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Auchenorrhyncha monitoring and proposal of management measures for potential pests on peach orchards in Beira Interior region

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Abstract

The Auchenorrhyncha suborder comprises several species considered to be pests of economically important crops whether as a result of the direct damage caused by their feeding process or through some species ability to act as vectors for plant pathogens such as viruses and phytoplasmas. Knowing this, the main goal of this study was the monitoring of the Auchenorrhyncha suborder for potential pests on peach orchards of the Beira Interior region, with a particular focus on the green leafhopper species *Asymmetrasca decedens* (Paoli), as well as potential vectors of the bacterium *Xylella fastidiosa* Wells *et al*.

The green leafhopper *Asymmetrasca decedens* was recorded in mainland Portugal in 2013, on the Beira Interior Region. As a highly polyphagous species, this leafhopper is associated with many infestations resulting in considerable damage in crops such as peach, plum, almond, cotton, among others. This species is capable of acting as a vector for 'Candidatus Phytoplasma Phoenicium', a pathogenic plant bacterium responsible for almond witches-broom disease that affects almond, peach, and other crops. *A. decedens* is also considered to be a potential vector for the phytoplasma that causes European Stone Fruit Yellow's disease which affects stone fruit trees. This leafhopper has a great potential as an emergent pest in this country, especially due to its significant resistance to conventional pesticides, and its wide range of hosts, that include economically important crops of the Beira Interior Region such as stone fruit, therefore posing as a threat to agriculture in Portugal.

Xylella fastidiosa is a xylem-limited vector-borne bacterium transmitted by some species of the Auchenorrhyncha suborder and is the causal agent of diseases that affect grapevine, olive, stone fruit, citrus and others. In 2013 this bacterium was detected in Italy associated with a disease known as Olive Quick Decline Syndrome, which had devastating effects in olive orchards in the region. Since then, *X. fastidiosa* has been found in other countries in Europe, including in Portugal, where it was reported for the first time in 2019. Due to the recent detection of *X. fastidiosa* in Portugal, the widespread presence of confirmed vectors, and the considerable variety of susceptible economically important crops, monitorization is imperative in order to avoid, contain and reduce the potential damage caused by the bacterium.

The captured individuals of the Auchenorrhyncha community were identified to the lowest possible taxonomic level, population dynamics were analyzed and environmental factors such as climatic variables were also studied to determine its influence on species variation. In total, 8140 individuals were captured on Póvoa de Atalaia and Louriçal do Campo orchards and 39 species of the Auchenorrhyncha community were detected. Two confirmed vectors species of *X. fastidiosa*, *Philaenus spumarius* (Linnaeus) and *Neophilaenus campestris* (Fallén), were found on both orchards in 2019. This detection highlights the need to continuously monitor these species considering the threat these could become if the current area affected by *X. fastidiosa* expanded. The dominant species on the orchards were *Empoasca solani* which presented a shorter and earlier distribution as the peak was registered on April 18th and *A. decedens* which gradually replaced the first and reached its abundance peak on September 20th. The data obtained regarding *A. decedens* abundance and distribution allowed the establishment of appropriate periods for the control of this species.

Keywords: *Asymmetrasca decedens*, *Xylella fastidiosa*, Beira Interior Region, stone fruit, pest management

Resumo alargado

Há muito tempo que as pragas constituem um desafio para a população humana quer por competirem pelos mesmos recursos quer pelo efeito prejudicial que podem representar para a saúde. Um dos mais importantes grupos de pragas são os insetos por serem responsáveis por consideráveis estragos na agricultura, a qual é essencial à alimentação humana, e o considerável impacto económico resultante. Vários fatores antropogénicos tais como o aumento de ambientes manipulados pelo Homem, como o caso de monoculturas, o aumento de transportes e mobilidade resultante da crescente globalização e outros fatores como alterações climáticas, têm contribuido para a presença e maior frequência de determinadas pragas. Ao longo dos anos a humanidade tem vindo a desenvolver métodos e estratégias de controlo para estas pragas de forma a conter ou limitar os estragos causados, incluindo programas de monitorização de forma a prever possíveis surtos e determinar a distribuição geográfica de determinadas espécies problemáticas.

A subordem Auchenorrhyncha inclui mais de 42000 espécies, sendo várias consideradas pragas agrícolas quer pelos danos causados diretamente pelo seu processo de alimentação quer por serem vetores de patogéneos de plantas como bactérias e vírus. Tendo isto em conta, o principal objetivo deste estudo foi a monitorização da comunidade Auchenorrhyncha em pomares de pêssegos na região da Beira Interior, com um particular destaque para a cigarrinha verde *Asymmetrasca decedens* (Paoli) e potenciais vetores da bactéria *Xyllela fastidiosa* Wells *et al*.

Asymmetrasca decedens encontra-se amplamente distribuída na região do Mediterrâneo e oeste asiático. Esta espécie foi detetada em Portugal na ilha da Madeira em 2004 e na região da Beira Interior em 2013. Trata-se de uma espécie altamente polífaga, com a capacidade de se alimentar de plantas herbáceas, arbustos e árvores, incluíndo várias espécies economicamente importantes como pêssego, ameixa, amêndoa, algodão, entre outros. Através do seu processo de alimentação esta cigarrinha provoca uma descoloração das folhas, conferindo-lhes uma aparência amarelada, podendo também causar deformações como o enrolamento das folhas especialmente nas regiões mais periféricas e necrose dos tecidos, conferindo-lhes um aspeto queimado, geralmente designado como “hopperburn”. *A. decedens* demonstra uma especial apetência por plantas mais jovens provocando-lhes estragos mais significativos. Para além dos estragos diretos causados, esta espécie também pode atuar como vetor de fitoplasmas, bacterias patogénicas associadas a doenças que afetam culturas economicamente importantes. Esta cigarrinha verde foi confirmada como vetor de “Candidatus Phytoplasma Phoenicium”, o fitoplasma responsável pela doença “Almond witches’ broom” que afeta amêndoa, pêssego, nectarina, entre outros. Este fitoplasma levou à morte de mais de 150000 amendoeiras no Líbano nos anos 90, tendo-se verificado os efeitos durante mais de duas décadas. *A. decedens* é também considerada um potencial vetor do fitoplasma responsável por “European Stone Fruit Yellow’s” que afeta várias espécies de prunóideas. Esta espécie tem o potencial de se tornar uma praga emergente em Portugal, quer pela resistência demonstrada a pesticidas convencionais o que dificulta o seu controlo, quer pela grande variedade de plantas hospedeiras que incluem prunóideas representando portanto uma ameaça à agricultura em Portugal, em particular na região da Beira Interior pois esta região é responsável por 45% da produção nacional de pêssego.

Xylella fastidiosa trata-se de uma bactéria limitada ao xilema das plantas e transmitida por espécies de insetos vetores da comunidade Auchenorrhyncha. Esta bactéria é responsável por várias doenças, podendo afetar vinha, oliveira, citrinos, prunóideas, entre outros. Apesar desta bactéria ter sido detetada no continente americano há mais de um século, *X. fastidiosa* foi detetada na Europa, em Itália, apenas em 2013, quando foi associada a uma doença conhecida como “Olive Quick Decline Syndrom”, a qual desencadeou efeitos devastadores em pomares de oliveiras da região. Desde a deteção, várias prospeções

têm sido realizadas na Europa, tendo esta bactéria sido detetada em Portugal, pela primeira vez, em 2019. Devido à recente presença, à existência de espécies vetoras confirmadas, e à considerável variedade de espécies economicamente importantes suscetíveis no país, a monitorização é fundamental para evitar, conter e reduzir os potenciais estragos provocados pela bactéria. Uma das doenças causadas por *X. fastidiosa*, "Peach Phony disease" provoca consideráveis estragos e reduções na produção de pêssego, tratando-se portanto de uma potencial ameaça à produção na região da Beira Interior se a atual área afetada por esta bactéria aumentar e se as potenciais espécies vetoras não forem devidamente monitorizadas. Embora as espécies vetoras sejam o principal fator envolvido na dispersão desta bactéria, o transporte de longa distância também pode contribuir para o aumento da área afetada através da movimentação de plantas afetadas. Por não existir nenhum método para o controlo desta bactéria, a principal forma de limitar a sua distribuição passa pelo controlo de espécies vetoras, podendo também ser removidas e destruídas plantas afetadas, ou selecionados cultivares que demonstrem uma maior tolerância a esta bactéria desenvolvendo menos sintomas.

Para a monitorização das espécies da comunidade Auchenorrhyncha na região da Beira Interior, foram feitas amostragens em 2019 ao longo de 30 semanas com recurso a armadilhas de cola amarelas em dois pomares desta região, Póvoa de Atalaia e Louriçal do Campo. Os indivíduos da comunidade Auchenorrhyncha detetados foram identificados até ao nível taxonómico mais baixo possível e quando não foi possível foram consideradas morfoespécies. No total, 8140 indivíduos foram capturados e 39 espécies de quatro famílias diferentes foram identificadas. Duas espécies de vetores de *Xylella fastidiosa* foram capturadas em ambos os pomares, *Philaenus spumarius* (Linnaeus) e *Neophilaenus campestris* (Fallén), embora poucos indíviduos de cada espécie tenham sido coletados. No entanto, confirma o risco de disseminação de *X. fastidiosa* nos pomares caso a área afetada por esta bactéria se expanda á região da Beira Interior. Outros vetores de fitoplasmas também foram detetados nos pomares, *Anaceratagallia laevis* (Ribaut), *Aphrodes makarovi* Zachvatkin, *Austroagallia sinuata* (Mulsant & Rey), *Dictyophara europaea* (Linnaeus), *Empoasca decipiens* Paoli, *Euscelidius variegatus* (Kirschbaum), *Laodelphax striatella* (Fallén), *Megophthalmus scrabipennis* Edwards, *Neoaliturus fenestratus* (Herrich-Shaffer), *Philaenus spumarius* e *Zyginidia scutellaris* (Herrich-Schaffer). A família Cicadellidae foi a mais comum representando 99.77% dos indivíduos capturados e a subfamília Typhlocybinae correspondendo a 95.84%. Foram detetadas duas espécies dominantes em ambos os pomares, *Asymmetrasca decedens* e *Empoasca solani* (Curtis), sendo que a primeira se destaca representanto 79.64% dos indivíduos capturados. Estas espécies demonstraram distribuições temporais quase opostas, o que pode ser justificado pelas variáveis climáticas estudadas que demonstraram correlações significativas opostas para as duas espécies. A temperatura mínima, média e máxima demonstrou uma forte correlação positiva com a abundância de *A. decedens* enquanto que para *E.solani* foi observada uma correlação negativa. Esta espécie também demonstrou uma forte correlação positiva com o número de horas de frio (<7ºC) e com a humidade relativa. *E. solani* revelou uma distribuição mais curta, apresentando o pico da sua abundância a 19 de abril, sendo depois gradualmente substituída por *A. decedens* cujo pico foi atingido a 20 de setembro. Vários inseticidas foram aplicados nos pomares, no entanto nenhum demonstrou um efeito considerável ou consistente na redução da densidade populacional da praga. Tal facto pode dever-se à resistência conhecida ou às datas de aplicação, mas merecia um estudo mais detalhado com maior número de armadilhas e monitorização de ninfas de *A. decedens*. Este estudo permitiu uma melhor compreensão da comunidade Auchenorrhyncha presente nos pomares de pessegueiros, detetar vetores de *X. fastidiosa* na região da Beira Interior salientando a importância da continuada monitorização dos mesmos, e obter informação relativa ao ciclo de vida e à variação da abundância de *A. decedens* permitindo estabelecer períodos adequados para o controlo desta espécie.

Palavras-chave: *Asymmetrasca decedens, Xylella fastidiosa,* Beira Interior, prunóideas, gestão de pragas

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Introductory note

The work presented in this thesis was developed under the project *Grupo Operacional PrunusFito*, PDR2020-101-031707.

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State of the Art

1.1 Pest Management

Pests have constituted a challenge to the human population for a long time, mainly due to the constant competition for the same resources such as food, fiber and shelter (Arif *et al*. 2017, FAO 2013, Geier 1966, Radcliffe *et al*. 2009). This competition intensified when humans started to cultivate plants and domesticate animals, and some of the earliest methods of pest control include the rotation and movement of crops (Dent 2000, Radcliffe *et al*. 2009).

The concept of pest is defined by FAO as "any species, strain or biotype of plant, animal or pathogenic agent injurious to plants and plant products, materials or environments and includes vectors of parasites or pathogens of human and animal disease and animals causing public health nuisance" (FAO 2017).

Through the twentieth and twentieth first centuries, the main control method for pests has been the chemical control, applications of pesticides with insecticidal, herbicidal or fungicidal properties. Despite the high effectiveness of utilized pesticides, as some of them were even described as being "miraculous", a few concerns began to arise throughout the twentieth century regarding the safety of these products, as shortly after, ecological repercussions started to appear (Metcalf 2012, Radcliffe *et al*. 2009). Examples of these backlashes are cases of insecticide resistance, accumulation of certain chemicals along the food chain, and chemical residues on vegetables and fruits (Dent 2000, Geier 1966, Radcliffe *et al*. 2009).

More recently, as a result of increased pest problems, different control methods and strategies have been developed leading to a more integrated approach in order to have further efficient results and reduce the ecological impact by diminishing and complementing chemical control with biological, physical or cultural approaches (Dent 2000, El-Shafie 2018, FAO 2019, Radcliffe *et al*. 2009). This lead to a multidisciplinary endeavor referred to as Integrated Pest Management, that according to FAO's definition "means the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human and animal health and the environment" (FAO 2017).

Other measures besides the direct control options are also crucial to slow the development of pest populations, for instance, prevention, monitoring, early diagnosis and forecasting. Monitoring harmful organisms is fundamental and should be done with adequate tools, such as field observations, regarding the occurrence of pests or the appearance of symptoms or damage on crops, and also diagnostic systems like traps, weather stations to assess meteorological or environmental factors, among others (Arakan & Canhilal 2004, Dent 2000, El-Shafie 2018, FAO 2017, Geier 1966, Radcliffe *et al.* 2009).

One of the most prominent and concerning pest groups are insects as these are responsible for substantial losses and damage in crops all around the world, as well as being able to act as vectors for human and animal diseases (El-Shafie 2018). Insects are the most diverse group of animals on earth making up over 75% of the world's animal species, having a remarkable adaptation capacity that has allowed them to colonize all habitats with the exception of the open ocean (Arif *et al*. 2017, El-Shafie 2018, FAO 2013). Insect numbers far exceed any other animal group, and the existing diversity allows them to play many important roles that directly impact humans and the environment. Some insect species are pollinators, others are parasitoids of harmful pests, organic matter decomposers, or even producers of valuable products such as honey, silk, or pharmacological compounds (Arif *et al*. 2017).

Insect pests have been defined as any insect in the wrong place, which means the same insect species might be a pest in a certain area and be completely harmless or even beneficial in others (Arif *et al*. 2017, FAO 2013, Mozaffarian 2018). These insect pests can cause damage to humans, crops and farm animals, and despite all the disturbance caused, they represent less than 0,5 percent of all known insect species (Dent 2000). Herbivorous insects are thought to be responsible for damaging over one fifth of the world's entire crop production every year (FAO 2013). One of the main reasons for the presence of insect pests is the existence of human manipulated environments, where crops are selected to increase its yield and productivity to meet the increasing food demand around the world (Radcliffe *et al*. 2009). These agricultural systems provide a highly favorable environment for insects, allowing a faster colonization, spread and growth, as the traits selected on these plants such as large size, nutritious value, all in a confined area, makes them more susceptible to infestations by insects (FAO 2013). This is the case of many monocultures which are highly suitable habitats for a pest but very unfavourable to the pest's natural enemies, creating perfect conditions for outbreaks (Dent 2000, Radcliffe *et al*. 2009, Stinner et al. 1983).

The increasing globalization and consequently the increase of trade and transport also accentuates the problems resulting from insect pests, as it facilitates the transport of certain pests species to new areas, where there might not exist natural enemies to control the expansion of these as there were in their native geographical area. Without the environmental constraints and in the absence of natural enemies, these exotic species become pests, potentially causing extensive damage (FAO 2011, 2013, 2019).

Considering all the factors involved in pest outbreaks and the continuous increase of mobility around the world, it became essential to develop monitoring programs for potentially problematic insect species, to assess the efficacy of control measures and their geographical distribution, and also to forecast and predict pest outbreaks (FAO 2011, Radcliffe *et al*. 2009). These strategies allow a more timed and directed approach, for example, defining the most effective period of action, leading to improvements and more accurate control measures (Dent 2000).

The sampling techniques used in monitoring insects have to be reliable, robust and an appropriate representation of insect abundance in the area of interest. The forecasting systems are becoming more sophisticated and allow the definition of thresholds for insecticide applications according to the level of infestation, the weather patterns, instead of a fixed schedule, which may reduce the number of applications of insecticides (Dent 2000, Radcliffe *et al*. 2009). These improvements help reduce downsides associated with chemical insecticides, while still reducing losses in crops and orchards. Nevertheless, there are always disadvantages associated with the use of these products, for instance, the elimination of competing species or pest parasitoids, insecticide resistance, secondary pest outbreaks, environmental contamination, residues, among others (Metcalf 2012, Radcliffe *et al*. 2009). To continually reduce the negative effects, other methods (cultural, physical, biological) should be applied if possible or available, however many alternative methods are not usually as effective in the short term, and if the damage is too devastating it might be the only solution with an immediate effect, therefore it is crucial to determine the correct timing, dosage, persistence and placement (Dent 2000, FAO 2011, FAO 2017, Myers et al. 1998, Radcliffe *et al*. 2009).

1.2 Auchenorrhyncha suborder

The Auchenorrhyncha, a suborder of Hemiptera, is subdivided into two groups, the planthoppers or infraorder Fulgoromorpha, and the leafhoppers or infraorder Cicadomorpha (Biedermann & Niedrighaus 2009). The Auchenorrhyncha suborder has over 42000 species described worldwide and is characterized by sap feeding insects (Mifsud *et al*. 2010), with a rostrum (feeding apparatus) arising on the ventral side from below the head, compound eyes situated laterally on the head, two pairs of wings, being the forewings usually larger or longer than the hind wings, and all species live in terrestrial habitats covered in plants, having a great jumping ability which is its main mode of locomotion (Dietrich 2005, Lehr 1988, Ossiannilsson 1978). These insects are hemimetabolous, and most of them are active during the day (Biedermann & Niedrighaus 2009, Ossiannilsson 1978).

As sap feeding insects, the Auchenorrhyncha suborder can utilize the xylem, floem and mesophyll as a food source (Mifsud *et al*. 2010). These insects can be monophagous, oligophagous or polyphagous depending on how specialized they are, which depends on the variety of plants they are able to feed on (Biedermann & Niedrighaus 2009). If a species is able to reproduce during at least one generation on a certain plant, these are considered hostplants of that species (Ossiannilsson 1978).

The Auchenorrhyncha suborder includes many species considered pests of cultivated or ornamental plants (Chaieb *et al*. 2011, Habib *et al*. 1972, Maugin & Sforza 2011, Ossiannilsson 1978, Raupach *et al*. 2002). The direct damage is a result of their feeding process, as their saliva can induce blockages on plant vascular tissues which may lead to deformations, discoloration of leaves, shortening of shoots, or even premature death of plants *(*Antonatos *et al*. 2019, Mozaffarian 2018). Examples of this kind of damage are, *Empoasca facialis* Milchelmore, a pest of cotton, peanuts, and others crops in the African continent, *Eupteryx atropunctata* (Goeze) that affects potatoes and beetroots and *Perkinsiella saccharicida* Kirkaldy, a concerning pest for sugarcane (Ossiannilsson 1978).

Besides the direct impact caused by some species of the Auchenorrhyncha suborder, the indirect damage caused to crops through the transmission of plant pathogens by vector species of this suborder is even more concerning as it causes greater economic impact *(*Antonatos *et al*. 2019). Some plant pathogenic viruses, as well as phytoplasmas, pathogenic plant bacteria, can be transmitted by vector species of the Auchenorrhyncha suborder (Ammar & Nault 2002, Nielson 1968). Both viruses and phytoplasmas can result in serious damage to certain plants, which may lead to devastating effects on economically important crops (Nielson 1968). For instance, *Neoaliturus tenellus* (Baker) is a leafhopper species that is able to transmit the Californian curly top virus which is responsible for several diseases and affects various important crops (Ossiannilsson 1978).

The infraorder Fulgoromorpha has over 12000 species described, and the infraorder Cicadomorpha has over 30000 species to date, and despite their economic importance resulting from the direct and indirect damage caused by some species, there are still many gaps in the information obtained so far about the Auchenorrhyncha suborder (Mifsud *et al*. 2010). To accurately determine species within this suborder, it is necessary to observe its morphological characteristics and genitalia. Usually the male genitalia allows a more precise identification, as opposed to the female genitalia that is much more conservative between species. The male genital structures are present on the 9th abdominal segment, on the genital capsule that contains the copulatory organs and appendages such as the aedeagus, styles, among others (Dietrich 2005, Ossiannilsson 1981).

1.3 *Asymmetrasca decedens*

Asymmetrasca decedens (Paoli) sometimes also referred to as *Empoasca decedens* Paoli, is a green leafhopper species that belongs to Suborder Homoptera (Auchenorrhyncha), Family Cicadellidae, and Subfamily Typhlocybinae (Coutinho *et al*. 2015, Ossiannilsson 1978, Ribaut 1936).

The adults of this leafhopper species are characterized by a greenish to yellowish colour, a narrow and slender body, and by its very small dimensions, having an overall length that ranges between 3 to 3.5mm, even though females commonly have larger dimensions in comparison with males (Al-Asady 2002, Coutinho *et al*. 2015, Pastore *et al.* 2004). These insects, in its adult stage, are very active and its main movement form is hopping due to its hind legs, despite being capable of flying as a result of having two pairs of membranous wings (Ossiannilsson 1978).

A. decedens is very identical to other green leafhoppers belonging to the same subfamily, Typhlocybinae, due to having similar colour patterns and dimensions, however it is possible to distinguish the several species considering the male genitalia as it differs considerably between the numerous species. *A. decedens* can be easily identified by the observation of the aedeagus, a male copulatory organ, which has a very characteristic L-like projection that is positioned laterally and marginally beneath the apex and can be seen with a stereomicroscope (Fig. 1.1) (Al-Asady 2002, Alvarado *et al*. 1994, Coutinho *et al*. 2015, Ossiannilsson 1978, Ribaut 1936).

Figure 1.1- Male genitalia of *Asymmetrasca decedens* (Author's original).

Asymmetrasca decedens is a hemi-metabolous species, as it performs an incomplete metamorphosis during the course of its development. The eggs are deposited on plant tissues or soil, and its development is controlled by external environmental factors such as temperature, photoperiod, among others (Alvarado *et al*. 1994, Torres *et al*. 2002). These eggs, when in adequate external conditions, hatch into nymphs that go through five different nymphal stages until they reach the adult form (Lehr 1988, Mifsud *et al*. 2010). The main difference between the nymphal and the adult stage is the absence of wings, even though nymphs possess wing pads that extend in the last nymphal instar developing to macropterous adults in *A. decedens* case. This species is multivoltine and therefore the number of generations that develop each year may vary up to 3-5 generations, mainly according to climatic conditions (Biedermann & Niedrighaus 2009, Ribault 1936, Zenner 2005).

1.3.1 Geographical distribution

The leafhopper species *Asymmetrasca decedens* has a very wide distribution across the palaearctic and oriental regions, mainly around the Mediterranean and West Asia (Aguim-Pombo & Freitas 2008, Coutinho *et al*. 2015, Torres *et al.* 2000). This species has been recorded in countries such as Egypt, Greece, Iran, Italy, Lebanon, Saudi Arabia, Slovenia, Spain, Tunisia, Turkey, among others (Abou-Jawdah *et al.* 2011, Alvarado *et al*. 1994, Chaieb *et al.* 2011, Habib *et al*. 1972, Jacas *et al.* 1997, Mozaffarian 2018). *A. decedens* was reported in Portugal, in 2004 on Madeira island and it is suspected that this species entered the island as a result of the transportation of ornamental plants (Aguim-Pombo & Freitas 2008, Freitas & Aguim-Pombo 2004, 2006). This leafhopper was also described in 2013, on mainland Portugal in the Beira Interior Region and it is thought that the species was introduced by the importation of plant trees from countries in the Mediterranean region, where *A. decedens* also occurs (Coutinho *et al*. 2015).

Many factors influence the distribution, expansion and populational density of this species, particularly climatic conditions, such as temperature, photoperiod, precipitation, and other factors like the use of fertilizers, irrigation, the availability of new host plants, among others (Jacas *et al.* 1997, Raupach *et al*. 2002, Torres *et al.* 2002). This leafhopper species in particular can complete its development if temperatures range between 12ºC and 27ºC, however a faster development is observed when exposed to higher temperatures, which can explain *A. decedens* preferential distribution around the Mediterranean region due to the more advantageous conditions (Torres *et al.* 2002). Despite having optimal/preferential conditions that allow this species to thrive in certain environments, *A. decedens* has demonstrated a considerable adaptation capacity, as it is able to colonize new regions successfully by expanding its plant host range (Freitas & Aguim-Pombo 2006).

1.3.2 Habitat and Feeding

Asymmetrasca decedens is a highly polyphagous species which means it is able to feed on numerous plants, ranging from shrubs and trees to herbaceous plants (Freitas & Aguim-Pombo 2006). This species usually feeds on plants sap and can generally be found on the lower surface of leaves (Coutinho *et al*. 2015, Jacas *et al*. 1997). These insects are usually encountered in higher populational densities in agricultural ecosystems such as orchards, pastures and greenhouses, having a particular propensity for younger plants (Chaieb *et al.* 2011). Because this species is highly polyphagous and has a wide range of food sources, *A. decedens* has therefore, a very high capacity to adapt to different environments, allowing it to easily survive and expand its distribution even when introduced to a new area, as it is able to adapt and colonize new hostplants (Freitas & Aguim-Pombo 2006, Nestel & Klein 1995). This ability, however, can be quite problematic as the species is able to cause damage and negatively affect many different important crops and food plants valuable to humans, that may result in very high economic impacts (Atakan 2009, Chaieb *et al.* 2011).

