DEPARTAMENTO DE BIOTECNOLOGÍA-BIOLOGÍA VEGETAL

ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA
AGRONÓMICA, ALIMENTARIA Y DE BIOSISTEMAS

UNIVERSIDAD POLITÉCNICA DE MADRID

**ECOLOGICAL DETERMINANTS OF *POTYVIRUS*
EMERGENCE IN WILD ECOSYSTEMS**

DOCTORAL THESIS

CRISTINA RODRÍGUEZ NEVADO

Master's Degree in Genetics and Cell Biology

2018

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Madrid, 2018

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EL PRESIDENTE

LOS VOCALES

EL SECRETARIO

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RESUMEN

Cada vez son más numerosas las evidencias que indican que existe una amplia diversidad de virus de plantas en los ecosistemas silvestres. Sin embargo, la mayoría de las especies de virus presentes en estos ecosistemas permanecen sin caracterizar. Es más, apenas existen datos sobre los factores que afectan al riesgo de infección y a la diversidad genética de las poblaciones de virus que infectan huéspedes silvestres, ambos relacionados con los procesos de emergencia de virus. Esta tesis analiza los factores ecológicos que determinan el riesgo de infección y la diversidad genética de las poblaciones de virus de plantas en los ecosistemas silvestres. En concreto, la tesis se centra en las especies de virus del género *Potyvirus* presentes en los dos ecosistemas silvestres más representativos del centro de la Península Ibérica: los bosques de ribera y los encinares. Estos análisis se basan en una caracterización detallada de los dos ecosistemas silvestres estudiados y de los potyvirus que se encuentran en ellos a lo largo de tres años: en localizaciones de ambos ecosistemas silvestres se ha analizado la prevalencia de potyvirus (como medida del riesgo de infección) y se han registrado las variaciones estacionales de diversos factores ecológicos y climáticos que caracterizan cada ecosistema. Los factores ecológicos que afectan a la prevalencia y a la diversidad genética poblacional de los potyvirus se identificaron a diferentes escalas taxonómicas (género vs. especie) y se consideró el efecto de la estrategia de vida del virus (especialista vs. generalista).

La comparación de los datos obtenidos en los dos ecosistemas estudiados indicó que la prevalencia de potyvirus fue generalmente más alta en los bosques de ribera, lo que se asoció con una mayor riqueza de especies de plantas y una mayor abundancia de plantas huéspedes, la mayoría de las cuales eran especies perennes. Este resultado está de acuerdo con las teorías que predicen que la identidad de las especies presentes en un ecosistema, y no solo su número, es un factor determinante del riesgo de infección por patógenos. Otros análisis presentados en esta tesis apoyan este resultado. Por ejemplo, cuando se estudió la asociación entre los factores ecológicos/climáticos y la prevalencia de potyvirus dentro de cada ecosistema se identificaron la composición de la comunidad vegetal, y la abundancia relativa y la densidad del/los huésped(es) como los factores más importantes que afectan a la prevalencia de potyvirus en los bosques de ribera y en los

encinares. Un análisis más preciso del papel de estos factores ecológicos en la prevalencia/riesgo de infección de potyvirus reveló que cada uno está involucrado en una etapa diferente de la emergencia viral: la composición de la comunidad vegetal determinaría el riesgo de que los potyvirus entren en el ecosistema. Una vez dentro del ecosistema, la abundancia relativa/densidad del huésped determinaría la dispersión de los potyvirus, probablemente a través de su efecto en la eficacia de transmisión de los virus. Por lo tanto, determinaría el grado de infección (prevalencia) en las poblaciones de huéspedes. Curiosamente, la gama de huéspedes de cada especie de potyvirus afecta a la importancia relativa de cada uno de estos factores ecológicos: la densidad del huésped es el principal predictor de la prevalencia de un virus especialista, mientras que la abundancia relativa del huésped lo es de la prevalencia de un virus generalista.

Los cambios en la epidemiología de los patógenos pueden dar como resultado modificaciones en la estructura genética en sus poblaciones. Por lo tanto, cambios en la prevalencia de los potyvirus pueden ir acompañados de variaciones en la diversidad genética de sus poblaciones. De hecho, el análisis de los factores ecológicos que afectan a la diversidad genética de las poblaciones de potyvirus en bosques de ribera y encinares indicaron que la abundancia relativa y la densidad del/los huésped(es) fueron factores determinantes de dicha diversidad genética. Además, junto con estos factores ecológicos, la prevalencia de los potyvirus también fue un predictor importante de la diversidad genética de las poblaciones virales. Los resultados de esta tesis revelan además que la importancia relativa de estos predictores depende de la gama de huéspedes de cada especie de virus: la abundancia relativa de los huéspedes fue el factor más determinante de la diversidad genética de las poblaciones de un virus generalista, mientras que la prevalencia fue el principal predictor de la diversidad genética en las poblaciones de un virus especialista. Los resultados de esta tesis también indican que, en los dos ecosistemas silvestres, otros factores ecológicos y climáticos, tal vez vinculados a la dinámica de las poblaciones de los vectores de los potyvirus, juegan un papel importante en la evolución de estos virus. Finalmente, esta tesis ha investigado por primera vez los patrones y procesos de especiación dentro del género *Potyvirus* en ecosistemas silvestres. Los resultados apoyan que el proceso de

diversificación genética de los potyvirus es compatible con eventos de especiación simpátrica en los ecosistemas estudiados.

En resumen, los resultados de esta tesis ofrecen información relevante sobre los factores determinantes de la emergencia de virus de plantas en ecosistemas silvestres y destacan la importancia de considerar la interacción entre la ecología, la epidemiología y la diversidad genética de las poblaciones de huéspedes y virus de plantas con el fin de comprender los procesos que conducen a la emergencia de virus de plantas en ecosistemas silvestres.

ABSTRACT

Increasing evidence indicates that viruses are widespread and highly diverse in wild ecosystems. Most of the virus species present in wild ecosystems however, remain uncharacterized. Moreover, in these ecosystems the factors affecting plant virus infection risk and population genetic diversity, two traits linked to virus emergence, are largely unknown. This thesis analyses the ecological determinants of plant virus infection risk and population genetic diversity in wild ecosystems. The thesis focuses on virus species of the genus *Potyvirus* present in the two most representative wild ecosystems in the centre of the Iberian Peninsula: riparian and evergreen oak forests. These analyses are based on a detailed characterization of these wild ecosystems and of the potyviruses that populate them: along three years, potyvirus prevalence (as a measure of infection risk), and seasonal data about ecological and climatic factors, were recorded in locations of both wild ecosystems. The ecological determinants affecting the prevalence and population genetic diversity of potyviruses were identified at different taxonomical scales (genus vs. species) and considering different virus life-history strategies (specialist vs. generalist).

Comparative analyses of potyvirus prevalence between ecosystems indicated that virus prevalence was generally higher in riparian forests, which was associated with higher plant species richness and abundance of host plants, most of which were perennial species. This result is in agreement with theories predicting that the identity of the species present in an ecosystem, and not only the number of species, is a key factor in determining pathogen infection risk. Further analysis reported in this thesis supports this result. For instance, analyses of the association between the ecological/climatic factors and the potyvirus prevalence within each ecosystem identified the plant community composition, the host relative abundance and the host density as the most important determinants of potyvirus prevalence in the two studied ecosystems. A fine study of the role of these ecological factors in potyvirus prevalence/infection risk revealed that they are involved in different stages of virus emergence: the plant community composition would determine the risk of potyvirus jump into the ecosystem. Once introduced in the ecosystem, host relative abundance/density would affect potyvirus invasion, likely affecting plant-to-plant transmission, and thus determining the extent of the infection (prevalence) in the host populations. Interestingly, the host range of each

potyvirus species appears to determine the relative importance of each of these ecological factors: host density was the chief predictor of the prevalence of a specialist virus, and host relative abundance was the major determinant of the prevalence of a generalist virus.

Epidemiological changes may result in genetic modifications in the pathogen population. Therefore, changes in potyvirus prevalence can be accompanied by variations in the virus population genetic diversity. Indeed, analyses of ecological factors affecting the genetic diversity of potyvirus populations in evergreen oak and riparian forests indicated that host relative abundance and density were also important determinants of such viral genetic diversification. Together with these ecological traits, prevalence was also a major predictor of virus population genetic diversity. As for potyvirus epidemiology, the results of this thesis reveal that the relative importance of these predictors depend on the virus species-specific host range: the population genetic diversity of a generalist virus was mainly determined by host relative abundance, whereas prevalence was the chief predictor of the population genetic diversity of a specialist virus. The results of this thesis also indicate that other ecological and climatic factors, perhaps linked to the dynamics of the virus vector populations, play an important role in the evolution of potyvirus populations in wild ecosystems. Finally, this thesis has investigated for the first time, the patterns and processes of speciation within the genus *Potyvirus* in wild ecosystems, demonstrating that the process of genetic diversification of potyviruses is compatible with sympatric speciation events in the studied ecosystems.

In summary, the results of this thesis provide relevant information on the determinants of plant virus emergence in wild ecosystems and highlight the importance of considering the interplay between the ecology, epidemiology and genetic diversity of plant host and virus populations in order to fully understand the processes that lead to plant virus emergence in wild ecosystems.

1. INTRODUCTION

1.1. Importance of plant viruses

Plant diseases are one of the major constraints on agricultural production worldwide, with a socioeconomic impact that can be comparable to those caused by human and animal diseases. Around 16% of global crop yields are reduced each year due to plant pathogens, which pose a serious risk to food security (Oerke, 2006; Savary et al., 2006). Although losses caused by plant viruses alone are difficult to estimate, plant viruses are considered the second most damaging crop pathogens only after fungi/oomycetes (Hsu, 2002). Indeed, according to some estimates plant viruses cause 7% of yield reductions in crops yearly (Oerke and Dehne, 2004). However, the economic impact of individual viral epidemics can be enormous. For instance, strains of *Cassava mosaic begomoviruses* cause 25 million tons of cassava losses every year in Africa, India and Sri Lanka; *Citrus tristeza virus* (CTV) is responsible for losses in citrus tree cultures estimated to over 100 million trees worldwide; and viruses affecting rice cultures result in yield losses estimated in more than US\$1.5 billion in South-East Asia alone (reviewed by Nicaise, 2014). The threat posed by plant virus diseases increases under the current conditions of climate change and global trade as these conditions favour the emergence of new diseases. Emerging infectious diseases, including those caused by plant viruses, can be defined as infections that have appeared in a new host population or have previously existed in a host population but are rapidly increasing in incidence or geographic range due to changes in its epidemiology (Woolhouse, 2002; 2005). The negative impact of diseases is greatest in the case of emerging ones, since they affect host populations that have not been previously challenged by the causal agent (Vurro et al., 2010; Woolhouse et al., 2005). Viruses account for half of the reported emerging infectious diseases from plants reported in the last four decades (Anderson et al., 2004). For instance, in the past 15 years *Pepino mosaic virus* (PepMV) (Hanssen and Thomma, 2010; Gómez et al., 2012) and *Tomato yellow leaf curl virus* (TYLCV) (Moriones and Navas-Castillo, 2000; Hanssen et al., 2010) have emerged in tomato crops causing devastating epidemics worldwide.

Besides being important crop pathogens, viruses are present in wild ecosystems (Cooper and Jones, 2006; Roossinck, 2010; Prendeville et al., 2012), in which they are highly diverse and widespread (Roossinck, 2012; Stobbe and Roossinck, 2014), and they may be important ecological agents. For instance, quantitative resistance of wild plants to viruses have been described (Gilbert, 2002; Pagán et al., 2010; Moreno-Pérez et al., 2014), suggesting that these may affect the host population composition. Also, viral infection can drastically reduce the number of individuals in the host populations by decreasing the competitive and/or the reproductive abilities of infected plants, shaping host population size (Anderson et al., 2004; Malmstrom et al., 2005b; Vijayan et al., 2017) and, in some extreme cases, leading to local extinction of wild host populations (Futayama-Noguchi, 2001; Futayama et al., 2001). Viruses of wild ecosystems may also be important for agroecosystems (**Figure 1.1**). The intensification of agriculture has caused that an increasing proportion of wild plant communities have been reduced to patches that lie now surrounded by agricultural lands (Stukenbrock and McDonald, 2008; Alexander et al., 2014). The border between wild ecosystems and agricultural ecosystems represents an agro-ecological interface where crop species are in closer proximity to wild species. Plant viruses may move across this interface and induce the emergence of new infectious diseases (Alexander et al., 2014; Roossinck and García-Arenal, 2015; Malmstrom and Alexander, 2016). In this context, plant viruses infecting wild hosts may be important causal agents of emerging diseases in crops due to their ubiquity, dispersal capacity by vectors and ability to colonize both crop and wild plant species (Pagán et al., 2012; Alexander et al., 2014). Indeed, virus incidence in wild plants has been traditionally studied because of the role of these plants as the reservoirs of crop pathogens (Pinel-Galzi et al., 2015).

Most of the current knowledge on plant-virus interactions comes from the study of viruses that cause diseases in crops. Despite the importance of plant viruses for the conservation of wild ecosystems and for the emergence of infectious diseases in crops, little is known on their epidemiology and evolution and on the associated ecological determinants (Pagán et al., 2016). The study of the ecological factors and the evolutionary processes that determine the epidemiology and genetic structure of virus populations in wild ecosystems may

contribute to develop more efficient strategies for controlling disease emergence, and to better understand the ecological role of viral infections in wild plant populations.

Figure 1.1. Virus interactions and virus movement at the agro-ecological interface. Numbers represent the relative amount known about each movement pathway (1=most, 4=least). Figure taken from [Alexander et al., 2014](#).

1.2. Ecological factors driving emergence of plant viruses

As for pathogens at large, virus emergence may be achieved through different processes, including spatial-spread to new environments, cross-species transmission, and increasing prevalence in a population where the pathogen is already present ([Hudson et al., 2008](#); [Holmes, 2009](#)). The process of pathogen (including viruses) emergence through spatial-spread and cross-species transmission is complex and comprises multiple phases: In a first phase, preexisting genetic variants from host populations in which the pathogen is well established (reservoir hosts) are transmitted into a new habitat or new host species (spillover). In a second phase, the pathogen must adapt to the new host or habitat establishing productive infections through a process of adaptation. Finally, the pathogen must develop effective between-host transmission mechanisms in the new host/habitat to establish sustainable transmission cycles ([Hudson et al., 2008](#); [Holmes, 2009](#)). Emergence can also occur in a host population in which the pathogen previously existed when the prevalence

of the pathogen rapidly increases due to changes in the underlying epidemiology.

Emergence generally occurs in the context of complex ecological systems, and involves interactions between pathogens, hosts and vectors. Thus, ecological factors determining the population dynamics of these three interactive partners may affect the process of emergence (Jones, 2009; Elena et al., 2014). Changes in the ecology of hosts, pathogens and vectors may favor increasing contact between host populations, and hence pathogen host jumps or spillovers, and/or increase pathogen between-host transmission and incidence. Indeed, such ecological changes have been associated with the emergence of plant pathogens in both crop and wild plant species (Anderson et al., 2004; Power and Mitchell, 2004; Woolhouse et al., 2005). Also, ecological factors can enhance pathogen prevalence eventually leading to emergence. Some examples of factors that cause increased pathogen prevalence in plants are the introduction of new, more efficient vector species (Anderson et al., 2004; Fargette et al., 2006; Morales, 2006) and changes in the plant host community that enhance the abundance of hosts that maximize pathogen replication and/or vector reproduction (Borer et al., 2009; Pagán et al., 2012; Rodelo-Urrego et al., 2013). In the last three decades, the increasing number of emerging pathogens has boosted the interest of researchers for understanding the ecological factors affecting infection/disease risk (i.e. the ability of pathogens to infect their host/cause disease) that may finally lead to emergence of infectious diseases (Anderson et al., 2004; Keesing et al., 2006). This has resulted in the development of a significant body of theoretical work aiming at predicting the conditions that increase/decrease infection/disease risk (Ostfeld and Keesing, 2012; Johnson et al., 2015). According to these works, ecological factors that are potential determinants of infection/disease risk can be largely grouped into biotic and abiotic factors.

1.2.1. Biotic factors

1.2.1.1. Species biodiversity

Changes in the host ecology have been identified among the most frequent causes of infection/disease risk (Morse and Schluederberg, 1990; Jones, 2009).

Because infectious diseases involve interactions between at least two species (host and pathogen), it has been proposed for a long time that ecosystem biodiversity will play a key role in infection risk ([Burdon and Chilvers, 1982](#)). Biodiversity is defined as the number and relative abundance of species and genotypes present in a given ecosystem ([Wilson, 1992](#)). Because different host species/genotypes are expected to vary in their susceptibility and ability to replicate or transmit specific pathogens, host diversity could play a central role in infection risk ([Keesing et al., 2010](#); [Ostfeld and Keesing, 2012](#)). Two contrasting hypotheses relate ecosystem species diversity (i.e. the number of species or species richness) and host abundance to disease risk. The “Amplification Effect” hypothesis predicts that species diversity will be positively correlated with infection risk, as increasing species diversity will result in increased abundance of host species, and therefore of inoculum sources for a focal host. Conversely, the “Dilution Effect” hypothesis posits that reduced species diversity increases the abundance of the focal host species, facilitating pathogen transmission and increasing infection risk. Thus, higher species diversity decreases the abundance of the focal host, resulting in a reduction in the number of contacts between susceptible and infected individuals and in lower infection risk ([Keesing et al., 2006](#); [Ostfeld and Keesing, 2012](#)).

Most experimental analyses on the effects of plant diversity and relative abundance on infection risk have focused on the effects of plant species richness on the prevalence of foliar fungal diseases (e.g. [Mitchell et al., 2002](#); [2003](#); [Roscher et al., 2007](#)). The majority of these studies indicated that higher species richness reduced infection risk due to a lower host density or to the differential ability of host species to transmit the pathogen (“Dilution Effect”). Investigations about the relationship between host species diversity and infection risk in plant-virus systems are also scarce and restricted to a handful of interactions ([Malmstrom et al., 2005a, 2005b](#); [Pagán et al., 2012](#); [Rodelo-Urrego et al., 2013](#)). Interestingly, these works yielded contradictory results: data on infection of *Cereal-* and *Barley yellow dwarf viruses* (C/BYDV) in *Poaceae* species supported the “Amplification Effect” ([Malmstrom et al., 2005a, 2005b](#)). However, the effect of plant species diversity on begomovirus infection risk in the focal host *Capsicum annuum* var. *glabriusculum* (or wild pepper)

accorded to the “Dilution Effect” ([Pagán et al., 2012](#); [Rodelo-Urrego et al., 2013](#)).

1.2.1.2. Community composition

Further developments of the dilution and amplification theories proposed that which of these effects occurs in a given ecosystem would depend on factors other than to host species diversity or abundance ([Randolph and Dobson, 2012](#); [Johnson et al., 2015](#)). For instance, the potential to increase infection risk and/or pathogen transmission might also depend on the identity of the species present in a given ecosystem (community composition) ([Seabloom et al., 2009a, 2009b](#)). If the most competent host species (i.e., those that better transmit the pathogen) are the most resilient to extinction, then infection risk will increase as biodiversity declines. On the other hand, if competent host species tend to disappear as diversity declines, then the loss of species diversity will tend to reduce infection risk. Also, if increasing species diversity leads to the introduction of new host species, the resulting scenario would be compatible with the “Amplification Effect”, whereas if new species were mostly non-hosts a “Dilution Effect” would be more likely ([Randolph and Dobson, 2012](#); [Johnson et al., 2015](#)). On this regard, the life cycle of the species present in an ecosystem could be a relevant trait to analyze ([Johnson et al., 2012](#)). Theoretical work has proposed that short-lived (annuals) hosts are better pathogen reservoirs than long-lived ones (perennials). Long-lived species are more likely to be challenged against pathogen infections during their life span, and therefore invest in developing defense mechanisms. Conversely, short-lived hosts will invest less in costly defenses against pathogens, so that they will be more susceptible to infection and/or will sustain larger vector populations, thus being effective reservoirs for infectious diseases ([Boots and Bowers, 2004](#); [Miller et al., 2007](#); [Hily et al., 2014](#); [Bruns et al., 2015](#)). The local abundance of long-lived hosts has been also associated with increased infection risk: long-lived hosts can increase pathogen spread rates and prevalence by serving as long-term sources of infection for susceptible individuals ([Herms and Mattson, 1992](#); [Borer et al., 2010](#)). This effect of the plant lifespan on infection risk may differ

between wild and agroecosystems as, at odds with most agroecosystems, wild ecosystems are dominated by perennials ([Malmstrom et al., 2011](#)).

Few studies have directly or indirectly investigated the importance of host plant life cycle on infection risk, and the majority was done under laboratory conditions. [Salvaudon et al., 2013](#) showed that long-lived genotypes of *Arabidopsis thaliana* were more tolerant to *Hyaloperonospora arabidopsidis*, indirectly showing that hosts with longer life spans were more likely to be better sources of pathogen inoculum. More directly, [Hily et al., 2014](#) demonstrated that long-lived *A. thaliana* genotypes were better reservoirs of *Cucumber mosaic virus* (CMV) than short-lived ones. There is also evidence that, in wild ecosystems, short-lived (annual) species may have a high reservoir potential of a generalist plant virus than long-lived (perennial) hosts ([Malmstrom et al., 2005b](#); [Alexander et al., 2017](#)). Therefore, the relationship between lifespan of plant species and pathogen infection risk remain only partially understood.

1.2.1.3. Host density and biomass

Because host density affects directly host-pathogen encounter rates, the density of the host plant populations can have a marked effect on the size and rate of increase of those of their pathogens. Indeed, an accepted axiom in plant pathology is that a greater host density would reduce the distance between susceptible hosts, facilitating the dispersion of pathogens and increasing infection/disease risk ([Burdon and Chilvers, 1982](#)). Agroecosystems are homogenous environments with higher host density that facilitates pathogen transmission between plants, driving the emergence of highly virulent, host-specialized plant pathogens compared to pathogens in wild ecosystems. Therefore, in agroecosystems host density is expected to have a central role in infection risk, and most evidence supporting this positive association between host density and infection risk comes from studies in agroecosystems ([Thrall and Burdon, 1999](#); [Burdon and Thrall, 2008](#); [Stukenbrock and McDonald, 2008](#)). However, it should be noted that evidences for a negative correlation between host density and disease risk also exist (reviewed by [Burdon and Chilvers, 1982](#)).

Few studies have addressed the association between plant density and infection risk in wild ecosystems. Many of these have recorded a positive association between the two traits (Alexander, 1984; Burdon et al., 1992; Knops et al., 1999; Mitchell et al., 2002; Cobb et al., 2010; Pagán et al., 2012). However, the influence of many interacting factors in the ecosystem makes it difficult to interpret the effects of host plant density on disease risk (Power, 1987). For example, the effects of species biodiversity and host density on disease risk are often difficult to differentiate (Keesing et al., 2010). On this regard, it has been shown that infection risk increased with biodiversity loss associated with increased host plant density (Knops et al., 1999; Cobb et al., 2010). The interpretation of most works in wild ecosystems is limited by the difficulty of disentangling these interactions, and only a few utilized methodologies to separate such effects (Pagán et al., 2012).

As for plant biomass, some authors have proposed that higher biomass may increase the prevalence of fungal pathogens because it favors conditions for pathogen development (Harvell et al., 2002). For viruses, larger plant biomass may favor contact between plants and promote the growth of vector populations increasing virus prevalence (Sacristán et al., 2004). However, very few works have empirically tested these ideas, and with contradictory results. While plant biomass was positively associated with fungal growth (McCartney, 1997) and with virus prevalence (Sacristán et al., 2004), other works failed in finding any effect of biomass on infection risk (Seabloom et al., 2010).

1.2.1.4. Pathogen host range

Emergence is not only influenced by host ecology, but also by pathogen ecology. One the major pathogen traits that could influence emergence is its host range, i.e., the number of species that a pathogen can infect. Pathogens can be divided into two groups according to their host range: generalists and specialists. Generalist pathogens infect many host species, often from taxonomically distant plant families (Whitlock, 1996), an ability that provides such pathogens with more chances of survival and transmission. On the other hand, specialists are able to infect one or few closely related host species, where these pathogens are highly transmissible and efficiently exploit host

resources (Futuyama and Moreno, 1988). Because biological fitness varies across environments, virus fitness shall vary across host species (Elena et al., 2014). As a consequence, it is often assumed that generalism comes at the cost of suboptimal virus transmission and host resource exploitation, as generalist viruses cannot maximize their fitness in all hosts. On the other hand, specialist pathogens will overcompete generalists in the host that the former can infect, but at the cost of a high dependency on the availability of such host (Pagán et al., 2016). Which of this two strategies is “selected” by a pathogen may deeply impact how host ecology affects infection risk. It has been proposed that the “Amplification Effect” would require a generalist pathogen that infects a wide host range. Otherwise, increasing of the number of species in an ecosystem would rarely increase the number of host species. Following this rationale, the more restricted the host range of the pathogen, the higher the “Dilution Effect”.

Remarkably, few studies have compared how host ecology affects the infection risk of generalist and specialist pathogens. For plant viruses, the relationship between biodiversity and disease risk has been analysed using the generalist C/BYDV, with results that are compatible with the “Amplification Effect” (Malmstrom et al., 2005a, 2005b; Borer et al., 2010). On the other hand, a decrease in biodiversity was associated with an increased risk of infection in wild pepper populations by two specialist viruses, the begomoviruses *Pepper golden mosaic virus* (PepGMV), and *Pepper huasteco yellowvein virus* (PHYVV) supporting “Dilution Effect” hypothesis (Pagán et al., 2012). However, in these wild pepper populations the relationship between the risk of infection by CMV, a generalist virus, and plant species diversity also accorded to the “Dilution Effect”. It should be noted that these works defined host range according to literature, and did not attempted to characterize the virus host range in the analysed ecosystems. It has been recently shown that virus host range may differ according to habitat, such that theoretical generalists may act as specialists in certain conditions (McLeish et al., 2017). Thus, in order to understand how pathogen host range affects infection risk, the host range of the pathogen under study in the analysed ecosystems should be known.

1.2.2. Abiotic factors

The process of pathogen emergence does not depend only on biotic factors. In an ecosystem, plant-virus-vector interactions occur also within an abiotic environmental context. Thus, it is expected that climatic conditions and other abiotic factors that define an ecosystem also have a fundamental role in infection dynamics ([Patz et al., 2000](#)). Moreover, the current situation of climate change likely alters the interaction between hosts, viruses and their vectors. Therefore, it is of particular importance to investigate how changes in climatic conditions affect pathogen infection risk ([Anderson et al., 2004](#)). However, environmental mediation of infection/disease risk is only partially understood. Temperature, relative humidity, rainfalls and edaphic factors have been proposed as relevant factors affecting pathogen emergence. Variation in the abiotic environment can mediate infection risk through: (i) their effect on host physiology, altering host defense responses and predisposing plants to infectious diseases, and (ii) their effect on population dynamics of vectors, promoting pathogen transmission ([Garret et al., 2006](#); [Kennelly et al., 2012](#)).

1.2.2.1. Effect of abiotic factors on host physiology

Host plants are susceptible to changes in abiotic environmental conditions and, if these changes result in weakened individuals, plants may be less able to fence against pathogen infections or vector attacks ([Kennelly et al., 2012](#)). It has long been recognized that temperature is one such abiotic factor ([Kassanis, 1957](#)). Research that focused on the molecular mechanisms underlying the effect of temperature on disease dynamics found that temperature determines the efficacy of plant defense mechanisms such as RNA silencing and genetic resistance. For example, antiviral resistance mechanisms based in protein-protein recognition of the pathogen by the plant are also temperature-dependent ([Canto and Palukaitis, 2002](#); [Pfitzner, 2006](#)). It is well known that temperature above 28°C results in the inactivation of the genetic resistance conferred by the N gene against *Tobacco mosaic virus* (TMV) ([Canto and Palukaitis, 2002](#)). Also, [Zhang et al., 2012](#) showed that higher temperatures resulted in increased replication of *Turnip crinkle virus* (TCV) in *Arabidopsis* plants due to the accumulation of loss-of-function mutations in proteins involved

in the RNA silencing-based defence. Other studies have shown that plants are more susceptible to viruses at low temperature (more severe symptoms) due to a less efficient activity of the RNA silencing-mediated plant defense (Tuttle et al., 2008). Other climatic conditions have been also shown to affect plant infection/disease dynamics. An example of rainfalls mediation of pathogen prevalence has been reported using C/BYDV-infected natural grasslands. This study showed an indirect effect of the rainfalls regime on both host fecundity and virus prevalence: in sites with lower precipitations, virus prevalence increased, and authors speculate that precipitations may have indirectly increased virus prevalence by increasing host stress (i.e., drought stress) beyond the stress level caused only by virus infection (Seabloom et al., 2009).

The impact of soil edaphic factors can also influence infection/disease risk by modulating traits of hosts and pathogens involved in pathogen spread. It has been shown that, after seeding, soil moist conditions and possibly soil type favor survival of CMV seed-infected plants. Higher seed survival would result in a greater source of primary virus inoculum available for acquisition and spread to susceptible plants by aphids (Bwyte et al., 1994; Bueso et al., 2017). Also, variability in soil moisture and/or soil type can limit nutrient availability and determine the diversity and composition of communities of plants in the ecosystems (Borer et al., 2014a, 2014b), therefore defining the number and identity of host species present in a given ecosystem.

1.2.2.2. Effect of abiotic factors on the vector population

Seasonal variation of temperature, relative humidity and rainfalls have been repeatedly reported to have a great effect in the abundance of aphid population and other virus vectors because of their impact on vector reproduction and ability to transmit viruses (Morales and Jones, 2004; Fajinmi et al., 2011). For example, it has been observed that seasonal changes in temperature and humidity affected the population size of thrips (*Frankliniella fusca* and *Frankliniella occidentalis*), vectors transmitting *Tomato spotted wilt virus* (TSWV): high humidity and low temperatures negatively affected thrips populations by killing larvae. This study also reported an increased flight activity of adult vectors at higher temperatures and lower relative humidity, which was

associated with higher TWSV prevalence ([Culbreath and Srinivasan, 2011](#)). Similar effects of temperature and relative humidity on the reproductive ability, mortality and flight activity of aphid populations have been reported (e.g. [Díaz and Fereres, 2005](#)).

Several works aimed at developing mathematical predictive models of pathogen emergence have recognized the importance of climatic factors in determining pathogen infection/disease risk. Many of these works showed that simulating vector population dynamics as a function of seasonal changes in temperature, humidity and rainfalls significantly improves prediction of disease emergence ([Mohasin and Chona, 2002](#); [Thackray et al., 2004](#); [Reynaud et al., 2009](#); [Chappell et al., 2013](#)). These models have been mostly developed for crop pathogens and very few works have analysed how co-occurring stress factors, (i.e., plant pathogen infection together with adverse climatic conditions), influence infection risk in wild ecosystems ([Sacristán et al., 2004](#); [Cooper and Jones, 2006](#)).

1.3. Genetic diversity of plant virus populations

The process of pathogen emergence, including viruses, does not only involves changes in pathogen epidemiology, but also in the genetic composition of the pathogen population. These changes are thought to be dependent of the ability of the virus to generate genetic diversity, as well as on ecological factors that may shape the genetic diversity of the virus population ([Elena et al., 2014](#)).

1.3.1. Generation of genetic diversity in plant virus populations

One of the defining characteristics of viruses is their high capacity to generate genetic diversity ([Holmes, 2009](#)). Genetic diversity is the essential component in a population that allows a species to evolve in an everchanging environment with shifting selection pressures. A fundamental step to reduce the number of emergent virus diseases, and a long-standing goal for evolutionary biologists, is to understand which factors modulate the genetic diversity of virus populations. As for most organisms, the two main mechanisms for generating genetic diversity in plant virus populations are mutation and recombination.

1.3.1.1. Mutation

The high capacity to generate genetic diversity derives from a combination of factors. The first major factor is the high mutation rates of plant viruses. Most plant viruses have RNA genomes that encode RNA-dependent RNA polymerases, which lack proofreading activity resulting in high rates of nucleotide misincorporation (Drake and Holland, 1999). Mutation rates in RNA plant viruses, first estimated for *Tobacco mosaic virus* (TMV) (Malpica et al., 2002), are in the range of 10^{-3} to 10^{-6} nucleotide substitutions per site per round of replication, similar to those reported for RNA viruses infecting bacteria or animals (Malpica et al., 2002; Sanjuán et al., 2009, 2010; Tomas and Elena, 2010). Thus, viral mutation rates are several orders of magnitude higher than those of their host plants, estimated to be around 10^{-9} (Kay et al., 2006). High mutation rates allow viruses to explore large portions of the mutational space. The higher the mutation rate the larger the probability to generate virus genotypes fitter in new environments. However, it has been well established that most mutations in RNA plant viruses are highly deleterious, with an important fraction being lethal, whereas neutral mutations are considerably less frequent (Carrasco et al., 2007; Hillung et al., 2015). In this scenario, high mutation rates may lead to a high burden of deleterious mutations (i.e., mutational load) in the virus populations and ultimately to extinction (Chao, 1990). Here, the second major factor associated with the high genetic diversity of virus populations becomes important: plant viruses generally have large population sizes (García-Arenal et al., 2001). For instance, the number of infectious units of TMV has been estimated as about 10^7 per infected mesophyll cell of experimentally infected tobacco plants (Harrison, 1956; Malpica et al., 2002), and the number of *Tobacco mild green mosaic virus* (TMGMV) particles in field-infected *Nicotiana glauca* leaves has been estimated as 10^{11} (Moya et al., 1993). Also, the effective population size of TMGMV in *N. glauca* was estimated to be of about 10^5 (Moya et al., 1993), and that of 12 potyviruses have been estimated to be in the order of 10^4 (Hughes, 2009). As a consequence, deleterious mutations have small chances to persist in the virus population, as these will be quickly purged by negative selection (Elena and Sanjuán, 2005). However, it should be noted that factors, such as variation in

replication potential among genotypes, differences in generation time among infected cells, and severe reductions in population size at various steps during the virus life cycle, might lead to effective population sizes (roughly, the number of individuals in the population that pass their genes to the next generation) much smaller than the census size of the population. Finally, the third major factor is the much shorter generation time of RNA viruses (minutes to hours) (Wu et al., 1994) than that of plants. Thus, virus evolution occurs in different time scales from that of their host plants.

1.3.1.2. Recombination

Besides mutation, genetic diversity in virus populations can also be generated by recombination, that is, the exchange of genomic fragments between genotypes (Simon-Loriere and Holmes, 2011). Recombination can occur between genotypes of the same or of different virus species. Recombination rates have been estimated for plant viruses to be between 10^{-5} and 10^{-8} (Froissart et al., 2005; Simon-Loriere and Holmes, 2011; Tromas et al., 2014), so that the contribution of recombination to the generation of genetic diversity would be in the same order as that of mutation. Recombination may represent an evolutionary advantage for viruses because (1) it can create fitter genotypes more rapidly than mutation and (2) it might purge deleterious mutations from virus populations, thereby preventing the decrease in overall fitness in clonal populations due to the accumulation of deleterious mutations (Muller's ratchet) (Pressing and Reanney, 1984; Chao, 1990; García-Arenal et al., 2001; Hull, 2014; Moya et al., 2004). Thus, genetic exchange by recombination may have important epidemiological implications of practical relevance, and it has been associated with host jumps, host range expansion, changes in virulence, breaking of host resistance and finally, the emergence of new viral plant diseases (Pita et al., 2001; Díaz-Pendon et al., 2010).

1.3.1.3. Processes that shape the genetic diversity of plant virus populations

Mutation and recombination are a consequence of the mechanisms of virus replication, and the resulting new genotypes are therefore in principle randomly generated (but see Bujarski, 2013 for exceptions). Central to understanding the

population genetic diversity of plant viruses is which mutations become fixed in the population and how fast they do so ([Duffy et al., 2008](#)) or, more generally, what determines the frequency in the populations of the genotypes generated through mutation and recombination. In the absence of migration, the number and frequency of these genotypes in the population (i.e., the genetic structure of the population) is the result of two different evolutionary processes: genetic drift and selection ([García-Arenal et al., 2001](#); [Hartl and Clark, 2007](#); [Acosta-Leal et al., 2011](#)).

Genetic drift occurs when populations of organisms are not large enough to ensure that each genotype will have progeny in the next generation. As a consequence, the genotypes passed into the next generation are randomly sampled from the mother population, regardless of their relative fitness. Genetic drift may be particularly relevant in plant virus populations during the severe reductions in their population size (population bottlenecks) that may occur along the virus life cycle, for instance, at the infection of a new host population, a new host plant, or new organs within a host plant ([Sacristán et al., 2003](#); [Gutiérrez et al., 2012](#); [Fabre et al., 2014](#)). Genetic drift reduces the genetic diversity of populations and increases the diversity among populations. Also, because the genotypes that start a new population are not selected according to their fitness, genetic drift counters the effects of selection ([García-Arenal et al., 2001](#); [Acosta-Leal et al., 2011](#)).

On the other hand, selection is a directional process by which genotypes that are fittest in a given environment will increase in frequency in the population (positive or diversifying selection), whereas less fit genotypes will decrease in frequency (negative or purifying selection). As is the case for genetic drift, selection results in a decrease of the population diversity and may also cause an increased diversity between populations, if under different selection pressures, so that the effects of selection and genetic drift are often difficult to distinguish. When selection has been differentiated from genetic drift, selection has been associated with every life history trait of plant viruses, such as survival in the environment due to higher structural stability of the virus particles ([Fraile et al., 2014](#)), adaptation to the host plant resulting in more effective within-host multiplication ([Hillung et al., 2015](#)), and adaptation to the transmission mode resulting in more efficient between-host transmission

(Hajimorad et al., 2011; Pagán et al., 2014). These processes are associated with the colonization of new geographical areas and have been proposed as a possible explanation to the emergence of viral diseases in crops (Marco and Aranda, 2005; Moriones and Navas-Castillo, 2008). Sequence analyses of plant virus genomes indicated that selection on the encoded proteins is mostly negative (García-Arenal et al., 2001). This implies that selection plays an important role to restrict diversity (Schneider and Roossinck, 2001).

Although there is a large number of studies that analyze the genetic diversity and structure of plant virus populations, very little is known about what environmental factors shape such genetic diversity and how they affect the process of emergence (Fraile and García-Arenal, 2016).

1.3.2. Ecological factors affecting virus population genetic diversity

Ecological changes that affect infection risk may also result in genetic changes in the virus population (Grenfell et al., 2004; Archie et al., 2009; Pybus and Rambaut, 2009). Indeed, theoretical elaborations hypothesize that biodiversity may determine the genetic diversity of pathogen populations, including viruses, through changes in the pathogen's epidemiology. According to this hypothesis, the loss of biodiversity would increase host abundance and density, which in turn would lead to higher transmission rates and a higher prevalence of pathogens (Keesing et al., 2006; Burdon and Thrall, 2008; Keesing et al., 2010; Ostfeld and Keesing, 2012). As a consequence of these epidemiological changes, pathogen populations would increase in size, accelerating their evolutionary rates (Scholle et al., 2013; Lanfear et al., 2014), which could ultimately lead to higher genetic diversity.

As shown in the previous sections, there is increasing evidence linking the loss of ecosystem biodiversity to higher transmission rates and prevalence of viruses and other pathogens (reviewed in Keesing et al., 2010; Ostfeld and Keesing, 2012; Alexander et al., 2014; Pagán et al., 2016). However, the understanding on how the loss of biodiversity modulates the genetic diversity of virus populations is scarce and relies mostly on indirect evidence (Holmes, 2009; Scholle et al., 2013; Lanfear et al., 2014). This is especially so in the case of plant viruses (Alexander et al., 2014), for which most of the existing evidence

comes from analyses of plant-virus interactions under different levels of ecosystem anthropization, which is also the major cause of biodiversity loss (Jones, 2009; Keesing et al., 2010). At odds with the hypothesis above, comparative genomics analyses of geminivirus species showed a reduction of virus genetic diversity and of recombination rates with increased ecosystem anthropization (Silva et al., 2011, 2012; Lima et al., 2013). On the other hand, inferences of divergence times for several groups of plant viruses have traced their radiation to the origin or the intensification of agriculture (Fargette et al., 2008; Duffy and Holmes, 2008; Gibbs et al., 2008; Pagán and Holmes, 2010, but see Lefeuvre et al., 2010; Yasaka et al., 2014). These works proposed that such bursts of genetic diversity could have co-occurred with the intrinsic loss of biodiversity characteristic of agroecosystems. Altogether, these results suggest that the effect of ecosystem anthropization, and the concurrent biodiversity loss, on the genetic diversity of plant viruses may depend on the relative importance of mutation and recombination. In agreement with this idea, Rodelo-Urrego et al., 2015 showed that, in wild pepper-infecting begomoviruses, recombination was more important as a mechanism of generating genetic diversity in habitats with higher biodiversity than in those with lower biodiversity. These apparently contradictory results mostly derive from analyses that combine viral sequences obtained from different hosts, and from different geographic locations. Hence, it is difficult to disentangle whether the reported changes in plant virus genetic diversity are indeed due to ecosystem biodiversity or the result of host and/or local adaptation processes. As a consequence, it is still unclear how biodiversity loss affects the genetic diversity of plant virus populations.

1.3.3. Virus emergence and speciation

The process of genetic diversification of the virus population may result in the emergence of new diseases caused by the appearance of new virus species (i.e. “speciation” events) (Huyse et al., 2005; Elena et al., 2011). Viral speciation can occur either sympatrically or allopatrically. Sympatric speciation would occur when viruses diversify and adapt to different niches within the same host species (maybe using different cell types, establishing different seasonalities, etc). In contrast, allopatric speciation represents the genetic diversification that

occurs in different host species, either by cross-species transmission (i.e., host jumping) or codivergence with hosts over extended time periods ([Kitchen et al., 2011](#)).

Evolutionary processes at two levels have been proposed to equally determine the mode of virus speciation: “microevolutionary” processes, that is, those that shape intra-species and intra-host genetic diversity, and “macroevolutionary” processes, i.e. those affecting inter-host or inter-species genetic diversification ([Huyse et al., 2005](#)). Both genetic and ecological factors are thought to shape these processes ([Huyse et al., 2005](#); [Pagán et al., 2016](#)). However, to date only a reduced number of studies have analysed speciation at both scales. Most of these works have focused in fungi and bacteria ([Buckling and Rainey, 2002](#); [Giraud et al., 2010](#)), and few in host-virus systems ([Pagán and Holmes, 2010](#); [Kitchen et al., 2011](#)). Thus, analysis of plant virus speciation and the role of ecological factors involved at both scales remains under investigated, particularly in wild ecosystems.

1.4. Wild ecosystems in the Iberian Peninsula

The Iberian Peninsula (582,000 km²) is located in the southwest corner of Europe. From a biogeographical perspective, the Iberian Peninsula can be divided into two regions with differentiated ecological features, Eurosiberian and Mediterranean ([Rivas-Martínez, 2004](#)) (**Figure 1.2**).

Figure 1.2. Biogeographic map of Europe ([Rivas-Martínez et al., 2004](#)).

The Eurosiberian region covers the northern third of the peninsula, which is under the influence of a mild and humid Atlantic climate. The Mediterranean region spans the remaining territory and is characterized by a Mediterranean climate with significantly drier and warmer conditions than the Eurosiberian region (Rivas-Martínez, 2004).

As a consequence of its biogeographical location, in the transition between the Eurosiberian and Mediterranean regions, the Iberian Peninsula has remarkable forest diversity. There is a wide knowledge of the Iberian ecosystems in terms of its vegetation structure and climate-based floristic composition (e.g. Gavilán and Fernández-González, 1997; Gavilán et al., 1998; Gavilán, 2005; Rivas-Martínez, 2007). Particular biotic strategies predominate in the forests of each of these two biogeographical regions (**Figure 1.3**).

Figure 1.3. Map of potential vegetation in Spain. Modified from “Mapa de Series de Vegetación de España” (Rivas-Martínez, 1987). Departamento de Geografía y Ordenación del Territorio. Universidad de Zaragoza.

The forests of the Eurosiberian region are determined by the need of overcoming the winter cold that constitutes the most unfavorable factor. Thus,

Eurosiberian forests are dominated by deciduous species. On the other hand, the conditions of the Mediterranean region favor complex mosaics of plant communities of evergreen, deciduous and/or mixed forests, scrublands or natural grasslands. In this region, rainfalls are irregularly distributed over time. Thus, xerophytic perennial plant species adapted to survive long periods of drought have achieved greater success (Blanco et al., 2005). Within the Mediterranean region, there are patches of Atlantic climate occupying particularly humid areas for microclimatic or edaphic reasons, specifically in mountain areas or river basins. These Atlantic patches have the capacity to allow the introgression of plant species typical of humid climatic regions into more arid ones (Stella et al., 2013) (Figure 1.3).

According to the Coordination of Information on the Environment (CORINE) Agency Land Cover Map of 2012 (Figure 1.4), more than a third (34%) of Spain territory is covered by croplands, while almost another third (26%) is covered by forested lands. Semi-natural vegetation, that includes shrub and/or herbaceous vegetation associations, represents 21% of the total, followed by pastures (13%), while the least common forms of cover are water areas (1%) and wetlands (0.2%).

Figure 1.4. Map and percentage of land cover types in Spain. According to CORINE (Coordination of Information on the Environment), 2012 (<https://www.eea.europa.eu>).

This thesis focused on the Mediterranean biogeographical region, which is the larger in the Iberian Peninsula (**Figure 1.3**). Specifically, the area studied is located in central Spain (Meseta). The Meseta is a large plateau situated at 600 m of elevation, and is traversed by the Spanish Sistema Central, a mountain range that divides the Duero and Tajo watersheds. As mentioned above, this region is under a Mediterranean-Continental climate, which is characterized by extreme temperatures and strong diurnal and seasonal thermal oscillations. Precipitations in this climate are irregular, these being more frequent in spring and autumn, and very scarce in summer with high rates of evaporation that leave the land arid ([Loidi, 2017](#)). The thesis focuses on two forest types in this region: evergreen oak forests and riparian forests. The former is the most representative and abundant forest in the Mediterranean region of the Iberian Peninsula; the later forest is representative of the Atlantic climate patches embedded in the Mediterranean region. Their distribution and their different environmental conditions influence the adaptive responses, structure and floristic composition of plant communities in both types of forests. Hence, they are characterized by diverse ecological conditions in terms of biodiversity, floristic composition and climatic conditions ([Blanco et al., 2005](#)) that, as mentioned previously, can potentially affect plant virus infection risk and virus population genetic diversity. Thus, these forests represent a suitable system to analyse how ecological conditions affect pathogen emergence. Moreover, both types of forests are frequently bordering crop fields (**Figure 1.4**). Thus, these two ecosystems are potential sources of inoculum for the emergence of diseases in the crops. Therefore, understanding the ecological factors that favor the emergence of viral diseases in evergreen oak and riparian forests may provide relevant information for the development of conservation policies, and also for the prevention of emerging diseases in crops.

1.4.1. Riparian forest

Riparian forests typically appear adjacent to riverbeds (**Figure 1.3**). The presence of the river causes important changes in the ecological conditions of the adjacent lands. The most remarkable one consists of a greater water availability, which leads to an increased relative humidity as a consequence of

higher evapotranspiration, higher soil moisture, and particular soil textures/pH. There are also changes in the thermal regime: the maximum temperatures are attenuated due to the higher energy consumption in the evapotranspiration process. These conditions allow riparian forests to avoid the consequences of the summer drought characteristic of the Mediterranean climate. As a consequence, in this forest lives vegetation typical of the Atlantic environments, along with the riparian vegetation of Mediterranean distribution (Blanco et al., 2005). This partly explains the high floristic diversity and the structural complexity of Mediterranean riparian forests (Stella et al., 2013).

The diversity of plant species found in riparian forests is generally organized according to the distance to the riverbed and therefore to the amount of water available. So that, the plant communities most specialized will happen on the water and near the riverside; families such as the *Ranunculaceae*, *Juncaceae*, *Cyperaceae* or grasses such as the reed (*Phragmites australis*) among others, constitute the dominant herbaceous stratum in this sector. Also, communities of clovers (e.g. *Trifolium pratense*, *Trifolium campestre*) develop together with other species such as *Rumex crispus*, *Rumex conglomeratus* and mints (e.g. *Mentha longifolia*). Among the vegetation of the shrub layer, members of the family *Rosaceae* such as *Crataegus*, *Rosa* or *Rubus* species are also important. Also near to the river course, arboreal and/or shrub vegetation (e.g., *Salix eleagnos*, *Salix cinerea*) resistant to flooding can be found contributing to the fixation of fluvial deposits. Finally, forests linked to conditions of greater climate stability on the riverbed, but less dependent on water, are composed mainly of deciduous trees as poplars (*Populus nigra*), willows (*Salix atrocinerea*), alders (*Alnus glutinosa*), ash (*Fraxinus angustifolia*), elm (*Ulmus minor*) and sometimes by Pyrenean oak (*Quercus pyrenaica*). Climbing plants play also an important role in the structure of riparian vegetation and reach great diversity and development due to the more humid environmental conditions. Some examples among this diversity are *Hedera helix*, *Humulus lupulus*, *Lonicera periclymenum* subsp. *hispanica*, *Rosa sempervirens*, *Rubus* spp., *Solanum dulcamara*, *Tamus communis* or *Vitis vinifera* subsp. *Sylvestris* (Blanco et al., 2005).

The riparian vegetation communities delay the speed of water in floods, protect the stability of adjacent lands, and enable the deposit of nutrients

transported by water, thus contributing to increase the soil fertility and the development of higher plant density and biomass. As a consequence, large parts of the remaining riparian forests of the Tajo and Duero rivers in central Spain are protected. Still, the lands of riparian influence are often used for the installation of orchards, pastures and arboreal/herbaceous crops of high yield, such that cultivated lands can be found near protected areas (Virgós, 2001).

1.4.2. Evergreen oak forest

Holm oak or evergreen oak (*Quercus ilex* L.) forests are widely distributed in the Mediterranean basin (Figure 1.5). Approximately 60% of this type of forest is located in the Iberian Peninsula where they represent the most characteristic forests (Ramírez and Díaz, 2008; Patón et al., 2010). In Spain, evergreen oaks dominate in about 3 million ha, i.e. 25% of the area where trees cover at least 10% of the ground (Rodà, 1999) (Figure 1.5). In the geographical areas dominated by evergreen oaks, plants have to cope with a selective pressure resulting from multiple climatic stresses: winter cold, irregularity in the rainfall distribution, and specially the coincidence of the period of maximum drought with the higher temperatures in summer. From the edaphic point of view, the lack of nutrients can also be a contributing stress factor. All these conditions have determined the morphological and physiological evolutive responses of the plant communities. These forests are formed mainly by broad-leaved sclerophyll vegetation, characterized by hard, evergreen foliage that is specially adapted to prevent moisture loss and to favor the storage of nutrients (Rodà, 1999).

The extreme conditions of the Mediterranean climate determine the structural and floristic composition of the evergreen oak forests in Central Spain. In this region, they are mainly monospecific forests with the dominance of holm oak trees in the arboreal stratum, which usually do not exceed 10m in average height. Beneath the evergreen oak forest canopy, the influence of climate also causes a marked reduction of the species diversity, resulting in lower plant biomass than in riparian forests (Blanco et al., 2005; Stella et al., 2013). Among the most typical and abundant species under the canopy are: *Daphne gnidium*, the wild asparagus (*Asparagus acutifolius*), the gum rockrose

(*Cistus ladanifer*), and *Thapsia villosa* (Blanco et al., 2005). The clearings of the evergreen oak forest have sandy soils and are very poor in organic matter. These clearings are occupied by different shrub formations adapted to sun exposure (heliophilous). Rockroses such as *Cistus ladanifer* in acid soils or *Cistus laurifolius* in mountain areas, and *Retama sphaerocarpa* in the most clayey soils becomes the predominant shrub element, being the most characteristic substitution stages of the evergreen oaks. When the soil degradation progresses, other shrubs such as the lavender (*Lavandula stoechas* subsp *pedunculata*) and thyme (*Thymus mastichina*) can be found along with the rockroses (Blanco et al., 2005). Also, clearings typically sustain Mediterranean annual plants. The most frequent species of these communities are *Tuberaria gutatta* and annual species of grasses, legumes and asteraceans that constitute the herbaceous stratum.

Figure 1.5. Distribution range of *Quercus ilex* in the Mediterranean basin (Blanco et al., 2005).

Evergreen oak forests have also great economic value, almost one million ha of the area occupied by these forests is used for firewood, and another 1.3 million ha are "dehesas", a savannah-type man-managed formation. In these formations, *Q. ilex* is considered as a fruit tree and has been selected for sweet acorn production, which is used to supplement feed for pigs (Pulido et al., 2001). In the remaining areas, evergreen oak forests can be regarded as woodlands that have undergone very low or no silvicultural management (Costa et al., 2011; Loidi, 2017), and that lay frequently adjacent

to cultivated fields. This thesis focuses in this second type of evergreen oak forests as they can be considered as largely wild ecosystems.

1.5. The genus *Potyvirus*

The genus *Potyvirus*, named after its type member, *Potato virus Y* (PVY), is the largest genus in the family *Potyviridae*, containing 90% of its species. Potyviruses are one of the two largest genera of plant viruses (represents ~30% of all known plant viruses), the other being the begomoviruses (King et al., 2012). Viruses of the genus *Potyvirus* are characterized by common biological and genomic features: they form cytoplasmic inclusion (CI) bodies, are transmitted by vectors, share a genome structure and have similar nucleotide and amino acid sequence in the coat protein. Species and strains of this genus are differentiated on the basis of natural host range, pathogenicity and cytopathology, mode(s) of transmission, antigenic properties and genome sequence relatedness (Barnett, 1992). Replication of these viruses appears to be typical for (+) ssRNA viruses, where the plus strand is transcribed to produce a (-) RNA strand, which then serves as template for the production of progeny (+) strand RNA molecules. This process probably occurs in the cytoplasm (Mäkinen and Hafrén, 2014).

