower *et al*. (2014) studied the predation of bark-foraging birds in ash forests (*Fraxinus* spp.) on invasive emerald ash borer (*Agrilus planipennis*). The native bark-foraging birds reduced the tree-level emerald ash borer densities by 85%, and these birds increased the intensity of predation in zones infected by emerald ash borer (~45%) compared with non-infected zones (~22%).

In my thesis I will focus on the natural biotic resistance on Portuguese vineyards.

1.2 Vineyards

Portugal has a strong tradition in grapevine production (Cunha et al., 2009). For example, 26% (74,000 ha) of the agricultural area in the Vinho Verde region (Northwest) is occupied by vineyards (Altieri & Nicholls, 2002). According to the International Organization of Vine and Wine (OIV), in 2014 Portugal was the seventh country with more vineyards in world. The three countries with more area are Spain (1018000 hectares), France (800000 hectares) and Italy (769hectares). The wine represents an important product for the economy of Portugal: for instance, in 2015 (http://www.ivv.min-agricultura.pt) the exportations of wine from Portugal to other countries represented a gain of 727 M€.

The main problem of the wine industry, is that vineyards are affected by diverse pests or diseases like viruses, mycoplasma, bacteria, insects, worms, arthropods, oomycetes and fungi (Delaunois et al., 2014). To combat these pests and diseases the producer's use chemical treatments (pesticides), and particularly fungicides (Delaunois et al., 2014), which can be sprayed more than 10 times per year on a single vineyard (Corio-Costet et al., 2011). Studies in French and Italian vineyards estimate that over 93000 and 125 tons of fungicides, respectively, can be sprayed per year on a single vineyard (Viel et al., 1998; Niccolucci et al., 2008) .

In monetary terms the use of pesticides is more expensive and harmful for the producer than the biocontrol. Pimentel (2005) analyzed the costs of pesticides and concluded that the use of pesticides causes $10 billion in environmental and social damages per year, $4 million of which on vineyards alone (Pimentel, 2005). The use of pesticides negatively affect the vineyards biodiversity and other biophysical properties, such as the soil and surface water retention (Delaunois et al., 2014), and can have direct negative consequences for human health, as some chemicals can pass to the wine (Viel et al., 1998; Delaunois et al., 2014).

1.2.1 Vineyards in Central Portugal

Portugal has one of the greatest concentrations of autochthonous castes in the entire world (n=290, Martins, 2009), and was divided in 13 wine regions (Fig.1). Each region has characteristic castes; for instance in the “Vinho Verde” region the traditional castes are the white castes that produced the green wine (Alvarinho, Loureiro and Trajadura). One the most emblematic wine regions is central Portugal, is the Bairrada. The most common red castes here are the Baga, Touriga Nacional and Jaen, and the most common white castes are Arinto, Maria Gomes (also called Fernão Pires in other regions) Bical and Cerceal (www.infovini.com).

Each caste have unique characteristics, such as color (red or white), grape size, number of grapes per bunch, sugar quantity, or acidity (Varandas et al., 2004; Keller, 2010). These factors influence the choice of producers because they create the wine flavour. In fact, each wine has a characteristic flavour derivative of used castes. For example, when one compares Baga with Touriga the differences are evident: Baga is a productive caste (with many bunchs and number of grapes per bunch), and grapes are small and acidic. The Touriga is a caste with many bunches but with few grapes per bunch (less productive) and with a higher sugar content (www.infovini.com; www.vinha.pt; www.ivv.min-agricultura.pt).

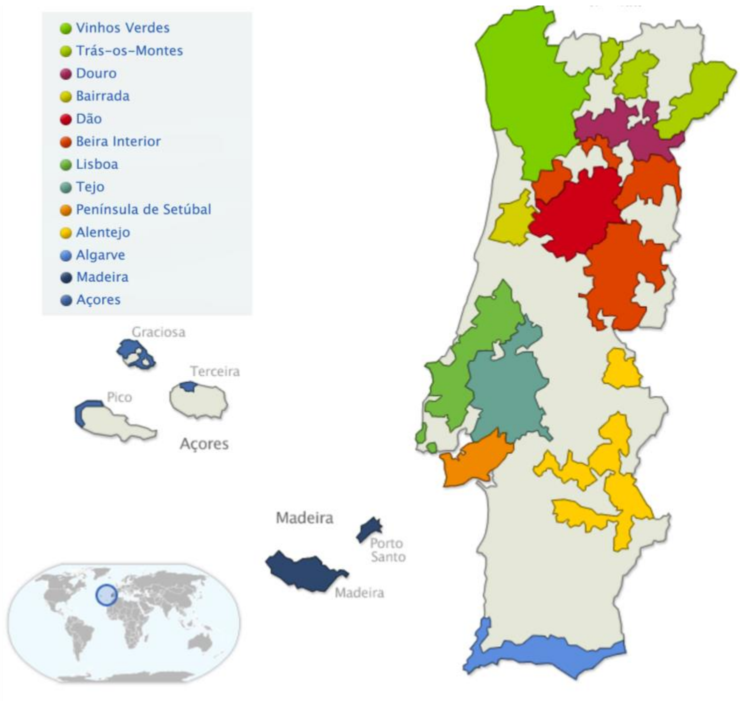

Fig. 1. Map of Portugal wine regions.

1.3 Birds in agro-ecosystems

Birds have a double function in agriculture areas; they can be pests (Canavelli et al. 2014) or biocontrol agents (Ceia & Ramos, 2014; Barbaro et al., 2016). In agriculture systems bird pests may attack a huge range of crops, like corn or sunflower and fruit crops in orchards or vineyards causing significant economic, social and conservation impacts (Bomford & Sinclair, 2002)(Trace et al., 2007; Canavelli et al., 2014).

Passeriformes prey on ripening grapes because they represent an abundant food source in late summer and autumn (Kross, Tylianakis, & Nelson, 2012). In Europe the species that cause the most damage in grapes are the common starling (*Sturnus vulgaris*), spotless starling (*Sturnus unicolor*), blackbird (*Turdus merula*), corvids and european robin (*Erithacus rubecula*) (De Grazio, 1978; Feare et al., 1992; Bomford & Sinclair, 2002;Tracey & Saunders, 2003; Anderson et al., 2014).

Bird damage varies between vineyards and years but in some cases about 50% of the production may be affected, which has severe economic costs (Fig.2) (Somers &

Morris, 2002a).

Fig. 2. Bunch strongly attacked by birds that ate nearly all fruits.

But the birds are not only pests, they can also act as biocontrol agents of other pests (Sekercioglu, 2006; Wenny et al., 2011; Maas et al. , 2015; Ceia & Ramos, 2014). The control of arthropods by insectivorous birds is a vastly valued service in different types of ecosystems (Sekercioglu, 2006). Many birds are specialized arthropod consumers in farmland areas (50% predominately consume insects and 75% occasionally) (Wenny et al., 2011). Ceia and Ramos described the role of insectivorous birds in pest control for oak woodlands. The oaks have various insect species that cause economic losses in different parts of the trees, these insect pests have various life cycle stages, so the authors compiled a list with twenty-six bird species that complement each other in space and time, predating most insect pest's life stages.

The most important study with biocontrol in agricultural areas was the control of the common vole (*Microtus arvalis*), the major agricultural pest in Europe (Paz et al., 2013). In this study, the authors installed nest-boxes in agro-ecosystem with the goal to increase the population of raptors, particularly common kestrel (*Falco tinnunculus*) and barn owl (*Tyto alba*), predators of the common vole. The introduction of nest-sites increased the populations of both predators leading to a decline in the vole population. In Australia, New Zealand Falcons (*Falco novaeseelandiae*) were introduced to control other birds consuming grapes (Eurasian blackbird, *Turdus merula*; song thrush, *Turdus philomelos* ; starling, *Sturnus vulgaris* and silvereye, *Zosterops lateralis*. Falcons reduced grape losses by 95% in relation to vineyards without falcons. Nest boxes were also used in California to increase the population of bluebirds (*Sialia Mexicana*), which increased he removal rates of sentinel larvae by 2.4 times (Jedlicka, Greenberg, & Letourneau, 2011).

All these studies were conducted with the main goal of contributing to reduce the use of chemicals in agriculture, mitigate the effects of agriculture practices and increase economic revenue of each crop. The economic importance of farmland birds was studied in coffee production in Jamaica, and the authors found that bird control of the insect pest *Hypothenemus hampei* ,contributed about 310$ per hectare per year (Johnson, Kellermann, & Stercho, 2010).

Throughout Europe, the populations of farmland birds are declining due to agriculture intensification, which includes heavy pesticide use that eliminate their arthropod diet species and have a direct toxic effect (Morris et al., 2001; Geiger et al., 2010; Mineau & Whiteside, 2013).

1.4 Insects in agro-ecosystems

Insects can also be both: pests and auxiliaries in agricultural areas (Bournier, 1976; Jonsson et al., 2008). Insects, together with plant diseases (bacteria and virus), are the factors that cause more significant losses in agriculture and forestry. Insects can affect the survival and productivity of crops in various ways (Fernandes, 1987), and many of them can cause strong economic impacts in crops. The vine attracts several pests that can attack all organs of the plant (Bournier, 1976). The most damaging pest is the Phylloxera, *Daktulosphaira vitifoliae* , a root pest, with a winged form, which in a few years destroyed 2,500,0000 ha of vineyards in western Europe (Bournier, 1976) (Fig.3A). Another root pest that causes several damages in vines is the *Vitacea polistiformis*, a lepidoptera, whose caterpillar can completely destroy entire vineyards (Clark et al., 1964). The insects that cause more significant damages directly on the grapes are the grape totrix, *Lobesia brotana* and grape tineid, *Eupoecilia ambiguella*. The caterpillar of these insects consume the grape, and their entry holes also favor fungi colonization (Bournier, 1976). Another arthropod pest group rarely mentioned are the thrips (Thysanoptera), that cause the epidermis necrosis and suberization. To control all such insect pests, most producers use insecticides. However, the use of chemicals reduces the pests but also their natural biocontrol agents,

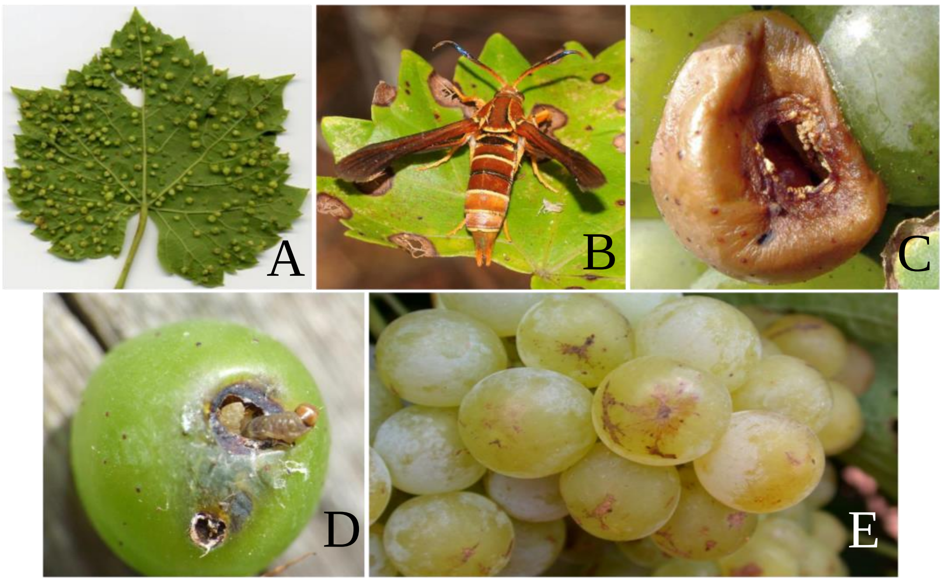

Fig. 3. Insect damages on vines, A) *Dactylosphaera vitifolli* in leafs; B) *Vitacea polistiformis*; C) *Eupoecilia ambiguella*; D) *Lobesia brotana*; E) Thrips.

which also represents a problem for the producer. For example, Phytoseiidae (Fig.4A) have a substantial impact on spider mites and eriophyid mites (mite pests of vines) in vineyards. The different phytoseiid life stages contribute to control these insect pests under their noxious levels in vineyards (Bostanian, Vincent & Isaacs, 2015). Coccinellidae also have a positive effect in controlling vineyard pests by preying upon mealybugs and phytophagous mites (Fig.4B) (Bostanian et al., 2015). The Phytoseiidae and Coccinellidae are predators, but parasitoids also have significant impacts in vines pests, namely the families Ichneumonidae, Tachinidae and Chalcidoidea (Fig.4). Other parasitoid group that have been introduced and successfully established in classical biological control programs are the Tachinidae. The Tachinidae is the parasitoid of the two most important pest of grapes, *Lobesia brotana* and *Eupoecilia ambiguella* (Martinez & Hoelmer, 2006).

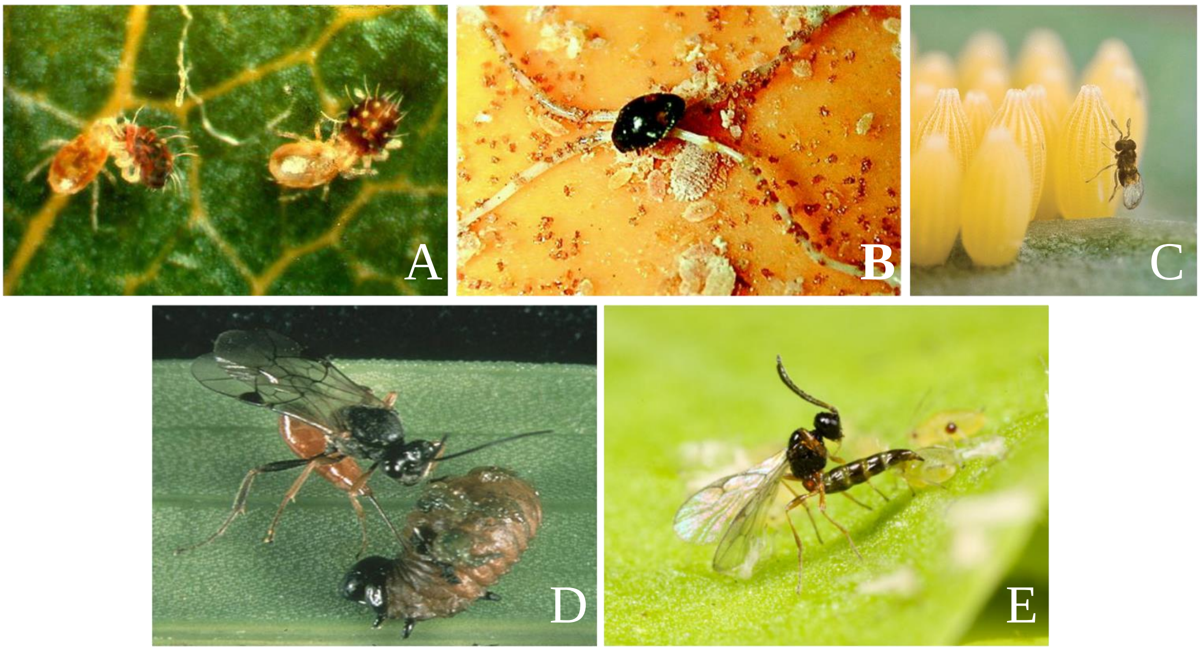

Fig. 4. A) Phytoseiidae preying upon red spider mites (Acari: Tetranychidae); B) Coccinellinidae preying a mealybug. C) Trichogramma parasiting the eggs of an insect pest; D) Ichneumonid parasiting a pest larvae; E) Tachinidae parasiting a pest aphide.