The small dimensions of *A. decedens* and its respective mouth structures do not allow this species to directly feed on the intracellular liquid of the parenchymal cells, unlike some other cicadellids, instead they feed almost continuously on the mesophyll cells of lower leaf veins of corresponding host plants (Coutinho *et al.* 2015). The limited use of the plant is associated with damage, typical marks and scores that can easily be confounded with other insect groups such as mites (Chaieb *et al.* 2011). These lesions sometimes lead to tissue necrosis or even more significant deformations (Alvarado *et al.* 1994).

A. decedens has been found on many different economically important crops in several countries and was already described on cotton, almond, vine, beetroot, beans, potatoes, eggplants, cherry, raspberry, peach, citrus, apricot, among others (Abou-Jawdah *et al.* 2014, Alvarado *et al*. 1994, Atakan 2009, Chaieb *et al.* 2011, Coutinho *et al*. 2015, Freitas & Aguim-Pombo 2006, Jacas *et al.* 1997, Torres et al. 1998, 2000). In the Beira Interior Region in mainland Portugal, this species has been associated with peach, plum and apricot orchards (Coutinho *et al*. 2015).

This leafhopper is more abundant during summer and harvest seasons, and a very high density of individuals can be observed when conditions are more favourable (Alvarado *et al*. 1994). Adults of this species spend winter in appropriate retreats, moving from deciduous to evergreens shrubs or trees, and plant litters, in order to avoid unfavourable conditions. When these conditions become favorable again, usually around spring, adults expand to other host plants, such as stone fruit trees, to lay eggs, where nymphs will hatch and feed on leaves of the plants they are on (Alvarado *et al*. 1994, Jacas *et al.* 1997, Nestel & Klein 1995).

1.3.3 Impact and damage on crops

Both adults and nymphs of *A. decedens* species are able to cause damage to crops due to their feeding process, as a result of continuously piercing and sucking on leaf cells (Demichelis *et al.* 2010). The leaves are certainly the most affected part of the plant, as these start to turn yellow and later become reddish, and curl around the edges, usually on the more peripheral regions of the leaf, which can also result in tissue necrosis, conferring the leaves a dry and burned appearance, usually known as 'hopperburn' (Fig.1.2) (Alvarado *et al*. 1994, Atakan 2009, Chaieb *et al.* 2011, Coutinho *et al*. 2015, Freitas & Aguim-Pombo 2006, Torres *et al*. 1998, 2000). Other effects include premature defoliation, reduced growth, stunting, fruit yellowing and deformations on plant tissues. The effects are always more devastating in younger plants as these become significantly weaker and often die (Alvarado *et al*. 1994). The damage caused by this leafhopper leads to decreases in yield and production and therefore can cause great economic losses (Freitas & Aguim-Pombo 2006).

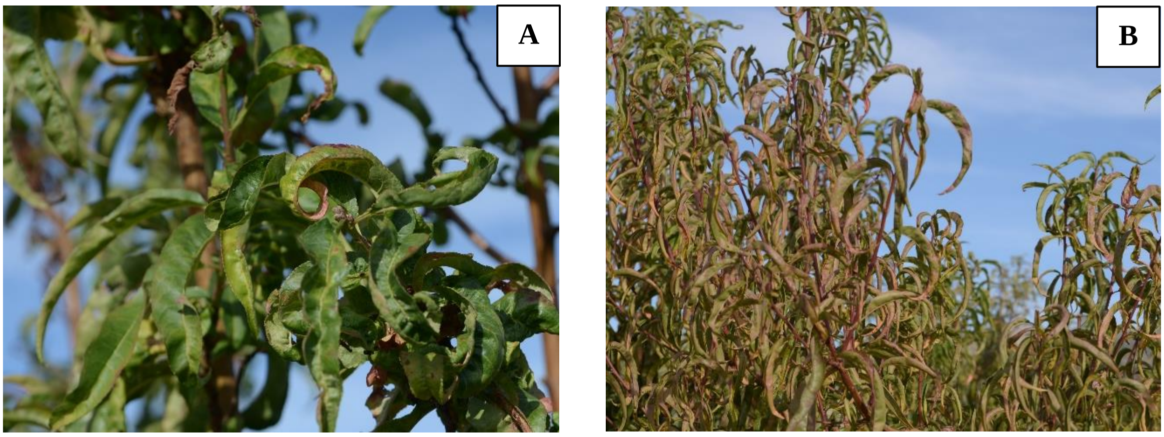

Figure 1.2- Characteristic symptoms caused by *Asymmetrasca decedens* feeding process on peach orchards: curled and yellowish leaves (A), with a dry and burned appearance (B). Originals by: J. Pereira Coutinho.

Apart from the direct damage caused by *A. decedens* on crops, the indirect disturbance caused by the species is also very concerning, as it is able to act as a vector for phytoplasmas. Phytoplasmas are plant pathogenic bacteria that lack cell walls and are associated with diseases that affect economically important crops (Abou-Jawdah *et al.* 2014, Salehi *et al.* 2018). These bacteria belong to the class Mollicutes and are limited to the phloem ducts of infected plants, therefore, to propagate and infect new plants it requires the transmission by phloem sap feeding insects such as *A. decedens* and other cicadellids (Salehi *et al.* 2018).

Other forms of transmission may occur, for instance, through the vegetative propagation of infected plants and also grafts. Even though some plants might be asymptomatic, the common symptoms associated with phytoplasmas include reduced leaf and fruit size, foliar yellowing and reddening, abnormal development of flowers, leaves and shoots, phloem necrosis and overall decline or stunting of infected plants (Salehi *et al.* 2018).

The phytoplasma *'Candidatus* Phytoplasma phoenicium' is associated with a lethal disease named almond witches'-broom disease, which belongs to the pigeon pea witches broom group (16SrIX), that affects peach, nectarine and almond (Abou-Jawdah *et al.* 2010, 2011, 2014, Dakhil *et al*. 2011, Lova *et al.* 2011). This disease is characterized by small yellowish leaves, proliferation, dieback and the appearance of witches'-broom on stems. The disease was responsible for killing over 150000 almond trees in Lebanon in the 1990's and the effects lasted for over two decades (Abou-Jawdah *et al.* 2011), affecting a wide range of places, from coastal areas to elevations that exceeded 1200m (Abou-Jawdah *et al.* 2014). Years later, also in Lebanon, this phytoplasma was also identified in association with an intense disease that rapidly spread and affected peach and nectarine trees (Lova *et al.* 2011). The mechanism of spread indicated the presence of a very efficient vector and *A. decedens* was the most abundant leafhopper species present in these orchards. *Ca.* P. phoenicium was detected on *A. decedens* salivary glands and through transmission trials this leafhopper was confirmed to be the responsible vector of *Ca.* P. phoenicium (Abou-Jawdah *et al.* 2014), which is the suspected agent for AlmWB disease that resulted on the devastating effect on almond orchards in Lebanon. This phytoplasma could be characterized as a quarantine pathogen considering it is associated with a lethal disease that affects three major stone fruit crops, has the potential to occupy several ecological niches and cannot be restrained by classical control measures, having only been reported in two countries so far, Iran and Lebanon. (Dakhil *et al*. 2011, Salehi *et al.* 2018).

'Candidatus Phytoplasma prunorum' is associated with European stone fruit yellows, a disease that affects trees of the genus *Prunus* such as peach, apricot, nectarine, plum, cherry, among others (Landi *et al*. 2010, Ludvikova *et al.* 2011, Marcone *et al.* 2010, Marcone *et al.* 2014, Martin *et al.* 2011, Pastore *et al.* 2004, Turk *et al.* 2011). This phytoplasma belongs to the apple proliferation (AP) phytoplasma group (16SrX) and is present around the world, but more commonly in Europe. This phytoplasma disease may cause very varied symptoms considering its wide range of hosts and its different susceptibilities and expressions (Marcone *et al.* 2010, Martin *et al.* 2011, Turk *et al.* 2008**)**. *A. decedens* is considered to be a potential vector for this disease as it has been demonstrated that some specimens showed the presence of the phytoplasma responsible for ESFY (Landi *et al.* 2010, Ludvikova *et al.* 2011, Turk *et al.* 2011).

1.3.4 Control methods

A. decedens has often been recorded as a pest in several countries as it has been responsible for causing considerable damage and consequently substantial economic losses, and as a result of its impact on important crops, many control methods have been tested and used in order to reduce or attenuate the devastation caused by this species (Alvarado *et al*. 1994, Freitas & Aguim-Pombo 2006, Kersting *et al*. 1997).

The monitoring of *A. decedens* is crucial for the successful implementation of control programs as well as to determine the appropriate and most effective timing for these control applications. This leafhopper is frequently monitored to determine its distribution, expansion and populational density in orchards, greenhouses, and other agricultural areas (Nestel & Klein 1995). This species and other leafhoppers can be monitored with a wide array of different traps and sampling techniques that measure the overall movement and activity, and can therefore be effective in estimating population dynamics, and predicting the incidence of plant pathogens that can be transmitted by these species (Kersting *et al*. 1997). An example of a commonly used sampling technique for leafhoppers and other insects, is the use of yellow sticky traps, chromothropic traps that rely on the response of insects to a certain color and are covered in glue or other viscous substances (Nestel & Klein 1995, Pastore *et al.* 2004). These are commonly used to estimate insect abundance and have been proven to be effective in monitoring leafhopper populations (Arakan & Canhilal 2004, Freitas & Aguim-Pombo 2006, Kersting *et al*. 1997, Rebelo & Quartau 1995).

Chemical control is the main control method for *A. decedens* and even though several chemicals have been tested so far, this species has shown a great resistance to normal insecticides (Coutinho *et al*. 2015, Freitas & Aguim-Pombo 2004, Grassi *et al.* 2008, Meisner *et al.* 1992). *A. decedens* populations are usually larger during the warmer seasons, mainly during summer, and some studies suggest the control of this pest should take place at the beginning of this season in order to have a greater impact (Alvarado *et al*., 1994; Torres *et al*., 1998; Grassi *et al*., 2008). To date no program of biological control is known for this species (Freitas & Aguim-Pombo 2006) as there is a lack of information regarding this species natural enemies. A study in Turkey described an ectoparasite *Erythraeus ankaraicus* Saboori (Acari: Erythraeidae), capable of affecting *A. decedens* nymphs (Gencsoylu 2007). Some species of *Aphelopus* genus (Hymenoptera: Dryinidae) and *Anagrus* genus (Hymenoptera: Mymaridae) have also been described as parasites for several leafhopper species belonging to the Typhlocybinae subfamily (Agboka *et al.* 2003, Hesami *et al.* 2004, Jervis 1980, Triapitsyn *et al.* 2010, Waloff & Jervis 1987). Agricultural services have recommended the use of various chemicals to control this species, ranging from insect growth regulators, which affect the synthesis of chitin and consequently insects cannot reach the adult stage and reproduce, to insecticides that affect the nervous system such as organophosphates, pyrethroids and nicotinoids (Grassi *et al*. 2008). The use of certain insecticides also depends on the affected crop, as different chemicals have been recommended for peach, almond, strawberries, among others (Freitas & Aguim-Pombo 2006). Thiamethoxam, thiacloprid, acetamiprid and margosan-O are examples of insecticides tested to control this species, the first three are nicotine based and were tested on raspberry and the latter is natural and biodegradable, and was tested on cotton (Grassi *et al*. 2008, Meisner *et al.* 1992).

1.4 Xylella fastidiosa

Xylella fastidiosa Wells *et al.* is a gram-negative gamma-proteobacterium of the family Xanthomonadaceae. This bacterium is xylem-limited and therefore an obligatory colonizer of plants. *X. fastidiosa* is a commensalist bacterium and a harmless endophyte in the majority of its host plants, however some of the existing bacterial genotypes are responsible for several different diseases that can affect a great number of economically important crops (Cao *et al.* 2011, Cornara *et al*. 2018, 2019a, EFSA 2018, EPPO 2018, Janse & Obradovic 2010, Morente *et al.* 2018b, Sicard *et al.* 2018, Tsagkarakis *et al.* 2018).

X. fastidiosa is a vector-borne pathogen and its plant to plant transmission is directly related to the presence, behavior and abundance of insect vectors in relation to healthy and infected plants *(*Antonatos *et al*. 2019, Kacar *et al.* 2017). The natural spread of this bacterium depends considerably on the dispersal of xylem-sap feeding insects which is the case of many Auchenorrhyncha species usually belonging to the infra order Cicadomorpha, namely the Aphrophoridae (spittlebugs), Cercopidae (froghoppers), and Cicadellidae (sharpshooters) families (Cornara *et al*. 2018, 2019a, 2019b, Dáder *et al.* 2019, Krugner *et al.* 2019, Lopes *et al.* 2014, Miranda *et al*. 2013, Saponari *et al.* 2014, Sicard *et al.* 2018).

According to the genetic data obtained to date, at least six subspecies of *X. fastidiosa* have been proposed, *fastidiosa, morus, multiplex, pauca, sandyi* and *tashke* (Chen *et al.* 2019, EFSA 2019a, Godefroid *et al.* 2019). Despite this division, these subspecies and the respective taxonomic boundaries are still being debated, as there has not been reached a consensus so far regarding the matter (EFSA 2018).

1.4.1 History and distribution

The bacterium *Xylella fastidiosa* was described and cultured for the first time in 1987 in the USA, as the cause of a grapevine disease described in 1892 by Newton Pierce, that was latter called Pierce's disease (PD). This disease had devastating consequences on vines in Southern California in the 1880's, having destroyed over 14000 ha of grapes, and it still remains a considerable problem and concern in the grape industry in southern USA. In 1890 a related disease named phony peach disease (PPD) was also recorded in the USA, and was the cause of several outbreaks years later, in 1929, 1951 and 1976 (Chen *et al.* 2019, Cornara *et al.* 2019a, Dáder *et al.* 2019, Godefroid *et al.* 2019, Janse & Obradovic 2010, Kruse *et al*. 2019, Ledbetter & Steven 2018, Lopes *et al.* 2014). In the early 1950s, a disease known as almond leaf scorch was first noticed in Los Angeles and California, and was responsible for devastating almond orchards from 1966 to 1976. In 1987 a disease similar to PD that affected citrus trees, named citrus variegated chlorosis (CVC) was described in Brazil and a few years later another disease was also reported, known as coffee leaf scorch (CLS), and both of these were confirmed to be caused by *X. fastidiosa* as well. Due to this bacterium's impact on the citrus industry, its genome was sequenced in 2000 by the research community in Brazil, becoming therefore the first sequenced plant pathogen. Other countries in South America have also reported diseases caused by this bacterium such as plum leaf scald (PLS), identified in Argentina and later spread to Paraguay (Cornara *et al.* 2019a, Dáder *et al.* 2019, Kruse *et al.* 2019, Miranda *et al.* 2013, Marucci *et al.* 2008).

The bacterium *X. fastidiosa* has also been reported in several Asian countries however not all have been confirmed and some were even refuted by further investigations. The bacterium was however confirmed in Iran and also Taiwan, where it was considered to be the causal agent of pear leaf scorch (PLS), and the first reports date back to the 1980's (Cornara *et al.* 2019a, Dáder *et al*. 2019, EPPO 2018).

Xylella fastidiosa was first detected in Europe in 2013, while investigating the etiology of a disease affecting olive trees (*Olea europaea*) in Italy. The disease was named olive quick decline syndrome (OQDS) as it was characterized by the rapid death of olive trees. OQDS was first detected in olive orchards on the west coast of the Salento Peninsula in the region of Apulia, considered to be one of the main olive-growing areas in Italy (Cornara *et al.* 2017a, Dáder *et al.* 2019, Godefroid *et al.* 2019, Martelli 2016, Santoiemma *et al.* 2019, Saponari *et al.* 2014, Sicard *et al.* 2018, Tsagkarakis *et al.* 2018). The finding of this bacterium lead to a large scale search through Europe, and other detections were made in areas such as Corsica, Tuscany, France, Spain and the Balearic islands, and more recently in Germany and Portugal (Cornara *et al*. 2018, 2019a, EFSA 2018, 2019a, 2019b, EPPO 2018).

X. fastidiosa' s potential to invade, establish and expand beyond its current range is somewhat predicted by the distribution of its respective vectors. Other factors include pathogen genotype, vector phenology, mobility and natural infectivity, plant phenology, plant water status, plants nutrient status, plant community composition, agricultural practices and climate (Cornara *et al.* 2017a, 2019a, EFSA 2018, Sicard *et al.* 2018).

Climate influences the distribution of vector populations, pathogen transmission and plant infection dynamics, among others (Cornara *et al.* 2017b, 2019a, EFSA 2019a). Studies have shown that elevated temperatures usually favor feeding rates, shorter incubation periods and higher survival rates of vector species. Precipitation has also been shown to be positively associated with higher populational densities of vectors and with the establishment and spread of *X. fastidiosa* (Sicard *et al.* 2018). Other studies have demonstrated that cold or freezing winter temperatures can affect the survival or even eliminate the bacterium in xylem vessels allowing plants to partially recover (Cao *et al.* 2011, EFSA 2018).

Anthropogenic factors may also lead to the introduction of vectors or pathogens to previously unaffected areas, this is the case of increasingly efficient means of transportation, the conversion of natural habitats to agroecosystems and monocultures, and also farming practices that situate susceptible host populations near each other (Cornara *et al.* 2017b, 2019a, Sicard *et al.* 2018).

Climate change may broaden the suitable geographic range, leading to the establishment of the bacterium in areas previously unsuitable, and to new potential epidemics of the bacterium, as well as an increase in the severity of *X. fastidiosa* induced diseases (Cornara *et al.* 2017b, 2019a, Godefroid *et al*. 2019). Estimating the potential distribution and impact of a pest species and its respective responses to climate change constitutes a pest control strategy as it allows an earlier detection and a timed planning of phytosanitary measures. Many species distribution models indicate the current geographical range of *X. fastidiosa* in Europe is small compared to the extent of climatically suitable areas, especially around the Mediterranean region (Cornara *et al.* 2017b, 2019a, Godefroid *et al.* 2019), considered to be at risk as the potentially affected areas also present other favorable conditions, such as vectors presence and wide range of suitable hosts, including economically important crops (Bosso *et al.* 2016, Chen *et al.* 2019, Cornara *et al.* 2017b, EFSA 2019a, Godefroid *et al.* 2019, Godefroid *et al*. 2019, Morente *et al*. 2018b).

1.4.2 Vectors and transmission

The introduction of a pathogen in a new region is often considered to be the main driver of infectious diseases, however in the case of an arthropod-borne pathogen it is required the presence of vectors for the establishment of the pathogen (Cornara *et al.* 2017a, 2019a). Considering *X. fastidiosa* is a xylem-inhabiting bacterium it requires the presence of xylem sap-feeding insects of the Auchenorrhyncha suborder to be transmitted. When an infected plant is susceptible, the bacteria moves systematically through the xylem vessels and therefore after a variable length of time depending on plant species, it becomes accessible for acquisition by the piercing-sucking insect vectors (Cornara *et al*. 2018, 2019a, EFSA 2018, EPPO 2018, Krugner *et al.* 2019, Lopes *et al*. 2014, Tsagkarakis *et al.* 2018).

Both nymphs and adults may act as vectors as they acquire the bacteria through their feeding process and are able to inoculate the pathogen to healthy plants right after acquisition. This bacterium does not infect the insects body systematically as it is restricted to the alimentary canal, on specific areas of the foregut, the precibarium and the cibarium, where it adheres and multiplies (Cornara *et al*. 2019a, EFSA 2018, EPPO 2018, Sicard *et al*. 2018, Ranieri *et al.* 2020). Nymphs lose infectivity with every stage which demonstrates that the transmission is not transstadial. On the contrary, adult vectors can transmit the bacterium to new host plants during their whole lifetime as it multiplies and persists in the vectors foregut, however it cannot be transovarially transmitted to its progeny (Cornara *et al.* 2019a). The most effective vectors responsible for the spread of *X. fastidiosa* are winged adults as these have a considerably high mobility. Besides the natural spread of the bacterium by insect vectors, long-range transport of the bacterium also occurs, mainly through the movement of infected plant materials as stated above (Cornara *et al*. 2018, 2019a, EFSA 2018, Sicard *et al.* 2018).

In North America, over 60 vector species have been documented to carry or to transmit this bacterium so far. Sharpshooters are considered to be the main vectors of *X. fastidiosa* in this region (Chen *et al.* 2019, Cornara *et al*. 2019b, Daugherty *et al.* 2011, Krugner *et al.* 2019), and the most important vector species of this bacterium to economically important crops in North America are: *Homalodisca vitripennis* (Germar), *Homalodisca insolita* (Walter), *Graphocephala atropunctata* (Signoret), *Graphocephala versuta* (Say), *Oncometopia nigricans (*Walker), *Oncometopia Orbona* (Fabricius), *Draeculacephala Minerva* (Ball), and *Cuerna costalis* (Fabricius) (Cornara *et al.* 2019a, Dáder *et al*. 2019, EFSA 2018). Considering the vectors associated with Pierce's disease in the region, *H. vitripennis* also known as the glassy winged sharpshooter, is regarded as the most important vector, due to its high mobility, high density, extreme polyphagy, lack of biological control organisms and wide geographical distribution. This species wide range of hosts allows the transmission of other *X. fastidiosa* caused diseases, therefore affecting several crops such as almond, peach, plum, among others (Cornara *et al.* 2019a, EFSA 2018, Janse & Obradovic 2010, Krugner *et al.* 2019, Kruse *et al.* 2019, Miranda *et al.* 2013).

In Europe, only a few species of sharpshooters exist, and these exhibit a limited distribution so far. Considering the data obtained to date, the meadow spittlebug *Philaenus spumarius* Linnaeus, has been identified as the main vector of *X. fastidiosa* in the epidemics described affecting olive orchards in Italy *(*Antonatos *et al*. 2019, Cornara *et al.* 2017a, 2017b, 2018, 2019a, 2019b, Dáder *et al.* 2019, Kacar *et al.* 2017, EFSA 2018, Janse & Obradovic 2010, Martelli 2016, Morente *et al.* 2018a, Santoiemma *et al.* 2019, Saponari *et al.* 2014, Saponari *et al.* 2019, Tsagkarakis *et al.* 2018, Ranieri *et al.* 2020).

P. spumarius belongs to the superfamily Cercopoidea and is considered a spittlebug due to the shell produced by nymphs that contains air bubbles introduced in a secretion produced by glands located between the 7th and 8th abdominal sternites (Cornara *et al*. 2018, Morente *et al.* 2018a). The spittle is used to maintain a microclimate favorable to nymphs. This species is widely distributed in the Palearctic regions and extending to Nearctic as well as temperate regions. It can be found in Europe, especially in countries surrounding the Mediterranean such as Italy, Spain, Greece and Portugal, and as a highly polyphagous insect, it is able to feed on a considerable variety of plants (Cornara *et al.* 2017a, 2017b, 2018, 2019a, 2019b, Dáder *et al*. 2019, EFSA 2018, Morente *et al.* 2018a, Ranieri *et a*l. 2020). *P. spumarius* is a univoltine species and overwinters as an egg. The species distribution depends on the distribution of suitable host plants (Cornara *et al*. 2018).

Other xylem-sap feeding insects besides *P. spumarius* have also been found in the affected orchards and are considered to be potential vectors, such as *Cicada orni* Linnaeus, *Neophilaenus campestris* (Fállen), *Neophilaenus lineatus* (Linnaeus). *Neophilaenus campestris* has been demonstrated to be a competent vector of the bacterium to olive in Italy, however very few individuals were collected in the olive canopies, and therefore the species was considered to have a minor importance in OQDS spread (Antonatos *et al*. 2019, Kacar *et al.* 2017, Cavalieri *et al.* 2019, Cornara *et al.* 2019a, 2019b, Dáder *et al*. 2019, EFSA 2018, Martelli 2016, Morente *et al.* 2018b, Tsagkarakis *et al.* 2018). This species is also widespread through some Mediterranean countries such as Italy, Spain and Portugal and might possibly be involved in transmitting *X. fastidiosa* to other host plants besides olive, therefore posing as a potential threat to these countries (Morente *et al.* 2018b). In Madrid, *P. spumarius* and *N. campestris* have been associated with olive groves where *X. fastidiosa* had been detected, however these were found in much lower densities than what was observed in southern Italy and the outbreak in this region is considered to be eradicated. Also in Spain, in Alicante region, *X. fastidiosa* has been identified in almond orchards and the bacterium was detected in both *P. spumarius* and *N. campestris* sampled in the infected orchards in the region. In the Balearic Islands was also reported the presence of the bacterium in cherry trees and both these species were the most abundant, yet none was proven to be infected. In 2016 an outbreak of *X. fastidiosa* was reported in Germany, however the infected plants were destroyed, and the outbreak is now considered eradicated. In this region many spittlebug species were registered including the predominant species *P. spumarius* and *N. campestris* (Cornara *et al.* 2019a, EFSA 2018, Morente *et al.* 2018b, Olmo *et al.* 2017).

1.4.3 Symptoms and impact

Xylella fastidiosa causes major plant diseases that usually result from the outcome of a complex interaction between the bacterium, the host plants, insect vectors and environmental conditions. Many of these diseases are characterized by symptoms similar to those caused by water stress (Cornara *et al.* 2019a, EFSA 2018, EPPO 2018, Sicard *et al.* 2018). While infected with the bacterium many plants may remain symptomless and serve as reservoirs in the environment, while for others, the infection leads to a quick death. The colonization and the time lapse between inoculation and symptom appearance in plants caused by this bacterium depends on several factors such as the pathogen genotype and the host plant affected (Cornara *et al.* 2017a, 2019a, Daugherty *et al*. 2011, EFSA 2018, EPPO 2018, Janse & Obradovic 2010).