1.5.1. Molecular biology

Potyvirus virions are flexuous filaments, non-enveloped, rod-shaped particles of 680–900 nm long and 11–15 nm wide, with helical symmetry. Virions contain a single molecule of linear, positive sense single-stranded RNA (ssRNA), about 10,000 nucleotides, surrounded by about 2,000 units of coat protein (CP). The RNA genome carries a VPg (viral protein genome-linked) protein covalently bound to its 5'-end, which is encoded in the virus genome. The 3'-end of the genome is polyadenylated with a variable number of adenosines. The genome is characterized by a single major open reading frame (ORF) that encodes a polyprotein of about 340-370 kilodalton (KDa), which is subsequently cleaved by three virus-coded proteinases into ten functional proteins (**Figure 1.6**): the first protein (P1), the helper component protease (HC-Pro), the third protein

(P3), the 6K1 protein, the cylindrical inclusion protein (CI), the 6K2 protein, the viral protein genome-linked (VPg), the small nuclear inclusion protein (NIa), the large nuclear inclusion protein (NIb) and coat protein (CP). Gene order and protein sequences are conserved throughout the family *Potyviridae* (King et al., 2012) (**Figure 1.6**). Two additional proteins, P3N-PIPO and P3N-ALT, are originated through frameshifts in the P3 cistron (Chung et al., 2008; Hagiwara-Komoda et al., 2016), although they are not present in all members of the genus.

Figure 1.6. Potyvirus genome organization: A, Schematic diagram of a potyvirus particle (Left) and negative contrast electron micrograph of particles of an isolate of *Plum pox virus* (Right) (From King et al., 2012). B, genomic map of members of the genus *Potyvirus* (modified from Rodríguez-Nevado et al., 2017): The blue box represents the polyprotein ORF. The alternate P3N-PIPO ORF, depicted by the small yellow box above the main reading frame, is putatively translated by +2 frameshift of the polyprotein ORF, and its product is expressed as a fusion with the N-terminal part of P3. Functions associated with the mature proteins proteolytically processed from the polyprotein are shown. Autocatalytic cleave sites of the HC-Pro and P1 gene products are indicated by green triangles, and NIa-Pro cleavage sites are marked by blue triangles. VPg, genome-linked viral protein covalently attached to the 5'-terminal nt.

Most of the mature virus proteins are multifunctional (**Figure 1.6**). The P1 corresponds to the N-terminal gene of the potyvirus genome and encodes the first of three viral proteinases, cleaving the polyprotein at the P1/HC-Pro junction (Verchot et al., 1992). Together with P3, P1 is the least conserved

protein among potyviruses, except for conserved amino acids found at the C-terminus of all potyvirus P1 proteins and which correspond to the proteinase catalytic domain. Recently, it has been demonstrated that the N-terminal region of P1 controls early replication of potyviruses ([Pasin et al., 2014](#)). It has been also shown that the P1/HC-Pro fusion carries a potential pathogenicity enhancer, which is involved in suppression of host defense ([Kasschau and Carrington, 1998](#); [Rajamäki et al., 2005](#); [Valli et al., 2007](#); [Pasin et al., 2014](#)). A new small ORF, called PISPO, has been identified inside the coding sequence of the P1-coding sequence of some potyviruses that infect sweet potato. This ORF could be translated by a frameshift similar to that involved in the expression of the PIPO ORF ([Clark et al., 2012](#); [Li et al., 2012](#)). HC-Pro is the second viral proteinase, cleaving the polyprotein at the HC-Pro/P3 junction ([Carrington et al., 1989](#)). HC-Pro protein is known to suppress RNA silencing and stimulate viral genome amplification and long-distance movement ([Kasschau et al., 2003](#); [Soitamo et al., 2011](#)). Recent results have revealed that HC-Pro is required to stabilize the CP for the proper yield and infectivity of potyviral progeny ([Valli et al., 2014](#)). Like P1, P3 has also been proposed to be involved in virus replication ([Cui et al., 2010](#)) and, together with 6K2, in virus movement and symptom development ([Spetz and Valkonen, 2004](#)). Based on the fact that the proteolytic splitting between P3 and 6K1 is not essential for virus infectivity, it has been hypothesized that P3–6K1, rather than P3 and 6K1, might be the main functional product, and its proteolytic processing would have a regulatory role ([Riechmann et al., 1995](#)). However, processing at the P3–6K1 junction still affected symptom expression, suggesting that 6K1 alone may have a relevant role in potyviral infection ([Waltermann and Maiss, 2006](#)). The CI protein is a multifunctional protein ([Sorel et al., 2014](#)). This protein has ATPase and RNA helicase activities, which are required for virus RNA replication ([Fernández et al., 1997](#)). The CI protein acts in collaboration with P3N–PIPO in aiding virus movement ([Wei et al., 2010](#)). Furthermore, the CI protein acts as a virulence factor for different resistance genes ([Sorel et al., 2014](#)). The 6K2 protein is an integral membrane protein associated to VPg in endoplasmic reticulum-derived membranes ([Schaad et al., 1997](#); [Léonard et al., 2004](#)) forming cytoplasmic vesicles which are viral replication sites ([Cotton et al., 2009](#)). Also, 6K2 protein is found as a polyprotein with the VPg and the NIa

protein (6K2–VPg–NIaPro product) in infected plants (Restrepo-Hartwig and Carrington, 1994). NIa is the largest protein and is partially processed to produce VPg and NIaPro (Dougherty and Parks, 1991). The free VPg is the major form of protein linked to the 5'-end of the genomic RNA although the complete NIa (VPg–NIaPro) has also been detected at the end of the RNA (Mathur and Savithri, 2012). When VPg is part of the NIa, it is localized both in the cytoplasm and in the nucleus of the infected cells (Beauchemin et al., 2007; Rajamäki and Valkonen, 2009) whereas when it is part of the 6K2–VPg–NIaPro product, VPg is targeted to membranous factories induced by the virus where it plays a key role in viral RNA replication (Beauchemin et al., 2007; Wei and Wang, 2008). VPg contains a nucleotide-binding motif and, when it is bound to the NIaPro domain, preferably in cis, has NTPase activity (Mathur and Savithri, 2012). The NIaPro is the protease domain responsible for the proteolytic processing of the central and C-terminal regions of the potyviral polyprotein (Adams et al., 2005). NIb is the RNA-dependent RNA polymerase, or RNA replicase, responsible for potyviral genome replication (Hong and Hunt, 1996). NIb could play be important for potyviral infection through the interaction with proteins that regulate diverse cellular processes in the nucleus of the infected cells (Xiong and Wang, 2013). Finally, the CP is the only structural protein. It is involved in the encapsidation of the viral RNA, but has also been implicated in genome amplification, movement, aphid transmission (Wylie et al., 2002) and in the regulation of viral RNA amplification (Bhat et al., 2013; Zhao et al., 2013)

1.5.2. Ecology, epidemiology and disease control

Potyriviruses have a worldwide distribution and infect species of most angiosperm families. Most potyriviruses infect dicotyledonous plants, with only a few of them infecting monocotyledonous hosts. Most virus species of the genus may cause a large range of macroscopic symptoms, which in many cases result in serious diseases of crops with high socio-economical relevance (e.g. Walsh and Jenner, 2002; Quenouille et al., 2013; Rybicki, 2015). In the Iberian Peninsula, potyriviruses are important crop pathogens, causing severe losses in commercially important plant species of various botanical families (Melgarejo et al., 2004) such as: *Asteraceae* and *Brassicaceae* (Moreno et al., 2004a),

Boraginaceae and *Fabaceae* (Luis-Arteaga et al., 1996), *Cucurbitaceae* (Luis-Arteaga et al., 1998; Moreno et al., 2004b), *Poaceaceae* (Achon and Sobrepere, 2001) or *Solanaceae* (Feres et al., 1993; Navas-Castillo et al., 1999). In these hosts, potyviruses induce visible symptoms, often causing stunting, chlorotic vein banding, mosaic mottling, necrosis, and/or distortion of leaves. In monocotyledonous hosts, potyviruses induce longitudinal chlorotic or necrotic streaks in the leaves. Flowers, seeds, and fruits are also affected by numerous potyviruses (Shukla et al., 1994). The nature and extent of symptoms for a specific host genotype depend on the particular virus strain, as well as on environmental conditions, probably through their influence on host physiology and development (Revers et al., 1999). There are examples of potyviruses involved in cases of synergism with other unrelated viruses, resulting in symptoms that are more severe than those induced by either virus alone (Pruss et al., 1997; Sikora et al., 1998).

Potyviruses are horizontally transmitted by aphids in a non-persistent manner and are transmissible experimentally by mechanical inoculation. Some viruses are also transmitted vertically through the seeds (King et al., 2012). Non-persistent virus transmission is characterized by very short acquisition and inoculation times of seconds to minutes. Thus, once an aphid has left a virus-infected source plant and begun feeding on an uninfected plant, its ability to transmit virus is short-lived (Ng and Perry, 2004). Usually, a number of aphid species are capable of transmitting a particular potyvirus, and thus the level of vector specificity is relatively low. Hence, in a given host population potyvirus epidemiology is determined by the predominant aphid species and ecological and climatic conditions (Feres et al., 1993; Sikora et al., 1998). The presence or absence of aphids may affect initial levels and distribution of potyviruses in a plant community, and subsequent vector activity will influence further distribution of the viruses within and between plant communities (Pleydell et al., 2018). Hence, in general, higher potyvirus prevalence will coincide with periods where vectors are abundant (Feres et al., 1993; Katis et al., 2006; Chatzivassiliou et al., 2016). Other factors that may favor potyvirus epidemics are the emergence of more aggressive virus strains (Larsen et al., 2008; Desbiez et al., 2009), the traffic of infected plant material in a global market (García et al., 2014), or the introduction of new hosts in a geographical area

that act as virus sources when in the proximities of susceptible crops ([Hobbs et al., 2000](#)).

Control of potyviral diseases is difficult. Insecticide sprays for aphids are used by farmers to control potyvirus spread. However, these are generally ineffective because of the rapid acquisition and inoculation periods required by aphid vectors, such that vectors transmit the virus before the insecticide acts. In some cases, insecticides can even speed up virus spread by increasing vector activity ([Dedryver et al., 2010](#)). Cross-protection using mild virus isolates that can protect plants against a severe isolate of the same virus, appears to be a more durable strategy for controlling potyvirus diseases ([Yarden et al., 2000](#); [Zhou, 2012](#)). However, there are a number of limitations in cross-protection use, which include practical and safety concerns ([Lecoq and Raccach, 2001](#)). The most effective and simplest method of control of potyvirus diseases is through the use of host plant resistance. This has been a useful approach and has provided stable resistance to several potyviruses ([Lecoq et al., 2004](#); [Gómez et al., 2009](#)). A less commonly used approach has been to breed crop cultivars with resistance to vectors, which has the potential added benefit of reducing losses associated with vector feeding damage ([Dogimont et al., 2010](#)). Methods of genetic engineering have been used to introduce genes for virus resistance obtained from novel sources. Some examples of this strategy include cultivated vegetables with coat protein-mediated resistance to several potyvirus species ([Lindbo and Falk, 2017](#)).

Besides the damage that potyviruses can cause in crops, some of them have been reported to infect weeds (e.g. [dos Santos Martins et al., 2016](#); [McLeish et al., 2017](#)) and wild plant species ([Malpica et al., 2006](#); [Pagán et al., 2010](#)), and symptoms induced by potyviruses in wild plant communities are not always obvious ([Rodríguez-Nevado et al., 2017](#)). Few studies have investigated potyvirus presence in wild ecosystems of the Iberian Peninsula, and such studies often focused in potyviruses known to be crop pathogens ([McLeish et al., 2017](#), but see [Rodríguez-Nevado et al., 2017](#)). These studies indicate that, in wild ecosystems, potyviruses may be important ecological agents influencing the dynamics of the populations of their hosts. In addition, given that agricultural lands occupy more than a third of Spain territory, evergreen oak forests and other natural ecosystems are often adjacent to agroecosystems (see **section**

1.4), which favours plant virus dispersal between both types of ecosystems (Alexander et al., 2014). Thus, potyviruses present in wild ecosystems may have the potential to cause epidemics in adjacent crops (McLeish et al., 2017; Rodríguez-Nevado et al., 2017). Despite this ecological and economic relevance, the factors affecting potyvirus infection risk and population genetic structure in wild ecosystems remain largely unexplored.

1.5.3. Genetic diversity and evolution

As previously stated, potyviruses comprise the largest and economically most important genus of plant RNA viruses. The genus *Potyvirus* characterized by a very large species and genetic diversity. Potyviruses are emerging in new geographic regions as potential epidemic pathogens (some recent examples in Rodríguez-Nevado et al., 2017 and Desbiez et al., 2017). For this reason, it is important to gain a better understanding of the origin, biogeographical diversification and evolution of potyviruses. Phylogenetic analyses based on the potyvirus CP gene for approximately half of all known potyvirus species indicated that the genus *Potyvirus* probably emerged from a virus of monocotyledonous plants about 6,600 years ago in Southwest Eurasia or North Africa, coinciding with the dawn of agriculture (Gibbs et al., 2008). Derived from the dating of the origin of potyviruses, it has been suggested that conditions generated by agriculture and a propitious climate enabled potyviruses and their vectors to spread, speciate and attain their current dominance (Gibbs et al., 2008).

Sequenced isolates has been obtained from experimental and natural populations in order to analyse how potyvirus populations generate genetic diversity. The evolutionary rate estimated for potyviruses is of 1.15×10^{-4} nucleotide substitutions/site/year (Gibbs et al., 2008), a value within the range of RNA viruses of both plants and animals (Duffy et al., 2008). This estimate derives from a data set containing sequences from potyvirus species in which recombination has not been detected. It is unclear the relative importance of recombination for the generation of genetic variation in potyvirus populations. Although in general, mutation plays a central role (Tromas and Elena, 2010), several studies have shown that recombination events are especially common

in some potyviruses ([Chare and Holmes, 2006](#)). Indeed, both intraspecies ([Bousalem et al., 2003](#); [Ohshima et al., 2002](#); [Moreno et al., 2004b](#)) and interspecies ([Desbiez and Lecoq, 2004](#); [Larsen et al., 2005](#); [Gell et al., 2015](#)) recombination events are involved in potyviral evolution. Experimental studies indicated that potyvirus populations are genetically stable and seem to evolve under strong purifying selection ([Schneider and Roossinck, 2001](#)). However, genetic drift may be important also in the evolution of potyvirus populations ([French and Stenger, 2003](#); [Hughes, 2009](#)). Despite all this effort in characterizing the genetic diversity of potyviruses, very little is known about what ecological factors determine changes in genetic diversity of potyvirus populations and on the relationship of these changes with virus emergence ([Rodríguez-Nevado et al., 2017](#)).

2. OBJECTIVES

The main objective of this thesis is to analyse the ecological determinants of plant virus infection risk and population genetic diversity in wild ecosystems, using as a model the virus species of the genus *Potyvirus* present in two wild ecosystems of the Iberian Peninsula: riparian and evergreen oak forests. To achieve this general objective, the following specific objectives will be addressed:

1. Ecological characterization of evergreen oak and riparian forest locations in the central plateau of the Iberian Peninsula; and identification of *Potyvirus* species, and analysis of their host range and prevalence in these ecosystems.
2. Analysis of the ecological factors affecting genus-wide potyvirus prevalence in evergreen oak and riparian forests.
3. Analysis of the ecological factors affecting potyvirus species-specific prevalence according to their host range: specialist (narrow host range) vs. generalist (wide host range).
4. Study of the ecological and epidemiological factors affecting the population genetic diversity, and the speciation mode, of the potyviruses present in riparian and evergreen oak forests at the genus-wide (macroevolution) level.
5. Study of the ecological and epidemiological factors affecting the population genetic diversity of the potyviruses present in riparian and evergreen oak forests at the species-specific (microevolution) level, and according to their host range.

3. MATERIALS AND METHODS

3.1. Plant sampling and data collection in wild ecosystems

3.1.1. Description of sampling locations

Field samplings were done in two different wild ecosystems: evergreen oak forests and riparian forests. Five locations of evergreen oak forests and five locations of riparian forests were visited during 2013, 2014 and 2015. The locations were distributed in a transect of 300 km (north-south) in the center of the Iberian Peninsula encompassing the Duero and Tajo watersheds and the provinces of Valladolid, Segovia, Madrid, and Toledo. The sampled locations are listed in **Table 3.1** and their location in the Iberian Peninsula is represented in **Figure 3.1**.

Table 3.1. Locations of Iberian evergreen oak forests and riparian forests visited during 2013, 2014 and 2015 in this thesis.

Ecosystem	Location ¹	Province	Latitude (N°)	Longitude (W°)	Elevation (m)
Evergreen oak forest	CARBONERO EL MAYOR (CEM)	Segovia	41.148	-4.318	928
Evergreen oak forest	EL PARDO (PAR)	Madrid	40.501	-3.740	616
Evergreen oak forest	MONTEGANCEDO (MON)	Madrid	40.403	-3.836	713
Evergreen oak forest	CENICIENTOS (CEN)	Madrid	40.259	-4.449	773
Evergreen oak forest	MARJALIZA (MAR)	Toledo	39.578	-3.922	853
Riparian forest	POLLOS (POL)	Valladolid	41.444	-5.148	679
Riparian forest	PALAZUELOS (PAL)	Segovia	40.921	-4.052	1083
Riparian forest	RASCAFRÍA (RAS)	Madrid	40.890	-3.884	1162
Riparian forest	EL ESCORIAL (ESC)	Madrid	40.574	-4.153	913
Riparian forest	SANTA CRUZ DE RETAMAR (SCR)	Toledo	40.279	-4.231	589

¹ Name of the nearest town and three-letter code (in parenthesis) indicating the name of the sampled locations.

The evergreen oak forest locations were chosen to be representative of this ecosystem. That is, to include natural woodland areas dominated by trees and shrubs and with clearings where herbaceous species are abundant. The riparian forest locations were chosen to be closer than three meters from the nearest riverside. The predominant tree species on the riverside was not considered a criterion for selecting these locations. Locations in this study were selected such that there was no evidence of human management.

Figure 3.1. Geographic distribution of the evergreen oak (green dots) and the riparian forest (blue dots) locations visited in this study. Color scale indicates the elevation gradient. Abbreviations for each location are as in Table 3.1.

3.1.2. Plant sampling design

At each location, a plot of 25x25 meters was defined, and within this plot a random-systematic sampling was performed. This sampling design consisted on selecting a random starting point in the plot and then collecting samples in a fixed, periodic interval called sampling interval ([Barbour et al., 1999](#)). In this work, the 25x25 plot was divided in a grid of 1x3 meters (**Figure 3.2**). At each of these rectangles or sampling intervals, plant material from the most abundant species was harvested. If the most abundant species was a tree or a shrub, at least three leaves from different branches of one individual of such species was collected. If the most abundant species was herbaceous, the stem and leaves of an individual were harvested. A total of 200 samples were collected at each location. Samplings were performed during the months of spring (April and May), summer (June and July) and autumn (October and November) to account

for seasonal differences in plant species composition and phenological stage, and in aphid activity. A total of 12,000 samples were collected in these surveys.

Figure 3.2. Sampling design performed in the monitored locations. A plot of 25x25 meters divided in a grid of 1m². Red rectangle indicates the spatial unit of sampling (3x1m interval), and the arrow indicates sampling direction. Green squares represent the eight quadrants of 1m² randomly selected for quantification of plant biomass (see [section 3.1.3](#)).

3.1.3. Monitoring and estimation of ecological factors

At each visit, the following information was collected in the five evergreen oak and the five riparian forests locations:

1) Plant biodiversity: In parallel with plant sampling, additional individuals representative of each collected plant species were harvested and inventoried in herbariums. These herbariums were used to identify the botanical family and species of each collected individual. Plant identification was performed by Professor Rosario G. Gavilán (Plant Biology Department, Universidad Complutense de Madrid, Madrid, Spain). This allowed determining the number

of species, and the number of individuals per species collected at each location and visit. Using this data, plant species richness and various diversity indexes that consider both species richness and evenness were calculated as measurements of plant species diversity, which was used as a proxy of plant biodiversity:

i) Species richness (S): Is the simplest way of measuring plant biodiversity, and it is defined as the number of plant species present in an ecosystem/location.

ii) Shannon's diversity index (H'): This diversity index provides more information about plant community composition than S, as it reflects the heterogeneity of a community based on two factors: the number of species present and their relative abundance (Shannon, 1948). In order to obtain the Shannon's index, the relative abundance of each species (p_i) is calculated, and then multiplied by its natural logarithm ($\ln p_i$). The resulting product is summed across species (S), and multiplied by -1. The higher the value of H', the greater the diversity:

$$H' = - \sum_{i=1}^n p_i \ln p_i$$

In this thesis, the relative abundance of a given plant species (p_i) was estimated as the number of individuals of the species i (n_i) relative to the total number of individuals collected at each location and visit ($n=200$):

$$p_i = n_i / n$$

iii) Shannon's equitability index (E_H): This is a modification of H' that allows exploring whether all the species have similar relative abundances or some of them exhibit pronounced dominance in the ecosystem/location. It is calculated by dividing H' by $H'_{\max}$ (here, $H'_{\max} = \ln S$). Equitability acquires a value between 0 (one species is dominant) and 1 (abundance evenness between species).

2) Life Cycle: Plants were classified according to their life cycle in two categories: perennial and annual plants. This information was obtained from Real Jardín Botánico (Consejo Superior de Investigaciones Científicas, CSIC) (<http://www.floraiberica.es/>).

3) Plant biomass: At each location and visit, eight quadrants of 1m² were randomly selected and photographed (**Figures 3.2 and 3.3**), and these photographs were used to calculate the plant coverage using Image J v1.5 (Schneider et al., 2012). Briefly, the green fraction of each photograph was selected using the "Threshold Color" option, and considered to be the area occupied by living plants. This provided an estimate of the vegetation coverage of each quadrant. Also, in the eight quadrants the maximum height of living plants was measured at three points across the diagonal of the quadrant (**Figure 3.3**). Values of plant coverage and height were used to calculate the apparent biovolume (V), which is the most common measure for aerial biomass (Castro et al., 1996). The plant biovolume at each quadrat was calculated as the mean of the three plant height measures multiplied by the vegetation coverage. Plant biovolume in the 25x25m plot (m³) was estimated by averaging values in the eight quadrants at each location and visit.

Figure 3.3. Schematic representation and examples of 1m² quadrants used to quantify plant biomass. (A) Red dots across the diagonal of the grey square indicate the three points in which plant height was measured. (B to D) Photographs of 1m² quadrants taken in the same location at different seasons.

4) Plant Density: This trait was estimated for each species using distance methods. These methods can be used to determine whether plants of the same

species are distributed randomly, regularly, or are clumped (Barbour et al., 1999). In this work, the Mean Euclidean nearest neighbor distance (ENN_MN) was used. ENN_MN was estimated using the spatial pattern analysis program Fragstats v4.2.1 (McGarigal et al., 2012). The ENN_MN is defined using simple Euclidean geometry as the mean of the shortest straight-line distance between an individual of a plant species and its nearest neighbor of the same species. The larger this distance, the lower the plant density. This index could be estimated because the sampling scheme described above allowed identifying the position of each sampled plant within the 25x25m plot.

5) Climatic variables: Data related with temperature (°C), relative humidity (%) and rainfalls (mm) at the months in which locations were visited were retrieved from the closest meteorological stations to each location, which were at a similar altitude (Table 3.2). This data was provided by the Agencia Estatal de Meteorología (AEMET, Spain, <http://www.aemet.es>).

Table 3.2. Meteorological stations from which climatic variables of evergreen oak and riparian forests locations were obtained.

Ecosystem	Location	Meteorological station	Indicative code ¹	Latitude (N°)	Longitude (W°)	Elevation (m)
Evergreen oak forest	CARBONERO EL MAYOR	Miguelañez	2482B	41.073	-4.223	870
Evergreen oak forest	EL PARDO	Ciudad Universitaria	3194U	40.27	-3.432	664
Evergreen oak forest	MONTEGANCEDO	Pozuelo de Alarcón	3194Y	40.265	-3.484	665
Evergreen oak forest	CENICIENTOS	Rozas de Puerto Real	3330Y	40.184	-4.291	890
Evergreen oak forest	MARJALIZA	Mora de Toledo	3254Y	39.411	-3.465	717
Riparian forest	POLLOS	Rueda	2507Y	41.252	-4.575	715
Riparian forest	PALAZUELOS	Segovia	2465	40.564	-4.073	1005
Riparian forest	RASCAFRÍA	El Pualar	3104Y	40.532	-3.531	1159
Riparian forest	EL ESCORIAL	Alpedrete	3268C	40.393	-4.010	924
Riparian forest	SANTA CRUZ DEL RETAMAR	Castillo Bayuela	3362Y	40.062	-4.405	560

¹Indicative code of the meteorological station according to AEMET.

6) Edaphic factors:

(i) Soil pH: at each location, soil pH was obtained from the "European Soil Data Center" (<http://eusoils.jrc.ec.europa.eu/library/data/pH/>). These values

were confirmed in soil samples collected at each location by determining the pH according to the protocol described by [Rojas \(1989\)](#).

(ii) *Soil moisture*: a soil moisture probe (TDR 100; Spectrum Tech Inc., Plainfield, IL, USA) was used to determine volumetric water content (VWC) of the soil to 12 cm deep. Measures were taken at the eight quadrants of 1m² randomly selected in each location. Four measurements were taken around each of the quadrants (one at each quadrat side) and data was averaged.

A summary of the value of each of the parameters recorded at each location and visit is provided in **Appendix 1, Table A1.1 and A1.2**.

3.1.4. Analysis of the sampling effort

The species richness captured by a given sampling scheme (sampling effort) can be visualized as the asymptote of an accumulation curve that represents the observed species richness relative to the number of sampled individuals. Once this asymptote is reached, additional sampling will not increase species richness ([Gotelli and Colwell, 2010](#)). The effectiveness of the sampling effort to represent the total species richness in the monitored locations of each ecosystem was analysed using these accumulation curves.

The number of individuals of each sampled species identified at each location and season during the monitored period was used to calculate the mean number of species and its standard deviation per sampling effort unit with the EstimateS 9.1 software ([Colwell, 2013](#)). The potential effect of the order in which the samples were collected was removed by randomizing 100 times their order in each dataset. Accumulation curves were plotted for each location of evergreen oak and riparian forests in each monitored season, and the asymptote values of the accumulation curves were compared with values of nonparametric estimators of species richness, which use information on the rare species in a sample to estimate the number of undetected species ([Gotelli and Chao, 2013](#)). The estimators used were the Chao 1 estimator, which accounts for the number of species that are represented only by one or two individuals, and the Abundance-based Coverage Estimator (ACE) that is based on the observed number of common and infrequent species (considering as infrequent

those species with 10 or fewer individuals). Because both estimators led to the same values, only results derived from the Chao 1 estimator are shown. ACE and Chao 1 richness estimators were computed along with log-linear 95% confidence intervals (CIs) (Chao, 1987). The CIs were used to estimate the precision of the richness estimates and to infer the statistical significance between mean observed and estimated species richness: evidence of statistical difference was considered when the observed mean species richness lied outside the 95% CIs of the estimated mean species richness (Schenker and Gentleman, 2001; Ramsey and Schafer, 2002).

3.2. Potyvirus detection and identification

3.2.1. RNA extraction from plant samples

Infection by potyvirus species was detected in total RNA preparations from plant material. The Cetyltrimethylammonium (CTAB) – Polyvinyl pyrrolidone (PVP) method (Chang et al., 1993) was selected for purification of total plant RNA. Since salt precipitation prevented efficient RNA purification of some plant species, the method was modified adjusting the amount of NaCl (5M) to 50µl per 500 µl of supernatant, instead of 100µl, during the final precipitation step with 2.5 volumes of cold absolute ethanol (-20°C). This method allowed efficient and consistent RNA purification from the majority of the plant species present in the studied ecosystems, including problematic tissues harboring large amounts of polysaccharides, polyphenols and other secondary metabolites.

The RNA integrity was verified after running an aliquot of the purified RNA in a denaturing 1.2% agarose gel, stained with ethidium bromide (EtBr), in Tris-acetate-EDTA (TAE) buffer. The RNA extraction yield and the presence of contaminants was determined by measuring absorbance (A) of the intact total RNA extracts at 230nm, 260nm and 280nm in a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Samples with yield greater than 10ng/µl (in a total volume of 30 µl), and A260/A280 and A260/A230 ratios between 1.7-2.0 and ~2.0, respectively, were considered to have good quantity and quality for further analysis.

3.2.2. Detection and identification of virus species of the genus *Potyvirus*

The presence of virus species within the genus *Potyvirus* was analysed by One-step SYBR Green-based real-time RT-PCR in the LightCycler® 480 Real-Time PCR System (Roche Diagnostics, Mannheim, Germany). For each run, 10 ng of total plant RNA were added to the Brilliant III SYBR® Green Ultra-Fast QRT-PCR Master Mix (Agilent Technologies, Santa Clara, CA, USA) following the manufacturer's recommendations. Universal primers NIb2F and NIb3R ([Zheng et al., 2010](#)) for species of the genus *Potyvirus* were used to amplify a region of ~350 nucleotides of the gene that encodes the NIb protein. The thermal profile consisted of a 5-min pre-incubation step at 65°C, 10-min RT step at 50°C and 5 s of *Taq* polymerase activation at 95°C, followed by 50 cycles of PCR at 95°C for 10 s (denaturation), 50°C for 20 s (annealing), and 72°C for 30 s (extension). Following amplification, a melting curve analysis, which determines the specific melting temperature (T_m) of an amplicon, was performed to verify that the obtained amplicons were of the expected size. This analysis consisted in a denaturation step at 95°C for 30 s, lowered to 60°C for 1 min, and followed by an incubation step in which the temperature was increased to 95°C at a rate of 0.11°C/s with continuous reading of fluorescence. T_m values were analyzed using the *T_m Calling analysis module* in LightCycler® 480 Software Version 1.5.

In all real-time RT-PCR analyses, RNA purified from *Turnip mosaic virus* isolate UK 1 (UK 1-TuMV), a well-characterized potyvirus species ([Sánchez et al., 2003](#)), was included as positive control. For that, UK 1-TuMV, kindly provided by Prof. Fernando Ponz (CBGP UPM-INIA, Spain), was propagated in Indian mustard [*Brassica juncea* (L.) Czern.] and viral particles were purified according to the protocol described in [Sánchez et al., 1998](#). Viral genomic RNA was extracted from purified virions by 1% SDS treatment and phenol-chloroform extraction with ethanol precipitation. Viral RNA was re-suspended in DEPC-water and kept at -80°C. Real-time RT-PCR negative controls included the reagent mix without RNA template. Real-time RT-PCR amplification was confirmed in 1.2% agarose gel. PCR products of the expected size were considered as positive detections of potyviruses. To confirm potyvirus presence, these products were purified using the StrataPrep DNA Gel Extraction Kit

(Agilent Technologies) and sequenced. Sequences were assembled using MEGA 6 (Tamura et al., 2013), and their percentage of nucleotide identity with the potyviral sequences available in GenBank was analysed using BLASTN (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Following the ICTV criteria (King et al., 2012), 350-nt NIb sequences with a nucleotide identity over 55% with any known species of the genus *Potyvirus* were considered as belonging to this genus, and sequences with nucleotide identity between 55% and 76% were considered as belonging to a non-previously described species of this genus (Adams et al., 2005).

It should be noted that, according to King et al., 2012, the demarcation criteria for potyvirus species is based upon the coat protein gene. However, in this thesis a fragment of the NIb was used as potyvirus species demarcation criterion. Thus, whether the NIb region of ~350 nucleotides contained sufficient phylogenetic information to identify different potyvirus species with the same accuracy than the CP gene was analysed. To do so, sequences of potyvirus isolates for which the 350-nt NIb fragment and the full-length CP genes were available (n=271) were retrieved from GenBank. These NIb and CP sequences were aligned and phylogenetic trees were constructed for each genomic region as described in **section 3.2.3**. Congruence between the phylogenies obtained with the NIb and the CP sequences was tested with CopyCat v.2.03 (Meier-Kolthoff et al., 2007). A total of 5 (2%) non-significant and 266 (98%) significant associations between phylogenies of the 350-nt NIb fragment and the CP sequences of potyvirus isolates were retrieved in the cophylogenetic analysis using a significance threshold of $P=0.050$. Thus, results indicated that the topology of the tree obtained with the 350nt region of the NIb gene was congruent with that obtained with the CP sequence (**Appendix 1, Table A1.3**). Hence, both genomic regions allowed identifying *Potyvirus* species with comparable accuracy.

3.2.3. Phylogenetic characterization of potyvirus species

Phylogenetic relationships between the potyvirus species detected in evergreen oak and riparian forests and the other members of the genus *Potyvirus* were inferred using Maximum Likelihood methods. To perform this analysis, NIb

sequences of the potyviruses detected in this thesis were aligned with those of representative isolates of all known potyviruses. Sequences were aligned using the MAFFT algorithm (Kato et al., 2002) and the alignment was refined using MUSCLE (Edgar, 2004), both integrated in GENEIOUS v.11 software (Kearse et al., 2012). Using this alignment, a phylogenetic tree was constructed using PhyML package v3.0 (Guindon et al., 2010) under the Tamura-Nei model of nucleotide substitution, with a Gamma distribution of substitution rates, as this was selected as the best-fitted nucleotide substitution model in jModelTest v. 2.1.10 (Darriba et al., 2012). The Nearest-Neighbor Interchanges (NNI) and Subtree Pruning and Regrafting (SPR) heuristic methods were used to estimate tree topology, and the best topology obtained was selected. The support for each internal branch of the phylogeny was estimated by the SH-like approach. This is a faster but equally accurate method than the bootstrap methods, and is recommended when a large number of sequences is analysed (Anisimova et al., 2011). The tree was visualized and edited in FigTree software v1.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Similar phylogenetic analyses were done using the CP gene. To do so, the CP gene sequence of each isolate was also obtained and used in phylogenetic analyses. For that, universal potyvirus primer NlbFor1 (Ha et al., 2008) and an Oligo(dT)₁₈ Primer (Life Technologies) were used to obtain the 3' end (900nt) of these isolates, and these fragments were sequenced as described above. Because NlbFor1 is a universal primer for *Potyvirus* species, specific primers for the CP of the three most prevalent virus species detected in this work were designed for some of the analyses presented in this thesis (see Results section). For that, the 900nt sequences were aligned as described above and scanned for conserved regions in order to design CP species-specific primers. The suitability of the designed primers for species-specific CP amplification was analyzed using OligoAnalyzer® Tool (<http://eu.idtdna.com/calc/analyser>). Amplifications were done by Reverse Transcription Polymerase Chain Reaction (RT-PCR). AffinityScript Multiple Temperature Reverse Transcriptase (Agilent Technologies) was used to produce the cDNA, which was used as a template for DNA amplification using the Biotools DNA Polymerase (Biotools, B&M Labs, S.A., Madrid, Spain) (Table 3.3). Unfortunately, not all the CP sequences of isolates for which the 350-nt

Nlb fragment was available could be obtained. Thus, in the Results section analyses with both the Nlb and the CP gene sequences are presented.

Table 3.3. Sequence of species-specific primers used to amplify the CP gene of the isolates of the most prevalent potyviruses obtained in this thesis.

Primer	Primer Sequences 5'–3'	Potyvirus species
WHMV-CPFor	AGAGAACTGGTGACAGTCCTGAGTT	<i>Wild hop mosaic virus</i>
WHMV-CPRev	CTGGCAAGACTCATGTCAATTTCTC	
MeRV-CPFor	CCAAAGCTTGAACAAGAGAGAATTGTTTCG	<i>Mediterranean ruda virus</i>
MeRV-CPRev	ACACCAAGCATGKTRTGCATAT	
CIYVV-CPFor	GATGACAGCCARATGAAATT	<i>Clover yellow vein virus</i>
CIYVV-CPRev	GGAGAATTTAAAGACGGATACTCTA	

3.3. Comparative genomics of potyviruses

3.3.1. Detection of recombination in potyvirus populations

Inter and intra-species recombination events in the CP gene and in the partial Nlb gene sequences were analyzed. These analyses were done using the sequences from potyvirus isolates obtained in this thesis (**Appendix 1, Table A1.4**), and also including reference sequences of these genes from all known potyviruses (**Appendix 1, Table A1.5**). Recombination was detected utilizing four different methods based on different assumptions (Posada, 2002) as implemented in the RDP4 package (<http://darwin.uvigo.es/rdp/rdp.html>): RDP, GENECONV, Bootscan, and Chimaera, and employing the default parameters and a Bonferroni correction *P*-value cut-off of 0.05 (Martin et al., 2015). Only recombination signals detected by all methods were considered as positive to minimize false positives. With this criterion, no recombinants were detected. Analyses using the more relaxed criterion of considering as recombinants those detected by two or more methods to minimize false negatives yielded the same result.

3.3.2. Analysis of nucleotide substitution rates

3.3.2.1. Robustness of temporal signal

To test the strength of the temporal signal in these data, essential to the accurate estimation of substitution rates, the BEAST analyses described in

section 3.3.2.2 were repeated on data sets in which sampling times were randomized such that they lack any temporal structure. Runs for randomized data were repeated 10 times. The mean and 95% HPDs of the substitution rate estimates for the randomized data were then compared with those obtained from the real data; major differences in these estimates indicate the presence of temporal structure.

3.3.2.2. *Estimation of substitution rates*

For the partial NIb and the CP data set, rates of nucleotide substitution per site were estimated using the Bayesian Markov Chain Monte Carlo (MCMC) method available in the BEAST v1.8.3 package ([Drummond and Rambaut, 2007](#)). The best-fit model of nucleotide substitution in each case was determined using jModelTest v. 2.1.10 ([Darriba et al., 2012](#)), and all data sets were subsequently run using the general time-reversible substitution model with invariant sites and a gamma distribution of among-site rate variation (GTR+I+ Γ_4). These sequence data were analyzed using a relaxed (uncorrelated, lognormal) molecular clock (see [Drummond et al., 2006](#)) and a Bayesian skyline model as a coalescent prior, as estimating demographic parameters was not the aim of this study. In all cases, the BEAST analyses were run until all relevant parameters converged, with 10% of the MCMC chains discarded as burn-in. Statistical confidence is represented by values for the 95% highest probability density (HPD).

3.3.3. *Analysis of potyvirus population genetic diversity*

The different parameters of potyvirus population genetic diversity were estimated based on the CP and partial NIb gene sequences. Virus population genetic diversity (π), as average pairwise nucleotide difference between sequences, and the average pairwise mean number of non-synonymous (d_N) and synonymous (d_S) nucleotide substitutions per site were estimated using MEGA 6 ([Tamura et al., 2013](#)) and the best-fitted nucleotide substitution model as determined by jModelTest v. 2.1.10 (**section 3.2.3**) ([Darriba et al., 2012](#)). Standard errors of each measure were based on 1,000 bootstrap replicates. d_N and d_S were also estimated by averaging values at individual codons using different methods implemented in the HYPHY program v.2.2 (SLAC, Single

Likelihood Ancestor Counting; FEL, Fixed Effects Likelihood; IFEL, Internal Fixed Effects Likelihood; REL, Random Effects Likelihood; FUBAR, Fast Unbiased Bayesian Approximation) (Kosakovsky et al., 2005; Murrell et al., 2013). Selection pressures were estimated as the d_N/d_S ratio. As comparable results were obtained with all these methods, only results of analyses using average pairwise values are shown. Finally, in each potyvirus population the number of haplotypes (H) and the haplotype diversity [$H_d = (1 - \sum x_i^2) n / (n - 1)$], where x_i is the frequency of an haplotype and n is the population sample size (Nei and Tajima, 1981)] were calculated using DnaSP v.5 (Librado and Rozas, 2009).

3.3.4. Analysis of the genetic structure of potyvirus populations

NIb- and CP-based bayesian trees were constructed using the Tamura-Nei substitution model with a Gamma distribution of nucleotide substitution rates. All analyses were run until relevant parameters converged, with 25% of the MCMC chains discarded as burn-in. These trees were constructed using MrBayes v3.2.6 (Ronquist et al., 2012). Maximum clade credibility (MCC) trees, with Bayesian posterior probability values providing a measure of the robustness of each node, were also summarized from the MrBayes tree samples using TreeAnnotator v1.8.3 available as part of the BEAST package (Drummond and Rambaut, 2007).

Association between potyvirus population geographic origin and host species, and the position of each virus isolate in the NIb and CP-based phylogenies was assessed using the Association Index (AI), the Parsimony Score (PS) and the Monophyletic Clade Size (MC) statistics. PS and AI measure the degree to which sequences from virus isolates that share the same geographic origin/host species cluster together across the entire tree topology. MC assesses the same association but for each specific geographic origin/host species. Statistical significance of these indexes was obtaining by calculating empirical distributions of the three association statistics from the credible sample (posterior distribution) of NIb-based phylogenetic trees constructed utilizing Bayesian Markov Chain Monte Carlo (MCMC) methods. Empirical distribution for each index, constructed with at least 2,000 trees, was compared with that resulting from randomizing 1,000 times the position of each

sequence in the Bayesian tree. The null hypothesis of random phylogeny-trait associations was rejected at a significance level of $P=0.05$. This analysis was performed using the BaTS software ([Parker et al., 2008](#)).

3.4. Biological and molecular characterization of a novel potyvirus in a wild ecosystem

3.4.1. Biological characterization of Mediterranean ruda virus (MeRV)

For the characterization of MeRV described below, tissue of one mountain rue plant sampled in El Pardo and infected with MeRV was selected. This plant tested negative for other species of the genus *Potyvirus* by RT-PCR. More importantly, analyses of the NGS sequence data obtained from total RNA extracts of this field-infected mountain rue plant did not detect any other viral sequence. Tissue of the infected field plant was grinded in 0.1 M phosphate buffer pH 7, 0.02% sodium diethyldi-thiocarbamate (DIECA), and used to inoculate *Nicotiana benthamiana* plants. After 20 days, *N. benthamiana* plants developed a systemic mosaic. MeRV virions were purified from these plants according to the protocol described by [Sánchez et al., 2013](#). Agarose gel electrophoresis of the virion preparation showed a single band, indicating that all virions in the preparation had the same size. Indeed, agarose gel electrophoresis of nucleic acid extracts from these virions showed a single band of ssRNA of about 9,000–10,000 nt. The same NIb nucleotide sequence amplified from the field-infected mountain rue plant was obtained from virion preparations using universal potyvirus primers. Together, these results indicated that a single virus species was transferred from mountain rue to *N. benthamiana* plants, and that this species belonged to the genus *Potyvirus*, discarding the possibility of mixed viral infection. This isolate was named MeRV-ParP17, and its virions were used for further characterization.

To determine the host range and the symptoms induced by MeRV infection, different plant species of the botanical families *Amaranthaceae*, *Asteraceae*, *Boraginaceae*, *Chenopodiaceae*, *Cistaceae*, *Cucurbitaceae*, *Fabaceae*, *Fagaceae*, *Poaceae*, *Rutaceae* and *Solanaceae* were used (**Table**

3.4). These species were chosen to include the most abundant species in the sampled evergreen oak forests (the ecosystem from which the virus was isolated), and the most common crops in the surrounding areas. For each plant species, four to seven plants (10-15 days old) were mechanically inoculated by applying purified virion RNA (100 ng/ul) in 0.1 M Na₂HPO₄ onto the first two completely expanded leaves dusted with carborundum. The inoculated plants were maintained in a greenhouse (20-25°C, and 16 h of light), and symptoms were recorded over an 8-week period. Tissue from systemically infected leaves of symptomatic and asymptomatic plants was collected 20 days post-inoculation and tested for potyvirus infection by real-time RT-PCR using specific primers qCPFor (5'-GACTGACTATAGTTTAGCGCGC-3') and qCPRev (5'-GCCTCTGATAGCTGCTGCTTTC-3') that amplify a 111-bp region of the MeRV CP gene.

Table 3.4. Plant families and species used to analyse MeRV host range.

Experimental host range	Natural host range ¹
<i>Amaranthaceae</i>	<i>Asteraceae</i>
<i>Gomphrena globosa</i>	<i>Santolina rosmarinifolia</i>
<i>Asteraceae</i>	<i>Taraxacum officinalis</i>
<i>Lactuca sativa</i>	<i>Boraginaceae</i>
<i>Chenopodiaceae</i>	<i>Anchusa azurea</i>
<i>Chenopodium amaranticolor</i>	<i>Cistaceae</i>
<i>Chenopodium quinoa</i>	<i>Cistus ladanifer</i>
<i>Cucurbitaceae</i>	<i>Fabaceae</i>
<i>Cucumis melo</i> *	<i>Anthyllis vulneraria</i>
<i>Cucumis sativus</i>	<i>Lupinus angustifolius</i>
<i>Fabaceae</i>	<i>Vicia villosa</i>
<i>Phaseolus vulgaris</i>	<i>Fagaceae</i>
<i>Pisum sativum</i>	<i>Quercus ilex</i>
<i>Solanaceae</i>	<i>Poaceae</i>
<i>Capsicum annuum</i> *	<i>Bromus rubens</i>
<i>Nicotiana benthamiana</i>	<i>Rutaceae</i>
<i>Nicotiana clevelandii</i>	<i>Ruta montana</i>
<i>Nicotiana tabacum</i> (Samsun)	
<i>Solanum lycopersicum</i> *	

¹ Most abundant plant species present in evergreen oak forests and coexisting with mountain rue. Asterisks indicate most common crops in the surrounding areas to monitored evergreen oak forests.

3.4.2. Sequencing of the MeRV genome

Total plant RNA of a single field-collected mountain rue (*Ruta montana* L.) from El Pardo, which tested positive for MeRV infection (MeRV-ParP17), was treated with TURBO DNA-free™ Dnase (Life Technologies, Carlsbad, CA, USA). After treatment, ribosomal RNA (rRNA) was removed using the Ribo-Zero™ Plant Leaf kit (Epicenter-Illumina, Madison, WI, USA). For genome sequencing, 3 µg of total RNA was used for library preparation and subjected to high-throughput Next Generation Sequencing (NGS) using the Illumina platform (HiSeq2000, 2 x 125bp length, at the Centre for Genomic Regulation, Barcelona, Spain), generating 20 million paired-end reads. Adapters and low-quality sequences from NGS data were removed using Seqtk (<https://github.com/lh3/seqtk/>). The resulting reads were assessed for sequence quality using FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). MeRV genome was assembled using a reference-guided read mapping to the phylogenetically nearest potyvirus species genome as determined in the phylogenetic characterization of potyvirus species (see section 3.2.3) utilizing Bowtie 2.0. Given that the reference genome was from a virus species different from MeRV, loose mapping criteria with the default parameters for “very-sensitive-local” alignment mode was used. This settings allowed constructing contigs using reads that do not perfectly match the reference genome (Langmead and Salzberg, 2012). Only reads with mapping quality (MAPQ) score ≥30 were considered correctly aligned (Li et al., 2008; Ruffalo et al., 2011). Integrative Genome Viewer (IGV) (Thorvaldsdóttir et al., 2013) was used to visualize the mapping reads and assess genome assembly. BLAST was used to identify and remove possible chimeric contigs contained in the alignment during the assembly. Mapping quality and sequencing coverage across the reference was evaluated in Qualimap 2 (Okonechnikov et al., 2016). The consensus sequence of the MeRV genome was extracted using the *Fasta Alternate Reference Maker* tool in the Genome Analysis ToolKit (GATK) 3.8 (McKenna et al., 2010).

The NGS-derived MeRV genomic sequence was confirmed using the same mountain rue plant RNA preparation as template for RT-PCR, utilizing different sets of specific primers. Primer pairs were designed to produce 12 fragments in such a way that adjacent fragments overlapped by at least 100 nt,

covering the potyvirus polyprotein (see list of primers in **Table 3.5**). Expand™ Reverse Transcriptase (Sigma-Aldrich, St. Louis, MO, USA) was used for retrotranscription and PCR Phusion® High-Fidelity DNA Polymerase (New England BioLabs, Beverly, MA, USA) for PCR amplification. The 5′ and 3′ ends of the virus were obtained by rapid amplification of cDNA ends (RACE) using Ambion™ FirstChoice® RLM RACE kit (Life Technologies). The fragments and ends were sequenced, and assembled with MEGA 6, revealing 100% nucleotide identity between overlapping fragments and with the NGS-derived nucleotide sequence. The same nucleotide sequence was also obtained using RNA from purified virions as template.

Table 3.5. Species-specific primers designed to obtain the full-length genomic sequence of ParP17-MeRV.

Primer	Sequence (5′–3′)	Position ¹
MERV-P1 5UTR For	CGTATCATCAGAGACCCGTG	100-119
MERV-P1 5UTR Rev	CACGTACGTGTTGGAGAGCC	314-333
MERV-P1For	CATGTGAAACTGATCTTAA	223-241
MERV-P1Rev	CTATTGCAAGGGAATAGTGC	1185-1204
MERV-For1	GTAGCTAGCTGTGGTTCGG	997-1015
MERV-Rev1	CCTTTGAGCGTTCCTCCGAC	1968-1987
MERV-For2	GCAACTTCGAAGCAGCAAAG	1853-1872
MERV-Rev2	CTGCACACTCCATGACCG	2812-2829
MERV-For3	GCGAGTTAGATCAGTGTTG	2753-2771
MERV-Rev3	CACCGCGGACTAGTATGTC	3709-3727
MERV-For4	CTAAGAGGGAGTCAGACAAC	3553-3572
MERV-Rev4	CTACACGTCCAAGTCTCTG	4522-4540
MERV-For5	GAAGGTGTCGTTGATTTCG	4420-4438
MERV-Rev5	GCATCGTGAGCTAACAATG	4529-5447
MERV-For6	GTGCGAGCTCAGTTGATGG	5278-5296
MERV-Rev6	GGACACTTGAAGATACCAT	6287-6305
MERV-For7	CATGCGCGATTACAATCC	6126-6143
MERV-Rev7	GTCCAACGATAATCGGGTTTG	7067-7087
MERV-For8	GCTAAATTCTTCAGGCCTC	6982-7000
MERV-Rev8	CTTGAGACCTAGTTCAGC	7954-7971
MERV-For9	GTAAGAGAGATTGACG	7844-7861
MERV-Rev9	CCGTCCATCATTGTCCAG	8826-8843
MERV-For10	GTATGATGGCGTGAAAGCG	8712-8730
MERV-Rev10	GTAGCACCTCACTAACAAG	9288-9306

¹ Positions according to the NGS-derived ParP17-MeRV nucleotide genomic sequence (Acc. N. MF953305).

The open reading frame of the MeRV polyprotein was identified with ORF Finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). PredictProtein (Rost et al., 2004) and the NCBI's conserved domain database (CDD)-Search service

(Marchler-Bauer et al., 2007) were used to identify the putative cleavage sites and the conserved sequence domains of the polyprotein. Molecular weight of the putative viral proteins was predicted from protein sequence by using the *Molecular Weight* tool (https://www.bioinformatics.org/sms/prot_mw.html). The genomic sequence of MeRV-ParP17 was deposited in GenBank under accession number MF953305.

3.4.3. Phylogenetic analyses of ParP17-MeRV

Phylogenetic relationships between ParP17-MeRV and the other members of the genus *Potyvirus* were analysed using the nucleotide and amino acid sequences of the viral polyprotein. To study these relationships a collection of the reference sequences of the potyviruses compiled from GenBank were obtained (**Appendix 1, Table A1.5**). Nucleotide and amino acid sequences of these reference potyviruses were aligned with the corresponding sequences of ParP17-MeRV using MUSCLE (Edgar, 2004). Phylogenetic trees were constructed utilizing Bayesian MCMC methods. Analysis were run as explained in **section 3.3.3**, but using the general time-reversible substitution model with invariant sites and a gamma distribution of among-site rate variation (GTR+I+ Γ_4) for nucleotide alignments, and the Whelan and Goldman (WAG) substitution model for amino acid alignment. These substitution models were the best fitted to the *Potyvirus* polyprotein alignment as detected by jModelTest v. 2.1.10. Maximum clade credibility (MCC) trees were summarized as in **section 3.3.3**.

Nucleotide identities (%) of the complete MeRV genome with those of the two phylogenetically closest virus species were compared using SimPlot v3.5.1 (Lole et al., 1999). Plots of nucleotide identity were obtained using the MeRV genome as the query sequence, and a sliding window of 200 nt that was moved across the alignment in steps of 20 nt.

3.5. Statistical analyses

All the statistical analyses described in this section were performed using the statistical software R v.3.4.1 (R Core Team, 2017), available in <http://www.r-project.org/>.

3.5.1. Comparison of floristic composition and potyvirus prevalence between seasons and ecosystems

The differences in the plant biodiversity, as species richness, between ecosystems and in potyvirus prevalence between seasons of the same sampling cycle (summer, autumn and spring) and between sampling cycles at each season, were compared using Chi-square tests (R-library: MASS) or Fisher Exact test as needed. When necessary, the Yates correction was applied. Differences in mean values of climatic/edaphic factors (temperature, relative humidity, rainfalls, soil pH and soil moisture) were compared using two-sided Z-tests (R-library: BSDA).

To determine the differences in the floristic composition between ecosystems, the squared Euclidean distance was used as a measure of dissimilarity between the identity, number and relative abundance of the plant species in each location. Ward's clustering method (Ward, 1963) was utilized to obtain clustering dendograms based on these Euclidean distances (R-library: vegan).