1.5 Objectives

In this study we analyze whether natural biotic resistance can reduce losses onto vineyards in the wine region of Bairrada, Central Portugal. Because different grape castes present a different physiology, we investigated the role of birds and insects, both as pests and natural biocontrol agents of different grape castes.

We analyzed the functional biodiversity of birds and insects (pests, auxiliaries and neutral) and assessed the fluctuation of these biodiversity groups during the period of grape formation. Finally, we evaluated the importance of vineyard shape on fruit losses, by assessing the influence of the distance to the vineyard edge on grape losses due to pests and natural biocontrol. This reasoning makes sense because we would expect a higher grape loss close to the edge because many birds and arthropods may use the edge as a refuge and shelter. On the other hand, a higher biocontrol may also occur close to the edge if predators and parasitoids are much more abundance on the edge.

2. Materials and methods

2.1 Study area

The study area was in “*Região Vitivinicula da Bairrada”,* in Central Portugal, extending between Águeda and Coimbra, which comprise an area of about 108000 hectares (Fig.5). The climate is typically Mediterranean with Atlantic influence, characterized by hot and dry summers and mild and humid winters.

The wine farms in this region are generally small and used as a monoculture. The total area occupied by vineyards are less than 10000 hectares, representing 9.26% of the region.

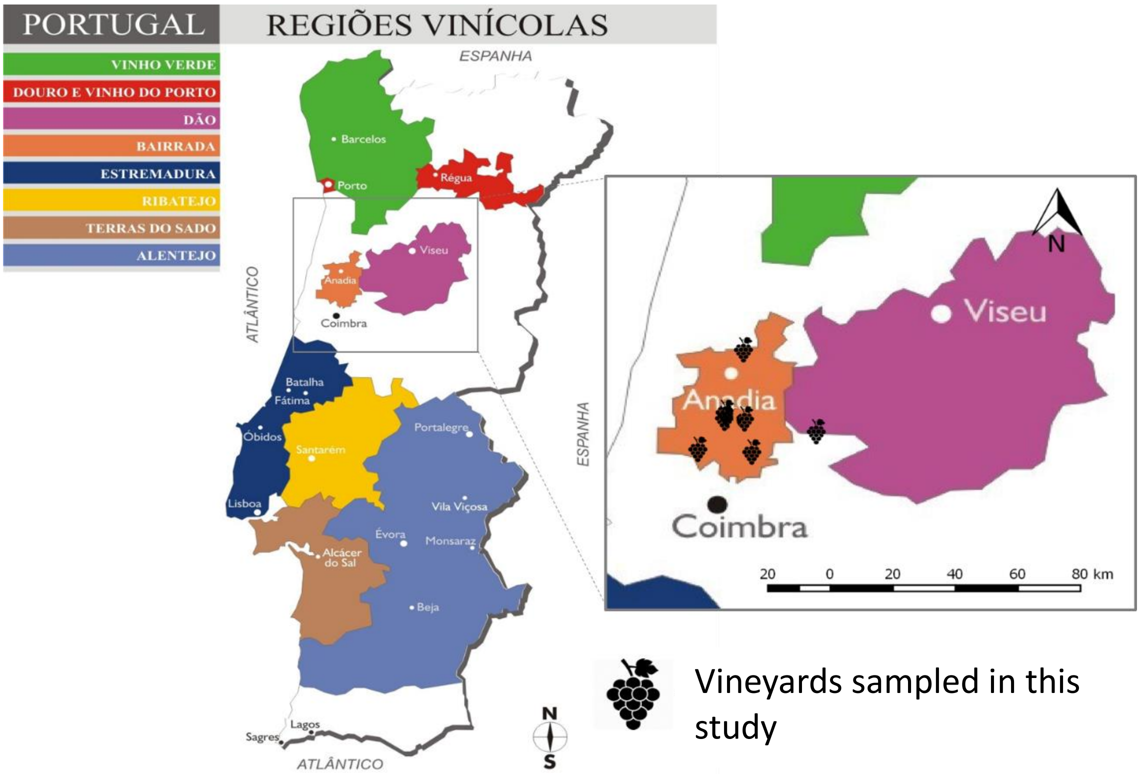

Fig. 5. Map of Portugal with the characteristic Wine regions. In detached the Bairrada region with the vineyards where sampling was conducted.

We selected six vineyards along the Bairrada region: One in North, two in South, two in center and one in East. (Fig. 5, Table 1). We choose four types of castes, two red: Touriga Nacional (hereafter referred to as Touriga, for simplicity) and Baga; and two white: Arinto and Chardonnay. The castes were chosen to be among the most common castes in the region and as representatives of red and white castes. We choose Baga in two vineyards, Arinto in three, Chardonnay in two and Touriga nacional was present in all vineyards but we selected it only in five vineyards.

In each vineyard, we selected 10 vines from each caste present, namely: 50 Touriga nacional vines (5 sites), 40 Arinto vines (4 sites), 20 chardonnay vines (2 sites), and 20 Baga vines (2 sites). Tis sampling characterized 60 white vines and 70 red vines. All selected vines were separated by a minimum distance of 30m, so that losses could be considered mostly independent (Williams & Martinson, 2000; Nicholls, Parrella, & Altieri, 2001; Chacoff & Aizen, 2006). Birds are also fairly restricted in their range when they attack a specific group of vines (Somers & Morris, 2002b; Barbaro & Battisti, 2011).

Table 1 Description of each vineyard.

Vineyard	Code	Coordinates	Castes sampled	Edge type	Area (ha)
Quinta da Aguieira-Aveleda	Aveleda	N 40º36’39.32 W 8º26’39.11	Chardonnay Touriga	Roads or houses	10.86
Caves Messias	Messias	N 40º21’27.81 W8º25’05.26	Baga Arinto Touriga	Mixed native forest and *Eucalyptus*	57.82
Caves de São João	S. Joao	N 40º21’54.91 W 8º34’02.12	Chardonnay Arinto	Mostly pines and some *Eucalyptus*	11.54
Caves de São Lourenço- Ideal Drinks	S. Lourenco	N 40º26’24.65 W 8º29’39.64	Touriga	Pines, oaks and road	10.22
Boas Quintas	Boas Quintas	N 40º24’26.86 W 8º13’56.99	Arinto Touriga	Pines and road	7.94
Estação Vitiviniculda da Bairrada	Estacao	N 40º26’23.56 W 8º26’19.06	Baga Arinto Touriga	Forest, fruit trees, shrubs and houses	2.62

2.2 Exclusion experiment

In order to analyze the effect of natural biodiversity on grape losses we performed exclusion experiments on selected vines. Three treatments were implemented: 1) bird's exclusions, 2) Bird and insect exclusions, and 3) control vines (no exclusion). In the birds treatment we protect the grapes against birds, with a metal net with a mesh of 19x19 mm (Fig.6A). These nets protected the bunches inside from bird predation. In the bird and insect exclusion treatment, grape bunches were protected with a fine plastic mesh of 1.9 x 1.9 mm (Fig.6B) that protected them both from bird and insect predation, while allowing bunch ventilation and growth. In each selected vine, three bunches were selected and randomly allocated to each of the three treatments.

Fig. 6 A) Plastic net used in insect exclusion; B) Metal net used in the bird exclusion.

All nets were installed in early June, when the unripe fruits were already formed but before they started to mature. At this stage, the grapes were sufficiently robust to endure the net installation and were not yet attacked by insects or birds. Immediately before the installation of each net, the number of forming grapes in each bunch was recorded.

The nets were in place during June, July and August, and verified regularly. By the end of August we removed the nets and registered the number of grape losses by each pest type: birds, insects and fungi. The cause of each grape loss was identified based on the marks left on the grapes, according to field guides and the farmer's experience (Fig. 7; Ellis & Nita, n.d.; Neves, n.d.; Isaacs et al., 2003; Carisse et al., 2006;; Hahn & Wold-burkness, 2008; Hoover et al., 2011; Mani, Shivaraju, & Kulkarni, 2014).

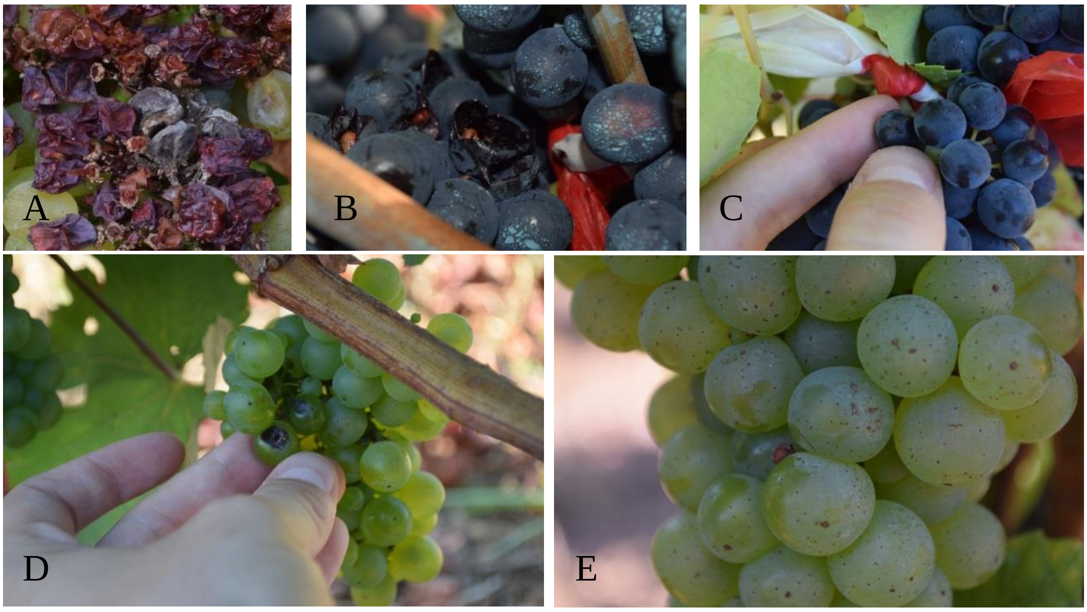

Fig. 7. Damage marks found on grapes. A) fungal damages; B) bird predation; C, D and E) different damage caused by insects.

2.3 Bird sampling

To evaluate the abundance of birds in each vineyard we conducted monthly census between June, July and August). Four census were performed per month per vineyard, between 7:00 and 10:00 (Fig. 8). Two sites with good visibility and at opposite edges were used in alternation in each vineyard. Each census lasted 5 minutes, during which all birds were identified both visually and acoustically, and their horizontal distance to the observer was estimated. Only birds within a radio of 50m from the census point were used in the analyses; which should represent the local bird abundance that potentially use the vineyard.

Bird species were divided into three groups: Auxiliary, Neutral, and Pest. These categories were adjusted every month in order to reflect bird feeding habits in relation to the available resources. For instance, when breeding (May-June) most species are largely insectivorous, and, by August species such as the blackbird can be largely frugivorous.

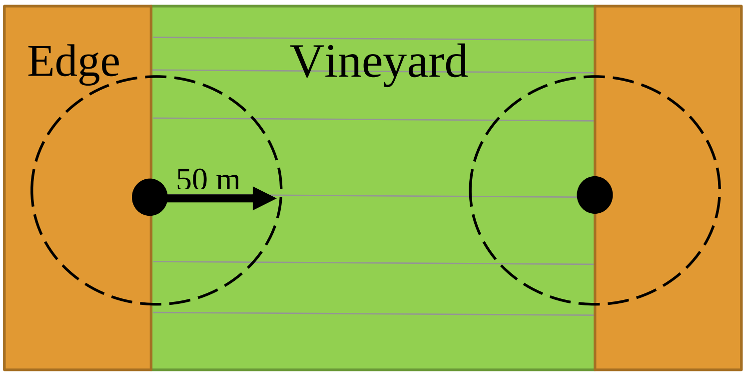

Fig. 7. Scheme of the bird census method used.

2.4 Insect sampling

To sample the insects in each vineyard, we used sticky yellow traps with 10x25cm (Fig. 8), commercially available from Koppert Horiver. The traps were sticky on both sides, and were suspended from the lower wire that connects the different vines along straight lines. Five traps were placed on each vineyard at 30-50 m apart. Traps were operated once per month during the duration of the experiment (June - August), and on each occasion they were removed after 5 days, and kept at 4-5 ºC until insect identification.

Fig. 8. Yellow traps used for insect sampling.

All insets were later extracted from the traps with a solvent, identified to family level, sorted according to their morphotipes and counted. Only three families of micro hymenoptera (Platygastridae, Diapriidae and Proctotrupidae) were grouped together due to their similar morphology.

All morphotypes were then measured from the fronts to the tip of the abdomen using a microscale under a binocular microscope. Antennae and wings extending beyond these points were not included in the total length measurement. Identification and nomenclature was based on identification keys (Borror and Delong, 1988; Quigley and Madge, 1988) and some books: Michael (2007) and Pereira et al. (2012).

The insects were divided into three groups regarding their main relation with agriculture crops, namely: auxiliary, neutral, and pests. This classification was temporally flexible (i.e. variable across months), in order to adapt to the changing roles of insets in relation to their life cycles and food availability.