When the bacterium invades the plants xylem vessels it blocks the transport of water and minerals, which generally leads to symptoms such as leaf scorch, foliage wilting and defoliation, chlorosis or bronzing along the leaf margin and dwarfing. The symptoms usually appear first on a few branches and latter spread to the entire plant and if the infection is severe it may lead to the death of infected plants (Cornara *et al.* 2019a, Daugherty *et al*. 2011, EPPO 2018). Over 300 plant species have been reported to be host plants to *X. fastidiosa*, however the bacterium does not appear to cause disease in many of these as some remain asymptomatic. The wide range of hosts of *X. fastidiosa* includes cultivated plants, forest species and ornamental plants, having therefore a considerable potential impact for agriculture, nurseries, orchards, and the environment (Cornara *et al.* 2019a, Dáder *et al.* 2019, Daugherty *et al.* 2011, EFSA 2018, EPPO 2018, Janse & Obradovic 2010).

X. fastidiosa is the causal agent for diseases like alfalfa dwarf, citrus variegated chlorosis, almond leaf scorch, bacterial leaf scorch of blueberry, bacterial leaf scorch of shade trees, coffee leaf scorch, olive leaf scorching and quick decline syndrome, Pierce's disease of grapes, phony peach disease and plum leaf scald (Cao *et al.* 2011, Chen *et al.* 2019, Cornara *et al.* 2019a, EFSA 2018, 2019a, EPPO 2018, Miranda *et al*. 2013, Marucci *et al.* 2008, Tsagkarakis *et al*. 2018).

Due to the high number of species affected by this bacterium and the elevated number of diseases caused by it, there is a great variety of symptoms and they can easily be confounded with others caused by different biotic or abiotic factors, including other pathogens, water deficiencies, pollutants, nutritional problems, environmental stresses, among others. Depending on the plant species and cultivar, symptoms from premature leaf drop, yellow discoloration, stunting, to reduction of production and dimension of fruits may occur (Cornara *et al.* 2019a, Janse & Obradovic 2010). One of the main crops produced in Beira Interior Region in Portugal is stone fruit such as peach and plum, both of which can be affected by diseases caused by *X. fastidiosa*. Phony peach disease is characterized by stunted shoots, denser and darker foliage and branches growing horizontally which gives trees a more rounded and compact shape. Flowers and leaves appear earlier and remain for a longer period of time than in healthy trees. Affected trees have fewer and smaller fruits and after some years the peach orchards become economically worthless. If the trees are infected before bearing it will never be productive. Plum leaf scald is characterized by the typical scorched appearance, as well as an earlier blossom season. Affected trees also become more debilitated making them more susceptible to other problems (Chen *et al*. 2019, Diekmann & Putter 1996, EFSA 2019a, EPPO 2019, Janse & Obradovic 2010, Martelli 2016, Miranda *et al.* 2013, Saponari *et al*. 2019, Wells *et al.* 1981).

In countries where the bacterium occurs, severe damage to important crops is observed which in turn leads to considerable direct and indirect economic impact in these areas. In California the current annual cost of Pierce's disease is estimated in 104 million US$, which includes both vine losses and prevention measures. In Brazil, the disease affecting citrus trees caused by *X. fastidiosa* is estimated to be responsible for the removal of over 100 million citrus trees since its discovery in 1987, and the current annual cost still surrounds 120 million US$. The region of Apulia in Italy was also heavily impacted a considerable area of olive orchards, exceeding over 5000 km^2 that corresponds to 1-3 millions of olive trees. The economic loss in Europe is considered to be quite substantial, however it is still impossible to estimate the value (Cornara *et al*. 2019a, EFSA 2018, Godefroid *et al*. 2019).

1.4.4 Management measures

X. fastidiosa is regulated in the EU as a quarantine organism as it meets the criteria assessed by EFSA such as a clear establishment of the pests identity, its presence in the EU territory, either under eradication or containment within a limited distribution, and the respective economic consequences caused by this organism presence in the EU territory. Official control measures have been applied as well as a reinforcement of survey activities (EFSA 2018, Martelli 2016, Saponari *et al*. 2014, Tsagkarakis *et al.* 2018).

There is no single method for the control of this bacterium and therefore it should be implemented considering each specific situation. Some of the currently used control methods include: plant removal of infected and surrounding plants, severe pruning of symptomatic plants in order to reduce and suppress disease symptoms, host plant resistance by selection of cultivars that show a higher tolerance to the pathogen and a reduced development of symptoms, control of insect vectors that can be achieved by insecticide applications on crops and soil tilling to kill nymphs on herbaceous plants (Cornara *et al*. 2018, Dáder *et al*. 2019, EFSA 2018, 2019a, Janse & Obradovic 2010, Saponari *et al*. 2019).

Considering that the chemical control of the bacterium is not possible, the only way to slow the spread of the diseases caused by this bacterium besides the chemical and biological control of vector species, is the use of resistant varieties as well as cultural and hygienic measures (Cao *et al.* 2011, Cornara *et al.* 2017a, EFSA 2018, 2019a, Janse & Obradovic 2010). To achieve the best outcome in the development of efficient control management practices, it is necessary a robust knowledge of the vector species, namely its biology and ecology. Characteristics of the environment such as habitat type, food resources, disturbances, crop management, removal of vegetation cover, and application of pesticides can affect the vector population growth (Cornara *et al*. 2018, EFSA 2018, 2019a, 2019b, Morente e*t al*. 2018a).

Biological control of vector species is highly limited. Natural enemy species of these vectors exist, for example *P. spumarius* adults can be attacked by *Verralia aucta* Fallenn, an endoparasitoid, or by fungi of the genus *Entomophora* sp., however the impact of these enemy populations seems to be reduced because they are neither very abundant nor very specific in the areas where the vector develops (Dáder *et al.* 2019, EFSA 2019a).

Chemical control of Cercopoidea is also relatively scarce as these species are not usually considered pests of crops with a high economic importance. Most methods of chemical control for this insect group target nymphs, however some synthetic pyrethroids and neonicotinoids have shown good results on adults of *P. spumarius* (Cornara *et al*. 2018, Dáder *et al*. 2019, EFSA 2019a, 2019b). Naturally derived insecticides are considerably less effective. Some studies demonstrated that under laboratory conditions, pyrethroids deltamethrin, sulfoxaflor and lambda-cyhalothrin were successful in controlling *P. spumarius* (Cornara *et al*. 2018, Dáder *et al*. 2019).

1.4.5 *Xylella fastidiosa* in Portugal

Xylella fastidiosa represents a serious threat to many countries in the Mediterranean region, including in Portugal, where the bacterium was detected in January of 2019 in Vila Nova de Gaia. The subspecies of the bacterium detected in the region was then identified as *X. fastidiosa* subsp. *multiplex*, and it is associated with at least 58 plant species/genus in Europe including important crops widely represented in Portugal. Not only does this country have economically important susceptible host plants, but vectors such as *P. spumarius* and *N. campestris* are also widespread. Olive, stone fruit, citrus and grapevine are some of the main crops in the country and can be seriously affected by diseases caused by this bacterium (EPPO 2019, Santoiemma *et al*. 2019, DGAV 2019a, 2019b).

Since the detection of *X. fastidiosa* in Portugal, intensive prospections have taken place in the surrounding areas and measures such as the removal and destruction of infected plants, and restrictions to the movement of susceptible plants have been applied. After the initial detection of the bacterium, 32 additional focal points were reported (Figure 1.3.), mainly in public areas and some private gardens. In all the detected cases, the same subspecies of the bacterium, *X. fastidiosa* subs. *multiplex* was identified (DGAV 2019a, 2019b, 2020).

In the affected area insecticides were applied in order to eliminate potential insect vectors present in the region to avoid the spread of the bacterium. Plants considered to be hosts of *X. fastidiosa* are not allowed to be planted in the area, and the already existing ones cannot be transported to the outside of the affected zone, nor commercialized. Continuous sampling, identification of susceptible host species, and sensibilization of the population in the affected area is also critical in order to have a more vigilant community and an easier access for official services in charge of prospection to better assess the efficacy of implemented measures (DGAV 2019a, 2019b, 2020).

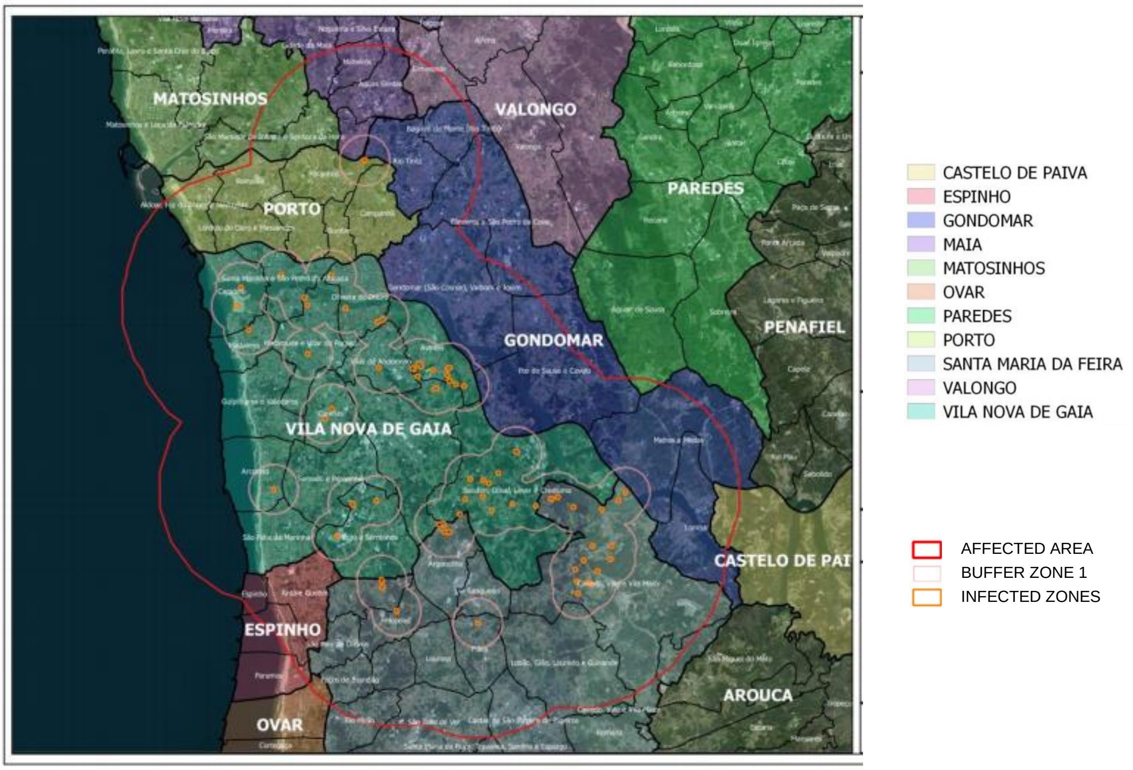

Fig. 1.3- Infected and buffer zones of the area affected by *Xylella fastidiosa* in Portugal in 29/1/2020 (DGAV, 2020).

1.5 Beira Interior Region

Beira Interior Region is situated on the central region of mainland Portugal and corresponds approximately to the districts of Castelo Branco and Guarda. This agricultural area has a wide variety of permanent cultures, such as vine and olive, as well as stone fruit trees (Lopes *et al.* 2018). This region is the main producing area for stone fruit trees at a national level, resulting from a set of natural resources and climatic advantages that are characteristic of this particular region (Simões *et al*. 2007).

Throughout the years there has been an increase in awareness regarding the need to ally agricultural production and environmental preservation. This is a growing problem all around the world because of the continuously increasing world population that demands a growth in agricultural production, however environmental sustainability has to be considered as well as the conservation of biodiversity, climate change adaptation, and other factors (Lopes *et al.* 2018). This led to a necessity of developing methods that allow a sustainable production, which in turn resulted in an approach called integrated production (Felix & Cavaco 2008). In recent years, most of the production on Beira Interior Region is a result of integrated production, as this approach has been replacing conventional agriculture, leading to greater environmental awareness, as well as an increase on technical knowledge of farmers that allows a more informed decision regarding phytosanitary interventions, fertilizer applications, among other important decisions about production (Felix & Cavaco 2008, Lopes *et al.* 2018, Simões *et al.* 2007). In this region, biological production is still very diminished, having a very reduced expression of only 2% (Lopes *et al.* 2018).

Cova da Beira is a region within Beira Interior with a considerable relevance on the production of stone fruit in the area, mainly peach and cherry, and over 70% of this area corresponds to the installation of new orchards, as only a much lesser area corresponds to valorization and renewal actions on already existing orchards (Lopes *et al*. 2018). The newly installed orchards are more susceptible to pests like *A. decedens,* as this species prefers younger or developing plants, and is able to cause a greater amount and severity of damage on these orchards, that will consequently lead to greater loss in production and thus a more significant economic impact (Chaieb *et al.* 2011).

1.5.1 Peach Orchards

The peach orchards in Beira Interior region represent approximately 45% of the national area occupied by the production of this particular stone fruit, corresponding to an area of over 1600 ha (Ferreira *et al*. 2017). This area is characterized by acid soils with low fertility that are mostly coarse in texture which translates to conditions of high permeability and aeration, considered to be highly favourable conditions to peach production, as it is very sensible to radicular asphyxiation that may result from floods or other phenomenon. This region is exposed to elevated temperatures and light hours, two factors that also favor the production of peach as well as other stone fruits (Simões *et al*. 2007).

Different cultivars of peach orchards can be found in the region and each cultivar produces fruits with different visual characteristics as well as taste, and may also have different maturation dates, however these characteristics also depend on climatic conditions and other factors regarding the cultural techniques used by each farmer. According to the maturation season of each cultivar, these can be divided in early cultivars, season cultivars and late cultivars. The existence of cultivars with different maturation seasons allows a larger commercialization period, as fruits can be sold during the entire summer season (Ferreira *et al*. 2017).

A common practice on peach orchards is green pruning, a technique adopted by the integrated production of peaches for the canopy volume condition. This technique allows a better aeration and a bigger absorption of solar radiation and consequently leads to a more intense red color and improves overall quality of the fruits (Dolinski *et al*. 2016, 2018, Trevisan *et al*. 2006). The severity of the pruning can vary according to other factors such as nitrogen application, as the removal of the branches leads to a better use of nitrogen by the remaining branches which stimulates the developing of flower buds (Dolinski *et al*. 2018)

The quality of peach fruits depends on many characteristics such as weight, external appearance, acidity, sugar content, firmness, among others. In turn, these components depend on external factors such as climatic conditions, applied treatments, load of fruits per tree, pruning, harvest season, and also on the impact of phytosanitary problems like diseases and pests, as these can lead to significant losses on quality and production and an increase of costs on control measures (Dolinski *et al*. 2018, Ferreira *et al*. 2017, Trevisan *et al*. 2006). Some examples of diseases affecting peach are brown rot, powdery mildew, X-disease, plum-pox, among others (Diekmann & Putter 1996, Kappel & Sholberg 2008, Marcone *et al*. 2014).

II Objectives

With this study aimed for the Auchenorrhyncha suborder monitoring, the focus lies on the leafhopper species *Asymmetrasca decedens,* an emergent pest in mainland Portugal and other potential pests, particularly potential vectors of *Xylella fastidiosa,* on Póvoa de Atalaia and Louriçal do Campo peach orchards, of the Beira Interior region.

In a more detailed manner, the goals of this study are:

- Monitoring the life cycle of *Asymmetrasca decedens* on peach orchards of the Beira Interior Region.
- Monitoring and identification of Auchenorrhyncha suborder species, specifically potential vectors of *Xylella fastidiosa*.
- Assess on different dates the effect of green pruning on the species populational density.
- Assess the effects of weather conditions on the species populational density.
- Inform the producers for the importance of an early installation of traps for the monitoring, following a preventive approach on these pests control.

III. Materials and Methods

3.1 Study area

The monitoring of the Auchenorrhyncha suborder took place on two peach orchards, Póvoa de Atalaia and Louriçal do Campo (Figure 3.1), property of Joaquim Martins Duarte & Filhos, on the Beira Interior Region, more specifically, on Cova da Beira in mainland Portugal. All the presented maps were rendered in QGIS version 3.10.5 and a summary of the sources and metadata used on the maps formulation is available in Appendix A. To the north of Louriçal do Campo orchard, other peach and apricot orchards exist, while to the south and west, a riparian gallery as well as some riverside vegetation (Ocreza river) can be found and to the east, a natural pasture along with a pine forest are present. Póvoa de Atalaia orchard is hedged by peach and cherry orchards on the north and south, while on the west another peach orchard and a forest are present, and to the east a water line and some riparian vegetation exist. This orchard is also characterized by the presence of some small ponds and open water reservoirs. A drip irrigation system is present on both orchards.

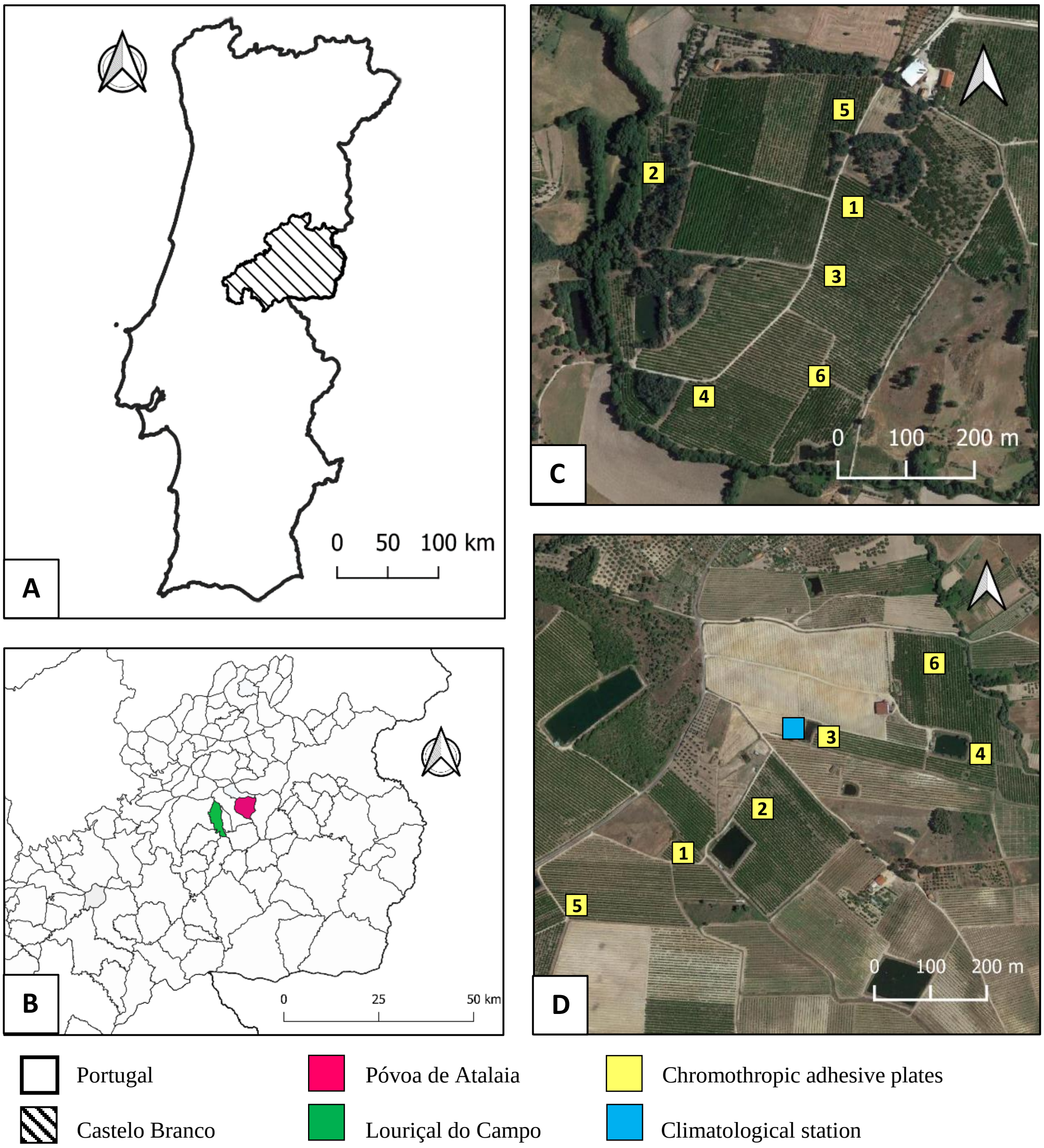

Fig. 3.1- Orchards location (A,B) and chromothropic traps placed in Louriçal do Campo (C) and Póvoa de Atalaia (D).

On both orchards exist a total of 18 different peach cultivars with a 4.5 m compass between the line and 2.5m on the line. On table 3.1 are presented the different cultivars on both orchards and the respective production season. The control of the adventitious flora is achieved on the line with the use of herbicides as well as through periodic cuts between the line, where a natural and permanent grassing-in is found as it allows a better control on soil erosion.

Green pruning, a cultural approach, is carried out occasionally during the months of June and July as presented in table 3.2, for the canopy volume condition on Louriçal do Campo and Póvoa de Atalaia orchards. This method was exercised on 24 to 30 trees surrounding the ones where the trap was placed on (including these) with the exception of the pruning exerted between 28th and 31st of July, where the whole cultivar was pruned. The cultivars in which the traps were placed are presented on table 3.1. Chemical control directed at specific pests was also applied on both orchards according to harvest season. The phytosanitary treatments applied on both orchards are presented on table 3.3.

Table 3.1 - Cultivars present on Louriçal do campo (LC) and Póvoa de Atalaia (PA) orchards and respective production season. P(1-6) - Traps number 1 to 6 from Póvoa de Atalaia; L(1-6) – Traps number 1 to 6 from Louriçal do Campo.

Cultivar	Production season	Orchard	Trap	Reference
'Andross'	Season	PA/LC	P1/L5	Ferreira *et al.* (2017); Nectalia (n.d)
'Catherine'	Season	PA/LC	L2	Ferreira *et al.* (2017); Nectalia (n.d)
'Diamond princess'	Season	PA	-	Ferreira *et al.* (2017)
'Extreme 460'	Season	LC	L1	Provedo (n.d)
'Extreme 486'	Late	LC	L3	Provedo (n.d)
'Honey Cascade'	Season	PA	-	Dalival (n.d)
'Honey Royal'	Season	PA	-	Nectalia (n.d); Dalival (n.d)
'Jerte'	Early	PA	P5	Provedo (n.d)
'Poblet'	Season	PA/LC	P3/L4	Provedo (n.d)
'Red Jim'	Late	PA	P2	Nectalia (n.d)
'Rich Lady'	Early	LC	-	Stoned Peach (2018)
'Royal Glory'	Early	LC	-	Ferreira *et al.* (2017)
'Royal Summer'	Season	LC	L6	Ferreira *et al.* (2017); Dalival (n.d)
'Summer Rich'	Season	LC	-	Ferreira *et al.* (2017)
'Summer Sun'	Early	LC	-	Nectalia (n.d)
'Sweet Dream'	Season	PA/LC	P4/P6	Ferreira *et al.* (2017); Dalival (n.d)
'Tardibelle'	Late	LC	-	Ferreira *et al.* (2017)
'Tirrenia'	Early	LC	-	Ferreira *et al.* (2017)

Table 3.2 - Green pruning on Louriçal do Campo and Póvoa de Atalaia orchards between June 21st 2019 and July 31st 2019.

Green Pruning			
Date	**Cultivar**	**Orchard**	**Traps**
21/jun	'Poblet'	Louriçal do Campo	L4
21/jun	'Jerte'	Póvoa de Atalaia	P5
24/jun	'Red Jim'	Póvoa de Atalaia	P2
24-25/jun	'Poblet'	Póvoa de Atalaia	P3
27/jun	'Royal Summer'	Louriçal do Campo	L6
01/jul	'Andross'	Louriçal do Campo	L5
28-31/jul	'Royal Summer'	Louriçal do Campo	L6

Table 3.3- Phytosanitary treatments applied on Louriçal do Campo and Póvoa de Atalaia orchards from February 7th 2019, to August 24th 2019.

Pharmaceutical Product	Active substance	Aplication date	Cultivars product(s) was(were) applied on	Enemy
Fungicide	Copper oxychloride	7 to 14/02/2019	All	Peach leaf curl; gumspot of stone fruit
	Thiram	2 to 3/03/2019		Peach leaf curl; gumspot of stone fruit
	Thiram	21 to 23/03/2019		Peach leaf curl; gumspot of stone fruit
	Difenoconazole	12 to 14/04/2019		Powdery mildew ; brown rot
	Sulfur	16/05/2019		Powdery mildew
	Sulfur	21 to 23/05/2019		Powdery mildew
	Penconazole	22 to 23/06/2019	Except Rich Lady, Royal Glory Royal Summer and Tirrenia	Powdery mildew
	Penconazole	22 to 26/06/2019	Except Diamond Princess	Powdery mildew
	Fluopyrame + Tebuconazole	08/07/2019	Catherine; Sweet Dream; Honey Royal; Summer Sun; Summer Rich	Brown rot
	Fluopyram + Tebuconazole	18/07/2019	Jerte	Brown rot
	Fluopyram + Tebuconazole	01/08/2019	Andross; Honey Cascade; Andross	Brown rot
	Difenoconazole	30/07 to 1/08/2019	Poblet	Brown rot
	Fluopyram + Tebuconazole	24/08/2019	Poblet; Tardibelle; Extreme 486	Brown rot
Insecticide	Flonicamid	21 to23/03/2019	All	Aphids
	Lambda-cyhalothrin	08/07/2019	Catherine; Summer Sun; Sweet Dream; Summer Rich; Honey Royal	Mediterranean fruit fly
	Lambda-cyhalothrin	18/07/2019	Jerte	Mediterranean fruit fly
	Deltamethrin + thiacloprid	29/07/2019	Extreme 460	Mediterranean fruit fly
	Lambda-cyhalothrin	01/08/2019	Andross; Honey Cascade	Mediterranean fruit fly
	Deltamethrin + thiacloprid	15/08/2019	Poblet	Mediterranean fruit fly
	Lambda-cyhalothrin	24/08/2019	Tardibelle; Extreme 486; Poblet	Mediterranean fruit fly
Acaricide	Acrinathrin	29/05/2019	Royal Glory; Rich Lady	Red spider mite
Herbicide	Glyphosate	2 to 4/4/2019	All	
	Ammonium Glufosinate	22 to 26/4/2019	New peach trees	

3.2 Sampling

As the PrunusFito project covers a period of over 3 years, the specimens sampling and collection for this dissertation occurred from April 4th to October 31st in 2019, as these dates would allow the conclusion of the master's dissertation in time. On each one of the peach orchards were placed 6 yellow chromothropic traps, more specifically yellow adhesive plates with dimensions of 22,5×20cm. These traps were collected and replaced weekly, performing a total of 360 yellow sticky traps. On Louriçal do Campo orchard, the traps were placed on the cultivars: "Extreme 460", "Catherine", "Extreme 486" , "Poblet", "Andross" and "Royal Summer". On Póvoa de Atalaia orchard, one trap was located on each of the cultivars: "Andross", "Red Jim", "Poblet", "Jerte" and two on "Sweet Dream".