3.5.2. Analysis of ecological factors affecting infection risk and genetic diversity of potyviruses

3.5.2.1. Analysis at potyvirus genus-wide level

Potyvirus prevalence was used as a measure of virus infection risk. Thus, in order to develop a predictive model that explained potyvirus infection risk, genus-wide potyvirus prevalence in evergreen oak and riparian forests was used as the response variable. Models were constructed utilizing supervised inductive Machine learning methods. Machine Learning (ML) is an area within the Artificial Intelligence field, which is focused on improving the performance of

a system solving a task through acquiring knowledge from the experience (Mitchell, 1997). In this thesis, datasets of classified (supervised) observations were used as the input in the ML algorithms, which retrieved mathematical patterns. These patterns allowed assigning a given class of the response trait to each observation in the dataset (inductive) and to predict the class of new data (classification). Building a ML model to predict potyvirus infection risk required to cover two steps, which are described in the following subsections.

3.5.2.1.1 Model construction

Genus-wide *Potyvirus* prevalence ML models were built using data of the first sampling cycle. For this, plant species at each location and visit were classified into two groups: Non-host plant species (in which potyvirus infection was never detected during the monitored period), and host species (in which potyvirus infection was detected at least once). Potyvirus prevalence in host species was categorized into four classes: uninfected host (i.e., host species for which no infected individuals were detected at a given visit/location), and infected hosts with low, medium and high potyvirus prevalence. In order to minimize biases due to imbalance in the response variable, discretization of prevalence values into the later three classes, was done such that it resulted in similar number of observations in each class. Multi-class classification models were built in which categorized prevalence was considered as the response variable. The following ecological factors were considered as predictors: location, season, plant family, plant species, plant life cycle, plant density and relative abundance, plant biomass, species richness, Shannon's index and Shannon's equitability index, soil pH, temperature, relative humidity and rainfalls in the sampled locations (minimal, maximal and average values) and number of host plants in each location. Categorical predictors with two or more (n) categories, were transformed in $n-1$ dichotomous variables such that one of the variables is taken as reference category and each of the variables created would enter in the model individually. Three ML algorithms adapted to support multi-class classification problems were run (R-library: RWeka): Multinomial Logistic Regression (Logistic), J48 Decision Tree (J48) and Random Forest.

Logistic regression is one of the most popular statistical methods for classification problems (Mertler and Reinhart, 2016). Unlike other statistical

methods (e.g. ordinary linear regression), logistic regression allows for response variables that have arbitrary distributions (as it is the case of this study), rather than normal distributions, and can be transformed to conform to different assumptions about the relationships between the predictors and the response variable. The J48 is an algorithm that allows both numeric and nominal variables to be evaluated. This kind of model generally works better in classification problems with categorical predictors that has large number of categories, and potentially establishes complex interactions with other predictors (Quinlan, 1996), as it is the case of this thesis. The J48 algorithm creates a simple decision tree from training dataset cases, which is easily interpretable as the structure of decisions in the classifying process can be graphically represented in such tree. The decision tree tries to recursively partition the dataset into subsets by evaluating the normalized Information Gain (difference in entropy) resulting from choosing a predictor to split the data from a node in the tree. The predictor with the highest Information Gain is used on every step to define each node. The process stops when no predictor can be found that increases the Information Gain. Random Forest (Breiman, 2001) is an ensemble of techniques because combines many independent decision trees, where each tree is trained with a different subset of predictors and tested on random samples from the whole original dataset. The final output is a combination of the predictions of the individual trees. Finally, ZeroR algorithm was used as the baseline for classification accuracy. ZeroR is the simplest classification method, which depends on the response variable and ignores all predictors. This algorithm simply predicts the majority class in the data. Classification models were considered valuable if they had better performance than this baseline algorithm (better classification than expected by chance) (Witten et al., 2016).

Each of these algorithms was applied to two different categorizations of the response variable: (i) one in which non-host species plus the four categories of hosts were considered, and (ii) other in which only the four host species categories were considered. Because in both categorization the data set was imbalanced (either the number of non-host species was much larger than the number of host species, or the number of uninfected host species was larger than the number of infected species), Synthetic Minority Over-sampling

Technique (SMOTE) ([Chawla et al., 2002](#)) was applied to increase the number of observations in minority classes of potyvirus prevalence.

3.5.2.1.2. Model evaluation

The performance (predictive power) of the ML models was evaluated by two approaches: (i) by means of five replicates of two-fold cross validation (5x2CV) ([Dietterich, 1998](#)). In this method, the dataset of the first sampling cycle (used to build the models) was randomly split into two datasets of equal size: training and testing datasets. Then, the former dataset was used to train the model, and the later dataset was used as an unseen test set to assess the model performance. This procedure was repeated five times and the performance measures were averaged. (ii) The model constructed with data from the first sampling cycle was challenged with the data of the second sampling cycle and performance was measured.

For evaluating the performance of the ML models, measures based on a confusion matrix were used: True Positives (TP), True Negatives (TN), False Positives (FP) and False Negatives (FN) were calculated. Performance was quantified as overall model accuracy, defined as $(TP+TN)/(TP+TN+FP+FN)$, which represents the rate of correct predictions of the ML models. In imbalanced data sets, measures derived from a confusion matrix are highly dependent on the predictions for the majority class. That is, high accuracy of the model may be just due to correct classification of the majority class, but minority classes may be poorly classified. Thus, calculations of the Area Under the Receiver Operating Characteristic curve (AUROC) were also used to assess model accuracy. Importantly, AUROC values are not affected by data imbalance. This measure allowed analyzing whether, despite the use of SMOTE techniques, the ML predictive power was still affected by data imbalance. ROC curves were obtained by plotting the True Positive rate (TPR), defined as $(TP)/(TP+FN)$, against the False Positive rate (FPR), defined as $(FP)/(FP+TN)$. After plotting the ROC curve, the area under the curve was calculated. Models with no better accuracy than obtained by chance have an AUROC=0.5, whereas highly accurate models are characterized by large areas under the ROC curve, reaching perfect accuracy when AUROC=1 ([Fawcett, 2004](#)). For minority classes of the response variable (classes of infected host

with low, medium and high prevalence), the True Positive rate (TPR) was also calculated in order to determine the accuracy in the prediction of the less represented prevalence classes.

The relative importance of the predictors considered by the ML models were also analysed: in Logistic Regression models, “Stepwise AIC” method was used for selection of the most relevant factors affecting potyvirus prevalence (R-library: `nnet`). Both stepwise-forward and stepwise-backward procedures were applied (R-library: `stats`): starting from null and full models, predictor variables were tested to be included or excluded at each step based on Akaike’s Information Criterion (AIC). The AIC penalizes for the complexity, dropping from the model those variables do not result in a lower AIC (indicative of a better fit to the data) ([Burnham and Anderson, 2004](#)). Sequential addition and/or deletion of predictor variables continues until no further variables can be dropped without a significant loss of fit, reaching the model with the best global fit but with the minimum number of predictors. Importance of every predictor variable in the models was calculated as the increase in Nagelkerke R^2 values (R^2_N) that each variable produces when it is added to the model ([Nagelkerke, 1991](#)) (R-library: `lmtest`). Logistic Regression models do not assume normality, linearity or homoscedasticity. However, these models assume independence of observations, and therefore collinearity is assumed to be minimal ([Hosmer et al., 2013](#)). Predictors were tested for collinearity by calculating the variance inflation factor (VIF). For all predictors VIF values were smaller than 5, indicating the absence of collinearity. The predictive power of the resulting Logistic Regression models were evaluated using R-library: `caret`, and Area under the ROC curve was estimated using R-library: `HandTill2001`. It should be noted that J48 models do not provide numerical information on the relative importance of each predictor variable. However, the relative importance of the predictor variables can be derived from the position, and the frequency of appearance (number of nodes), of each predictor in the tree. Also, a single optimal tree is generated, when it is possible that other combination of predictors yield a model with similarly predictive power. At odds, Random Forest combine multiple independent decision trees to analyse the relative importance of each predictor and are therefore more robust. In Random Forest models the importance of each predictor is calculated from randomized tree

ensembles and ranked based on Mean Decrease Impurity (MDI) measures (Louppe et al., 2013), which works similarly to Information Gain. These metrics measure the contribution of each predictor variable in decreasing the overall entropy of the ML model: the higher the increase in ML model entropy when a given predictor is eliminated from the model, the higher the relative contribution of such predictor to the accuracy of the model. These measures are normalized in a range from 0 to 1: the higher the MDI of a given predictor variable, the more relevant such variable is.

3.5.2.2. Analysis at potyvirus species-specific level

Mixed effect multiple regression models were used to analyse the association between ecological factors, and species-specific potyvirus prevalence and population genetic diversity parameters (Burnham and Anderson, 2004). This approach, rather than ML methods, was chosen because the reduced size of the species-specific dataset resulted in ML models with low accuracy. On the other hand, species-specific datasets were large enough for the mixed approach to construct accurate models. In these analyses continuous values of the dependent variable was used and normal distribution of data was analysed by Shapiro-Wilk normality test (R-library: stats). We considered the following factors as predictors of potyvirus prevalence: host plant density and relative abundance, species richness, plant biomass, temperature, relative humidity and precipitations in the sampled locations (minimal, maximal and average values), and season. The same ecological factors, with the addition of species-specific potyvirus prevalence, were used as predictors of virus population diversity parameters. A set of models that included a global model containing all ecological factors as fixed predictors (except season that was considered as covariate), and nested models that contained all possible combinations of these predictors, were fitted for each response variable using general linear mixed models (R-library: asreml3). Models were constructed using a simultaneous autoregressive variance-covariance matrix to account for time-dependency and covariation between predictor variables. Global and nested models were ranked according to AIC, and the model with the lowest AIC score was selected as the best-ranked model. The relative importance of the predictors included in each model was calculated by residual maximum likelihood (R-library: asreml3).

4. RESULTS

4.1. Characterization of evergreen oak forest and riparian forests in the Iberian Peninsula

This thesis focuses in two wild ecosystems in the central plateau of the Iberian Peninsula: evergreen oak forests and riparian forests. These ecosystems were chosen because they are the most representative, and have the higher ecological value, among those present in this geographical area. In addition, both ecosystems are of particular agronomical interest as they are often adjacent to agroecosystems, which favors plant virus dispersal between wild and agroecosystems. Therefore, evergreen oak and riparian forests may be sources of virus epidemics in crops. Finally, *a priori* these ecosystems represent two extremes of a continuum among the ecosystems located in the Iberian Peninsula in terms of ecological and climatic conditions. For instance, plant species of evergreen oak forests are adapted to the extreme conditions of the Mediterranean-Continental climate, characterized by warm and dry summer seasons, and lower but mild temperatures and more abundant rainfalls in spring and autumn. The water shortage during summer season may compromise nutrient availability on plant communities in evergreen oak forests limiting plant biodiversity and biomass (Rodà, 1999). On the other hand, plant communities in riparian forest are under the influence of the nearby rivers, which results in more stable temperatures and higher relative humidity throughout the year. Under these conditions, riparian forests are generally characterized by moist and nutrient-rich soils associated with higher plant biodiversity and biomass, and a different floral composition than evergreen oak forests (**Figure 4.1**). These ecological and climatic factors may be determinants of pathogen infection risk and population genetic diversity (Anderson et al., 2004; Archie et al., 2009; Johnson et al., 2015). If these differences in ecological and climatic conditions between evergreen oak and riparian forests hold true in the locations monitored in this thesis, the selected ecosystems would represent a suitable system to analyse how the above-mentioned factors affect plant virus infection risk and population genetic diversity. Thus, in this chapter, differences in floral composition, ecological factors and climatic variables among locations monitored in this work are analysed.

Figure 4.1. Examples of the landscape in summer, autumn and spring of evergreen oak forests (Cenicientos) and riparian forests (Rascafría) locations visited in this thesis.

4.1.1. Analysis of plant biodiversity and floristic composition

Plant biodiversity was quantified as species richness. Thus, for a meaningful analysis of plant biodiversity, plant sampling needs to be representative of the number of plant species (species richness) present in the analysed locations of each ecosystem. To test if this was so, an estimation of plant species richness as a function of the number of samples collected at each location and season was obtained using the non-parametric Chao 1 method (S_{est}) (see **section 3.1.4**). Estimates were compared with the observed plant species richness derived from the data collected during the field samplings (S_{obs}). Using this data, accumulation curves of the number of samples to the number of plant species collected were constructed for each location and season during the first sampling cycle (2013-2014) in both ecosystems. The asymptotic values of S_{est} and S_{obs} curves were calculated as a measure of expected and observed plant species richness, respectively. In most locations and seasons, the 95% confidence intervals (CIs) for asymptotic S_{est} contained the mean of the asymptotic S_{obs} values, indicating that there was no significant difference between both observed and expected values in locations of evergreen oak (**Figure 4.2 and Table 4.1**) and riparian forests (**Figure 4.3 and Table 4.2**).

Figure 4.2. Observed (S_{obs} , black) and expected (S_{est} , Chao 1 estimator, red) accumulation curves of plant species richness in the studied locations of evergreen oak forest for the monitored seasons during the 2013-2014 field samplings. The x-axis is the number of individuals sampled at each location (in this thesis, $N=200$) and the y-axis is the number of plant species collected according to sample size. Plotted values are means of 100 randomizations.

Table 4.1. Plant biodiversity in the studied evergreen oak forest locations during 2013-2014 field samplings. Observed and expected plant species richness, and Shannon's diversity and equitability indexes are shown.

Location	Season	S_{obs} (95% CI)*	S_{est} (95% CI)*	H'	E_H
Carbonero el Mayor	Summer 2013	17 (13.88–20.12)	19.98 (17.35–42.45)	2.55 ± 0.00	0.88
	Autumn 2013	18 (15.02–20.98)	19.49 (18.18–30.42)	2.19 ± 0.00	0.76
	Spring 2014	14 (12.64–15.36)	14.00 (14.08–15.09)	2.31 ± 0.00	0.88
Cenicientos	Summer 2013	20 (19.06–20.94)	20.00 (20.00–21.45)	2.82 ± 0.00	0.94
	Autumn 2013	25 (24.38–25.62)	25.00 (26.23–26.58)	2.78 ± 0.00	0.86
	Spring 2014	27 (23.73–30.27)	28.99 (27.28–40.91)	2.94 ± 0.00	0.89
El Pardo	Summer 2013	24 (20.19–27.81)	25.49 (24.15–39.01)	2.95 ± 0.00	0.93
	Autumn 2013	15 (11.91–18.09)	17.99 (15.35–40.37)	2.26 ± 0.00	0.84
	Spring 2014	26 (22.96–29.04)	27.49 (28.18–38.42)	2.86 ± 0.00	0.88
Marjaliza	Summer 2013	31 (28.95–33.05)	31.74 (31.08–38.19)	3.14 ± 0.00	0.91
	Autumn 2013	19 (18.31–19.69)	19.00 (19.93–20.34)	2.45 ± 0.00	0.83
	Spring 2014	26 (24.77–27.23)	26.17 (26.01–29.58)	2.96 ± 0.00	0.91
Montegancedo	Summer 2013	24 (22.43–25.57)	24.25 (24.01–28.78)	2.96 ± 0.00	0.93
	Autumn 2013	22 (18.22–25.78)	23.49 (22.15–37.01)	2.62 ± 0.00	0.85
	Spring 2014	25 (22.97–27.03)	25.60 (25.05–32.08)	2.86 ± 0.00	0.92

S_{obs} : observed plant species richness. S_{est} : expected plant species richness based on the Chao 1 estimator. H' : Shannon's diversity index. E_H : Shannon's equitability index.

* values are mean and 95% confidence intervals based in 100 randomizations from pooled location-specific individuals ($N=200$).

Figure 4.3. Observed (S_{obs} , black) and expected (S_{est} , Chao 1 estimator, red) accumulation curves of plant species richness in the studied locations of riparian forest for the monitored seasons during the 2013-2014 field samplings. The x-axis is the number of individuals sampled at each location (in this thesis, $N=200$) and the y-axis is the number of plant species collected according to sample size. Plotted values are means of 100 randomizations.

Table 4.2. Plant biodiversity in the studied riparian forest locations during 2013-2014 field samplings. Observed and expected plant species richness, and Shannon's diversity and equitability indexes are shown.

Location	Season	S_{obs} (95% CI)*	S_{est} (95% CI)*	H'	E_H
El Escorial	Summer 2013	29 (26.97–31.03)	29.60 (29.05–36.08)	3.15 ± 0.00	0.94
	Autumn 2013	28 (25.47–30.53)	29.24 (28.26–37.48)	2.90 ± 0.00	0.87
	Spring 2014	34 (31.26–36.74)	35.42 (34.19–44.52)	3.23 ± 0.00	0.92
Rascafría	Summer 2013	38 (36.88–39.12)	38.14 (38.01–41.23)	3.47 ± 0.00	0.95
	Autumn 2013	28 (26.58–29.42)	28.00 (28.00–31.09)	3.00 ± 0.00	0.90
	Spring 2014	28 (26.96–29.04)	28.12 (28.01–30.96)	2.95 ± 0.00	0.89
Palazuelos	Summer 2013	31 (27.71–34.29)	32.99 (31.28–44.91)	3.17 ± 0.00	0.92
	Autumn 2013	32 (24.75–39.25)	48.42 (36.23–95.65)	2.92 ± 0.00	0.84
	Spring 2014	26 (23.95–28.05)	26.75 (26.08–33.20)	2.84 ± 0.00	0.87
Pollos	Summer 2013	32 (27.57–36.43)	36.18 (32.79–54.24)	3.15 ± 0.00	0.91
	Autumn 2013	32 (28.28–35.72)	34.98 (32.54–48.39)	3.00 ± 0.00	0.86
	Spring 2014	35 (30.64–39.36)	39.48 (35.97–55.61)	3.09 ± 0.00	0.87
Santa Cruz de Retamar	Summer 2013	29 (27.33–30.67)	29.43 (29.03–34.55)	3.14 ± 0.00	0.93
	Autumn 2013	27 (22.10–31.90)	32.22 (27.99–54.48)	2.75 ± 0.00	0.80
	Spring 2014	30 (22.30–37.70)	43.93 (32.91–96.79)	2.96 ± 0.00	0.87

S_{obs} : observed plant species richness. S_{est} : expected plant species richness based on the Chao 1 estimator. H' : Shannon's diversity index. E_H : Shannon's equitability index.

* values are mean and 95% confidence intervals based in 100 randomizations from pooled location-specific individuals ($N=200$).

The same conclusion was obtained when observed and expected plant species richness was compared using the data of the second sampling cycle (2014-2015) for locations of evergreen oak (**Appendix 2, Figure A2.1 and Table A2.1**) and riparian forests (**Appendix 2, Figure A2.2 and Table A2.2**). Hence, this analysis indicated that the sampling effort performed in this thesis was sufficient to obtain a representative estimate of the plant species richness in the monitored ecosystems and seasons.

Next, we studied the differences in plant species richness between evergreen oak and riparian forests. Because when the two sampling cycles were analysed separately equivalent results were obtained, data on the analyses of the two cycles together are shown. Contingency tests using plant species richness as variable indicated that, within each ecosystem, the number of plant species did not vary between seasons ($\chi^2 < 3.29$; $P > 0.069$). However, in every season the number of plant species present in riparian forests was significantly higher than in evergreen oak forests ($\chi^2 > 30.24$; $P < 1 \times 10^{-5}$) (**Tables 4.1 and 4.2 and Figure 4.4**). The Shannon's diversity and equitability indexes were also higher in riparian than in evergreen oak forests, indicating that the number of plant species was higher and more evenly distributed (**Tables 4.1 and 4.2**).

Figure 4.4. Seasonal variation in the number of plant species identified at riparian and evergreen oak forests, and number of common species to both ecosystem (2013-2015).

To characterize plant biodiversity in a given ecosystem, it is important to consider both the species richness and the species identity. A percentage of 96% (5731/6000) of the total plant individuals sampled in the monitored locations were identified at the species level in riparian forests and 98%

(5877/6000) in evergreen oak forests (**Appendix 2, Tables A2.3 and A2.4**). Common plant species to both ecosystems represented approximately one third (30-40%) of total plant species collected in evergreen oak forests, and one fifth (20-24%) of those collected in riparian forests (**Figure 4.4**). This suggested that the identity of the species present in each ecosystem, and therefore their floristic composition, was different. To analyse this possibility, Ward's dendrograms were constructed using data on species richness, identity and relative frequency at each sampled location of evergreen oak and riparian forests (**Figure 4.5**). These dendrograms indicated that floristic composition at each location varied across seasons. In spite of this variation, dendrograms indicated that in every season locations always grouped according to ecosystem. Thus, evergreen oak and riparian forests had different floristic compositions.

Figure 4.5. Cluster analysis of the monitored locations according to plant species identity as depicted by the Ward's dendrogram method. The vertical axis represents Euclidean distance between locations, and colours indicate the ecosystem to which each location is assigned (Evergreen oak forests: green; Riparian forests: blue). CEM: Carbonero el Mayor, CEN: Cenicientos, ESC: El Escorial, MAR: Marjaliza, MON: Montegancedo, PAL: Palazuelos, PAR: El Pardo, POL: Pollos, RAS: Rascafría, SCR: Santa Cruz de Retamar.

As part of the information collected on plant identity, the type of life cycle (annual or perennial) of each plant species was determined (**Appendix 2, Tables A2.3 and A2.4**). Using this information, the proportion of plant species in each ecosystem with different life cycles was analysed. Contingency tests indicated that, in evergreen oak forests, there were similar proportions of perennial and annual plants in summer and spring (45-49% vs. 51-55%, respectively, $\chi^2 < 1.62$; $P > 0.203$), but in autumn there were more perennial than annual plants (59% vs. 41%, $\chi^2 = 5.78$; $P = 0.016$). In riparian forests the majority of plant species were perennials in every season (67-74% vs. 26-33%, $\chi^2 > 21.78$; $P < 1 \times 10^{-5}$) (**Figure 4.6**).

Figure 4.6. Seasonal proportion of annual and perennial plants in the studied ecosystems.

Differences in plant species richness and floristic composition may result in differences in the amount of plant biomass at each ecosystem (Blanco et al., 2005). Thus, the overall and seasonal plant biomasses at each ecosystem were calculated (**Table 4.3**). When Z-tests were used to compare the mean values of plant biomass between ecosystems, overall, plant biomass was larger in riparian than in evergreen oak forests ($V = 0.119 \pm 0.10$ vs. 0.056 ± 0.05 m³; $Z = 2.17$, $P = 0.030$), and similar trends were observed when each season was analysed separately ($Z > 1.99$; $P < 0.046$) (**Table 4.3**). Thus, differences in plant composition and biodiversity between ecosystems were accompanied by differences in plant biomass.

Table 4.3. Mean values of plant biomass (m^3) in evergreen oak and riparian forests locations.

Ecosystem/Location	Season		
	Summer	Autumn	Spring
Evergreen oak forest			
Carbonero el Mayor	0.054 ± 0.06	0.025 ± 0.00	0.019 ± 0.00
Cenicientos	0.007 ± 0.01	0.009 ± 0.00	0.081 ± 0.00
El Pardo	0.011 ± 0.00	0.046 ± 0.00	0.065 ± 0.03
Marjaliza	0.153 ± 0.17	0.047 ± 0.04	0.065 ± 0.06
Montegancedo	0.026 ± 0.02	0.061 ± 0.02	0.177 ± 0.15
Average*	0.050 ± 0.06	0.038 ± 0.02	0.081 ± 0.06
Riparian forest			
El Escorial	0.270 ± 0.15	0.197 ± 0.00	0.141 ± 0.02
Palazuelos de Eresma	0.063 ± 0.02	0.018 ± 0.02	0.075 ± 0.00
Pollos	0.121 ± 0.02	0.081 ± 0.02	0.137 ± 0.01
Rascafría	0.341 ± 0.21	0.037 ± 0.00	0.213 ± 0.04
Santa Cruz de Retamar	0.010 ± 0.01	0.038 ± 0.04	0.043 ± 0.04
Average*	0.161 ± 0.14	0.074 ± 0.07	0.121 ± 0.07

*Biomass values based in grouping data from all locations of the ecosystem (m^3).

Values are mean $\pm$ standard deviations based in data registered between 2013 and 2015.

4.1.2. Analysis of variation in abiotic factors between riparian and evergreen oak forests

4.1.2.1. Climatic factors

The following climatic factors were recorded in the monitored locations: monthly maximum, minimum and mean temperature ($^{\circ}\text{C}$), maximum, minimum and mean relative humidity (%), and rainfalls (mm) in the summer, autumn and spring months. In evergreen oak forests, analysis of variation in these climatic factors among seasons showed that summer was the driest season, registering the least rainfalls ($Z > 5.17$; $P < 1 \times 10^{-5}$) and the lowest values of relative humidity ($Z > 2.46$; $P < 0.014$) (**Table 4.4 and Figure 4.7**). Autumn was the wettest season with the highest values of rainfalls ($Z > 2.55$; $P < 0.011$) and relative humidity ($Z > 3.08$; $P < 0.002$). Summer had the highest maximum, mean and minimum temperatures ($Z > 4.69$; $P < 1 \times 10^{-5}$), with no significant differences between spring

and autumn ($Z < 0.86$; $P > 0.220$) (**Table 4.4** and **Figure 4.7**). In riparian forests, summer was also the driest season, with the lowest values of rainfalls ($Z > 4.29$; $P < 1 \times 10^{-5}$) and relative humidity ($Z > 2.17$; $P < 0.030$) and the highest temperatures ($Z > 2.05$; $P < 0.041$). Autumn had higher temperatures and relative humidity than spring ($Z > 2.05$; $P < 0.041$). No differences were observed in the rainfalls between spring and autumn ($Z = 1.00$, $P = 0.315$) (**Table 4.4** and **Figure 4.7**).

Table 4.4. Averaged seasonal values for the climatic factors recorded in the monitored locations.

Climatic factor	Summer		Autumn		Spring	
	Evergreen oak forest	Riparian forest	Evergreen oak forest	Riparian forest	Evergreen oak forest	Riparian forest
$T_{\min}$	9 ± 2.48	7 ± 3.25	3 ± 2.75	3 ± 2.91	2 ± 1.52	1 ± 2.48
T_{mean}	21 ± 2.14	20 ± 2.77	15 ± 1.59	15 ± 1.44	13 ± 1.26	13 ± 2.07
$T_{\max}$	34 ± 1.77	33 ± 2.22	27 ± 1.85	28 ± 1.40	26 ± 1.78	26 ± 2.15
$RH_{\min}$	14 ± 2.32	14 ± 1.41	25 ± 3.29	23 ± 5.35	17 ± 3.36	17 ± 1.72
RH_{mean}	40 ± 4.38	43 ± 5.37	63 ± 6.55	64 ± 3.21	54 ± 6.08	57 ± 4.19
$RH_{\max}$	91 ± 4.61	95 ± 2.44	97 ± 3.92	99 ± 0.76	96 ± 4.30	98 ± 1.07
Rainfalls	85 ± 36.63	176 ± 90.98	493 ± 246.80	521 ± 237.10	283 ± 81.15	387 ± 348.03

$T_{\min}$, T_{mean} , $T_{\max}$: Average of monthly maximum, minimum and mean temperatures ($^{\circ}\text{C}$) in each season.

$RH_{\min}$, RH_{mean} , $RH_{\max}$: Average of monthly maximum, minimum and mean relative humidity (%) in each season. Rainfalls: Average of total rainfalls (mm) in each season.

Values are mean $\pm$ standard deviations based in data registered between 2013-2015.

Seasonal differences between evergreen oak and riparian forests in climatic factors were also compared. No significant differences were found in the minimum, mean and maximum seasonal temperatures between both ecosystems ($Z < 1.23$; $P > 0.113$). Similarly, maximum, minimum and mean relative humidity and rainfalls did not differ between ecosystems ($Z < 2.06$; $P > 0.039$ and $Z < 0.92$; $P > 0.358$ respectively). The only exceptions were that summer rainfall was larger in riparian forest ($Z = 2.06$; $P = 0.039$), accompanied also by larger maximum relative humidity in this season ($Z = 2.06$; $P = 0.040$) (**Table 4.4** and **Figure 4.7**). Thus, at odds with the ecological factors analysed in **section 4.1.1**, climatic factors did not generally varied between evergreen oak and riparian forests.

Figure 4.7. Seasonal differences in climatic variables between ecosystems. Asterisks indicate statistically significant differences.

4.1.2.2. *Edaphic factors*

Besides climatic factors, variation in the edaphic factors soil pH and moisture between ecosystems were also analysed. For soil pH, no differences between seasons were detected, as soil pH remained constant throughout the monitored period ($Z < 0.49$; $P > 0.628$) (**Table 4.5**). Z-tests indicated no significant differences in the soil pH values between both ecosystems ($Z = 0.98$; $P = 0.327$) and between locations of the same ecosystem ($Z < 0.96$; $P > 0.359$).

Table 4.5. Averaged values of soil pH in the monitored locations.

Evergreen oak forest locations	pH ¹	Riparian forest locations	pH ¹
Carbonero el Mayor	5.5 ± 0.00	El Escorial	5.5 ± 0.00
Cenicientos	4.5 ± 0.00	Palazuelos	5.5 ± 0.00
El Pardo	6.0 ± 0.00	Pollos	5.0 ± 0.00
Marjaliza	6.0 ± 0.00	Rascafría	5.5 ± 0.00
Montegancedo	6.0 ± 0.00	Santa Cruz de Retamar	5.5 ± 0.00

¹Values are mean ± standard deviation based in data registered between 2013 and 2015.

Volumetric water content (VWC) of the soil was used as measure of soil moisture in each location and season. In this case, Z-tests indicated that average soil moisture was significantly higher in riparian forests than in evergreen oak forests in every season ($Z > 4.91$; $P < 1 \times 10^{-5}$). Significant differences were also registered between seasons in each ecosystem: highest soil moisture was registered in autumn, whereas driest soils were found in summer in both ecosystems ($Z > 3.23$; $P < 0.001$).

Table 4.6. Averaged values of soil moisture registered in the monitored locations.

Ecosystem/Location	Season		
	Summer	Autumn	Spring
Evergreen oak forest			
Carbonero el Mayor	2.60 ± 0.47	6.58 ± 0.77	3.59 ± 0.46
Cenicientos	1.28 ± 0.35	8.79 ± 1.51	1.65 ± 0.92
El Pardo	–	9.13 ± 1.71	3.58 ± 0.71
Marjaliza	–	3.36 ± 1.14	2.78 ± 0.77
Montegancedo	1.66 ± 0.39	10.80 ± 4.30	–
Average*	1.81 ± 0.68	7.73 ± 3.44	2.90 ± 0.79
Riparian forest			
El Escorial	5.04 ± 1.77	19.74 ± 3.21	12.11 ± 9.17
Palazuelos	6.95 ± 0.47	22.94 ± 3.33	9.99 ± 0.62
Pollos	–	–	13.13 ± 12.09
Rascafría	3.75 ± 1.25	20.48 ± 3.08	8.01 ± 1.13
Santa Cruz de Retamar	2.76 ± 0.31	9.80 ± 2.77	1.64 ± 0.41
Average*	4.87 ± 2.34	18.24 ± 5.89	8.98 ± 7.66

*Volumetric water content values based in data of all locations in the ecosystem (%). Missing values (–). Values are mean ± standard deviations based in data registered between 2013 and 2015.

4.2. Analysis of genus-wide potyvirus prevalence in wild ecosystems of the Iberian Peninsula

Current understanding of plant virus biodiversity and host interactions is largely restricted to viruses isolated from crops. However, recent studies have shown that plant viruses are widespread and highly diverse in wild ecosystems (Roossinck, 2012; Stobbe and Roossinck, 2014). Few studies however have investigated the genetic diversity of plant virus populations in wild ecosystems and their prevalence in native wild hosts (Malpica et al., 2006; Pagán et al., 2010; Rodríguez-Nevado et al., 2017). In this thesis, the presence of species of the genus *Potyvirus* was investigated in evergreen oak and riparian forests of the Iberian Peninsula. This is the largest genus of plant viruses and contains species that are major crop pathogens (e.g. Walsh and Jenner, 2002; Quenouille et al., 2013), together with species that have been reported to infect wild hosts (Malpica et al., 2006; Rodríguez-Nevado et al., 2017). This suggests that potyviruses may be important ecological agents in wild ecosystems (perhaps including evergreen oak and riparian forests), and with potential to cause epidemics in adjacent crops.

In this chapter, the association between the ecological factors that characterize evergreen oak and riparian forests (analysed in **section 4.1**) and potyvirus prevalence is analyzed, with the ultimate goal of building a predictive model of virus prevalence in both ecosystems. Multivariate models aiming at predicting virus prevalence in cultivated hosts have been shown to represent a powerful tool to predict viral diseases in crops (Thackray et al., 2004). However, examples of similar models in wild ecosystems are scarce, especially for plant viruses (Pagán et al., 2012). In addition, these studies generally used linear or multiple linear regressions and focused on the effect of few ecological variables in the prevalence of a single viral species infecting one or few focal hosts (Pagán et al., 2016). However, relationships between ecological factors and virus prevalence are not necessarily linear, and considering only part of the virus host range may miss relevant interactions for virus prevalence. To broaden the scope of previous works, here the role of a larger variety of factors

as predictors of potyvirus prevalence in evergreen oak and riparian ecosystems is analysed (see **section 3.5.2.1.1**), and the complete host range of the identified potyviruses is considered. Also, Machine Learning-based methods (ML) are used to build predictive models of genus-wide potyvirus prevalence. ML methods have the advantage that they model complex nonlinear relationships without having to satisfy the assumptions required by traditional statistical methods (Olden et al., 2008; Thessen, 2016). For the purpose of this thesis, these methods allow studying complex relationships, as they consider both the effect of host-virus interactions and the influence of the environment in virus epidemiology.

4.2.1. Potyvirus prevalence and host abundance in evergreen oak and riparian forests

To analyze potyvirus prevalence, 83% (9,934/12,000) of the samples collected in this thesis were used. For the rest of the samples, appropriate RNA extracts could not be obtained because the nature or the preservation state of the sample did not allow it (i.e., samples from plant species that were rich in phenolic compounds or polysaccharides, or samples that were poorly preserved). Infection by potyvirus species was detected in spring, summer and autumn during the monitored period, and prevalence was calculated as the percentage of infected individuals relative to the total number of analysed plants.

Average potyvirus prevalence ranged between 1.06% and 3.43% in evergreen oak forests, and between 2.43% and 7.56% in riparian forests, depending on the season and the sampling year (**Figure 4.8A and B**). In the first sampling year (2013-2014), the comparison of potyvirus prevalence in evergreen oak forests between seasons indicated that prevalence was significantly higher in autumn than in summer (3.35% vs. 1.06%, $\chi^2=7.04$; $P=0.008$), with intermediate prevalence values in spring that did not differ from those in the other two seasons (1.87%, $\chi^2<2.52$; $P>0.112$). In riparian forests, the highest potyvirus prevalence was observed in summer (7.56%, $\chi^2>8.14$; $P<0.004$), whereas no significant differences were observed between autumn and spring (2.62% and 4.17%, respectively, $\chi^2=2.39$; $P=0.122$) (**Figure 4.8A**).

In the second sampling year (2014-2015), the highest potyvirus prevalence in evergreen oak forests was detected in summer (3.43%, $\chi^2 > 4.37$; $P < 0.036$), whereas no significant differences were observed between autumn and spring (1.72% vs. 1.27%, $\chi^2 = 0.35$; $P = 0.551$). No significant differences between seasons were observed in riparian forests during this sampling year ($\chi^2 < 0.79$; $P > 0.373$) (**Figure 4.8B**).

Figure 4.8. Seasonal potyvirus prevalence and host plant abundance in evergreen oak forests (green) and riparian forests (blue) during the 2013-2014 sampling cycle (A and C), and during the 2014-2015 sampling cycle (B and D). Asterisks indicate significantly different potyvirus prevalence between ecosystems.

Differences in potyvirus prevalence at each season between sampling years were also analyzed. Fluctuations in potyvirus prevalence were observed only in summer: 7.56% vs. 3.13% ($\chi^2 = 16.36$; $P < 1 \times 10^{-5}$) in riparian forests, and 1.06% vs. 3.43% ($\chi^2 = 7.97$; $P = 0.005$) in evergreen oak forests. There were no significant changes in virus prevalence between years in the other seasons ($\chi^2 < 3.58$; $P > 0.058$) (**Figure 4.8A and B**). The comparison between ecosystems indicated that in the first sampling cycle, potyvirus prevalence was significantly higher in riparian forests than in evergreen oak forests in spring (4.17% vs. 1.87%, respectively, $\chi^2 = 6.33$; $P = 0.012$) and summer (7.56% vs. 1.06%, $\chi^2 = 33.36$; $P < 1 \times 10^{-5}$), but not in autumn (2.62% vs. 3.35%, $\chi^2 = 0.413$; $P = 0.520$) (**Figure 4.8A**). In the second sampling cycle, the comparison of seasonal

changes in potyvirus prevalence between ecosystems showed largely similar results than in the first sampling year: In spring, virus prevalence was significantly higher in riparian than in evergreen oak forests (3.22% vs. 1.27%, $\chi^2=7.26$; $P=0.007$), and no significant differences were observed in autumn (1.72% vs. 2.43%; $\chi^2=0.80$; $P=0.371$). The exception was the potyvirus prevalence in summer that, at odds with the first sampling cycle, did not differ between ecosystems (3.43% vs. 3.13%, $\chi^2=0.050$; $P=0.822$) (**Figure 4.8B**).

The analysis of potyvirus prevalence allowed identifying virus host species in evergreen oak and riparian forests. Thus, changes in host plant abundance – as the number of host species relative to total number of plant species – between seasons, sampling cycle and ecosystems were analyzed. Host plant abundance in evergreen oak forests ranged between 16.39% (10/61) and 28.30% (15/53), and in riparian forests between 33.33% (38/114) and 47.17% (41/87), depending on the season and the sampling cycle (**Figure 4.8C and D**). In the first sampling cycle, the comparison between seasons showed that host abundance was similar in summer, autumn and spring both in evergreen oak forests (22.54%, 28.30% and 24.64%, respectively, $\chi^2<0.27$; $P>0.600$) and in riparian forests (47.13%, 34.74% and 38.10%, $\chi^2<2.39$; $P>0.121$) (**Figure 4.8C**). Similar results were obtained in the second sampling cycle: 26.79%, 16.39% and 20.59% ($\chi^2<1.31$; $P>0.252$) in evergreen oak forests, and 33.33%, 33.65% and 36.46% ($\chi^2<0.11$ $P>0.743$) in riparian forests (**Figure 4.8D**). Also, in each ecosystem host plant abundance was similar in both sampling cycles ($\chi^2<3.38$, $P>0.066$) (**Figure 4.8C and D**).

The comparison of the abundance of host plants between ecosystems indicated that, in the first sampling cycle, host abundance in summer was higher in riparian than in evergreen oak forests (47.13% vs. 22.54%, $\chi^2=9.21$, $P=0.002$) (**Figure 4.8C**), co-occurring with the higher potyvirus prevalence in the former ecosystem (**Figure 4.8A**). In the other two seasons, there were no significant differences in host abundance between ecosystems ($\chi^2<2.84$; $P>0.092$). In the second sampling cycle, host abundance was higher in riparian than in evergreen oak forests both in autumn (33.65% vs. 16.39%, $\chi^2=4.94$, $P=0.026$) and in spring (36.46% vs. 20.59%, $\chi^2=4.06$, $P=0.044$) (**Figure 4.8D**). Higher host abundance in riparian forest during spring co-occurred with higher

potyvirus prevalence in this ecosystem (**Figure 4.8B**). No differences in host abundance between ecosystems were observed in summer ($\chi^2=0.48$; $P=0.49$).

Finally, differences in the proportion of host plant species at each ecosystem according to their life cycle (annual or perennial) were also analysed. In evergreen oak forests, there was similar proportion of annual and perennial hosts at every season and sampling cycle ($\chi^2<0.20$; $P>0.654$). At odds, in riparian forests the majority of host species were perennials ($\chi^2>31.25$; $P<1\times10^{-5}$) (**Figure 4.9**).

Figure 4.9. Seasonal proportion of annual and perennial host plants in evergreen oak forests (green) and riparian forests (blue) during the 2013-2014 (A) and the 2014-2015 (B) sampling cycles.

4.2.2. Ecological factors affecting *Potyvirus* genus-wide prevalence

ML methods were applied to build a model that best explained genus-wide potyvirus prevalence in evergreen oak and riparian forests. The following ecological factors were used as predictors: location of the sampled area, season, plant family, plant species, plant life cycle, plant density and relative abundance, plant biomass, plant species richness (S), Shannon's index (H') and Shannon's equitability index (E_H), soil pH, minimum, maximum and average values of temperature and relative humidity and rainfalls and number of host plants in each location.

Non-normal distribution of the potyvirus prevalence data due to high proportion of non-host and non-infected plants prevented building accurate regression models using potyvirus prevalence as a continuous variable ($r\leq 0.35$; $P\geq 0.350$). Therefore, ML models were built considering genus-wide potyvirus

prevalence as a categorical variable. To do so, plant species at each location and visit were classified into two major groups: Non-host plant species (in which potyvirus infection was never detected during the monitored period), and host species (in which potyvirus infection was detected in at least one individual at one of the visits). Analysis of the frequency distribution of potyvirus prevalence in plant species of both riparian and evergreen oak forests, showed a high percentage of non-host species (56% and 69%, respectively) (**Figure 4.10**). At each location and visit, host species were further divided into two groups: (i) Uninfected hosts (i.e., samples from plant species that are hosts but with no infected individuals at a given visit/location), which constituted the second most abundant class in the distribution (33% and 25%) (**Figure 4.10**). (ii) Infected hosts (i.e., host species with at least one infected individual at a given visit/location). Finally, infected hosts were distributed into three prevalence classes: low, medium and high potyvirus prevalence (**Figure 4.10**). These three classes were created by calculating the frequency distribution of potyvirus prevalence across infected hosts and estimating the terciles of the distribution such three classes with similar number of observations in each prevalence class were created. Importantly, this categorical classification allowed minimizing biases due to imbalance in the number of instances at each category of prevalence. Potyvirus prevalence thresholds for each of these classes are shown in **Table 4.7**.

Figure 4.10. Histograms of potyvirus prevalence frequency distribution in riparian forest (blue) and evergreen oak forest (green).

Table 4.7. Thresholds used to categorize infected hosts in low, medium and high prevalence classes in the studied ecosystems.

Riparian forest	Evergreen oak forest
0% < low prevalence < 15.00%	0% < low prevalence < 9.09%
15.00% ≤ medium prevalence < 33.33%	9.09% ≤ medium prevalence < 18.18%
31.33% ≤ high prevalence ≤ 100%	18.18% ≤ high prevalence ≤ 100%

Unfortunately, similar balancing between prevalence classes and non-host/uninfected classes could not be attained. Non-hosts constituted the majority class representing between 54%-72% of the observations, followed by uninfected hosts that represented between 22-34%. Minority classes corresponded to infected hosts with low, medium and high potyvirus prevalence (**Table 4.8**). Thus, for the construction of the models, these imbalances were minimized by applying Synthetic Minority Over-sampling Technique (SMOTE) (Nitesh et al., 2002), which increases the number of observations in the minority classes of potyvirus prevalence in the training dataset (**section 3.5.2.1.1**).

ML models were constructed and trained using datasets containing information collected during the first sampling cycle (training set). The performance (predictive power) of the models was evaluated by means of five replicates of two-fold cross validation (5x2CV) on training sets. Also, the model constructed with data from the first sampling cycle was challenged with the data of the second sampling cycle (validation set), and performance was measured (**section 3.5.2.1.2**). **Table 4.8** shows the distribution of plant species in evergreen oak and riparian forests across the five categories of potyvirus prevalence in the training and validation data sets.

Table 4.8. Number and relative percentage (in parenthesis) of plant species in each class of potyvirus prevalence in the training and validation datasets used to build ML models.

Riparian forest datasets			Evergreen oak forest datasets		
Prevalence class	Training set ¹	Validation set ¹	Prevalence class	Training set ¹	Validation set ¹
<i>Non-host</i>	249 (54%)	257 (58%)	<i>Non-host</i>	222 (67%)	228 (72%)
<i>Uninfected host</i>	147 (31%)	153 (34%)	<i>Uninfected host</i>	94 (28%)	70 (22%)
<i>Low prevalence</i>	21 (5%)	12 (3%)	<i>Low prevalence</i>	5 (1%)	6 (2%)
<i>Medium prevalence</i>	21 (5%)	10 (2%)	<i>Medium prevalence</i>	6 (2%)	6 (2%)
<i>High prevalence</i>	21 (5%)	13 (3%)	<i>High prevalence</i>	6 (2%)	5 (2%)
TOTAL	459	445	TOTAL	333	315

¹Datasets containing information collected during the first sampling cycle (Training set) and the second sampling cycle (Validation set).

4.2.2.1. *Complete ML models of genus-wide potyvirus prevalence*

ML models were built using the above-defined classes of potyvirus prevalence as the response variable (**Table 4.8**). The following ecological factors were considered as predictors of potyvirus prevalence in the studied ecosystems: location, season, plant family, plant species, plant life cycle, plant density and relative abundance, plant biomass, species richness (S), Shannon's index (H') and Shannon's equitability index (E_H), soil pH, minimum, maximum and seasonal averaged values of temperature, relative humidity and rainfalls and number of host plant species in each location (**Table 4.9**). Three different ML algorithms were run: Multinomial Logistic Regression (Logistic), J48 Decision Tree (J48) and Random Forest. In order to compare ML algorithms, the ZeroR algorithm was also run to represent the baseline for classification accuracy. Classification models were considered valuable if they had better performance than the baseline algorithm (better classification than expected by chance).

Table 4.9. Mean values of continuous variables used as predictors in ML models for potyvirus prevalence in the studied ecosystems.

Predictors	Riparian forests		Evergreen oak forests	
	Training set	Validation set	Training set	Validation set
Plant density (m)	5.48 ± 5.24	5.69 ± 8.64	5.49 ± 4.95	4.13 ± 3.51
Relative abundance (%)	3.51 ± 3.23	3.51 ± 3.47	4.82 ± 4.56	5.76 ± 6.51
Biomass (m ³)	0.15 ± 0.14	0.10 ± 0.07	0.06 ± 0.06	0.06 ± 0.07
S	31.00 ± 3.80	29.17 ± 6.48	21.91 ± 5.26	21.30 ± 4.42
H'	3.03 ± 0.20	2.93 ± 0.29	2.72 ± 0.30	2.45 ± 0.30
E_H	0.89 ± 0.05	0.86 ± 0.04	0.88 ± 0.05	0.81 ± 0.06
HPS	13.50 ± 3.70	12.38 ± 3.89	8.50 ± 1.87	5.86 ± 2.41
T _{min} (°C)	3.04 ± 3.38	4.33 ± 4.31	3.73 ± 3.30	5.43 ± 4.01
T _{med} (°C)	15.32 ± 3.57	16.31 ± 4.00	16.12 ± 3.62	17.65 ± 4.27
T _{max} (°C)	28.20 ± 3.56	29.71 ± 3.48	28.15 ± 3.52	29.71 ± 4.05
RH _{min} (%)	18.37 ± 4.93	18.49 ± 5.69	18.92 ± 5.40	19.00 ± 6.34
RH _{med} (%)	54.21 ± 8.67	56.56 ± 10.35	50.33 ± 8.69	52.08 ± 13.46
RH _{max} (%)	96.50 ± 3.05	97.24 ± 1.91	93.78 ± 4.79	94.50 ± 5.15
Rainfalls (mm)	370.72 ± 288.85	351.98 ± 280.93	179.08 ± 130.50	326.29 ± 276.86
Soil pH	5.25 ± 0.35	5.25 ± 0.35	5.33 ± 0.76	5.33 ± 0.76

Mean ± standard deviation of continuous predictor variables in the first sampling cycle (Training set) and the second sampling cycle (Validation set). S: Species richness; H': Shannon's index; E_H : Shannon's equitability index; HPS: number of host plants at a given location; T_{max}, T_{min}, T_{mean}: Average of monthly maximum, minimum, and mean temperatures at a given season/location; RH_{max}, RH_{min}, RH_{mean}: Average of monthly maximum, minimum, and mean relative humidity at a given season/location; Rainfalls: Average of total rainfalls in a season.

Overall performance of the ML algorithms was evaluated using three measures: the Accuracy, the True Positive rate (TPR) and the area under the ROC curve (AUROC) (see **section 3.5.2.1.2**). In riparian forests, comparison between ML model performances showed that, in general, all three performed better than the baseline, regardless of cross-validation methods or a validation data set were used (**Table 4.10**). According to cross-validation methods, Logistic had the worst accuracy and AUROC values (Accuracy=47%, AUROC=72%, and the highest TP rate (TPR=25%) (**Table 4.10**). Also, this model had a drop in accuracy when it was challenged against the validation dataset (Accuracy=30%, AUROC=67% and TPR=17%). On the other hand, and according to cross-validation methods, J48 and Random Forest models had better performance than Logistic, except for the TPR (Accuracy=75% and 72%, AUROC=84% and 74%, and TPR=13% and 15%, respectively) (**Table 4.10**). These two ML models presented more robust values than Logistic when compared with the performance on the validation dataset (Accuracy=86% and 81%, AUROC=91% and 92%, and TPR=11% and 22%). Indeed, J48 and Random forest generally showed better performances on the validation dataset than in cross-validation analyses (**Table 4.10**). Thus, the built models have good predictive power not only in subsets of data from the same sampling cycle used to construct them (cross validation), but, more importantly, in a different dataset than that used to build the model (validation set).

Table 4.10. Performance measures (% of successfully classified instances) of ML models to predict potyvirus prevalence in riparian forests.

	5x2CV training set ¹				Validation set ²			
	ZeroR	Logistic	J48	Random Forest	ZeroR	Logistic	J48	Random Forest
Accuracy	54	47	75	72	58	30	86	81
AUROC	50	72	84	74	50	67	91	92
TPR	0	25	13	15	0	17	11	22

¹ Models were evaluated by means of 5 replications of two-fold cross validation (5x2CV) on the training dataset (first sampling cycle).

² Models were challenged with dataset containing information collected during the second sampling cycle. AUROC: Area under the ROC curve (AUROC); TPR: True Positive rate. Values are shown as percentages.

In evergreen oak forests, the three models showed similar performances than in riparian forests (**Table 4.11**). The exceptions were: (i) that J48 and

Random forest did not increase values of the AUROC curve when challenged with the validation dataset. Rather, the AUROC values were similar to those of the ZeroR baseline model (**Table 4.11**); and (ii) that AUROC values of the Logistic model were equal or better than in the other two models (**Table 4.11**).

Table 4.11. Performance measures (% of successfully classified instances) of ML models to predict potyvirus prevalence in evergreen oak forests.

	5x2CV training set ¹				Validation set ²			
	ZeroR	Logistic	J48	Random Forest	ZeroR	Logistic	J48	Random Forest
Accuracy	67	73	75	85	72	60	94	92
AUROC	50	89	75	92	50	80	51	65
TPR	0	19	11	17	0	31	20	20

¹ Models were evaluated by means of 5 replications of two-fold cross validation (5x2CV) on the training dataset (first sampling cycle).

² Models were challenged with dataset containing information collected during the second sampling cycle. AUROC: Area under the ROC curve (AUROC); TPR: True Positive rate. Values are shown as percentages.

In the analyses above, all predictor variables were kept in the model (complete model). However, it would be possible that not all predictors have the same relative importance in the accuracy of the model, such that simpler models may have similar or even better performance than the complete model. To test this possibility, different approaches were performed depending on the ML model:

(i) For Logistic models, a stepwise model selection approach was used to compare the accuracy of models built using all possible combinations of the predictors. The best-ranked model explaining potyvirus prevalence in riparian forests contained plant species, plant relative abundance and season as predictors (**Table 4.12**). This model explained 95% of the variance in the response variable, plant species having much higher relative importance (98%) than the other two predictors (**Table 4.12**). The best model in evergreen oak forests contained plant species, averaged rainfalls, averaged relative humidity and plant biomass. This model explained 99% of the variance, plant species having much higher relative importance than other predictors (96%) (**Table 4.12**). Thus, results indicated that plant identity was the most relevant factor in predicting potyvirus prevalence. Indeed, models in which plant species was the only predictor did not perform worse than the best-ranked model (**Table 4.12**). Also, the analysis of the predictive power of these models indicated that,

overall, their accuracy, AUROC and TPR values were equally good or even higher than in the complete models (compare **Table 4.10, 4.11 and 4.12**), although it should be noted that these models performed worse in the validation dataset than in the training dataset (**Table 4.12**). In both ecosystems, no other combination of factors yielded a model that closely competed with the best-ranked one (**Appendix 3, Table A3.1**).

4.12. Model fitting and performance of ML models for predicting potyvirus prevalence.

Model structure*	Model fitting ¹		Model performance on 5x2CV training set ²			Model performance on validation set ²		
	AIC	R ² _N	Acc	AUROC	TPR	Acc	AUROC	TPR
<i>Riparian forests</i>								
PSP (98)+PRA (1)+SEA (1)	1407.29	0.95	92	97	54	83	72	15
PSP (100)	1446.74	0.93	87	94	25	86	67	12
<i>Evergreen oak forests</i>								
PSP (96)+RAF (2)+RH _{med} (1)+V (1)	906.96	0.99	95	98	26	91	70	20
PSP (100)	921.93	0.95	96	97	27	94	68	20

*The relative importance (%) of each predictor variable is shown in parenthesis.

¹ Model fitting measures: Akaike's Information Criterion (AIC) and Nagelkerke R² (R²_N).

² Model performance measures: rate of correct predictions of the model (Acc: Accuracy), Area under the ROC curve (AUROC), True Positive rate (TPR). Values are shown as percentages.

PSP: Plant species; PRA: Plant relative abundance; SEA: Season; RAF: Rainfalls; RH_{med}: Average relative humidity; V: Plant biomass.