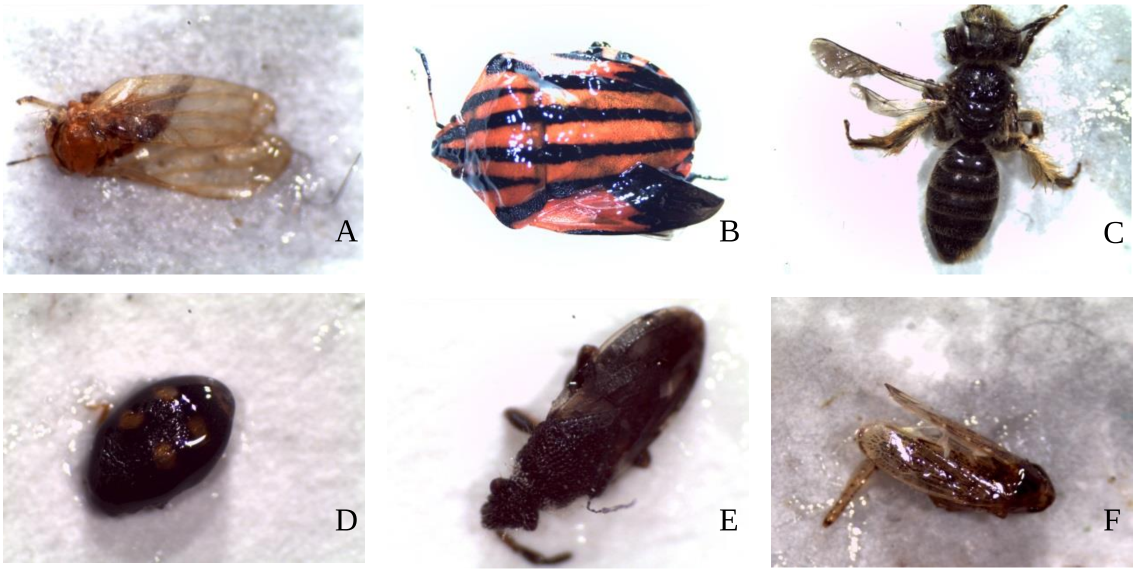

Fig. 9. Insects found on the yellow traps. *A) Psocoptera; B Pentatomidae; C) Apidae; D) Coccinellidae; E) Lygaeidae; F) Cicadellidae.* All the images were taken in a Leica EZ4E Microscope.

2.5 Statistical analysis

Grape losses were quantified in terms of percentage of losses, in relation to the initial number of grapes present in each bunch (i.e. losses= N. grapes$_{final}$ - N. grapes$_{initial}$ / N. Grapes$_{initial}$ *100$_{)}$.

To evaluate whether grape loss by birds and insets differed among caste types and caste color we used Generalized Linear Mixed Models (GLMM), with caste (Arinto, Chardonnay, Baga and Touriga nacional) and color (red and white) as fixed factors, vineyard as a random factor and distance to the edge as a co-variable. We preformed four GLMMs, two for bird fruit losses using the mean of fruit losses by birds in the control treatment, one for the castes (Touriga, Baga, Arinto and Chardonnay) and other for the color (red and white). The other two GLMMs were for insect fruit losses and the response variable was the mean number of fruit losses by insects.

To analyze if the differences between castes were significant we performed a General Linear Hypothesis followed by a Tuckey multiple comparisons test. This test consists in estimating the mean of a multivariate normal or to test some hypotheses concerning the mean vector using a Tuckey multiple comparisons test, creating confidence intervals for all pairwise differences between factor level means while controlling for the family error rate.

To see the importance of natural biodiversity in controlling grape losses we preformed various linear regression with percentage of fruit losses per bunch (response variable), with the three types of insects and birds individually (pest, auxiliary and neutral), as the explanatory variables.

We further estimated total insect biomass from insect-length to biomass general allometric equations (Roger *et al*. 1977):

$$\text{biomass (mg)} = 0.0305 * L^{3.62}$$

$$\text{(L= lenght (cm))}$$

Whenever possible, the equation parameters were adjusted to each family, following Sample *et al.*, (1993) (See Annex) .

All the analyses were performed in R v3.05 (R Core team 2016), using packages ggplot2, Rmisc , lmerTest and multcomp.

3.Results

3.1 Grape losses

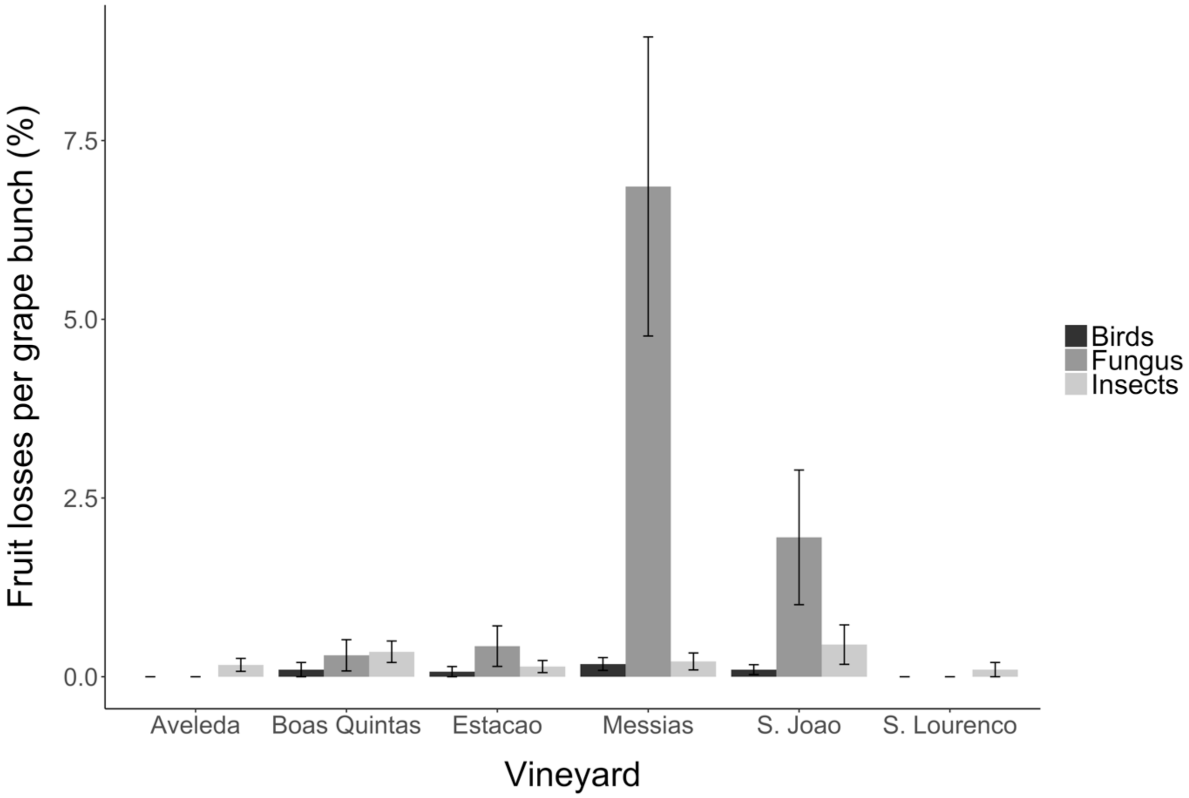

Fig. 10. Mean percentage of fruit losses due to birds, fungi and insect activity per vineyard. Error bars represent the standard error.

Overall, grape losses were low or negligible on most vineyards (Mean= 0.59%). The vineyard with a higher proportion of fruit losses was, by far, Messias (7.5%), where the percentage of fruit losses by fungi reached 7%, more than all losses on all other vineyards put together. All other vineyards had little losses, and particularly those of São Lourenço and Aveleda, where there were no documented losses due to birds or fungi, and only 1% of the fruits were lost due to insect activity (Fig.10). The percentage losses in all vineyards by fungi was 2.01%, by birds was 0.08% and insects 0.24%.

In relation to insect losses (Fig.11), the Chardonnay was the caste with the higher percentage of losses (0.43%) and the Touriga was the caste with the lower percentage of losses (0.08%). The caste color with higher percentage of losses was the white (Arinto and Chardonnay), these caste color had 0.7% of fruit losses by insects, whereas the red casts (Baga and Touriga) had 0.5%.

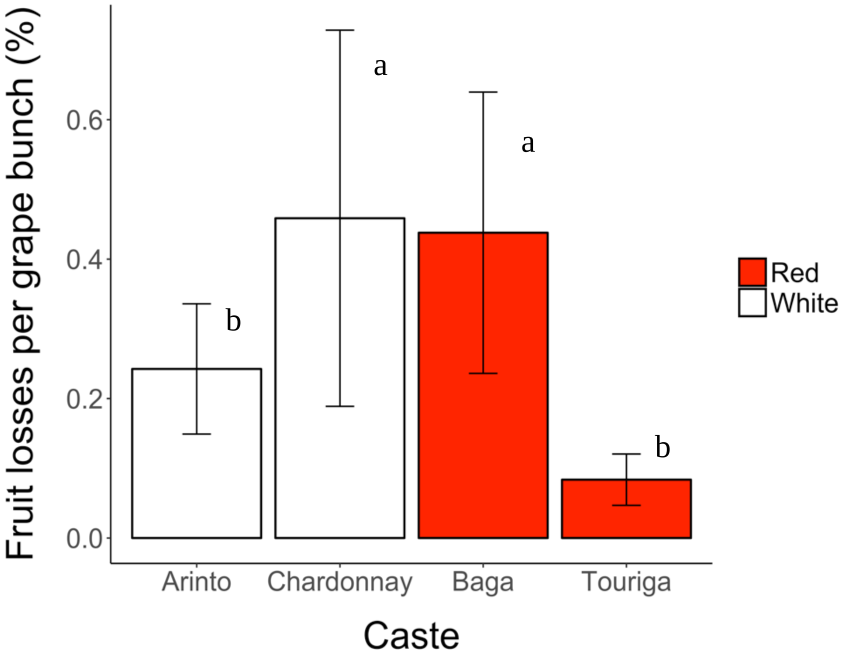

Fig. 11. Percentage of fruit losses by insects per caste. Bars with the same letters do not differ significantly between them. Error bars represent the standard error.

The GLMM show that fruit loss by insects in the control bunches differed almost significantly among castes ($F_{3,117}$=8.5, p=0.06). Namely, with Chardonnay and Baga showing slightly higher losses than both Arinto and Touriga nacional (Fig.12, Table 2).

Table 2. Summary of the General Linear Hypothesis model testing the effect of caste on fruit loss by insects. The model compares all caste-pairs. Significant results are highlighted in bold.

	Estimate	Std. Error	z value	Pr(>|z|)
Baga - Arinto	2.1236	0.6032	3.520	**0.00234**
Chardonnay - Arinto	1.1071	0.4342	2.550	**0.04961**
Touriga - Arinto	-0.7690	0.5116	-1.503	0.42227
Chardonnay - Baga	-1.0165	0.6597	-1.541	0.40016
Touriga - Baga	-2.8926	0.7638	-3.787	**< 0.001**
Touriga - Chardonnay	0 -1.8761	0.5882	-3.190	**0.00719**

In relation to fruit losses by birds (Fig.13) the GLMM did not show a significant effect of castes ($F_{3,117}=4.8$, $p=0.12$).

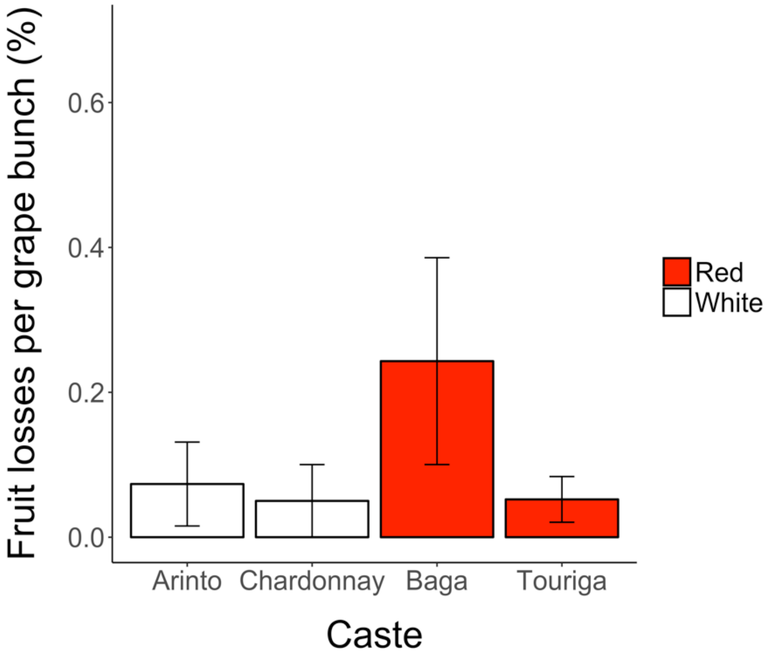

Fig. 12. Mean percentage of fruit losses by birds per caste. Error bars represent the standard error

Despite the differences between some castes regarding losses by insects, the GLMM showed that color did not influence fruit losses due to insects ($F_{1,119}=0.46$, $p=1$) or birds ($F_{1,119}=5.67$, $p=0.12$).

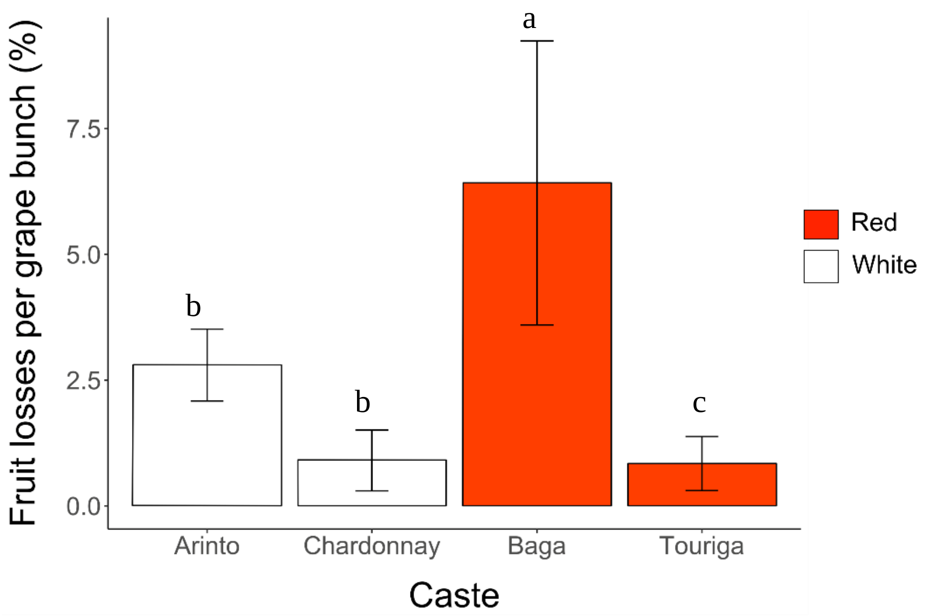

Fig. 13. Mean percentage of fruit losses by fungi per caste. Error bars represents the standard error. Bars with the same letter did not differ significantly between them.

The GLMM show that fruit loss by fungus differed significantly among castes ($F_{3,365}$=67.06, p=0.003).

The Baga showing higher losses than the other three castes. Arinto and Chardonnay castes showing higher losses than the Touriga Nacional (Fig. 13, Table 3).

Table 3. Summary of the General Linear Hypothesis model testing the effect of caste on fruit loss by fungi. The model compares all caste-pairs. Significant results are highlighted in bold.