The sampling was carried out by members of Appizêzere, the host institution of the PrunusFito Project.

The captured insects of the Auchenorrhyncha suborder were in its adult stage and were removed from the yellow sticky traps with petroleum to dissolve the glue in a fume hood. The samples were preserved at room temperature in eppendorfs with 70% alcohol and labelled with the sampling details, date, place, and identifying number, until the morphological identification.

3.3 Morphological identification

All Auchenorrhyncha were analyzed in this study as several species are considered to be pests of economically important crops, being responsible for serious damage to certain species of cultivated plants (Ossiannilsson 1978). The Auchenorrhyncha specimens were sorted and identified to the lowest possible taxonomic level using several identification keys, listings and books (Biedermann & Niedrighaus 2009, Bluemel *et al.* 2014, Dietrich 2005, Giustina 1989, Nielson 1968, Dmitriev 2003-present, Ossiannilsson 1978, Quesne 1960, 1965, 1969, Quesne & Payne 1981, Ribaut 1936, 1952, Zenner *et al.* 2005). The specimens that could not be identified to the species level were separated into morphospecies based on the similarity of morphological characteristics.

In order to identify the collected specimens, morphological characteristics were observed as well as the male specimens genitalia, considering that most identification keys are based on male genital characters that tend to be more variable as opposed to the female genitalia that is considered to be more conservative. Females were also identified to the lowest possible taxonomic level and if all the males in a genus correspond to a single species, the females of that same genus were considered to belong to the same species after a careful observation of its morphological characteristics. If more than one species was present within the same genus, morphospecies were established for the females within that genus and these were named after the genus or subfamily followed by “sp.” and a letter corresponding to the morphotype (example: Deltocephalinae sp. A). Considering the impossibility of a morphological distinction between females as well as damaged specimens of Empoascini (Typhlocybinae) and in order to avoid underestimating the number of captured individuals in the data treatment, the number of Empoascini that could not be identified was distributed by the tribe species that existed in the sample proportionally to the number of males captured of each species.

To better observe the male genitalia of the several specimens, a microscopic preparation is required. To prepare the male genitalia, a posterior section of the abdomen, the pygofer, is removed with a dissection needle by gently separating it from the rest of the body. This section is then placed in a small container with hot potassium hydroxide (KOH 10%), for 20 to 40 seconds for the smaller and lighter colour specimen samples, and for around two minutes for the larger or darker samples, in order to increase its transparency. This process allows an easier removal of the soft tissue parts as well as a better observation of the sclerotized pieces of the genitalia. The section of the abdomen is then removed from the KOH 10% solution and placed in a drop of glycerin in order to separate the genital parts from the remaining tissue. After separating the genitalia, it was assembled on a glass slide with glycerin, and sealed with nail polish. The prepared specimens were then identified using the stereomicroscope OLYMPUS SZX7 TR30 and a L1100 A binocular optical microscope, by a careful observation of the morphological characteristics and the male genitalia.

The remaining parts of the samples were conserved on eppendorfs with 70% alcohol on the Entomology lab (FCUL).

3.4 Photographic record

For the photographic record of the whole body, images from several perspectives of the collected specimens were taken using the stereomicroscope Zeiss SteREO Lumar V.12 equipped with The Imaging Source digital camera Zeiss Axiocam 503 and the AxioVision 4.91.1 software. The images obtained from the specimens were acquired at multiple focal distances in an automized manner and subjected to focus stacking with the tool "Extended Focus" by the controlling software, using the Wavelets method. For the photographic record of the male genitalia, the widefield fluorescence microscope OLYMPUS BX51 coupled with The Imaging Source DFK 23U274 camera with the Micro-Manager 2.0 gamma software was used. To scale and process the images of the genitalia, ImageJ V1.8.0_172 was employed. Some of the obtained genitalia images were subjected to *focus stacking* with the *plugin* "Extended Depth of Field" (Foster *et al.* 2004) and the Wavelets method. This allows the stacking of images that were acquired manually at multiple focal distances. The photographic record of whole body images was done prior to the record of the male genitalia as the second requires a partial destruction of the specimens.

3.5 Data Analysis of the Auchenorrhyncha community

3.5.1 Abundance, Dominance and Frequency

In order to characterize the Auchenorrhyncha community on Póvoa de Atalaia and Louriçal do Campo orchards, species dominance and frequency was calculated for each orchard. Dominance indicates the percentage of individuals of a given species in relation to the total number of individuals of all species. Species were categorized as "dominant", "influential" or "nondominant" if its dominance was > 10, 5-10 or < 5%, respectively. Frequency indicates the proportion of dates in which a given species occurs regarding the total number of sampling dates. Regarding the values obtained for frequency, species were classified as "constant", "secondary" or "accidental" if frequency values were > 50, 25-50 or < 25% respectively (Tsagkarakis *et al.* 2018). The overall species abundance was analyzed for each orchard as well as the average abundance of the species considered to be either dominant or constant on both orchards. For either dominant or constant species that exhibited considerably different average means for both orchards, a Wilcoxon signed rank test was conducted to determine if the samples from the two orchards were statistically different from each other, as this test allows to test for paired nonparametric data (Woolson 2008).

3.5.2 Species accumulation and rarefaction curves

Species accumulation curves were determined to evaluate the sufficiency of the sampling effort on the determination of the number of Auchenorrhyncha species existing on the studied orchards. The species accumulation curves record the cumulative number of species detected on a particular environment as a function of the cumulative effort expended searching for them. In the data obtained for these orchards, the species accumulation curves were determined as a function of the number of samples obtained, considered as the set of individuals and species detected on all of the collected traps for a given orchard in a certain sampling date.

Considering the interpretation of accumulation curves is influenced by the order in which the samples are added, rarefaction curves were also determined based on the number of individuals as well as on samples. Rarefaction curves consist on an interpolated estimate of the average species accumulation curve and the respective variation from random resampling techniques without replacement, thus removing the effect of the order of samples on the curve shape. For the statistical analyses was used the program R version 3.6.3 (R Core Team, 2020) implemented in RStudio version 1.2.5033 (RStudio Team, 2019). Both the species accumulation and rarefaction curves were computed using the *specaccum* function of the 'vegan' package (Oksanen *et al*. 2019). 1000 permutations were considered in the generation of rarefaction curves, as the greater the number of permutations used, the better the estimate of the variation around the average curve value and the greater the confidence in comparing rarefaction curves.

3.5.3 Estimating specific richness

In order to estimate the species richness of the Auchenorrhyncha found on both orchards, three non-parametric estimators (Chao2, Jackknife1 and Jackknife2) were used due to the lack of assumptions regarding the species abundance distribution (Gotelli & Colwell, 2011). These non-parametric estimators perform better accuracy, precision and bias wise than other extrapolated asymptotic functions or other parametric estimators. Chao2 is ranked as one of the estimators with an highest overall bias while Jackknife1 as one of the estimators with an highest overall accuracy (Walker & Moore, 2005). The specific richness estimators Chao2 (Equation 3.1), Jackknife1 (Equation 3.2) and Jackknife 2 (Equation 3.3) can be calculated with the equations presented below:

$$S_{Chao\,2} = S_{Obs} + \frac{(q1)^2}{2(q2)}\left(\frac{n-1}{n}\right) \quad \textbf{(3.1)}$$

$$S_{Jackknife\,1} = S_{Obs} + q1\left(\frac{n-1}{n}\right) \quad \textbf{(3.2)}$$

$$S_{Jackknife\,2} = S_{Obs} + \left[\frac{q1(2n-3)}{n} - \frac{q2(n-2)^2}{n(n-1)}\right] \quad \textbf{(3.3)}$$

where $\mathbf{S_{Obs}}$ is the observed specific richness (total number of species present in the samples); **q1** is the number of species present on a single sample (*singletons*); **q2** is the number of species present on exactly two samples (*doubletons*); and **n** is the total number of the set of samples. To calculate the values of these estimators was used the *specpool* function from the 'vegan' package (Oksanen *et al.* 2019).

The *Percentage of True Richness* (PTR, Equation 3.4) was also calculated for each estimator and orchard combination:

$$PTR = \frac{S_{Est}}{S_{Obs}} \times 100 \quad \textbf{(3.4)}$$

where $\mathbf{S_{Obs}}$ is the observed richness and $\mathbf{S_{Est}}$ corresponds to the richness calculated by each estimator (S_{Chao2}, $S_{Jackknife1}$, $S_{Jackknife2}$).

3.5.4 Relation between climatic variables and green leafhopper populations

The meteorological data was provided by Anabela Barateiro (Appizêzere). The data set includes 12 variables that were measured daily : minimum, average and maximum temperature, minimum, average and maximum relative humidity, average solar radiation, average and maximum wind velocity, total precipitation, number of hours of cold (below 7ºC), and number of hours of wet leaf. This data was registered on Póvoa de Atalaia climatological station (7°24'26.09"W, 40°4'15.52"N) based on Póvoa de Atalaia orchard. As this climatological station is located near Louriçal do Campo orchard (9,98km) the data obtained from Póvoa de Atalaia station was used for both orchards. Considering that the supplied meteorological data corresponds to daily measurements and the Auchenorrhyncha captures were performed with a weekly frequency, the meteorological data was assembled weekly (Appendix – Table

B.1). The form in which the meteorological data was aggregated was different for several variables as presented in Table 3.4.

Table 3.4 –Weekly data aggregation of daily meteorological data with the respective abbreviation.

Daily meteorological data	Unit	Weekly agregation Statistic	Variable abbreviation
Minimum temperature	ºC	Minimum	TMin
Average temperature	ºC	Average	TAve
Maximum temperature	ºC	Maximum	TMax
Minimum relative humidity	%	Minimum	HRMin
Average relative humidity	%	Average	HRAve
Maximum relative humidity	%	Maximum	HRMax
Average solar radiation	W/M^2	Average	RadAve
Average wind speed	m/s	Average	WSAve
Maximum wind speed	m/s	Maximum	WSMax
Total precipitation	Mm	Average	TotPrec
Number of hours of cold (<7ºC)	NA	Average	NHCold
Number of hours of wet leaf	NA	Average	NHWetLeaf

The weekly aggregated data regarding the climatic variables was used in order to study the interdependence between the populational dynamics of the dominant species of green leafhoppers on the studied orchards and the respective climatic variables with the most impact on these species variation. The data regarding the green leafhoppers corresponds to the weekly time series of the average number of individuals per orchard for each of the dominant green leafhopper species. This interdependence was analyzed through the estimate of the cross correlation and its significance between time series for *lags* between 0 and 7 weeks previous to sampling as the development form egg hatching to adult emergence varies between 2 and 7 weeks (Jacas *et al.* 1997, Torres *et al.* 2002). Both the most significant cross correlations were identified as well as the corresponding *lag* value for which the correlation value was stronger for each variable. The cross correlations were calculated with the *ccf* function from R’s base package.

IV. Results and Discussion

4.1 Auchenorrhyncha community

In total, 8140 individuals were collected on both orchards and 39 species belonging to 4 different families were identified: Aphrophoridae, Cicadellidae, Delphacidae and Dictyopharidae. (Table 4.1 and 4.2, Appendix - C). Louriçal do Campo orchard presented both a larger species diversity as 36 species were identified, as well as a greater number of individuals as 4729 specimens were collected. On Póvoa de Atalaia orchard 27 species were encountered, and 3411 individuals were detected.

Table 4.1 – Auchenorrhyncha abundance, dominance and frequency of the collected specimens on Póvoa de Atalaia orchard. N: abundance, number of captured individuals, D (%); Dominance, proportion of a species abundance in relation to the abundance of all species; F(%): Frequency, proportion of dates a species is found on.

Suborder	Family	Subfamily	Genus/Species	N	D(%)	F(%)
Cicadomorpha	Aphrophoridae	-	*Neophilaenus campestris* (Fallén)	3	0.09	10.00
			Philaenus spumarius (Linnaeus)	1	0.03	3.33
	Cicadellidae	Agalliinae	*Agallia consobrina* Curtis	70	2.05	36.67
			Anaceratagallia laevis (Ribaut)	35	1.03	36.67
			Austroagallia sinuata (Mulsant & Rey)	4	0.12	13.33
		Aphrodinae	*Aphrodes makarovi* Zachvatkin	15	0.44	20.00
		Deltocephalinae	*Deltocephalinae* sp.A	1	0.03	3.33
			Euscelidius variegatus (Kirschbaum)	6	0.18	16.67
			Psammotettix sp. A	2	0.06	6.67
			Psammotettix sp. B	1	0.03	3.33
		Macropsinae	*Macropsis cerea* Germar	1	0.03	3.33
		Typhlocybinae	*Alebra* sp.	1	0.03	3.33
			Arboridia sp.	8	0.23	16.67
			Asymmetrasca decedens (Paoli)	2790	81.79	93.33
			Empoasca decipiens Paoli	2	0.06	6.67
			Empoasca solani (Curtis)	362	10.61	60.00
			Eupteryx filicum (Newman)	1	0.03	3.33
			Eupteryx sp.	1	0.03	3.33
			Ribautiana cruciata (Ribaut)	1	0.03	3.33
			Ribautiana debilis (Douglas)	1	0.03	3.33
			Ribautiana sp.	1	0.03	3.33
			Zygina lunaris (Muslant & Rey)	1	0.03	3.33
			Zygina nivea (Muslant & Rey	2	0.06	3.33
			Zygina ordinaria (Ribaut)	45	1.32	56.67
			Zyginidia scutellaris (Herrich-Shaffer)	52	1.52	46.67
Fulgoromorpha	Delphacidae	Delphacinae	*Laodelphax striatella* (Fallén)	3	0.09	10.00
	Dictyopharidae	-	*Dictyophara europaea* (Linnaeus)	1	0.03	3.33

The family with the greatest representation in the samples from both orchards is the Cicadellidae family which represents 99.77% of the captured individuals, being the Typhlocybinae the most represented subfamily corresponding to 95.84% of the collected specimens. Regarding species dominance, *Asymmetrasca decedens* (Paoli) stood out representing 79.64% of the captured individuals, followed by *Empoasca solani* (Curtis) with 13.45%. In regard to species frequency, 5 species were considered constant as these appeared in over 50% of the sampling dates: *A. decedens*, *Zygina ordinaria* (Ribaut), *E. solani, Agallia consobrina* Curtis, and *Zyginidia scutellaris* (Herrich-Schaffer) (Appendix C).

Table 4.2 - Auchenorrhyncha abundance, dominance and frequency of the collected specimens on Louriçal do Campo orchard. N: abundance, number of captured individuals, D (%); Dominance, proportion of a species abundance in relation to the abundance of all species; F(%): Frequency, proportion of dates a species is found on.

Suborder	Family	Subfamily	Genus/Species	N	D(%)	F(%)
Cicadomorpha	Aphrophoridae	-	*Neophilaenus campestris* (Fallén)	1	0.02	3.33
			Philaenus spumarius (Linnaeus)	3	0.06	6.67
	Cicadellidae	Agalliinae	*Agallia consobrina* Curtis	93	1.97	56.67
			Anaceratagallia laevis (Ribaut)	42	0.89	36.67
			Austroagallia sinuata (Mulsant & Rey)	10	0.21	13.33
		Aphrodinae	*Aphrodes makarovi* Zachvatkin	12	0.25	10.00
		Deltocephalinae	*Deltocephalinae* sp. A	1	0.02	3.33
			Deltocephalinae sp. B	1	0.02	3.33
			Euscelidius variegatus (Kirschbaum)	2	0.04	6.67
			Euscelis sp.	2	0.04	6.67
			Fieberiella florii (Stal)	1	0.02	3.33
			Neoaliturus fenestratus (Herrich-Shaffer)	4	0.08	6.67
			Platymetopius guttatus Fieber	2	0.04	6.67
			Psammotettix sp. A	2	0.04	6.67
			Psammotettix sp. B	5	0.11	13.33
			Psammotettix sp. C	2	0.04	6.67
		Dorycephalinae	*Eupelix cuspidata* (Fabricius)	4	0.08	13.33
		Macropsinae	*Macropsis cerea* Germar	1	0.02	3.33
		Megophthalminae	*Megophthalmus scrabipennis* Edwards	1	0.02	3.33
		Typhlocybinae	*Alebra* sp.	2	0.04	6.67
			Arboridia sp.	2	0.04	6.67
			Asymmetrasca decedens (Paoli)	3693	78.09	93.33
			Empoasca decipiens Paoli	1	0.02	3.33
			Empoasca solani (Curtis)	733	15.50	6667
			Eupteryx sp.	1	0.02	3.33
			Fruticidia bisignata (Mulsant & Rey)	1	0.02	3.33
			Fruticidia sanguinosa (Rey)	1	0.02	3.33
			Ribautiana cruciata (Ribaut)	1	0.02	3.33
			Ribautiana debilis (Douglas)	3	0.06	10.00
			Typhlocybinae sp. A	1	0.02	3.33
			Zygina lunaris (Mulsant & Rey)	5	0.11	16.67
			Zygina nivea (Mulsant & Rey)	2	0.04	6.67
			Zygina ordinaria (Ribaut)	61	1.29	70.00
			Zyginidia scutellaris (Henry-Shaffer)	26	0.55	43.33
Fulgoromorpha	Delphacidae	Delphacinae	*Laodelphax striatella* (Fallén)	1	0.02	3.33
			Metadelphax propinqua (Fieber)	6	0.13	10.00

The dominant species in Louriçal do Campo orchard were *A. decedens* and *E. solani* representing 78.09% and 15.5% of the captured individuals respectively. Both these species were also considered to be constant on this orchard in conjunction with *A. consobrina* and *Z. ordinaria*, even though *A. decedens* was certainly the most constant species as it appeared in 93.33% of the sampling dates. On this orchard, 4 constant species were detected, as well as 2 secondary and 30 accidental.

On Póvoa de Atalaia orchard, *A. decedens* and *E. solani* were also the most dominant species, representing 81.79% and 10.61% respectively. On this orchard 3 constant species were detected, *A. decedens, E. solani* and *Z. ordinaria,* 3 secondary and 21 accidental.

Several of the detected species are considered vectors or potential vectors of phytoplasmas capable of affecting and causing damage to many different crops such as vine, apple, stone fruit, potato, carrot, among others. This is the case of *Neoaliturus fenestratus* (Herrich-Shaffer), which has been demonstrated to carry aster yellows (AY), stolbur (Stol), 'Candidatus Phytoplasma solani' and 'Candidatus Phytoplasma asteris'. *Megophthalmus scrabipennis* Edwards, *Laodelphax striatella* (Fallén), *Anaceratagallia laevis* (Ribaut) and *Austroagallia sinuata* (Mulsant & Rey) were also established as species able to carry AY phytoplasma. Other species like *Zyginidia scutellaris* have also been demonstrated to be infected with the stolbur phytoplasma while *Aphrodes makarovi* Zachvatkin and *Philaenus spumarius* (Linnaeus) tested positive for '*Ca*. P. solani'. *Dictyophara europaea* (Linnaeus) has been proven to be able to carry and transmit the *Flavescence dorée* (FD) phytoplasma. The FD phytoplasma was also confirmed to be transmitted by *Euscelidius variegatus* (Kirschbaum) under laboratory conditions, and this species has tested positive for AY phytoplasma and is a confirmed vector of '*Ca*. P. asteris'. *Empoasca decipiens* Paoli is also a confirmed vector of '*Ca*. P. asteris' (Batlle *et al.* 2000, Bosco *et al.* 1997, Bozbuga & Elekçioglu 2008, Filippin *et al.* 2009, Landi *et al*. 2013, Lessio & Alma 2008, Orenstein *et al*. 2003, Orságová *et al* 2011, Safarova *et al.* 2018, Weintraub & Beanland 2006). Most of these species are presented in Appendix D photographic records.

Two of the captured species, *Philaenus spumarius* and *Neophilaenus campestris* (Fallén), are certainly worth noting despite being captured in relatively low numbers (4 individuals of each species) (Fig. 4.1 and 4.2). Both these species are xylem sap feeding insects and therefore considered to be vectors for *Xylella fastidiosa*, a xylem inhabiting bacterium recently detected in Portugal, responsible for causing diseases affecting several important crops including peach. Both species have been demonstrated to carry and transmit the bacterium, acting as competent vectors for *X. fastidiosa* (Cavalieri *et al.* 2019, Cornara *et al.* 2019a).

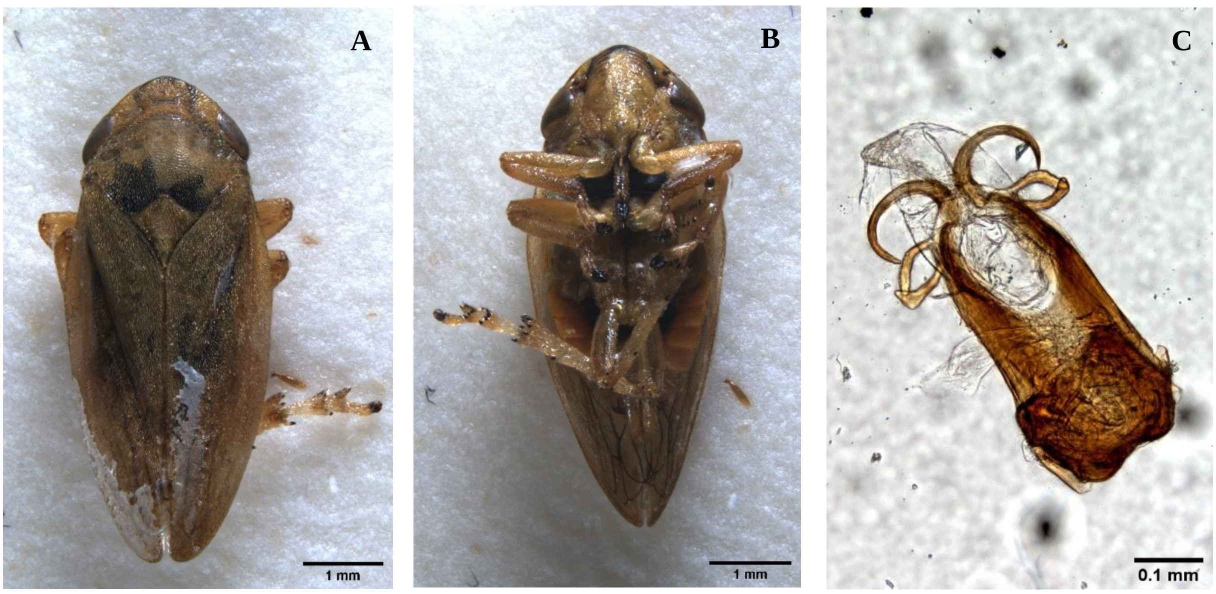

Figure 4.1 – *Philaenus spumarius* (Linnaeus) adults dorsal view (A), ventral view (B) and male genitalia (C). Author's original.

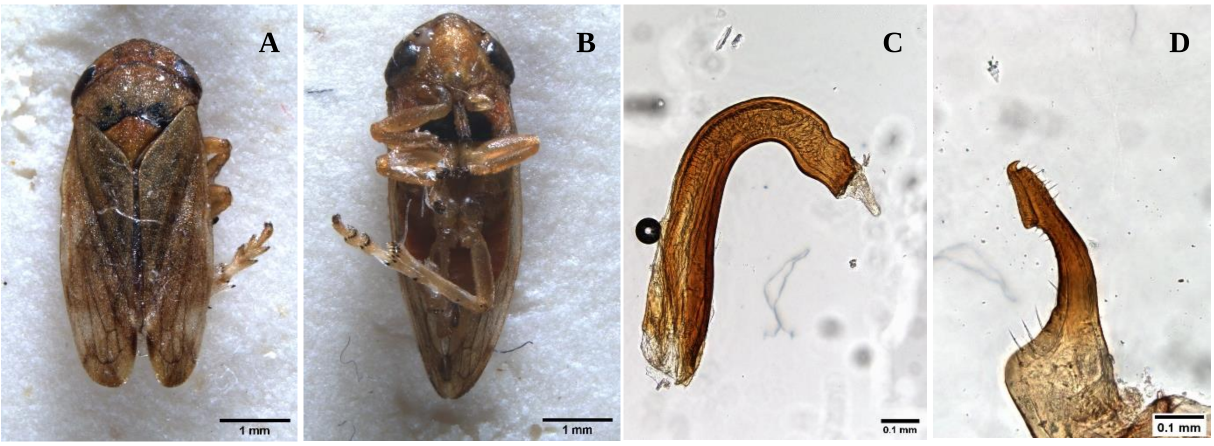

Figure 4.2 – *Neophilaenus campestris* (Fallén) adults dorsal view (A), ventral view (B) and male genitalia (C,D). Author's original.