(ii) Models obtained with the J48 method were also analysed. J48 methods create a single decision tree from the training dataset. The tree represents the classification rules for the prediction of potyvirus prevalence and includes a root node from where the tree starts, and internal nodes connected by two or more branches. To which of these branches a given observation is assigned depends on threshold values of the predictor defining the node (see **Figure 4.11**). The tip nodes are known as *leaf nodes*, and the probability of being assigned to a given potyvirus prevalence category according to the predictors and threshold values that define the tree.

Figure 4.11 illustrates the decision tree for potyvirus prevalence classes in riparian forests, which summarizes the best predictors such that the deeper the node in the tree defined by a given predictor, and the higher the number of nodes defined by that predictor, the higher its relative importance. The trained

model classified correctly 91%, 90% and 92% of infected hosts with low, medium and high prevalence, respectively (**Figure 4.11**). Also, the model correctly assigned 100% of non-hosts and 93% of uninfected hosts. Of the predictors evaluated, J48 identified plant species as the best discriminator between prevalence classes (root node) (**Figure 4.11**). Moreover, presence/absence of certain host species was also identified as a relevant determinant of potyvirus incidence. For instance, *Conopodium subcarneum* (73%) and *Trifolium pratense* (25%) defined infected hosts with low prevalence, *Dactylis glomerata* (97%) defined host with medium prevalence, and *Lamium album* (90%) defined hosts species with high prevalence. Plant relative abundance was a relevant predictor of high virus prevalence when *Humulus lupulus* was the infected host, and its relative abundance was above 1.59% (**Figure 4.11**).

Figure 4.11. Condensed J48 Decision Tree of potyvirus prevalence in riparian forests. Defining predictors and categories (for categorical predictors) or threshold values (for continuous predictors) of each node are represented. Leaf nodes indicate the predicted prevalence class, the percentage of the total number of instances assigned to that prevalence class (in parenthesis) and the percentage of instances assigned to that prevalence class at the specific leaf node (out of the parenthesis). Dashed line group plant species that identified non-hosts that have been collapsed in the tree.

The J48 decision tree for potyvirus prevalence in evergreen oak forests training correctly classified 99% of uninfected host and non-hosts, 94% of infected hosts with low and medium prevalence and 98% of hosts with high prevalence (**Figure 4.12**). Again, this model identified plant species as the best predictor of prevalence class. (*Leontodon longirostris* defined all instances

classified as infected hosts with low prevalence from Marjaliza. Also, *Tolpis umbellata* defined infected hosts with medium prevalence when minimum relative humidity was higher than 15%. Finally, *Ruta montana* defined most cases of infected hosts with high prevalence (**Figure 4.12**).

Figure 4.12. Condensed J48 Decision Tree of potyvirus prevalence in evergreen oak forests. Defining predictors and categories (for categorical predictors) or threshold values (for continuous predictors) of each node are represented. Leaf nodes indicate the predicted prevalence class, the percentage of the total number of instances assigned to that prevalence class (in parenthesis) and the percentage of instances assigned to that prevalence class at the specific leaf node (out of the parenthesis). Dash line represents rest of classification rules that were associated to plant species. Dashed line group plant species that identified non-hosts that have been collapsed in the tree.

(iii) As mentioned in **section 3.5.2.1.2**, J48 Decision Trees provide less robust results than Random Forest models. Thus, to confirm the results obtained with J48 and Logistic models, the relative importance of each predictor in determining potyvirus prevalence was analysed by Random Forest models and ranked based on Mean Decrease Impurity (MDI) measures. In both ecosystems, plant family and species were the most relevant predictors (MDI>0.75) of potyvirus prevalence (**Figure 4.13**). The remaining predictors had similar relative importance (MDI=0.35-0.5) in both ecosystems (**Figure 4.13**).

Figure 4.13. Relative importance of the predictors of potyvirus prevalence according to Mean Decrease Impurity (MDI) values from 100 Random Forest trees. Trees were generated for the riparian forests (blue) and evergreen oak forests (green) datasets. Values are normalized in a range from 0 to 1. The higher the MDI of a predictor, the more relevant the predictor is.

In summary, the utilized ML methods constructed models with high accuracy, in both the training and the validation data sets. These models detected the identity of the plant species present in an ecosystem as the major (and often the unique) predictor of potyvirus prevalence, regardless of the ML method used. The dominance of plant species identity as a predictor of potyvirus prevalence could mask the effect of other factors that may also be important for virus prevalence. Thus, ML models were constructed excluding the predictors associated with plant identity (plant species and plant family).

4.2.2.2. ML models of genus-wide potyvirus prevalence excluding plant identity

The performance of the Logistic, J48 and Random Forest methods was assessed as in the previous section. Comparison between model performances showed that in both ecosystems, the three ML methods had performance scores close or even lower than the baseline (ZeroR) model in both the training and the validation datasets (**Table 4.13 and 4.14**).

Table 4.13. Model fitting and performance of ML models for predicting potyvirus prevalence in riparian forests excluding plant identity.

	5x2CV training set ¹				Validation set ²			
	ZeroR	Logistic	J48	Random Forest	ZeroR	Logistic	J48	Random Forest
Accuracy	54	47	46	47	58	42	42	41
AUROC	50	61	57	59	50	60	58	59
TPR	0	28	26	26	0	40	38	34

¹ Models were evaluated by means of 5 replications of two-fold cross validation (5x2CV) on the training dataset (first sampling cycle).

² Models were challenged with dataset containing information collected during the second sampling cycle. AUROC: Area under the ROC curve (AUROC); TPR: True Positive rate. Values are shown as percentages.

Table 4.14. Model fitting and performance of ML models for predicting potyvirus prevalence in evergreen oak forests excluding plant identity.

	5x2CV training set ¹				Validation set ²			
	ZeroR	Logistic	J48	Random Forest	ZeroR	Logistic	J48	Random Forest
Accuracy	67	62	60	58	72	17	39	61
AUROC	50	58	54	58	50	47	51	65
TPR	0	5	6	0	0	19	34	30

¹ Models were evaluated by means of 5 replications of two-fold cross validation (5x2CV) on the training dataset (first sampling cycle).

² Models were challenged with dataset containing information collected during the second sampling cycle. AUROC: Area under the ROC curve (AUROC); TPR: True Positive rate. Values are shown as percentages.

The relative importance of the predictors considered by these models were also analysed:

(i) Using model selection method for Logistic models, in riparian forests the best-ranked models had Accuracy and TPR values that were not significantly higher than those of the complete models, were validated with the training or the validation datasets (55% and 5% vs. 58% and 3%). However, AUROC values were significantly higher in the simpler than in the complete model (72% vs. 61% and 72% vs. 60%, for the training or the validation datasets, respectively), indicating better predictive power (**Table 4.15**). The best-ranked model explained 24% of the variance in the potyvirus prevalence in riparian forests (**Table 4.15**). Similar results were obtained in evergreen oak forests. Also, the most relevant ecological factors identified in Logistic models were largely the same in both ecosystems. Plant relative abundance had the highest relative importance (46% and 80% in riparian and evergreen oak forests, respectively), and plant life cycle was the second most important factors

(relative importance: 25% and 20% in riparian and evergreen oak forests, respectively). In riparian forests, the number of host species (relative importance: 21%) and rainfalls (relative importance: 8%) were also included in the models (**Table 4.15**). In riparian forest, a logistic model including the same factors as the best-ranked one but excluding rainfalls had similar predictive power (**Appendix 3, Table A3.2**). In evergreen oak forests, no other model closely competed with the best ranked one (**Appendix 3, Table A3.2**).

Table 4.15. Model fitting and performance of ML models for predicting potyvirus prevalence excluding plant identity.

Model structure	Model fitting ¹		Model performance on 5x2CV training set ²			Model performance on validation set ²		
	AIC	R ² _N	Acc	AUROC	TPR	Acc	AUROC	TPR
<i>Riparian forests</i>								
PRA (46)+PLC (25)+HPS (21)+RAF (8)	914.75	0.24	55	72	5	58	72	3
<i>Evergreen oak forests</i>								
PRA (80)+PLC (20)	516.05	0.15	67	71	0	62	60	0

* The relative importance (%) of each predictor variable is shown in parenthesis.

¹ Model fitting measures: Akaike's Information Criterion (AIC) and Nagelkerke R² (R²_N).

² Model performance measures: rate of correct predictions of the model (Acc: Accuracy), Area under the ROC curve (AUROC), True Positive rate (TPR). Values are shown as percentages.

PRA: Plant relative abundance; PLC: Plant life cycle; HPS: number of host plant species; RAF: Rainfalls.

(ii) J48 models generated large and complex decision trees. Because of the difficulty in summarizing this tree, only the raw tree is provided in **Appendix 3, Figures A3.1 and A3.2**. In riparian forests, plant relative abundance was the predictor with the highest relative importance, as it was located in the root node and defined the highest number of nodes in the tree (36 out of 164 nodes). Plant density and number of host plant species were also relevant predictors, as they defined the same number of nodes in the tree than plant relative abundance (36 out of 164 nodes) (**Appendix 3, Figure A3.1**). Other ecological factors also appeared in the tree (season, plant life cycle, species richness, plant biomass, E_H, T_{mean}, T_{max}, RH_{min}, and rainfalls), but defined a small number of nodes (less than 10 out of 164 each) (**Appendix 3, Figure A3.1**). In evergreen oak forests, season was the predictor defining the root node (**Appendix 3, Figure A3.2**). Plant density (defining 16 out of 64 nodes), and plant relative abundance (12 out of 64 nodes) were also important predictors of potyvirus prevalence as they defined the largest number of nodes (**Appendix 3,**

Figure A3.2). (**Appendix, Figure A3.2).** Other factors that appeared in the tree were plant life cycle, number of host plant species, plant biomass, E_H , T_{min} and RH_{max} , with low relative importance (defined less than 5 out of 64 nodes each).

(iii) Finally, according to Random Forest methods the predictors with the higher relative importance (higher MDI values) were plant density and relative abundance ($MDI > 0.5$). All other predictors presented MDI values lower than 0.4 (**Figure 4.14**). Note that plant life cycle, one of the predictors with higher relative importance in Logistic models, was also classified among the top five predictors of potyvirus prevalence by Random Forest methods.

Figure 4.14. Relative importance of the predictors of potyvirus prevalence according to Mean Decrease Impurity (MDI) values from 100 Random Forest trees. Trees were generated for the riparian forests (blue) and evergreen oak forests (green) datasets, excluding plant identity traits as predictors. Values are normalized in a range from 0 to 1. The higher the MDI of a predictor, the more relevant the predictor is.

In summary, excluding plant identity traits reduced the accuracy of ML models in predicting potyvirus prevalence, but allowed identifying other ecological factors that also affect such prevalence. All three ML models identified plant relative abundance as a predictor, and most models also

indicated that plant density and plant life cycle (annual or perennial) have a role in determining potyvirus prevalence in riparian and evergreen oak forests.

4.2.2.3. Role of ML predictors in determining genus-wide potyvirus prevalence

Results obtained in **sections 4.2.2.1 and 4.2.2.2** showed that ML models with the best predictive power contained plant species as the most important predictor. These models predicted mostly non-host and uninfected hosts categories (high Accuracy and AUROC), but showed lower predictive power for infected hosts with low, medium and high prevalence (low TPR). Interestingly, although ML models excluding plant identity traits had lower Accuracy and AUROC, had higher TPR values. These results suggested that: (i) complete ML models, particularly when plant species identity is considered, could be more powerful to predict presence/absence of potyvirus infection; and (ii) factors other than plant identity may be relevant to predict the intensity of potyvirus infection (i.e., the level of prevalence). In order to investigate these questions, models that contained plant identity as unique predictor or a combination of host relative abundance, plant density were constructed to predict the two major classes: non-host and host plant species.

4.2.2.3.1. ML models of genus-wide potyvirus presence/absence

To analyse the ecological factors affecting the presence/absence of potyviruses in the studied ecosystems, the categories of virus prevalence were simplified into two categories: Non-hosts, which constituted again the majority class representing between 54%-72% of the observations, and host plant species (the sum of uninfected and infected hosts), which represented between 28%-46% of the observations (**Table 4.8**).

In riparian forests, the three ML models showed robust performance in both cross-validation and validation analyses. Logistic model had lower accuracy when it was challenged against the validation set than in cross-validation analyses (Accuracy=76% and 94%, AUROC=84% and 98%, respectively). J48 and Random Forest showed similar or better performances on the validation dataset than in cross-validation analyses (Accuracy=98% and

97%; AUROC=99% and 99% and Accuracy=92% and 93%, AUROC=99% and 98%, respectively) (**Table 4.16**).

Table 4.16. Performance measures (% of successfully classified instances) of ML models to predict host species in riparian forests.

	5x2CV training set ¹				Validation set ²			
	ZeroR	Logistic	J48	Random Forest	ZeroR	Logistic	J48	Random Forest
Accuracy	54	94	97	93	55	76	98	92
AUROC	50	98	99	98	50	84	99	99
TPR	54	94	97	93	55	76	98	92

¹ Models were evaluated by means of 5 replications of two-fold cross validation (5x2CV) on the training dataset (first sampling cycle).

² Models were challenged with dataset containing information collected during the second sampling cycle. AUROC: Area under the ROC curve (AUROC); TPR: True Positive rate. Values are shown as percentages.

In evergreen oak forests, the three models showed similar performance scores than in riparian forests: Logistic model had a high predictive power that was worse in validation than in cross-validation analyses (Accuracy=70% and 97%, AUROC=92% and 99%, respectively) (**Table 4.17**). Although J48 performance values were similar to those of the ZeroR baseline model in cross-validation analyses, predictive power was maximal when this model was challenged against the validation set (Accuracy=67% and 100%, AUROC=49% and 100%, respectively). Random Forest models showed very high predictive power, and similar or better performances on the validation dataset than in cross-validation analyses (**Table 4.17**).

Table 4.17. Performance measures (% of successfully classified instances) of ML models to predict host species in evergreen oak forests.

	5x2CV training set ¹				Validation set ²			
	ZeroR	Logistic	J48	Random Forest	ZeroR	Logistic	J48	Random Forest
Accuracy	67	97	67	97	72	70	100	99
AUROC	49	99	49	99	50	92	100	100
TPR	67	97	67	97	72	70	100	99

¹ Models were evaluated by means of 5 replications of two-fold cross validation (5x2CV) on the training dataset (first sampling cycle).

² Models were challenged with dataset containing information collected during the second sampling cycle. AUROC: Area under the ROC curve (AUROC); TPR: True Positive rate. Values are shown as percentages.

Remarkably, the high performance of the ML models predicting presence/absence of potyvirus prevalence was achieved by including plant species identity as the sole predictor, with no other ecological factor being relevant in predicting virus prevalence. Thus, the species present in a given ecosystem determine the likelihood of potviruses to enter in such ecosystem.

4.2.2.3.2. *ML models of genus-wide potyvirus prevalence level*

ML models considering only observations in the low, medium or high potyvirus prevalence classes were constructed. Note that here TPR and accuracy represent the same measure, and thus have the same values.

In riparian forests, comparison between ML model performances showed that, all three performed better than the baseline, regardless of cross-validation method or a validation data set were used (**Table 4.18**). According to cross-validation methods, Random Forest had the best accuracy and AUROC values (Accuracy=68%, AUROC=82%). This model had worse predictive power when it was challenged against the validation dataset (Accuracy=63%, AUROC=67%). Logistic and J48 models had similar performance measures in cross-validation analyses (Accuracy=63%, AUROC=76% and Accuracy=60%, AUROC=75%, respectively). These models also had a drop in accuracy when they were challenged against the validation dataset (**Table 4.18**).

Table 4.18. Performance measures (% of successfully classified instances) of ML models to predict host potyvirus prevalence in riparian forests.

	5x2CV training set ¹				Validation set ²			
	ZeroR	Logistic	J48	Random Forest	ZeroR	Logistic	J48	Random Forest
Accuracy	32	63	60	68	34	46	63	63
AUROC	46	76	75	82	50	66	66	67
TPR	32	64	60	68	34	46	63	63

¹ Models were evaluated by means of 5 replications of two-fold cross validation (5x2CV) on the training dataset (first sampling cycle).

² Models were challenged with dataset containing information collected during the second sampling cycle. AUROC: Area under the ROC curve (AUROC); TPR: True Positive rate. Values are shown as percentages.

In general, ML models had low predictive power in evergreen oak forests when cross-validation methods were used (**Table 4.19**). However, all three models performed better than the baseline, Random Forests also having the best performance in cross-validation analysis (Accuracy=35%,

AUROC=43%). In this case, the predictive power was significantly better when the model was challenged against the validation dataset (Accuracy=76%, AUROC=91%). Logistic and J48 models had low performance scores in cross-validation analyses but their predictive power was higher when the validation dataset was used (Accuracy=29% and 41%, AUROC=50% and 65%; Accuracy=24% and 65%, AUROC=37% and 79%, respectively).

Table 4.19. Performance measures (% of successfully classified instances) of ML models to predict host potyvirus prevalence in evergreen oak forests.

	5x2CV training set ¹				Validation set ²			
	ZeroR	Logistic	J48	Random Forest	ZeroR	Logistic	J48	Random Forest
Accuracy	12	29	24	35	35	41	65	76
AUROC	18	50	37	43	50	65	79	91
TPR	12	29	24	35	35	41	65	77

¹ Models were evaluated by means of 5 replications of two-fold cross validation (5x2CV) on the training dataset (first sampling cycle).

² Models were challenged with dataset containing information collected during the second sampling cycle. AUROC: Area under the ROC curve (AUROC); TPR: True Positive rate. Values are shown as percentages.

As in previous sections, the relative importance of the predictors considered by each model were studied:

(i) Using model selection method for Logistic models, in riparian forests the best-ranked model explained 85% of the variance, plant relative abundance having higher relative importance (67%) than the other predictors in the model: rainfalls, species richness, season, host plant density and plant biomass, which had relative importances lower than 10% (**Table 4.20**). In evergreen oak forests, the best-ranked model explained 99% of the variance. This model contained four predictors: number of host plant species had higher relative importance (32%), followed by plant relative abundance (26%) and host plant density (25%), and finally rainfalls (17%) (**Table 4.20**). No other model closely competed with the best-ranked ones (**Appendix 3, Table A3.3**). Regardless of the ecosystem, the Accuracy and AUROC values of these models were higher than those of the complete models that excluded plant identity traits (compare **Tables 4.13 and 4.14** with **Tables 4.18 and 4.19**).

4.20. Model fitting and performance of ML models for predicting host potyvirus prevalence.

Model structure*	Model fitting ¹		Model performance on 5x2CV training set ²			Model performance on validation set ²		
	AIC	R ² _N	Acc	AUROC	TPR	Acc	AUROC	TPR
Riparian forests								
PRA (67)+RAF (9)+S (7)+SEA (7)+HPD (6)+V(4)	79.71	0.85	75	91	75	71	84	70
Evergreen oak forests								
HPS (32)+PRA (26)+HPD (25)+RAF(17)	20.06	0.99	94	99	93	70	83	69

*The relative importance (%) of each predictor variable is shown in parenthesis.

¹ Model fitting measures: Akaike's Information Criterion (AIC) and Nagelkerke R² (R²_N).

² Model performance measures: rate of correct predictions of the model (Acc: Accuracy), Area under the ROC curve (AUROC), True Positive rate (TPR). Values are shown as percentages.

PRA: Plant relative abundance; RAF: Rainfalls; S: Species richness; SEA: Season; HPD: Host plant density; V: Plant biomass; HPS: number of host plant species.

(ii) **Figure 4.15** illustrates the J48 decision tree for host potyvirus prevalence classes in riparian forests. The model classified correctly 67%, 90% and 95% of infected hosts with low, medium and high prevalence, respectively (**Figure 4.15**). The first major predictor in the decision tree was plant relative abundance (root node). The same predictor, but with different threshold values, also defined 55% and 63% of infected hosts with high and medium prevalence, respectively (**Figure 4.15**). Plant density and T_{max} were also important predictors in the decision tree: T_{max} defined 45% of infected hosts with high prevalence, and plant density defined 100% and 37% of infected hosts with low and medium prevalence, respectively.

Figure 4.15. Condensed J48 Decision Tree of host potyvirus prevalence in riparian forests. Defining predictors and categories (for categorical predictors) or threshold values (for continuous predictors) of each node are represented. Leaf nodes indicate the predicted prevalence class, the percentage of the total number of instances assigned to that prevalence class (in parenthesis) and the percentage of instances assigned to that prevalence class at the specific leaf node (out of the parenthesis).

In evergreen oak forests, plant relative abundance was also the most relevant predictor in the J48 decision tree for host potyvirus prevalence classes (**Figure 4.16**), followed by plant density, which defined between 40-60% and 67% of infected hosts with high and medium prevalence, depending on threshold values (**Figure 4.16**). Finally, plant life cycle defined 100% of infected hosts with low prevalence when plants were annuals and 33% of hosts with medium prevalence when plants were perennials (**Figure 4.16**).

Figure 4.16. Condensed J48 Decision Tree of host potyvirus prevalence in evergreen oak forests. Defining predictors and categories (for categorical predictors) or threshold values (for continuous predictors) of each node are represented. Leaf nodes indicate the predicted prevalence class, the percentage of the total number of instances assigned to that prevalence class (in parenthesis) and the percentage of instances assigned to that prevalence class at the specific leaf node (out of the parenthesis).

(iii) Finally, according to Random Forest methods, in both ecosystems the predictors with the higher relative importance (higher MDI values) were plant density and relative abundance (MDI=0.70-0.85). Plant life cycle was also ranked as the third most important predictor in riparian (MDI=0.5) and evergreen oak (MDI=0.75) forests. All other predictors presented lower MDI values (**Figure 4.17**).

These results confirmed that ML models identified plant relative abundance as the most important predictor and showed that plant density and plant life cycle are also important determinants of potyvirus prevalence in the studied ecosystems. Moreover, models that contained these factors improved

their predictive power when were used to predict level of prevalence on infected hosts plant species. Thus, once potyviruses enter riparian and evergreen oak forests, factors other than plant species identity are the best predictors of the level of virus prevalence.

Figure 4.17. Relative importance of the predictors of host potyvirus prevalence according to Mean Decrease Impurity (MDI) values from 100 Random Forest trees. Trees were generated for the riparian forests (blue) and evergreen oak forests (green) datasets, excluding plant identity traits as predictors. Values are normalized in a range from 0 to 1. The higher the MDI of a predictor, the more relevant the predictor is.

Bivariate analyses indicated that in riparian forests, there was a negative association between the relative abundance of host plant species and potyvirus prevalence ($r=-0.25$; $P=1\times 10^{-5}$) (**Figure 4.18A**), whereas there was a weak positive correlation between host plant density and potyvirus prevalence ($r=0.10$; $P=0.002$) (**Figure 4.18C**). In evergreen oak forests however, the relationship between host plant relative abundance and plant density with potyvirus prevalence followed the same trend than in riparian forests, but the association between these traits was not significant ($r<0.09$; $P>0.103$) (**Figures 4.18B and D**).

Figure 4.18. Bivariate relationships between host potyvirus prevalence and the best predictor variables identified in ML models. Regressions of plant relative abundance (A) and (B), and plant density (C) and (D) on potyvirus prevalence in riparian forests (blue) and evergreen oak forests (green) are represented.

4.3. Analysis of species-specific potyvirus prevalence in wild ecosystems in the Iberian Peninsula

In section 4.2, ecological factors affecting genus-wide potyvirus prevalence in evergreen oak and riparian forests were analysed. Although these analyses yield relevant information to understand virus epidemiology in the monitored ecosystems, for certain virus species some ecological factor may be more important than others, according to the specific characteristics of such virus species. Thus, analyses at the genus-wide level may mask the differential importance of a given ecological factor at the species-specific level (Jones, 2009). It has been proposed that one of the traits that may influence the relative importance of ecological factors in virus prevalence is the virus host range (Malpica et al., 2006; Pagán et al., 2012; McLeish et al., 2017). For instance, for a generalist virus (i.e., that with a wide host range), host density might be the key ecological factor: as a significant number of plant species would be hosts, the relevant factor for virus prevalence is how close are plants to each other. On the other hand, for a specialist virus (i.e., that with a narrow host range), plant species richness might be the relevant factor: as few species are virus hosts, the number of plant species would determine the chances for the virus to find a susceptible plant (Pagán et al., 2012; García-Arenal and Fraile, 2013; Johnson et al., 2015). Following this rationale, in the present chapter the importance of ecological factors in determining potyvirus species-specific prevalence is analysed. The analyses focus in two of the potyvirus species detected in evergreen oak and riparian forests: one that follows a specialist strategy and another that follows a generalist strategy.

4.3.1. Identification of potyvirus species

Potyvirus species present in evergreen oak and riparian forests were identified by sequencing the PCR products obtained with the universal primers described by Zhang et al., 2010, which correspond to a 350nt fragment of the NIb (see **section 3.2.2** of Materials and Methods). A total of 87 NIb fragments from the same number of potyvirus isolates were obtained. Using this sequence data set, analyses of nucleotide sequence identity between the amplified fragments and

the same region of the genome from potyvirus sequences available in GenBank (**Table 4.21**), and Maximum Likelihood-based inference of phylogenetic relationships between these sequences, were performed (**Figure 4.19**). Virus species identification was confirmed by sequencing the CP gene of the same isolates for which the 350nt NIb fragment was obtained, and following the same procedure (**Table 4.22 and Figure 4.20**).

Table 4.21. Nucleotide sequence identity of the four identified virus species with the phylogenetically closest potyvirus species based on the 350nt NIb fragment.

Identified potyvirus species	N	Phylogenetically closest potyvirus	Sequence identity (%) [*]
<i>Clover yellow vein virus</i>	14	<i>Clover yellow vein virus</i>	95-97
<i>Endive necrotic mosaic virus</i>	2	<i>Endive necrotic mosaic virus</i>	79
Mediterranean ruda virus	40	<i>Bean yellow mosaic virus</i>	74-76
		<i>Clover yellow vein virus</i>	54-56
Wild hop mosaic virus	31	<i>Papaya ringspot virus</i>	73-78

N: number of sequences.

^{*}Range of nucleotide identity between the sequenced isolates and representative sequences of the phylogenetically closest potyvirus species.

Both nucleotide NIb-based nucleotide identity and phylogenetic analyses indicated the presence of four potyvirus species:

(i) 14 sequences had 95-97% nucleotide identity (**Table 4.21**), and clustered in the same monophyletic group in the phylogenetic tree (**Figure 4.19**), with the available sequences of *Clover yellow vein virus* (CIYVV) and were therefore categorized as belonging to CIYVV. Similar identity (94-97%, **Table 4.22**) and phylogenetic position (**Figure 4.20**) were obtained in the analysis of CP gene.

(ii) Two sequences shared 79% nucleotide identity with the unique NIb sequence of *Endive necrotic mosaic virus* (ENMV) available in GenBank (**Table 4.21**) and clustered together with this reference sequence (**Figure 4.19**), and were identified as belonging to ENMV. This result could not be confirmed, as the CP gene of these two virus isolates could not be obtained.

Figure 4.19. Maximum likelihood phylogenetic tree based on the nucleotide sequence of a 350nt fragment of the NIb gene. Asterisks indicate nodes with SH-like support of ≥ 0.80 . The tree is mid-point rooted. Phylogenetic subgroups of potyviruses defined by Shukla et al., 1994 were used as reference and indicated in coloured branches. BCMV: *Bean common mosaic virus*; ChiVMV: *Chilli veinal mottle virus*; PRSV: *Papaya ringspot virus*; PVY: *Potato virus Y*; SCMV: *Sugarcane mosaic virus*; SPFMV: *Sweet potato feathery mottle virus*; TuMV: *Turnip mosaic virus*. Expansions of the phylogeny contain the group of virus sequences in which potyvirus isolates sequenced in this study (underlined) clustered. CIYVV: *Clover yellow vein virus*; ENMV: *Endive necrotic mosaic virus*; MeRV: *Mediterranean ruda virus*; WHMV: *Wild hop mosaic virus*.

(iii) 31 partial NIb sequences had 73-78% nucleotide identity with *Papaya ringspot virus* (PRSV) (Table 4.21) and clustered in a monophyletic group closely related, but separated, to the existing PRSV NIb sequences (Figure 4.19). As most isolates had nucleotide identities below 76%, they were considered as belonging to a non-previously described species of the genus *Potyvirus* (Adams et al., 2005). Analyses of the CP gene of these isolates indicated a nucleotide identity of 71-73% with the reference sequence of *Zucchini yellow fleck virus* (ZYFV) (Table 4.22), for which no NIb sequence was available. ZYFV was the phylogenetically closest potyvirus (Figure 4.20), PRSV being the second closest. As the species demarcation criteria for potyviruses based on CP sequence identity is lower than 76% (King et al., 2012), this result confirmed that this group of 31 sequences belonged to a no-described virus

species provisionally named as Wild hop mosaic virus (WHMV). The name was chosen because this species was detected with the highest prevalence in hop plants (*Humulus lupulus*) of wild ecosystems (see potyvirus host range in **section 4.3.2**).

(iv) The remaining 40 partial NIb sequences showed 74-76% nucleotide identity with sequences of *Bean yellow mosaic virus* (BYMV) isolates available in GenBank (**Table 4.21**). However, in the NIb-based phylogenetic analysis these 40 sequences formed a cluster closely related to CIYVV sequences (**Figure 4.19**) with which they shared 54-56% nucleotide identity (**Table 4.21**). The position of these 40 partial NIb sequences in the tree with respect to BYMV could not be determined because nodes containing BYMV sequences were not statistically supported. However, the analysis of the CP sequences was more consistent: it showed a nucleotide identity of 70% between the 40 sequenced CPs and the BYMV reference CP sequences (**Table 4.22**). Also, both groups of sequences grouped in different, but closely related, monophyletic groups (**Figure 4.20**). According to ICTV rules, these results indicated that the 40 sequences were from a novel species of the genus *Potyvirus* that was provisionally named Mediterranean ruda virus (MeRV) because this species was detected exclusively in mountain rue plants (*Ruta montana*) (see potyvirus host range in **section 4.3.2**).

Table 4.22. Nucleotide sequence identity of three identified virus species with the phylogenetically closest potyvirus species based on the CP.

Identified potyvirus species	N	Phylogenetically closest potyvirus	Sequence identity (%) [*]
<i>Clover yellow vein virus</i>	12	<i>Clover yellow vein virus</i>	94-97
Mediterranean ruda virus	40	<i>Bean yellow mosaic virus</i>	70
		<i>Clover yellow vein virus</i>	66-67
Wild hop mosaic virus	4	<i>Zucchini yellow fleck virus</i>	71-73
		<i>Papaya ringspot virus</i>	70

N: number of sequences.

^{*}Range of nucleotide identity between the sequenced isolates and representative sequences of the phylogenetically closest potyvirus species.

Figure 4.20. Maximum likelihood phylogenetic tree based on the nucleotide sequence of the CP gene. Asterisks indicate nodes with SH-like support of ≥ 0.80 . The tree is mid-point rooted. Phylogenetic subgroups of potyviruses defined by Shukla et al., 1994 were used as reference and indicated in coloured branches. BCMV: *Bean common mosaic virus*; ChiVMV: *Chilli vein mottle virus*; PRSV: *Papaya ringspot virus*; PVY: *Potato virus Y*; SCMV: *Sugarcane mosaic virus*; SPFMV: *Sweet potato feathery mottle virus*; TuMV: *Turnip mosaic virus*. Expansion of the phylogeny section contains the group of virus sequences in which potyvirus isolates of this study (underlined) are clustered. CIYVV: *Clover yellow vein virus*; BYMV: *Bean yellow mosaic virus*; MeRV: *Mediterranean ruda virus*; ZYFV: *Zucchini yellow fleck virus*; WHMV: *Wild hop mosaic virus*.

4.3.2. Potyvirus species-specific host range and prevalence

The host range of the identified potyvirus species was determined. Two virus species (ENMV and MeRV) were found in a single host species, whereas the other two (CIYVV and WHMV) were detected infecting plant species from different botanical families (**Table 4.23**). The two viruses with broader host ranges had a broader geographical distribution than virus species with a narrower host range (**Table 4.23**).

Table 4.23. Host range and geographical distribution of potyvirus species identified in the studied ecosystems.

Potyvirus species	Ecosystem	Location	Host species
CIYVV	Riparian forest	El Escorial	<i>Trifolium campestre</i> (Fabaceae)
			<i>Trifolium pratense</i> (Fabaceae)
		Palazuelos	<i>Rumex pulcher</i> (Polygonaceae)
			<i>Trifolium campestre</i> (Fabaceae)
			<i>Trifolium pratense</i> (Fabaceae)
ENMV	Riparian forest	Santa Cruz de Retamar	<i>Andryala ragusina</i> (Asteraceae)
MeRV	Evergreen oak forest	El Pardo	<i>Ruta montana</i> (Rutaceae)
WHMV	Evergreen oak forest	Marjaliza	<i>Cistus albidus</i> (Cistaceae)
		El Escorial	<i>Trifolium pratense</i> (Fabaceae)
			<i>Trifolium campestre</i> (Fabaceae)
			<i>Trifolium pratense</i> (Fabaceae)
		Pollos	<i>Ecballium elaterium</i> (Cucurbitaceae)
			<i>Humulus lupulus</i> (Cannabaceae)
			<i>Humulus lupulus</i> (Cannabaceae)
		Santa Cruz de Retamar	<i>Salix atrocinerea</i> (Salicaceae)

CIYVV: Clover yellow vein virus; ENMV: Endive necrotic mosaic virus; MeRV: Mediterranean ruda virus; WHMV: Wild hop mosaic virus

CIYVV was detected infecting three plant species in two locations of riparian forest (**Table 4.23**): In El Escorial, CIYVV infected the perennial plant *Trifolium pratense* and the annual plant *Trifolium campestre*, and in Palazuelos the virus infected these two hosts plus the perennial plant *Rumex pulcher*. The prevalence of CIYVV ranged between 4.17% and 28.57% in these two locations (**Appendix 4, Table A4.1**). ENMV was found occasionally infecting individuals of the perennial plant *Andryala ragusina* in the riparian forest of Santa Cruz de Retamar, with prevalence between 10.00% and 11.50% (**Appendix 4, Table A4.2**). The remaining two viruses (MeRV and WHMV) accounted for the largest fraction of infected plant species. MeRV was detected in the perennial plant *Ruta montana* in the evergreen oak forests of El Pardo (**Table 4.23**), and showed a prevalence of 18.18-73.91% (see **Table 4.24**). WHMV infected mostly perennial plant species collected in riparian forests (**Table 4.23**): *Trifolium pratense* in El Escorial and Palazuelos; *Humulus lupulus* in Pollos and Santa Cruz de Retamar; *Ecballium elaterium* in Pollos; and *Salix atrocinerea* in Santa Cruz de Retamar. WHMV was also detected in the annual plant *Trifolium*

campestre at Palazuelos (**Table 4.23**). In these hosts, prevalence of WHMV ranged between 5.41% and 100% during the monitored period (see **Table 4.27**). Exceptionally (one single plant), the virus was found in the evergreen oak forest of Marjaliza infecting the perennial plant *Cistus albidus*. Overall, potyvirus species identified in this thesis mostly infected perennial plants, with host range and prevalence varying according to virus species, geographical location and season.

Thus, the identified virus species could be classified into two groups according to their host range: MeRV and EMNV behaved as specialists, whereas WHMV and CIYVV behaved as generalists. To further study how the ecological factors recorded in evergreen oak and riparian forests could differentially influence virus prevalence according to the virus host range, further analyses focused in the most prevalent representative species of each strategy: MeRV as a specialist virus, and WHMV as a generalist virus.

4.3.3. Ecological factors affecting MeRV prevalence

4.3.3.1. Analysis of MeRV prevalence

As mentioned in the previous section, MeRV behaved as a specialist virus, as its host range was limited to the perennial plant mountain rue that was present only in evergreen oak forests. Indeed, the virus was highly prevalent in this host plant. In order to perform a more accurate analysis of the ecological determinants of MeRV prevalence, additional samplings were performed in locations of evergreen oak forests during a third sampling year (summer and autumn 2015 and spring 2016). Infection by MeRV was detected in every season during this third sampling year, as it was in the previous two years (**Table 4.24**). In total, MeRV prevalence was analysed in 98% (301/306) of the mountain rue plants collected in evergreen oak forests between summer 2013 and spring 2016. The virus was detected in 123/301 (41%).

Table 4.24. Season-specific average values of MeRV prevalence and of ecological/climatic factors measured in evergreen oak forest location.

Parameter	Sampling cycle 1			Sampling cycle 2			Sampling cycle 3		
	Summer 2013	Autumn 2013	Spring 2014	Summer 2014	Autumn 2014	Spring 2015	Summer 2015	Autumn 2015	Spring 2016
N	13	30	15	23	48	33	68	38	33
Prevalence	46.15	33.33	40.00	73.91	31.25	18.18	32.35	71.05	45.45
Relative abundance	0.07	0.15	0.08	0.12	0.24	0.16	0.34	0.19	0.16
Host plant density	2.94	2.36	2.36	2.54	2.43	2.32	2.39	2.41	2.46
V	0.01	0.05	0.05	0.01	0.04	0.08	0.00	0.10	0.02
S	24	15	26	25	18	27	9	18	19
T_{max}	34.47	24.90	28.37	36.30	29.10	25.85	40.70	22.90	31.45
T_{min}	8.80	-1.45	2.87	11.33	5.93	0.70	18.20	-2.20	14.51
T_{mean}	22.40	12.70	14.50	23.70	16.90	12.50	29.00	11.40	23.00
RH_{max}	85.67	90.00	89.00	86.33	89.33	88.50	83.00	89.00	89.00
RH_{min}	17.67	23.00	18.67	17.67	26.33	18.00	14.00	21.00	19.00
RH_{mean}	39.00	53.00	46.00	37.00	57.00	49.00	49.00	55.00	48.00
Rainfalls	95.00	216.00	230.00	42.00	419.00	348.00	94.00	136.00	289.00

N: Total number of analysed plants per population.

Prevalence: percentage of infected plants of mountain rue (*Ruta montana*).

V: Plant biomass (m³).

S: Species richness.

T_{max}, T_{min}, T_{mean}: Average of monthly maximum, minimum and mean temperatures (°C) in a season.

RH_{max}, RH_{min}, RH_{mean}: Average of monthly maximum, minimum and mean relative humidity (%) in a season.

Rainfalls: Average of total rainfalls (mm) in a season.

MeRV prevalence in mountain rue plants varied across seasons between 18% in spring 2015 to 74% in summer 2014 (**Table 4.24**). Average MeRV prevalence did not vary between sampling cycles ($\chi^2 < 2.21$, $P > 0.137$). However, during the first and second cycle the incidence was higher in summer than in spring and autumn ($\chi^2 > 11.43$, $P < 1 \times 10^{-5}$), whereas in the third sampling cycle the highest incidence occurred in autumn ($\chi^2 > 4.79$, $P < 0.029$) (**Table 4.24**). Cultivated fields of tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.) located near to the sampled locations of evergreen oak forests were also visited to address the possibility that MeRV could also infect crops. Melon (*Cucumis melo* L.) fields were also included in the surveys, as, together with pepper and tomato, this was the major cultivated plant in the area (**Table 4.25**). At least two fields from each crop were visited in summer, spring and autumn, and at each visit between 20 and 50 individuals were sampled. For that, three leaves of different branches from one out of every three plants were collected along a fixed itinerary. MeRV was never detected in melon, tomato and pepper plants.

Table 4.25. Locations of crop fields in the Iberian Peninsula visited between 2013 and 2016.

Location	Ecosystem	Latitude	Longitude	Elevation (m)
Cortijo de San Isidro	Cultivated/Melon	40.054	-3.580	494
Villamanrique de Tajo	Cultivated/Melon	40.083	-3.216	559
Cortijo de San Isidro	Cultivated/Tomato	40.056	-3.579	496
Ciruelos de Pradales	Cultivated/Tomato	41.439	-3.705	1161
Cortijo de San Isidro	Cultivated/Pepper	40.055	-3.579	495
Ciruelos de Pradales	Cultivated/Pepper	41.439	-3.703	1164

4.3.3.2. *Analysis of ecological factors affecting MeRV prevalence*

To further understand the ecological determinants of MeRV prevalence in evergreen oak forest, the following ecological factors were considered as predictors: host plant density and relative abundance, species richness, plant biomass, temperature and relative humidity (minimal, maximal and average values), and rainfall in the sampled locations (**Table 4.24**). The lower number of observations in this data set, as compared with that used in **section 4.2**, prevented analysing the association between the ecological factors and MeRV prevalence using ML methods. Instead, multiple regression model selection analyses were used. In these analyses, the continuous values of the dependent variable were used as data followed a normal distribution (using the Shapiro-Wilk normality test, $W=0.90$, $P>0.267$) (at odds with data used in **section 4.2**), and season was included as a covariate to account for the dependency of MeRV prevalence over time (**Table 4.24**). The best-ranked model contained plant density as the only predictor, this model closely competed with that containing rainfall as the only predictor (**Table 4.26**). The other models showed poorer predictive power ($\Delta_i > 2$).

Table 4.26. Model selection analyses for MeRV prevalence. Best-ranked models are shown.

Model structure [*]	$r^{\dagger}$	logLik	AIC [‡]	$\Delta_i^{\S}$	$\omega_i^{\P}$
HPD (100)	0.68*	-22.44	52.87	0.00	0.52
RAF (100)	-0.68*	-22.51	53.03	0.16	0.48

^{*}The relative importance (%) of each predictor variable is shown in parenthesis.

[†]Correlation coefficient. Asterisks indicate significant correlations ($P<0.05$).

[‡]Akaike's Information Criterion.

[§] Δ_i is the difference between the AIC of a given model and that of the best-ranked model, and quantifies how models compete (best-ranked model: $\Delta_i=0$; substantial empirical support: $\Delta_i=1-2$; considerable less support: $\Delta_i=2-7$; and no support: $\Delta_i>10$) (Burnham and Anderson, 2002).

[¶]AIC model weight as $\omega_i = \exp(-0.5\Delta_i) / \sum \exp(-0.5\Delta_i)$. The larger the ω_i , the greater the likelihood of the model relatively to the competing models. Maximum $\omega_i=1$.

HPD: Host plant density; RAF: Rainfalls.

Bivariate analyses indicated that there was a positive association between mountain rue density and MeRV prevalence ($r=0.68$; $P=0.046$) (Figure 4.21A), whereas rainfall was negatively associated with MeRV prevalence ($r=-0.68$; $P=0.052$) (Figure 4.21B).

Figure 4.21. Bivariate relationships between MeRV prevalence and the best predictor variables identified by model selection analyses. Regressions of host density (A) and rainfall (B) on MeRV prevalence are represented.

4.3.4. Ecological factors affecting WHMV prevalence

4.3.4.1. Analysis of WHMV prevalence

WHMV infected six different host plant species from taxonomically distant plant families in four riparian forest locations (Table 4.27). Additional samples of WHMV hosts were also collected in locations where the virus was present for a third sampling cycle in order to increase the time span of the study (Table 4.27). Infection by WHMV was detected in every season during the monitored period and prevalence was calculated as the percentage of infected plants relative to the total number of plants collected from a given host species. WHMV presence was analysed in 454 host plant samples (6 to 141 samples, depending on the host species). At odds with MeRV, WHMV was not detected in every season and at every location during the monitored period. WHMV prevalence varied across seasons between 8.33% in summer 2015 to 100% in spring 2014 and summer 2015. Overall, higher average prevalence was recorded in summer and spring ($\chi^2=9.70$, $P<0.002$) than in autumn ($\chi^2<3.46$, $P>0.063$). Average WHMV prevalence varied significantly between the first and the second cycles ($\chi^2=5.40$, $P=0.020$), but there were not significant differences between these cycles and third sampling cycle ($\chi^2<3.23$, $P>0.072$) (Table 4.27).

Table 4.27. Prevalence of WHMV in riparian forests during the monitored period.

	EL ESCORIAL		PALAZUELOS				POLLOS				SANTA CRUZ DE RETAMAR			
	<i>Trifolium pratense</i>		<i>Trifolium pratense</i>		<i>Trifolium campestre</i>		<i>Humulus lupulus</i>		<i>Ecballium elaterium</i>		<i>Humulus lupulus</i>		<i>Salix atrocinerea</i>	
Season	N	WHMV (%) ¹	N	WHMV (%) ¹	N	WHMV (%) ¹	N	WHMV (%) ¹	N	WHMV (%) ¹	N	WHMV (%) ¹	N	WHMV (%) ¹
Sampling cycle 1														
Summer 2013	10	0.00	15	0.00	8	0.00	6	66.67	0	–	1	0.00	2	50.00
Autumn 2013	17	0.00	18	0.00	7	0.00	0	–	0	–	0	–	1	0.00
Spring 2014	13	0.00	21	0.00	6	0.00	1	0.00	2	100.00	4	50.00	2	0.00
Sampling cycle 2														
Summer 2014	12	0.00	20	0.00	0	–	4	25.00	3	–	1	0.00	3	0.00
Autumn 2014	18	11.11	24	0.00	20	0.00	14	0.00	0	–	0	–	2	0.00
Spring 2015	22	22.73	37	5.41	14	21.43	11	63.64	0	–	2	50.00	2	0.00
Sampling cycle 3														
Summer 2015	12	8.33	4	0.00	1	0.00	1	0.00	1	100.00	0	–	0	–
Autumn 2015	18	0.00	4	0.00	0	–	4	0.00	0	–	14	7.14	2	0.00
Spring 2016	19	15.79	8	0.00	0	–	19	10.53	0	–	4	25.00	0	–

N: Total number of analysed plants per population.

¹ Prevalence calculated as percentage of WHMV-infected plants relative to the total number of plants analysed from a given host species.

Table 4.28. Average value of the ecological and climatic factors used as predictors of WHMV prevalence.

Season	Location ¹	Relative abundance (%)	Plant density	Biomass (m ³)	S	T _{max}	T _{min}	T _{mean}	RH _{max}	RH _{min}	RH _{mean}	Rainfalls
Sampling cycle 1												
Summer 2013	ESC	5.50	2.98	0.38	29.00	32.95	7.55	20.43	88.75	13.50	39.50	159.00
Autumn 2013	ESC	8.50	3.29	0.10	28.00	27.07	2.43	14.77	99.67	19.00	59.00	434.67
Spring 2014	ESC	7.00	3.46	0.13	34.00	27.07	2.47	13.43	98.67	18.67	50.00	431.00
Summer 2013	PAL	18.00	5.95	0.08	31.00	31.10	5.58	18.33	94.25	14.25	46.50	216.00
Autumn 2013	PAL	40.70	6.82	0.01	32.00	25.93	0.07	13.07	98.67	17.00	63.67	277.33
Spring 2014	PAL	13.50	5.29	0.08	26.00	24.27	0.97	11.90	99.67	15.67	57.67	349.00
Summer 2013	POL	3.00	6.98	0.11	32.00	32.48	6.60	18.68	96.25	14.25	49.00	222.50
Autumn 2013	POL	0.00	0.00	0.10	32.00	26.30	-0.80	12.90	99.00	31.50	65.50	299.00
Spring 2014	POL	1.00	13.15	0.13	35.00	25.50	1.23	12.67	99.00	18.00	56.33	166.00
Summer 2013	SCR	1.22	5.66	0.02	29.00	35.30	9.85	23.13	95.00	14.00	37.50	107.00
Autumn 2013	SCR	0.00	0.00	0.01	27.00	27.37	5.10	16.60	99.00	23.00	60.33	419.33
Spring 2014	SCR	2.00	2.91	0.06	30.00	27.97	4.50	15.30	98.00	17.00	52.67	315.33
Sampling cycle 2												
Summer 2014	ESC	6.00	3.45	0.17	31.00	34.27	9.33	22.27	93.33	13.67	36.33	211.33
Autumn 2014	ESC	9.00	3.49	0.20	26.00	29.65	3.65	15.20	99.50	30.00	62.67	594.50
Spring 2015	ESC	11.00	3.18	0.15	32.00	27.53	1.27	13.70	96.00	16.33	53.00	230.67
Summer 2014	PAL	20.00	3.91	0.04	23.00	32.57	6.63	20.13	96.33	12.00	42.33	138.67
Autumn 2014	PAL	34.50	3.27	0.03	30.00	27.50	4.50	14.97	98.67	24.67	63.00	507.00
Spring 2015	PAL	28.00	3.54	0.07	21.00	24.70	-0.73	11.87	98.33	15.33	61.00	232.00
Summer 2014	POL	2.00	5.44	0.13	35.00	34.57	7.33	20.47	93.33	12.67	44.33	80.00
Autumn 2014	POL	7.00	3.85	0.06	31.00	28.37	3.83	15.40	98.00	27.33	67.33	365.00
Spring 2015	POL	5.50	3.45	0.14	32.00	27.63	-0.73	12.57	97.00	14.67	60.50	101.33
Summer 2014	SCR	0.50	0.00	0.00	34.00	36.13	13.00	24.20	95.00	14.00	36.33	45.33
Autumn 2014	SCR	0.00	0.00	0.06	27.00	29.90	7.90	17.40	99.00	23.00	67.67	1031.33
Spring 2015	SCR	1.00	16.03	0.01	17.00	29.60	3.90	15.80	98.00	17.00	55.00	319.33
Sampling cycle 3												
Summer 2015	ESC	6.00	2.80	0.44	25.00	39.17	13.83	26.07	88.33	12.00	37.92	124.67
Autumn 2015	ESC	9.00	2.99	0.01	26.00	26.07	3.00	15.17	98.67	20.33	60.83	364.00
Spring 2016	ESC	7.50	3.19	0.31	39.00	28.38	13.93	21.16	97.33	17.50	51.50	330.83
Summer 2015	PAL	2.50	15.50	0.00	29.00	24.70	-0.73	11.87	95.67	11.67	–	320.67
Autumn 2015	PAL	1.00	14.00	0.00	29.00	24.73	2.10	13.50	99.33	17.33	–	208.67
Spring 2016	PAL	3.50	5.17	0.03	31.00	14.16	4.48	9.32	–	–	–	152.00
Summer 2015	POL	7.50	2.79	0.34	41.00	37.73	9.57	22.73	92.67	10.33	52.67	188.00
Autumn 2015	POL	6.00	3.23	0.04	28.00	25.37	0.33	13.23	98.00	22.00	66.42	415.33
Spring 2016	POL	9.50	4.13	0.26	39.00	27.6	11.76	19.67	98.00	16.33	58.42	133.67

¹Locations are designed with a three-letter code: ESC, El Escorial; POL, Pollos; SCR, Santa Cruz de Retamar.

S: Species richness

T_{max}, T_{min}, T_{mean}: Average of monthly maximum, minimum and mean temperatures (°C) in a season.

RH_{max}, RH_{min}, RH_{mean}: Average of monthly maximum, minimum and mean relative humidity (%) in a season.

Rainfalls: Average of total rainfalls (mm) in a season.

(–) Missing data.

4.3.4.2. *Analysis of ecological factors affecting WHMV prevalence*

To further understand the ecological determinants of WHMV prevalence in riparian forest, the following ecological factors were considered as predictors: host plant density and relative abundance, species richness, plant biomass, temperature and relative humidity (minimal, maximal and average values), and rainfall in the sampled locations. For this analysis, multiple regression model selection analyses were used. However, note that, at odds with MeRV, WHMV was detected in more than one location of riparian forests. Thus, for multiple regression analyses location-specific values in each season and year were used (**Table 4.28**). The best-ranked model contained plant relative abundance, mean temperature, species richness and plant biomass as predictors ($r=0.79$, $P=0.002$), with plant relative abundance having the higher relative importance (63%) (**Table 4.29**). The other predictors had much lower relative importance ($<18\%$). Ten models closely competed with the best-ranked one ($\Delta_i < 2$). All of these models had similar predictive power than the best-ranked one ($r=0.81-0.73$, $P<0.006$), and included plant relative abundance and mean temperature as predictors. Plant relative abundance had always the highest relative importance (46 to 72%) ($r=0.63$, $P=0.012$) (**Table 4.29**). Some models contained relative humidity and rainfalls as predictors, but always with low relative importance (**Table 4.29**).

Bivariate analyses indicated that there was a negative association between the relative abundance of host plant species and WHMV prevalence ($r=-0.43$; $P=0.008$) (**Figure 4.22A**), whereas plant biomass, mean temperature and species richness were not significantly associated with WHMV prevalence ($r<0.16$; $P>0.118$) (**Figure 4.22B-D**).

If the two virus species in which this section is focused are taken as representatives of a specialist and a generalist strategy, the results of this section indicate that the ecological factors determining virus prevalence depend on their host range. The chief predictor of MeRV (specialist) prevalence is the density of host plants, whereas the relative abundance of host plants is the major predictor of WHMV (generalist) prevalence.

Table 4.29. Model selection analyses for WHMV prevalence. Best-ranked models are shown ($\Delta_i < 2$).

Model structure*	$r^†$	logLik	AIC‡	$\Delta_i^§$	$\omega_i^ $
PRA (63)+T _{mean} (18)+S (13)+V (5)	0.79*	-60.67	133.35	0	0.17
PRA (47)+T _{mean} (17)+S (16)+HPD (15)+V (5)	0.81*	-60.04	134.07	0.73	0.12
PRA (51)+T _{mean} (22)+S (10)+RH _{min} (13)+V (5)	0.81*	-60.19	134.38	1.03	0.10
PRA (63)+T _{mean} (18)+S (16)+RH _{max} (3)	0.78*	-61.33	134.65	1.31	0.09
PRA (58)+T _{mean} (19)+S (13)+V (5)+RH _{mean} (5)	0.80*	-60.35	134.70	1.36	0.09
PRA (46)+S (19)+T _{mean} (16)+HPD (16)+RH _{max} (3)	0.80*	-60.46	134.93	1.58	0.08
PRA (60)+T _{mean} (18)+S (14)+V (5)+RH _{max} (3)	0.80*	-60.47	134.95	1.60	0.08
PRA (53)+T _{mean} (24)+RH _{min} (17)+V (6)	0.77*	-61.52	135.04	1.69	0.07
PRA (57)+T _{mean} (19)+S (10)+RFA (9)+V (5)	0.80*	-60.52	135.04	1.70	0.07
PRA (72)+T _{mean} (20)+V (8)	0.73*	-62.63	135.26	1.91	0.07
PRA (67)+S (17)+T _{mean} (16)	0.73*	-62.67	135.33	1.99	0.06

*The relative importance (%) of each predictor variable is shown in parenthesis.