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
Baga - Arinto	0.72858	0.07498	9.717	**< 0.001**
Chardonnay - Arinto	0.19383	0.15146	1.280	0.54663
Touriga - Arinto	-1.28391	0.16525	-7.770	**< 0.001**
Chardonnay - Baga	-0.53475	0.16903	-3.164	**0.00732**
Touriga - Baga	-2.01249	0.16548	-12.161	**< 0.001**
Touriga - Chardonnay	-1.47774	0.22405	-6.595	**< 0.001**

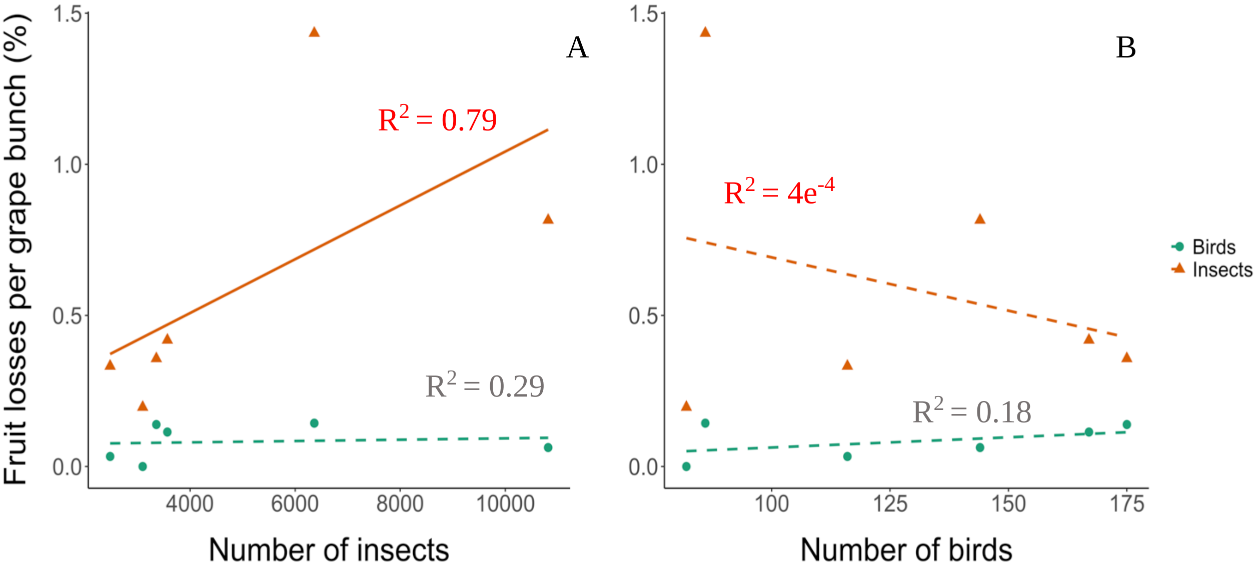

Fig. 14 Relationships between the total number of insects (A) and birds (B) and the mean fruit losses caused both by birds and insects per vineyard. The solid regression lines represent significant relationships.

Insect abundance was positively related to a 79% grape loss increase by insects (Fig.15A; $r_s = 0.89$; $p = 0.03$), while losses by birds were not affected by insect abundance ($r_s = 0.54$; $p = 0.2$). Similarly, bird abundance did not significantly affect grape losses by birds ($r_s = -0.46$; $p = 0.35$) or by insects (Fig.14B, $r_s = 0.02$, $p=1$). The increase of insect pests did not significantly affect grape losses by insects (Fig.14A, $r_s = 0.09$, $p = 0.92$). The increase of auxiliary insects did not significantly affect grape losses by insects (Fig.15B, $r_s = -0.2$, $p=0.71$). When for all regressions, the insect biomass was used instead of number of insects, all regressions were non-significant.

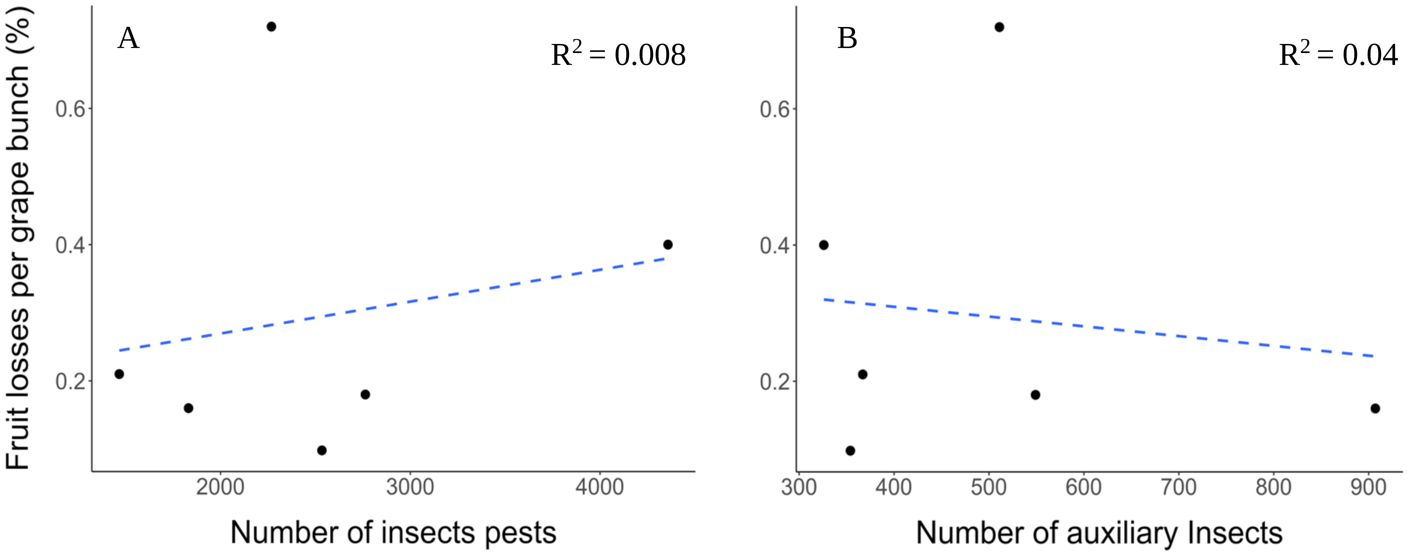

Fig. 15. Relation between the total number of insect's pests (A) and auxiliary (B) and the mean of fruit losses caused by insects per vineyard. The dashed regression lines were non-significant regressions. Each point represents one vineyard.

In relation to the number of birds, the increase in the number of bird pest individuals did not significantly affect the losses by birds (Fig.16A, $r_s = 0.66$, $p = 0.18$). Similarly, the increase in the number of auxiliary birds did not significantly affect the losses by birds (Fig.16B, $r_s = 0.02$, $p = 1$). Also, the increase of auxiliary birds did not significantly affect the abundance of insects (Fig.16C, $r_s=0.26$, $p=0.41$).

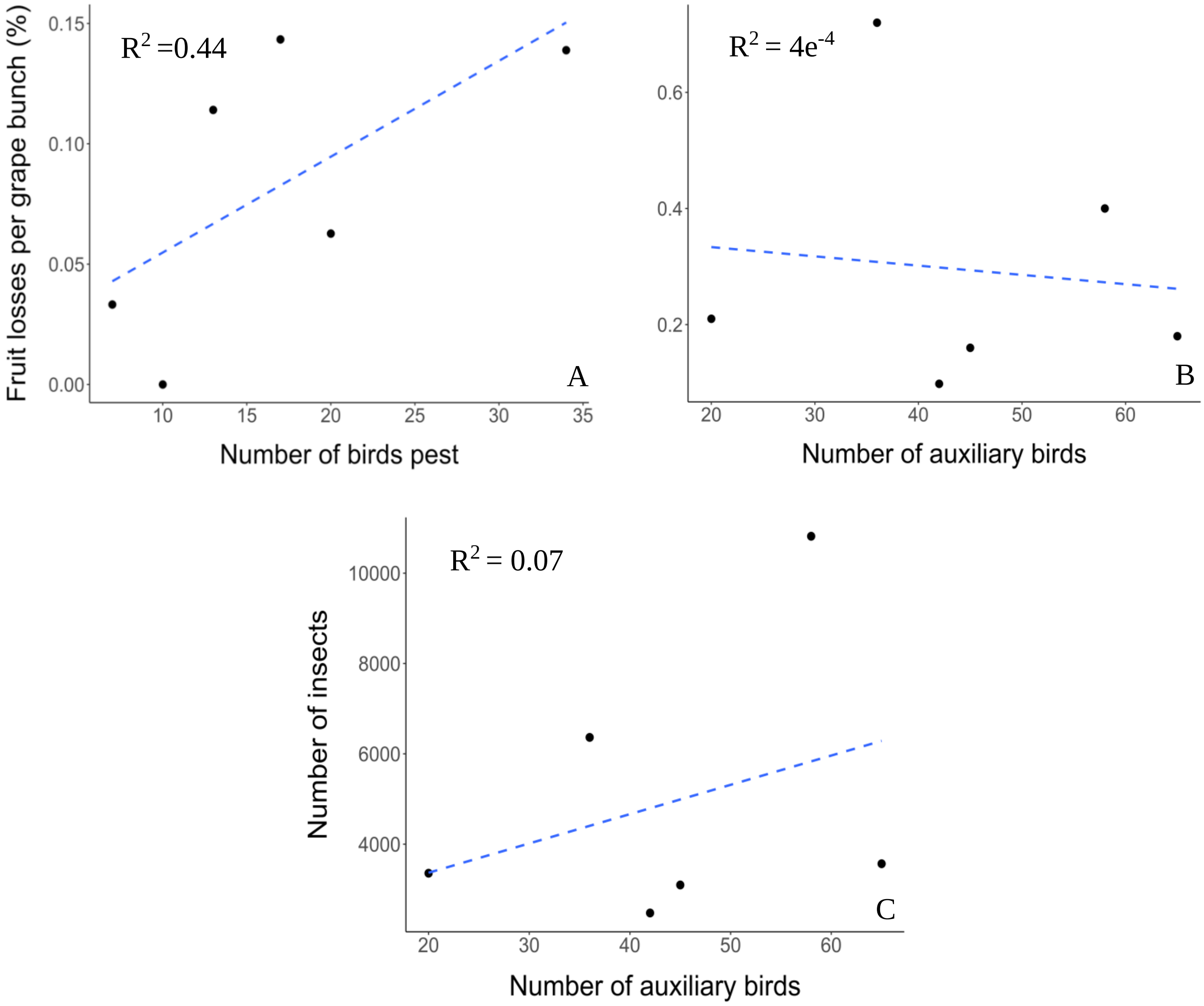

Fig. 16. A) Regression of total bird pests with mean of grapes lost to birds per bunch. The bird pests were the frugivorous birds; B) Regression of number of auxiliary birds with mean of grapes lost to insects per bunch. The auxiliary birds were the insectivorous birds. C) Regression of number of auxiliary birds with total number of insects. The dashed regression lines were non-significant regressions. Each point represents one vineyard.

3.2 Vineyard biodiversity

3.2.1 Bird abundance

The vineyard with more birds was Estação vitivínicola da Bairrada (1.62 individuals per census), and the vineyard with less birds was São João (0.76 individuals per census). The difference in bird numbers per census between the three months was very small: 0.93, 1.21 and 1.14 individuals, respectively for June, July and August. In terms of functional biodiversity most birds were neutral (1.58 individuals per census), and only 0.79 birds per census were pests. In July the number of neutral birds per census was higher than in the other two months (2.01 birds on July, 1.3 and 1.42 in June and August, respectively). The number of pest birds did not vary much along the season (4.25 individuals in June, 5 in July and 4.83 in August), same occurs for auxiliary (0.65 in June, 0.83 in July and 0.88 in August) (Fig.17; Appendix 2).

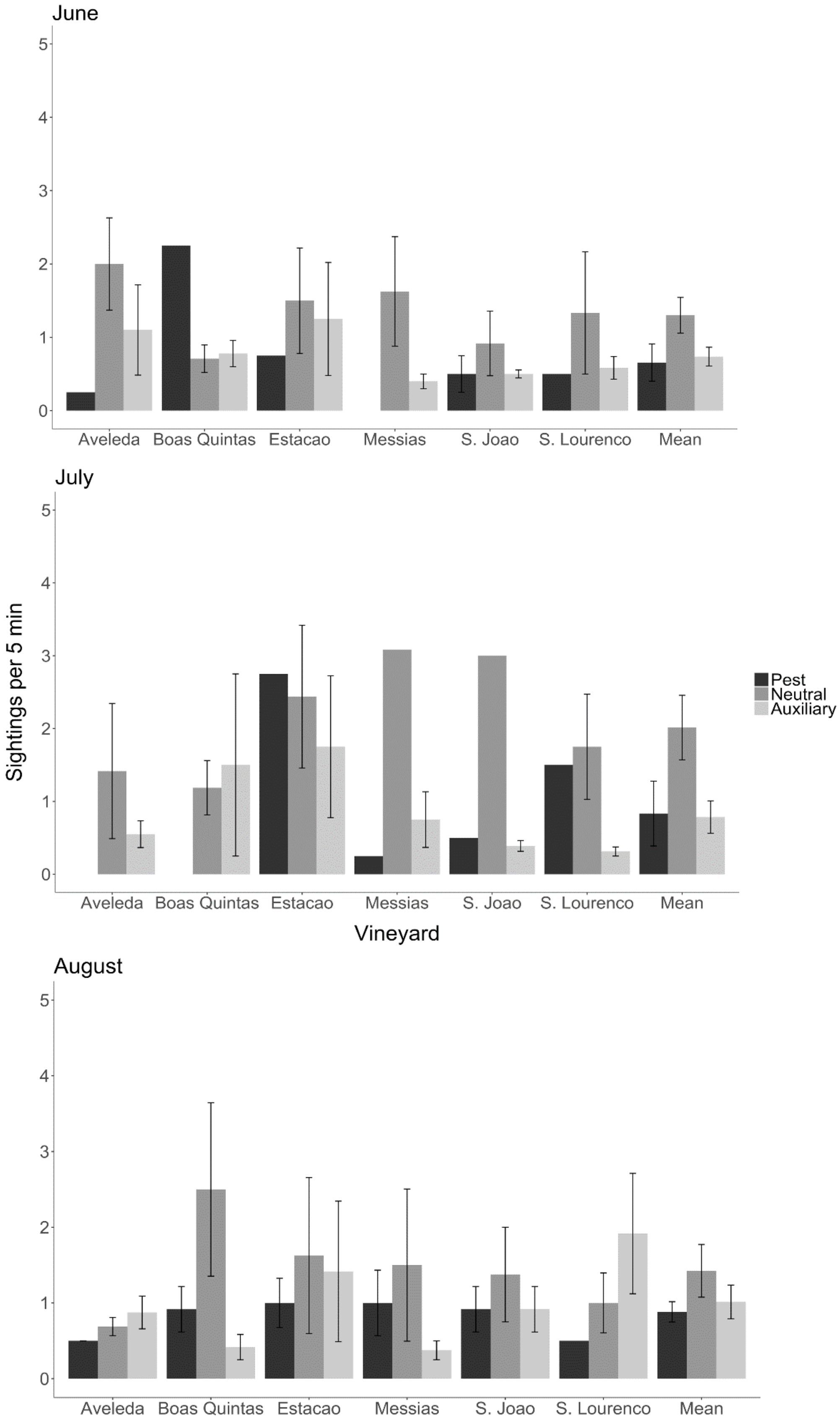

Fig. 17. Bird sightings per census in each vineyard during the study period. Error bars represent the standard error.

