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Impact of *Vat* resistance in melon on viral epidemics and genetic structure of virus populations

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Highlights

- We evaluated the melon *Vat*-mediated resistance to virus in eight field trials.
- *Vat* always reduced the epidemics of CABYV.
- The reduction on CMV epidemics was irregular.
- *Vat* had a limited impact on WMV epidemics.
- *Vat* had no effect on the genetic structure of CABYV and WMV populations.

Abstract

Cultivar choice is at the heart of cropping systems and resistant cultivars should be at the heart of disease management strategies whenever available. They are the easiest, most efficient and environmentally friendly way of combating viral diseases at the farm level. Among the melon genetic resources, *Vat* is a unique gene conferring resistance to both the melon aphid *Aphis gossypii* and the viruses it carries. The ‘virus side’ of this pleiotropic phenotype is seldom regarded as an asset for virus control. Indeed, the effect of *Vat* on virus epidemics in the field is expected to vary according to the composition of aphid populations in the environment and long-term studies are needed to draw a correct trend. Therefore, the first objective of the study was to re-evaluate the potential of *Vat* to reduce viral diseases in melon crops. The second objective was to investigate the potential of *Vat* to exert a selection pressure on virus populations. We monitored the epidemics of *Cucurbit aphid-borne yellows virus* (CABYV), *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) in two melon lines having a common genetic background, a resistant line (R) and a susceptible line (S), in eight field trials conducted in southeastern France between 2011 and 2015. *Vat* had limited impact if any on WMV epidemics probably because *A. gossypii* is not the main vector of WMV in the field, but a favorable impact on CMV, yet of variable intensity probably related to the importance of *A. gossypii* in the total aphid population. *Vat* had a significant impact on CABYV epidemics with mean incidence reduction exceeding 50% in some trials. There was no effect of *Vat* on the structure of virus populations, both for the non-persistent WMV transmitted by numerous aphid species and for the persistent CABYV transmitted predominantly by *A. gossypii*.

Keywords: *Aphis gossypii*, *Cucumis melo*, genetic resistance, Gompertz model, selection pressure

1. Introduction

Pathogenic plant viruses generate economic losses by adversely affecting plant growth and reproduction, causing death of host tissues and plants, sterility, reduction of yield or quality, increased susceptibility to other stresses, loss of aesthetic value, quarantine and eradication of infected plants, as well as the cost of implementing control methods (Bos, 1982; Waterworth and Hadidi, 1998). Although crop losses are complex to assess, it was estimated that about 4% of global crop production is lost due to pathogenic plant viruses (Popp and Hantos, 2011).

To prevent viral diseases, plants have evolved diverse resistance mechanisms (Soosaar *et al.*, 2005). Recessive resistance is often related to mutation(s) in one of the plant genes required by the virus to complete a particular step of its infectious cycle (Caranta and Dogimont, 2008). Dominant resistance is mostly related to genes encoding nucleotide-binding leucine-rich repeat (NLR) proteins that recognize virus avirulence factors through ‘gene-for-gene’ interactions (Boualem *et al.*, 2016). The recognition is followed by a rapid induction of programmed cell death at the site of virus inoculation and in the adjacent cells, which manifests as a hypersensitive response (HR). NLR genes usually confer a narrow resistance spectrum restricted to a single virus species. The *Vat* gene in melon (*Cucumis melo*) is an exception. *Vat* confers resistance to both the melon aphid *Aphis gossypii* and the viruses it carries (Boissot *et al.*, 2016a). This pleiotropic phenotype was first evidenced in the early 1980s (Pitrat and Lecoq, 1980, 1982). *Vat* resistance to virus is triggered by an effector present in the saliva of the aphid

transmitting the virus (Boissot *et al.*, 2016a) and is efficient against any non-persistently aphid-transmitted virus inoculated by *A. gossypii* (Lecoq *et al.*, 1980).