†Correlation coefficient. Asterisks indicate significant correlations ($P < 0.05$).

‡Akaike's Information Criterion.

§ Δ_i is the difference between the AIC of a given model and that of the best-ranked model, and quantifies how models compete (best-ranked model: $\Delta_i = 0$; substantial empirical support: $\Delta_i = 1-2$; considerable less support: $\Delta_i = 2-7$; and no support: $\Delta_i > 10$) (Burnham and Anderson, 2002).

||AIC model weight as $\omega_i = \exp(-0.5\Delta_i) / \sum \exp(-0.5\Delta_i)$. The larger the ω_i , the greater the likelihood of the model relatively to the competing models. Maximum $\omega_i = 1$.

PRA: Plant relative abundance; T_{mean}: Average temperature; S: Species richness; HPD: Host plant density; V: Plant biomass; RH_{min}: Minimum relative humidity; RH_{max}: Maximum relative humidity; RFA: Rainfalls.

Figure 4.22. Bivariate relationships between WHMV prevalence and the best predictor variables identified by model selection analyses. Regressions of plant relative abundance (A), plant biomass (B), mean temperature (C) and species richness (D) on WHMV prevalence are represented.

4.4. Analysis of potyvirus genus-wide genetic diversity in wild ecosystems in the Iberian Peninsula

In the previous sections, the ecological determinants of potyvirus prevalence were analyzed at the genus and at the species level. These determinants, and their relative importance, varied depending on the taxonomical scale (genus vs. species) of the analysis and on the life strategy (specialist vs. generalist) of the virus species studied. Epidemiological changes may result in genetic modifications in the pathogen population ([Grenfell et al., 2004](#); [Archie et al., 2009](#); [Pybus and Rambaut, 2009](#)). Therefore, changes in potyvirus prevalence can be accompanied by variations in the virus population genetic diversity. As for virus epidemiology, evolutionary processes that shape virus population genetic diversity may take place at two levels: (i) macroevolutionary processes, which affect inter-species genetic diversification; and (ii) microevolutionary processes, which shape intra-species genetic diversity. RNA viruses, as are potyviruses, may differ greatly in their modes of macro and microevolution ([Kitchen et al., 2011](#)), and ecological factors are thought to shape these processes ([Huyse et al., 2005](#)). However, little is known about which and how ecological factors shape virus population genetic diversity at both evolutionary scales ([Huyse et al., 2005](#); [Rodelo-Urrego et al., 2015](#)).

To address these questions, in the following sections: (i) the effect of potyvirus prevalence and ecosystem ecological factors on the genetic diversity and selection pressures in potyvirus populations present in evergreen oak and riparian forests of the Iberian Peninsula at the genus-wide (macroevolutionary scale) level is investigated. (ii) The population genetic structure of the potyvirus population is analysed in order to understand the modes of virus speciation in the studied wild ecosystems.

4.4.1. Genetic diversity of potyviruses

Given that sequences of a sole potyvirus species (MeRV) were collected in evergreen oak forests, in this ecosystem genus-wide and species-specific analyses are equivalent. Thus, the genetic diversity at the genus *Potyvirus* level

was studied only in riparian forests, and species-specific analyses of the ecological factors affecting MeRV population genetic diversity are presented in **section 4.5**. As the potyvirus populations of riparian forests were genetically structured according to geographical location (see **section 4.4.4** for details), evolutionary parameters (i.e., genetic diversity and selection pressures parameters) were quantified for potyvirus populations at each location. Both the partial NIb and the CP genes sequence datasets were used. The following parameters were considered as measures of genetic diversity: the number of haplotypes (H), the haplotype diversity (H_d), the average nucleotide genetic diversity (π), and the number of synonymous (d_S) and non-synonymous (d_N) substitutions per site (**Table 4.30**). The ratio between the latter two parameters was used as a measure of selection pressures in the virus population (**Table 4.30**).

When the partial NIb sequence data set was used, potyvirus population haplotype number, haplotype diversity, genetic diversity and number of non-synonymous and synonymous substitutions per site varied between locations ($H=3-9$; $H_d=0.733-0.933$; $\pi=0.012-0.231$, $d_N=0.000-0.198$; $d_S=0.128-0.808$). The virus populations at El Escorial and Palazuelos had the highest values in all genetic diversity parameters, and Pollos the lowest (**Table 4.30**). In addition, the potyvirus populations in the four locations monitored were under purifying selection ($d_N/d_S < 1$). Purifying selection was weakest in the virus population of El Escorial, and strongest in the virus population of Pollos (**Table 4.30**).

Data obtained using the CP gene sequence could be only obtained for two locations: Pollos and Palazuelos. For El Escorial and Santa Cruz de Retamar, less than three sequences were available and values could not be determined (**Table 4.30**). Potyvirus population haplotype number, haplotype diversity, genetic diversity and number of non-synonymous and synonymous substitutions per site varied between locations ($H=3-9$; $H_d=0.978-1.000$; $\pi=0.020-0.029$, $d_N=0.015-0.019$; $d_S=0.022-0.226$), but in a much narrower interval than for the NIb (**Table 4.30**). The potyvirus population at Palazuelos had the higher value for all the estimated parameters (**Table 4.30**). Analyses also indicated that the CP gene was generally under purifying selection, this being much stronger in the virus population of Palazuelos than in the population of Pollos (**Table 4.30**).

Table 4.30. Partial Nlb gene- and CP gene-based number of haplotypes (H), haplotype diversity (H_d), genetic diversity (π), number of non-synonymous and synonymous substitutions (d_N and d_S) per site and d_N/d_S ratio of genus-wide potyvirus populations in riparian forest locations.

Location	N	H	$H_d \pm SE$	$\pi \pm SE$	$d_N \pm SE$	$d_S \pm SE$	$d_N/d_S \pm SE$
<i>Nlb</i>							
ESC	11	8	0.927 ± 0.066	0.231 ± 0.038	0.198 ± 0.039	0.738 ± 0.092	0.269 ± 0.102
PAL	16	9	0.933 ± 0.035	0.216 ± 0.031	0.163 ± 0.036	0.808 ± 0.079	0.202 ± 0.086
POL	13	6	0.833 ± 0.071	0.012 ± 0.004	0.000 ± 0.000	0.128 ± 0.038	0.000 ± 0.038
SCR	6	3	0.733 ± 0.155	0.209 ± 0.041	0.139 ± 0.042	0.535 ± 0.078	0.261 ± 0.088
<i>CP</i>							
ESC	2	2	ND	ND	ND	ND	ND
PAL	10	9	0.978 ± 0.054	0.029 ± 0.004	0.019 ± 0.006	0.226 ± 0.029	0.086 ± 0.030
POL	3	3	1.000 ± 0.272	0.020 ± 0.005	0.015 ± 0.007	0.022 ± 0.010	0.650 ± 0.018
SCR	1	1	ND	ND	ND	ND	ND

N: number of sequences; SE: standard error; ND: Not determined.

4.4.2. Analysis of recombination and nucleotide substitution rates of potyvirus populations

To investigate which were the mechanisms contributing to the generation of genetic diversity of the potyvirus populations present in riparian forests, recombination and nucleotide substitution rates were estimated.

Inter and intra-species recombination events in the CP gene and in the partial Nlb gene sequences were analysed. These analyses were done using only the potyvirus sequences obtained in this thesis (**Appendix 1, Table A1.4**), and also including reference sequences from all known potyviruses (**Appendix 1, Table A1.5**). No recombination events were detected in any of the analyses, either between isolates of the same viral species or between isolates of different species. This result indicates that, at least for the analyzed regions of the potyvirus genome, the main mechanisms generating genetic diversity was mutation. Thus, nucleotide substitution rates at the genus level were estimated for potyviruses of riparian forests.

The presence of temporal signal in the data utilized to estimate nucleotide substitution rates is essential to the accurate estimation of such substitution rates. Thus, the strength of temporal signal in the potyvirus partial Nlb and the CP sequence datasets was first assessed. To do so, the BEAST

analyses described below were repeated on data sets in which sampling times were randomized so that temporal structure was disrupted. Runs for randomized data were repeated 10 times (see **section 3.3.2.1 of Materials and Methods**). The mean and 95% HPDs of the substitution rate estimates for the randomized data were then compared with those obtained from the real data. In all cases, estimates of the mean substitution rate differed by at least one order of magnitude between the real and randomized data sets (**Figure 4.23**). Also, the 95% HPD values for all of the randomized controls excluded the mean substitution rates estimated for the real data, indicating that they are significantly different. In addition, the lower 95% HPD values in the randomized data sets differed by at least two orders of magnitude from the corresponding mean estimates, and hence strongly indicated insufficient temporal structure. Far narrower 95% HPD results were observed for the real data. Hence, the sequence data analysed here contain sufficient temporal structure for reliable estimation.

Figure 4.23. Estimates of the rate of nucleotide substitutions per site in the partial Nlb and the CP genes sequence data sets, using the real (blue squares) and randomized (red squares) dates of isolation, with 95% HPD values shown.

Because sampling time spanned only three sampling cycles, rates of nucleotide substitutions per site per day (subs/site/day) were estimated. Nucleotides substitution rates based on the partial Nlb sequence were of 9.01×10^{-6} subs/site/day (95% HPD: $8.22 \times 10^{-7} - 7.19 \times 10^{-5}$). Substitution rates based on the CP gene were in the same range: 5.35×10^{-6} subs/site/day (95% HPD: $1.27 \times 10^{-6} - 1.22 \times 10^{-5}$). These values roughly corresponded to 3.29×10^{-3} subs/site/year (95% HPD: $3.00 \times 10^{-4} - 2.62 \times 10^{-2}$), and 1.95×10^{-4} subs/site/year (95% HPD: $4.63 \times 10^{-5} - 4.45 \times 10^{-4}$), for the Nlb and the CP genes, respectively.

Table 4.3.1. Average values of potyvirus prevalence and of ecological factors measured in riparian forests locations.

Season	Location ¹	Prevalence ²	Relative abundance	Plant density	V	S	T _{max}	T _{min}	T _{mean}	RH _{max}	RH _{min}	RH _{mean}	Rainfalls
Sampling cycle 1													
Summer 2013	ESC	0.00	9.50	4.08	0.38	29.00	32.95	7.55	20.43	88.75	13.50	39.50	159.00
Autumn 2013	ESC	0.00	8.50	3.29	0.10	28.00	27.07	2.43	14.77	99.67	19.00	59.00	434.67
Spring 2014	ESC	7.69	7.00	3.46	0.13	34.00	27.07	2.47	13.43	98.67	18.67	50.00	431.00
Summer 2013	PAL	4.35	18.00	5.95	0.08	31.00	31.10	5.58	18.33	94.25	14.25	46.50	216.00
Autumn 2013	PAL	8.33	40.70	6.82	0.01	32.00	25.93	0.07	13.07	98.67	17.00	63.67	277.33
Spring 2014	PAL	0.00	13.50	5.29	0.08	26.00	24.27	0.97	11.90	99.67	15.67	57.67	349.00
Summer 2013	POL	66.67	3.00	6.98	0.11	32.00	32.48	6.60	18.68	96.25	14.25	49.00	222.50
Autumn 2013	POL	–	0.00	0.00	0.10	32.00	26.30	-0.80	12.90	99.00	31.50	65.50	299.00
Spring 2014	POL	66.67	1.00	13.15	0.13	35.00	25.50	1.23	12.67	99.00	18.00	56.33	166.00
Summer 2013	SCR	18.18	8.54	5.75	0.02	29.00	35.30	9.85	23.13	95.00	14.00	37.50	107.00
Autumn 2013	SCR	0.00	5.50	4.46	0.01	27.00	27.37	5.10	16.60	99.00	23.00	60.33	419.33
Spring 2014	SCR	33.33	3.00	5.09	0.06	30.00	27.97	4.50	15.30	98.00	17.00	52.67	315.33
Sampling cycle 2													
Summer 2014	ESC	0.00	6.00	3.45	0.17	31.00	34.27	9.33	22.27	93.33	13.67	36.33	211.33
Autumn 2014	ESC	11.11	12.5	4.07	0.20	26.00	29.65	3.65	15.20	99.50	30.00	62.67	594.50
Spring 2015	ESC	22.22	13.5	3.95	0.15	32.00	27.53	1.27	13.70	96.00	16.33	53.00	230.67
Summer 2014	PAL	0.00	20.00	3.91	0.04	23.00	32.57	6.63	20.13	96.33	12.00	42.33	138.67
Autumn 2014	PAL	0.00	34.5	3.27	0.03	30.00	27.50	4.50	14.97	98.67	24.67	63.00	507.00
Spring 2015	PAL	19.61	28.00	3.54	0.07	21.00	24.70	-0.73	11.87	98.33	15.33	61.00	232.00
Summer 2014	POL	14.29	2.00	5.44	0.13	35.00	34.57	7.33	20.47	93.33	12.67	44.33	80.00
Autumn 2014	POL	0.00	7.00	3.85	0.06	31.00	28.37	3.83	15.40	98.00	27.33	67.33	365.00
Spring 2015	POL	63.64	5.50	3.45	0.14	32.00	27.63	-0.73	12.57	97.00	14.67	60.50	101.33
Summer 2014	SCR	0.00	8.50	3.66	0.00	34.00	36.13	13.00	24.20	95.00	14.00	36.33	45.33
Autumn 2014	SCR	0.00	2.50	8.41	0.06	27.00	29.90	7.90	17.40	99.00	23.00	67.67	1031.33
Spring 2015	SCR	14.29	8.00	12.92	0.01	17.00	29.60	3.90	15.80	98.00	17.00	55.00	319.33

¹Locations are designed with a three-letter code: ESC, El Escorial; POL, Pollos; SCR, Santa Cruz de Retamar.

²Percentage of infected plants relative to the total number of plants analysed from all host species present in each location.

S: Species richness

V: Plant biomass (m³).

T_{max}, T_{min}, T_{mean}: Average of monthly maximum, minimum and mean temperatures (°C) in a season.

RH_{max}, RH_{min}, RH_{mean}: Average of monthly maximum, minimum and mean relative humidity (%) in a season.

Rainfalls: Average of total rainfalls (mm) in a season.

4.4.3. Analysis of ecological factors affecting the genus-wide potyvirus population genetic diversity

The ecological factors (**Table 4.31**) affecting genus-wide potyvirus population H_d , π , d_N , d_S , and d_N/d_S within riparian forests locations were analysed using multiple regression model selection analyses. The partial Nlb sequence dataset was used in this analysis. Note that, as for datasets used in **sections 4.3 and 4.4**, datasets utilized in the present section had much smaller sample size than those in **section 4.2** and were normally distributed ($W=0.92$; $P=0.391$). The following factors were used as predictors: host plant density and relative abundance, species richness, plant biomass, temperature and relative humidity (minimal, maximal and average values), and rainfall in the sampled locations. Also, potyvirus prevalence was added as an additional predictor (**Table 4.31**). Potyvirus prevalence in each location was calculated as the percentage of infected plants relative to the total number of plants analysed from all host species present in each location. Host plant relative abundance and density were calculated accordingly. The best-ranked model explaining H_d contained plant relative abundance and plant biomass as the most important predictors ($r=0.94$; $P=1 \times 10^{-5}$). In this model plant relative abundance had higher relative importance than plant biomass (41% and 29%, respectively) (**Table 4.32**). Bivariate analyses indicated a positive association between these two predictors and H_d ($r=0.33$; $P=0.038$) (**Figure 4.24A and B**). The best-ranked models predicting potyvirus genetic diversity (π), and d_N , contained rainfalls and plant relative abundance as the most important predictors (relative importance ranged between 30%-44%) ($r>0.85$; $P<0.006$) (**Table 4.32**). Bivariate analysis showed a positive relationship between these predictors and the studied parameters ($r>0.33$; $P<0.039$), although the association with plant relative abundance was only marginally significant ($r=0.27$; $P<0.067$) (**Figure 4.24C-F**). The best-ranked models predicting d_S contained again plant relative abundance and rainfalls as the most important predictors ($r=0.93$; $P=1 \times 10^{-5}$) (**Table 4.32**), both factors showing a positive association with d_S (**Figure 4.24G and H**). The best-ranked models predicting d_N/d_S ($r=0.72$; $P=0.008$) contained potyvirus prevalence and rainfalls as the most important predictors (**Table 4.32**). Bivariate analysis showed a negative relationship between d_N/d_S and prevalence ($r=-$

0.53; $P=0.004$), whereas the association with rainfalls was positive ($r=0.35$; $P=0.032$) (Figure 4.24I and J). No other combination of factors yielded a model that closely competed with the best-ranked one (Appendix 5, Table A5.1).

Table 4.32. Model selection analyses for potyvirus population haplotype diversity (H_d), genetic diversity (π), number of synonymous (d_s) and non-synonymous (d_N) substitutions per site, and selection pressures (d_N/d_s). Model structures included host plant density, host relative abundance, plant species richness and plant biomass; and temperature, relative humidity and rainfall in the sampled locations (minimal, maximal and average values) as predictors, and season as covariate. Potyvirus prevalence was also included as predictor of virus evolution parameters. Best-ranked models are shown.

Model structure*	$r^{\dagger}$	logLik	AIC [‡]	$\Delta_i^{\S}$	$\omega_i^{\P}$
H_d					
PRA (41)+V (29)+HPD (11)+T _{mean} (10)+RH _{mean} (9)	0.94*	33.44	-52.89	0.00	0.32
PRA (47)+V (35)+T _{mean} (10)+RH _{mean} (9)	0.93*	32.06	-52.13	0.76	0.22
PRA (40)+V (28)+HPD (11)+T _{mean} (9)+RH _{mean} (8)+RFA (3)	0.95*	34.01	-52.03	0.86	0.21
π					
RAF (36)+PRA (32)+S (16)+RH _{mean} (12)+T _{mean} (4)	0.85*	24.46	-34.91	0.00	0.33
PRE (36)+RAF (23)+PRA (18)+S (12)+RH _{mean} (7)+T _{mean} (4)	0.87*	25.11	-34.23	0.68	0.24
RAF (33)+PRA (29)+S (18)+RH _{mean} (11)+HPD (5)+T _{mean} (4)	0.86*	24.87	-33.74	1.17	0.18
d_N					
RAF (44)+PRA (30)+RH _{mean} (11)+S (10)+T _{mean} (5)	0.87*	27.52	-41.04	0.00	0.16
RAF (40)+PRA (26)+S (11)+RH _{mean} (10)+HPD (8)+T _{mean} (5)	0.88*	28.52	-41.03	0.01	0.16
PRE (36)+RAF (28)+PRA (17)+S (7)+T _{mean} (6)+RH _{mean} (6)	0.88*	28.51	-41.02	0.02	0.16
d_s					
PRA (45)+RAF (25)+S (9)+HPD (8)+RH _{mean} (8)+T _{mean} (5)	0.93*	15.54	-15.08	0.00	0.16
PRE (32)+PRA (29)+RAF (17)+S (6)+T _{mean} (6)+RH _{mean} (5)+HPD (5)	0.94*	16.51	-15.02	0.07	0.15
PRE (35)+PRA (31)+RAF (18)+RH _{mean} (6)+S (5)+T _{mean} (5)	0.93*	15.44	-14.89	0.19	0.14
d_N/d_s					
PRE (49)+RAF (39)+V (12)	0.72*	17.85	-25.69	0.00	0.10
RAF (44)+S (20)+PRA (19)+RH _{mean} (13)+T _{mean} (4)	0.79*	19.64	-25.28	0.41	0.08
PRE (44)+RAF (37)+V (14)+HPD (5)	0.74*	18.50	-25.69	0.69	0.07

*The relative importance (%) of each predictor variable is shown in parenthesis.

[†]Correlation coefficient. Asterisks indicate significant correlations ($P<0.05$).

[‡]Akaike's Information Criterion.

[§] Δ_i is the difference between the AIC of a given model and that of the best-ranked model, and quantifies how models compete (best-ranked model: $\Delta=0$; substantial empirical support: $\Delta=1-2$; considerable less support: $\Delta=2-7$; and no support; $\Delta>10$) (Burnham and Anderson, 2002).

[¶]AIC model weight as $\omega_i = \exp(-0.5\Delta_i) / \sum \exp(-0.5\Delta_i)$. The larger the ω , the greater the likelihood of the model relatively to the competing models. Maximum $\omega_i=1$.

PRA: Plant relative abundance; V: Plant biomass; HPD: Host plant density; T_{mean}: Average temperature; RH_{mean}: Average relative humidity; RAF: Rainfalls; PRE: Prevalence; S: Species richness.

Figure 4.24. Bivariate relationships between potyvirus population evolutionary parameters and the best predictors. The associations between potyvirus population haplotype diversity (A and B), population genetic diversity (C and D), number of non-synonymous (E and F) and synonymous mutations per site (G and H) and overall selection pressures d_N/d_s (I and J) and best predictors are represented.

It is also important to indicate that models competing with the best ranked one that explained d_N and d_S contained potyvirus prevalence as the most important predictor (relative importance ranged between 32% and 36%) (Table 4.32). Potyvirus prevalence showed a negative association with both d_N and d_S ($r > -0.60$; $P < 0.002$) (Figure 4.25A and B).

Figure 4.25. Bivariate relationships between potyvirus population evolutionary parameters and the best predictors in models competing with the best-ranked one. The associations of potyvirus population d_N (A), and population d_S (B) with potyvirus prevalence are represented.

4.4.4. Analysis of potyvirus speciation modes

To determine the respective roles of allopatric *versus* sympatric speciation, we assessed the strength of clustering by host species and location of sample origin within the interspecific potyvirus phylogeny. If allopatric speciation were the dominant process, we would expect no significant association between phylogeny and host species, and perhaps between phylogeny and geography. In contrast, if sympatric speciation were the most important macroevolutionary process in these data, we would expect a significant association between phylogeny and both the host species and the location of sampling (Kitchen et al., 2011).

The population genetic structure of the potyvirus species according to geographical location and host of origin of each isolate was analysed through association analyses. The degree of association between the geographical origin of each sequenced isolate and the host species, and the position of the corresponding NIb sequence in the phylogeny was investigated using Maximum-likelihood trees (Figures 4.26 and 4.27), and estimating the

Association Index (AI), Parsimony Score (PS) and Monophyletic Clade Size (MC) statistics. These analyses allowed exploring the modes of potyvirus speciation in the two ecosystems considered in this thesis.

Figure 4.26. Maximum-likelihood phylogeny based on the NIb region representing the association between potyvirus phylogeny and geographical location. Asterisks indicates nodes with a bootstrap support ≥ 0.90 .

Both the AI and PS statistics revealed a significant association between the interspecies phylogeny of the *Potyvirus* and the plant host each virus species naturally infects ($P < 0.01$); hence, virus species that have the same geographical origin tend to cluster together on the tree (**Figure 4.26**). Similarly, a statistically significant association was observed between the potyvirus phylogeny and the host that each virus infects ($P < 0.01$ in both tests) (**Figure**

4.27), such that virus species that infect plant hosts in the same geographic location tend to cluster together. Together, these data suggest that viral speciation tends to occur within the same host species and in the same geographic area, which is compatible with genetic diversification through sympatric speciation.

Association analyses were also done using the CP sequence dataset with comparable results (Appendix 5, Figure A5.1 and A5.2).

Figure 4.27. Maximum-likelihood phylogeny based on the NIb region representing the association between potyvirus phylogeny and host plant species. Asterisks indicates nodes with a bootstrap support ≥ 0.90 .

4.5. Analysis of potyvirus species-specific genetic diversity in wild ecosystems in the Iberian Peninsula

If the ecological factors that shape virus prevalence differ between specialist and generalists, the ecological determinants of virus population genetic diversity could also vary according to the virus host range (e.g. [Hillung et al., 2015](#); [Cuevas et al., 2016](#)). Phylogenetic analyses in RNA viruses also suggest that the virus host range affects the speciation mode at the microevolutionary scale ([Kitchen et al., 2011](#)). However, the interplay between ecological factors, virus host range and population genetic diversity/structure has been seldom analysed in wild ecosystems.

These questions are addressed in this section by focusing in two of the virus species identified in evergreen oak and riparian forests: MeRV and WHMV. As described in previous sections, in the analysed ecosystems the first is a specialist, whereas the second is a generalist virus.

4.5.1. Analysis of the population genetic diversity of a specialist potyvirus in wild ecosystems of the Iberian Peninsula

Given that MeRV was identified as a new potyvirus species, the virus was genetically and biologically characterized prior to analysing the effect of ecological factors in the genetic diversity of MeRV populations.

4.5.1.1. Analysis of the complete nucleotide sequence of MeRV

The MeRV genomic sequence was obtained by NGS sequencing and confirmed by RT-PCR. The virus full-length genome sequence consists of 9,560nt excluding the 3' terminal poly(A) tail. As for all members of the genus *Potyvirus*, the MeRV-ParP17 genome has a single ORF encoding a polyprotein of 3,077 amino acids with an estimated molecular weight of 350 kDa. This ORF encodes all typical proteins, consensus cleavage sites and conserved catalytic domains present in the potyviruses (**Figure 4.28**).

Figure 4.28. Schematic representation of the genomic organization of MeRV and of the nucleotide identity of the virus with the phylogenetically closer species. The position of each gene is indicated in relation to the sequence of the isolate MeRV-ParP17. The blue box represents the polyprotein ORF. The alternate P3N-PIPO ORF is depicted by the small yellow box above the main reading frame. The corresponding predicted molecular weights in kilodalton (KDa) are indicated below each polyprotein cistron. Autocatalytic cleave sites of the HC-Pro and P1 gene products are indicated by green triangles, and NIa-Pro cleavage sites are marked by blue triangles. Conserved amino acids at each cleavage site according to [Adams et al., 2005](#) are shown above each triangle. The panel below plots the nucleotide identities between MeRV-ParP17 genome and those of the phylogenetically nearest potyviruses: *Bean yellow mosaic virus* (BYMV) and *Clover yellow vein virus* (CIYVV). Each point plotted is the per cent identity within a sliding window of 200nt wide centred on the position plotted, with a step size between points of 20nt (Kimura two-parameter analysis).

The P1 protein, a serine protease, contains the strictly conserved catalytic triad His₂₀₈-(X₈)-Asp₂₁₇-(X₃₀)-Ser₂₄₈ with the conserved GHSG₂₄₆₋₂₄₉ motif around the active serine site. In the HC-Pro, the protease active site residues are located at Cys₃₄₄, contained in the conserved sequence GYCH₃₄₂₋₃₄₅, and at His₄₁₇. Also, a conserved cysteine-rich region was identified in the N-terminal region of HC-Pro stretching from Cys₂₇ to Cys₅₈. HC-Pro motifs involved in aphid transmission (RITC₅₂₋₅₅ and PTK₃₁₀₋₃₁₂), genome replication (IGN₁₅₂₋₁₅₄) and systemic movement (CCC₂₉₂₋₂₉₄) ([Urcuqui-Inchima et al., 2001](#)) are also conserved in MeRV-ParP17. The P3 cistron contains the P3N-PIPO

ORF. The 5' end of this ORF starts with the highly conserved motif among potyviruses G₂A₆ and it has a length of 70 codons, terminating in a UAG stop codon at nucleotides 672 to 674 of the P3 cistron. The cylindrical inclusion (CI) protein cistron includes the conserved RNA helicase motifs located between residues 85 and 359, and the NIa contains the conserved His₄₆, Asp₈₁, Cys₁₅₁, His₁₆₇ cysteine protease catalytic tetrad. Conserved sequence motifs in RNA-dependent-RNA polymerases of positive strand RNA viruses were also identified in the MeRV-ParP17 NIb protein. Finally, the CP cistron contains a NAG motif, essential for aphid transmission in some potyviruses (Wylie et al., 2002), and located at residues 10-12 in the N-terminal region of the CP. The Arg₁₇₀ and Asp₂₁₄ residues, involved in virion assembly and cell-to-cell movement (Dolja et al., 1994, 1995), are located in the conserved core region of the MeRV-ParP17 CP. The virus 5' untranslated region (UTR) is 149 nt long, an average length for potyviruses (<200nt). The 3' UTR is 180 nt long excluding the poly(A)-tail and is rich in AU segments, which is a common feature among potyviruses.

The complete nucleotide sequence of the MeRV-ParP17 polyprotein was aligned with those of available fully sequenced potyviruses (n=107) (**Appendix 1, Table A1.5**). The percentage of nucleotide identity of the MeRV-ParP17 genome with that of the other potyviruses ranged from 50% to 66%, showing highest identity with bean yellow mosaic virus (BYMV) (65%) and clover yellow vein virus (CIYVV) (66%). Accordingly, Bayesian phylogenetic trees revealed that MeRV-ParP17 clustered together with BYMV and CIYVV (**Figure 4.29**). Following the approach described by Mbanzibwa et al., 2011 SimPlot analyses were performed to compare the percentage of nucleotide identity between MeRV-ParP17 and BYMV/CIYVV across the whole genome (**Figure 4.28**). When each cistron was analysed individually, the MeRV-ParP17 P1 and P3 cistrons showed the lowest nucleotide identity with the same cistrons of BYMV and CIYVV (**Figure 4.28**). The remaining cistrons showed a sequence identity with the corresponding ones of BYMV and CIYVV ranging on average between 57% and 69%. Equivalent results were obtained using the amino acid sequences.

Figure 4.29. Bayesian phylogeny based on the coding nucleotide sequence of potyviruses. Asterisks indicate nodes with posterior probabilities of ≥ 0.90 . The tree is mid-point rooted. Branch lengths are drawn to a scale of nucleotide substitutions per site. Phylogenetic subgroups of potyviruses defined by Shukla et al., 1994 are used as reference and indicated in coloured branches. BCMV: *Bean common mosaic virus*; ChiVMV: *Chilli vein mottle virus*; PRSV: *Papaya ringspot virus*; PVY: *Potato virus Y*; SCMV: *Sugarcane mosaic virus*; SPFMV: *Sweet potato feathery mottle virus*; TuMV: *Turnip mosaic virus*. Expansion of the phylogeny section contains the group of virus sequences in which MeRV is clustered.

4.5.1.2. *MeRV host range*

The MeRV-ParP17 host range was determined by inoculating purified virions of MeRV-ParP17 into 10 wild species belonging to seven botanical families, which represent the most abundant species in evergreen oak forests, and 13 plant species of six botanical families, including *Cucurbitaceae* and *Solanaceae* species that are commonly cultivated or found in the area surrounding the surveyed evergreen oak forests. The results are shown in **Table 4.33**.

None of the 10 wild species from evergreen oak forests were hosts of MeRV, except its original reservoir mountain rue (6/6 plants infected), in which virus infection was asymptomatic. Only three *Solanaceae* species were infected by MeRV-ParP17: *N. benthamiana* (5/5), *C. annuum* (4/6) and *S. lycopersicum* (6/6), infection in these three species causing mild mosaic symptoms. There was no variation in susceptibility across hosts ($\chi^2=0.81$, $P=0.668$).

Table 4.33. Host range and symptoms of MeRV-ParP17.

Plant species	Systemic infection ¹	Systemic symptoms
<i>Amaranthaceae</i>		
<i>Gomphrena globosa</i>	0/6	-
<i>Asteraceae</i>		
<i>Lactuca sativa</i>	0/6	-
<i>Santolina rosmarinifolia</i> *	0/6	-
<i>Taraxacum officinalis</i> *	0/6	-
<i>Boraginaceae</i>		
<i>Anchusa azurea</i> *	0/7	-
<i>Chenopodiaceae</i>		
<i>Chenopodium amaranticolor</i>	0/6	-
<i>Chenopodium quinoa</i>	0/5	-
<i>Cistaceae</i>		
<i>Cistus ladanifer</i> *	0/6	-
<i>Cucurbitaceae</i>		
<i>Cucumis melo</i>	0/5	-
<i>Cucumis sativus</i>	0/5	
<i>Fabaceae</i>		
<i>Anthyllis vulneraria</i> *	0/5	-
<i>Lupinus angustifolius</i> *	0/6	-
<i>Phaseolus vulgaris</i>	0/5	-
<i>Pisum sativum</i>	0/6	-
<i>Vicia villosa</i> *	0/6	-
<i>Fagaceae</i>		
<i>Quercus ilex</i> *	0/4	-
<i>Poaceae</i>		
<i>Bromus rubens</i> *	0/6	-
<i>Rutaceae</i>		
<i>Ruta montana</i>	6/6	Asymptomatic
<i>Solanaceae</i>		
<i>Capsicum annuum</i> (<i>Dulce Italiano</i>)	4/6	Mild mosaic
<i>Nicotiana benthamiana</i>	5/5	Mild mosaic
<i>Nicotiana clevelandii</i>	0/5	-
<i>Nicotiana tabacum</i> (<i>Samsun</i>)	0/5	-
<i>Solanum lycopersicum</i>	6/6	Mild mosaic

¹ Values are number of infected over inoculated plants. Asterisks indicate plant species present in evergreen oak forests and coexisting with mountain rue (*Ruta montana*).

4.5.1.3. Analysis of MeRV population genetic diversity

All the MeRV sequenced isolates were collected at El Pardo. The lack of isolates from other locations and/or hosts prevented to analyze the genetic structure of this virus population according to these factors. Hence, to test whether changes in ecological factors and MeRV prevalence across seasons affected the genetic diversity of the virus population, the CP sequence of 69 MeRV isolates from this location, collected along three growth cycles were utilized (**Table 4.34**). Using this sequence data set, the number of haplotypes (H), the haplotype diversity (H_d), the average nucleotide genetic diversity (π), and the number of synonymous (d_S) and non-synonymous (d_N) substitutions per site of the MeRV population at each visit were estimated. MeRV population haplotype number, haplotype diversity, genetic diversity and number of non-synonymous and synonymous substitutions per site greatly varied between

seasons ($H=2-9$; $H_d=0.50-1.00$; $\pi=0.001-0.007$; $d_N=0.001-0.006$; $d_S=0.001-0.053$) (**Table 4.34**). The highest values in all genetic diversity parameters were obtained in the first and third sampling cycles. Averaged measures of d_N/d_S indicated that MeRV populations were under purifying selection ($d_N/d_S < 1$) in the first and third sampling cycles, while neutral evolution seems to be responsible for the changes in MeRV in the second sampling cycle ($d_N/d_S = 1$). Note that this is due to the extremely high d_N/d_S value in Spring 2015.

Table 4.34. Number of haplotypes (H), haplotype diversity (H_d), genetic diversity (π), and number of non-synonymous and synonymous substitutions (d_N and d_S) per site and d_N/d_S of the El Pardo MeRV population based on the CP gene.

Season	N	H	$H_d \pm SE$	$\pi \pm SE$	$d_N \pm SE$	$d_S \pm SE$	$d_N/d_S \pm SE$
Sampling cycle 1							
Summer 2013	7	6	0.952 ± 0.036	0.006 ± 0.002	0.005 ± 0.004	0.037 ± 0.012	0.135 ± 0.009
Autumn 2013	8	7	0.964 ± 0.027	0.007 ± 0.002	0.006 ± 0.004	0.050 ± 0.016	0.120 ± 0.012
Spring 2014	5	4	0.900 ± 0.072	0.004 ± 0.002	0.001 ± 0.000	0.028 ± 0.010	0.035 ± 0.000
Cycle ¹	20	16	0.974 ± 0.014	0.007 ± 0.002	0.015 ± 0.007	$0.072 \pm 1 \times 10^{-5}$	0.097 ± 0.011
Sampling cycle 2							
Summer 2014	9	6	0.889 ± 0.030	0.004 ± 0.002	0.002 ± 0.002	0.043 ± 0.015	0.047 ± 0.002
Autumn 2014	7	4	0.714 ± 0.068	0.006 ± 0.002	0.004 ± 0.003	0.022 ± 0.009	0.181 ± 0.003
Spring 2015	4	2	0.500 ± 0.133	0.001 ± 0.001	0.003 ± 0.002	0.001 ± 0.000	3.000 ± 0.020
Cycle ¹	20	12	0.932 ± 0.020	0.005 ± 0.001	0.009 ± 0.020	0.066 ± 0.004	1.076 ± 0.020
Sampling cycle 3							
Summer 2015	4	4	1.000 ± 0.089	0.007 ± 0.002	0.006 ± 0.004	0.042 ± 0.014	0.143 ± 0.004
Autumn 2015	10	4	0.778 ± 0.029	0.004 ± 0.001	0.002 ± 0.002	0.045 ± 0.014	0.044 ± 0.002
Spring 2016	15	9	0.848 ± 0.023	0.006 ± 0.002	0.006 ± 0.004	0.053 ± 0.014	0.113 ± 0.004
Cycle ¹	29	16	0.931 ± 0.016	0.006 ± 0.001	0.018 ± 0.009	0.098 ± 0.024	0.100 ± 0.009

¹ Average of the three seasons of a sampling cycle.

N: number of sequences; SE: standard error.

4.5.1.4. Analysis of ecological factors affecting MeRV population genetic diversity

The ecological factors affecting MeRV population H_d , π , d_N , d_S and d_N/d_S were analysed using multiple regression model selection analyses (**Table 4.35**). The same variables as for predicting potyvirus species-specific prevalence and adding MeRV prevalence as an additional predictor (data of predictor variables is in **Section 4.3, Table 4.24**) were utilized. The best-ranked model explaining H_d contained prevalence as the only predictor ($r=0.79$; $P=0.011$) (**Table 4.35**). This model closely competed with that including MeRV prevalence and plant species richness as main predictors ($r=0.73$; $P=0.055$), virus prevalence having much higher relative importance than plant species richness (89.8% and 10.2%,

respectively). The model containing MeRV prevalence and host relative abundance ($r=0.70$; $P=0.046$) also showed $\Delta_i < 2$, with virus prevalence having again the highest relative importance (99.1%) (**Table 4.35**). Bivariate analyses indicated a positive association between H_d and virus prevalence (**Figure 4.30A**).

Table 4.35. Model selection analyses for MeRV haplotype diversity (H_d), genetic diversity (π), number of synonymous (d_s) and non-synonymous (d_n) substitutions per site, and selection pressures (d_n/d_s). Model structures included host plant density, host relative abundance, plant species richness and plant biomass; and temperature, relative humidity and rainfall in the sampled locations (minimal, maximal and average values) as predictors, and season as covariate. MeRV prevalence was also included as predictor of virus evolution parameters. Best-ranked models are shown.

Model structure*	$r^{\dagger}$	logLik	AIC ‡	$\Delta_i^{\S}$	$\omega_i^{\P}$
H_d					
PRE (100)	0.79*	7.60	-9.21	0.00	0.47
PRE (90) + S (10)	0.73*	7.19	-8.38	0.83	0.30
PRE (99) + PRA (1)	0.70*	8.22	-7.67	1.51	0.22
π					
PRE (77) + S (23)	0.74*	35.62	-63.23	0.00	0.47
PRE (100)	0.64*	36.35	-62.15	1.08	0.27
PRE (99) + RAF (1)	0.43	37.00	-61.99	1.24	0.25
d_n					
PRE (80) + S (20)	0.70	45.44	-82.89	0.00	0.71
S (100)	-0.65	45.54	-81.09	1.80	0.29
d_s					
PRE (75) + S (25)	0.82*	29.60	-49.21	0.00	0.56
PRE (100)	0.69*	29.72	-47.43	1.78	0.23
PRE (72) + S (17) + RAF (11)	0.83	29.88	-47.30	1.91	0.21
d_n/d_s					
PRE (85) + S (15)	0.83*	30.34	-50.68	0.00	0.56
PRE (78) + S (13) + RAF (9)	0.84*	30.44	-48.88	1.80	0.23
PRE (100)	0.68*	30.12	-48.75	1.93	0.21

*The relative importance (%) of each predictor variable is shown in parenthesis.

† Correlation coefficient. Asterisks indicate significant correlations ($P < 0.05$).

‡ Akaike's Information Criterion.

§ Δ_i is the difference between the AIC of a given model and that of the best-ranked model, and quantifies how models compete (best-ranked model: $\Delta_i = 0$; substantial empirical support: $\Delta_i = 1-2$; considerable less support: $\Delta_i = 2-7$; and no support; $\Delta_i > 10$) (Burnham and Anderson, 2002).

¶ AIC model weight as $\omega_i = \exp(-0.5\Delta_i) / \sum \exp(-0.5\Delta_i)$. The larger the ω_i , the greater the likelihood of the model relatively to the competing models. Maximum $\omega_i = 1$.

PRE: MeRV prevalence; S: Plant species richness; PRA: Plant relative abundance; RAF: Rainfalls.

The model best explaining MeRV population genetic diversity (π) contained MeRV prevalence and plant species richness as predictors ($r=0.74$; $P=0.022$), prevalence having higher relative importance (77.2%) (**Table 4.35**). This model closely competed with that containing MeRV prevalence as the sole predictor ($r=0.64$; $P=0.042$) (**Table 4.35 and Figure 4.30B**), or this variable together with rainfall ($r=0.43$; $P=0.541$). However, the later model did not show a significant association between the predictor(s) and the response variable (**Table 4.35**). None of the tested models accurately predicted d_N ($P \geq 0.106$). On the other hand, the best-ranked models predicting d_S and d_N/d_S contained MeRV prevalence as predictor, solely ($r \geq -0.68$; $P \leq 0.018$) (**Figures 4.30C,D**) or in combination with plant species richness and rainfall ($r \geq 0.83$; $P < 0.050$), MeRV prevalence having always much higher predictive power (relative importance $\geq 72\%$) than the other two predictors (relative importance $\leq 25\%$) (**Table 4.35**).

Figure 4.30. Bivariate relationships between MeRV population evolutionary parameters and the best predictor variable. In each case, best predictor variable was identified by model selection analyses. Regressions of MeRV population haplotype diversity (A), population genetic diversity (B), number of synonymous mutations per site (C) and overall selection pressures d_N/d_S (D) on MeRV prevalence are represented.

Thus, overall, MeRV prevalence is the best predictor of the genetic diversity and selection pressures in the virus population.

4.5.2. Analysis of the population genetic diversity of a generalist potyvirus in wild ecosystems of the Iberian Peninsula

4.5.2.1. Analysis of WHMV population genetic diversity

WHMV was detected in four riparian forest locations. Unfortunately, it was not possible to obtain enough number of CP sequences of WHMV isolates at each location to analyse the population genetic diversity. Hence, at odds with MeRV, analysis of WHMV population genetic diversity was performed using the partial-Nlb gene sequence of the 40 WHMV isolates collected along three sampling cycles. However, note that both genomic regions contain similar phylogenetic information (see **section 3.2.2** and **Appendix 1, Table A1.3**). As WHMV populations were genetically structured according to geographical location (see **section 4.5.2.3** below), genetic diversity and selection pressures parameters were quantified at each location. As WHMV was not detected in every season during the monitored period, not enough sequences were obtained at each visit to perform seasonal estimates of genetic diversity parameters.

WHMV population haplotype number, haplotype diversity, genetic diversity and number of non-synonymous and synonymous substitutions per site varied between locations ($H=3-10$; $H_d=0.800-0.982$; $\pi=0.011-0.033$, $d_N=0.000-0.066$; $d_S=0.109-0.233$). The WHMV populations at El Escorial and Santa Cruz de Retamar had the highest values in all genetic diversity parameters (**Table 4.36**). In addition, the WHMV populations in El Escorial and Palazuelos were under purifying selection ($d_N/d_S < 1$), which was stronger in the WHMV populations at Pollos and Santa Cruz de Retamar (**Table 4.36**).

Table 4.36. Number of haplotypes (H), haplotype diversity (H_d), genetic diversity (π), number of non-synonymous and synonymous substitutions (d_N and d_S) per site and selection pressures (d_N/d_S) of WHMV populations in riparian forests based on the partial Nlb gene sequence.

Location	N	H	$H_d \pm SE$	$\pi \pm SE$	$d_N \pm SE$	$d_S \pm SE$	$d_N/d_S \pm SE$
ESC	11	10	0.982 ± 0.046	0.033 ± 0.007	0.066 ± 0.017	0.233 ± 0.073	0.285 ± 0.072
PAL	5	3	0.800 ± 0.164	0.016 ± 0.005	0.005 ± 0.005	0.109 ± 0.035	0.044 ± 0.035
POL	17	8	0.882 ± 0.047	0.011 ± 0.003	0.000 ± 0.000	0.150 ± 0.046	0.000 ± 0.046
SCR	6	5	0.933 ± 0.122	0.027 ± 0.008	0.000 ± 0.000	0.135 ± 0.040	0.000 ± 0.040

¹Locations are designed with a three-letter code: ESC, El Escorial; PAL: Palazuelos; POL, Pollos; SCR, Santa Cruz de Retamar.

N: number of sequences; H: number of haplotypes; H_d : haplotype diversity; π : genetic diversity; SE: standard error.

4.5.2.2. Analysis of ecological factors affecting WHMV population genetic diversity

The ecological factors affecting WHMV population H_d , π , d_N , d_S and d_N/d_S were analysed using multiple regression model selection analyses (**Table 4.37**). The same variables as for predicting potyvirus species-specific prevalence and adding WHMV prevalence as an additional predictor (data of predictor variables is in **Section 4.3, Tables 4.27 and 4.28**) were utilized. The best-ranked model explaining H_d contained plant relative abundance and rainfalls as the most important predictors ($r=0.78$; $P=0.007$). In this model plant relative abundance had similar relative importance than rainfalls (30% and 26%, respectively) (**Table 4.37**). Bivariate analysis showed a negative relationship between plant relative abundance and H_d ($r=-0.25$; $P=0.042$), whereas the association with rainfalls was positive but not significant ($r=0.14$; $P=0.142$) (**Figure 4.31A,B**). The best-ranked models predicting WHMV genetic diversity (π) contained rainfalls and virus prevalence as the most important predictors ($r=0.71$; $P=0.003$), rainfalls having higher relative importance than virus prevalence (50% and 27%, respectively). Bivariate analysis showed a positive association between rainfalls and π ($r=0.29$; $P=0.025$), whereas the association with WHMV prevalence was negative ($r=-0.25$; $P=0.042$) (**Figure 4.31C,D**). The best-ranked models predicting WHMV population d_N and d_N/d_S contained plant biomass and WHMV prevalence as the most important predictors, plant biomass having the highest relative importance (46%) ($r>0.78$; $P=0.002$) (**Table 4.37**). Bivariate analyses indicated a positive association between plant biomass and the studied parameters ($r>0.32$; $P<0.017$) (**Figure 4.31E,I**), whereas the association with virus prevalence was negative and only marginally significant ($r<-0.21$; $P>0.062$) (**Figure 4.31F,J**). The best-ranked model predicting d_S contained plant biomass and rainfalls as the most important predictors ($r=0.81$; $P=0.012$), the former predictor having the highest relative importance (46%) (**Table 4.37**). Bivariate analyses showed a positive association between these two predictors and the studied parameters, although the association was only significant with plant biomass ($r=0.44$; $P=0.004$) (**Figure 4.31G,H**).

No other combination of factors yielded a model that closely competed with the best-ranked one (**Appendix 6, Table A6.1**).

Table 4.37. Model selection analyses for WHMV population haplotype diversity (H_d), genetic diversity (π), number of synonymous (d_s) and non-synonymous (d_N) substitutions per site, and selection pressures (d_N/d_s). Model structures included host plant density, host relative abundance, plant species richness and plant biomass; and temperature, relative humidity and rainfall in the sampled locations (minimal, maximal and average values) as predictors, and season as covariate. WHMV prevalence was also included as predictor of virus evolution parameters. Best-ranked models are shown.

Model structure	$r^{\dagger}$	logLik	AIC ‡	$\Delta_i^{\S}$	$\omega_i^{\P}$
H_d					
PRA (30)+RAF (26)+PRE (12)+RH _{mean} (12)+T _{mean} (11)+V (9)	0.78*	37.97	-59.94	0.00	0.55
PRA (29)+RAF (26)+PRE (12)+RH _{mean} (12)+T _{mean} (11)+V (8)+S (2)	0.79*	38.15	-58.30	1.64	0.24
PRA (30)+RAF (26)+ RH _{mean} (12)+PRE (11)+T _{mean} (11)+V (8)+HPD (2)	0.78*	37.97	-57.94	2.00	0.20
π					
RAF (50)+PRE (27)+RH _{mean} (17)+T _{mean} (6)	0.71*	66.22	-120.44	0.00	0.25
RAF (50)+PRE (28)+RH _{mean} (22)	0.67*	65.16	-120.33	0.11	0.24
RAF (46)+PRE (28)+RH _{mean} (16)+PRA (4)+T _{mean} (6)	0.71*	66.47	-118.94	1.50	0.12
d_N					
V (46)+PRE (21)+RAF (17)+RH _{mean} (9)+T _{mean} (7)	0.78*	49.73	-85.46	0.00	0.38
V (41)+PRE (22)+RAF (17)+RH _{mean} (10)+T _{mean} (7)+S (3)	0.80*	50.49	-84.99	0.47	0.30
V (45)+PRE (19)+RAF (16)+RH _{mean} (10)+T _{mean} (7)+PRA (3)	0.79*	49.97	-83.94	1.52	0.18
d_s					
V (46)+RAF (13)+PRE (12)+RH _{mean} (9)+T _{mean} (8)+S (8)+PRA (4)	0.81*	43.62	-69.25	0.00	0.46
V (57)+RAF (11)+PRE (10)+RH _{mean} (9)+T _{mean} (8)+ PRA (5)	0.77*	42.35	-68.70	0.55	0.35
V (44)+RAF (13)+PRE (10)+RH _{mean} (9)+T _{mean} (8)+S (7)+HPD (5)+PRA (4)	0.81*	43.79	-67.57	1.67	0.20
d_N/d_s					
V (45)+PRE (23)+RAF (16)+RH _{mean} (9)+T _{mean} (7)	0.79*	25.37	-36.74	0.00	0.41
V (41)+PRE (23)+RAF (16)+RH _{mean} (9)+T _{mean} (8)+S (3)	0.80*	25.98	-35.96	0.78	0.28
V (43)+PRE (19)+RAF (16)+RH _{mean} (10)+T _{mean} (7)+PRA (5)	0.79*	25.43	-34.85	1.89	0.16

*The relative importance (%) of each predictor variable is shown in parenthesis.

† Correlation coefficient. Asterisks indicate significant correlations ($P < 0.05$).

‡ Akaike's Information Criterion.

§ Δ_i is the difference between the AIC of a given model and that of the best-ranked model, and quantifies how models compete (best-ranked model: $\Delta_i = 0$; substantial empirical support: $\Delta_i = 1-2$; considerable less support: $\Delta_i = 2-7$; and no support; $\Delta_i > 10$) (Burnham and Anderson, 2002).

¶ AIC model weight as $\omega_i = \exp(-0.5\Delta_i) / \sum \exp(-0.5\Delta_i)$. The larger the ω_i , the greater the likelihood of the model relatively to the competing models. Maximum $\omega_i = 1$.

PRA: Plant relative abundance; PRE: WHMV prevalence; RAF: Rainfalls; RH_{mean}: Average relative humidity; T_{mean}: Average temperature; V: Plant biomass; HPD: Host plant density; S: Species richness.

Figure 4.31. Bivariate relationships between WHMV population evolutionary parameters and the best predictors. The associations between potyvirus population haplotype diversity (A and B), population genetic diversity (C and D), number of non-synonymous (E and F) and synonymous mutations per site (G and H) and overall selection pressures d_N/d_S (I and J) and best predictors are represented.

4.5.2.3. Analysis of WHMV speciation mode

Both AI and PS statistics revealed a significant association between the WHMV phylogeny and the location where the sequences were collected ($P < 1 \times 10^{-5}$). MC statistic indicated that this result was due to the stronger clustering of the sequences collected in El Escorial, Pollos and Santa Cruz de Retamar ($P < 0.01$) (Figure 4.32).

Figure 4.32. Maximum-likelihood phylogeny based on the NIb region representing the association between WHMV phylogeny and geographical location. Asterisks indicates nodes with a bootstrap support ≥ 0.90 .

In addition, AI and PS statistics showed a significant genetic structure of the WHMV populations according to host species ($P < 1 \times 10^{-5}$). In this case, according to the MC statistics, the significance was due to the stronger clustering of the virus sequences collected from *Trifolium pratense* and *Humulus lupulus* ($P < 0.01$) (Figure 4.33).

Figure 4.33. Maximum-likelihood phylogeny based on the NIb region representing the association between WHMV phylogeny and host plant species. Asterisks indicates nodes with a bootstrap support ≥ 0.90 .

These data suggest that WHMV speciation tends to occur within the same host species and in the same geographic area, which is compatible with genetic diversification through sympatric speciation, at least for certain hosts.

5. DISCUSSION

Most of our knowledge on plant-virus interactions comes from the study of viruses that cause diseases in crops. Therefore, our understanding of plant viruses might be biased towards those interactions that have the most obvious agro-economic consequences. Indeed, until recently weedy and wild plants were considered of interest only as source/reservoir of virus inoculum for crops (Cooper and Jones, 2006; Jones, 2009; Alexander et al., 2014). The advent of next generation sequencing (NGS) has allowed exploration of the virome of a number of different ecosystems (e.g., Delwart, 2007; Hurwitz and Sullivan, 2013), including wild plant communities (Roossinck, 2012; Stobbe and Roossinck, 2014). These studies have shown that plant-virus interactions in wild ecosystems are far more diverse than in agricultural ecosystems: in wild plants virus infections are common, and virus diversity and identity appear to be different from that in crops (Roossinck, 2012; Stobbe and Roossinck, 2014). Indeed, NGS analyses in wild plants have uncovered large (previously) unknown virus diversity (Roossinck, 2011). These observations suggest that plant viruses are important ecological agents in wild plant communities. Thus, the detailed genomic and biological characterization of viral diversity in wild ecosystems, and the analysis of their population dynamics is central to fully understand plant–virus interactions, and will yield information on the processes that lead to disease emergence in these ecosystems (Pagán et al., 2016). However, most of the virus species present in wild ecosystems remain uncharacterized (Roossinck, 2012; Stobbe and Roossinck, 2014), and the determinants of their epidemiology and evolution are largely unknown (Pagán et al., 2016).