3.2.2 Insect abundance

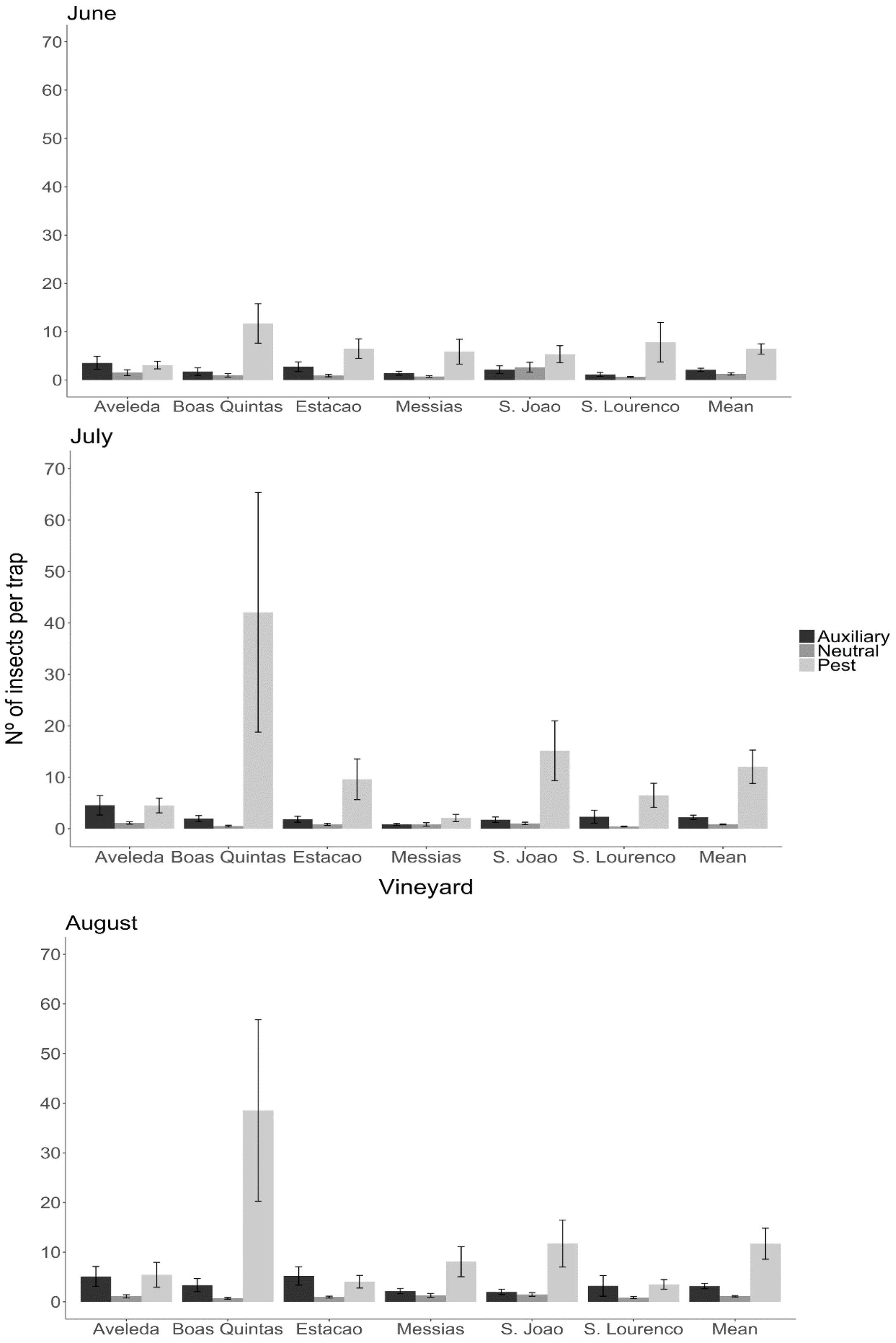

Fig. 18 . Abundance of insects per trap in each vineyard during the study period. Error bars represent the standard error.

The vineyard with more insects was Boas Quintas (16.19 individuals per trap) and the vineyard with less insects was Messias (3.02 insects per trap). In terms of functional biodiversity in vineyards most insects were pests (8.19 individuals per trap), and fewer insects were neutrals (1.2 individuals per trap). The number of auxiliary insects was 2.46 individuals per trap. In all months the number of pest insects was much higher than the other two insect types. In June the number of insects were 6.44 individuals for insect pests, 1.24 individuals for neutral insects and 2.11 individuals for auxiliary insects. In July the number of insect pests were 12.04 individuals, the auxiliary insects were 02.22 and the neutral insects were 0.84 individuals. In August the insect pests were 11.69 individuals, neutral insects were 1.12 and auxiliary insects were 3.15 individuals. The number of insects increased throughout the season: 3.93 insect individuals for June, 6.28 for July and 6.46 for August (Fig.18; Appendix 3).

3.2.3 Insect biomass

The vineyard with more biomass of insects was São João (13.72 mg per trap) and the vineyard with lower insect biomass was São Lourenço (4.51 mg per trap). The biomass variation among the three months was small: 8.56, 9.97 and 9.18 mg, respectively for June, July and August. In terms of functional biodiversity in vineyards the neutral insects comprised the major biomass (18.96 mg per trap), which was much higher than the auxiliaries (10.34 mg) and pests (2.25 mg). In June the biomass of neutral insects was 19.81 mg, of the auxiliaries 6.27 mg and of the pests 2.73 mg. In July the biomass of neutral insects was 18.21 mg, of the auxiliaries 14.85 mg and of the pests 1.84 mg. In August the tendency was maintained: the biomass of neutral insects was the highest (18.86 mg) and the biomass of pest insects was the smallest (2.18 mg) (Fig.19; Appendix 4).

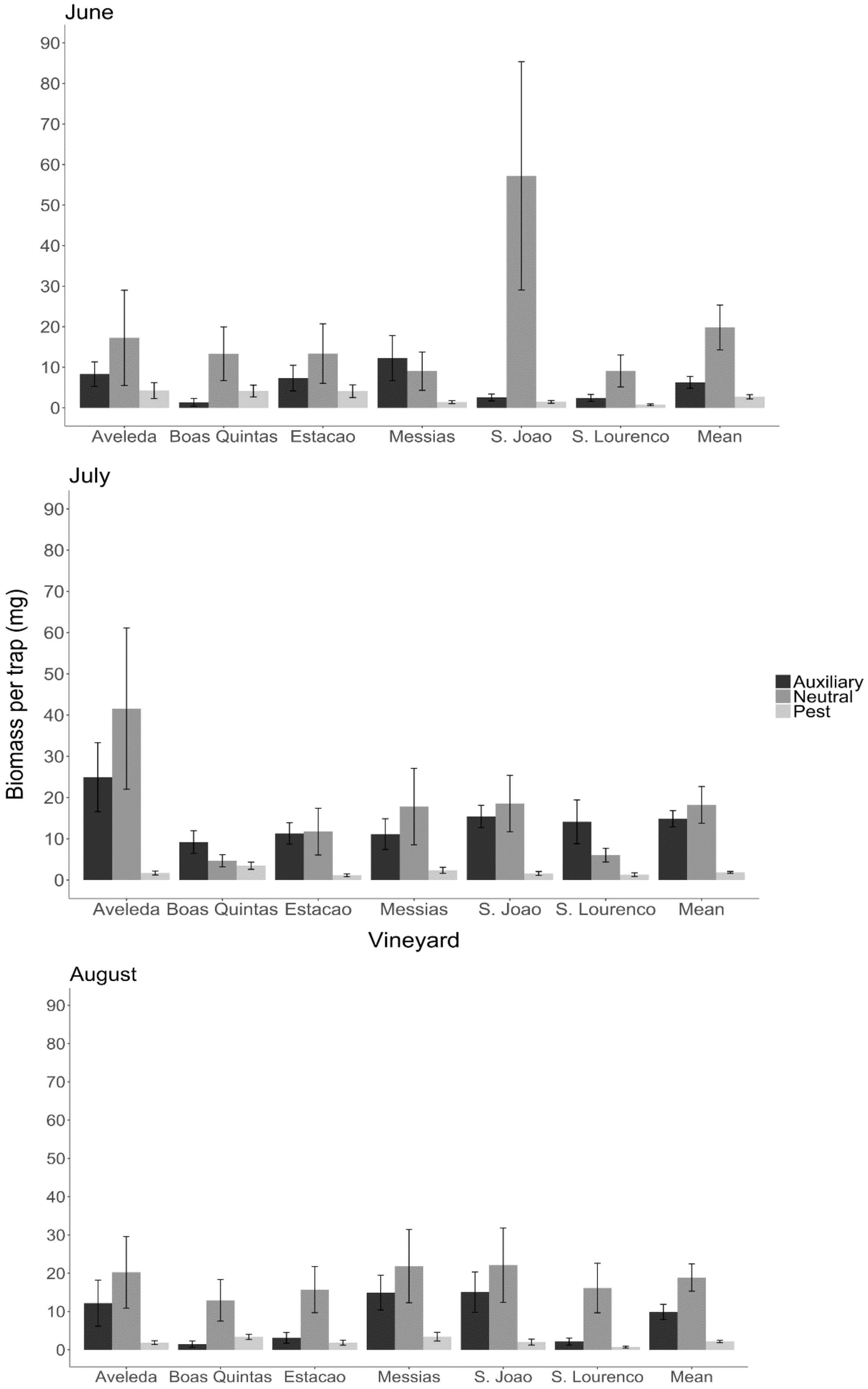

Fig. 19. Insect biomass per trap in each vineyard for the three study months. Error bars represent the standard error.

3.3 Distance to the edge and fruit losses

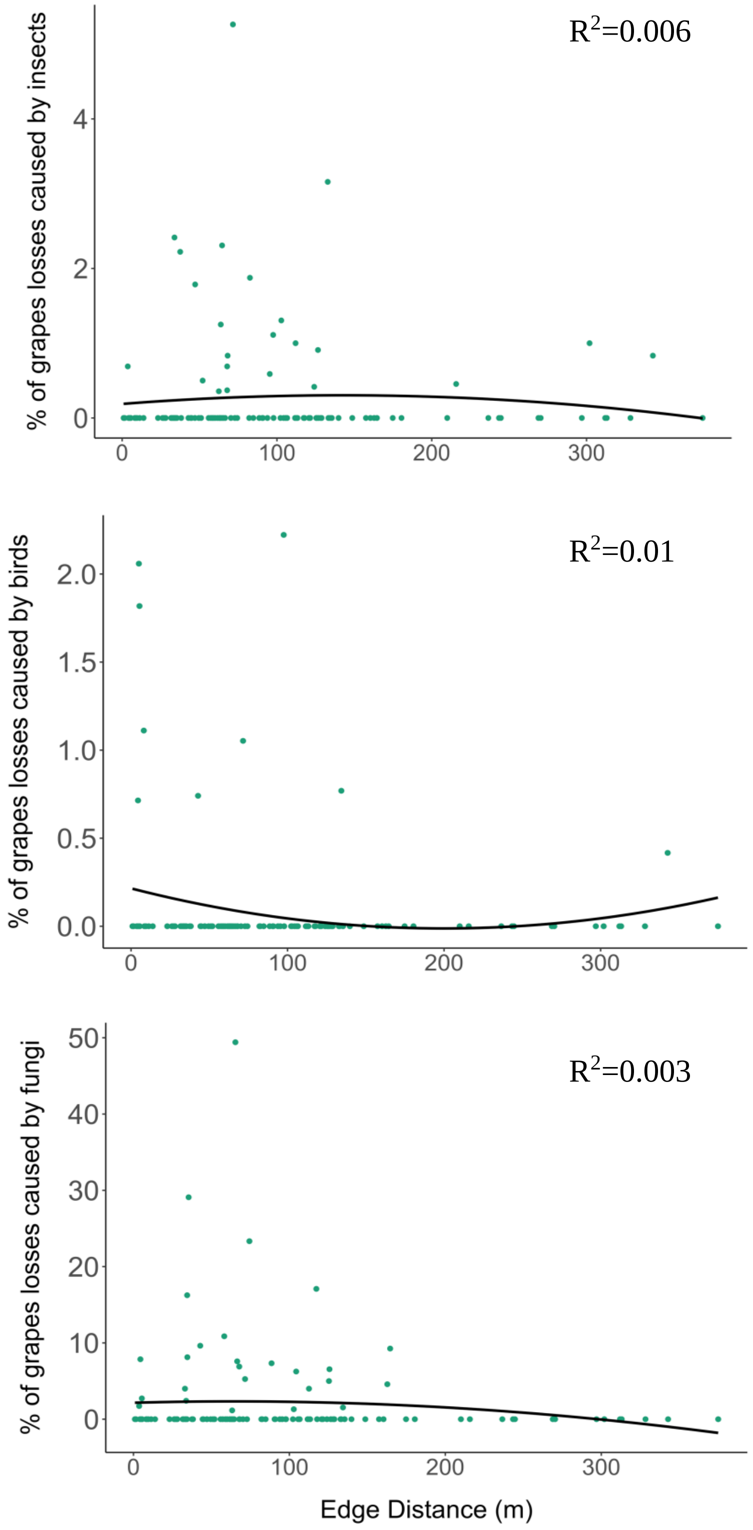

Fig. 20. Relationship between the proportion of grapes lost due to insects, birds and fungi on each bunch in relation to the distance to the edge of the vineyard. Each point represents a vine.

Grape losses were independent of the distance to the vineyard edge (Insects: r_s= 0.08, p=0.38, Birds: r_s = -0.12, p= 0.20 and Fungi: r_s = -0.06, p=0.50).

4.Discussion

This study shows that most grape losses in vineyards of Bairrada region were due to fungi (9,5%), while losses due to birds and insects were almost negligible (0.45% and 1.42% respectively) in all vineyards. The insects and birds did not show an overall preference by grape color, but insects caused more losses on the castes Chardonnay (white) and Baga (red). This caste grape preference should be taken into account in future studies to understand why insects may be more attracted to certain vineyards, and for future non-chemicals experiments and understanding the dynamics of infesting grape insects in the vineyards (Witzgall, 2001; Rigamonti et al., 2011). Unfortunately, organically grown vineyards were not available in the region and therefore chemical spraying was used in all vineyards sampled. We expect that the positive effects of the biotic resistance conferred by natural biodiversity is probably more important in the absence of pesticide treatments. Our results suggest that the chemical use in the region is actually effective as losses by insects and birds were negligible and losses with fungi were relatively low. However, this form of agriculture is known to affects the biodiversity and by consequence the ecosystems services. The biocontrol is one of the services considered most at risk (Geiger et al., 2010). The new agricultural practices are characterized by a reduction of environmental impact without compromising production. This model is called integrated production (Perini & Susi, 2004), and this model takes into account the biodiversity role in agricultural areas, so more policies have been implemented to protect this biodiversity (Mccracken, 2011). The increase of biodiversity levels is related with an increase of natural enemies, which favors the biotic resistance hypothesis (Letourneau & Bothwell, 2008). A study in France in different types of arable farm showed that a 42% reduction in the use of pesticides did not affect wine production on 77% of the farms, and in 59% this reduction actually proved to be more profitable for the cultures (Lechenet et al., 2017). The European Authorities have been developing more laws with the objective of applying these environmental polices (Donald et al., 2002; Schrijver, 2016).