Considering the samples obtained from both orchards, 5 species were considered to be either dominant and/or constant (Fig. 4.3), *A. consobrina, A. decedens, E.solani, Z. ordinaria* and *Z. scutellaris*. *A. decedens* presented an average abundance of 22 individuals per trap in Louriçal do Campo and 17 individuals in Póvoa de Atalaia. *E. solani* also presented a higher abundance in Louriçal do Campo, 6 individuals per trap, as opposed to Póvoa de Atalaia where on average 3 individuals per trap were found. *A. consobrina, Z. ordinaria* and *Z. scutellaris* demonstrated a similar abundance on both orchards however these species appeared with an abundance lower than 2 individuals per trap. Seeing that both *A. decedens* and *E. solani* presented considerably different average means and standard errors (Fig. 4.3), an Wilcoxon signed rank test was conducted to determine if this variation was significatively different between both orchards, and for both species the obtained p-value was ≤ 0.05, indicating that there was a statistically significant difference between both orchards for these two species.

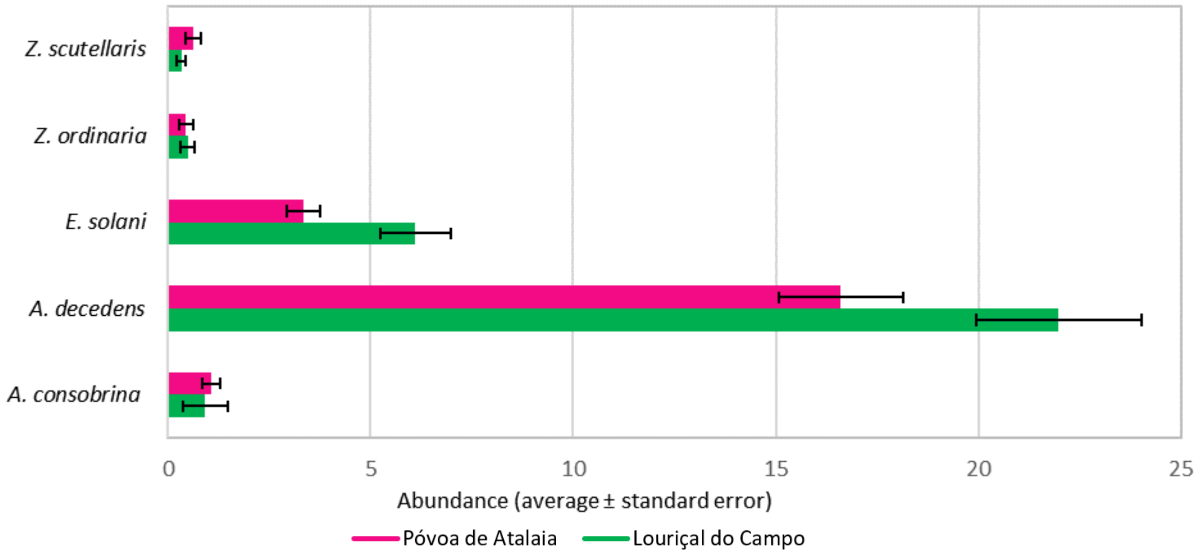

Figure 4.3 – Average abundance of dominant and/or constant species with the associated standard error on both orchards.

The species rarefaction curves for both the sampling effort (dates) and abundance did not demonstrate an asymptotic tendency on either orchards (Fig.4.4), where the increase in species richness would become lesser as the number of sampling dates or abundance would increase, leading to a curve with a very low slope. Considering that this asymptotic tendency had not been reached as a result of the sampling effort, it likely means that this effort was not sufficient to detect several species of the Auchenorrhyncha community. This deduction is also supported by the estimation of specific richness according to 3 different estimators, Chao2, Jackknife 1 and Jackknife 2, all of which estimated a higher number of species than the observed in the sampling. A reasonable error is associated with the estimator Chao2 for Póvoa de Atalaia orchard and therefore considerations have only been made regarding the other more reliable estimators as these present a lesser variation associated with the estimate. Jackknife 1 estimator indicated that the specific richness on this orchard is estimated to be 40 while Jackknife 2 estimates 50 species, likely meaning that between 13 and 22 species have been left undetected.

The specific richness estimates for Louriçal do Campo orchard were also more elevated than the observed richness on this orchard. Chao2 estimated that at least 44 species exist on the orchard while Jackknife 1 and Jackknife 2 estimated 49 and 52 species respectively, which indicates that between 8 and 16 species were left undetected. According to these values, neither of the orchards sampling effort seems to have been sufficient, however the sampling effort on Louriçal do Campo orchard was more elevated as it presented a higher species diversity and less species were left undetected, which indicates a better characterization of the Auchenorrhyncha community for this orchard. The species richness estimate indicated by the estimator Jackknife 2 is highly similar between both orchards which seems to suggest that both these orchards might have a similar number of species, which is not unlikely given the considerable proximity and similarity between the two peach orchards. Several factors might be responsible for the undetected species, such as an insufficient sampling period and effort as it only covered the months from April to October, and only 6 traps were placed on each orchard and none on the surroundings, as well as the diversity variation through the year and between different years, species phenology and different ecological niches, as the traps were only placed on the peach trees, and also the potentially low abundance of individuals of certain species .

Table 4.3 - Auchenorrhyncha specific richness estimation for both orchards according to Chao2, Jackknife1 and Jackknife2 estimators based on incidence data.

Orchard	SObs	Chao2		Jackknife1		Jackknife2	
		SEst ± SE	PTR(%)	SEst ± SE	PTR(%)	SEst	PTR(%)
LC	36	44.17+5.94	123	48.57+4.67	135	51.69	144
PA	27	67.84+37.26	251	39.57+3.75	146	49.90	184

S_{Obs} – Observed specific richness; S_{Est} – Estimated specific richness; SE – Standard error; PTR – Percentage of True Richness; LC – Louriçal do Campo; PA – Póvoa de Atalaia.

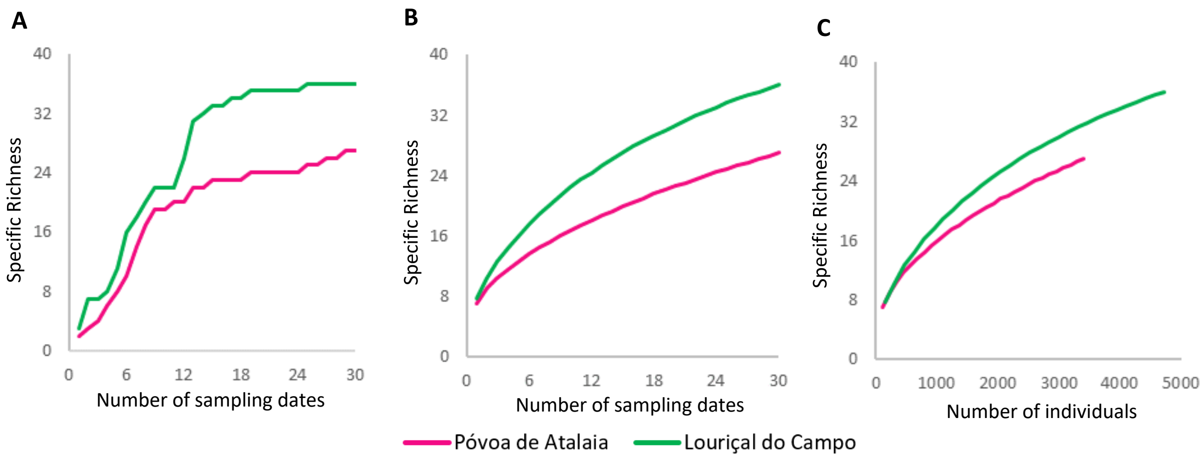

Figure 4.4 – Species accumulation and rarefaction curves for both studied orchards. **A –** Species accumulation curves based on the sampling dates in a chronological order. **B-** Species rarefaction curves based on sampling effort, here considered as the number of sampling dates. **C-** Species rarefaction curves based on the number of individuals.

4.2. Auchenorrhyncha abundance variation throughout the sampling period

During the sampling period a considerable fluctuation was observed in the Auchenorrhyncha abundance as presented in figure 4.5. Both orchards conferred a substantial variation temporally, as in Louriçal do Campo orchard the number of captured individuals variated between 40 and 351, and in Póvoa de Atalaia between 14 and 329. These minimums and maximums occurred on the same dates on both orchards, April 12th and September 20th, respectively.

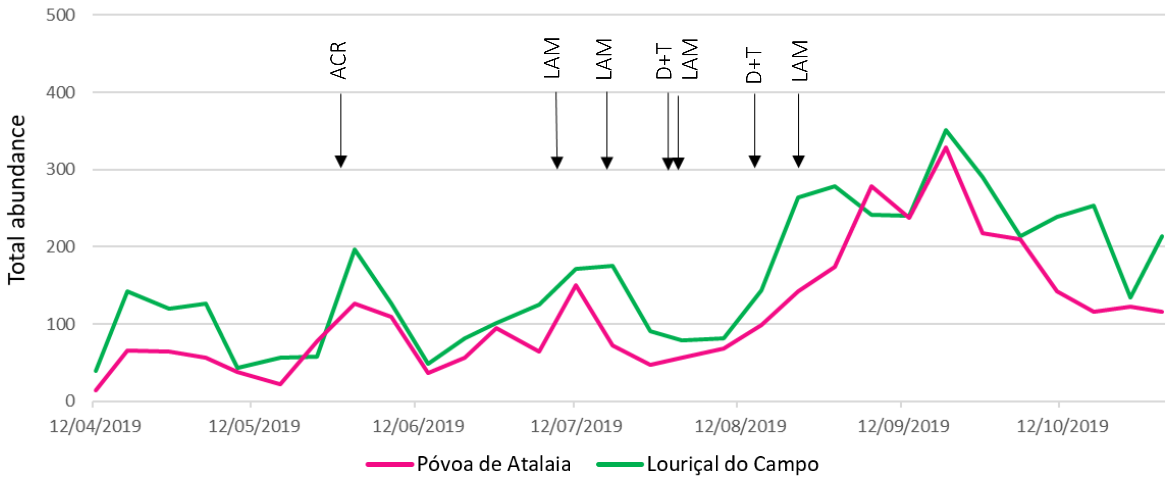

Figure 4.5 – Auchenorrhyncha total abundance through the sampling period for both orchards and phytosanitary treatments applied. ACR: Acrinathrin; D+T: Deltamethrin + Thiacloprid; LAM: Lambda-cyhalothrin

In the first sampling collection, on April 12th, was registered the lowest number of individuals for the entire sampling period on both orchards; one week later on April 19th, a first peak was observed and it was more evident on Louriçal do Campo orchard were 143 individuals were detected as opposed to Póvoa de Atalaia orchard were only 66 individuals were registered. The following weeks denote a slight and slow decrease on the number of captured individuals until a more sudden increase leads to a second peak on both orchards on May 31st, registering 197 on Louriçal do Campo and 127 on Póvoa de Atalaia followed by an abrupt decrease for two consecutive weeks. This sudden decrease partially coincides with the application of Acrinathrin, however this pharmaceutical product was only applied on the cultivars "Royal Glory " and "Rich Lady " both of which are only present on Louriçal do Campo orchard and neither of these cultivars had traps. Acrinathrin is a contact and ingestion acaricide and insecticide, and is a pyrethroid that acts on the nervous system as a sodium channel modulator (Mendes & Cavaco 2016). Considering the decline was observed on both orchards, alternative factors other than the applied acaricide are more likely to have influenced the decrease in the number of captured individuals. Other factors may include insect movement between orchards and the respective surroundings, handling of agricultural machinery, potential natural enemies, among others.

On July 12th, a third peak was reached registering 171 individuals for Louriçal do Campo and 151 for Póvoa de Atalaia. In the following week a sudden decrease was observed in Póvoa de Atalaia orchard as well as a stabilization on Louriçal do Campo, both of which may have been caused by the application of Lambda-cyhalothrin. This phytosanitary product is a contact and ingestion insecticide that acts on the nervous system as a modulator on sodium channels (Mendes & Cavaco 2016). Lambda cyhalothrin is a pyrethroid and in this particular case, was applied targeting the Mediterranean fruit fly, however other insect species can be affected. The insecticide was applied on the cultivars "Catherine", "Honey Royal" and "Sweet dream" in Póvoa de Atalaia, which include two traps, and in Louriçal do Campo, on the cultivars “Catherine”, “Summer Rich”, “Summer Sun” and “Sweet Dream” that includes one trap. This could potentially explain the larger decrease on Póvoa de Atalaia orchard as two cultivars containing traps were affected by the pesticide while on Louriçal do Campo only one trap was on the area affected by the insecticide and therefore denoted a considerably smaller effect. One week later, on July 19th a decrease occurred in the number of captured individuals for Louriçal do Campo orchard which coincides with the second application of Lambda-cyhalothrin, however on this date the insecticide was only applied on cultivar “Jerte” that only exists on Póvoa de Atalaia orchard and therefore other factors are more likely to be responsible for this decrease.

On August 1st both orchards presented a low number of captured individuals which coincided with the application of Deltamethrin and Thiacloprid on cultivar “Extreme 460” in Louriçal do Campo where one trap was located, which could potentially explain the low number of captures observed the coming week. Deltamethrin is a contact and ingestion insecticide, a pyrethroid that acts on the nervous system as a sodium channel modulator while Thiacloprid is a systemic insecticide that acts by contact and ingestion as a neonicotinoid that affects the nervous system as an antagonist of the nicotinic receptor of acetylcholine (Mendes & Cavaco 2016). Both these insecticides were also targeting the Mediterranean fruit fly. Lambda-cyhalothrin was also applied in “Honey Cascade” on Louriçal do Campo and “Andross” cultivar on both orchards, which contain traps, however the following weeks demonstrate a considerable increase on the number of captures which could indicate these insecticides only acted for a short period of time. On August 15th, Deltamethrin and Thiacloprid were applied on cultivar “Poblet” on both orchards but no effect is visible as the captures continue to increase the coming weeks. On August 24th, Lambda-cyhalothrin was applied on “Extreme 486”, “Tardibelle ” and “Poblet”, in Louriçal do Campo, where two traps were present therefore potentially explaining the slight reduction on the increase of captures, and also on cultivar “Poblet” in Póvoa de Atalaia were one trap was present, however no effect was observed. After this date no other insecticides was applied considering most of the harvesting season ended shortly after. The number of captures continued to increase until reaching the maximum on September 20th, as 351 individuals were captured on Louriçal do Campo and 329 on Póvoa de Atalaia. The following weeks denoted an overall decrease until October 31st when the sampling period ended. Despite the overall decrease, the number of captured individuals was still higher than 100 on both orchards, and on the last capture in Louriçal do Campo an increase was registered reaching 214 individuals. Considering the sampling ended on October 31st, the variation of the Auchenorrhyncha community on both orchards remains incomplete.

On another approach, green leafhoppers, in particular, *Asymmetrasca decedens* and *Empoasca solani*, were also analyzed as these were the most common during the sampling period. The total species abundance resembles the one of *E.solani* during the first weeks, which is then gradually replaced by *A. decedens* (Fig 4.5). After July 5th, the total abundance of the Auchenorrhyncha community almost completely matches *A. decedens* abundance as these species is by far the most dominant and constant one, as referred previously. This difference/variation between *E. solani* and *A.decedens* could be related to different ecological requirements for each species, which may occur to minimize competition between them.

E. solani average abundancy curve (Fig.4.6B) registered a maximum on April 18th after which a relatively slow and steady decrease occurred until reaching a minimum on May 31st. This minimum coincides with the application of Acrinathrin, however during the weeks following the application an increase is observed on the number of captures. This product was not applied on any cultivar with traps on either orchards, which likely indicates other factors were responsible for the variation observed on *E. solani* captures. A second and smaller peak occurred on June 27th in Póvoa de Atalaia and July 12th in Louriçal do Campo, after which the number of captures decreased and remained low or null until the

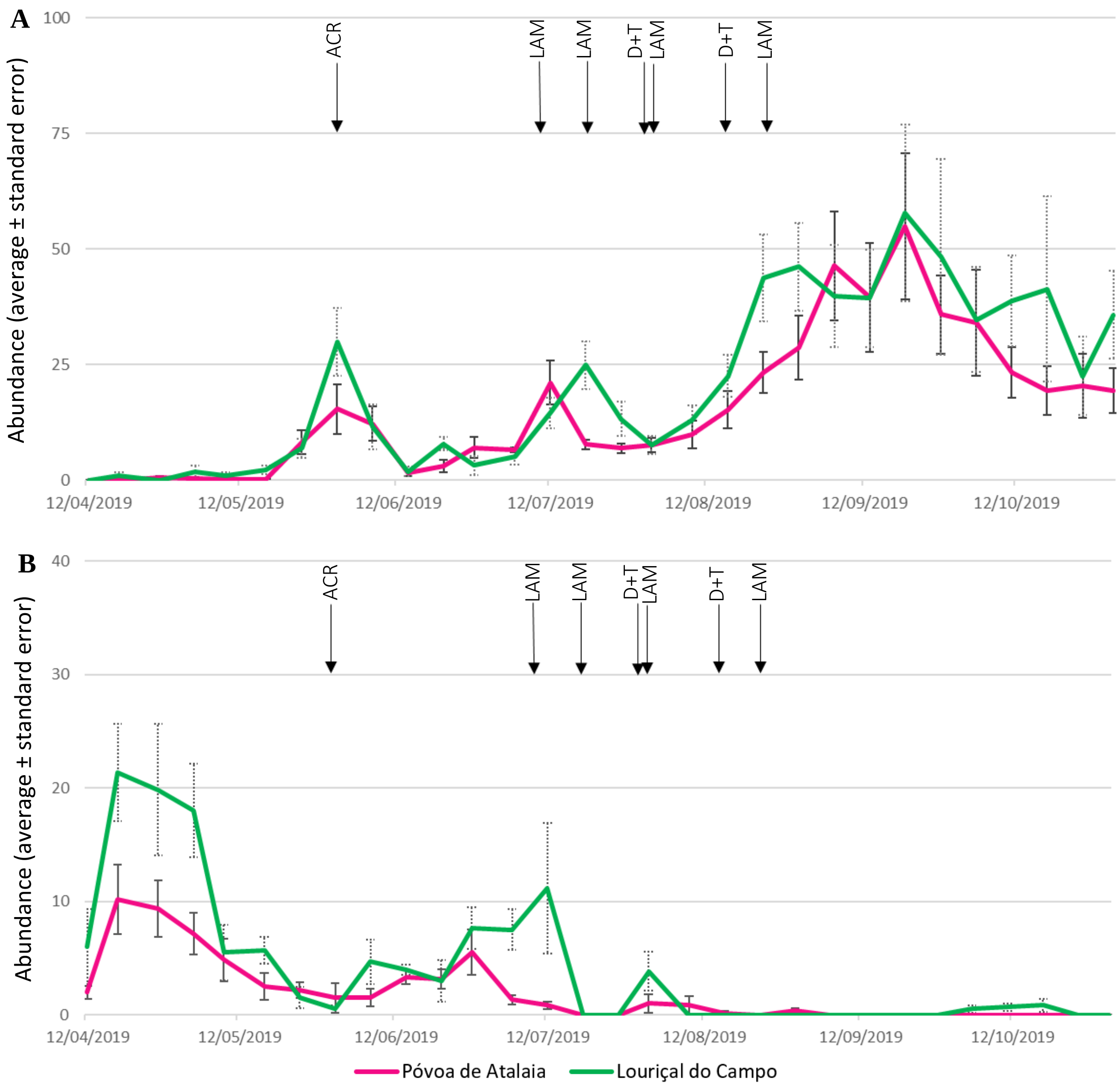

Figure 4.6 – *Asymmetrasca decedens* (Paoli) (A) and *Empoasca solani (*Curtis) (B) average abundance through the sampling period for both orchards and phytosanitary treatments applied. ACR: Acrinathrin; D+T: Deltamethrin + Thiacloprid; LAM: Lambda-cyhalothrin

end of the sampling period. The decrease observed on Louriçal do Campo orchard coincides with the application of Lambda-cyhalothrin that was applied on three cultivars, one of which included a trap, as referred previously, and therefore could possibly explain the registered decrease in the number of captures.

The average abundancy curve for *A. decedens* demonstrates a very low number of captures until May 17th when these started to increase reaching a peak on May 31st (Fig.4.6A). The following decrease could be explained by the application of Lambda-cyhalothrin as stated previously. Considering that during the remaining of the sampling period *A. decedens* average abundance curve highly resembles the total Auchenorrhyncha abundance curve (Fig.4.6A and Fig.4.5), the factors influencing the variation and the effects or lack of them resulting from the application of insecticides were the same as referred previously for the total Auchenorrhyncha community. As the sampling collection ended on October 31st, it was not possible to determine when *A. decedens* populations would leave/disappear from either orchards. Some studies (Alvarado *et al*. 1994, Jacas *et al*. 1997) indicate this species migrates to preferential host plants such as peach, among others, when conditions are more favourable, which explains the increase of captures at the end of spring/beginning of summer. When conditions become more unfavourable, these move to appropriate retreats such as deciduous/evergreens shrubs or trees which explains the overall decrease observed on *A. decedens* captures during the month of October. As no traps were placed on the surroundings of either orchard, it was not possible to collect further information on the movement and activity of *A. decedens* or the remaining Auchenorrhyncha community. Seeing that more orchards, and different types of vegetation involve the studied orchards, if traps were placed in more areas including these surroundings, data regarding species migration between these areas could potentially lead to a better understanding of the Auchenorrhyncha community's dynamics.

Green pruning, as referred previously, is a technique adopted by the integrated production of peaches for the canopy volume condition as it allows a better aeration and a bigger absorption of solar radiation and consequently leads to a more intense color and improves overall quality of fruits. This technique was used on 3 cultivars on each of the studied orchards as presented in table 3.2. Figure 4.7 represents the variation of the total abundance of the Auchenorrhyncha community during the sampling period and the application of the cultural approach known as green pruning. It was hypothesized that this cultural approach could possibly have an influence on the Auchenorrhyncha population and therefore a potential use as a cultural method for the control of this suborder. By analyzing figure 4.7, the carrying out of the green pruning did not seem to have any impact on the variation of the Auchenorrhyncha abundance as none of the executed pruning seemed to cause any change alteration in the trajectory on the number of captures. As observed in figure 4.7, when the pruning was executed on Póvoa de Atalaia orchard on June 21st, 24th and 25th, the number of captures was increasing and this tendency remained after the pruning until June 27th where a small decrease occurred during the succeeding week, followed by a new increase on captures the week after. On Louriçal do Campo orchard, the pruning was executed on June 21st, 27th and July 1st, where the number of captures was increasing every week, and continued increasing on the weeks following the pruning, as no visible changes seemed to occur on the captures. From July 28th to July 31st the pruning was carried out on the entire cultivar, however the number of captures remained almost the same the following week, after which started to increase. The cultural approach did not seem to cause an impact on the variation of the Auchenorrhyncha community, however few traps were used to monitor the effect between the areas where the pruning occurred and the areas where no pruning was executed, as well as other factors potential influence on the number of captures.

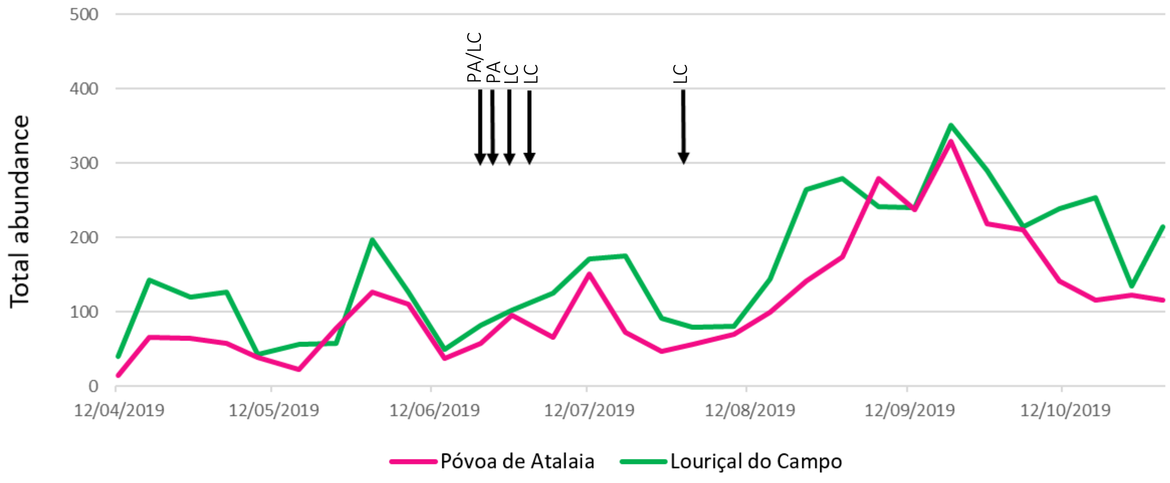

Figure 4.7 - Auchenorrhyncha total abundance through the sampling period for both orchards and the respective execution of the cultural approach green pruning. PA/LC: Green pruning carried out on both orchards; PA: Green pruning carried out on Póvoa de Atalaia orchard; LC: Green pruning carried out on Louriçal do Campo orchard.

4.3. Relation between climatic variables and green leafhopper populations

The occurrence periods for *Asymmetrasca decedens* and *Empoasca solani* were different, as *E. solani* exhibited shorter and earlier appearance, and *A. decedens* appeared latter and with a higher abundance. Considering the life cycle of insects is affected by climatic variables, an attempt was made to determine how some climatic variables influenced both these species populations on Póvoa de Atalaia and Louriçal do Campo orchards (Fig.4.8).