To address these questions, this thesis analyses the ecological determinants of plant virus prevalence and population genetic diversity in two wild ecosystems, which accounts for the largest area of wild ecosystems in the Iberian Peninsula: evergreen oak and riparian forests. The thesis uses the species of the genus *Potyvirus* as a model, as this genus is the largest of plant viruses and various species within the genus have been reported to infect plant species commonly found in the ecosystems studied. Through surveys done between 2013 and 2016, and using state-of-the-art bioinformatics approaches, the present work provides information on the prevalence, host range and population genetic diversity of the potyvirus species detected in evergreen oak

and riparian forests. At different taxonomical scales (genus vs. species) and considering different virus life-history strategies (specialist vs. generalist) this thesis identifies the ecological determinants affecting the prevalence and population genetic diversity of potyviruses.

5.1. Characterization of evergreen oak forest and riparian forests in the Iberian Peninsula

For this thesis, the locations of evergreen oak and riparian forests selected were chosen such that there was no evidence of human intervention and could be considered as wild ecosystems. However, large areas of the Iberian Peninsula, and often those adjacent to the monitored locations, have been under human management for long periods of time. Thus, it could be possible that the floristic composition of the forest locations utilized in this thesis had been indirectly influenced by the changes in the vegetation of the adjacent human-managed fields. For instance, plant species predominant in the nearby managed fields may have invaded the evergreen oak and riparian forests, altering the typical flora of these forests. Because human management generally leads to a homogenization of the flora, it could also be possible that its indirect influence in wild ecosystems may result in the reduction of the differences in biodiversity and floristic composition between the two analysed ecosystems that has been generally reported ([Blanco et al., 2005](#); [Loidi, 2017](#)). However, the analyses of the floristic composition in the monitored locations (**section 4.1**) indicated that it differed between both ecosystems, and the typical species that characterize the plant communities of riparian and evergreen oak forests in Central Spain were present ([Blanco et al., 2005](#); [Loidi, 2017](#)). Differences in plant community composition between both ecosystems were also accompanied by different proportion of annual and perennial plant species. Riparian forests supported a predominance of perennials, while annual plants accounted for 40-50% of the species present in evergreen oak forests. The highest percentage of annuals in the later ecosystem agrees with previous observations in Mediterranean evergreen oak forests, as annuals are best adapted to the seasonal regime of mild moist winters and dry summers that

characterize the climate where these forests predominate (Archibold, 1995; Bruno et al., 2014). In addition, biodiversity was significantly higher in riparian forests than in evergreen oak forests, as expected (Blanco et al., 2005; Loidi, 2017). This higher plant biodiversity was associated with higher biomass in riparian forests than in evergreen oak forests, as is characteristic of these types of ecosystems (Blanco et al., 2005). Therefore, the locations selected for this study are representative of the two ecosystems under study. This conclusion is also supported by the analyses of sampling effort, which indicate that the sampling scheme used here captured most, and in many cases all, the species diversity in the monitored locations (see **Figure 4.2 and 4.3**).

At odds with differences in biodiversity, floristic composition and plant biomass, climatic factors (temperature, relative humidity and rainfalls) did not generally vary between evergreen oak and riparian forests. Previous works have reported differences in climatic conditions between evergreen oak and riparian forests (Blanco et al., 2005; Loidi, 2017). There are two possible explanations for this contradiction that are not mutually exclusive: (i) because the evergreen oak and riparian forests selected here are both in an area of Mediterranean climate, climatic conditions are similar. Note that the Mediterranean climate is characterized by large variations in the climatic conditions within the same season, which results in large standard errors of average climatic parameters that may mask differences between seasons/ecosystems. (ii) Because the stations providing climatic parameters were not exactly in the monitored locations, the data may have not fully capture the microclimatic conditions of riparian forests that extend near the river streams (Naiman and Decamps, 1997). In this case, rainfalls would be the less affected parameter, as stations were close enough to the locations and at a similar altitude to have a sufficiently good measure of this parameter. Indeed, rainfalls was the climatic parameter in which differences between ecosystems were more obvious (see **Table 4.4**). Likely, temperature and relative humidity in riparian forest locations were more poorly capture by the nearby climatic stations. Indeed, other relative humidity measures recorded *in situ*, as was the case of soil moisture, showed higher values in riparian that in evergreen oak forest. Interestingly, soil moisture has been shown to be a meaningful indicator

of the climatic conditions in riparian forests ([Rodà, 1999](#)), and may compensate for the lack of precision in other climatic variables.

5.2. Ecological determinants of genus-wide potyvirus infection risk in wild ecosystems

In this thesis, potyvirus prevalence was used as a proxy of infection risk. Potyvirus infection was detected at every season and sampling year in evergreen oak and riparian forests, indicating that potyvirus infection occur consistently in both ecosystems. Potyvirus prevalence fluctuated between seasons and years, but on average infection prevalence was of 3.80% in riparian forests and of 2.09% in evergreen oak forests. This prevalence is smaller than that reported in other wild plant populations, which indicated that an average of 10% of individuals in a population are infected (reviewed by [Prendeville et al., 2012](#)). However, note that this estimate refers to plants infected by any virus, and this thesis focused in a specific virus genus. The few reports of species-specific prevalence in wild plant populations have often report values closer to those reported here (e.g. [Sacristán et al., 2004](#); [Cooper and Jones, 2006](#); [Tugume et al., 2008](#); [McLeish et al., 2017](#)).

Potyvirus prevalence was generally higher in riparian than in evergreen oak forests, which was associated with higher plant species richness and abundance of host plants in the former ecosystem. Interestingly, this result would support the "Amplification Effect" hypothesis: as the number of total species in the ecosystem increases, the number of host species increases leading to increased pathogen prevalence, and therefore infection risk ([Keesing et al., 2006](#); [Ostfeld and Keesing, 2012](#)). This is in disagreement with accumulating evidence in a variety of animal-pathogen ([LoGiudice et al., 2003](#); [Keesing et al., 2010](#); [Roche and Guégan 2011](#)) and plant-pathogen ([Mitchell et al., 2003](#); [Hass et al., 2011](#); [Pagán et al., 2012](#); [Civitello et al., 2015](#)) systems, which support the "Dilution Effect" hypothesis that states that higher species diversity decreases the abundance of hosts, resulting in a reduction in the number of contacts between susceptible and infected individuals and therefore

in lower pathogen prevalence (Keesing et al., 2006; Ostfeld and Keesing, 2012).

The conditions in which dilution and amplification effects are more likely to occur, and the spatial scale at which such effects manifest, are still under debate (Randolph and Dobson, 2012; Johnson et al., 2013; Johnson et al., 2015). For instance, as the relationship between biodiversity and pathogen prevalence will often depend strongly on spatial scale, different effects could be expected at the local analysis or at the community scale (Johnson et al., 2015). Indeed, recent work has shown that virus prevalence in plant populations of different habitats conforms the “Dilution” or the “Amplification Effects” depending on the spatial scale of the analysis. In these plant populations, the prevalence–diversity relationship was habitat specific, showing both amplification and dilution effects at the habitat spatial scale, whereas only an amplification effect was detected at the larger ecosystem spatial scale (McLeish et al., 2017). Similarly, the analyses of this thesis, which are done at the ecosystem spatial scale, also agree with the “Amplification Effect” hypothesis.

In addition, the prevalence–diversity relationship may be driven by the identity of the particular species present in the ecosystems/communities than diversity *per se* (Randolph and Dobson, 2012). On this regard, the life cycle of the plant species present in an ecosystem could be a relevant trait to understand how biodiversity determines pathogen prevalence (Johnson et al., 2012). Theoretical work has proposed that annual hosts are better pathogen reservoirs than perennial ones: perennial species are more likely to be challenged against pathogen infections during their life span, and therefore invest in developing defense mechanisms. Conversely, short-lived hosts will invest less in costly defenses against pathogens, so that they will be more susceptible to infection and/or will sustain larger vector populations, thus being effective reservoirs for infectious diseases (Miller et al., 2007; Bruns et al., 2015). At odds, the percentage of perennial hosts was much larger in riparian than in evergreen oak. Moreover, in riparian forests there was a higher percentage of perennial hosts than of total plant perennial species, indicating a preference of potyviruses for infecting plants with longer life cycles. However, the above-mentioned theoretical models considered resistance as the single major defence mechanisms. Mathematical models considering both resistance

and tolerance predicted that higher abundance of perennial hosts would be associated with increased pathogen prevalence: perennial hosts would be more likely to evolve tolerance, rather than resistance, which would increase pathogen spread rates and prevalence by serving as long-term sources of infection for susceptible individuals (Herms and Mattson, 1992; Boots and Bowers, 2004; Borer et al., 2010). Our results would be compatible with this prediction, and are also in agreement with studies showing that virus prevalence increases with higher abundance of perennial hosts (Borer et al., 2010; Malmstrom et al., 2005b; Seabloom et al., 2013, but see Alexander et al., 2017). Thus, the results of this thesis show the importance of considering not only the number of species/relative abundance, but also their identity, to understand the infection risk, and consequently the emergence, of virus epidemics (Randolph and Dobson, 2012; Keesing et al., 2006; Johnson et al., 2015).

Potyvirus prevalence in riparian and evergreen oak forests, two ecosystems that greatly differ in their ecology, appears to be determined by the species richness and identity in terms of their life cycle. However, are these factors also important determinants of virus prevalence over time within the same ecosystems, or are there other relevant ecological/climatic factors? In order to answer this question, the association between the ecological factors that characterize evergreen oak and riparian forests locations and the ecosystem-specific potyvirus prevalence was analysed using Machine Learning (ML) based-methods. Complete ML models including all predictors of potyvirus prevalence considered in this thesis performed better than the baseline model and generally showed high predictive power regardless of the method used (Tables 4.10 and 4.11). The three utilized methods identified the identity of the plant species present in the ecosystem as the major (and often the solely) determinant of potyvirus prevalence in both evergreen oak and riparian forests. The presence/absence of host species such as *H. lupulus*, *T. pratense* or *R. montana* (many of the perennial) defined the different levels of potyvirus prevalence, whereas the presence/absence of non-host species defined the scenarios of no virus presence. As stated above, early theory on the relationship between biodiversity and infection risk predicted that species richness would be the more relevant factor in defining this relationship (Keesing

et al., 2006; Ostfeld and Keesing, 2012). More recent elaborations incorporated the identity of the species in the ecosystem as a key factor in determining infection risk: if higher species richness results in higher number of host species infection risk would be higher, and if higher species richness is due to a larger number of non-host species infection risk would decrease (Randolph and Dobson, 2012; Johnson et al., 2015). The results of this thesis support the key role of species identity even over species richness in determining infection risk, and are also in agreement with previous works indicating that plant virus prevalence is associated with the presence of certain species in the ecosystem, and not only with a general increment in the ecosystem species richness (Malpica et al., 2006; McLeish et al., 2017).

Because the overly high importance of plant species as determinant of virus infection risk could mask the effect of other factors, ML models excluding plant species identity traits as predictor were constructed. Although these models differed in the factors identified as the most important predictors of potyvirus prevalence and in the relative importance of these predictors, some general patterns arise. All ML models identified plant relative abundance as a major predictor of potyvirus prevalence in the two studied ecosystems, and bivariate relationships between potyvirus prevalence and host relative abundance indicated a (weak) negative association between these traits. This result would be in agreement with theory attributing to host relative abundance a key role in infection risk and/or pathogen transmission (Keesing et al., 2006; Ostfeld and Keesing, 2012). However, this theory predicts a positive association between host relative abundance and infection risk, whereas the opposite relationship was observed in riparian and evergreen oak forests (**Figure 4.18**). This difference with theoretical predictions would be explained if: (i) the best potyvirus hosts are those that appear less frequently in the studied ecosystems. This seems to be the case for host species of other plant viruses such as CMV in several habitats in the Iberian Peninsula (McLeish et al., 2017). (ii) Virus infection increases host mortality such that higher virus prevalence results in larger decrease of the host population size (and therefore of host relative abundance). Although metagenomics analyses in wild ecosystems have shown that virus infection generally does not result in the expression of disease symptoms (Prendeville et al., 2012; Roossinck, 2012), some examples exist of

plant viruses that cause obvious diseases, affect plant population sizes and plant ecosystem composition (Power and Mitchell, 2004; Malmstrom et al., 2005a; Power et al., 2011; Rua et al., 2011; Rodelo-Urrego et al., 2013; Prendeville et al., 2014). This could be the case of potyviruses infecting the ecosystems studied in this thesis, especially for evergreen oak forests (see **section 5.3** for further discussion). (iii) Plant isolation favours infection. Indeed, several studies have reported a negative correlation between plant density and virus prevalence, which was attributed to migratory aphids landing preferentially on isolated large plants (A'Brook 1964; Way and Heathcote, 1966).

The second factor generally detected by ML models as predictor of potyvirus infection risk was host density, with bivariate analyses shown no (evergreen oak forests) or weak (riparian forests) positive association between these traits. The importance of this factor in the ML models constructed in this thesis supports the generally assumed idea that higher plant density increases infection risk (Burdon and Thrall, 2008). The aggregated distribution of host plants within each location may favor virus transmission by aphids, and consequently may increase infection risk. This is also in agreement with previous work in other plant virus-wild host interactions that also identified host density as a key factor for plant virus infection risk (Malmstrom et al., 2005a; Borer et al., 2010; Pagán et al., 2012; Rodelo-Urrego et al., 2013). Finally, the third predictor commonly detected by all ML methods was plant life cycle, with the presence of perennial plants favouring higher potyvirus prevalence. This would be in agreement with results discussed in **section 5.1**. Also, the importance of perennial plants as potyvirus hosts fits with other of the results discussed in this section. For instance, perennial hosts tend have less relative abundance than annual hosts in the analysed ecosystems, especially given that they have larger adult sizes (our observations). This would be compatible with the negative relationship between potyvirus prevalence and host relative abundance. Also, perennial hosts usually form aggregated clusters, which would explain at least in part the positive association between host density and potyvirus prevalence.

It should be noted that ML models that excluded plant species identity traits did not accurately predicted potyvirus infection risk in any ecosystem, showing performance scores close or even lower than the baseline model in

both training and validation sets (**Tables 4.13 and 4.14**). The comparison of ML models predicting potyvirus presence/absence and potyvirus intensity of infection yielded an explanation for this result. In ML models predicting potyvirus presence/absence, including only plant virus identity as predictor was enough to accurately predict potyvirus presence. On the other hand, for models predicting virus intensity the most relevant factors were host relative abundance and density. Thus, the identity of the species present in an ecosystem defines the chances for a virus to enter in such ecosystems, with other ecological factors poorly predicting non-host or uninfected prevalence categories. Once the virus entered the ecosystems (i.e., only categories of virus prevalence are considered), the capacity of the virus to invade the ecosystem depends mostly on other ecological factors. Interestingly, this pattern holds for two ecosystems with very different ecological characteristics, which argues in favour of the generality of the observed patterns.

5.3. Ecological determinants of species-specific potyvirus infection risk in wild ecosystems

Ecological factors that affect genus-wide *Potyvirus* prevalence in evergreen oak and riparian forests yield relevant information about virus epidemiology in the monitored ecosystems. Nevertheless, for certain virus species some ecological factors may be more important than others, according to the specific characteristics of such virus species (Jones, 2009). Previous works highlighted the importance of knowing the host range of the pathogen(s) under study to understand how host and virus ecology affect virus prevalence and hence, infection risk (Malmstrom et al., 2005a; Borer et al., 2010; Pagán et al., 2012; McLeish et al., 2017). However, it is rare to find in the literature an analysis of this subject in which the pathogen host range is fully characterized in a given ecosystem (but see McLeish et al., 2017). In this thesis the importance of ecological factors in determining *Potyvirus* species-specific prevalence according to the virus host range was analysed.

Identification of the potyviruses present in wild plants of both ecosystems indicated the presence of novel species of genus *Potyvirus*. Discovery of novel

species is common in virus surveys using metagenomics methods, but because in this type of studies the samples often represent pools of many hosts, little or no connection can be made to the virus host(s) (Roossinck and García-Arenal, 2015; Roossinck, 2015). The methodology used in this thesis allowed to fully characterize the natural host range of the known and novel potyvirus species present in the monitored ecosystems. Four viral species were identified in this thesis, of which CIYVV and ENMV, are known potyvirus species that have been commonly found infecting cultivated species. ENMV is present in the Mediterranean basin in a large set of wild and cultivated plants, mainly of the family *Asteraceae* (Desbiez et al., 2017). In this thesis, sequences related to this potyvirus species were identified infecting a non-previously known host species in the family *Asteraceae*. Phylogenetic analyses of all available ENMV NIb nucleotide sequences hosts separated isolates from wild hosts and from cultivated ones. Failure in amplifying the CP gene of the ENMV isolates from wild hosts prevented confirming this observation. However, results suggest that ENMV isolates of wild and cultivated hosts are genetically different. CIYVV is one of the most prevalent potyviruses in legumes, causing important economic losses in crops and pastures worldwide (Andrade et al., 2007). In riparian forests of the Iberian Peninsula, CIYVV has been detected in legumes (*Trifolium* species) and host species of other families (*Polygonaceae*), which has not been reported as CIYVV host to date. Both NIb and CP sequences of CIYVV identified in this study clustered in the same monophyletic group than available sequences of this virus. The analysis of this thesis also identified two new potyvirus species (MeRV and WHMV). Host range of MeRV was limited to the perennial plant *Ruta montana* in a location of evergreen oak forests, being highly prevalent in this host plant. Finally, WHMV had a broader host range and geographic distribution, infecting mostly perennial plant species from different botanical families collected in locations of riparian forests (*Cannabaceae*, *Cucurbitaceae*, *Fabaceae* and *Salicaceae*) and evergreen oak forests (*Cistaceae*). Hence, this study significantly extended the knowledge of natural hosts of known viruses of the genus *Potyvirus* in wild ecosystems.

In the analyzed ecosystems, MeRV and EMNV behaved as specialists, because they infected a single host species; whereas WHMV and CIYVV were detected infecting plant species from different botanical families thus, behaved

as generalists. In order to study how the ecological factors recorded in evergreen oak and riparian forests could differentially influence virus prevalence according to virus host range, this thesis focused in the most prevalent virus representative of each strategy: MeRV as a specialist virus, and WHMV as a generalist virus.

MeRV would represent one of the first examples of specialist plant viruses native of wild ecosystems, as most plant viruses described in wild perennial plants naturally infect broader host ranges ([Cooper and Jones, 2006](#)). Specialist viruses are often extremely well adapted to their host(s). This means that these pathogens are highly transmissible and efficiently exploit host resources to maximize its fitness ([Woolhouse et al., 2001](#); [García-Arenal and Fraile, 2013](#)). Theoretical models of host adaptation assume that pathogen transmission is positively correlated with its multiplication and negatively correlated with its virulence. In turn, multiplication is positively associated with virulence, which lead to a trade-off between transmission and virulence. Thus, these models predict that pathogen fitness would be optimal at intermediate levels of virulence ([Anderson and May, 1982](#)). Despite behaving as specialist, MeRV infection did not induced any apparent symptom in mountain rue, neither in the field nor in the greenhouse. Similarly, infections by any of the other potyviruses detected in evergreen oak forests, which also showed narrow host ranges, were also asymptomatic. This would be in agreement with previous studies that generally reported a lack of obvious symptoms associated with virus infections in wild plants ([Cooper and Jones, 2006](#); [Roossinck, 2010](#); [Prendeville et al., 2012](#)). The apparent absence of symptoms in MeRV-infected mountain rue plants could be explained if: (i) MeRV is not optimally adapted to mountain rue, which is unlikely given the high prevalence of the virus; (ii) adaptation leads to reduced virulence; and (iii) negative effects of virus infection are not associated with plant growth but with other host traits. Some of our results support the later possibility. Given that infected plants cannot clear infection, and that mountain rue is a perennial plant, we would expect relatively homogeneous virus prevalence across seasons if virus adaptation would lead to reduced virulence ([Fraile et al., 2017](#)). However, MeRV prevalence variable even within the same sampling cycle. This suggests that MeRV infection could have an effect on plant survival. Optimal temperature for seed germination of

species from the *Ruta* genus is about 30°C (Mguis et al., 2011), and consequently young plants are more abundant in summer. Given that maximum MeRV prevalence occurs in this season and decays afterward, it could be hypothesized that MeRV-induced mortality could be more frequent in young individuals. In support of this hypothesis, additional surveys of mountain rue plants in evergreen oak forests indicated that MeRV was less prevalent in older plants (i.e., more than a year-old and at least one flowering period) (40%, 6/15 plants) than in younger plants (i.e., less than a year-old and not flowered) (71%, 5/7 plants). This indicates that not every young infected individual reaches the adult stage. Hence, our data strongly suggests that MeRV is an important modulator of the population dynamics of its host. On the other hand, this does not seem to be the case in crop hosts, as we failed in detecting the virus in pepper and tomato fields. Therefore, although a potential threat for these crops, MeRV infection in pepper and tomato is rare in the surveyed areas.

Multivariate models indicated that host plant density was the major predictor of MeRV infection risk, with a positive association between plant density and MeRV prevalence. This relationship has been discussed in the previous section for genus-wide potyvirus prevalence. Results for MeRV also support theory attributing a key role in infection risk to host density (Keesing et al., 2006; Ostfeld and Keesing, 2012). Again, species diversity was not a good predictor of MeRV infection risk in wild mountain rue populations of evergreen oak forests and no significant association between host density/abundance of mountain rue and species richness in evergreen oak forests was found ($r \leq 0.15$; $P \geq 0.699$). The poor predictive power of species richness and host relative abundance could be explained in this case to the absence of alternative host species for MeRV in evergreen oak forests and to the low levels of natural variation in plant species diversity/relative abundance across a homogeneous plant community. Finally, rainfall was negatively associated with MeRV prevalence. The rainfalls regime might have an effect on host physiology (Kennelly et al., 2012), increasing host susceptibility to virus infection and therefore virus prevalence (Seabloom et al., 2009). Alternatively, higher MeRV prevalence was generally observed in summer, when rainfall is lower and density of aphids (the main vectors of potyviruses) peaks (Nebreda et al., 2004; Mondal et al., 2016). Although MeRV transmission and the specific MeRV aphid

vector species involved have not been characterized here, these results would be compatible with the maximum activity of the associated MeRV vectors. This highlights the relevance of considering factors associated with virus transmission to fully understand the determinants of infection risk.

For the generalist virus WHMV, the best-ranked model predicting virus prevalence contained host relative abundance as the main predictor. There was a negative association between the host relative abundance and infection risk. As discussed in the previous section, this could be due to: (i) virus infection increases host mortality, (ii) the best hosts are less frequent plants, and (iii) plant isolation favours increased prevalence through attracting virus vectors. (A'Brook 1964; Way and Heathcote, 1966; Malmstrom et al., 2005a; Power et al., 2011; Prendeville et al., 2014; McLeish et al., 2017). At odds with MeRV, WHMV infection-induced mortality was not analysed, and this thesis has no data to determine whether mortality effects have a role in the negative association between host relative abundance and WHMV prevalence. On the other hand, the best hosts of WHMV in terms of prevalence level (*H. lupulus*, *E. elaterium*) are rare species in riparian forests, which could explain why WHMV prevalence is higher in host with low relative abundance. Host species that are rare at larger spatial scales (e.g., the ecosystem scale) might act as hosts when generalist viruses can infect them in the absence of other hosts within their range, or because of heterogeneity in transmission among host species (Streicker et al., 2013) and fine-scale variation in vector ecology (Hamer et al., 2011). Also, rare host species have been acknowledged as important hosts of spillover dynamics when the ecological structure of the surrounding flora and close phylogenetic relatedness are strong predictors of prevalence (Parker et al., 2015). The observation that rare hosts determine the prevalence of WHMV also agrees with other experimental analyses indicating that hosts with low abundance determine the prevalence of other generalist viruses such as CMV in other habitats of the Iberian Peninsula (McLeish et al., 2017). Interestingly, the importance of low abundant hosts for WHMV prevalence may also explain why species richness and plant biomass are secondary predictors of WHMV prevalence: higher species richness would increase the probability of rare host to appear (and thus the trend towards a positive association between virus prevalence and species richness shown in **Figure 4.22**). Also, the appearance

of this host species, whose size is generally above the average of the plants in the ecosystem, would result in larger plant biomass.

Thus, the analyses of the factors driving potyvirus species-specific prevalence in evergreen oak and riparian forests highlight the importance of considering the interplay between virus host range and host ecology in order to fully understand virus epidemiology, and provide insights on the most relevant ecological determinants of virus infection dynamics.

5.4. Potyvirus genus-wide population genetic diversity in wild ecosystems

Potyvirus populations in riparian forests showed considerable haplotype and genetic diversity in the NIb gene, whereas the CP gene tended to be more conserved. According to the results of this thesis, recombination played a minor role in generating such genetic diversity. This would be at odds with previous analyses in several potyvirus species, which indicated that recombination was involved in the evolution of these species (e.g., [Revers et al., 1996](#); [Wylie and Jones, 2009](#); [Kehoe et al., 2014](#); [Yasaka et al., 2017](#)). Notably, the selected genomic fragments have been shown to undergo very little intra- and inter-specific recombination processes ([Kehoe et al., 2014](#); [Yasaka et al., 2017](#)), which may explain the results of this thesis. Alternatively, it has been shown that in certain potyvirus species, and in other plant viruses, recombination hotspots tend to be distributed such that complete genes are exchanged ([Pagán and Holmes, 2010](#); [Yasaka et al., 2017](#)), in what has been termed “modular evolution” ([Botstein, 1980](#)). Given that the NIb and the CP gene sequence data sets were analysed separately, recombination breakpoints in-between genes could not be detected. Regardless of the potential causes of the lack of recombination events in the analysed potyvirus populations, it seems clear that, in the sequenced genomic fragments, the main mechanism for generating genetic diversity is mutation. Estimates of short-term nucleotide substitution rates of potyviruses were obtained in this thesis. The substitution rates reported in this work may represent over-estimates as, due to the short sampling time span considered, transitory mutations that will not be fixed in the

virus population were considered. However, certain temporal structure was detected (**Figure 4.23**), and substitution rates were in the same range than those estimated for other plant viruses, and for RNA viruses ([Pagán and García-Arenal, 2018](#)) in general ([Duffy et al., 2008](#)).

The ecological factors affecting the evolution of potyviruses in riparian and evergreen oak forests were also analysed. The most important factors predicting virus evolutionary parameters were host plant relative abundance and rainfalls and, at a lesser extent, plant biomass. Host relative abundance, rainfalls and plant biomass were positively associated with potyvirus evolutionary parameters. Two virus species, CIYVV and WHMV accounted for most infections of riparian potyviruses. These two viruses have overlapping host ranges, and common hosts (i.e., *Trifolium* species) are abundant in riparian forests. Larger host relative abundance may reflect larger host populations, which is often associated with an increase in the plant population genetic diversity ([Frankham, 1996](#); [Leimu et al., 2006](#)). Higher host genetic diversity has been reported to positively correlate with pathogen population genetic diversity in wild ecosystems ([Rodríguez-Nevado et al., 2018](#)), which would explain the role of host relative abundance in the potyvirus genetic diversity. In addition, a possible explanation for the positive association between rainfalls and biomass with potyvirus population genetic diversity might be that these ecological and climatic variables indirectly affect potyvirus genetic diversity, perhaps by influencing the viral vector population. For instance, larger plant biomass, which in riparian forests generally coincides with the season of higher rainfalls, has been associated with larger aphid population sizes ([Mahdavi-Arab et al., 2014](#); [Turley and Johnson, 2015](#)). Larger population sizes may result in multiple viruliferous aphids landing on the same plant, such that plant-to-plant transmission bottlenecks, which are generally strong for plant viruses ([Moury et al., 2007](#); [Betancourt et al., 2008](#)), would be weaker increasing virus population genetic diversity.

Finally, this thesis has, for the first time, shed light on the patterns and processes of speciation within the genus *Potyvirus* in wild ecosystems. In particular, our observation of a statistically significant association between phylogeny and both host specificity and geographical origin suggests that the potyvirus species present in evergreen oak and riparian forests may have

arisen within the same host species and in a restricted geographic area, as is expected under a process of sympatric speciation. Such a predominance of sympatric speciation may be expected, given that both plants and the aphid vectors that transmit plant viruses have a limited ability to move large geographic distances and that the anthropogenic factors that would assist allopatric speciation (such as increased transportation of plants) likely have little effects on the ecosystems analysed that have not undergone human management. However, it is important to note that the lack of information on the host range of the potyviruses present in the studied ecosystems in other geographical regions/habitats, particularly for the newly identified virus species, may be biasing this analysis against the detection of allopatric processes. Equivalent phylogenetic studies of speciation processes in other families of RNA viruses have proven to be informative, and mostly indicated speciation through sympatric processes both for plant ([Pagán and Holmes, 2010](#)) and animal ([Kitchen et al., 2011](#)) hosts.

5.5. Potyvirus species-specific population genetic diversity in wild ecosystems

Changes in MeRV prevalence were accompanied by variations in the virus population genetic and haplotype diversities. Viral populations may modify their genetic diversity by changing fixation rates, population sizes, and/or selection pressures ([Moya et al., 2000](#)). In this thesis, MeRV fixation rates were not estimated. However, given that the observed fluctuations in MeRV population genetic diversity occurred in short periods of time, and that genus-wide potyvirus fixation rates did not depart from values generally reported for RNA viruses, it is unlikely that these fluctuations are associated with changes in fixation rates. In turn, multiple regression model analyses indicated that MeRV prevalence was the best predictor of population genetic/haplotype diversity (higher virus genetic/haplotype diversities were observed at increasing prevalence). Higher virus prevalence results in increasing pathogen population sizes ([Burdon and Thrall, 2008](#)). Therefore, our results suggest that MeRV genetic diversity is likely modulated by fluctuations in virus population size. This

would be in agreement with theoretical elaborations predicting that higher population sizes may lead to higher genetic diversity (Scholle et al., 2013; Lanfear et al., 2014). Interestingly, MeRV prevalence was the best predictor of d_S , both variables showing a positive association, but not of d_N . Accordingly, selection pressures, measured as d_N/d_S , were negatively associated with virus prevalence. This indicates that neutral evolution, rather than adaptive selection, might be responsible for the changes in MeRV population genetic diversity associated with fluctuations in virus prevalence. Similar results have been obtained in other plant virus populations infecting wild hosts (Lima et al., 2013; Rodelo-Urrego et al., 2015).

The results of the model selection analyses for WHMV population genetic diversity are more difficult to interpret. The most important factors predicting virus population haplotype and genetic diversity were host plant relative abundance and rainfalls. In addition, the best predictor of d_N , d_S and d_N/d_S was plant biomass (Table 4.37). At odds with the associations at the genus level found for riparian potyviruses, host relative abundance was negatively associated with WHMV genetic diversity parameters. On the other hand, rainfalls and plant biomass were positively associated with this virus population trait. As stated in the previous section, a possible explanation for these results is that the ecological and climatic variables identified in model selection analyses do not directly affect virus genetic diversity. Rather, host relative abundance, rainfalls and biomass may indirectly affect WHMV genetic diversity through influencing the viral vector population. Larger plant biomass and higher rainfalls would promote larger aphid population sizes (Mahdavi-Arab et al., 2014; Turley and Johnson, 2015), which may result in multiple viruliferous aphids landing on the same plant, such that plant-to-plant transmission bottlenecks would be weaker, increasing virus population genetic diversity without necessarily affecting virus prevalence (Bergstrom et al., 1999). This would also explain the general low predictive power of virus prevalence on population genetic diversity parameters. Note that, as stated above, the most relevant WHMV hosts are rare species in riparian forests, which, for a given number of viruliferous aphids and all else being equal, would increase the chances of multiple landings in the same host plant. This would be also compatible with the higher virus genetic diversity observed in the less abundant

hosts (**Figure 4.31**). However, it must be acknowledged that all these hypotheses are highly speculative, as it is out of the scope of this thesis to analyse the relationship between vector population traits and potyvirus population genetic diversity. In all these models, WHMV prevalence was a secondary predictor, suggesting that virus population size is not a primary determinant of WHMV evolution. Moreover, when WHMV prevalence affected virus population genetic diversity (specifically d_N) or selection pressures (d_N/d_S), the association between these traits was negative, suggesting that WHMV hosts exert a strong purifying selection on the virus population.

Finally, and despite differences in the ecology and epidemiology of MeRV and WHMV, the analysis of their speciation modes also indicated that these virus species diverged through processes of sympatric speciation, suggesting again that this might be a general trend among potyviruses. Further analyses in other potyvirus species, and including both wild and cultivated hosts, would help to test the generality of this conclusion.

5.6. Limitations of the study

Some cautionary comments, however, are called upon the results of this thesis. First, ML models were constructed with data from a single sampling cycle and validated with data from a second one. This is a short life span and might be well possible that yearly variation in ecological and climatic conditions not reflected in this study could reduce the accuracy of the ML models developed here. However, the models built with data of the first sampling cycle performed well in the data from the second sampling cycle, which showed seasonal variation in ecological parameters as compared with the first sampling cycle. Moreover, preliminary analyses with data of a third sampling cycle also indicated good performance of the ML models. Thus, although the predictive models constructed during this thesis can be clearly improved, they appear to be robust against yearly variation in ecological and climatic conditions. Second, the analyses of the association between potyvirus prevalence and ecological factors are based on the data from ten locations, and the analyses of the association between potyvirus evolutionary parameters and

ecological/epidemiological factors are based on data from a subset of these locations. We are aware that this might represent a small sample size. However, it was enough to detect significant, and in many cases strong, correlations between the studied parameters. Third, although we conclude that MeRV is a specialist virus and WHMV is a generalist virus, we cannot discard that these viruses are able to infect host species that, due to their low frequency, have been insufficiently sampled. We believe, however, that this is unlikely given the sampling effort done in this work (over 10,000 samples). Fourth, although the best-ranked models explained a large proportion of the variation in potyvirus infection risk and population genetic diversity, other factors not considered here could also play a role in MeRV evolution and epidemiology. For instance, host genetic diversity or vector population dynamics has been also reported as important determinants of virus disease risk and population genetic diversity in wild ecosystems ([Pagán et al., 2012](#); [Rodelo-Urrego et al., 2015](#); [Rodríguez-Nevado et al., 2018](#)). Unfortunately, the lack of information on the genomic sequence of most potyvirus hosts identified in this thesis prevented including this variable in our analyses. Analyses in other host-pathogen system would help to test the generality of our observations. Fifth, the analysis of the effect of MeRV infection on host mortality is based on the differential virus prevalence between adult and young plants. It should be noted that these results could be also explained if virus titer in adult plants would be much lower than in younger ones, such that it could lead to an underestimation of virus prevalence in adults. However, no significant differences in MeRV accumulation were observed between young and adult plants ($F_{1,8}=0.63$; $P=0.450$).

5.7. Concluding remarks

In summary, this thesis analyses the ecological and climatic factors that determine the prevalence and genetic diversity of potyviruses in two wild ecosystems of the Iberian Peninsula. These analyses are based on a detailed characterization of the ecosystems and virus populations under study. The results indicate that the floristic composition of the ecosystem, and the relative abundance and the density of hosts play a fundamental role in the prevalence

of potyviruses. The former factor determines the entry of potyviruses in the ecosystem, whereas the later two factors play a role in the extent of the infection (i.e., in the level of prevalence). Thus, these ecological factors play a role in potyvirus emergence at different stages of this process. The host range of each potyvirus species determines the relative importance of each of these ecological factors. Host relative abundance and density are also important determinants of the level of genetic diversification in potyvirus populations, which suggest a link between the epidemiological changes that lead to virus emergence and genetic changes in the virus populations. The results of this thesis also indicate that other ecological and climatic factors, perhaps linked to the dynamics of the virus vector populations, play an important role in the evolution of potyvirus populations in wild ecosystems. Finally, in the studied ecosystems the process of genetic diversification of potyviruses is compatible with sympatric speciation events. Altogether, the results of this thesis provide noble information on the determinants of plant virus emergence in wild ecosystems, and highlight the importance of considering the interplay between the ecology, epidemiology and genetic diversity of plant host and virus populations in order to fully understand plant virus emergence.

6. CONCLUSIONS

This thesis analysed the ecological determinants of the infection risk of species of the genus *Potyvirus* present in riparian and evergreen oak forests of the Iberian Peninsula. These analyses aimed at understanding the ecological determinants of plant virus emergence in wild ecosystems, which are central to fully understand plant-virus interactions but are still poorly characterized.

1. The locations monitored in this thesis conform to the ecological characteristics of typical riparian and evergreen oak forests of the Iberian Peninsula and are therefore, representative of these wild ecosystems. A sampling effort of 200 plants/location is in general sufficient to fully capture the floristic composition of each location.
2. Virus prevalence was generally higher in riparian than in evergreen oak forests, which was associated with higher plant species richness and abundance of host plants, most of which were perennial species. This result supports the “Amplification Effect” hypothesis and highlights the importance of considering the identity of the species present in an ecosystem, and not only the number of plant species, in order to characterize diversity-prevalence relationships.
3. The plant community composition determines the risk of potyvirus jump into the studied wild ecosystems. Once introduced in the ecosystem, host relative abundance/density chiefly predict the extent of potyvirus invasion (prevalence), likely affecting plant-to-plant transmission. As these two processes are linked to different stages of pathogen emergence, different components of plant diversity (identity/relative abundance) determine different stages of emergence.
4. The main ecological factors that determine potyvirus prevalence in the studied wild ecosystems depend on the virus host range. The prevalence of a strict specialist is mostly determined by the density of host plants. On the other hand, the prevalence of a generalist is determined by the relative abundance of hosts. Thus, the epidemiology of potyviruses in their native wild ecosystems depends on virus life history traits.

5. The ecological factors that determine the population genetic diversity of the potyviruses detected in riparian and evergreen oak forests at the genus-wide level are the same than those determining virus epidemiology. Thus, the ecological factors that affect potyvirus epidemiology promote genetic modifications in the virus population.
6. Higher population genetic diversity of a specialist potyvirus native of wild ecosystems is associated with higher virus prevalence. This larger genetic diversity appears to be due to larger accumulation of synonymous mutations. This suggests that neutral evolution, perhaps associated with virus population size, rather than host adaptation likely determines the genetic diversity of a specialist potyvirus.
7. The population genetic diversity of a generalist potyvirus increases with lower host relative abundance, and higher rainfalls and biomass. There is no obvious mechanism by which these factors may directly affect virus genetic diversity. However, it can be hypothesized that host relative abundance, rainfalls and biomass may indirectly affect virus genetic diversity through influencing the viral vector population.
8. The patterns of genus-wide and species-specific genetic diversification of potyviruses in wild ecosystems are compatible with sympatric speciation.

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APPENDIX

APPENDIX 1

Materials and methods

Table A1.1. Ecological and climatic parameters recorded at each location and visit in riparian forests during 2013-2015.

Location	Parameter	Summer 2013	Autumn 2013	Spring 2014	Summer 2014	Autumn 2014	Spring 2015
El Escorial	Plant density (m)*	4.66 ± 2.22	6.03 ± 4.89	5.22 ± 4.28	5.08 ± 3.55	4.15 ± 3.26	4.13 ± 3.28
	Relative abundance (%)*	3.45 ± 2.23	3.57 ± 3.57	2.94 ± 2.35	3.23 ± 2.49	3.85 ± 3.20	3.13 ± 2.78
	Biomass (m ³)	0.38	–	0.13	0.17	0.20	0.15
	S	29	28	34	31	26	32
	H'	3.15	2.90	3.23	3.16	2.93	3.13
	E _H	0.94	0.87	0.92	0.92	0.90	0.90
	HPS	15	13	15	18	8	16
	T _{min} (°C)	7.55	2.43	2.47	9.33	3.65	1.27
	T _{med} (°C)	20.43	14.77	13.43	22.27	15.20	13.70
	T _{max} (°C)	32.95	27.07	27.07	34.27	29.65	27.53
	RH _{min} (%)	13.50	19.00	18.67	13.67	30.00	16.33
	RH _{med} (%)	39.50	59.00	50.00	36.33	62.67	53.00
	RH _{max} (%)	88.75	99.67	98.67	93.33	99.50	96.00
	Rainfalls (mm)	159.00	434.67	431.00	211.33	594.50	230.67
	Soil pH	5.50	5.50	5.50	5.50	5.50	5.50
	Soil moisture (%)*	–	–	–	5.04 ± 1.77	19.74 ± 3.01	12.11 ± 9.17
Palazuelos	Plant density (m)*	7.15 ± 5.13	7.35 ± 9.17	7.96 ± 9.18	13.06 ± 13.36	12.25 ± 20.65	10.21 ± 22.30
	Relative abundance (%)*	3.23 ± 2.31	3.13 ± 3.82	3.81 ± 3.71	4.35 ± 3.97	3.33 ± 3.93	4.76 ± 5.47
	Biomass (m ³)	0.08	0.01	0.08	0.04	0.03	0.07
	S	31	32	26	23	30	21
	H'	3.17	2.92	2.82	2.76	2.83	2.50
	E _H	0.92	0.84	0.87	0.88	0.83	0.82
	HPS	15	14	14	13	13	12
	T _{min} (°C)	5.58	0.07	0.97	6.63	4.50	-0.73
	T _{med} (°C)	18.33	13.07	11.90	20.13	14.97	11.87
	T _{max} (°C)	31.10	25.93	24.27	32.57	27.50	24.70
	RH _{min} (%)	14.25	17.00	15.67	12.00	24.67	15.33
	RH _{med} (%)	46.50	63.67	57.67	42.33	63.00	61.00
	RH _{max} (%)	94.25	98.67	99.67	96.33	98.67	98.33
	Rainfalls (mm)	216.00	277.33	349.00	138.67	507.00	232.00
	Soil pH	5.50	5.50	5.50	5.50	5.50	5.50
	Soil moisture (%)*	–	–	–	6.95 ± 0.47	22.94 ± 3.11	9.99 ± 0.62

*Mean±standard deviation for all plant species collected at each location and season.

S: Species richness; H': Shannon's index; E_H: Shannon's equitability index; HPS: number of host plant species; T_{max}, T_{min}, T_{mean}: Average of monthly maximum, minimum, and mean temperatures at a given season/location; RH_{max}, RH_{min}, RH_{mean}: Average of monthly maximum, minimum, and mean relative humidity at a given season/location; Rainfalls: Average of total rainfalls in a season; Soil moisture: Average of measures taken at eight quadrants of 1m² randomly selected in each location.

Table A1.1. Continued.

Location	Parameter	Summer 2013	Autumn 2013	Spring 2014	Summer 2014	Autumn 2014	Spring 2015
Pollos	Plant density (m)*	4.83 ± 5.77	4.85 ± 5.61	3.27 ± 2.92	4.44 ± 4.40	4.39 ± 4.11	2.78 ± 2.17
	Relative abundance (%)*	3.13 ± 2.52	3.08 ± 3.46	2.65 ± 3.05	2.86 ± 2.37	3.23 ± 3.32	3.13 ± 2.62
	Biomass (m ³)	0.11	0.10	0.13	0.13	0.06	0.14
	S	32	32	35	35	31	32
	H'	3.15	2.97	3.09	3.26	2.98	3.14
	E _H	0.91	0.86	0.87	0.92	0.87	0.90
	HPS	15	12	13	10	12	8
	T _{min} (°C)	6.60	-0.80	1.23	7.33	3.83	-0.73
	T _{med} (°C)	18.68	12.90	12.67	20.47	15.40	12.57
	T _{max} (°C)	32.48	26.30	25.50	34.57	28.37	27.63
	RH _{min} (%)	14.25	31.50	18.00	12.67	27.33	14.67
	RH _{med} (%)	49.00	65.50	56.33	44.33	67.33	60.50
	RH _{max} (%)	96.25	99.00	99.00	93.33	98.00	97.00
	Rainfalls (mm)	222.50	299.00	166.00	80.00	365.00	101.33
	Soil pH	5.00	5.00	5.00	5.00	5.00	5.00
	Soil moisture (%)*	–	–	–	–	–	13.13 ± 12.09
Rascafría	Plant density (m)*	6.51 ± 3.24	4.94 ± 4.20	7.33 ± 6.06	4.53 ± 4.00	5.80 ± 6.28	3.85 ± 4.15
	Relative abundance (%)*	2.63 ± 1.57	3.48 ± 3.47	3.57 ± 3.38	2.63 ± 2.25	3.13 ± 2.76	2.78 ± 3.64
	Biomass (m ³)	0.49	–	0.18	0.19	0.04	0.24
	S	38	28	28	38	32	36
	H'	3.47	3.00	2.95	3.30	3.12	3.01
	E _H	0.95	0.90	0.89	0.91	0.90	0.84
	HPS	20	16	14	15	15	16
	T _{min} (°C)	2.38	-1.45	-0.90	2.37	1.40	-3.80
	T _{med} (°C)	15.33	13.80	9.50	16.87	14.15	9.70
	T _{max} (°C)	29.18	26.50	22.93	30.40	28.95	23.90
	RH _{min} (%)	16.50	16.50	19.33	16.33	18.50	19.67
	RH _{med} (%)	50.50	64.00	61.00	47.67	69.00	62.00
	RH _{max} (%)	97.25	97.00	97.67	96.33	98.50	97.00
	Rainfalls (mm)	363.50	466.50	1334.67	216.00	814.50	392.67
	Soil pH	5.50	5.50	5.50	5.50	5.50	5.50
	Soil moisture (%)*	–	–	–	3.75 ± 1.25	20.48 ± 2.88	8.01 ± 1.13

*Mean±standard deviation for all plant species collected at each location and season.

S: Species richness; H': Shannon's index; E_H: Shannon's equitability index; HPS: number of host plant species; T_{max}, T_{min}, T_{mean}: Average of monthly maximum, minimum, and mean temperatures at a given season/location; RH_{max}, RH_{min}, RH_{mean}: Average of monthly maximum, minimum, and mean relative humidity at a given season/location; Rainfalls: Average of total rainfalls in a season; Soil moisture: Average of measures taken at eight quadrants of 1m² randomly selected in each location.

Table A1.1. Continued.

Location	Parameter	Summer 2013	Autumn 2013	Spring 2014	Summer 2014	Autumn 2014	Spring 2015
Santa Cruz de Retamar	Plant density (m)*	4.31 ± 2.17	5.16 ± 5.68	3.24 ± 2.37	4.82 ± 6.32	4.10 ± 4.32	4.97 ± 5.33
	Relative abundance (%)*	3.45 ± 2.32	3.48 ± 4.31	3.33 ± 3.53	2.94 ± 5.04	3.70 ± 3.88	5.88 ± 7.27
	Biomass (m ³)	0.02	0.01	0.06	0.00	0.06	0.01
	S	29	27	30	34	27	17
	H'	3.14	2.64	2.96	2.80	2.86	2.24
	E _H	0.93	0.80	0.87	0.79	0.87	0.79
	HPS	16	8	10	13	12	7
	T _{min} (°C)	9.85	5.10	4.50	13.00	7.90	3.90
	T _{med} (°C)	23.13	16.60	15.30	24.20	17.40	15.80
	T _{max} (°C)	35.30	27.37	27.97	36.13	29.90	29.60
	RH _{min} (%)	14.00	23.00	17.00	14.00	23.00	17.00
	RH _{med} (%)	37.50	60.33	52.67	36.33	67.67	55.00
	RH _{max} (%)	95.00	99.00	98.00	95.00	99.00	98.00
	Rainfalls (mm)	107.00	419.33	315.33	45.33	1031.33	319.33
	Soil pH	5.50	5.50	5.50	5.50	5.50	5.50
	Soil moisture (%)*	–	–	–	2.76 ± 0.31	9.80 ± 2.59	1.64 ± 0.41

*Mean ± standard deviation for all plant species collected at each location and season.

S: Species richness; H': Shannon's index; E_H: Shannon's equitability index; HPS: number of host plant species; T_{max}, T_{min}, T_{mean}: Average of monthly maximum, minimum, and mean temperatures at a given season/location; RH_{max}, RH_{min}, RH_{mean}: Average of monthly maximum, minimum, and mean relative humidity at a given season/location; Rainfalls: Average of total rainfalls in a season; Soil moisture: Average of measures taken at eight quadrants of 1m² randomly selected in each location.

Table A1.2. Ecological and climatic parameters recorded at each location and visit in evergreen oak forests during 2013-2015.

Location	Parameter	Summer 2013	Autumn 2013	Spring 2014	Summer 2014	Autumn 2014	Spring 2015
Carbonero el Mayor	Plant density (m)*	3.51 ± 1.91	5.75 ± 7.14	4.95 ± 3.57	6.38 ± 4.03	4.91 ± 3.85	4.38 ± 4.00
	Relative abundance (%)*	5.88 ± 4.34	5.56 ± 7.77	7.14 ± 6.02	6.25 ± 8.76	5.56 ± 9.19	5.88 ± 8.84
	Biomass (m ³)	0.09	0.03	0.02	0.01	0.02	0.02
	S	18	18	14	16	18	17
	H'	2.55	2.19	2.31	2.12	2.05	2.05
	E _H	0.88	0.76	0.88	0.76	0.71	0.72
	HPS	6	7	6	7	4	4
	T _{min} (°C)	4.27	-0.70	0.97	6.57	3.00	-1.20
	T _{med} (°C)	16.97	13.20	12.00	20.27	13.15	12.10
	T _{max} (°C)	31.17	25.50	24.60	33.23	25.55	26.13
	RH _{min} (%)	14.67	24.00	18.33	14.33	29.50	17.67
	RH _{med} (%)	48.67	65.67	57.33	43.00	70.00	62.00
	RH _{max} (%)	95.33	98.67	98.00	96.00	99.00	98.33
	Rainfalls (mm)	96.00	660.00	244.00	156.00	674.00	190.67
	Soil pH	5.50	5.50	5.50	5.50	5.50	5.50
Cenicientos	Soil moisture (%)*	–	–	–	2.60 ± 0.47	6.58 ± 0.77	3.59 ± 0.46
	Plant density (m)*	7.57 ± 7.27	6.14 ± 4.32	6.08 ± 6.64	4.75 ± 6.01	4.59 ± 4.73	5.15 ± 3.34
	Relative abundance (%)*	5.00 ± 3.03	3.98 ± 4.53	3.69 ± 3.23	4.55 ± 6.86	3.70 ± 6.21	4.55 ± 4.26
	Biomass (m ³)	0.01	0.00	–	0.00	0.01	0.08
	S	20	25	27	22	27	22
	H'	2.82	2.77	2.93	2.37	2.53	2.72
	E _H	0.94	0.86	0.89	0.77	0.77	0.88
	HPS	7	7	8	7	4	7
	T _{min} (°C)	7.80	3.67	2.47	9.30	5.57	1.53
	T _{med} (°C)	19.43	13.97	12.17	20.90	14.60	12.53
	T _{max} (°C)	31.33	25.13	24.13	32.60	27.00	25.17
	RH _{min} (%)	14.00	27.67	24.67	15.33	30.33	19.33
	RH _{med} (%)	44.00	66.00	61.00	41.33	75.00	62.00
	RH _{max} (%)	94.00	100.00	100.00	98.33	100.00	100.00
	Rainfalls (mm)	84.50	516.67	353.33	57.33	989.00	355.00
	Soil pH	4.50	4.50	4.50	4.50	4.50	4.50
	Soil moisture (%)*	–	–	–	1.28 ± 0.35	8.79 ± 1.41	1.65 ± 0.92

*Mean±standard deviation for all plant species collected at each location and season.

S: Species richness; H': Shannon's index; E_H: Shannon's equitability index; HPS: number of host plant species; T_{max}, T_{min}, T_{mean}: Average of monthly maximum, minimum, and mean temperatures at a given season/location; RH_{max}, RH_{min}, RH_{mean}: Average of monthly maximum, minimum, and mean relative humidity at a given season/location; Rainfalls: Average of total rainfalls in a season; Soil moisture: Average of measures taken at eight quadrants of 1m² randomly selected in each location.

Table A1.2. Continued.