4.1 Grape losses

We observed a very low proportion of grape losses by birds and insects, but other studies report a higher percentage of grape loss attributed to insects and birds. For example Kross et al. (2012) in their experiment occurred in Marlborough region (New Zeeland), register in some cases 3.5% of grape damaged by birds and Bournier (1976) cited that crop losses can reach 15%, caused by *Platynota stultana* (Lepidoptera: Tortricidae) in California (Bournier, 1976; Kross et al., 2012).

The fact that the percentage of grape losses attributed to fungi was much higher than the other two types of losses could be largely attributed to the vineyards of Caves Messias. There are two possible explications for this: 1) Net installation and handling of grape bunches could have resulted in some grape damage (which contributes for fungus colonization, and it is well known that grape damage, and development of insect colonies are usually higher in infected bunches than in undamaged bunches (Bellí et al., 2007); 2) The different hardness and thickness of grape varieties should also influence grape losses (Bellí et al., 2007). The four vineyards with fungi losses (Boas Quintas, Estação, Messias and São João) had the caste Arinto, which presents a slim cuticle that is more susceptible to fungi colonization (www.adegaalmeirim.pt). The Messias vineyard had another caste with a slim cuticle, Baga, which is susceptible to fungi colonization (http://www.signatureimports.com/PortugueseWineGlossary.pdf), so the Messias vineyard had two castes more susceptible to fungi colonization increasing the percentage of grape losses by fungi. Overall, the fungi damage is usually associated with particular skin grape types (Bellí et al., 2007).

Contrarily to some previous studies, we did not register a significant influence of caste color on bird and insect grape losses. Some studies have showed a significant influence of fruit color on fruit feeding by both birds (Whitney, 2005; Gagetti et al., 2016) and insects (Takahara & Takahashi, 2016). This contradiction might be explained by the very lower number of grape losses detected in our study. The biochemical composition of grapes is also other factor that influences the preference of grapes by birds and insects; in fact, the results of our General Linear Hypothesis and multiple comparisons analyses for insects demonstrated a preference by Chardonnay and Baga grape varieties over Arinto and Touriga. Grape sugar levels are known to influence the insect and bird feeding preferences (Galvan et al., 2008; Saxton et al., 2009), unfortunately we did not measure sugar content in this study. A study showed that *Lobesia brotana* is more likely to lay eggs on castes with high skin sugar content (Loureiro and Trajadura castes)(Varandas et al., 2004). The chemical and tactile characteristics of castes may also play an important role in grape losses by insects; for example castes present differences in nutrients, which may affect the choice of insects like the *Lobesia brotana* that selects Pinot Noir, Chardonnay Chasselas and Grenache castes to oviposit the eggs, and this selection affects the development time of larvae (Moreau, Benrey, & Thiéry, 2006; Moreau et al., 2008).

In summary, there are insects which select certain grape varieties due to skin, color, biochemical and physical characteristics, (Galvan et al., 2008) and this may be taken into account to plan better biocontrol plans, for example georeferencing the areas with castes that are preferred by insects, and installing nest sites for insectivorous birds or realizing natural predator of pests in these zones, thus mitigating the losses in critical vineyard areas and decreasing the costs of implementing these programs.

Few studies evaluated grape losses by birds. One exception is the study of Anderson et al. (2013), were North American farmers were asked for their perception regarding bird losses. The authors concluded that bird losses were relatively small across the five states analyzed (Mean=5.9%; Max 9.2%) which partly agrees with our empirical results (Anderson et al., 2013). Similar results were obtained in South Africa with bird losses varying between 1 and 5% (Dignon, 2013).

There are considerably more studies evaluating grape losses due to insects, although most of them are focused on the effects of a single insect pest (Hoffman & Dennehy, 1987; Moschos, 2005). In Brazil they registered 4% of grape losses in the whole country (Oliveira et al., 2014). Other study analyzed the losses caused by *Lobesia brotana* in grapes, and authors register 5.7% of losses in some years (Hoffman & Dennehy, 1987). Our insect losses (1.42%) are relatively lower than those of previous studies, bur the losses depend on many factors, like the year, the localization of the vine or the stage of grape development, and since we do not have a similar study that analyses only some vines and extrapolated for the vineyard we cannot compare a level of losses with rigor. More studies of this type will be need to evaluate the real percentage of losses caused by natural biodiversity in vineyards.

4.2 Relation between losses and natural biodiversity

In contrast with our expectations, we could not detect any effect of natural biodiversity on pest control. We showed that vineyards with more insects had more losses, as most insects sampled in the vineyards were pests (74.5%). Interestingly, the proportion of auxiliary insects was also very low, likely due to the use of pesticides hindering their potential role as biocontrol agents. Such a negative relationship between insecticide toxicity and the abundance of biocontrol agents (spiders, lacewings, carabids and parasitoids) has also been observed by Thomson and Hoffmann (2006) in Victoria (Australia).

Our bird census revealed that the number of bird pests and the number of bird auxiliaries were similar. It is well known that birds' feeding behavior changes along the grape season. Early in the season, when breeding, birds feed more on insects, which are also crucial dietary items for their offspring during their early stages of development to ensure growth and survival (Herrmann & Anderson, 2007). The reproductive season of the majority of the bird species corresponded to the first months of our experiment; therefore in June – July the birds were presumably feeding mostly on insects and in August – September they could start feeding on grapes which became ripe and available for consumption. In our dataset, vineyeards with a higher density of bird pests had considerably more grape losses (Fig. 16A), however, this relationship was not statistically significant due to the high heterogeneity of the data, the overall low effect of birds, and the small number of vineyards sampled., In contrast, other studies shown much higher consumption of grapes by flocks of starlings (Stevenson & Virgo, 1971; Curtis et al., 1994), This low consumption may be due to the type of vineyards in Bairrada region. Contrary to California and Alentejo vineyards the Bairrada region consists of a heterogeneous habitat with landscapes that provide shelter, breeding sites and feeding zones for birds (Pithon et al. , 2016), without the need to feed heavily on grapes..

4.3 Edge effect

We did not find any effect of the distance to the edge of the vineyard on the proportion of grape losses. Although we can observe a pattern in the three type of losses (birds, insects and fungi), most losses were in first 100m into the vineyard.

The grape losses caused by birds tended to decline with increasing distances from the edge (Somers & Morris, 2002b). Most avian species only visit the vineyards occasionally for feeding, flying out from the edge where cover is better (Pithon et al., 2016). So the vines closest to the edge should be visited more often by frugivorous birds, given the reduction in the distance they need to fly from surrounding perches at the edge habitat and protective cover against predators in those areas (Stevenson & Virgo, 1971; Somers & Morris, 2002; Saxton et al., 2004). Another factor that may affect grape losses is the type of edge, which may explain the abundance and diversity of birds (Pithon et al., 2016). Therefore, the edge type may influence the number of frugivorous and insectivorous birds, because some edges can have sufficient food for the frugivorous birds, without the need to visit the nearby vineyards, whereas other edge types may be poor in food resources forcing the birds to enter more often into the vineyard.

Like bird losses the losses caused by insects tended to decline with the increasing distances from the edge (Hoffman & Dennehy, 1987). The insects are also more common at the edge than in the vineyards interior (Williamson & Johnson, 2005; Sciarretta & Trematerra, 2014; Steel et al., 2017), so the insect pests tend to infect the vineyards closer to the edge. But the edge is also important for the auxiliaries insects (Nicholls et al., 2001; Rand, Tylianakis, & Tscharntke, 2006; Thomson & Hoffmann, 2010), which first predate/parasite the insect pests that are closer to the edge (Thomson & Hoffmann, 2013). Therefore, the grape losses caused by insects is presumably dependent on the ratio of the number of pests and auxiliary insects at the edge.

4.4. Conclusion

Our study reveals that grapes losses due to birds and insects are very small in the Bairrada region, contrary to our expectations. Even with this low number of losses the results show a preference of insects by Chardonnay and Baga castes. These differences should be explained by the physical and chemical characteristics of these castes. The color in this study apparently did not influence caste choice by native biodiversity. The fungi were the most important cause of grape losses, and the two castes with a slim skin, Baga and Arinto, were more susceptible to colonization by fungus.

In our study the vast majority of the insects were pests and not natural biocontrol agents, and the same occurred with birds. When comparing grape losses with the biological control provided by insects the birds found in vineyards, presumably the low number of biocontrol agents was not sufficient to reduce the number of insect pests. Despite this, our results reveal a small number of natural enemies and knowing the pesticides negative effects in this biological functional group, the use of pesticides seems to influence the biocontrol in vineyards. So, the productivity of vines may still be higher reducing the use of pesticides and implementing biocontrol programs of increasing/conserving the natural enemies in the vineyards, reducing the money spent on pesticides that can be used in these biocontrol programs. In a medium term this strategy may be even more sustainable, reducing more the use of pesticides, increasing the auto biocontrol of vineyards by natural enemies and decreasing the ecological footprint that this type of agriculture has in the environment. But to test if the natural biodiversity has an important role in the biocontrol in vineyards vineyard without pesticides should be included in future studies. The edge effect did not show a significant relation with grape losses although a pattern for greater losses in the first 100 meters was apparent. For the future, more studies about the influence of edge in losses of grapes will be necessary to mapping the critical zones of vineyards, and apply more effectively potential biocontrol programs. Finally, a good knowledge of the grape varieties and their characteristics proves important to know if a certain variety is more susceptible to losses, and why particular pests may prefer such variety as opposed to others.

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5.1 Webgraphy

http://www.revistadevinhos.pt/artigos/show.aspx?seccao=reportagens&artigo=15459& - site consulted in 22/03/2017

http://www.oiv.int/ - site consulted in 22/03/2017

http://www.ivv.min-agricultura.pt/np4/125/- site consulted in 22/03/2017 and in 22/04/2017

http://www.infovini.com/- site consulted in 22/04/2017

https://www.vinha.pt/- site consulted in 22/04/2017 https://www.census.gov/population/international/ -site consulted in 30/05/2017

5.2 References for figures

Fig.1. Image adapted by the site http://www.infovini.com/pagina.php?codNode=18012- site consulted in 05/03/2017

Fig. 2. Image taken by the site http://www.omafra.gov.on.ca/IPM/english/grapes/diseases-and-disorders/b-damage.html- site consulted in 1/06/2017

Fig. 3. Image created from a set of images taken from the site:
A) https://entomologytoday.org/2015/06/11/galls-insects-behind-the-weird-growths-on-plants/#jp-carousel-4127 - site consulted in 05/03/2017
B) http://bugguide.net/node/view/692105- site consulted in 05/03/2017
C) https://www.cropscience.bayer.com/en/crop-compendium/pests-diseases-weeds/pests/eupoecilia-ambiguella- site consulted in 05/03/2017
D) https://gd.eppo.int/taxon/POLYBO/photos- site consulted in 05/03/2017
E) Photo taken by Mauro Nereu

Fig. 4. Image created from a set of images taken from the site:
A) http://stankova.net/research.htm - site consulted in 05/03 /2017
B) Image taken in the book Arthropod Management in Vineyards: Pests, Approaches, and Future Directions
C) http://www.ciidae.com/1349/trichogramma-sp-2 - site consulted in 05/03/2017
D) https://www.forestryimages.org/browse/detail.cfm?imgnum=1445011- site consulted in 05/06/2017
E) http://www.biocomes.eu/biological-control/biological-control-examples/ - site consulted in 05/03/2017
Fig 5. Image created in QGis using the shape image taken in site http://www.adegaalmeida.com.br/blog/mapa-do-vinho-portugal/
Fig. 6. Photos taken by Mauro Nereu
Fig. 7. Photos taken by Mauro Nereu
Fig. 9. Photos taken by Mauro Nereu

Appendices

Appendix 1

Table 4. Insect biomass equations per family:

Family	Formula
Agromizydae	Biomass= $0.0221.L^{3.18}$
Aphidiae	Biomass= $0.0308.L^{4.9}$
Apidae	Biomass= $0.027.L^{4.28}$
Chloropidae	Biomass= $0.0215.L^{3.37}$
Chrysomelidae	Biomass= $0.0217.L^{2.43}$
Chrysopidae	Biomass= $0.027.L^{4.28}$
Cicadellidae	Biomass= $0.0256.L^{3.74}$
Coccinelidae	Biomass= $0.0387.L^{4.93}$
Curculionidae	Biomass= $0.0249.L^{3.25}$
Dermestidae	Biomass= $0.0249.L^{3.25}$
Elateridae	Biomass= $0.0293.L^{4.6}$
Formicidae	Biomass= $0.0292.L^{4.72}$
Lygalidae	Biomass= $0.0308.L^{4.78}$
Micro heminopteros	Biomass= $0.027.L^{4.28}$
Miridae	Biomass= $0.0149.L^{2.26}$
Mordellidae	Biomass= $0.0249.L^{3.25}$
Muscidae	Biomass= $0.0263.L^{3.62}$
Parasita heminoptero	Biomass= $0.027.L^{4.28}$
Pieridae	Biomass= $0.0312.L^{5.04}$
Sphecidae	Biomass= $0.027.L^{4,28}$
Syrphidae	Biomass= $0.0263.L^{3.62}$
Tenebridiae	Biomass= $0.012.L^{0.043}$
Tephritidae	Biomass= $0.02221.L^{3.18}$
Tipulidae	Biomass= $0.0221.L^{3.68}$
Tortricidae	Biomass= $0.0312.L^{5.04}$

For the families Acaridae, Araneidae, Buprestidae, Pentatomidae, Psocoptera and Thysanoptera we use the general formula for all the insects.