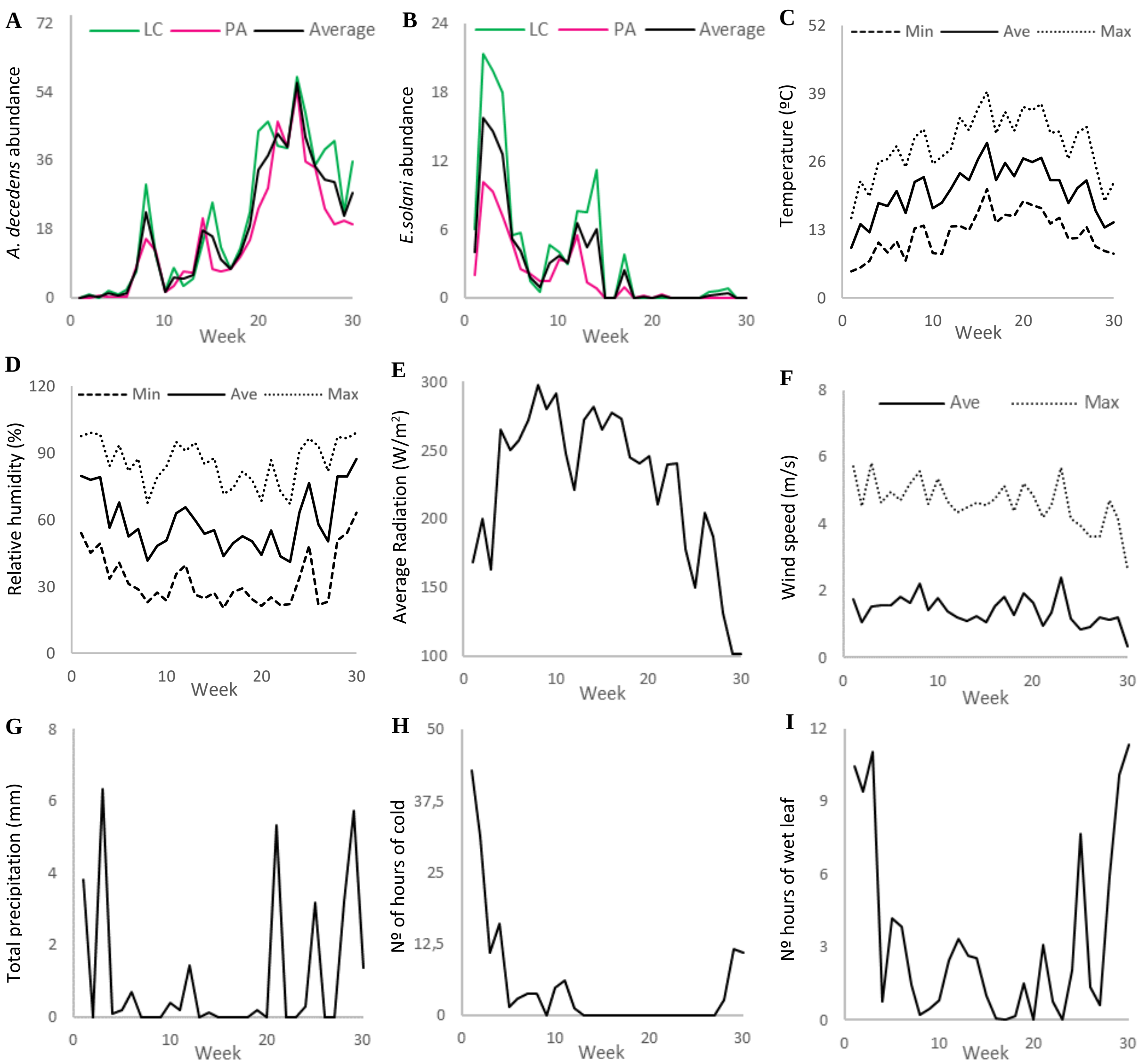

Figure 4.8 – Total abundance of *Asymmetrasca decedens* (Paoli) (A), *Empoasca decedens* (Curtis) (B) and climatic factors: temperature (C), relative humidity (D), average radiation (E), wind velocity (F), total precipitation (G), number of hours of cold (H) and number of hours wet leaf (I) for both the orchards Louriçal do Campo (LC) and Póvoa de Atalaia (PA).

On a first analysis, *A decedens* and *E. solani* seem to present an almost opposite distribution as the increase of the first seems to follow the decrease of the second as observable in figure 4.8. As referred previously this may occur in order to diminish the competition between the two species. The different distribution of both species may indicate that either different climatic variables influence these species or the same climatic variables may influence both species in different ways.

The obtained values for the cross correlations (Appendix– Table B.2 and B.3) between *A. decedens* and *E. solani* populations and the corresponding *lags* are presented in figures 4.9 and 4.10. The lags corresponding to the correlation coefficients peaks are presented on table 4.4.

Most of the tested climatic variables displayed correlation coefficients that differed significantly from zero ($p \leq 0.05$) for *A. decedens*, with the exception of the average wind speed and total precipitation. Some of the remaining variables only presented a correlation coefficient significantly different from zero for one of the orchards which was the case of the average radiation for Louriçal do Campo, and other for the average of both orchards such as maximum wind speed, minimum and average relative humidity. The climatic variables that present the strongest significant correlations for *A. decedens* abundance were the minimum, average and maximum temperature, with a particular highlight for the minimum temperature which seems to be the most influential variable. Other studies had previously pointed out that higher temperatures favored the development of this species, as the increase of temperature seems to lead to an increase in the abundance of this leafhopper. A prior study (Torres *et al*. 2002) demonstrated that *A. decedens* would complete its development and life cycle if temperatures ranged between 12 and 27ºC. This study also concluded that within this range of temperatures, the higher the temperature, the faster the development occurred for this leafhopper, ranging from 56 days at 12ºC to 11 days at 27ºC. The *lags* corresponding to the peaks of the correlation coefficient for the average abundance of *A. decedens* were 2 weeks for the number of hours of cold (<7ºC), 4 weeks for the minimum temperature and the number of hours of wet leaf, 4 and 5 weeks for Póvoa de Atalaia and the average of both orchards, and for Louriçal do Campo respectively, for the maximum relative humidity and 6 weeks for the average and maximum temperature. The maximum relative humidity and the number of hours of cold present a negative correlation with the abundance of *A. decedens*, which indicates the increase on either the relative humidity or the number of hours of cold negatively impacts the abundance of this leafhopper, likely leading to a decrease on the species populational density.

Table 4.4 - *Lags* corresponding to the maximum values of Pearson's correlation coefficients that differ significantly from zero ($p \leq 0.05$) regarding the average abundance of the dominant species of green leafhoppers (on the orchards LC - Louriçal do Campo; PA - Póvoa de Atalaia; and the average of both orchards) and the studied climatic variables with *lags* between 0 and 7 weeks previous (hence the *lag*'s negative value) to the sampling.

Species	***Asymmetrasca decedens***						***Empoasca solani***					
Orchards	LC		PA		Both		LC		PA		Both	
Variable	lag	ρ	lag	ρ	lag	ρ	lag	ρ	lag	P	lag	ρ
Minimum temperature	-4	0.627	-4	0.612	-4	0.637	-1	-0.568	-1	-0.673	-1	-0.615
Average temperature	-6	0.560	-6	0.544	-6	0.568	-1	-0.575	-1	-0.657	-1	-0.614
Maximum temperature	-6	0.543	-6	0.511	-6	0.543	-1	-0.567	-1	-0.650	-1	-0.606
Minimum relative humidity	-	-	-	-	-4	-0.367	-1	0.425	-1	0,441	-1	0.459
Average relative humidity	-	-	-	-	-4	-0.377	-1	0.488	-1	0.429	-1	0.481
Maximum relative humidity	-5	-0.407	-4	-0.453	-4	-0.438	-1	0.465	-1	0.393	-1	0.455
Average radiation	0	-0.383	-	-	-	-	-	-	-	-	-	-
Average wind speed	-	-	-	-	-	-	-	-	-	-	-	-
Maximum wind speed	0	-0.422	-	-	0	-0.402	-	-	-	-	-	-
Total precipitation	-	-	-	-	-	-	-	-	-	-	-	-
Number of hours of cold	-2	-0.472	-2	-0.437	-2	-0.469	-1	0.786	-1	0.858	-1	0.826
Number of hours of wet leaf	-4	-0.406	-4	-0.372	-4	-0.401	-1	0.570	-1	0.507	-1	0.564

Several climatic variables also exhibited correlation coefficients that differ significantly from zero ($p \leq 0.05$) for *E. solani*. This was the case for minimum, average and maximum temperature, minimum, average and maximum relative humidity, number of hours of cold (<7ºC), number of hours of wet leaf, and maximum wind speed although the later was only significantly different from zero on Louriçal do Campo orchard. The average radiation, average wind speed and total precipitation did not seem to significantly impact the abundance of *E. solani*. Unlike *A. decedens* that increased in abundance with the increase of temperature, *E. solani* presents an opposite relation as with the increase of temperature, a decrease occurs on the species abundance hence the negative values for the correlation coefficients presented for these variables. The number of hours of cold (<7ºC) was the climatic variable that displayed the most elevated and significant correlation coefficient, indicating that the larger number of hours in which the temperatures remained below 7ºC, the greater the abundance of *E. solani*. Minimum, average and maximum relative humidity as well as the number of hours of wet leaf present a positive correlation with the abundance of *E. solani*, indicating these increase on any of these variables leads to an increase on the species abundance as opposed to *A. decedens* where these variables negatively influenced the species abundance. For *E. solani*, the most significant *lags* corresponding to the peaks obtained for the correlation values were 1 week for all the studied variables that significantly impacted the species abundance. This suggests the climatic variables have the greatest impact in *E. solani* abundance the previous week of a certain sampling date, this may however be result from the more elevated numbers of *E. solani* on the first weeks of the sampling period.

Other factors besides climatic variables, such as the cultivars present on the orchards, the existence of water lines or small water reservoirs, and the surrounding orchards and vegetation are also likely to influence the abundance and distribution of species of the Auchenorrhyncha suborder such as *A. decedens* and *E. solani*. Even though no traps were placed on the orchards surroundings, it is important to analyze whether the variation of captures spatially and temporally on both orchards for each of the traps influences the species abundance.

As referred previously, *E. solani* presented an earlier distribution and was then gradually replaced by *A. decedens* on both orchards as it is also observable in figures 4.11 and 4.12. The greatest number of captures in Louriçal do Campo occurred in trap 1, as this adhesive plate captured almost twice as much as any other trap on the orchard (Fig. 4.11). The higher abundance of both the dominant species on this trap could potentially have been influenced by either the type or height of the vegetation close to the area where the trap was placed. On Póvoa de Atalaia orchard, the trap with the most captures was number 5 (Fig. 4.12), however several small open water reservoirs are scattered across the orchard and not much vegetation seems to be close to any of the traps which complicates the analyses. A greater number of traps on the orchards as well as on the orchards surroundings including the neighboring vegetation would allow a better understanding of the species distribution as some species such as *A. decedens* overwinter in other host plants and when conditions are more favourable colonize the orchards (Alvarado *et al*. 1994, Coutinho *et al*. 2015, Jacas *et al.* 1997).

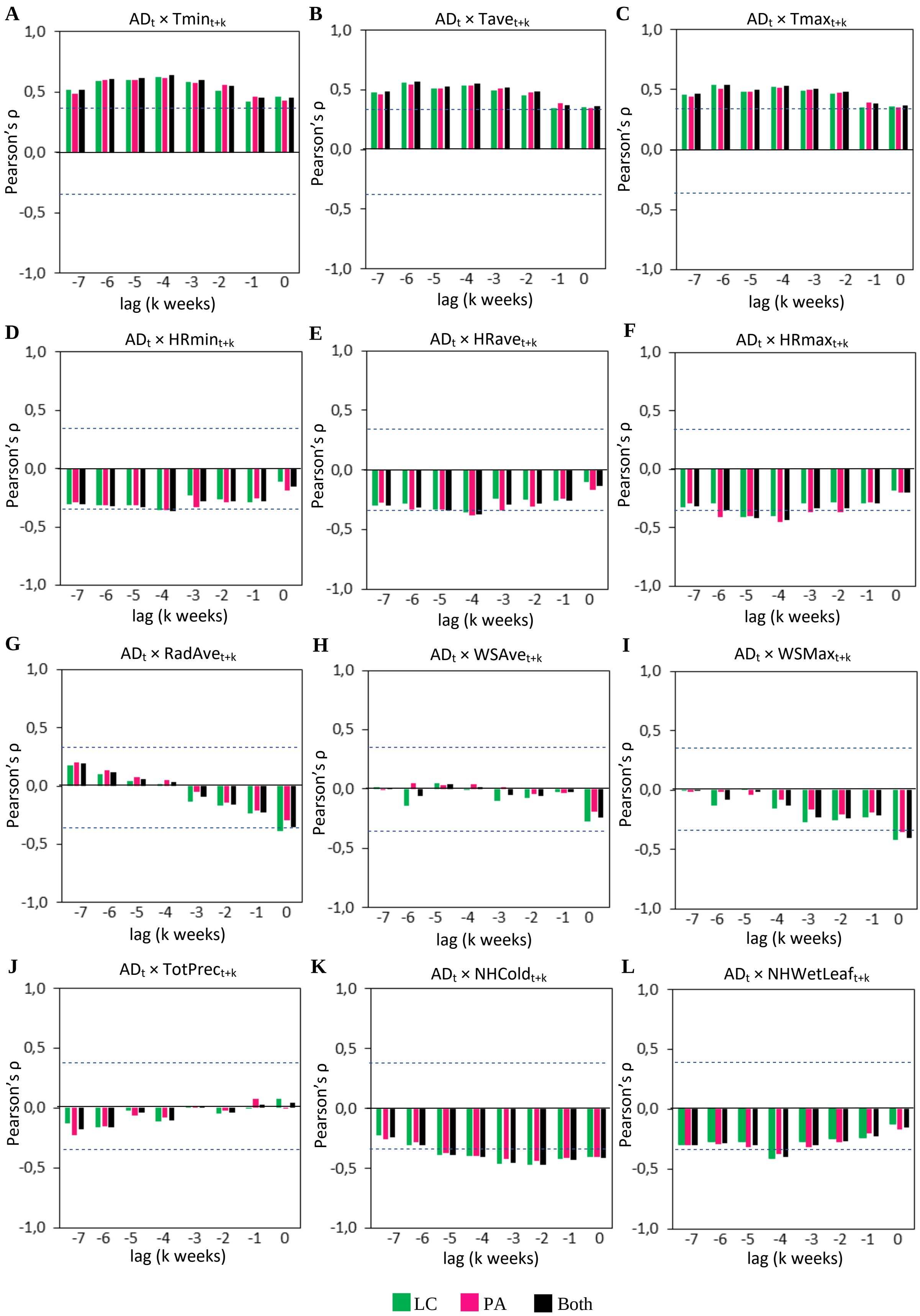

Figure 4.9 - Correlation graphics between *Asymmetrasca decedens* (Paoli) population and minimum, average and maximum temperature (A-C), minimum, average and maximum relative humidity (D-F), average radiation (G), average and maximum wind speed (H e I), o total precipitation (J), number of hours of cold (<7º)(K) number of hours of wet leaf (L) for Louriçal do Campo (LC), Póvoa de Atalaia (PA) and the average of both orchards (Both).

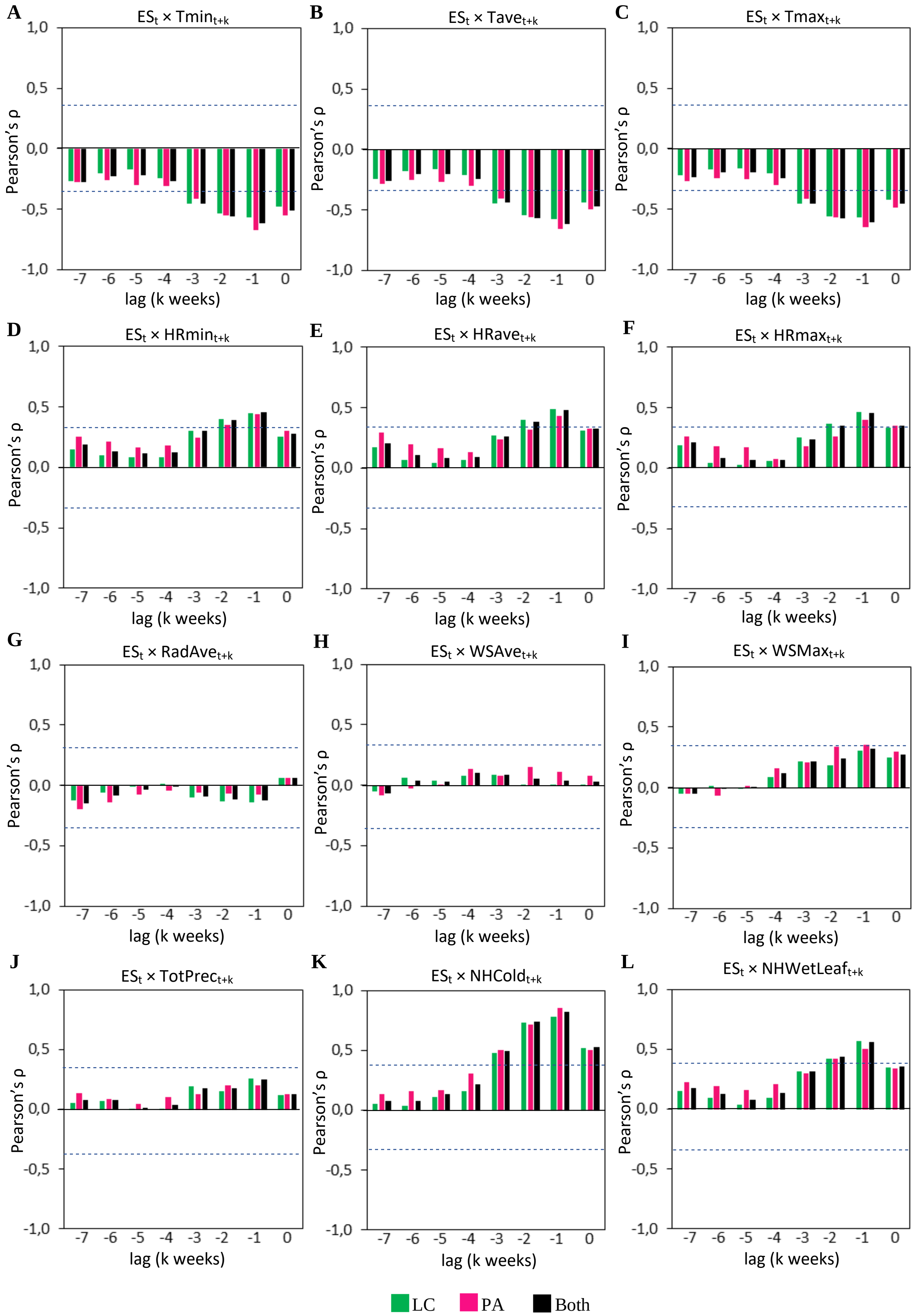

Figure 4.10 - Correlation graphics between *Empoasca solani* (Curtis) population and minimum, average and maximum temperature (A-C), minimum, average and maximum relative humidity (D-F), average radiation (G), average and maximum wind speed (H e I), o total precipitation (J), number of hours of cold (<7º)(K) number of hours of wet leaf (L) for Louriçal do Campo (LC), Póvoa de Atalaia (PA) and the average of both orchards (Both).

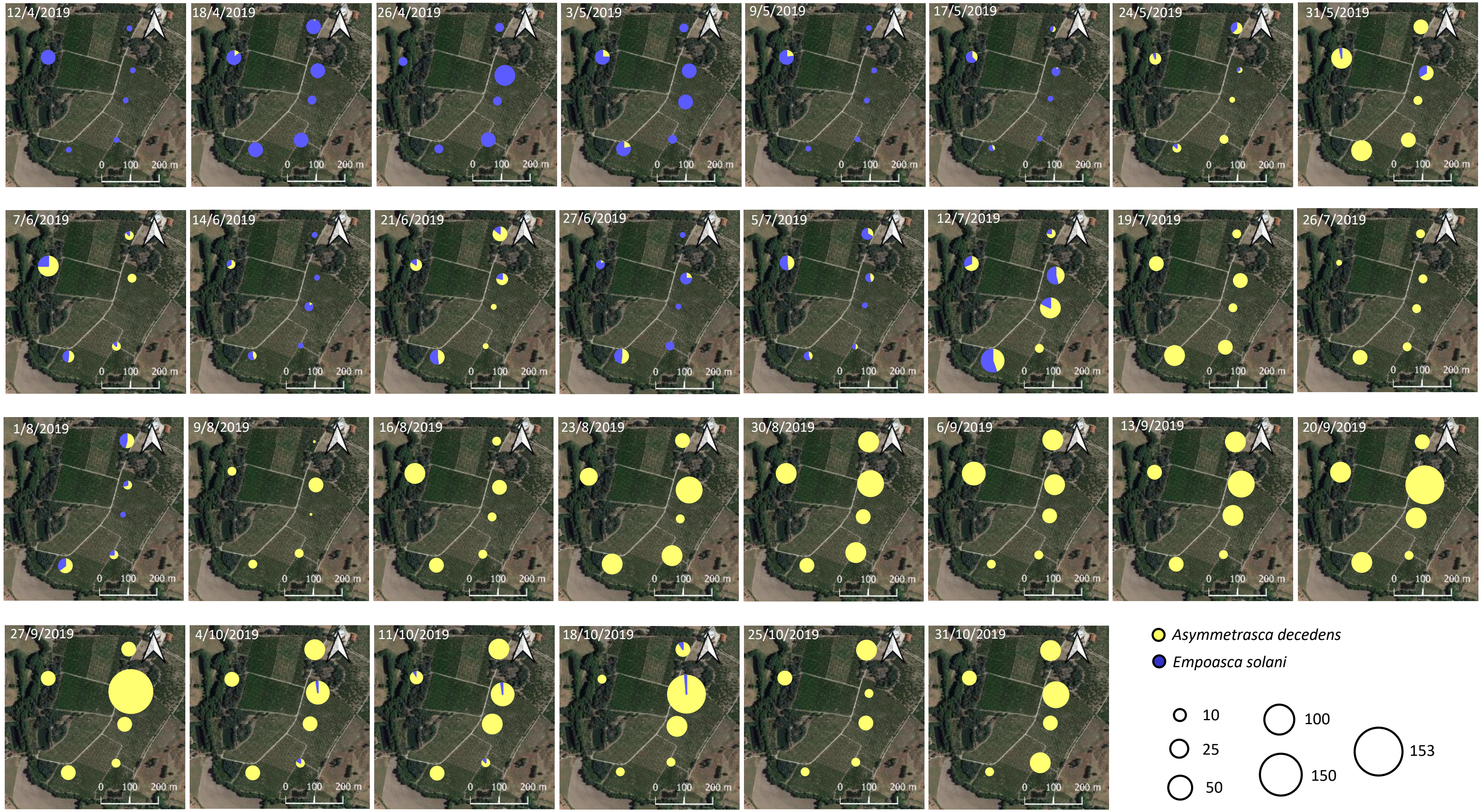

Figure 4.11 – Captures of *Asymmetrasca decedens* (Paoli) and *Empoasca solani* (Curtis) on Louriçal do Campo orchard throughout the sampling period.

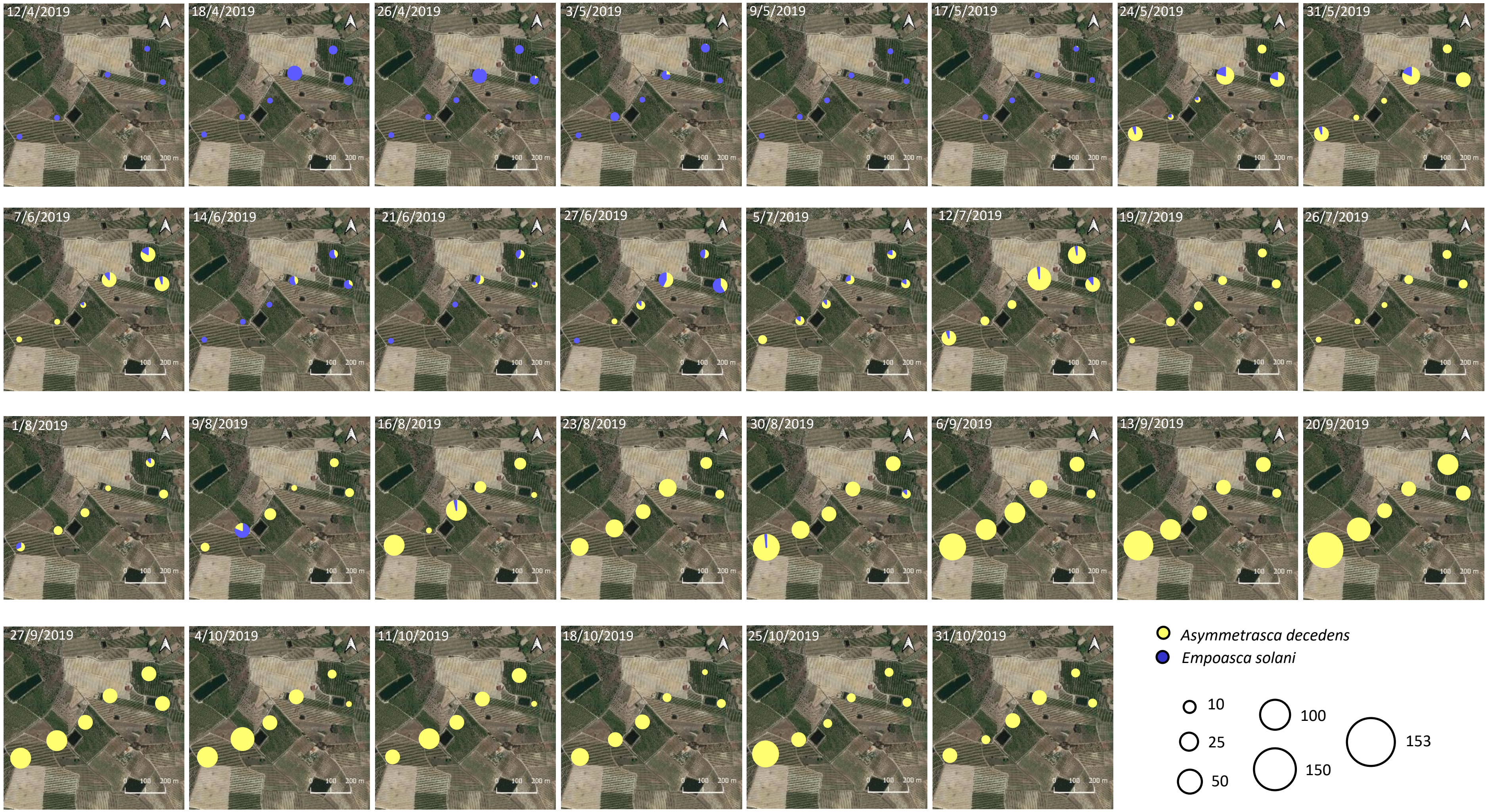

Figure 4.12 –Captures of *Asymmetrasca decedens* (Paoli) and *Empoasca solani* (Curtis) on Póvoa de Atalaia orchard throughout the sampling period.