The melon aphid *A. gossypii* is widespread across all continents. The species is structured in host races (Boissot *et al.*, 2016a). Clones belonging to the Cucurbit host race are able to colonize melon crops, causing leaf-curling, stunting and even plant death when colonization is intense. *A. gossypii* is also a known vector for over 50 plant viruses (Blackman and Eastop, 2000). In particular, it is an efficient vector of four viruses frequently observed on melon crops in France: *Cucurbit aphid-borne yellows virus* (CABYV, Polerovirus, Luteoviridae), *Cucumber mosaic virus* (CMV, Cucumovirus, Bromoviridae), *Watermelon mosaic virus* (WMV, Potyvirus, Potyviridae) and *Zucchini yellow mosaic virus* (ZYMV, Potyvirus, Potyviridae). CABYV induces yellowing of the older leaves, flower abortion and reduced number of fruits per plant. It is restricted to phloem tissues of infected plants and requires long aphid feeding periods (several hours to days) to be acquired and inoculated efficiently (persistent non-propagative transmission). This requirement is only met through feeding by a colonizing aphid species, which is the case for *A. gossypii* on melon crops. CMV, WMV and ZYMV cause mosaic symptoms on leaves, plant stunting and yield reduction (Lecoq and Desbiez, 2012). Severe infections of WMV and ZYMV also induce leaf deformation and mosaic on fruit, and ZYMV is also known to induce marbling and hardening of the flesh of fruit. CMV, WMV and ZYMV are acquired and inoculated during brief feeding probes (less than 1 min) in the epidermal cells (non-persistent transmission). This matches the behaviour of transient aphids which alight, probe and fly away if the plant is not a suitable host. Thus, *A. gossypii* but also many other non-colonizing aphid species can be involved in the spread of these three latter viruses in melon crops.

Vat-mediated resistance has been early used in breeding programmes and the first melon cultivar carrying *Vat* (Margot) was listed in the French official catalog of varieties in 1987.

Since then, more than a hundred cultivars reported as resistant to *A. gossypii* have been released in France and 80% of the melon crops cultivated in southeastern France are believed to carry *Vat* (Boissot *et al.*, 2016a). Although adapted clones have been characterized (Boissot *et al.*, 2016b), these cultivars still represent an efficient option to control *A. gossypii*. On the contrary, the ‘virus side’ of *Vat* pleiotropic phenotype is seldom regarded as an asset for virus control. In controlled conditions, *Vat* was shown to confer resistance to CMV, WMV and ZYMV when inoculated by *A. gossypii* (Lecoq *et al.*, 1979, 1980; Boissot *et al.*, 2016b) but *Vat*-carrying plants remained susceptible when viruses were inoculated mechanically or by other aphid species including *A. craccivora*, *A. fabae* and *Myzus persicae* (Lecoq *et al.*, 1979, 1980). The effect of *Vat* on virus epidemics in the field has been poorly documented so far (Boissot *et al.*, 2016a). It is expected to vary according to the composition of aphid populations in the environment but long-term studies are needed to draw an accurate trend. Therefore, the first objective of our study was to re-evaluate the potential of *Vat* to reduce viral epidemics in melon crops. Moreover, the effect of a virus resistance triggered by aphids on virus population genetic diversity in the field is not known. So, our second objective was to investigate the potential of *Vat* to exert selection pressure on virus populations.

2. Materials and methods

2.1. Field experiments

Eight field experiments were conducted at INRA St Paul experimental station in Avignon (southeastern France, 43°54'53N, 4°52'59E) between 2011 and 2015 (Table 1). Charentais-type melon plants were from two quasi-isogenic lines, a resistant line (R) and a susceptible line (S). The R line was derived from a cross between PI 161375, a *Chinensis* line carrying *Vat*, and a *Cantalupensis* line. The F₁ was backcrossed with a Charentais-type cultivar that served as recurrent parent; progenies were selected for *Vat* resistance for the next backcross. Fifteen backcrosses were done, the last 8 with the Charentais T line which served as susceptible line in this study. Charentais T does not carry any known gene of resistance to virus. Seedlings were prepared in an insect-proof greenhouse three weeks before planting. Depending on the trial, plants at the 1-3 leaf stage were planted in late April or late May on dark brown plastic mulch with drip irrigation. Early plantings were protected from wind damage with Agryl P17 fleece (Fiberweb France, Biesheim) for 11-15 days. Each treatment consisted of one plot of 120 to 240 plants (0.5 m plant spacing) organized in 6 to 16 rows (1.5 m row spacing) depending on the trial.

2.2. Plant sampling and virus detection

In order to establish virus dynamics, melon plots were sampled at regular intervals, up to 11 times during the cropping season. The sampling campaign generally started one week after planting or fleece removal in order to detect early onset of virus infection. For trial P13, samplings were delayed due to cold post-planting weather conditions slowing down crop

growth. A stratified random sampling design involving 24 to 40 plants per modality was established for each trial. Depending on the trial, strata consisted of one or two rows, and 4 to 5 plants were randomly chosen in each stratum. These tagged plants served for virus monitoring throughout the trial. Expanding leaves were sampled at each sampling date. It was assumed that information obtained from an expanding leaf will accurately reflect the status of the entire plant. CABYV, CMV, WMV and ZYMV were detected by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) in four separate tests with specific polyclonal antisera produced in our laboratory. Virus detection was considered positive when the absorbance at 405 nm (Multiskan EX, Thermo Electron Corporation) was above 3 times that of the healthy controls. ZYMV was detected only in 2012, so it was not included in further analyses.