Appendix 2

Table 5. Summary of birds in June

Vineyards	Type	Birds per census	Mean
Aveleda	Frugivores	0.25	0.65
Boas Quintas	Frugivores	2.25	
Estacao	Frugivores	0.75	
Messias	Frugivores	0	
S. Joao	Frugivores	0.5	
S. Lourenco	Frugivores	0.5	
Aveleda	Granivores	2	1.3
Boas Quintas	Granivores	0.7083333	
Estacao	Granivores	1.5	
Messias	Granivores	1.625	
S. Joao	Granivores	0.9166667	
S. Lourenco	Granivores	1.3333333	
Aveleda	Insectivores	1.1	0.77
Boas Quintas	Insectivores	0.7777778	
Estacao	Insectivores	1.25	
Messias	Insectivores	0.4	
S. Joao	Insectivores	0.5	
S. Lourenco	Insectivores	0.5833333	

Table 6. Summary of birds in July

Vineyard	Type	Birds per census	Mean
Aveleda	Frugivores	0	0.83

Boas Quintas	Frugivores	0	
Estacao	Frugivores	2.75	
Messias	Frugivores	0.25	
S. Joao	Frugivores	0.5	
S. Lourenco	Frugivores	1.5	
Aveleda	Granivores	1.42	2.01
Boas Quintas	Granivores	1.19	
Estacao	Granivores	2.44	
Messias	Granivores	3.08	
S. Joao	Granivores	3	
S. Lourenco	Granivores	1.75	
Aveleda	Insectivores	0.55	0.78
Boas Quintas	Insectivores	1.5	
Estacao	Insectivores	1.75	
Messias	Insectivores	0.75	
S. Joao	Insectivores	0.39	
S. Lourenco	Insectivores	0.3125	

Table 7. Summary of birds in August

Vineyard	Type	Birds per census	Mean
Aveleda	Frugivores	0.5	0.88
Boas Quintas	Frugivores	0.92	
Estacao	Frugivores	1	
Messias	Frugivores	1	
S. Joao	Frugivores	0.92	
S. Lourenco	Frugivores	0.5	
Aveleda	Granivores	0.69	1.42
Boas Quintas	Granivores	2.5	

Estacao	Granivores	1.63	
Messias	Granivores	1.5	
S. Joao	Granivores	1.38	
S. Lourenco	Granivores	1	
Aveleda	Insectivores	0.88	1.01
Boas Quintas	Insectivores	0.42	
Estacao	Insectivores	1.42	
Messias	Insectivores	0.375	
S. Joao	Insectivores	0.92	
S. Lourenco	Insectivores	1.92	

Appendix 3

Table 8. Summary of insects in June

Vineyard	Type	Insects per trap	Mean
Aveleda	Auxiliary	3.55	2.11
Boas Quintas	Auxiliary	1.76	
Estacao	Auxiliary	2.76	
Messias	Auxiliary	1.40	
S. Joao	Auxiliary	2.13	
S. Lourenco	Auxiliary	1.18	
Aveleda	Neutral	1.53	1.24
Boas Quintas	Neutral	0.97	
Estacao	Neutral	0.92	
Messias	Neutral	0.73	
S. Joao	Neutral	2.65	
S. Lourenco	Neutral	0.64	
Aveleda	Pest	3.09	6.44
Boas Quintas	Pest	11.71	
Estacao	Pest	6.50	
Messias	Pest	5.86	
S. Joao	Pest	5.34	
S. Lourenco	Pest	7.83	

Table 9. Summary of insects in July

Vineyard	Type	Insects per trap	Mean
Aveleda	Auxiliary	4.56	2.22
Boas Quintas	Auxiliary	1.95	
Estacao	Auxiliary	1.83	
Messias	Auxiliary	0.83	
S. Joao	Auxiliary	1.74	
S. Lourenco	Auxiliary	2.33	
Aveleda	Neutral	1.11	0.84
Boas Quintas	Neutral	0.52	
Estacao	Neutral	0.83	
Messias	Neutral	0.83	
S. Joao	Neutral	1.03	
S. Lourenco	Neutral	0.44	
Aveleda	Pest	4.50	12.04
Boas Quintas	Pest	42.08	
Estacao	Pest	9.61	
Messias	Pest	2.09	
S. Joao	Pest	15.17	
S. Lourenco	Pest	6.49	

Table 10. Summary of insects in August

Vineyard	Type	Insects per trap	Mean

Aveleda	Auxiliary	5.11	3.15
Boas Quintas	Auxiliary	3.36	
Estacao	Auxiliary	5.2	
Messias	Auxiliary	2.16	
S. Joao	Auxiliary	1.98	
S. Lourenco	Auxiliary	3.22	
Aveleda	Neutral	1.11	1.12
Boas Quintas	Neutral	0.71	
Estacao	Neutral	0.95	
Messias	Neutral	1.28	
S. Joao	Neutral	1.48	
S. Lourenco	Neutral	0.86	
Aveleda	Pest	5.43	11.69
Boas Quintas	Pest	38.54	
Estacao	Pest	4.0470588	
Messias	Pest	8.0827586	
S. Joao	Pest	11.7354839	
S.Lourenco	Pest	3.5125	

Appendix 4

Table 11. Summary of insect’s biomass in June

Vienyard	Type	Insects biomass per trap	Mean
Aveleda	Auxiliary	8.32	6.24
Boas Quintas	Auxiliary	1.36	
Estacao	Auxiliary	7.32	
Messias	Auxiliary	12.27	
S. Joao	Auxiliary	2.56	
S. Lourenco	Auxiliary	2.43	

Aveleda	Neutral	17.26	19.81
Boas Quintas	Neutral	13.33	
Estacao	Neutral	13.38	
Messias	Neutral	9.041	
S. Joao	Neutral	57.21	
S. Lourenco	Neutral	9.10	
Aveleda	Pest	4.25	2.73
Boas Quintas	Pest	4.14	
Estacao	Pest	4.11	
Messias	Pest	1.41	
S. Joao	Pest	1.48	
S. Lourenco	Pest	0.76	

Table 12. Summary of insect’s biomass in July

Vineyard	Type	Insects biomass per	Mean
Aveleda	Auxiliary	24.95	14.85
Boas Quintas	Auxiliary	9.21	
Estacao	Auxiliary	11.30	
Messias	Auxiliary	11.14	
S. Joao	Auxiliary	15.41	
S. Lourenco	Auxiliary	14.12	
Aveleda	Neutral	41.57	18.21
Boas Quintas	Neutral	4.66	
Estacao	Neutral	11.74	
Messias	Neutral	17.80	
S. Joao	Neutral	18.54	
S. Lourenco	Neutral	6.03	
Aveleda	Pest	1.69	1.84

Boas Quintas	Pest	3.47	
Estacao	Pest	1.14	
Messias	Pest	2.35	
S. Joao	Pest	1.59	
S. Lourenco	Pest	1.30	

Table 13. Summary of insect's biomass in August

Vineyard	Type	Insects biomass per trap	Mean
Aveleda	Auxiliary	12.19	9.89
Boas Quintas	Auxiliary	1.45	
Estacao	Auxiliary	3.10	
Messias	Auxiliary	14.92	
S. Joao	Auxiliary	15.07	
S. Lourenco	Auxiliary	2.14	
Aveleda	Neutral	20.24	18.86
Boas Quintas	Neutral	12.92	
Estacao	Neutral	15.72	
Messias	Neutral	21.85	
S. Joao	Neutral	22.12	
S. Lourenco	Neutral	16.14	
Aveleda	Pest	1.88	2.18
Boas Quintas	Pest	3.39	
Estacao	Pest	1.88	
Messias	Pest	3.42	
S. Joao	Pest	2.02	
S. Lourenco	Pest	0.68	

Appendix 5

Aerial images of the study sites, including the sampled vines, and the castes' distribution.

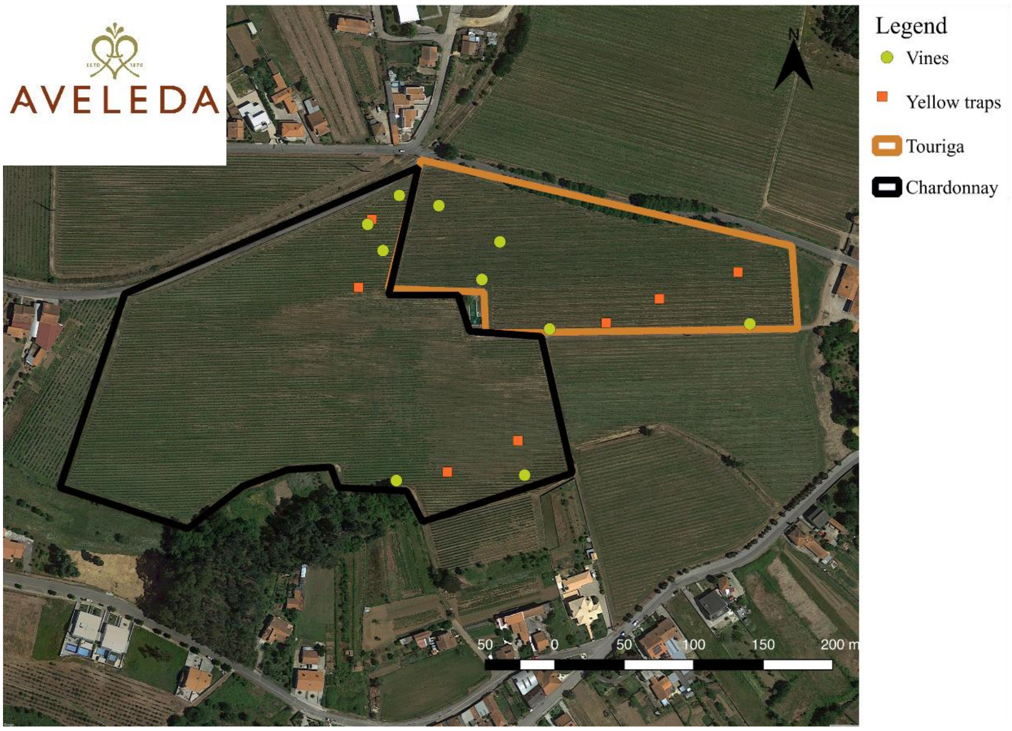

Fig. 21. Aveleda vineyard with the localization of vines (circles), yellow traps (squares) and the castes.

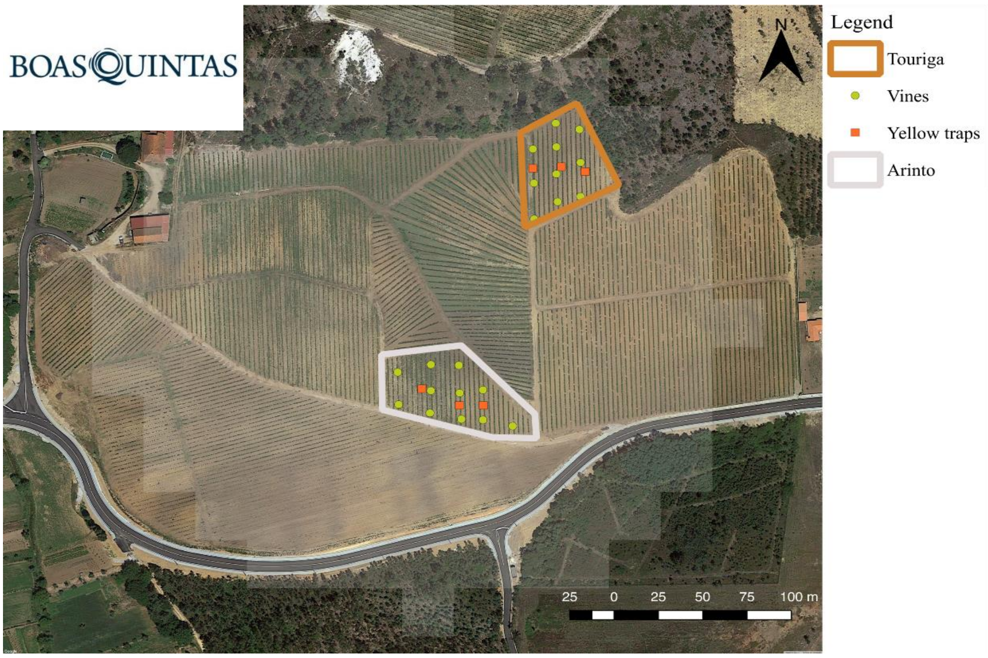

Fig. 22. Boas Quintas vineyard with the localization of vines (circles), yellow traps (squares) and the castes.

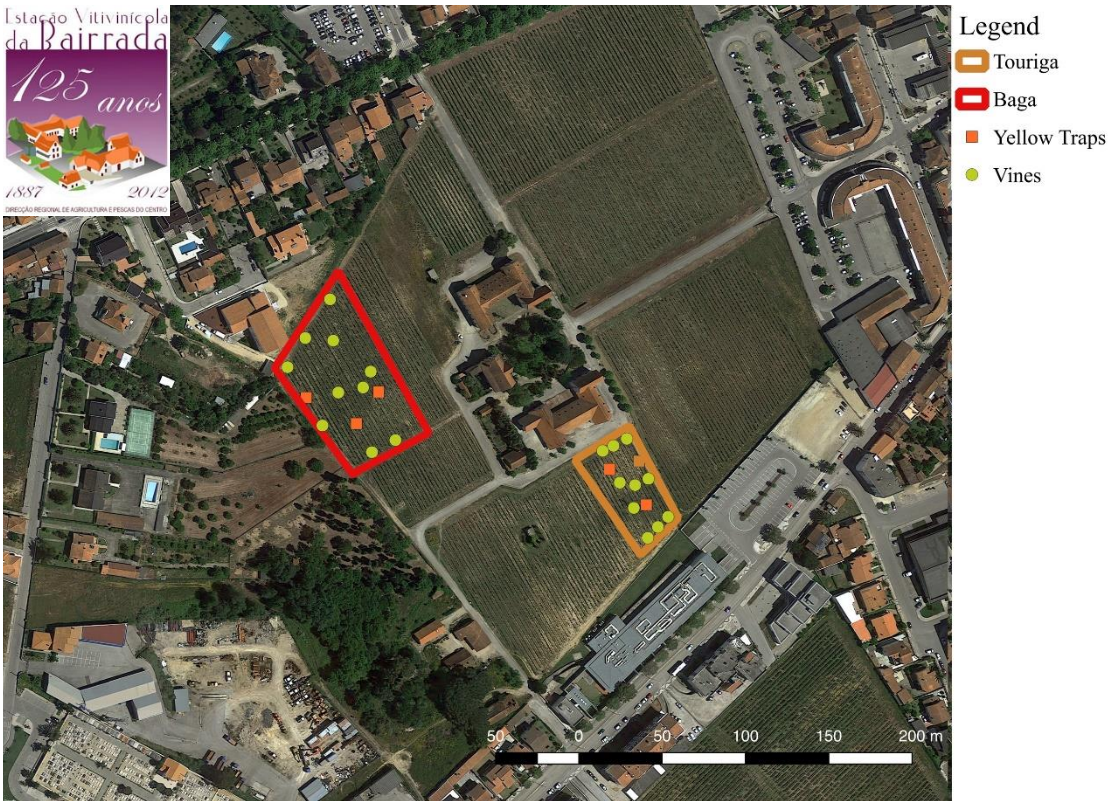

Fig. 23. Part of Estação Vitivinicula da Bairrada vineyard with the localization of vines (circles), yellow traps (squares) and the castes.