4.4 Data comparison with previous year

As referred before, this master's dissertation is part of the PrunusFito project which covers several years, and during the previous year (2018) a similar data collection occurred which in turn also lead to a master's dissertation (Guerreiro 2020). As both master's dissertations aimed to monitor the Auchenorrhyncha community on the peach orchards Póvoa de Atalaia and Louriçal do Campo, and the same variables were studied, a comparison between both years may lead to a better and more complete understanding of the information obtained and consequently a greater insight on the Auchenorrhyncha community and its respective variation, as well as on potential management measures and improvements for further studies in order to obtain a most accurate representation of this community. To note that in Guerreiro (2020) a shorter sampling period occurred (April 11th to September 7th) and only four yellow sticky traps were placed on each orchard on a weekly basis.

Comparing the Auchenorrhyncha community of both years, 8140 individuals were captured during 2019 while 4783 were collected in 2018. This difference is easily explained by the larger sampling period and greater number of traps per orchard as stated previously. All the species of the Auchenorrhyncha community detected on the peach orchards in either of the studied years are presented in Table 4.5. Several species were detected on only one of the years as observable in Table 4.5 which could be explained by the small number of specimens captured for each of these species, therefore likely indicating a low abundance, making them somewhat likely to go undetected on other years. Overall, 52 species were detected on both years, 39 during 2019 and 33 in 2018. The higher number of species in 2019 is possibly explained by the larger sampling period and number of traps. Unlike the first year, where Louriçal do Campo presented a higher number of captured individuals despite the lower number of species detected, during the second year this orchard presented both a larger abundance of individuals as well as a higher diversity of species.

Overall, on both years, the Cicadellidae family was the most representative one, making up 98.95% of all the collected specimens on the first year and 99.77% on the second. The same was verified for the most predominant subfamily on both years, Typhlocybinae, representing 89.36% and 95.84% respectively. The most dominant species were also the same on both years, *Asymmetrasca decedens* with 54.67% and 79.64% in 2018 and 2019 respectively, and *Empoasca solani* with 27.58% and 13.45%. The higher proportion of *A. decedens* relative to *E. solani* in 2019 compared with 2018, is mainly explained due to the larger sampling period which allowed the capture of this species individuals for a greater amount of time as opposed to the previous year where the sampling ended before *A. decedens* reached its peak. An additional explanation for the greater abundance of *A. decedens* in 2019 could be the more elevated minimum temperature, as well as the lower maximum and average relative humidity during the same sampling period. All these variables presented a significant correlation with the abundance of *A. decedens*, particularly the minimum temperature which gave the strongest correlation for this species. If the average minimum temperature is compared for the same sampling period on both years, a difference of over 3ºC is observable as the first year had an average minimum temperature of 9.72ºC and the second year an average minimum of 12.93ºC. The average minimum temperature for the second year is also above the limit temperature of 12ºC after which the development of *A. decedens* can occur as demonstrated in other studies (Torres *et al.* 2002). The decrease in the proportion of *E. solani* during the second year could also be explained by the slight decrease in total number of hours of cold (<7ºC), which was the variable with the strongest correlation for the species, and also the increase in the minimum temperature which had a negative correlation with *E.solani* abundance as opposed to *A. decedens.*

Table 4.5 – Auchenorrhyncha taxa detected on the orchards Póvoa de Atalaia (PA) and/or Louriçal do Campo (LC) on the year 2018 and/or 2019.

Genus/Species	PA 2018	LC 2018	PA 2019	LC 2019
Agallia consobrina Curtis	V	V	V	V
Alebra sp.			V	V
Alnetoidia alneti (Dahlbom)		V		
Anaceratagallia laevis (Ribaut)	V	V	V	V
Aphrodes makarovi Zachvatkin	V	V	V	V
Arboridia sp.			V	V
Arboridia parvula (Boheman)	V			
Asymmetrasca decedens (Paoli)	V	V	V	V
Austroagallia sinuata (Mulsant & Rey)			V	V
Cercopis intermedia Kirschbaum		V		
Cicadella viridis (Linnaeus)		V		
Deltocephalinae sp. A	V		V	V
Deltocephalinae sp. B				V
Dictyophara europaea (Linnaeus)			V	
Empoasca decipiens Paoli	V	V	V	V
Empoasca solani (Curtis)	V	V	V	V
Errastunus ocellaris (Fallén)	V			
Eupelix cuspidata (Fabricius)				V
Eupteryx sp.			V	V
Eupteryx filicum (Newman)			V	
Euscelidius variegatus (Kirschbaum)	V	V	V	V
Euscelis sp.				V
Fieberiella florii (Stal)				V
Fruticidia bisignata (Mulsant & Rey)	V	V		V
Fruticidia sanguinosa (Rey)				V
Hautpidia maroccana (Melichar)	V	V		
Hyalesthes obsoletus Signoret	V			
Jacobiasca lybica (Bergevin & Zanon)	V			
Laodelphax striatella (Fallén)	V	V	V	V
Lindbergina aurovittata (Douglas)	V			
Macropsis cerea Germar			V	V
Megophthalmus scrabipennis Edwards	V	V		V
Metadelphax propinqua (Fieber)	V	V		V
Neoaliturus fenestratus (Herrich-Shaffer)	V	V		V
Neophilaenus campestris (Fallén)			V	V
Philaenus spumarius (Linnaeus)	V	V	V	V
Phlepsius intricatus (Herrich – Schäffer)	-	-	-	-
Platymetopius guttatus Fieber				V
Psammotettix sp. A	V		V	V
Psammotettix sp. B			V	V
Psammotettix sp. C				V
Ribautiana cruciata (Ribaut)	V	V	V	V
Ribautiana debilis (Douglas)	V		V	V
Ribautiana sp.	V		V	
Sardius argus (Marshall)	V	V		
Sophonia orientalis (Matsumura)	-	-	-	-
Typhlocybinae sp. A	V			V
Zygina lunaris (Mulsant & Rey)	V	V	V	V
Zygina nivea (Mulsant & Rey)	V	V	V	V
Zygina ordinaria (Ribaut)	V	V	V	V
Zyginidia scutellaris (Herrich-Shaffer)	V	V	V	V

In 2018, a potential vector and two confirmed vectors of *Xylella fastidiosa* were detected on the orchards, *Cercopis intermedia* Kirschbaum, *Philaenus spumarius* and *Cicadella viridis* (Linnaeus), even though the later was only recently confirmed to carry and transmit the pathogen with a low efficiency (Bodino *et al.* 2019). The following year, two confirmed vectors were also detected on the orchards, *Philaenus spumarius* and *Neophilaenus campestris*. Considering the vector species for *X. fastidiosa* already detected on the orchards, it is certainly important to continue monitoring these species as well as the potential expansion of the bacterium to other areas as it could cause significant damage to the peach orchards in the region as it is responsible for Peach Phony disease, which could lead to considerable decreases in production.

Taking into account the specific richness estimated for Louriçal do Campo orchard in 2018 indicated that between 26 and 29 species were likely to exist on the orchard, and the following year 36 species were detected on the same orchard and the same estimators indicated that between 44 and 52 species were likely to exist on the orchard, it could lead to the likelihood that the sampling in 2018 was not sufficient to correctly estimate the number of species. The used estimators become more accurate with the increase in the number of samples, and considering that in 2019, 30 samples (for the 30 weeks of the sampling period) were obtained in comparison with the 22 from the previous year, it is probable that the estimates obtained with the data from 2019 are more precise and accurate and therefore a closer representation of the Auchenorrhyncha community. For Póvoa de Atalaia, a similar number of species was obtained on both years and a similar estimate was also obtained for both as the estimators indicated between 43 and 55 species for the first year and between 40 and 50 for the second. Despite the similar estimates obtained for this orchard, neither of the orchards sampling effort seems to have been sufficient as indicated by the rarefaction curves obtained for the later year.

The temporal distribution of the two dominant species of green leafhoppers observed on the orchards was similar on both years, as *E. solani* presented and earlier distribution and was then gradually replaced by *A. decedens* that reached its peak in September 20th on the second year. As the sampling period of the previous year ended on September 14th, it was not possible to determine when the peak would reach its maximum, however this species abundance was increasing considerably in the final weeks of the sampling period exhibiting a similar increase as observed in the following year. The data collection in 2019 led to a more complete insight into these species and remaining Auchenorrhyncha community distribution and variation, however a longer sampling period would still be recommended in order to determine when these species would disappear from the orchards, which was not possible on either of the studied sampling periods for both years. The overall Auchenorrhyncha community also presented an identical distribution on both years, with slight differences that may have occurred from a considerable number of variables, such as changes that occurred on the studied climatic variables between both years, different dates of insecticide applications, among others.

Regarding the insecticides applied on the orchards on either of the two studied years, three different types were used: pyrethroids, neonicotinoids and organophosphates. Pyrethroids affect primarily sodium, chloride and calcium channels, while neonicotinoids affect insects central nervous system as these bind to the nicotinic acetylcholine receptors, and organophosphates lead to the inhibition of acetylcholinesterase (Ensley 2018, Gupta *et al.* 2017, Hladik *et al*. 2018, Matsuda *et al.* 2020). It was not possible to determine the effect of the organophosphates applied on the orchards as these were not utilized on any of the areas with traps and therefore did not allow to determine its effect. The same happened for some of the applied neonicotinoids, which also did not permit to determine the applications outcome, while for others no considerable effect was observable on the Auchenorrhyncha community abundance. Some of the pyrethroid applications seem to suggest that there could be an effect resulting from these substances on the Auchenorrhyncha abundance, however this was not consistent in all the applications, and therefore other factors could be involved on the variations observed. It was not possible to confirm that any of the applied insecticides considerably affected the abundance of the captured Auchenorrhyncha. A greater number of traps on the orchards would allow a better comprehension of the potential effects of these insecticides.

No effect seemed to occur on the Auchenorrhyncha community abundance as a result of the cultural approach know as green pruning on either of the studied years. During the first year only one of the traps was located on the area affected by the executed pruning on August 14th and a lesser number of trees was pruned, however an increase was registered in the number of captures during the succeeding weeks. During the following year, green pruning was executed earlier, in June and July, on more traps as well as more trees were pruned around the traps, and once again, the following weeks did not lead to a decrease in the number of captures, and most of them even led to a slight increase. Considering the data obtained so far, this cultural approach did not seem to have any impact on the Auchenorrhyncha community abundance. Taking into account the pruning did not present any effect on the abundance of the target species and it partly debilitates the affected trees it would be recommended that the pruning occurred earlier, for example in June and July as opposed to August, as the number of individuals of the Auchenorrhyncha community increases considerably in August and September and these also weaken the trees, and therefore it would be suggested that this strategy should not coincide with the period of higher abundance of the Auchenorrhyncha community in order to decrease the impact on the peach orchards.

Some of the studied climatic variables did not present significant correlations for one of the years despite presenting for the other, especially for *E.solani*, where most of the climatic variables that presented a significant correlation in 2018 were almost the opposite to the variables that presents a significant correlation the following year. This could be partly explained by the shorter sampling period in 2018 because some of the variables did not present as much variation within the studied period, for example the overall minimum, average and maximum temperature only increased during the studied period of that year, while on the succeeding year, a similar increase was observe but due to the larger sampling period, all these variables also showed a decrease and therefore this variation could differently influence the correlation obtained for the same species. Another possible explanation is that some of the correlations observed do not necessarily mean causality, as it could be masked by other variables not considered in the study. Despite some differences, the minimum, average and maximum temperature showed a strong significant correlation with *A. decedens* on both years, which had already been shown in previous studies. Considering the data obtained in 2019, as it presents a more complete and likely more accurate representation, both species seem to be influenced by the same variables in opposing ways, as the variables that present a positive correlation for one species present a negative for the other, which explains *A. decedens* and *E.solani* different distribution.

Taking into account the placement of traps on the orchards, not all are comparable as traps 5 and 6 of both orchards did not exist the previous year. This does not allow an accurate comparison between both years as in the case of Póvoa de Atalaia orchard, trap 5 had the most captures on the second year however this trap was not placed the previous year which made it unattainable to establish a pattern. In Louriçal do Campo, on the first year traps 2 and 4 had the higher number of captures which was not verified the following year, as trap 1 had significantly more captures. This variation might be the result of several different factors not considered in the study including the potential existence of enemy species, changes in surrounding vegetation as well as surrounding cultivars, among others.

4.5 Management measures

Considering the potential impact caused by some species of the Auchenorrhyncha community present on the studied orchards, such as the damage caused by *A. decedens* feeding process, the possible transmission of phytoplasmas by this species, as well as the presence of vector species for *Xylella fastidiosa* it is highly important to analyze the data obtained so far in order to determine what information would be relevant to determine the most appropriate and effective management measures for these pests and what information is still necessary to acquire in order to complete the knowledge obtained so far.

Even though the information obtained regarding *A. decedens* abundance on the orchards allowed to determine the peak of this species abundance on both orchards, the sampling period still was not sufficient to determine when the species would leave the orchards. As stated previously, several studies demonstrated that *A. decedens* migrates to evergreen shrubs/trees when the conditions become more unfavourable, and despite the overall decrease in this species abundance observed during the month of October, a considerable number of individuals were still being captured when the sampling ended and therefore it was not possible to determine the period in which this phenomenon would occur. To obtain this information it would be recommended that the sampling period should be extended, and further traps should be placed on the orchards surroundings in order to better detect the species migration, as these leave the orchards. Coutinho *et al.* (2015) referred this species presence on common alder (*Alnus glutinosa* Gaertner) which existed on the surrounding of stone fruit orchards in Beira Interior region. The sampling on the surrounding regions of the orchards could be helpful to determine this species winter refuges as well as the orchards colonization routes during spring which would be useful to determine earlier management measures to be applied to this species. To better determine the orchards colonization in spring and to follow a more preventive approach, it would also be recommended the species monitoring to start a few weeks earlier as well as the placement of traps on the orchards surroundings.

This species has also been shown to prefer younger or developing plants as well as being able to cause more significant damage to younger trees in some previous studies (Alvarado *et al.* 1994, Chaieb *et al.* 2011). This study did not allow the observation of this species preference as the orchards had trees from different cultivars with different ages. To properly obtain information regarding this aspect it would be advised to place traps on the same cultivars with different ages and the same treatments, and observe if this influenced the species abundance. It would not be recommended to compare different cultivars with different ages as each cultivar may present different characteristics that may also influence the species distribution including a greater or lesser resistance/ susceptibility to the damage caused by *A. decedens*.

To date no biological control program exists for *A. decedens,* as not much information is available regarding this species natural enemies. In order to determine and acquire further information about potential enemies existing on the orchards a greater number and variety of traps would be recommended both on the orchards as well as on the surroundings. This study did not provide any information regarding potential enemies as only the Auchenorrhyncha community was identified, however no adult green leafhopper was found parasitized, which does not exclude to possibility of the parasitism of earlier stages such as nymphs and eggs, as these were not captured with the traps used for this study.

A cultural approach known as green pruning was tested during the sampling period as a possible control measure for *A. decedens*, however none of the information with the data obtained in the study seems to indicate this method had any effect on the species abundance. Despite being unlikely to constitute a potential control technique considering the data obtained so far, it would be advised to execute this method earlier, mainly in June or July, in order to avoid the period most affected by *A. decedens* as both debilitate the affected trees.

Currently most of the management measures for *A. decedens* consist on insecticide applications. On this study, acrinathrin, lambda cyhalothrin and deltamethrin were applied on the orchards however none of them targeted this species, and it was not possible to conclude without doubt that any of them had a considerable effect on its abundance, which could be a result of the products application timing, the low number of traps on the orchards and the lack of monitoring for other life stages of *A. decedens*. Thiacloprid was also applied on the orchards, and this insecticide had been shown to have a great efficacy against this species in a previous study in raspberry plantations (Grassi *et al*. 2008), however on the current study no considerable effect was observed. This may be due to different active ingredient concentrations between the treatment applied on both studies as well as the application of the insecticide in only one cultivar per application in the current study which could mask the effect of the treatment on this species abundance. Another likely explanation is that this insecticide targets nymphs and the current study did not allow the monitoring of this species nymphal stages and a correct application timing is required to maximize the persistence and activity of the treatment. The recommended timing for this insecticide application is during the peak of the first generation of nymphs which in the referred study occurred in May (Grassi *et al*. 2008), which is not the case for the current study as this insecticide was applied in August. Some other insecticides have been tested for this species in previous studies (Grassi *et al*. 2008, Meisner *et al*. 1992) such as the nicotinoids thiamethoxam, acetamiprid as well as the natural and biodegradable margosan-O, and also showed a favorable outcome regarding the species control. Despite the effective treatment resulting from the application of the referred neonicotinoids, an important side effect was observed as the number of mites significantly increased after the treatment which is also a response suspected for similar chloronicotinoid compounds as well as pyrethroids such as lambda-cyhalothrin and others (Grassi *et al*. 2008, Li & Harmsen 1993, Shanks *et al.* 1991). The application of lambda-cyhalothrin did sometimes coincide with some decreases or reductions on the increase in the number of captures in the orchards, and even though this insecticide did not target the green leafhopper species, in may lead to an increase in Acari (mites) as a common side effect of pyrethroids (Li & Harmsen 1993), and as stated previously, a mite ectoparasite (*Erythraeus ankaraicus*) has been described for *A. decedens* (Gencsoylu 2007). If the application of lambda-cyhalothrin did increase the number of mites, and potentially some of these affect *A. decedens*, it could explain some of the reductions on the number of captures following this insecticide's application. It would therefore be recommended to also monitor Acari populations to detect possible enemies of *A. decedens* and to observe if an increase occurred on mites following lambda-cyhalothrin applications. The previously referred insecticides thiamethoxam and acetamiprid, act as antagonists for acetylcholine nicotinic receptor and both present the same 14 days security interval recommended for peach orchards in the legislation approved for Portugal (Mendes & Cavaco 2016). Despite being approved in Portugal, recent studies have demonstrated that thiamethoxan, thiacloprid and acetamiprid, as well as other neonicotinoids, had considerably prejudicial effects on pollinators affecting locomotion, foraging, colony health, among others (Alkassab & Kirchner 2016, Shencheng *et al.* 2020, Tosi *et al.* 2017). Margosan-O which contains azadirachtin also showed a high mortality on nymphs as well as a repellent effect on the adults if sprayed on leaves (Meisner *et al*. 1992). Azadirachtin is included on the guide of pharmaceutical products that are allowed to be sold in this country, and therefore some studies could be conducted to test its efficacy on *A. decedens* control. This insecticide was demonstrated to have a low persistence on the environment and low toxicity on mammals (Mpumi *et al*. 2016). The recommended safety period for this product on peach orchards is three days and it acts as an insect growth regulator by disrupting ecdysone, an insect molting hormone (Mendes & Cavaco 2016). Considering the studies conducted so far (Grassi *et al*. 2008, Meisner *et al*. 1992) the most adequate combat strategy for this species should be directed to target nymphs at the end of spring/beginning of summer, as shortly after adults begin to appear.

Considering the species captured on the orchards during this study and the one from the previous year, 3 confirmed vectors, *Philaenus spumarius, Cicadella viridis, Neophilaenus campestris,* and 1 potential vector, *Cercopis intermedia,* for *X. fastidiosa* were detected. Even though these species were captured in low numbers it certainly highlights the need to monitor these insects due to its capability to transmit the bacterium responsible for diseases capable of affecting stone fruit such as peach phony disease that could considerably reduce the orchards production and lead to economic losses. To date, no control program targeting this bacterium exist, so the main form to stop the spread of *X. fastidiosa* is the control and monitoring of vector species. Some physical measures such as the removal of affected plants, severe pruning and selection of cultivars displaying a higher resistance can also take place. Considering the chemical control for Cercopoidea is limited as these species do not usually constitute a threat to important crops, it is highly important to gain a robust knowledge of the vectors species and to test insecticides with a potential effect on its control (Cao *et al.* 2011, Cornara *et al*. 2018, Dáder *et al*. 2019, EFSA 2018, 2019a, Janse & Obradovic 2010, Morente e*t al*. 2018a, Saponari *et al*. 2019). Most of the tested insecticides so far target nymphs and some have shown positive results under laboratory conditions in controlling *P. spumarius*, considered the bacterium's main vector in Europe (Cornara *et al*. 2018, Dáder *et al*. 2019, EFSA 2019a, 2019b). Regarding the biological control of vectors species very little information is available. Natural enemies of *P. spumarius* have been described however these did not seem to significantly impact this species abundance. (Dáder *et al.* 2019, EFSA 2019a).

V. Conclusions

In this study, 8140 individuals were captured, 4729 in Louriçal do Campo where 36 species were detected and 3411 in Póvoa da Atalaia where 27 species were found. Overall, on both peach orchards 39 different species were encountered, 2 of which are confirmed vectors of *Xylella fastidiosa* (*Philaenus spumarius* and *Neophilaenus campestris*) as well as vectors of phytoplasmas (*Anaceratagallia laevis, Aphrodes makarovi, Asymmetrasca decedens, Austroagallia sinuata, Dictyophara europaea, Empoasca decipiens, Euscelidius variegatus, Laodelphax striatella, Megophthalmus scrabipennis, Neoaliturus fenestratus, Philaenus spumarius* and *Zyginidia scutellaris*). Cicadellidae was the most dominant family representing 99.77% of the captured individuals while Typhlocybinae was the most common subfamily counting 95.84%. *Asymmetrasca decedens* and *Empoasca solani* were the most abundant species captured on both orchards, even though the first stands out representing 79.64% of the captures. The used specific richness estimators indicate that between 44 and 52 species likely exist on Louriçal do Campo orchard, and between 40 and 50 for Póvoa de Atalaia orchard.

A. decedens and *E. solani*, the two dominant green leafhopper species, present almost opposite temporal distributions as *E. solani* peak occurs earlier, on April 19th and slowly decreases being gradually replaced by *A. decedens* that reached its abundance peak on September 20th after which displayed an overall decrease until the end of the sampling period which did not allow to determine when this species would leave the orchards. Regarding the studied climatic variables, temperature showed a strong positive correlation with *A. decedens* abundance while a negative correlation was observed for *E. solani*. This species also showed a strong positive correlation with the number of hours of cold (<7ºC) and relative humidity.

Acrinathrin, lambda cyhalothrin, deltamethrin and thiacloprid were applied on the orchards and even though no considerable effect was observable, the low number of traps, the application timing, the lack of monitoring for nymphal stages could have interfered or masked the efficacy of these insecticides. It would be recommended the application of thiacloprid occurred earlier in order to target the first generation of nymphs and to test the efficacy of Azadirachtin which also targets nymphs and repels adults. Green pruning had been proposed as a possible control measure for *A. decedens*, however it did not seem to have any effect on its abundance.

This study contributed to better characterize the Auchenorrhyncha community, particularly the species *A. decedens* and *E. solani*, as well as to the detection of *X. fastidiosa* vectors on the peach orchards in the Beira Interior region which alerts for the need of future studies regarding these species monitoring. Some questions are left unanswered, including the species occurrence and variation on the orchards surroundings, the detection of potential enemies for these species, the monitoring of the remaining life stages of *A. decedens* as well as the assessment of the damage caused by this species regarding the cultivars affected as well as the cultivars age, when and how this species enters and leaves the orchards and what surrounding plants act as winter reservoirs for the species and the effect of certain insecticides in its control.

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Appendix A

Table A.1 – Data acquired for the maps assembling with the respective source and metadata.

Data	Source	Metadata
Portugal administrative limits: CAOP Continente -Carta Administrativa de Portugal Continental 2018	dgTerritório - www.dgterritorio.pt	Shapefile (shp); Geographic referencing system ETRS89/TM06-PT, Referencing ellipsoid GRS80, Cartographic projection, Mercator transverse, minimum rigor associated with scale 1:25000
Land Use and Occupation Charter: COS - Carta de Uso e Ocupação do Solo 2015		Shapefile (shp); Geographic referencing system ETRS89/TM06-PT, Referencing ellipsoid GRS80, Cartographic projection, Mercator transverse, minimum cartographic unit (MCU) of 1 ha

Appendix B

Table B.1 - Meteorological data from Póvoa de Atalaia station, from April 4th 2019 to October 31st 2019 grouped weekly.