Location	Parameter	Summer 2013	Autumn 2013	Spring 2014	Summer 2014	Autumn 2014	Spring 2015
El Pardo	Plant density (m)*	4.29 ± 2.90	2.86 ± 2.01	5.77 ± 5.37	3.93 ± 2.87	3.78 ± 4.33	3.77 ± 3.24
	Relative abundance (%)*	4.17 ± 2.77	6.67 ± 6.86	3.85 ± 3.70	4.00 ± 3.32	5.56 ± 7.28	3.70 ± 4.48
	Biomass (m ³)	0.01	0.05	0.05	0.01	0.05	0.08
	S	24	15	26	25	18	27
	H'	2.95	2.26	2.86	2.89	2.25	2.74
	E _H	0.93	0.84	0.88	0.90	0.78	0.83
	HPS	9	6	11	9	5	7
	T _{min} (°C)	8.80	-1.45	2.87	11.33	5.93	0.70
	T _{med} (°C)	22.37	12.70	14.50	23.70	16.93	12.45
	T _{max} (°C)	34.47	24.90	28.37	36.30	29.10	25.85
	RH _{min} (%)	17.67	23.00	18.67	17.67	26.33	18.00
	RH _{med} (%)	38.75	52.67	46.33	37.00	56.67	48.50
	RH _{max} (%)	85.67	90.00	89.00	86.33	89.33	88.50
	Rainfalls (mm)	94.67	215.50	229.67	42.33	418.50	348.00
	Soil pH	6.00	6.00	6.00	6.00	6.00	6.00
	Soil moisture (%)*	–	–	–	–	9.13 ± 1.60	3.58 ± 0.71
Marjaliza	Plant density (m)*	6.37 ± 5.47	5.38 ± 4.51	5.47 ± 4.39	2.79 ± 2.06	3.12 ± 3.96	5.89 ± 5.11
	Relative abundance (%)*	3.20 ± 2.69	5.26 ± 5.99	3.85 ± 3.16	5.56 ± 7.32	4.35 ± 5.91	4.17 ± 4.58
	Biomass (m ³)	0.25	0.08	0.02	0.00	0.02	0.11
	S	31	19	26	18	23	24
	H'	3.14	2.45	2.96	2.26	2.42	2.71
	E _H	0.91	0.83	0.91	0.78	0.77	0.85
	HPS	7	7	10	7	4	8
	T _{min} (°C)	9.95	3.90	3.80	12.37	6.90	2.30
	T _{med} (°C)	21.93	15.83	14.87	24.17	17.17	12.50
	T _{max} (°C)	33.78	27.23	27.50	35.73	29.63	24.90
	RH _{min} (%)	11.50	21.33	14.67	12.33	23.67	13.50
	RH _{med} (%)	39.00	59.67	48.00	33.67	62.00	54.50
	RH _{max} (%)	89.00	99.00	93.00	86.00	97.33	98.50
	Rainfalls (mm)	133.50	153.33	162.00	54.67	354.67	405.00
	Soil pH	6.00	6.00	6.00	6.00	6.00	6.00
	Soil moisture (%)*	–	–	–	–	3.36 ± 1.06	2.78 ± 0.77

*Mean±standard deviation for all plant species collected at each location and season.

S: Species richness; H': Shannon's index; E_H: Shannon's equitability index; HPS: number of host plant species; T_{max}, T_{min}, T_{mean}: Average of monthly maximum, minimum, and mean temperatures at a given season/location; RH_{max}, RH_{min}, RH_{mean}: Average of monthly maximum, minimum, and mean relative humidity at a given season/location; Rainfalls: Average of total rainfalls in a season; Soil moisture: Average of measures taken at eight quadrants of 1m² randomly selected in each location.

Table A1.2. Continued.

Location	Parameter	Summer 2013	Autumn 2013	Spring 2014	Summer 2014	Autumn 2014	Spring 2015
Montegancedo	Plant density (m)*	3.96 ± 2.37	4.98 ± 3.01	4.53 ± 3.65	4.89 ± 5.73	5.32 ± 5.90	4.31 ± 3.03
	Relative abundance (%)*	4.17 ± 2.69	4.55 ± 5.26	4.21 ± 3.80	5.88 ± 5.53	6.67 ± 6.40	3.85 ± 3.08
	Biomass (m ³)	0.04	0.05	0.07	0.01	0.08	0.28
	S	24	22	25	17	15	26
	H'	2.96	2.62	2.96	2.41	2.27	2.95
	E _H	0.93	0.85	0.92	0.85	0.84	0.90
	HPS	7	6	7	6	2	6
	T _{min} (°C)	6.10	1.27	2.10	9.90	3.53	3.80
	T _{med} (°C)	20.88	14.80	14.03	22.70	15.87	15.20
	T _{max} (°C)	33.83	27.20	27.93	35.50	29.70	29.33
	RH _{min} (%)	11.25	20.67	17.00	12.33	24.00	13.00
	RH _{med} (%)	38.25	59.00	49.67	36.00	64.67	53.00
	RH _{max} (%)	88.75	98.00	95.00	90.67	98.00	98.00
	Rainfalls (mm)	74.00	366.67	307.33	56.00	582.00	239.33
	Soil pH	6.00	6.00	6.00	6.00	6.00	6.00
	Soil moisture (%)*	–	–	–	1.66 ± 0.39	10.80 ± 4.30	–

*Mean±standard deviation for all plant species collected at each location and season.

S: Species richness; H': Shannon's index; E_H: Shannon's equitability index; HPS: number of host plant species; T_{max}, T_{min}, T_{mean}: Average of monthly maximum, minimum, and mean temperatures at a given season/location; RH_{max}, RH_{min}, RH_{mean}: Average of monthly maximum, minimum, and mean relative humidity at a given season/location; Rainfalls: Average of total rainfalls in a season; Soil moisture: Average of measures taken at eight quadrants of 1m² randomly selected in each location.

Table A1.3. Phylogenetic congruence between the phylogenies obtained with the Nlb and the CP sequences of potyvirus isolates.

Virus name	Potyvirus isolate	GenBank Acc. number	P value*
<i>Arracacha mottle virus</i>	AMoV_C-17	NC_018176	0.001
<i>Algerian watermelon mosaic virus</i>	AWMV_H4	EU410442	0.001
<i>Basella rugose mosaic virus</i>	BaRMV_AC	DQ821938	0.825
	BaRMV_BR	DQ821939	0.798
<i>Banana bract mosaic virus</i>	BBrMV	DQ851496	0.001
	BBrMV_TRY	HM131454	0.001
<i>Bean common mosaic necrosis virus</i>	BCMNV_Michigan	NC_004047	0.001
	BCMNV_NL5	HQ229993	0.001
	BCMNV_NL8	HQ229994	0.001
	BCMNV_PV0413	HG792063	0.001
	BCMNV_TN1	HQ229995	0.001
<i>Bean common mosaic virus</i>	BCMV_CD031	KM051430	0.001
	BCMV_CT	KM076650	0.001
	BCMV_DXH025	KJ807812	0.001
	BCMV_HB	AB863630	0.001
	BCMV_MB	KC832502	0.001
	BCMV_MS1	EU761198	0.001
	BCMV_NL1	L15331	0.001
	BCMV_NL4	L21766	0.001
	BCMV_PV0915	HG792064	0.001
	BCMV_R	AJ312437	0.001
	BCMV_Y	AJ312438	0.001
<i>Bidens mottle virus</i>	BiMoV_B12	EU250210	0.001
	BiMoV_B3	EU250212	0.001
	BiMoV_B4	EU250211	0.001
	BiMoV_HL	EU250214	0.001
	BiMoV_WF	EU250213	0.001
<i>Brugmansia suaveolens mottle virus</i>	BsMoV_Bs-Campinas	AB551370	0.001
<i>Blue squill virus A</i>	BSVA_KP1	JN052072	0.001
	BSVA_SW3	JQ807999	0.001
<i>Beet mosaic virus</i>	BtMV_BtMV-Wa	AY206394	0.823
	BtMV_Inner Mongolia	DQ674263	0.793
	BtMV_Xinjiang	DQ674264	0.784
<i>Bean yellow mosaic virus</i>	BYMV_GB32A	HG970854	0.004
	BYMV_IbG	AB079887	0.003
	BYMV_M11	AB079886	0.003
<i>Cowpea aphid-borne mosaic virus</i>	CABMV_BR1	HQ880242	0.001
	CABMV_MG-Avr	HQ880243	0.001
	CABMV_RR3	KM597165	0.001
	CABMV_RR4	KM655833	0.001
	CABMV_Z	AF348210	0.001
<i>Canna yellow streak virus</i>	CaYSV	GQ421689	0.001
	CaYSV_BZ2	KM882642	0.001
	CaYSV_NC1	KM882644	0.001
	CaYSV_NC5	KM882648	0.001
	CaYSV_OK	KM882640	0.001
<i>Celery mosaic virus</i>	CeMV_California	HQ676607	0.001
<i>Chilli ringspot virus</i>	ChiRSV-HN/14	JN008909	0.001
	ChiRSV-HN/Yingzhou	JQ234922	0.001
<i>Chilli veinal mottle virus</i>	ChiVMV_Ca	AJ972878	0.001
	ChiVMV-Ch-Jal	GU170807	0.001
	ChiVMV_Wenchang	GQ98131	0.001
	ChiVMV_YN-tobacco	JX088636	0.001
<i>Clover yellow vein virus</i>	CIYVV_CYVV	HG970870	0.001
	CIYVV_Gm	KF975894	0.001
	CIYVV_No.30	AB002698	0.001
<i>Cocksfoot streak virus</i>	CSV	AF499738	0.001
	CSV_CSV630wytham	EU119422	0.001
<i>Daphne mosaic virus</i>	DapMV	DQ299908	0.039
<i>Dasheen mosaic virus</i>	DMV_Caladium	AF048981	0.001
	DMV_M13	AJ298033	0.001
	DMV_T10	KJ786965	0.001

*A total of 5 (2%) non-significant and 266 (98%) significant associations between phylogenies of the Nlb and the CP sequences of potyvirus isolates were retrieved in the cophylogenetic analysis using a significance threshold of $P = 0.05$ (CopyCat v.2.03; [Meier-Kolthoff et al., 2007](#)).

Table A1.3. Continued.

Virus name	Potyvirus isolate	GenBank Acc. number	P value*
<i>East asian passiflora virus</i>	EAPV_SY071	AB690448	0.001
	EAPV_SY072	AB690449	0.001
	EAPV_SY101	AB690451	0.001
	EAPV_YM102	AB690452	0.001
	EAPV_YW101	AB690455	0.001
<i>Fritillary virus Y</i>	FVY_Panan	AM039800	0.001
<i>Habenaria mosaic virus</i>	HaMV_Ha-1	AB818538	0.001
<i>Hardenbergia mosaic virus</i>	HarMV_57.1	HQ161080	0.001
	HarMV_57.2	HQ161081	0.001
	HarMV_MD2	KJ152152	0.001
	HarMV_MD3	KJ152153	0.001
	HarMV_MD4A	KJ152154	0.001
	HarMV_MD4C	KJ152156	0.001
	HarMV_MD4D	KJ152157	0.001
	HMV_PHYS/H	AM184113	0.001
<i>Henbane mosaic virus</i>	JYMV	AB027007	0.001
	JYMV_FX1	KJ789141	0.002
	JYMV_SG1	KJ789140	0.001
	JYMV_WX1	KJ789138	0.002
	JYMV_WX3	KJ789139	0.001
<i>Konjak mosaic virus</i>	KoMV_KoMV-F	AB219545	0.008
<i>Lily mottle virus</i>	LMoV_Bate5	JN127341	0.001
	LMoV_ML61	AB570195	0.002
	LMoV_Sb	AJ564636	0.002
	LMoV_SMi	AM048875	0.001
	LMV_AF199	KF268955	0.001
<i>Lettuce mosaic virus</i>	LMV_Br6	KJ161174	0.001
	LMV_CL574	KJ161183	0.001
	LMV_ES16	KJ161185	0.001
	LMV_FR25	KJ161186	0.001
	LMV_Ham6	KJ161187	0.001
	LMV_LMV-Cr	KF268956	0.001
	LMV_Muju	KF955619	0.001
	LMV_Tn517	KJ161192	0.001
	LMV_Yar	KJ161194	0.001
	LMV_Yuhang	AJ306288	0.001
	LuMV_LU2	EU847625	0.004
	LYSV_1A3I	AB194623	0.001
	LYSV_3mEI7	AB194621	0.001
<i>Lupine mosaic virus</i>	LYSV_AG1	JX429967	0.001
	LYSV_Bate4	JN127340	0.001
	LYSV_SG2	JX429965	0.001
	LYSV_SW10	KF597285	0.001
	LYSV_SW9	KF597284	0.001
	LYSV_W-Ku	AB194622	0.001
	LYSV_Yuhan	AJ307057	0.001
	MDMV_Arg	DQ973169	0.001
	MDMV_Italy	JX185302	0.001
	MWMV_TN057	EF579955	0.001
<i>Moroccan watermelon mosaic virus</i>	NDV_Marjiniup 2	JQ395041	0.001
	NDV_Zhangzhou	AM182028	0.001
<i>Narcissus degeneration virus</i>	NLSYV_Marjiniup8	KC691259	0.001
	NLSYV_Zhangzhou	JQ326210	0.001
<i>Narcissus late season yellows virus</i>	NYSV_Marjiniup 3	JQ395042	0.001
	NYSV_Zhangzhou	EU430294	0.001
	NYSV_ZZ-2	JQ911732	0.001
<i>Narcissus yellow stripe virus</i>	OrMV_Bate9	JN127345	0.010
	OrMV_KP	JQ807997	0.014
	OrMV_OMV-O	D00615	0.018
	OrMV_SW3.1	JQ807995	0.016
	OrMV_SW3.3	JQ807996	0.012

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Table A1.3. Continued.

Virus name	Potyvirus isolate	GenBank Acc. number	P value*
<i>Onion yellow dwarf virus</i>	OYDV_13.L.Se	KF623538	0.001
	OYDV_2.T.Se	KF623539	0.001
	OYDV_5.L	KF623535	0.001
	OYDV_6.L.Se	KF623537	0.001
	OYDV_8.T	KF623536	0.001
	OYDV_O.70	KF623541	0.001
	OYDV_OYDV-Se	JX433019	0.001
	OYDV_Yuhang	AJ510223	0.001
<i>Panax virus Y</i>	PanVY_2	GQ916624	0.001
<i>Peanut mottle virus</i>	PeMoV_Australian	X73422	0.001
	PeMoV_Habin	KF977830	0.001
	PeMoV_M	AF023848	0.001
<i>Pennisetum mosaic virus</i>	PenMV_AZ1	JX070145	0.001
	PenMV_AZGL2	JX070143	0.001
	PenMV_B	AY642590	0.001
	PenMV_C	DQ977725	0.001
	PenMV_CD1	JX070152	0.001
	PepMoV_California	M96425	0.001
<i>Pepper mottle virus</i>	PepMoV_Florida	AF501591	0.001
	PepSMV	AM181350	0.001
<i>Pepper severe mosaic virus</i>	PkMV_MD	JQ609096	0.001
<i>Pokeweed mosaic virus</i>	PkMV_MS-FR03	JX291161	0.001
	PkMV_PA	JQ609095	0.001
<i>Papaya leaf-distortion mosaic virus</i>	PLDMV	NC_005028	0.001
	PLDMV_HaiNan-DF	JX974555	0.001
	PLDMV_P	AB088221	0.001
	PLDMV_Taiwan-CZ	JX416282	0.001
<i>Plum pox virus</i>	PPV_48-922	AY912058	0.001
	PPV_EI Amar	AM157175	0.001
	PPV_Fantasia	AY912056	0.001
	PPV_J4c	EU117116	0.001
	PPV_Penn12	EF640939	0.001
	PPV_Penn5	EF640933	0.001
	PPV_PS	AJ243957	0.001
	PPV_Vulcan	AY912057	0.001
	PRSV_E2	KC345609	0.001
	PRSV_HNVb	KF791028	0.001
<i>Papaya ringspot virus</i>	PRSV_P-5-19	EU882728	0.001
	PRSV_R3	KJ746106	0.001
	PRSV_W	AY010722	0.001
	PRSV_W-1	DQ374153	0.001
	PSbMV_DPD1	NC_001671	0.003
	PSbMV_L1	AJ252242	0.001
	PSbMV_NY	X89997	0.001
	PStV_Blotch	U05771	0.001
<i>Peanut stripe virus</i>	PStV_SN-Nib3	AF200624	0.001
	PToMV_Cuzqueno2	EU495235	0.001
<i>Peru tomato mosaic virus</i>	PToMV_PPK11	AJ516010	0.001
	PToMV_PPK13	NC_004573	0.001
	PVA_143-PVA	GU144321	0.001
<i>Potato virus A</i>	PVA_B11	NC_004039	0.001
	PVMV_ns1	FJ617225	0.001
<i>Pepper veinal mottle virus</i>	PVMV_P	AB126178	0.001
	PVV_DV-42	AJ243766	0.001
<i>Potato V virus</i>	PVV_KER.LAL.P	KC433411	0.001
	PVY_Adgen	AJ890348	0.001
<i>Potato virus Y</i>	PVY_Chile3	FJ214726	0.001
	PVY_JVW-186	KF770835	0.001
	PVY_LW	AJ890349	0.001
	PVY_HR1	FJ204166	0.001
	PVY_Wilga MV99	HE608963	0.001
	PWV_Gld-1	AB761400	0.001
<i>Passion fruit woodiness virus</i>	PWV-MU	HQ122652	0.001

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Table A1.3. Continued.

Virus name	Potyvirus isolate	GenBank Acc. number	P value*
<i>Sugarcane mosaic virus</i>	SCMV	DQ141605	0.001
	SCMV_Ohio	JX188385	0.001
	SCMV_Seehausen	JX185303	0.001
<i>Soybean mosaic virus</i>	SMV_G7A	FJ640982	0.001
	SMV_P	AJ507388	0.001
<i>Sweet potato feathery mottle virus</i>	SPFMV_O-Arg	KF386013	0.001
	SPFMV_RC-Arg	KF386014	0.001
	SPFMV_S	NC_001841	0.001
<i>Sweet potato virus C</i>	SPVC_Arg	KF386015	0.001
	SPVC_C1	GU207957	0.001
	SPVC_IL	JX489166	0.001
<i>Sweet potato virus G</i>	SPVG_GWB-G	JN613805	0.001
	SPVG_HG167	KM014814	0.001
	SPVG_IS103	KM014815	0.001
	SPVG_Jesus Maria	NC_018093	0.001
	SPVG_WT325	KF790759	0.001
	SPVG_Z01001	JN613806	0.001
<i>Sorghum mosaic virus</i>	SrMV_GX	KJ541740	0.001
	SrMV_H	U57358	0.001
	SrMV_Xiaoshan	NC_004035	0.001
	SrMV_Yuhang	AJ310198	0.001
<i>Sunflower chlorotic mottle virus</i>	SuCMoV_Chlorotic ringspots	GU181200	0.001
	SuCMoV_Common	GU181199	0.001
<i>Shallot yellow stripe virus</i>	SYSV_onion	AM267479	0.001
	SYSV_ZQ1	AJ865077	0.001
	SYSV_ZQ2	AJ865076	0.001
<i>Telosma mosaic virus</i>	TeMV_GX1	KJ789129	0.001
	TeMV_Hanoi	DQ851493	0.001
<i>Tobacco etch virus</i>	TEV	M15239	0.001
	TEV_HAT-AU	KM282187	0.001
	TEV_Mex21	KM282188	0.002
	TEV_N	KM282189	0.001
	TEV_TEV7DA	DQ986288	0.001
<i>Thunberg fritillary mosaic virus</i>	TFMV_Ningbo	HM584812	0.004
	TFMV_Panan-2	AJ885005	0.001
<i>Tomato necrotic stunt virus</i>	ToNSTV_MX5	JX846918	0.001
	ToNSTV_MX9354	JQ314463	0.001
<i>Turnip mosaic virus</i>	TuMV_UK1	AB194787	0.001
	TuMV_C1	AF394601	0.001
	TuMV_CAR37	DQ648592	0.001
	TuMV_CAR39	EF374098	0.001
	TuMV_CAR51	HQ637383	0.001
	TuMV_TW	AF394602	0.001
	TVBMV_HN39	EU734432	0.001
<i>Tobacco vein banding mosaic virus</i>	TVBMV_JX	JN630471	0.001
	TVBMV_LW	JN630473	0.001
	TVBMV_PY	JN630469	0.001
	TVBMV_SDYS1	HQ396791	0.001
	TVBMV_YN9.1	KF444434	0.001
	TVBMV_YND	EF219408	0.001
	TVBMV_YY	JN630472	0.001
<i>Tobacco vein mottling virus</i>	TVMV	X04083	0.001
	TVMV_S	U38621	0.001
<i>Verbena virus Y</i>	VVY_Michigan	EU564817	0.001
<i>Watermelon mosaic virus</i>	WMV_A08-160	JF273465	0.001
	WMV_C05-463	JF273458	0.001
	WMV_C06-257	JF273463	0.001
	WMV_C07-349	JF273461	0.001
	WMV_Cg09-640	JF273467	0.001
	WMV_Fr	AY437609	0.001
<i>Wild potato mosaic virus</i>	WPMV	AJ437279	0.001
<i>Wild tomato mosaic virus</i>	WTMV_Laichau	DQ851495	0.001
	WTMV_XC-1	KM114869	0.001
<i>Wisteria vein mosaic virus</i>	WVMV_Beijing	AY656816	0.001

*A total of 5 (2%) non-significant and 266 (98%) significant associations between phylogenies of the NIb and the CP sequences of potyvirus isolates were retrieved in the cophylogenetic analysis using a significance threshold of $P = 0.05$ (CopyCat v.2.03; [Meier-Kolthoff et al., 2007](#)).

Table A1.3. Continued.

Virus name	Potyvirus isolate	GenBank Acc. number	P value*
<i>Yam bean mosaic virus</i>	YBMV_SR	JN190431	0.001
<i>Yam mild mosaic virus</i>	YMMV_Brazil	JX470965	0.001
	YMMV_GH6_NC_1	KJ125473	0.001
	YMMV_SSY_FX_1	KJ125474	0.001
	YMMV_TG_NH_1	KJ125475	0.001
	YMMV_YB3_NC_1	KJ125476	0.001
	YMMV_ZY_XZ_1	KJ125479	0.001
<i>Yam mosaic virus</i>	YMV_Ivory Coast	NC_004752	0.003
<i>Zantedeschia mild mosaic virus</i>	ZaMMV_TW	AY626825	0.001
<i>Zucchini tigré mosaic virus</i>	ZTMV_E11045	KC345608	0.001
	ZTMV_Q10	KC345605	0.001
	ZTMV_Re01-25	KC345607	0.001
	ZTMV_VET-026	KC345606	0.001
<i>Zucchini yellow mosaic virus</i>	ZYMV_B	AY188994	0.001
	ZYMV_KR-PA	AY278998	0.001
	ZYMV_KR-PE	AY278999	0.001
	ZYMV_KR-PS	AY279000	0.001
	ZYMV_TW-TN3	AF127929	0.001

*A total of 5 (2%) non-significant and 266 (98%) significant associations between phylogenies of the NIB and the CP sequences of potyvirus isolates were retrieved in the cophylogenetic analysis using a significance threshold of $P = 0.05$ (CopyCat v.2.03; [Meier-Kolthoff et al., 2007](#)).

Table A1.4. List of potyvirus isolates obtained in this thesis (2013-2016).

Isolate name	Acc N°	Virus ¹	Sequenced	Location ²	Host	Year
CIYVV-ESC091_Tpr_PRI_2014	Unpublished	CIYVV	350-nt Nlb/CP	ESC	<i>Trifolium pratense</i>	2014
CIYVV-ESC103_Tca_PRI_2015	Unpublished	CIYVV	350-nt Nlb/CP	ESC	<i>Trifolium campestre</i>	2014
CIYVV-ESC131_Tpr_PRI_2015	Unpublished	CIYVV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2014
CIYVV-PAL014_Tpr_PRI_2015	Unpublished	CIYVV	350-nt Nlb/CP	PAL	<i>Trifolium pratense</i>	2015
CIYVV-PAL015_Tpr_PRI_2015	Unpublished	CIYVV	350-nt Nlb/CP	PAL	<i>Trifolium pratense</i>	2015
CIYVV-PAL022_Tpr_PRI_2015	Unpublished	CIYVV	350-nt Nlb/CP	PAL	<i>Trifolium pratense</i>	2015
CIYVV-PAL027_Tca_OTO_2013	Unpublished	CIYVV	350-nt Nlb/CP	PAL	<i>Trifolium campestre</i>	2013
CIYVV-PAL030_Rpu_OTO_2013	Unpublished	CIYVV	350-nt Nlb/CP	PAL	<i>Rumex pulcher</i>	2013
CIYVV-PAL033_Tpr_VER_2013	Unpublished	CIYVV	350-nt Nlb/CP	PAL	<i>Trifolium pratense</i>	2013
CIYVV-PAL040_Tca_PRI_2015	Unpublished	CIYVV	350-nt Nlb	PAL	<i>Trifolium campestre</i>	2015
CIYVV-PAL046_Tca_OTO_2013	Unpublished	CIYVV	350-nt Nlb/CP	PAL	<i>Trifolium campestre</i>	2013
CIYVV-PAL061_Tpr_OTO_2013	Unpublished	CIYVV	350-nt Nlb/CP	PAL	<i>Trifolium pratense</i>	2013
CIYVV-PAL078_Rpu_OTO_2013	Unpublished	CIYVV	350-nt Nlb/CP	PAL	<i>Rumex pulcher</i>	2013
CIYVV-PAL092_Tpr_PRI_2015	Unpublished	CIYVV	350-nt Nlb/CP	PAL	<i>Trifolium pratense</i>	2015
ENMV-SCR099_Ara_PRI_2015	Unpublished	ENMV	350-nt Nlb	SCR	<i>Andryala ragusina</i>	2015
ENMV-SCR177_Ara_VER_2013	Unpublished	ENMV	350-nt Nlb	SCR	<i>Andryala ragusina</i>	2013
MeRV-PAR003_Rmo_PRI_2015	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2015
MeRV-PAR005_Rmo_VER_2013	MF953320	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR005_Rmo_VER_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR006_Rmo_OTO_2013	MF953306	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR006_Rmo_OTO_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR006_Rmo_PRI_2014	MF953325	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR006_Rmo_PRI_2014	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2014
MeRV-PAR007_Rmo_VER_2013	MF953319	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR007_Rmo_VER_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR008_Rmo_OTO_2015	MF953350	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR008_Rmo_PRI_2015	MF953343	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR008_Rmo_PRI_2015	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2015
MeRV-PAR009_Rmo_OTO_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR009_Rmo_OTO_2015	MF953355	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR009_Rmo_PRI_2014	MF953323	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR011_Rmo_OTO_2015	MF953351	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR012_Rmo_VER_2014	MF953331	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR013_Rmo_PRI_2015	MF953342	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR013_Rmo_PRI_2015	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2015
MeRV-PAR014_Rmo_OTO_2015	MF953356	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR017_Rmo_PRI_2015	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2015
MeRV-PAR024_Rmo_PRI_2015	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2015
MeRV-PAR027_Rmo_PRI_2015	MF953345	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR027_Rmo_PRI_2015	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2015
MeRV-PAR027_Rmo_VER_2013	MF953316	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR027_Rmo_VER_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR033_Rmo_PRI_2016	MF953360	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR033_Rmo_VER_2013	MF953314	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR034_Rmo_VER_2014	MF953332	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR037_Rmo_PRI_2014	MF953324	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR037_Rmo_PRI_2014	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2014
MeRV-PAR037_Rmo_VER_2014	MF953333	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR040_Rmo_OTO_2015	MF953352	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR041_Rmo_PRI_2015	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2015
MeRV-PAR042_Rmo_OTO_2015	MF953353	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR043_Rmo_OTO_2013	MF953307	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR043_Rmo_OTO_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR046_Rmo_VER_2013	MF953318	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR046_Rmo_VER_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR047_Rmo_OTO_2015	MF953354	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR060_Rmo_PRI_2016	MF953361	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR060_Rmo_VER_2014	MF953334	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR061_Rmo_PRI_2016	MF953362	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR061_Rmo_VER_2015	MF953347	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR063_Rmo_PRI_2014	MF953322	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR063_Rmo_PRI_2014	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2014
MeRV-PAR063_Rmo_PRI_2016	MF953363	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR064_Rmo_OTO_2013	MF953308	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR064_Rmo_OTO_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR065_Rmo_OTO_2013	MF953309	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR065_Rmo_OTO_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR065_Rmo_PRI_2016	MF953364	MeRV	CP	PAR	<i>Ruta montana</i>	2016

¹Virus name: CIYVV: *Clover yellow vein virus*; ENMV: *Endive necrotic mosaic virus*; MeRV: *Mediterranean ruda virus*; WHMV: *Wild hop mosaic virus*

²Locations are designed with a three-letter code: ESC, El Escorial; MAR: Marjaliza; PAL: Palazuelos; PAR: El Pardo; POL, Pollos; SCR, Santa Cruz de Retamar.

Table A1.4. Continued.

Isolate name	Acc N°	Virus ¹	Sequenced	Location ²	Host	Year
MeRV-PAR066_Rmo_OTO_2013	MF953310	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR066_Rmo_OTO_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR066_Rmo_OTO_2015	MF953357	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR067_Rmo_OTO_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR068_Rmo_OTO_2015	MF953358	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR068_Rmo_VER_2015	MF953349	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR069_Rmo_OTO_2013	MF953311	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR069_Rmo_OTO_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR076_Rmo_PRI_2016	MF953365	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR081_Rmo_VER_2014	MF953327	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR084_Rmo_PRI_2016	MF953366	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR087_Rmo_OTO_2015	MF953359	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR088_Rmo_VER_2013	MF953317	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR088_Rmo_VER_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR089_Rmo_OTO_2013	MF953312	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR089_Rmo_OTO_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR089_Rmo_PRI_2016	MF953367	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR089_Rmo_VER_2015	MF953346	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR090_Rmo_PRI_2014	MF953321	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR090_Rmo_PRI_2014	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2014
MeRV-PAR096_Rmo_PRI_2016	MF953368	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR097_Rmo_PRI_2016	MF953369	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR111_Rmo_PRI_2016	MF953370	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR113_Rmo_OTO_2014	MF953335	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR114_Rmo_OTO_2013	MF953313	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR114_Rmo_OTO_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-PAR115_Rmo_OTO_2014	MF953336	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR116_Rmo_OTO_2014	MF953337	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR118_Rmo_VER_2014	MF953326	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR119_Rmo_OTO_2014	MF953338	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR120_Rmo_OTO_2014	MF953339	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR122_Rmo_PRI_2016	MF953371	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR124_Rmo_PRI_2016	MF953372	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR125_Rmo_PRI_2016	MF953373	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR130_Rmo_VER_2015	MF953348	MeRV	CP	PAR	<i>Ruta montana</i>	2015
MeRV-PAR135_Rmo_VER_2014	MF953328	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR136_Rmo_VER_2014	MF953330	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR137_Rmo_VER_2014	MF953329	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR140_Rmo_OTO_2014	MF953340	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR142_Rmo_OTO_2014	MF953341	MeRV	CP	PAR	<i>Ruta montana</i>	2014
MeRV-PAR166_Rmo_PRI_2016	MF953374	MeRV	CP	PAR	<i>Ruta montana</i>	2016
MeRV-PAR183_Rmo_VER_2013	MF953315	MeRV	CP	PAR	<i>Ruta montana</i>	2013
MeRV-PAR183_Rmo_VER_2013	Unpublished	MeRV	350-nt Nlb	PAR	<i>Ruta montana</i>	2013
MeRV-ParP17	MF953344	MeRV	Full genome	PAR	<i>Ruta montana</i>	2015
WHMV-ESC004_Tpr_PRI_2016	Unpublished	WHMV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2016
WHMV-ESC031_Tpr_PRI_2016	Unpublished	WHMV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2016
WHMV-ESC037_Tpr_PRI_2015	Unpublished	WHMV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2015
WHMV-ESC042_Tpr_PRI_2015	Unpublished	WHMV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2015
WHMV-ESC057_Tpr_PRI_2015	Unpublished	WHMV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2015
WHMV-ESC081_Tpr_OTO_2014	Unpublished	WHMV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2014
WHMV-ESC094_Tpr_VER_2015	Unpublished	WHMV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2015
WHMV-ESC101_Tpr_PRI_2015	Unpublished	WHMV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2015
WHMV-ESC107_Tpr_PRI_2015	Unpublished	WHMV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2015
WHMV-ESC129_Tpr_PRI_2016	Unpublished	WHMV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2016
WHMV-ESC127_Tpr_OTO_2014	Unpublished	WHMV	350-nt Nlb	ESC	<i>Trifolium pratense</i>	2014
WHMV-MAR043_Cal_PRI_2014	Unpublished	WHMV	350-nt Nlb	MAR	<i>Cistus albidus</i>	2014
WHMV-PAL025_Tpr_PRI_2015	Unpublished	WHMV	350-nt Nlb	PAL	<i>Trifolium pratense</i>	2015
WHMV-PAL039_Tca_PRI_2015	Unpublished	WHMV	350-nt Nlb	PAL	<i>Trifolium campestre</i>	2015
WHMV-PAL040_Tca_PRI_2015	Unpublished	WHMV	350-nt Nlb	PAL	<i>Trifolium campestre</i>	2015
WHMV-PAL057_Tca_PRI_2015	Unpublished	WHMV	350-nt Nlb	PAL	<i>Trifolium campestre</i>	2015
WHMV-PAL059_Tpr_PRI_2015	Unpublished	WHMV	350-nt Nlb	PAL	<i>Trifolium pratense</i>	2015
WHMV-POL002_Hlu_PRI_2015	Unpublished	WHMV	350-nt Nlb/CP	POL	<i>Humulus lupulus</i>	2015
WHMV-POL004_Hlu_PRI_2015	Unpublished	WHMV	350-nt Nlb	POL	<i>Humulus lupulus</i>	2015
WHMV-POL005_Hlu_PRI_2015	Unpublished	WHMV	350-nt Nlb	POL	<i>Humulus lupulus</i>	2015
WHMV-POL008_Hlu_PRI_2015	Unpublished	WHMV	350-nt Nlb/CP	POL	<i>Humulus lupulus</i>	2015
WHMV-POL009_Eel_PRI_2014	Unpublished	WHMV	350-nt Nlb	POL	<i>Ecballium elaterium</i>	2014
WHMV-POL009_Hlu_VER_2014	Unpublished	WHMV	350-nt Nlb	POL	<i>Humulus lupulus</i>	2014

¹Virus name: CIYVV: *Clover yellow vein virus*; ENMV: *Endive necrotic mosaic virus*; MeRV: *Mediterranean ruda virus*; WHMV: *Wild hop mosaic virus*

²Locations are designed with a three-letter code: ESC, El Escorial; MAR: Marjaliza; PAL: Palazuelos; PAR: El Pardo; POL, Pollos; SCR, Santa Cruz de Retamar.

Table A1.4. Continued.

Isolate name	Acc N°	Virus ¹	Sequenced	Location ²	Host	Year
WHMV-POL014_Hlu_PRI_2016	Unpublished	WHMV	350-nt Nlb	POL	<i>Humulus lupulus</i>	2016
WHMV-POL016_Hlu_VER_2013	Unpublished	WHMV	350-nt Nlb	POL	<i>Humulus lupulus</i>	2013
WHMV-POL019_Hlu_PRI_2015	Unpublished	WHMV	350-nt Nlb/CP	POL	<i>Humulus lupulus</i>	2015
WHMV-POL020_Hlu_PRI_2015	Unpublished	WHMV	350-nt Nlb	POL	<i>Humulus lupulus</i>	2015
WHMV-POL022_Eel_VER_2015	Unpublished	WHMV	350-nt Nlb	POL	<i>Ecballium elaterium</i>	2015
WHMV-POL024_Hlu_PRI_2016	Unpublished	WHMV	350-nt Nlb	POL	<i>Humulus lupulus</i>	2016
WHMV-POL042_Hlu_VER_2013	Unpublished	WHMV	350-nt Nlb	POL	<i>Humulus lupulus</i>	2013
WHMV-POL090_Eel_PRI_2014	Unpublished	WHMV	350-nt Nlb	POL	<i>Ecballium elaterium</i>	2014
WHMV-POL118_Hlu_VER_2013	Unpublished	WHMV	350-nt Nlb	POL	<i>Humulus lupulus</i>	2013
WHMV-POL192_Hlu_VER_2013	Unpublished	WHMV	350-nt Nlb	POL	<i>Humulus lupulus</i>	2013
WHMV-POLP93_Hlu_PRI_2015	Unpublished	WHMV	350-nt Nlb	POL	<i>Humulus lupulus</i>	2015
WHMV-SCR018_Hlu_OTO_2015	Unpublished	WHMV	350-nt Nlb	SCR	<i>Humulus lupulus</i>	2015
WHMV-SCR077_Hlu_PRI_2014	Unpublished	WHMV	350-nt Nlb/CP	SCR	<i>Humulus lupulus</i>	2014
WHMV-SCR077_Hlu_PRI_2016	Unpublished	WHMV	350-nt Nlb	SCR	<i>Humulus lupulus</i>	2016
WHMV-SCR101_Hlu_PRI_2014	Unpublished	WHMV	350-nt Nlb	SCR	<i>Humulus lupulus</i>	2014
WHMV-SCR152_Hlu_PRI_2015	Unpublished	WHMV	350-nt Nlb	SCR	<i>Humulus lupulus</i>	2015
WHMV-SCR98_Sat_VER_2013	Unpublished	WHMV	350-nt Nlb	SCR	<i>Salix atrocinerea</i>	2013

¹Virus name: CIYVV: *Clover yellow vein virus*; ENMV: *Endive necrotic mosaic virus*; MeRV: *Mediterranean ruda virus*; WHMV: *Wild hop mosaic virus*

²Locations are designed with a three-letter code: ESC, El Escorial; MAR: Marjaliza; PAL: Palazuelos; PAR: El Pardo; POL, Pollos; SCR, Santa Cruz de Retamar.

Table A1.5. Species of the genus *Potyvirus* compiled from GenBank used in the phylogenetic analyses.

GenBank Acc. number	Virus name
NC_010736	<i>Algerian watermelon mosaic virus</i>
NC_014905	<i>Apium virus Y</i>
NC_018176	<i>Arracacha mottle virus</i>
NC_025821	<i>Asparagus virus 1</i>
NC_009745	<i>Banana bract mosaic virus</i>
NC_009741	<i>Basella rugose mosaic virus</i>
NC_004047	<i>Bean common mosaic necrosis virus</i>
NC_003397	<i>Bean common mosaic virus</i>
NC_003492	<i>Bean yellow mosaic virus</i>
NC_005304	<i>Beet mosaic virus</i>
NC_023014	<i>Bidens mosaic virus</i>
NC_014325	<i>Bidens mottle virus</i>
NC_019415	<i>Blue squill virus A</i>
NC_030847	<i>Brazilian weed virus Y</i>
NC_014536	<i>Brugmansia suaveolens mottle virus</i>
NC_021196	<i>Calla lily latent virus</i>
NC_030794	<i>Callistephus mottle virus</i>
NC_013261	<i>Canna Yellow Streak Virus</i>
NC_025254	<i>Carrot thin leaf virus</i>
NC_027210	<i>Catharanthus mosaic virus</i>
NC_015393	<i>Celery mosaic virus</i>
NC_016044	<i>Chilli ringspot virus</i>
NC_005778	<i>Chilli veinal mottle virus</i>
NC_003536	<i>Clover yellow vein virus</i>
NC_003742	<i>Cocksfoot streak virus</i>
NC_020072	<i>Colombian datura virus</i>
NC_004013	<i>Cowpea aphid-borne mosaic virus</i>
NC_008028	<i>Daphne mosaic virus</i>
NC_003537	<i>Dasheen mosaic virus</i>
NC_021197	<i>Donkey orchid virus A</i>
NC_007728	<i>East Asian Passiflora virus</i>
NC_034273	<i>Endive necrotic mosaic virus</i>
NC_031339	<i>Euphorbia ringspot virus</i>
NC_014064	<i>Freesia mosaic virus</i>
NC_010954	<i>Fritillary virus Y</i>
NC_021786	<i>Habenaria mosaic virus</i>
NC_015394	<i>Hardenbergia mosaic virus</i>
NC_017967	<i>Hippeastrum mosaic virus</i>
NC_030236	<i>Impatiens flower break potyvirus</i>
NC_018833	<i>Iranian johnsongrass mosaic virus</i>
NC_029076	<i>Iris severe mosaic virus</i>
NC_000947	<i>Japanese yam mosaic virus</i>
NC_029051	<i>Jasmine ringspot virus</i>
NC_003606	<i>Johnsongrass mosaic virus</i>
NC_007913	<i>Konjac mosaic virus</i>
NC_004011	<i>Leek yellow stripe virus</i>
NC_003605	<i>Lettuce mosaic virus</i>
NC_005288	<i>Lily mottle virus</i>
NC_014898	<i>Lupine mosaic virus</i>
NC_003377	<i>Maize dwarf mosaic virus</i>
NC_009995	<i>Moroccan watermelon mosaic virus</i>
NC_008824	<i>Narcissus degeneration virus</i>
NC_023628	<i>Narcissus late season yellows virus</i>
NC_011541	<i>Narcissus yellow stripe virus</i>
NC_005029	<i>Onion yellow dwarf virus</i>
NC_019409	<i>Ornithogalum mosaic virus</i>
NC_014252	<i>Panax virus Y</i>
NC_005028	<i>Papaya leaf-distortion mosaic potyvirus</i>
NC_001785	<i>Papaya ringspot virus</i>
NC_014790	<i>Passion fruit woodiness virus</i>

Table A1.5. Continued.

GenBank Acc. number	Virus name
NC_001671	<i>Pea seed-borne mosaic virus</i>
NC_002600	<i>Peanut mottle virus</i>
NC_007147	<i>Pennisetum mosaic virus</i>
NC_001517	<i>Pepper mottle virus</i>
NC_008393	<i>Pepper severe mosaic virus</i>
NC_011918	<i>Pepper veinal mottle virus</i>
NC_014327	<i>Pepper yellow mosaic virus</i>
NC_004573	<i>Peru tomato mosaic virus</i>
NC_001445	<i>Plum pox virus</i>
NC_018872	<i>Pokeweed mosaic virus</i>
NC_004039	<i>Potato virus A</i>
NC_004010	<i>Potato virus V</i>
NC_001616	<i>Potato virus Y</i>
NC_003399	<i>Scallion mosaic virus</i>
NC_007433	<i>Shallot yellow stripe virus</i>
NC_004035	<i>Sorghum mosaic virus</i>
NC_002634	<i>Soybean mosaic virus</i>
NC_003398	<i>Sugarcane mosaic virus</i>
NC_014038	<i>Sunflower chlorotic mottle virus</i>
NC_034208	<i>Sunflower ring blotch virus</i>
NC_001841	<i>Sweet potato feathery mottle virus</i>
NC_020896	<i>Sweet potato latent virus</i>
NC_017970	<i>Sweet potato virus 2</i>
NC_014742	<i>Sweet potato virus C</i>
NC_018093	<i>Sweet potato virus G</i>
NC_026615	<i>Tamarillo leaf malformation virus</i>
NC_009742	<i>Telosma mosaic virus</i>
NC_007180	<i>Thunberg fritillary virus</i>
NC_001555	<i>Tobacco etch virus</i>
NC_009994	<i>Tobacco vein banding mosaic virus</i>
NC_001768	<i>Tobacco vein mottling virus</i>
NC_017824	<i>Tomato necrotic stunt virus</i>
NC_002509	<i>Turnip mosaic virus</i>
NC_017977	<i>Vallota speciosa virus</i>
NC_025250	<i>Vanilla distortion mosaic virus</i>
NC_010735	<i>Verbena virus Y</i>
NC_006262	<i>Watermelon mosaic virus</i>
NC_004426	<i>Wild potato mosaic virus</i>
NC_009744	<i>Wild tomato mosaic virus</i>
NC_007216	<i>Wisteria vein mosaic virus</i>
NC_016441	<i>Yam bean mosaic virus</i>
NC_019412	<i>Yam mild mosaic virus</i>
NC_004752	<i>Yam mosaic virus</i>
NC_011560	<i>Zantedeschia mild mosaic virus</i>
NC_023175	<i>Zucchini tigre mosaic virus</i>
NC_003224	<i>Zucchini yellow mosaic virus</i>

APPENDIX 2

Characterization of evergreen oak forest and riparian forests in the Iberian Peninsula

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Figure A2.1. Observed (S_{obs} , black) and expected (S_{est} , Chao 1 estimator, red) accumulation curves of plant species richness in the studied locations of evergreen oak forest for the monitored seasons during the 2014-2015 field samplings. The x-axis is the number of individuals sampled at each location (in this thesis, $N=200$) and the y-axis is the number of plant species collected according to sample size. Plotted values are means of 100 randomizations.

Table A2.1. Plant biodiversity in the studied evergreen oak forest locations during 2014-2015 field samplings. Observed and expected plant species richness, and Shannon's diversity and equitability indexes are shown.

Location	Season	S_{obs} (95% CI)*	S_{est} (95% CI)*	H'	E_H
Carbonero el Mayor	Summer 2014	16 (15.21–16.79)	16.00 (16.71–17.23)	2.12 ± 0.00	0.76
	Autumn 2014	18 (15.70–20.30)	18.75 (18.07–26.42)	2.05 ± 0.00	0.71
	Spring 2015	17 (11.75–22.25)	21.98 (17.77–48.99)	2.07 ± 0.00	0.72
Cenicientos	Summer 2014	22 (19.96–24.04)	22.75 (22.08–29.20)	2.37 ± 0.00	0.77
	Autumn 2014	27 (21.59–32.41)	33.97 (28.44–60.60)	2.53 ± 0.00	0.77
	Spring 2015	22 (18.82–25.18)	24.99 (22.35–47.61)	2.72 ± 0.00	0.88
El Pardo	Summer 2014	25 (21.30–28.70)	27.49 (25.37–41.91)	2.89 ± 0.00	0.90
	Autumn 2014	18 (13.79–22.21)	21.32 (18.50–39.97)	2.25 ± 0.00	0.78
	Spring 2015	27 (22.10–31.90)	32.22 (27.99–54.48)	2.74 ± 0.00	0.83
Marjaliza	Summer 2014	18 (12.68–23.32)	22.98 (18.77–49.99)	2.26 ± 0.00	0.78
	Autumn 2014	23 (17.29–28.71)	31.96 (24.99–63.27)	2.42 ± 0.00	0.77
	Spring 2015	24 (21.97–26.03)	24.60 (24.05–31.08)	2.71 ± 0.00	0.85
Montegancedo	Summer 2014	17 (13.31–20.69)	18.49 (17.15–32.01)	2.41 ± 0.00	0.85
	Autumn 2014	15 (11.36–18.64)	16.49 (15.15–30.01)	2.27 ± 0.00	0.84
	Spring 2015	26 (22.36–29.64)	27.99 (26.25–41.94)	2.95 ± 0.00	0.90

S_{obs} : observed plant species richness. S_{est} : expected plant species richness based on the Chao 1 estimator. H' : Shannon's diversity index. E_H : Shannon's equitability index.

*values are mean and 95% confidence intervals based in 100 randomizations from pooled location-specific individuals ($N=200$).

Figure A2.2. Observed (S_{obs} , black) and expected (S_{est} , Chao 1 estimator, red) accumulation curves of plant species richness in the studied locations of riparian forest for the monitored seasons during the 2014-2015 field samplings. The x-axis is the number of individuals sampled at each location (in this thesis, $N=200$) and the y-axis is the number of plant species collected according to sample size. Plotted values are means of 100 randomizations.

Table A2.2. Plant biodiversity in the studied riparian forest locations during 2014-2015 field samplings. Observed and expected plant species richness, and Shannon's diversity and equitability indexes are shown.

Location	Season	S_{obs} (95% CI)*	S_{est} (95% CI)*	H'	E_H
El Escorial	Summer 2014	31 (29.33–32.67)	31.43 (31.03–36.55)	3.16 ± 0.00	0.92
	Autumn 2014	26 (20.31–31.69)	30.98 (26.77–57.99)	2.93 ± 0.00	0.90
	Spring 2015	32 (26.18–37.82)	38.97 (33.33–68.37)	3.13 ± 0.00	0.90
Rascafría	Summer 2014	38 (31.34–44.66)	49.19 (40.64–85.50)	3.30 ± 0.00	0.91
	Autumn 2014	32 (29.02–34.98)	33.66 (32.23–43.93)	3.12 ± 0.00	0.90
	Spring 2015	36 (31.71–40.29)	40.48 (37.03–55.53)	3.01 ± 0.00	0.84
Palazuelos	Summer 2014	23 (21.77–24.23)	23.17 (23.01–26.58)	2.76 ± 0.00	0.88
	Autumn 2014	30 (25.84–34.16)	33.98 (30.80–49.72)	2.83 ± 0.00	0.83
	Spring 2015	21 (16.77–25.23)	24.73 (21.63–42.92)	2.50 ± 0.00	0.82
Pollos	Summer 2014	35 (32.78–37.22)	35.85 (35.09–42.94)	3.26 ± 0.00	0.92
	Autumn 2014	31 (26.83–35.17)	34.98 (31.80–50.72)	2.98 ± 0.00	0.87
	Spring 2015	32 (27.57–36.43)	36.18 (32.79–54.24)	3.14 ± 0.00	0.90
Santa Cruz de Retamar	Summer 2014	34 (29.65–38.35)	38.48 (34.97–54.61)	2.80 ± 0.00	0.79
	Autumn 2014	27 (24.60–29.40)	28.00 (27.11–35.94)	2.86 ± 0.00	0.87
	Spring 2015	17 (15.43–18.57)	17.25 (17.01–21.78)	2.24 ± 0.00	0.79

S_{obs} : observed plant species richness. S_{est} : expected plant species richness based on the Chao 1 estimator. H' : Shannon's diversity index. E_H : Shannon's equitability index.

*values are mean and 95% confidence intervals based in 100 randomizations from pooled location-specific individuals ($N=200$).

Table A2.3. Identified plant families and species in evergreen oak forest populations during 2013-2015 field samplings.

Plant Family	Plant species	Life Cycle
<i>Anacardiaceae</i>	<i>Pistacia terebinthus</i>	perennial
<i>Apiaceae</i>	<i>Anthriscus caucalis</i>	annual
	<i>Daucus carota</i>	perennial
	<i>Eryngium campestre</i>	perennial
	<i>Pimpinella villosa</i>	perennial
	<i>Scandix pecten-veneris</i>	annual
	<i>Thapsia villosa</i>	perennial
	<i>Torilis leptophylla</i>	annual
<i>Araliaceae</i>	<i>Hedera helix</i>	perennial
<i>Asparagaceae</i>	<i>Asparagus acutifolius</i>	perennial
<i>Asteraceae</i>	<i>Andryala arenaria</i>	annual
	<i>Andryala integrifolia</i>	perennial
	<i>Andryala ragusina</i>	perennial
	<i>Aster arvensis</i>	annual
	<i>Bellis perennis</i>	perennial
	<i>Bombycilaena discolor</i>	annual
	<i>Carduus tenuiflorus</i>	perennial
	<i>Centaurea melitensis</i>	annual
	<i>Centaurea paniculata</i>	perennial
	<i>Conyza andryala</i>	annual
	<i>Crepis capillaris</i>	annual
	<i>Crepis taraxacifolia</i>	perennial
	<i>Crupina vulgaris</i>	annual
	<i>Filago arvensis</i>	annual
	<i>Helichrysum stoechas</i>	perennial
	<i>Hieracium</i> sp.	perennial
	<i>Lactuca serriola</i>	annual
	<i>Lactuca virosa</i>	annual
	<i>Leontodon longirostris</i>	annual
	<i>Phagnalon saxatile</i>	perennial
	<i>Picris echioides</i>	perennial
	<i>Santolina rosmarinifolia</i>	perennial
	<i>Scolymus hispanicus</i>	perennial
	<i>Senecio jacobaea</i>	perennial
	<i>Sonchus oleraceus</i>	annual
	<i>Taraxacum officinale</i>	perennial
	<i>Tolpis umbellata</i>	annual
<i>Boraginaceae</i>	<i>Anchusa azurea</i>	perennial
	<i>Anchusa undulata</i>	perennial
	<i>Buglossoides arvensis</i>	annual
	<i>Echium plantagineum</i>	annual
	<i>Echium</i> sp.	annual
	<i>Echium vulgare</i>	annual
	<i>Heliotropium europaeum</i>	annual
	<i>Heliotropium europaeum</i>	annual
<i>Brassicaceae</i>	<i>Brassica barrelieri</i>	annual
	<i>Diplotaxis virgata</i>	annual
	<i>Iberis ciliata</i>	perennial
	<i>Malcolmia patula</i>	annual
	<i>Malcolmia triloba</i>	annual
<i>Campanulaceae</i>	<i>Campanula rapunculus</i>	perennial
	<i>Jasione montana</i>	annual
<i>Caryophyllaceae</i>	<i>Arenaria montana</i>	perennial
	<i>Holosteum umbellatum</i>	annual
	<i>Petrorhagia nanteuillii</i>	annual

Table A2.3. Continued.

Plant Family	Plant species	Life Cycle
<i>Caryophyllaceae</i>	<i>Silene colorata</i>	annual
	<i>Silene gallica</i>	annual
	<i>Stellaria media</i>	annual
<i>Cistaceae</i>	<i>Cistus albidus</i>	perennial
	<i>Cistus ladanifer</i>	perennial
	<i>Cistus laurifolius</i>	perennial
	<i>Cistus salviifolius</i>	perennial
	<i>Halimium umbellatum</i>	perennial
	<i>Halimium viscosum</i>	perennial
	<i>Tuberaria guttata</i>	annual
<i>Convolvulaceae</i>	<i>Convolvulus arvensis</i>	perennial
<i>Crassulaceae</i>	<i>Sedum forsterianum</i>	perennial
	<i>Sedum sediforme</i>	perennial
	<i>Umbilicus rupestris</i>	perennial
<i>Cucurbitaceae</i>	<i>Ecballium elaterium</i>	perennial
<i>Cupressaceae</i>	<i>Juniperus oxycedrus</i>	perennial
<i>Dipsacaceae</i>	<i>Pterocephalidum diandra</i>	annual
<i>Fabaceae</i>	<i>Anthyllis cornicina</i>	annual
	<i>Anthyllis lotoides</i>	annual
	<i>Anthyllis vulneraria</i> subsp. <i>gandogeri</i>	annual
	<i>Astragalus hamosus</i>	annual
	<i>Astragalus pelecinus</i>	annual
	<i>Biserrula pelecinus</i>	annual
	<i>Coronilla scorpioides</i>	annual
	<i>Cytisus scoparius</i>	perennial
	<i>Hymenocarpus cornicina</i>	annual
	<i>Hymenocarpus lotoides</i>	annual
	<i>Lathyrus angulatus</i>	annual
	<i>Lathyrus cicera</i>	annual
	<i>Lotus corniculatus</i>	perennial
	<i>Lupinus angustifolius</i>	annual
	<i>Lupinus hispanica</i>	annual
	<i>Lupinus</i> sp.	annual
	<i>Ornithopus compressus</i>	annual
	<i>Retama sphaerocarpa</i>	perennial
	<i>Trifolium angustifolium</i>	annual
	<i>Trifolium arvense</i>	annual
	<i>Trifolium campestre</i>	annual
	<i>Trifolium cherleri</i>	annual
	<i>Trifolium hirtum</i>	annual
	<i>Trifolium scabrum</i>	annual
	<i>Trifolium</i> sp.	annual
	<i>Trifolium stellatum</i>	annual
	<i>Trifolium striatum</i>	annual
	<i>Trigonella monspeliaca</i>	annual
	<i>Vicia disperma</i>	annual
	<i>Vicia lutea</i>	annual
	<i>Vicia villosa</i>	annual
<i>Fagaceae</i>	<i>Quercus coccifera</i>	perennial
	<i>Quercus ilex</i>	perennial
<i>Fumariaceae</i>	<i>Fumaria officinalis</i>	annual
<i>Geraniaceae</i>	<i>Erodium cicutarium</i>	annual
	<i>Geranium dissectum</i>	annual
	<i>Geranium molle</i>	annual
	<i>Geranium robertianum</i>	perennial
	<i>Geranium rotundifolium</i>	perennial

Table A2.3. Continued.