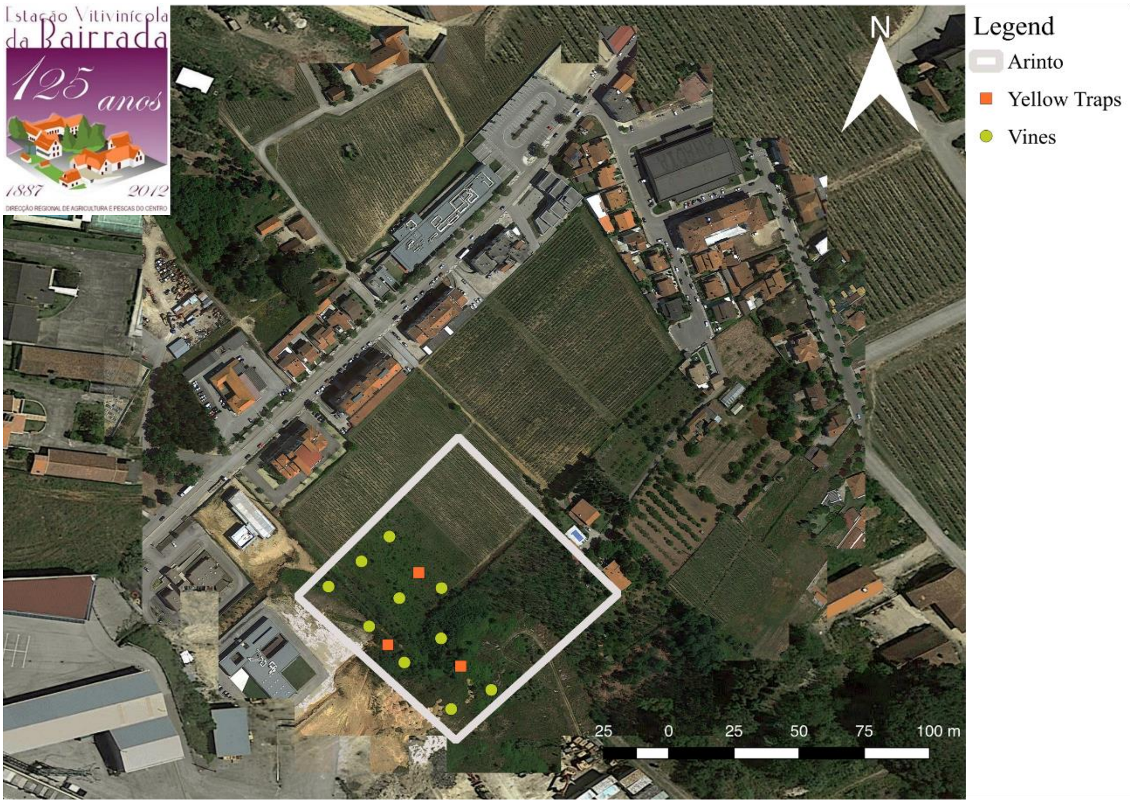

Fig. 24. Part of Estação Vitivinicula da Bairrada vineyard with the localization of vines (circles), yellow traps (squares) and the castes.

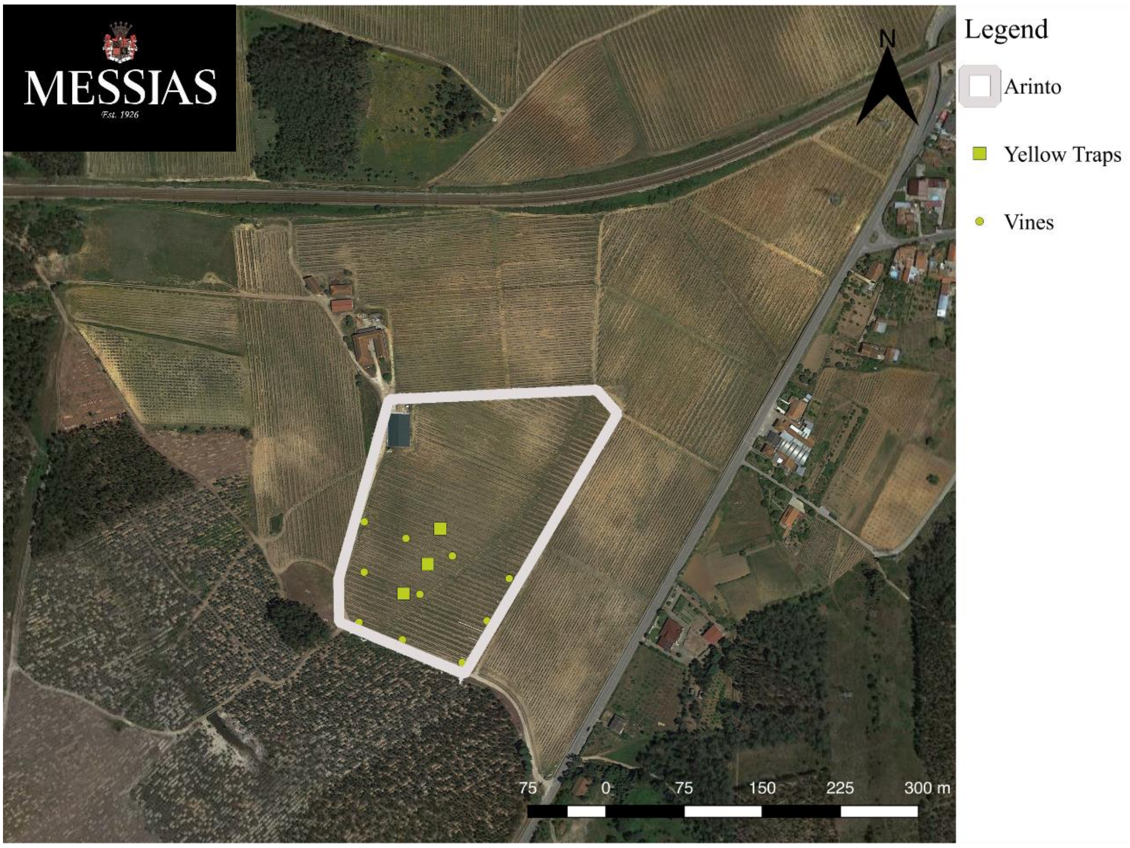

Fig. 26. Part of Messias vineyard with the localization of vines (circles), yellow traps (squares) and the castes.

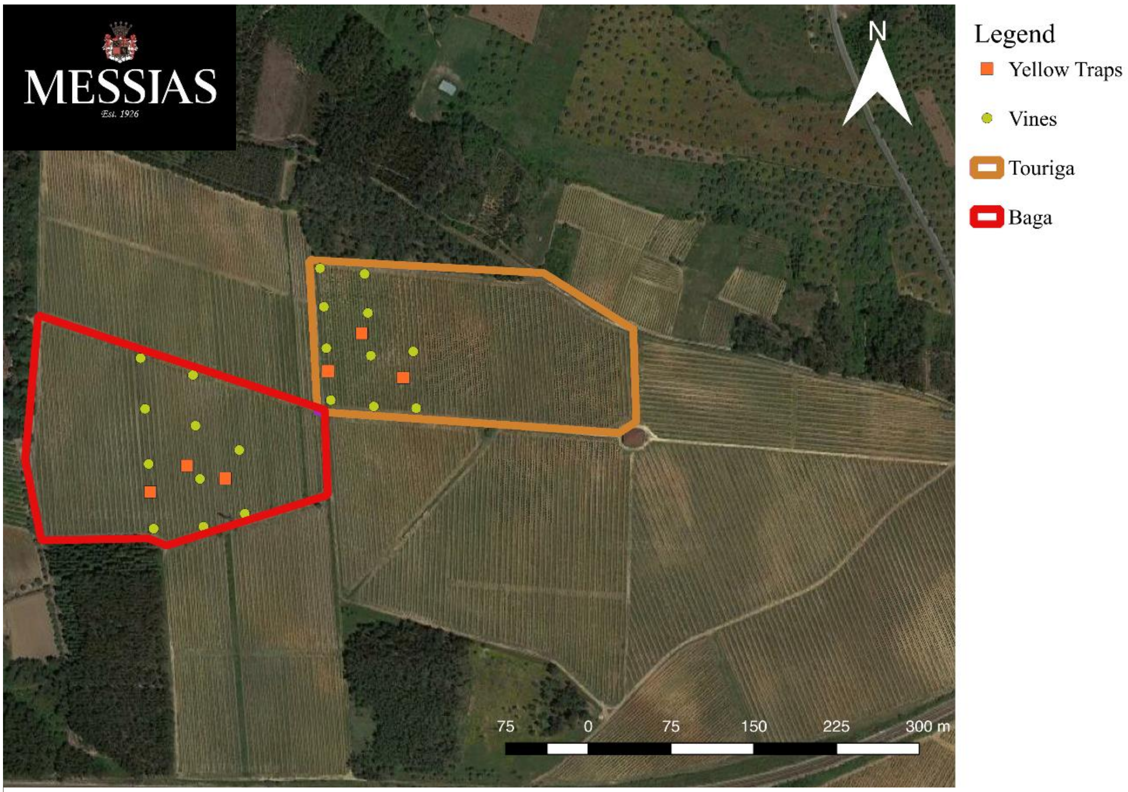

Fig. 25

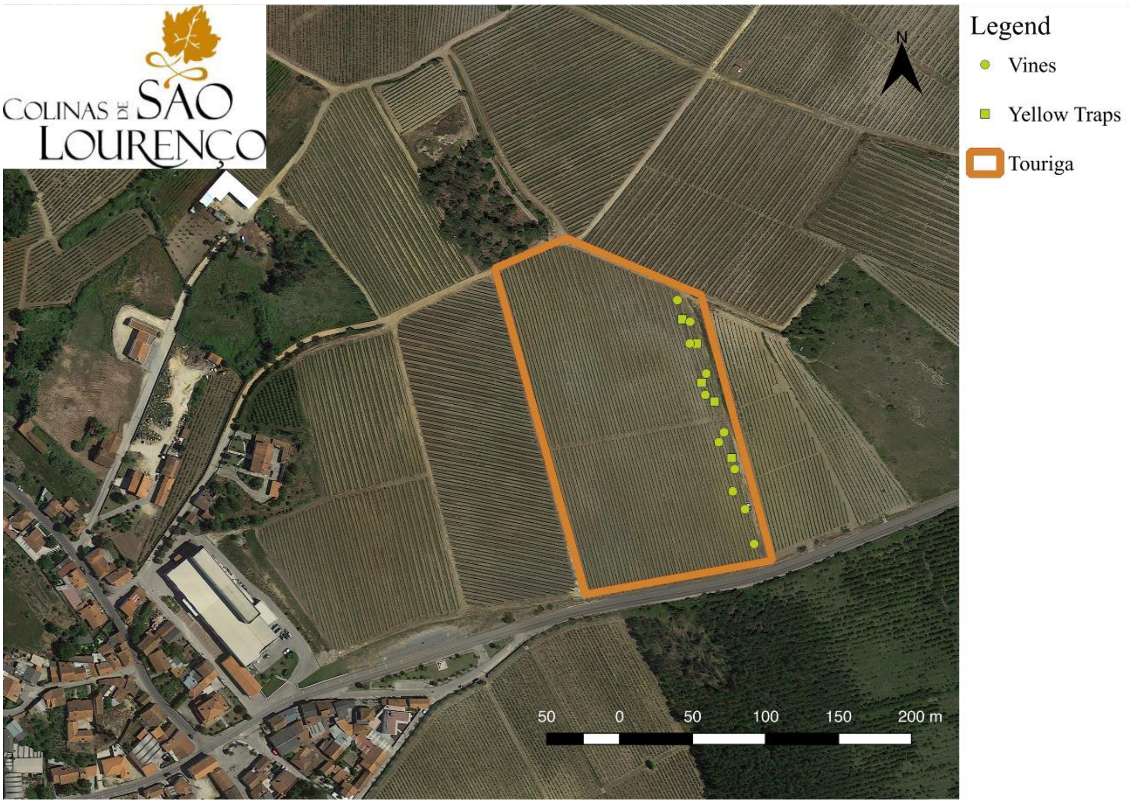

Fig. 27 Ideal Drinks (Colinas de São Lourenço) vineyard with the localization of vines (circles), yellow traps (squares) and the castes.

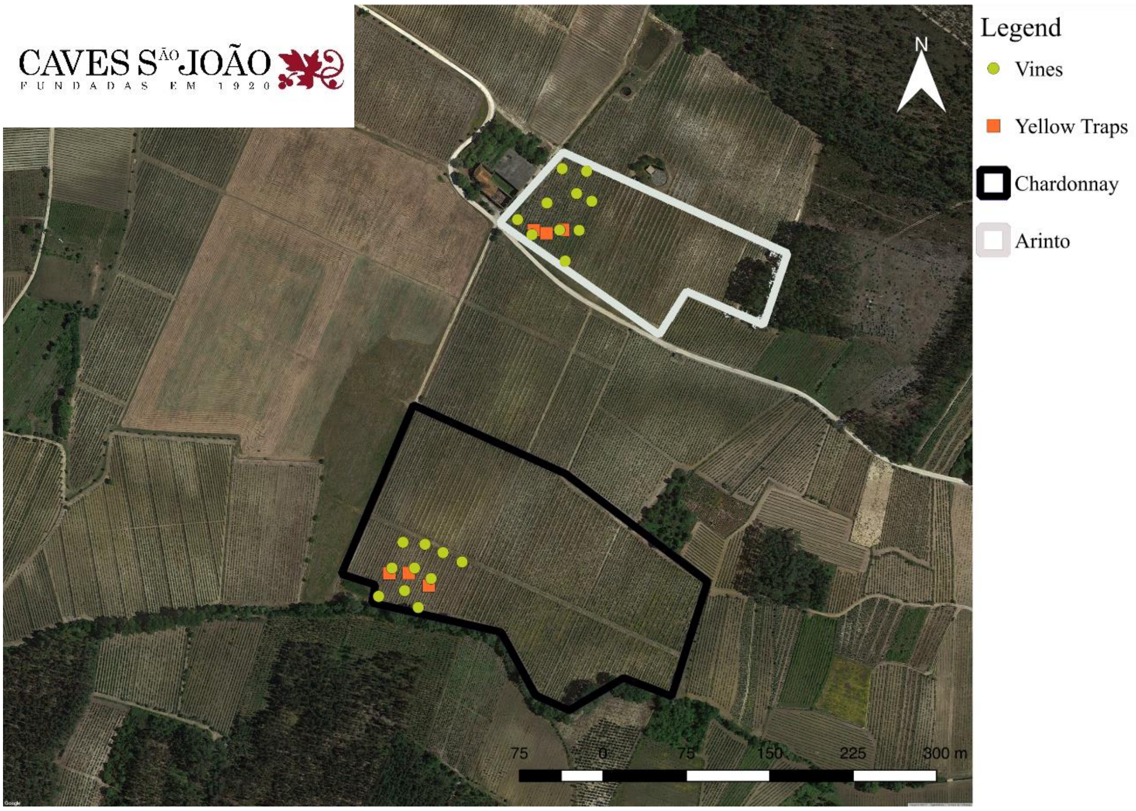

Fig. 28 São João vineyard with the localization of vines (circles), yellow traps (squares) and the castes.