Placement date	Removal date	TMin	TAve	TMax	HRMin	HRAve	HRMax	RadAve	WSAve	WSMax	TotPrec	NHCold	NHWetLeaf
4-Apr	12-Apr	5.04	9.54	15.24	54.13	79.69	97.63	168.16	1.74	5.71	3.82	42.85	10.44
12-Apr	18-Apr	5.79	14.04	22.31	45.12	77.88	99.12	200.03	1.05	4.53	0.00	31.30	9.40
18-Apr	26-Apr	7.01	12.53	19.34	49.33	79.15	98.20	163.38	1.51	5.81	6.35	11.02	11.03
26-Apr	3-May	10.57	18.07	25.95	33.52	56.35	84.16	265.14	1.55	4.62	0.09	16.04	0.76
3-May	9-May	8.63	17.50	26.50	40.72	67.81	93.56	249.97	1.56	4.94	0.20	1.45	4.19
9-May	17-May	10.77	20.41	28.99	31.18	52.50	81.93	257.43	1.81	4.70	0.68	2.94	3.82
17-May	24-May	7.07	16.21	24.93	28.79	55.89	87.37	272.00	1.64	5.21	0.00	3.66	1.47
24-May	31-May	13.24	22.13	30.67	22.92	41.64	67.66	298.05	2.22	5.58	0.00	3.77	0.21
31-May	7-Jun	13.75	23.06	32.21	27.21	48.32	79.22	280.21	1.40	4.61	0.00	0.00	0.45
7-Jun	14-Jun	8.56	17.14	25.61	23.61	50.78	84.06	291.13	1.78	5.36	0.40	4.75	0.81
14-Jun	21-Jun	8.36	18.17	26.94	35.56	62.92	94.95	247.27	1.39	4.67	0.20	5.97	2.45
21-Jun	27-Jun	13.62	20.83	28.32	39.63	65.61	90.99	221.09	1.20	4.34	1.43	1.21	3.35
27-Jun	5-Jul	13.72	23.79	34.44	26.30	59.73	94.56	272.57	1.09	4.50	0.00	0.00	2.64
5-Jul	12-Jul	12.92	22.49	31.99	24.64	53.72	84.95	281.76	1.24	4.62	0.11	0.00	2.56
12-Jul	19-Jul	16.40	26.46	36.17	27.10	55.28	87.65	265.44	1.06	4.56	0.00	0.00	0.99
19-Jul	26-Jul	20.75	29.59	39.37	20.34	43.63	71.54	277.57	1.53	4.73	0.00	0.00	0.07
26-Jul	1-Aug	14.37	22.47	31.39	27.56	49.55	74.09	273.19	1.80	5.13	0.00	0.00	0.01
1-Aug	9-Aug	15.86	25.77	35.56	29.20	52.60	81.79	244.75	1.29	4.38	0.00	0.00	0.18
9-Aug	16-Aug	15.64	23.24	31.83	24.31	50.25	78.01	240.99	1.91	5.21	0.17	0.00	1.49
16-Aug	23-Aug	18.41	26.64	36.47	21.38	44.15	68.47	245.93	1.63	4.84	0.00	0.00	0.00
23-Aug	30-Aug	17.71	25.92	35.85	25.05	55.18	86.93	210.47	0.96	4.20	5.33	0.00	3.10
30-Aug	6-Sep	17.10	26.75	37.06	21.54	43.59	72.64	239.67	1.35	4.55	0.00	0.00	0.76
6-Sep	13-Sep	14.21	22.47	31.49	22.14	41.06	67.05	240.58	2.41	5.67	0.00	0.00	0.00
13-Sep	20-Sep	15.27	22.48	31.76	33.93	63.36	90.91	178.16	1.16	4.16	0.29	0.00	2.07
20-Sep	27-Sep	11.27	18.11	26.51	48.28	76.50	96.56	149.96	0.86	3.94	3.17	0.00	7.66
27-Sep	4-Oct	11.43	20.97	31.50	21.67	57.91	93.13	204.58	0.93	3.63	0.00	0.00	1.34
4-Oct	11-Oct	13.57	22.39	32.68	23.15	50.30	81.91	186.86	1.21	3.61	0.00	0.00	0.60
11-Oct	18-Oct	9.80	16.67	25.34	50.76	79.37	97.25	131.71	1.11	4.70	3.20	2.64	5.91
18-Oct	25-Oct	8.95	13.45	18.46	54.10	79.39	96.59	101.95	1.19	4.17	5.74	11.52	10.07
25-Oct	31-Oct	8.42	14.43	21.97	63.15	87.32	99.15	101.86	0.33	2.65	1.37	10.96	11.31

Climatic variables: TMin - minimum temperature, TAve - average temperature, TMax - maximum temperature, HRMin - minimum relative humidity, HRAve - average relative humidity, HRMax - maximum relative humidity, RadAve - average solar radiation, WSAve - average wind speed, WSMax - maximum wind speed, TotPrec - total precipitation, NHCold - number of hours of cold (Temperature < 7ºC), NHWetLeaf - number of hours of wet leaf.

Table B.2 – Pearson's correlation coefficient for the average abundance of *Asymmetrasca decedens* (for the orchards Louriçal do Campo and Póvoa de Atalaia and for the average of both orchards) and the studied climatic variables with lags between 0 and 7 weeks previous to sampling. Values in **bold** correspond to a correlation significantly different from 0 ($p \leq 0.05$). Values in grey correspond to the stronger significant correlations within each climatic variable and the respective lag.

Orchard	Lag	TMin	TAve	TMax	HRMin	HRAve	HRMax	RadAve	WSAve	WSMax	TotPrec	NHCold	NHWetLeaf
Louriçal do Campo	-7	**0.522**	**0.480**	**0.457**	-0.309	-0.302	-0.325	0.180	0.020	-0.004	-0.129	-0.221	-0.291
	-6	**0.593**	**0.560**	**0.543**	-0.316	-0.282	-0.292	0.103	-0.139	-0.134	-0.161	-0.304	-0.268
	-5	**0.599**	**0.512**	**0.481**	-0.318	-0.329	**-0.407**	0.039	0.053	0.01	-0.019	**-0.387**	-0.269
	-4	**0.627**	**0.541**	**0.522**	-0.357	-0.357	**-0.404**	0.019	-0.005	-0.159	-0.112	**-0.393**	**-0.406**
	-3	**0.585**	**0.500**	**0.490**	-0.229	-0.240	-0.293	-0.132	-0.101	-0.270	0.012	**-0.465**	-0.269
	-2	**0.507**	**0.458**	**0.465**	-0.263	-0.253	-0.287	-0.170	-0.078	-0.258	-0.049	**-0.472**	-0.245
	-1	**0.423**	0.345	0.350	-0.288	-0.255	-0.290	-0.236	-0.025	-0.232	-0.007	**-0.425**	-0.234
	0	**0.458**	0.356	**0.364**	-0.111	-0.098	-0.181	**-0.383**	-0.271	**-0.422**	0.073	**-0.402**	-0.124
Póvoa de Atalaia	-7	**0.484**	**0.465**	**0.443**	-0.288	-0.277	-0.289	0.204	-0.003	-0.012	-0.228	-0.259	-0.304
	-6	**0.596**	**0.544**	**0.511**	-0.312	-0.330	**-0.410**	0.137	0.047	-0.011	-0.154	-0.284	-0.296
	-5	**0.599**	**0.514**	**0.481**	-0.316	-0.332	**-0.405**	0.075	0.034	-0.039	-0.066	**-0.368**	-0.317
	-4	**0.612**	**0.535**	**0.514**	-0.356	**-0.378**	**-0.453**	0.052	0.04	-0.082	-0.082	**-0.400**	**-0.372**
	-3	**0.579**	**0.510**	**0.498**	-0.332	-0.337	**-0.369**	-0.047	0.015	-0.161	0.005	**-0.421**	-0.315
	-2	**0.561**	**0.482**	**0.475**	-0.289	-0.304	**-0.368**	-0.140	-0.041	-0.207	-0.023	**-0.437**	-0.276
	-1	**0.463**	**0.389**	**0.395**	-0.256	-0.242	-0.282	-0.207	-0.030	-0.186	0.076	**-0.415**	-0.204
	0	**0.426**	0.344	0.354	-0.190	-0.164	-0.203	-0.296	-0.193	-0.352	-0.001	**-0.403**	-0.170
Average of both orchards	-7	**0.518**	**0.486**	**0.463**	-0.307	-0.298	-0.316	0.196	0.010	-0.008	-0.179	-0.244	-0.305
	-6	**0.611**	**0.568**	**0.543**	-0.322	-0.312	-0.354	0.121	-0.057	-0.081	-0.162	-0.303	-0.288
	-5	**0.616**	**0.527**	**0.495**	-0.327	-0.339	**-0.416**	0.057	0.044	-0.013	-0.042	**-0.389**	-0.299
	-4	**0.637**	**0.553**	**0.533**	**-0.367**	**-0.377**	**-0.438**	0.036	0.016	-0.127	-0.101	**-0.407**	**-0.401**
	-3	**0.599**	**0.520**	**0.508**	-0.284	-0.292	-0.337	-0.095	-0.050	-0.227	0.008	**-0.458**	-0.299
	-2	**0.549**	**0.484**	**0.484**	-0.283	-0.285	-0.334	-0.160	-0.062	-0.241	-0.039	**-0.469**	-0.267
	-1	**0.454**	**0.375**	**0.382**	-0.281	-0.257	-0.295	-0.228	-0.027	-0.216	0.031	**-0.432**	-0.227
	0	**0.457**	**0.361**	**0.370**	-0.152	-0.132	-0.197	-0.352	-0.242	**-0.402**	0.040	**-0.414**	-0.150

Climatic variables: TMin - minimum temperature, TAve - average temperature, TMax - maximum temperature, HRMin - minimum relative humidity, HRAve - average relative humidity, HRMax - maximum relative humidity, RadAve - average solar radiation, WSAve - average wind speed, WSMax - maximum wind speed, TotPrec - total precipitation, NHCold - number of hours of cold (Temperature < 7ºC), NHWetLeaf - number of hours of wet leaf.

Table B.3 – Pearson's correlation coefficient for the average abundance of *Empoasca solani* (for the orchards Louriçal do Campo and Póvoa de Atalaia and for the average of both orchards) and the studied climatic variables with lags between 0 and 7 weeks previous to sampling. Values in **bold** correspond to a correlation significantly different from 0 ($p \leq 0.05$). Values in grey correspond to the stronger significant correlations within each climatic variable and the respective lag.

Orchard	Lag	TMin	TAve	TMax	HRMin	HRAve	HRMax	RadAve	WSAve	WSMax	TotPrec	NHCold	NHWetLeaf
Louriçal do Campo	-7	-0.271	-0.242	-0.217	0.152	0.173	0.191	-0.122	-0.055	-0.055	0.051	0.052	0.148
	-6	-0.202	-0.179	-0.172	0.100	0.068	0.044	-0.060	0.063	0.014	0.073	0.038	0.092
	-5	-0.172	-0.161	-0.161	0.087	0.041	0.024	-0.013	0.040	-0.001	0.001	0.109	0.038
	-4	-0.240	-0.213	-0.204	0.088	0.066	0.058	0.017	0.081	0.09	0.004	0.164	0.098
	-3	**-0.454**	**-0.449**	**-0.451**	0.300	0.268	0.251	-0.102	0.085	0.214	0.189	**0.483**	0.315
	-2	**-0.535**	**-0.545**	**-0.557**	**0.399**	**0.397**	**0.37**	-0.133	0.004	0.187	0.153	**0.732**	**0.423**
	-1	**-0.568**	**-0.575**	**-0.567**	**0.452**	**0.488**	**0.465**	-0.141	0.006	0.302	0.262	**0.786**	**0.57**
	0	**-0.479**	**-0.439**	**-0.423**	0.256	0.309	0.336	0.064	0.008	0.247	0.118	**0.519**	0.352
Póvoa de Atalaia	-7	-0.278	-0.285	-0.268	0.254	0.296	0.26	-0.197	-0.085	-0.050	0.133	0.134	0.226
	-6	-0.264	-0.249	-0.242	0.213	0.194	0.183	-0.137	-0.027	-0.065	0.088	0.159	0.196
	-5	-0.301	-0.270	-0.253	0.166	0.166	0.170	-0.079	0.003	0.012	0.043	0.169	0.163
	-4	-0.309	-0.302	-0.303	0.182	0.135	0.077	-0.046	0.138	0.159	0.099	0.307	0.206
	-3	**-0.412**	**-0.409**	**-0.412**	0.248	0.238	0.185	-0.061	0.079	0.204	0.128	**0.500**	0.297
	-2	**-0.552**	**-0.562**	**-0.57**	0.351	0.322	0.264	-0.071	0.151	0.334	0.204	**0.717**	**0.426**
	-1	**-0.673**	**-0.657**	**-0.65**	**0.441**	**0.429**	**0.396**	-0.078	0.111	0.353	0.200	**0.858**	**0.507**
	0	**-0.548**	**-0.498**	**-0.486**	0.299	0.329	0.348	0.058	0.08	0.300	0.124	**0.502**	0.344
Average of both orchards	-7	-0.275	-0.259	-0.236	0.186	0.206	0.214	-0.149	-0.065	-0.054	0.08	0.079	0.176
	-6	-0.226	-0.205	-0.197	0.136	0.108	0.087	-0.085	0.037	-0.009	0.078	0.079	0.126
	-5	-0.218	-0.201	-0.197	0.116	0.082	0.068	-0.037	0.032	0.005	0.016	0.132	0.079
	-4	-0.268	-0.247	-0.241	0.122	0.09	0.065	-0.005	0.103	0.115	0.036	0.215	0.135
	-3	**-0.453**	**-0.44**	**-0.451**	0.302	0.264	0.234	-0.092	0.087	0.215	0.175	**0.499**	0.317
	-2	**-0.558**	**-0.568**	**-0.578**	**0.394**	**0.384**	0.348	-0.118	0.05	0.238	0.174	**0.743**	**0.435**
	-1	**-0.615**	**-0.614**	**-0.606**	**0.459**	**0.481**	**0.455**	-0.126	0.037	0.323	0.249	**0.826**	**0.564**
	0	**-0.514**	**-0.47**	**-0.456**	0.276	0.324	0.348	0.062	0.032	0.270	0.124	**0.525**	0.360

Climatic variables: TMin - minimum temperature, TAve - average temperature, TMax - maximum temperature, HRMin - minimum relative humidity, HRAve - average relative humidity, HRMax - maximum relative humidity, RadAve - average solar radiation, WSAve - average wind speed, WSMax - maximum wind speed, TotPrec - total precipitation, NHCold - total number of hours of cold (Temperature < 7ºC), NHWetLeaf - number of hours of wet leaf.

Appendix C

Table C.1 - Auchenorrhyncha abundance, dominance and frequency of all the collected specimens on both orchards. N: abundance, number of captured individuals, D (%); Dominance, proportion of a species abundance in relation to the abundance of all species; F(%): Frequency, proportion of dates a species is found on.

Suborder	**Family**	**Subfamily**	**Genus/Species**	**N**	**D(%)**	**F(%)**
Cicadomorpha	Aphrophoridae	-	*Neophilaenus campestris* (Fallén)	4	0.05	13.33
			Philaenus spumarius (Linnaeus)	4	0.05	10.00
	Cicadellidae	Agallinae	*Agallia consobrina* Curtis	163	2.00	60.00
			Anaceratagallia laevis (Ribaut)	77	0.95	40.00
			Austroagallia sinuata (Mulsant & Rey)	14	0.17	23.33
		Aphrodinae	*Aphrodes makarovi* Zachvatkin	27	0.33	23.33
		Deltocephalinae	*Deltocephalinae* sp.A	2	0.02	6.67
			Deltocephalinae sp. B	1	0.01	3.33
			Euscelidius variegatus (Kirschbaum)	8	0.10	20.00
			Euscelis sp.	2	0.02	6.67
			Fieberiella florii (Stal)	1	0.01	3.33
			Neoaliturus fenestratus (Herrich-Shaffer)	4	0.05	6.67
			Platymetopius guttatus Fieber	2	0.02	6.67
			Psammotettix sp. A	4	0.05	10.00
			Psammotettix sp. B	6	0.07	13.33
			Psammotettix sp. C	2	0.02	6.67
		Dorycephalinae	*Eupelix cuspidata* (Fabricius)	4	0.05	13.33
		Macropsinae	*Macropsis cerea* Germar	2	0.02	6.67
		Megophtalminae	*Megophthalmus scrabipennis* Edwards	1	0.01	3.33
		Typhlocybinae	*Alebra* sp.	3	0.04	10.00
			Arboridia sp.	10	0.12	20.00
			Asymmetrasca decedens (Paoli)	6483	79.64	96.67
			Empoasca decipiens Paoli	3	0.04	10.00
			Empoasca solani (Curtis)	1095	13.45	66.67
			Eupteryx filicum (Newman)	1	0.01	3.33
			Eupteryx sp.	2	0.02	6.67
			Fruticidia bisignata (Mulsant & Rey)	1	0.01	3.33
			Fruticidia sanguinosa (Rey)	1	0.01	3.33
			Ribautiana cruciata (Ribaut)	2	0.02	6.67
			Ribautiana debilis (Douglas)	4	0.05	13.33
			Ribautiana sp.	1	0.01	3.33
			Typhlocybinae sp. A	1	0.01	3.33
			Zygina lunaris (Mulsant & Rey)	6	007	20.00
			Zygina nivea (Mulsant & Rey)	4	0.05	10.00
			Zygina ordinaria (Ribaut)	106	1.30	76.67
			Zyginidia scutellaris (Herrich-Shaffer)	78	0.96	60.00
Fulgoromorpha	Delphacidae	Delphacinae	*Laodelphax striatella* (Fallén)	4	0.05	13.33
			Metadelphax propinqua (Fieber)	6	0.07	10.00
	Dictyopharidae	-	*Dictyophara europaea* (Linnaeus)	1	0.01	3.33

Appendix D

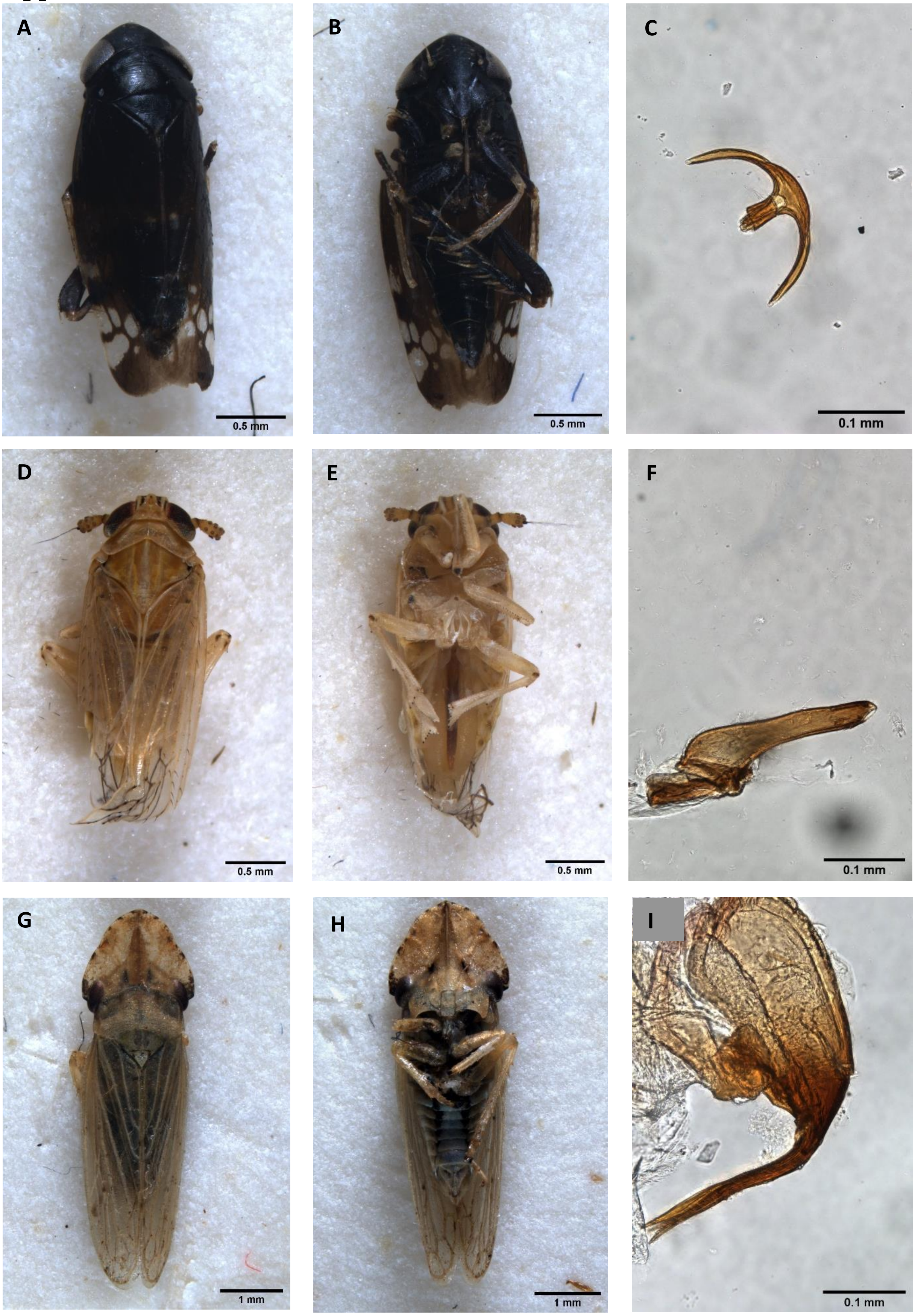

Figure D.1 – Male *Neoaliturus fenestratus* dorsal (A) and ventral (B) view and genitalia (C); Male *Metadelphax propinqua* dorsal (D) and ventral (E) view and genitalia (F); Male *Eupelix cuspidata* dorsal (G) and ventral (H) view and genitalia (I); Author's original.

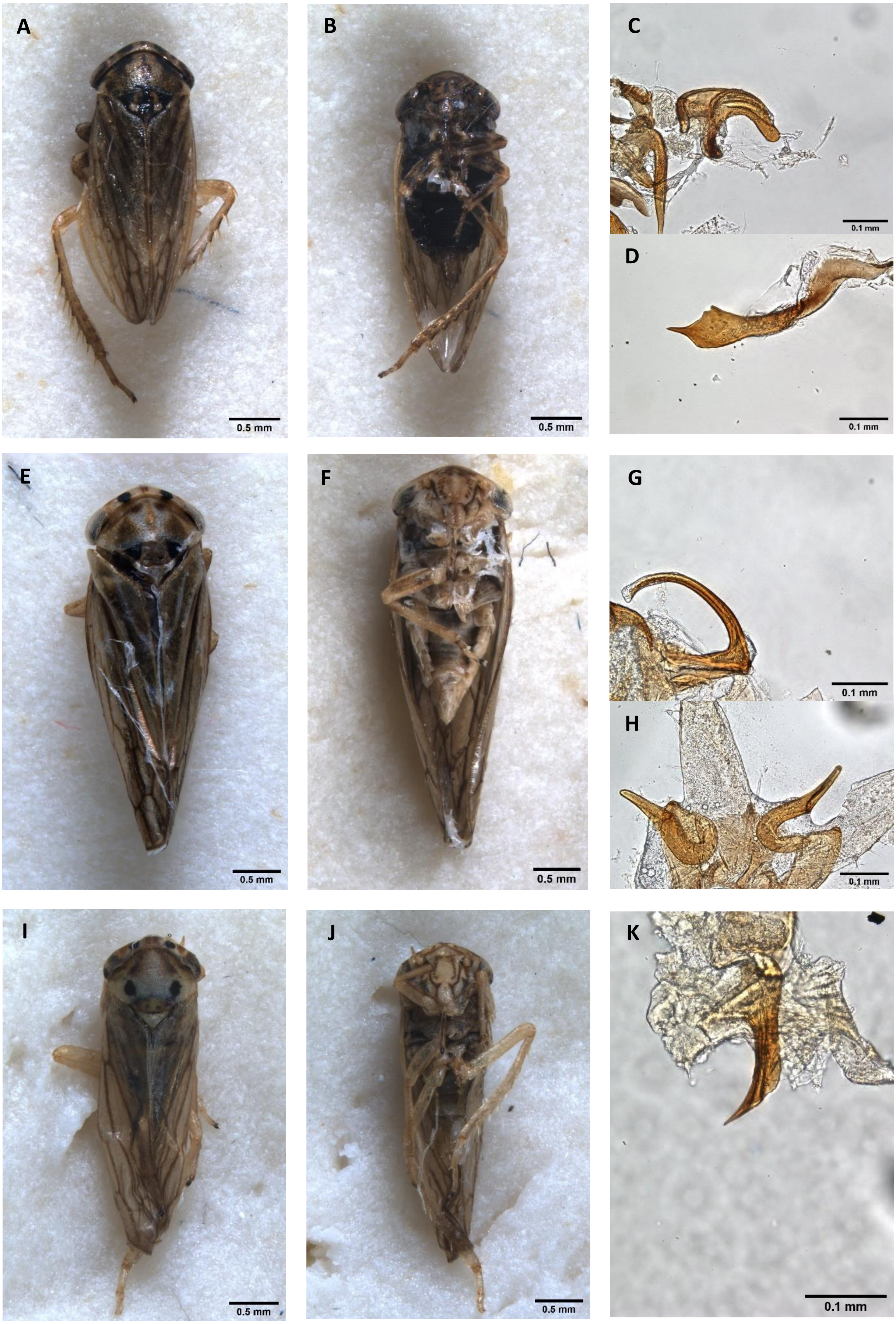

Figure D.2 – Male *Anaceratagallia laevis* dorsal (A) and ventral (B) view and genitalia (C,D); Male *Agallia consobrina* dorsal (E) and ventral (F) view and genitalia (GH); Male *Austroagallia sinuata* dorsal (I) and ventral (J) view and genitalia (K); Author's original.

Figure D.3 – Male *Aphrodes makarovi* dorsal (A) and ventral (B) view and genitalia (C); Male *Macropsis cerea* dorsal (D) and ventral (E) view and genitalia (FG); Male *Dictyophara europaea* dorsal (H) and ventral (I) view and genitalia (J) Author's original.

Figure D.4 – Male dorsal view of: *Zygina lunaris* (A,D), *Zygina ordinaria* (B), *Zygina nivea* (C), *Alebra* sp. (E), *Eupterix* sp. (F), *Platymetopius guttatus* (G), *Fieberiella florii* (H), *Euscelidius variegaus* (I,J), *Megophthalmus scrabipennis* (K), *Laodelphax striatella* (L). Author's original.