Plant Family	Plant species	Life Cycle
Lamiaceae	<i>Lavandula stoechas</i> subsp. <i>pedunculata</i>	perennial
	<i>Thymus mastichina</i>	perennial
Linaceae	<i>Linum narbonense</i>	perennial
Moraceae	<i>Ficus carica</i>	annual
Oleaceae	<i>Fraxinus angustifolia</i>	perennial
Pinaceae	<i>Pinus pinea</i>	perennial
Plantaginaceae	<i>Plantago bellardii</i>	annual
	<i>Plantago coronopus</i>	perennial
	<i>Plantago lagopus</i>	annual
	<i>Plantago lanceolata</i>	perennial
Poaceae	<i>Anthoxanthum odoratum</i>	annual
	<i>Avena barbata</i>	annual
	<i>Avena sterilis</i>	annual
	<i>Brachypodium sylvaticum</i>	perennial
	<i>Briza maxima</i>	annual
	<i>Bromus diandrus</i>	annual
	<i>Bromus madritensis</i>	perennial
	<i>Bromus mollis</i>	annual
	<i>Bromus rubens</i>	annual
	<i>Bromus tectorum</i>	perennial
	<i>Carex</i> sp.	perennial
	<i>Corynephorus fasciculatus</i>	annual
	<i>Cynodon dactylon</i>	perennial
	<i>Dactylis glomerata</i>	perennial
	<i>Dactylis hispanica</i>	perennial
	<i>Holcus lanatus</i>	perennial
	<i>Melica magnolia</i>	perennial
	<i>Piptatherum miliaceum</i>	perennial
	<i>Poa pratensis</i>	perennial
	<i>Taeniatherum caput-medusae</i>	annual
	<i>Vulpia myuros</i>	annual
Polygonaceae	<i>Rumex acetosa</i>	perennial
	<i>Rumex angiocarpus</i>	perennial
	<i>Rumex bucephalophorus</i>	annual
	<i>Rumex gallicus</i>	annual
Ranunculaceae	<i>Ranunculus bulbosus</i>	perennial
	<i>Ranunculus ollisiponensis</i>	perennial
Rosaceae	<i>Sanguisorba minor</i> subsp. <i>spachiana</i>	perennial
Rubiaceae	<i>Galium aparine</i>	annual
	<i>Sherardia arvensis</i>	annual
Rutaceae	<i>Ruta montana</i>	perennial
Scrophulariaceae	<i>Bellardia trixago</i>	annual
	<i>Linaria sparteae</i>	annual
	<i>Parentucellia latifolia</i>	annual
Thymelaceae	<i>Daphne gnidium</i>	perennial
Violaceae	<i>Viola kitaibeliana</i>	annual

Table A2.4. Identified plant families and species in Riparian forest populations during 2013-2015 field samplings.

Plant Family	Plant species	Life Cycle
<i>Apiaceae</i>	<i>Anthriscus caucalis</i>	annual
	<i>Conopodium subcarneum</i>	perennial
	<i>Daucus carota</i>	perennial
	<i>Eryngium campestre</i>	perennial
	<i>Myrrhoides nodosa</i>	annual
	<i>Peucedanum</i> sp.	perennial
	<i>Physospermum cornubiense</i>	perennial
	<i>Thapsia villosa</i>	perennial
	<i>Torilis arvensis</i>	annual
	<i>Torilis japonica</i>	annual
<i>Apocynaceae</i>	<i>Vinca minor</i>	perennial
	<i>Vincetoxicum nigrum</i>	perennial
<i>Araceae</i>	<i>Arum italicum</i>	perennial
<i>Araliaceae</i>	<i>Hedera helix</i>	perennial
<i>Aristolochiaceae</i>	<i>Aristolochia longa</i>	perennial
	<i>Aristolochia paucinervis</i>	perennial
<i>Asparagaceae</i>	<i>Asparagus acutifolius</i>	perennial
<i>Asteraceae</i>	<i>Achillea filipendulina</i>	perennial
	<i>Andryala arenaria</i>	annual
	<i>Andryala integrifolia</i>	perennial
	<i>Andryala ragusina</i>	perennial
	<i>Artemisia absinthium</i>	perennial
	<i>Aster arvensis</i>	annual
	<i>Bellis perennis</i>	perennial
	<i>Carduus tenuiflorus</i>	perennial
	<i>Centaurea calcitrapa</i>	perennial
	<i>Centaurea melitensis</i>	annual
	<i>Chondrilla juncea</i>	perennial
	<i>Cichorium intybus</i>	perennial
	<i>Conyza bonariensis</i>	annual
	<i>Conyza canadensis</i>	annual
	<i>Filago arvensis</i>	annual
	<i>Helichrysum stoechas</i>	perennial
	<i>Hieracium murorum</i>	perennial
	<i>Hieracium pilosella</i>	perennial
	<i>Hieracium sabaudum</i>	perennial
	<i>Jasonia tuberosa</i>	perennial
	<i>Lactuca serriola</i>	annual
	<i>Lactuca virosa</i>	annual
	<i>Lapsana communis</i>	annual
	<i>Leontodon longirostris</i>	annual
	<i>Senecio jacobaea</i>	perennial
	<i>Tanacetum corymbosum</i>	perennial
	<i>Tanacetum parthenium</i>	perennial
	<i>Tanacetum vulgare</i>	perennial
	<i>Taraxacum officinale</i>	perennial
<i>Boraginaceae</i>	<i>Anchusa azurea</i>	perennial
	<i>Anchusa undulata</i>	perennial
	<i>Buglossoides arvensis</i>	annual
	<i>Cynoglossum officinale</i>	perennial
	<i>Echium vulgare</i>	annual
	<i>Myosotis arvensis</i>	annual
	<i>Pentaglottis sempervirens</i>	perennial
<i>Symphytum tuberosum</i>	<i>Symphytum tuberosum</i>	perennial
<i>Brassicaceae</i>	<i>Alliaria petiolata</i>	perennial

Table A2.4. Continued.

Plant Family	Plant species	Life Cycle
<i>Brassicaceae</i>	<i>Brassica barrelieri</i>	annual
	<i>Capsella bursa-pastoris</i>	annual
	<i>Cardamine hirsuta</i>	annual
	<i>Eruca vesicaria</i>	annual
	<i>Malcolmia littorea</i>	perennial
	<i>Malcolmia patula</i>	annual
	<i>Malcolmia triloba</i>	annual
	<i>Sisymbrium austriacum</i>	perennial
	<i>Sisymbrium irio</i>	annual
<i>Cannabaceae</i>	<i>Humulus lupulus</i>	perennial
<i>Caprifoliaceae</i>	<i>Lonicera hispanica</i>	perennial
	<i>Lonicera periclymenum</i>	perennial
	<i>Lonicera xylosteum</i>	perennial
<i>Caryophyllaceae</i>	<i>Arenaria montana</i>	perennial
	<i>Cerastium arvense</i>	perennial
	<i>Dianthus armeria</i>	annual
	<i>Holosteum umbellatum</i>	annual
	<i>Lychnis flos-cuculi</i>	perennial
	<i>Petrorhagia nanteuillii</i>	annual
	<i>Saponaria officinalis</i>	perennial
	<i>Silene alba</i>	perennial
	<i>Silene mellifera</i>	perennial
	<i>Silene vulgaris</i>	perennial
	<i>Stellaria graminea</i>	perennial
	<i>Stellaria media</i>	annual
<i>Chenopodiaceae</i>	<i>Chenopodium album</i>	annual
	<i>Chenopodium opulifolium</i>	annual
<i>Convolvulaceae</i>	<i>Calystegia sepium</i>	perennial
	<i>Convolvulus arvensis</i>	perennial
<i>Cucurbitaceae</i>	<i>Bryonia dioica</i>	perennial
	<i>Ecballium elaterium</i>	perennial
<i>Cyperaceae</i>	<i>Carex broteriana</i>	perennial
	<i>Carex elata</i> subsp. <i>reuteriana</i>	perennial
	<i>Cyperus longus</i>	perennial
	<i>Scirpus holoschoenus</i>	perennial
<i>Dioscoreaceae</i>	<i>Tamus communis</i>	perennial
<i>Fabaceae</i>	<i>Adenocarpus complicatus</i>	perennial
<i>Fabaceae</i>	<i>Astragalus glycyphyllos</i>	perennial
	<i>Astragalus hamosus</i>	annual
	<i>Cytisus scoparius</i>	perennial
	<i>Genista florida</i>	perennial
	<i>Lathyrus angulatus</i>	annual
	<i>Lathyrus aphaca</i>	annual
	<i>Lathyrus cicera</i>	annual
	<i>Lathyrus pratensis</i>	perennial
	<i>Lotus corniculatus</i>	perennial
	<i>Lupinus angustifolius</i>	annual
	<i>Medicago littoralis</i>	annual
	<i>Medicago minima</i>	annual
	<i>Medicago sativa</i>	perennial
	<i>Melilotus indicus</i>	annual
	<i>Ornithopus compressus</i>	annual
	<i>Retama sphaerocarpa</i>	perennial
	<i>Trifolium arvense</i>	annual
	<i>Trifolium campestre</i>	annual
	<i>Trifolium glomeratum</i>	annual
	<i>Trifolium pratense</i>	perennial
	<i>Trifolium repens</i>	perennial

Table A2.4. Continued.

Plant Family	Plant species	Life Cycle
<i>Fabaceae</i>	<i>Trifolium scabrum</i>	annual
	<i>Vicia disperma</i>	annual
	<i>Vicia hybrida</i>	annual
	<i>Vicia lathyroides</i>	annual
	<i>Vicia orobus</i>	perennial
	<i>Vicia sativa</i>	annual
	<i>Vicia tenuifolia</i>	perennial
	<i>Vicia villosa</i>	annual
<i>Fagaceae</i>	<i>Quercus pyrenaica</i>	perennial
<i>Fumariaceae</i>	<i>Fumaria officinalis</i>	annual
	<i>Fumaria parviflora</i>	annual
<i>Geraniaceae</i>	<i>Erodium cicutarium</i>	annual
	<i>Geranium dissectum</i>	annual
	<i>Geranium molle</i>	annual
	<i>Geranium robertianum</i>	perennial
	<i>Geranium rotundifolium</i>	perennial
<i>Guttiferae</i>	<i>Hypericum perforatum</i>	perennial
<i>Juglandaceae</i>	<i>Juglans regia</i>	perennial
<i>Lamiaceae</i>	<i>Clinopodium vulgare</i>	perennial
	<i>Lamium album</i>	perennial
	<i>Lamium purpureum</i>	annual
	<i>Lycopus europaeus</i>	perennial
	<i>Marrubium vulgare</i>	perennial
	<i>Mentha longifolia</i>	perennial
	<i>Mentha pulegium</i>	perennial
	<i>Mentha rotundifolia</i>	perennial
<i>Lamiaceae</i>	<i>Nepeta caerulea</i>	perennial
	<i>Nepeta cataria</i>	perennial
	<i>Nepeta nepetella</i>	perennial
	<i>Origanum virens</i>	perennial
	<i>Salvia verbenaca</i>	perennial
	<i>Satureja montana</i>	perennial
	<i>Teucrium fruticans</i>	perennial
	<i>Teucrium scorodonia</i>	perennial
<i>Liliaceae</i>	<i>Asphodelus albus</i>	perennial
<i>Lythraceae</i>	<i>Lythrum salicaria</i>	perennial
<i>Malvaceae</i>	<i>Malva sylvestris</i>	perennial
	<i>Malva tournefortiana</i>	perennial
<i>Myrsinaceae</i>	<i>Lysimachia vulgaris</i>	perennial
<i>Oleaceae</i>	<i>Fraxinus angustifolia</i>	perennial
	<i>Ligustrum vulgare</i>	perennial
<i>Onagraceae</i>	<i>Epilobium lanceolatum</i>	perennial
<i>Oxalidaceae</i>	<i>Oxalis acetosella</i>	perennial
<i>Papaveraceae</i>	<i>Papaver rhoeas</i>	annual
<i>Pinaceae</i>	<i>Pinus pinea</i>	perennial
<i>Plantaginaceae</i>	<i>Plantago lanceolata</i>	perennial
	<i>Plantago media</i>	perennial
<i>Poaceae</i>	<i>Anthoxanthum odoratum</i>	annual
	<i>Arrhenatherum elatius</i>	perennial
	<i>Avena barbata</i>	annual
	<i>Brachypodium dystachion</i>	annual
	<i>Brachypodium sylvaticum</i>	perennial
	<i>Bromus diandrus</i>	annual
	<i>Bromus madritensis</i>	perennial
	<i>Catapodium rigidum</i>	annual
	<i>Cynosurus cristatus</i>	perennial
	<i>Cynosurus echinatus</i>	annual
	<i>Dactylis glomerata</i>	perennial

Table A2.4. Continued.

Plant Family	Plant species	Life Cycle
<i>Poaceae</i>	<i>Dactylis hispanica</i>	perennial
	<i>Elymus caninus</i>	perennial
	<i>Festuca arundinaceae</i>	perennial
	<i>Festuca durandoi</i>	perennial
	<i>Hordeum murinum</i>	annual
	<i>Lolium rigidum</i>	annual
	<i>Paspalum dilatatum</i>	perennial
	<i>Phleum pratense</i>	perennial
	<i>Phragmites australis</i>	perennial
	<i>Piptatherum miliaceum</i>	perennial
	<i>Poa pratensis</i>	perennial
	<i>Stipa gigantea</i>	perennial
	<i>Taeniatherum caput-medusae</i>	annual
<i>Polygonaceae</i>	<i>Rumex acetosa</i>	perennial
<i>Polygonaceae</i>	<i>Rumex bucephalophorus</i>	annual
	<i>Rumex conglomeratus</i>	perennial
	<i>Rumex crispus</i>	perennial
	<i>Rumex induratus</i>	perennial
	<i>Rumex pulcher</i>	perennial
<i>Ranunculaceae</i>	<i>Clematis vitalba</i>	perennial
	<i>Nigella gallica</i>	annual
	<i>Nigella nigellastrum</i>	perennial
	<i>Ranunculus bulbosus</i>	perennial
	<i>Ranunculus montserratii</i>	perennial
	<i>Ranunculus ollisiponensis</i>	perennial
	<i>Thalictrum flavum</i>	perennial
<i>Rosaceae</i>	<i>Crataegus monogyna</i>	perennial
	<i>Geum silvaticum</i>	perennial
	<i>Malus</i> sp.	perennial
	<i>Potentilla anserina</i>	perennial
	<i>Potentilla argentea</i>	perennial
	<i>Prunus spinosa</i>	perennial
	<i>Rosa obtusifolia</i>	perennial
	<i>Rubus</i> sp.	perennial
	<i>Rubus ulmifolius</i>	perennial
<i>Rubiaceae</i>	<i>Cruciata glabra</i>	perennial
	<i>Cruciata laevipes</i>	perennial
	<i>Galium aparine</i>	annual
	<i>Galium aparine</i> subsp. <i>spurium</i>	annual
	<i>Galium odoratum</i>	perennial
	<i>Galium parisiense</i>	annual
	<i>Galium rotundifolium</i>	perennial
	<i>Galium scabrum</i>	perennial
	<i>Galium verum</i>	annual
<i>Salicaceae</i>	<i>Populus nigra</i>	perennial
	<i>Populus</i> sp.	perennial
	<i>Salix atrocinerea</i>	perennial
	<i>Salix eleagnos</i>	perennial
	<i>Salix triandra</i>	perennial
<i>Saxifragaceae</i>	<i>Saxifraga granulata</i>	perennial
<i>Scrophulariaceae</i>	<i>Verbascum sinuatum</i>	perennial
	<i>Verbascum</i> sp.	perennial
	<i>Veronica officinalis</i>	perennial
<i>Simaroubaceae</i>	<i>Ailanthus altissima</i>	annual
<i>Solanaceae</i>	<i>Solanum dulcamara</i>	perennial
	<i>Solanum nigrum</i>	annual
<i>Ulmaceae</i>	<i>Ulmus pumila</i>	perennial

Table A2.4. Continued.

Plant Family	Plant species	Life Cycle
<i>Urticaceae</i>	<i>Urtica dioica</i>	perennial
	<i>Urtica urens</i>	annual
<i>Valerianaceae</i>	<i>Valerianella coronata</i>	annual
<i>Violaceae</i>	<i>Viola kitaibeliana</i>	annual
	<i>Viola odorata</i>	perennial
	<i>Viola riviniana</i>	perennial

APPENDIX 3

Analysis of genus-wide potyvirus prevalence in wild ecosystems in the Iberian Peninsula

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Table A3.1. Logistic models predicting potyvirus prevalence closely competing with the best-ranked one ($\Delta i < 2$), and/or the best model with considerable less support ($\Delta i > 2$).

Model structure*	Model fitting		Model performance on 5x2CV training set ³			Model performance on validation set ³		
	AIC ¹	Δi ²	Acc	AUROC	TPR	Acc	AUROC	TPR
Riparian forests								
PSP (98)+ PRA (1)+SEA (1)	1407.29	0.00	92	97	54	83	72	15
PSP (99) + PRA (1)	1428.66	21.37	ND	ND	ND	ND	ND	ND
Evergreen oak forests								
PSP (96)+ RAF (2)+RH _{med} (1)+V (1)	906.96	0.00	95	98	26	91	70	20
PSP (98)+ RAF (1)+RH _{med} (1)	916.00	9.04	ND	ND	ND	ND	ND	ND

*The relative importance (%) of each predictor variable is shown in parenthesis.

¹Akaike's Information Criterion.

² Δi is the difference between the AIC of a given model and that of the best-ranked model, and quantifies how models compete (best-ranked model: $\Delta i = 0$; substantial empirical support: $\Delta i = 1-2$; considerable less support: $\Delta i = 2-7$; and no support; $\Delta i > 10$) (Burnham and Anderson, 2002).

³Model performance measures: rate of correct predictions of the model (Acc: Accuracy), Area under the ROC curve (AUROC), True Positive rate (TPR). Values are shown as percentages. Measures calculated for models with $\Delta i < 2$. ND: Not determined.

PSP: Plant species; PRA: Plant relative abundance; SEA: Season; RAF: Rainfalls; RH_{med}: Average relative humidity; V: Plant biomass.

Table A3.2. Logistic models predicting potyvirus prevalence excluding plant identity closely competing with the best-ranked one ($\Delta i < 2$), and/or the best model with considerable less support ($\Delta i > 2$).

Model structure*	Model fitting		Model performance on 5x2CV training set ³			Model performance on validation set ³		
	AIC ¹	Δi ²	Acc	AUROC	TPR	Acc	AUROC	TPR
Riparian forests								
PRA (46)+PLC (25)+HPS (21)+RAF (8)	914.75	0.00	55	72	5	58	72	3
PRA (48)+PLC (22)+HPS (30)	915.75	1.00	47	71	5	54	66	0
PRA (61)+PLC (39)	932.52	17.77	ND	ND	ND	ND	ND	ND
Evergreen oak forests								
PRA (80)+PLC (20)	516.05	0.00	67	71	0	62	60	0
PRA (100)	518.24	2.19	ND	ND	ND	ND	ND	ND

*The relative importance (%) of each predictor variable is shown in parenthesis.

¹Akaike's Information Criterion.

² Δi is the difference between the AIC of a given model and that of the best-ranked model, and quantifies how models compete (best-ranked model: $\Delta i = 0$; substantial empirical support: $\Delta i = 1-2$; considerable less support: $\Delta i = 2-7$; and no support; $\Delta i > 10$) (Burnham and Anderson, 2002).

³Model performance measures: rate of correct predictions of the model (Acc: Accuracy), Area under the ROC curve (AUROC), True Positive rate (TPR). Values are shown as percentages. Measures calculated for models with $\Delta i < 2$. ND: Not determined.

PRA: Plant relative abundance; PLC: Plant life cycle; HPS: number of host plant species; RAF: Rainfalls.

Table A3.3. Logistic models predicting host potyvirus prevalence closely competing with the best-ranked one ($\Delta i < 2$), and/or the best model with considerable less support ($\Delta i > 2$).

Model structure*	Model fitting		Model performance on 5x2CV training set ³			Model performance on validation set ³		
	AIC ¹	Δi ²	Acc	AUROC	TPR	Acc	AUROC	TPR
<i>Riparian forests</i>								
PRA (67)+RAF (9)+S (7)+SEA (7)+HPD (6)+V(4)	79.71	0.85	75	91	75	71	84	70
PRA (70)+RAF (10)+S (7)+SEA (7)+HPD (6)	82.89	3.18	ND	ND	ND	ND	ND	ND
PRA (77)+S (8)+HPD (7)+SEA (6)	91.75	12.04	ND	ND	ND	ND	ND	ND
PRA (84)+S (9)+HPD (7)	93.25	13.54	ND	ND	ND	ND	ND	ND
PRA (90)+S (10)	95.62	15.91	ND	ND	ND	ND	ND	ND
PARA (100)	98.73	19.02	ND	ND	ND	ND	ND	ND
<i>Evergreen oak forests</i>								
HPS (32)+PRA (26)+HPD (25)+RAF(17)	20.06	0.00	94	99	93	70	83	69
HPS (39)+PRA (31)+HPD (30)	28.76	8.70	ND	ND	ND	ND	ND	ND
PRA (51)+HPD (49)	37.31	17.25	ND	ND	ND	ND	ND	ND
HPD (100)	39.00	18.94	ND	ND	ND	ND	ND	ND

*The relative importance (%) of each predictor variable is shown in parenthesis.

¹Akaike's Information Criterion.

² Δi , is the difference between the AIC of a given model and that of the best-ranked model, and quantifies how models compete (best-ranked model: $\Delta i=0$; substantial empirical support: $\Delta i=1-2$; considerable less support: $\Delta i=2-7$; and no support; $\Delta i>10$) (Burnham and Anderson, 2002).

³Model performance measures: rate of correct predictions of the model (Acc: Accuracy), Area under the ROC curve (AUROC), True Positive rate (TPR). Values are shown as percentages. Measures calculated for models with $\Delta i < 2$.

ND: Not determined.

PRA: Plant relative abundance; RAF: Rainfalls; S: Species richness; SEA: Season; HPD: Host plant density; V: Plant biomass; HPS: number of host plant species.

Figure A3.1. Raw J48 Decision Tree of potyvirus prevalence in riparian forests excluding plant identity. In parenthesis: The first number is the total number of instances reaching the leaf. The second number is the number of those instances that are misclassified. PRA: Plant relative abundance; PLC: Plant life cycle; HPS: number of host plant species; RAF: Rainfalls; S: Species richness; SEA: Season; HPD: Host plant density; V: Plant biomass; HPS: number of host plant species.

Classifier Model
J48 pruned tree

```

PRA <= 4
| HPS <= 16.096609
| | V <= 0.19338
| | | EH <= 0.93
| | | | PRA <= 1.594748
| | | | | PLC = perenne
| | | | | S <= 32.988912
| | | | | | HPS <= 15.468774
| | | | | | SEA = Summer: Non-host (14.53/5.0)
| | | | | | SEA = Spring
| | | | | | HPS <= 12: Uninfected host (7.0/2.0)
| | | | | | HPS > 12
| | | | | | | HPD <= 9.0554: Non-host (10.0)
| | | | | | | HPD > 9.0554
| | | | | | | HPD <= 10.414642: Uninfected host (2.0)
| | | | | | | HPD > 10.414642: Non-host (6.0/2.0)
| | | | | | SEA = Autumn
| | | | | | PRA <= 0.609756: Non-host (25.25/6.79)
| | | | | | PRA > 0.609756
| | | | | | | PRA <= 1.231199: Uninfected host (14.32/5.16)
| | | | | | | PRA > 1.231199: Non-host (9.56/2.0)
| | | | | | HPS > 15.468774
| | | | | | HPD <= 1.360822: Uninfected host (6.95/0.79)
| | | | | | HPD > 1.360822: Non-host (4.0/1.0)
| | | | | S > 32.988912
| | | | | PRA <= 1.231199
| | | | | | PRA <= 0.609756: Non-host (7.77/2.0)
| | | | | | PRA > 0.609756
| | | | | | | V <= 0.129182: Non-host (4.53/1.0)
| | | | | | | V > 0.129182: Uninfected host (7.0/2.0)
| | | | | | PRA > 1.231199
| | | | | | | HPD <= 6.4065: Non-host (4.0)
| | | | | | | HPD > 6.4065: High prevalence (4.0/2.0)
| | | | | | PLC = anual: Non-host (38.9/6.79)
| | | | PRA > 1.594748
| | | | | S <= 28.250285
| | | | | | SEA = Summer: Uninfected host (0.0)
| | | | | | SEA = Spring
| | | | | | | HPD <= 8.4013
| | | | | | | HPD <= 5.600244
| | | | | | | HPD <= 4.395034: Uninfected host (2.0)
| | | | | | | HPD > 4.395034: Non-host (2.0)
| | | | | | | HPD > 5.600244: Uninfected host (7.0)
| | | | | | | HPD > 8.4013: Non-host (3.0)
| | | | | | SEA = Autumn
| | | | | | | PRA <= 3.5: Non-host (17.64/5.74)
| | | | | | | PRA > 3.5: Uninfected host (5.95/1.79)
| | | | | S > 28.250285
| | | | | PRA <= 2.394846
| | | | | | V <= 0.098726: Uninfected host (12.0/4.0)
| | | | | | V > 0.098726
| | | | | | | SEA = Summer: High prevalence (5.0)
| | | | | | | SEA = Spring: Non-host (5.0/1.0)
| | | | | | | SEA = Autumn: High prevalence (0.0)
| | | | | | PRA > 2.394846
| | | | | | RHmax <= 92.430273: High prevalence (4.07/0.93)
| | | | | | RHmax > 92.430273: Non-host (56.93/20.73)
| | | | | EH > 0.93: High prevalence (6.0/1.0)
| | V > 0.19338
| | | S <= 29.072572
| | | | SEA = Summer
| | | | | V <= 0.373111: High prevalence (7.0)
| | | | | V > 0.373111
| | | | | | HPD <= 4.0503
| | | | | | HPD <= 3.398344: Uninfected host (4.0/1.0)
| | | | | | HPD > 3.398344: High prevalence (6.0)
| | | | | | HPD > 4.0503: Non-host (14.0/6.0)
| | | | SEA = Spring: Non-host (0.0)

```

```

| | | SEA = Autumn: Non-host (8.6/4.19)
| | | S > 29.072572: High prevalence (38.0)
| HPS > 16.096609
| | HPS <= 19.970434
| | | PRA <= 2.85216: High prevalence (30.0)
| | | PRA > 2.85216
| | | Tmed <= 158.663189: Medium prevalence (12.0)
| | | Tmed > 158.663189
| | | Tmin <= 43.754301
| | | RHmax <= 96.126623: High prevalence (3.5/0.5)
| | | RHmax > 96.126623: Medium prevalence (3.5)
| | | Tmin > 43.754301: High prevalence (10.0)
| HPS > 19.970434
| | PRA <= 2.049082: Non-host (21.0/8.0)
| | PRA > 2.049082
| | | PRA <= 2.728369: High prevalence (7.0/1.0)
| | | PRA > 2.728369: Medium prevalence (23.0/5.0)
PRA > 4
| HPS <= 16.096609
| | HPD <= 5.3624
| | | PLC = perenne
| | | tRainfalls <= 430.505004
| | | pH <= 5.004543
| | | | PRA <= 5.246646: Non-host (3.0/1.0)
| | | | PRA > 5.246646
| | | | RHmax <= 97.481608
| | | | HPD <= 3.415964: Non-host (2.0)
| | | | HPD > 3.415964: Uninfected host (3.0)
| | | | RHmax > 97.481608
| | | | SEA = Summer: Uninfected host (0.0)
| | | | SEA = Spring
| | | | HPD <= 3.0541: Non-host (2.0/1.0)
| | | | HPD > 3.0541: Uninfected host (3.0)
| | | | SEA = Autumn
| | | | HPD <= 3.225308: Non-host (2.0/1.0)
| | | | HPD > 3.225308: Low prevalence (3.0/1.0)
| | | pH > 5.004543
| | | | PRA <= 5.672662
| | | | S <= 32.459422
| | | | HPD <= 4.686559
| | | | PRA <= 4.905576: High prevalence (11.0/2.0)
| | | | PRA > 4.905576
| | | | | PRA <= 5.487805: Uninfected host (2.0/1.0)
| | | | | PRA > 5.487805: Non-host (2.9/1.0)
| | | | HPD > 4.686559: Low prevalence (7.0/1.0)
| | | | S > 32.459422
| | | | S <= 35.955022: Medium prevalence (6.0)
| | | | S > 35.955022: Low prevalence (2.0)
| | | PRA > 5.672662
| | | | HPS <= 10.820632: Uninfected host (2.9/0.9)
| | | | HPS > 10.820632
| | | | S <= 35.001138: Low prevalence (112.9/8.0)
| | | | S > 35.001138
| | | | RHmin <= 17.011769: Medium prevalence (3.0)
| | | | RHmin > 17.011769: Low prevalence (2.0)
| | | RAF > 430.505004
| | | Tmed <= 150.728161
| | | HPD <= 3.5099
| | | | S <= 31: Uninfected host (11.9/3.9)
| | | | S > 31: Low prevalence (4.0/1.0)
| | | | HPD > 3.5099: Non-host (11.9/3.0)
| | | Tmed > 150.728161: High prevalence (3.0)
| | PLC = anual
| | | PRA <= 4.905576: High prevalence (3.0/1.0)
| | | PRA > 4.905576: Non-host (18.48/3.1)
| HPD > 5.3624
| | S <= 32.007819
| | | EH <= 0.934789
| | | | HPD <= 5.920273
| | | | PRA <= 5.5: Non-host (5.0)
| | | | PRA > 5.5: Medium prevalence (2.0)
| | | HPD > 5.920273
| | | | HPD <= 6.8278
| | | | PRA <= 5.795902
| | | | S <= 29.495309: Uninfected host (3.0/1.0)
| | | | S > 29.495309: Medium prevalence (2.0)
| | | | PRA > 5.795902: Uninfected host (3.0)
| | | | HPD > 6.8278: Non-host (5.0/2.0)
| | | EH > 0.934789: Uninfected host (2.0/1.0)
| | S > 32.007819: Medium prevalence (6.0)
| HPS > 16.096609

```

```

| | PLC = perenne
| | | HPD <= 4.211434
| | | | RAF <= 362.701727: Low prevalence (9.0)
| | | | RAF > 362.701727
| | | | V <= 0.359783: Medium prevalence (2.0)
| | | | V > 0.359783: Uninfected host (2.0/1.0)
| | | HPD > 4.211434
| | | | Tmed <= 206.954058
| | | | RHmin <= 16.923399
| | | | Tmax <= 288.895616: Low prevalence (4.27)
| | | | Tmax > 288.895616: Medium prevalence (19.29/3.55)
| | | | RHmin > 16.923399: Medium prevalence (62.44/4.18)
| | | | Tmed > 206.954058: Low prevalence (5.0/1.0)
| | PLC = anual: Non-host (3.0/1.0)
Number of Leaves :      85
Size of the tree :     164

```

Figure A3.2. Raw J48 Decision Tree of potyvirus prevalence in evergreen oak forests excluding plant identity. In parenthesis: The first number is the total number of instances reaching the leaf. The second number is the number of those instances that are misclassified. PRA: Plant relative abundance; PLC: Plant life cycle; HPS: number of host plant species; RAF: Rainfalls; S: Species richness; SEA: Season; HPD: Host plant density; V: Plant biomass; HPS: number of host plant species.

Classifier Model
J48 pruned tree

```

-----
SEA = Summer
| PRA <= 6.579382: Non-host (93.0/22.0)
| PRA > 6.579382: Uninfected host (23.0/10.0)
SEA = Spring
| EH <= 0.877891: Medium preValence (25.0)
| EH > 0.877891
| | Tmed <= 151.388866
| | | PRA <= 3.669536
| | | | HPD <= 3.8973: Non-host (20.0)
| | | | HPD > 3.8973
| | | | | PLC = perennial: Non-host (28.0/6.0)
| | | | | PLC = annual
| | | | | PRA <= 1.578947: Uninfected host (6.0/1.0)
| | | | | PRA > 1.578947: Non-host (14.0/6.0)
| | | PRA > 3.669536
| | | | PLC = perennial
| | | | | HPD <= 3.5515
| | | | | HPD <= 3.2746: Non-host (11.0/2.0)
| | | | | HPD > 3.2746: Uninfected host (5.0/1.0)
| | | | | HPD > 3.5515
| | | | | V <= 0.069941: Medium preValence (24.86/4.86)
| | | | | V > 0.069941: Non-host (4.14/1.0)
| | | PLC = annual
| | | | PRA <= 9.700688
| | | | | HPD <= 4.709645
| | | | | HPS <= 9: Non-host (6.0)
| | | | | HPS > 9
| | | | | | HPD <= 4.173278: Uninfected host (6.0/2.0)
| | | | | | HPD > 4.173278: Non-host (2.0)
| | | | | HPD > 4.709645: Uninfected host (5.0)
| | | | PRA > 9.700688
| | | | | EH <= 0.882782: Low preValence (3.0/1.0)
| | | | | EH > 0.882782: Uninfected host (2.0)
| | Tmed > 151.388866: Medium preValence (16.0)
SEA = Autumn
| PRA <= 8.754965
| | HPS <= 7.067873
| | | pH <= 5.978282
| | | | HPS <= 6.983032: High preValence (5.0)
| | | | HPS > 6.983032
| | | | | PRA <= 2.666667
| | | | | V <= 0.007117: Non-host (14.0)
| | | | | V > 0.007117
| | | | | V <= 0.026251: High preValence (4.0)
| | | | | V > 0.026251: Non-host (10.0/1.0)
| | | | PRA > 2.666667
| | | | | V <= 0.016274
| | | | | | HPD <= 4.3232
| | | | | | HPD <= 3.4277: Non-host (2.0/1.0)
| | | | | | HPD > 3.4277: High preValence (10.0/2.0)
| | | | | | HPD > 4.3232: Non-host (3.0/1.0)
| | | | | V > 0.016274: Uninfected host (4.0/1.0)
| | | pH > 5.978282: Non-host (46.0/13.0)
| | HPS > 7.067873: High preValence (34.0)
| PRA > 8.754965
| | PLC = perennial
| | | RHmax <= 95.652663: High preValence (13.26/1.26)
| | | RHmax > 95.652663
| | | | HPD <= 3.033525: Non-host (5.0/1.0)
| | | | HPD > 3.033525: Uninfected host (2.0)
| | PLC = annual
| | | Tmin <= 1.524347: Uninfected host (3.74/0.74)
| | | Tmin > 1.524347: Low preValence (53.0/1.0)
Number of Leaves : 33
Size of the tree : 64

```

APPENDIX 4

Analysis of species-specific potyvirus prevalence in wild ecosystems in the Iberian Peninsula

---

Table A4.1. Prevalence of CIYVV in host plants analyzed in locations monitored in this study.

Season	EL ESCORIAL				PALAZUELOS					
	<i>Trifolium campestre</i>		<i>Trifolium pratense</i>		<i>Trifolium campestre</i>		<i>Trifolium pratense</i>		<i>Rumex pulcher</i>	
	N	CIYVV (%) ¹	N	CIYVV (%) ¹	N	CIYVV (%) ¹	N	CIYVV (%) ¹	N	CIYVV (%) ¹
Sampling cycle 1										
Summer 2013	7	0.00	10	0.00	8	0.00	15	6.67	10	0.00
Autumn 2013	0	–	17	0.00	7	28.57	24	4.17	35	5.71
Spring 2014	0	–	13	7.69	6	0.00	21	0.00	0	–
Sampling cycle 2										
Summer 2014	0	–	12	0.00	0	–	20	0.00	17	0.00
Autumn 2014	7	0.00	18	0.00	20	0.00	24	0.00	25	0.00
Spring 2015	5	20.00	22	4.55	14	7.14	37	10.81	0	–

N: Total number of analysed plants per population.

¹ Prevalence calculated as percentage of CIYVV-infected plants relative to the total number of plants analysed from a given host species.

Table A4.2. Prevalence of ENMV in host plants analyzed in locations monitored in this study.

Santa Cruz de Retamar		
<i>Andryala ragusina</i>		
Season	N	ENMV (%)¹
Sampling cycle 1		
Summer 2013	8	11.50
Autumn 2013	0	–
Spring 2014	0	–
Sampling cycle 2		
Summer 2014	0	–
Autumn 2014	0	–
Spring 2015	10	10.00

N: Total number of analysed plants per population.

¹ Prevalence calculated as percentage of ENMV-infected plants relative to the total number of plants analysed from a given host species.

APPENDIX 5

Analysis of potyvirus genus-wide genetic diversity in wild ecosystems in the Iberian Peninsula

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Table A5.1. Model selection analyses for potyvirus population haplotype diversity (H_d), genetic diversity (π), number of synonymous (d_S) and non-synonymous (d_N) substitutions per site, and selection pressures (d_N/d_S). Model structures included host plant density, host relative abundance, plant species richness and plant biomass; and temperature, relative humidity and rainfall in the sampled locations (minimal, maximal and average values) as predictors, and season as covariate. Potyvirus prevalence was also included as predictor of virus evolution parameters. Best-ranked models are shown ($\Delta_i < 2$).

Model structure*	$r^{\dagger}$	logLik	AIC [‡]	$\Delta_i^{\S}$	$\omega_i^{\P}$
H_d					
PRA (41)+ V (29)+ HPD (11)+ T _{mean} (10)+ RH _{mean} (9)	0.94*	33.44	-52.89	0	0.32
PRA (47)+ V (35)+ T _{mean} (10)+ RH _{mean} (9)	0.93*	32.06	-52.13	0.76	0.22
PRA (40)+ V (28)+ HPD (11)+ T _{mean} (9)+ RH _{mean} (8)+ RFA (3)	0.95*	34.01	-52.03	0.86	0.21
PRA (34)+ V (30)+ HPD (10)+ T _{mean} (10)+ RH _{mean} (9)+ PRE (7)	0.95*	34.01	-52.03	1.88	0.13
PRA (42)+ V (26)+ HPD (10)+ T _{mean} (10)+ RH _{mean} (9)+ S (4)	0.95*	33.50	-51.01	1.93	0.12
π					
RAF (36)+ PRA (32)+ S (16)+ RH _{mean} (12)+ T _{mean} (4)	0.85*	24.46	-34.91	0.00	0.33
PRE (36)+ RAF (23)+ PRA (18)+ S (12)+ RH _{mean} (7)+ T _{mean} (4)	0.87*	25.11	-34.23	0.68	0.24
RAF (33)+ PRA (29)+ S (18)+ RH _{mean} (11)+ HPD (5)+ T _{mean} (4)	0.86*	24.87	-33.74	1.17	0.18
RAF (38)+ PRA (26)+ S (14)+ RH _{mean} (10)+ V (8)+ T _{mean} (4)	0.85*	24.49	-32.98	1.93	0.13
PRE (46)+ RFA (32)+ V (15)+ HPD (7)	0.80*	22.46	-32.93	1.98	0.12
d_N					
RAF (44)+ PRA (30)+ RH _{mean} (11)+ S(10)+ T _{mean} (5)	0.87*	27.52	-41.04	0.00	0.16
RAF (40)+ PRA (26)+ S(11)+ RH _{mean} (10)+ HPD (8)+ T _{mean} (5)	0.88*	28.52	-41.03	0.01	0.16
PRE (36)+ RAF (28)+ PRA (17)+ S(7)+ T _{mean} (6)+ RH _{mean} (6)	0.88*	28.51	-41.02	0.02	0.16
PRE (34)+ RAF (26)+ PRA (15)+ S (8)+ RH _{mean} (6)+ HPD (6)+ T _{mean} (5)	0.90*	29.37	-40.74	0.30	0.14
PRE (41)+ RAF (31)+ PRA (18)+ T _{mean} (5)+ RH _{mean} (5)	0.86*	27.04	-40.08	0.96	0.10
RAF (43)+ PRA (28)+ RH _{mean} (11)+ S (9)+ T _{mean} (5)+ V (4)	0.87*	27.87	-39.73	1.31	0.08
PRE (35)+ RAF (28)+ PRA (16)+ S (7)+ T _{mean} (6)+ RH _{mean} (6)+ V (2)	0.89*	28.69	-39.38	1.66	0.07
RAF (51)+ PRA (35)+ RH _{mean} (10)+ T _{mean} (4)	0.82*	25.61	-39.23	1.81	0.07
RAF (41)+ PRA (22)+ S (10)+ RH _{mean} (9)+ HPD (9)+ T _{mean} (5)+ V (4)	0.89*	28.53	-39.05	1.99	0.06
d_S					
PRA (45)+ RAF (25)+ S (9)+ HPD (8)+ RH _{mean} (8)+ T _{mean} (5)	0.93*	15.54	-15.08	0.00	0.16
PRE (32)+ PRA (29)+ RAF (17)+ S (6)+ T _{mean} (6)+ RH _{mean} (5)+ HPD (5)	0.94*	16.51	-15.02	0.07	0.15
PRE (35)+ PRA (31)+ RAF (18)+ RH _{mean} (6)+ S (5)+ T _{mean} (5)	0.93*	15.44	-14.89	0.19	0.14
PRA (51)+ RAF (28)+ RH _{mean} (9)+ S (8)+ T _{mean} (5)	0.92*	14.35	-14.69	0.39	0.13
PRA (46)+ RAF (28)+ RH _{mean} (9)+ S (7)+ T _{mean} (5)+ V (5)	0.93*	15.22	-14.44	0.64	0.11
PRE (33)+ PRA (29)+ RAF (18)+ T _{mean} (6)+ RH _{mean} (6)+ S (5)+ V (3)	0.94*	16.08	-14.16	0.93	0.10
PRA (38)+ RAF (26)+ HPD (9)+ S (8)+ RH _{mean} (8)+ V (6)+ T _{mean} (5)	0.93*	15.75	-13.50	1.58	0.07
PRE (38)+ PRA (32)+ RAF (19)+ T _{mean} (5)+ RH _{mean} (5)	0.91*	13.71	-13.41	1.67	0.07
PRE (30)+ PRA (26)+ RAF (17)+ HPD (6)+ S (6)+ T _{mean} (6)+ RH _{mean} (5)+ V (4)	0.94*	16.62	-13.25	1.84	0.06

*The relative importance (%) of each predictor variable is shown in parenthesis.

[†]Correlation coefficient. Asterisks indicate significant correlations ($P < 0.05$).

[‡]Akaike's Information Criterion.

[§] Δ_i is the difference between the AIC of a given model and that of the best-ranked model, and quantifies how models compete (best-ranked model: $\Delta_i = 0$; substantial empirical support: $\Delta_i = 1-2$; considerable less support: $\Delta_i = 2-7$; and no support: $\Delta_i > 10$) (Burnham and Anderson, 2002).

[¶]AIC model weight as $\omega_i = \exp(-0.5\Delta_i) / \sum \exp(-0.5\Delta_i)$. The larger the ω_i , the greater the likelihood of the model relatively to the competing models. Maximum $\omega_i = 1$.

PRA: Plant relative abundance; PRE: WHMV prevalence; RAF: Rainfalls; RH_{mean}: Average relative humidity; T_{mean}: Average temperature; RH_{mean}: Average relative humidity; V: Plant biomass; HPD: Host plant density; S: Species richness.

Table A5.1. Continued.

Model structure*	$r^{\dagger}$	logLik	AIC [‡]	$\Delta_i^{\S}$	$\omega_i^{\P}$
dN/ds					
PRE (49)+ RAF (39)+ V (12)	0.72*	17.85	-25.69	0.00	0.10
RAF (44)+ S (20)+ PRA (19)+ RH _{mean} (13)+ T _{mean} (4)	0.79*	19.64	-25.28	0.41	0.08
PRE (44)+ RAF (37)+ V (14)+ HPD (5)	0.74*	18.50	-25.69	0.69	0.07
PRE (64)+ RAF (36)	0.65*	16.40	-24.80	0.89	0.07
PRE (44)+ V (12)+ T _{mean} (3)+ RH _{mean} (4)+ RAF (37)	0.78*	19.38	-24.75	0.94	0.06
PRE (34)+ HPD (5)+ V (10)+ S (13)+ T _{mean} (3)+ RH _{mean} (5)+ RAF (30)	0.83	21.27	-24.53	1.16	0.06
PRE (39)+ HPD (5)+ V (14)+ T _{mean} (3)+ RH _{mean} (3)+ RAF (36)	0.80	20.18	-24.36	1.34	0.05
PRE (54)+ S (17)+ RAF (29)	0.69*	17.14	-24.29	1.41	0.05
PRE (35)+ PRA (9)+ S (15)+ T _{mean} (4)+ RH _{mean} (8)+ RAF (29)	0.80	20.14	-24.28	1.41	0.05
PRE (46)+ V (12)+ RH _{mean} (2)+ RAF (39)	0.73*	18.13	-24.26	1.44	0.05
PRE (39)+ V (8)+ S (13)+ T _{mean} (4)+ RH _{mean} (5)+ RAF (32)	0.80	20.12	-24.23	1.46	0.05
PRE (45)+ S (17)+ T _{mean} (4)+ RH _{mean} (5)+ RAF (29)	0.77*	19.06	-24.13	1.57	0.05
PRE (46)+ V (9)+ S (13)+ RAF (33)	0.72*	17.97	-23.95	1.75	0.04
PRE (46)+ PRA (7)+ V (11)+ RAF (37)	0.72*	17.96	-23.93	1.76	0.04
PRE (40)+ HPD (5)+PRA (6)+ V (13)+ RAF (36)	0.76*	18.91	-23.81	1.88	0.04
HPD (5)+ PRA (16)+ S (22)+ T _{mean} (4)+ RH _{mean} (13)+ RAF (41)	0.79	19.91	-23.81	1.88	0.04
PRE (49)+ V (12)+ T _{mean} (1)+ RAF (37)	0.72*	17.88	-23.75	1.94	0.04
PRE (41)+ HPD (5) + V (14)+ RH _{mean} (2)+ RAF (38)	0.76*	18.87	-23.73	1.96	0.04

*The relative importance (%) of each predictor variable is shown in parenthesis.

[†]Correlation coefficient. Asterisks indicate significant correlations ($P < 0.05$).

[‡]Akaike's Information Criterion.

[§] Δ_i is the difference between the AIC of a given model and that of the best-ranked model, and quantifies how models compete (best-ranked model: $\Delta=0$; substantial empirical support: $\Delta=1-2$; considerable less support: $\Delta=2-7$; and no support; $\Delta > 10$) (Burnham and Anderson, 2002).

[¶]AIC model weight as $\omega = \exp(-0.5\Delta_i) / \sum \exp(-0.5\Delta_i)$. The larger the ω , the greater the likelihood of the model relatively to the competing models. Maximum $\omega_i = 1$.

PRA: Plant relative abundance; PRE: WHMV prevalence; RAF: Rainfalls; RH_{mean}: Average relative humidity; T_{mean}: Average temperature; RH_{mean}: Average relative humidity; V: Plant biomass; HPD: Host plant density; S: Species richness.

Figure A5.1. Maximum-likelihood phylogeny based on the CP region representing the association between potyvirus phylogeny and geographical location. Asterisks indicates nodes with a bootstrap support ≥ 0.90 .

Figure A5.2. Maximum-likelihood phylogeny based on the CP region representing the association between potyvirus phylogeny and host plant species. Asterisks indicates nodes with a bootstrap support ≥ 0.90 .

APPENDIX 6

Analysis of potyvirus species-specific genetic diversity in wild ecosystems in the Iberian Peninsula

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Table 4.37. Model selection analyses for WHMV population haplotype diversity (H_d), genetic diversity (π), number of synonymous (d_s) and non-synonymous (d_N) substitutions per site, and selection pressures (d_N/d_s). Model structures included host plant density, host relative abundance, plant species richness and plant biomass; and temperature, relative humidity and rainfall in the sampled locations (minimal, maximal and average values) as predictors, and season as covariate. WHMV prevalence was also included as predictor of virus evolution parameters. Best-ranked models are shown ($\Delta_i < 2$).

Model structure	$r^{\dagger}$	logLik	AIC [‡]	$\Delta_i^{\S}$	$\omega_i^{\P}$
H_d					
PRA (30)+RAF (26)+PRE (12)+RH _{mean} (12)+T _{mean} (11)+V (9)	0.78*	37.97	-59.94	0.00	0.55
PRA (29)+RAF (26)+PRE (12)+RH _{mean} (12)+T _{mean} (11)+V (8)+S (2)	0.79*	38.15	-58.3	1.64	0.24
PRA (30)+RAF (26)+ RH _{mean} (12)+PRE (11)+T _{mean} (11)+V (8)+HPD (2)	0.78*	37.97	-57.94	2.00	0.20
π					
RAF (50)+PRE (27)+RH _{mean} (17)+T _{mean} (6)	0.71*	66.22	-120.44	0.00	0.25
RAF (50)+PRE (28)+RH _{mean} (22)	0.67*	65.16	-120.33	0.11	0.24
RAF (46)+PRE (28)+RH _{mean} (16)+PRA (4)+T _{mean} (6)	0.71*	66.47	-118.94	1.50	0.12
RAF (45)+PRE (24)+RH _{mean} (23)+ S (8)	0.68*	65.37	-118.74	1.70	0.11
RAF (50)+PRE (27)+RH _{mean} (17)+T _{mean} (6)+V (1)	0.71*	66.32	-118.64	1.80	0.10
RAF (49)+PRE (25)+RH _{mean} (17)+T _{mean} (6)+HPD (2)	0.71*	66.23	-118.45	1.99	0.09
RAF (44)+PRE (23)+RH _{mean} (17)+T _{mean} (8)+S (9)	0.71*	66.22	-118.44	2.00	0.09
d_N					
V (46)+PRE (21)+RAF (17)+RH _{mean} (9)+T _{mean} (7)	0.78*	49.73	-85.46	0.00	0.38
V (41)+PRE (22)+RAF (17)+RH _{mean} (10)+T _{mean} (7)+S (3)	0.80*	50.49	-84.99	0.47	0.30
V (45)+PRE (19)+RAF (16)+RH _{mean} (10)+T _{mean} (7)+PRA (3)	0.79*	49.97	-83.94	1.52	0.18
V (41)+PRE (18)+RAF (17)+RH _{mean} (10)+ HPD (7)+T _{mean} (7)	0.78*	49.76	-83.52	1.94	0.14
d_s					
V (46)+RAF (13)+PRE (12)+RH _{mean} (9)+T _{mean} (8)+S (8)+PRA (4)	0.81*	43.62	-69.25	0.00	0.46
V (57)+RAF (11)+PRE (10)+RH _{mean} (9)+T _{mean} (8)+ PRA (5)	0.77*	42.35	-68.7	0.55	0.35
V (44)+RAF (13)+PRE (10)+RH _{mean} (9)+T _{mean} (8)+S (7)+HPD (5)+PRA (4)	0.81*	43.79	-67.57	1.67	0.20
d_N/d_s					
V (45)+PRE (23)+RAF (16)+RH _{mean} (9)+T _{mean} (7)	0.79*	25.37	-36.74	0.00	0.41
V (41)+PRE (23)+RAF (16)+RH _{mean} (9)+T _{mean} (8)+S (3)	0.80*	25.98	-35.96	0.78	0.28
V (43)+PRE (19)+RAF (16)+RH _{mean} (10)+T _{mean} (7)+PRA (5)	0.79*	25.43	-34.85	1.89	0.16
V (40)+PRE (20)+RAF (15)+ RH _{mean} (9)+HPD (7)+T _{mean} (7)	0.79*	25.39	-34.77	1.96	0.15

*The relative importance (%) of each predictor variable is shown in parenthesis.

[†]Correlation coefficient. Asterisks indicate significant correlations ($P < 0.05$).

[‡]Akaike's Information Criterion.

[§] Δ_i is the difference between the AIC of a given model and that of the best-ranked model, and quantifies how models compete (best-ranked model: $\Delta_i = 0$; substantial empirical support: $\Delta_i = 1-2$; considerable less support: $\Delta_i = 2-7$; and no support: $\Delta_i > 10$) (Burnham and Anderson, 2002).

[¶]AIC model weight as $\omega_i = \exp(-0.5\Delta_i) / \sum \exp(-0.5\Delta_i)$. The larger the ω_i , the greater the likelihood of the model relatively to the competing models. Maximum $\omega_i = 1$.

PRA: Plant relative abundance; PRE: WHMV prevalence; RAF: Rainfalls; RH_{mean}: Average relative humidity; T_{mean}: Average temperature; V: Plant biomass; HPD: Host plant density; S: Species richness.