2.3. Analyses of virus epidemics

In a first step, we analyzed the effect of *Vat* on the infection probability of a plant by a given virus with a generalized linear model (procedure GENMOD of SAS software, version 9.3 for Windows, SAS Institute Inc., Cary, NC, USA). The dependent variable (presence/absence of a given virus in a sample) being binary, the model was based on a binomial distribution and a logit link function. Explanatory variables were *Vat* resistance and sampling time.

In a second step, we analyzed virus dynamics and examined the effect of *Vat* on their features. Binary variables were pooled by modality and date to calculate virus incidence (number of infected plants divided by the number of sampled plants) and to establish virus progress curves. Virus dynamics having a characteristic S-shape, nonlinear model fitting was applied to incidence data (procedure NLIN of SAS software). Among the models commonly used to characterize the temporal dynamics of plant viruses (Nutter, 1997), the Gompertz function was chosen as its three parameters (α , β , γ) make sense biologically for interpretation of the data:

$$y_t = \gamma e^{-\beta e^{-\alpha t}}$$

y_t is the incidence, expressed as a percentage, at time t ;

t is the thermal time expressed in degree-days (basis 12°C, temperature below which melon growth is stopped);

α is linked to disease progress: when α increases, the epidemic is more rapid;

β is linked to disease onset: when β increases, the epidemic is delayed;

γ is the asymptote, i.e., the carrying capacity: when γ increases, maximum incidence is raised;

α , β and γ are positive parameters, γ is bounded above by 100.

The area under the disease progress curve (AUDPC) was calculated for each virus dynamic. Values were standardized by dividing by the total duration of the monitoring in each trial, in order to approximate mean incidence over the epidemic (Nutter, 1997). On the basis of their mean incidence, epidemics were categorized as mild [0-20%], intermediate [21-40%], severe [41-60%] or extreme [61-100%].

For each virus, the effect of *Vat* on the three parameter estimates and mean incidence was investigated through a nonparametric sign test performed between R and S values (XLSTAT, version 2015.4.01, Addinsoft, Paris).

2.4. Molecular analyses

Molecular analyses focused on CABYV and WMV. Approximately 20 ELISA-positive samples were selected for each virus, each year and each condition (R/S). However, there were some variations in this number (from 9 to 39) in relation to the dynamics of the epidemics in each case (Table 2).

Total RNAs were extracted from ELISA-positive samples with TRI-reagent® following the manufacturer's recommendations. Fragments of the coat-protein (CP) coding region were

amplified using specific primers. For CABYV, a 536-nt fragment in the CP and movement protein (MP) coding region was amplified with primers CABYV-CE9 (Kassem *et al.*, 2007) and CABYV-KN-3' (5'-CCGTTCCCCTTG TAGAGGAT-3'). For WMV, primers W-VVIAM-5' and WMV-3' were used (Desbiez *et al.*, 2009), yielding a 811-nt fragment encompassing the N-terminal variable region of the CP. The amplified fragments were sent for direct sequencing to Genoscreen (Lille, France). One sequence from each haplotype was submitted to GenBank.

Sequences were aligned with ClustalW included in MEGA6.06 (Tamura *et al.*, 2013). Reference GenBank sequences were added for the analyses. The best substitution model was selected with MEGA. The absence of recombination signal in the different virus fragments analyzed was checked using 6 different recombination detection methods included in RDP4.0 (Martin *et al.*, 2015). Distance and maximum-likelihood trees were built with MEGA using a bootstrap resampling (n= 500 bootstraps). Haplotypic networks were drawn with Network 5.0.0.0 (<http://www.fluxus-engineering.com/>). Cumulated haplotypic frequencies 2011-2015 were compared between R and S melons with a paired t-test and a two-sided paired Wilcoxon nonparametric test using the Rcmdr package of R software (<https://www.r-project.org/>). Haplotypic richness in R and S melons was estimated for CABYV and WMV on the 2011-2015 cumulated data using Abundance-based Coverage Estimator (ACE) (Chao and Lee, 1992) calculated with SpadeR (<https://chao.shinyapps.io/SpadeR/>).

3. Results

3.1. Effect of *Vat* on virus epidemics

The experimental setup consisted of eight melon field trials conducted in southeastern France between 2011 and 2015. CABYV, CMV and WMV were consistently observed every year. ZYMV was only observed in 2012 and consequently was not included in further analyses. Virus disease progress curves were successfully described by the Gompertz model in all cases except one (trial P11 where CMV incidence was 2.5% in the R melons at the end of the cropping cycle).

CABYV epidemics on S melons were variable in earliness and intensity (Figure 1). Disease onset mostly occurred during the three weeks following planting but infected plants could occasionally be detected as early as seven days after planting (P11), or conversely, 35 days after planting (V13). Disease progress varied from extremely rapid (V11) to slow (V12). Both earliness and progress rate get integrated in the calculation of the mean incidence (Table 3). Except one extreme epidemic (P11), CABYV epidemics were categorized as intermediate to severe.

Vat significantly reduced the probability of infection of a plant by CABYV in all trials (Table 4). In terms of effect on the virus dynamics, this translated into a significant reduction of parameter α of the Gompertz model (Table 3), meaning that CABYV incidence progress was slower on R melons than on S melons. The effects on parameter β (linked to disease onset: when β increases, the epidemic is delayed) and parameter γ (carrying capacity: maximum incidence at harvest) were not significant, but *Vat* resulted in delayed disease onset by 6-14 days and/or reduced disease incidence at harvest stage, in 5/8 trials. *Vat* significantly reduced

CABYV mean incidence (Table 3). In almost all trials, *Vat* permitted to shift from one epidemic category to the one below, with an average 37% reduction in mean incidence (range 12-57%). CMV epidemics on S melons were seldom intermediate: they were either mild, or severe to extreme (Figure 2, Table 3). For the latter, disease onset mostly occurred during the two weeks following planting whereas delayed onsets (36-49 days after planting) mostly led to mild epidemics. Interestingly, when CABYV epidemics developed rapidly (V11, P11), CMV onset was late and progress was slow. *Vat* significantly reduced the probability of infection of a plant by CMV in 6/8 trials (Table 4). None of the Gompertz model parameter estimates were significantly affected by *Vat* although presence of the gene resulted in a 7-8 day delay in disease onset and/or a reduced incidence at harvest (Table 3). *Vat* significantly reduced CMV mean incidence permitting classification to one epidemic category below that of comparable plants without the *Vat* gene in half of the trials, with an average 31% reduction (range 5-83%). The amount of reduction due to *Vat* was inversely proportional to mean incidence: for severe to extreme epidemics, the reduction induced by *Vat* was only 14%.

WMV was generally the latest virus to be observed on S melons (Figure 3). Except in P15 where disease onset was noticed 15 days after planting, WMV symptoms were first observed after 7 weeks of cropping. Epidemics were mostly mild to intermediate. *Vat* had no significant decrease effect on the probability of infection of a plant by WMV except in P14 (Table 4). For this particular trial, *Vat* delayed disease onset by 8 days and reduced disease progress. Except for P14, no change in epidemic category was observed (Table 3). An opposite trend, i.e., increase in viral incidence on *Vat*-carrying plants, was even observed in V12, P11 and P13.

3.2. Effect of *Vat* on virus populations

Preliminary data based on partial capsid gene sequences showed that the molecular variability of CMV was extremely low (data not shown) and ZYMV was present only in 2012. As a consequence, molecular analyses focused on CABYV and WMV.

For CABYV, 193 sequences were obtained, 79 for R and 114 for S plots (Table 2). Thirteen different haplotypes were characterized (Genbank KY586119-KY586131), including 5 haplotypes detected in only one plant each (singletons). Multiple infections of plants with two haplotypes differing by 1 or 2 mutations were observed in 13 cases. The overall variability of the virus was low over the 5 years (mean distance between haplotypes = 0.007). Most haplotypes differed by only 1 to 3 mutations in the sequenced fragment, with the exception of the more divergent haplotype G observed in 2011, 2013 and 2015 (Figure 4a). There was no evidence for recombination in the sequenced region. The same major haplotypes were present in both R and S plots (Figure 4b) and the differences in frequencies were not significant between the plots ($p=0.14$, paired t-test). Haplotypic richness estimators (ACE) in R (9-42 haplotypes) and S (13-61 haplotypes) plots were largely overlapping, showing that the estimated total number of haplotypes was not significantly different between the two conditions. Comparison with isolates from throughout the world showed that all isolates from the assay were closely related to the original CABYV isolate from southwestern France (Lecoq *et al.*, 1992) as well as to isolates from Egypt, Tunisia, Spain, Serbia and Czech Republic (Figure 5).

For WMV, 299 partial sequences were obtained, 153 for R and 146 for S plots (Table 2). Twenty-seven different haplotypes were present in the 712-nt sequences used for the analysis, including 13 singletons (Figure 6) (Genbank KY586092-KY586118). The estimated total number of haplotypes was not significantly different between R (24-141 haplotypes) and S (18-44 haplotypes) melons. WMV populations in the trials belonged to four different molecular groups and subgroups already known to be present in southeastern France (Desbiez *et al.*, 2009). Group CL was detected in one R plant in 2011, and at a low frequency in 2013, both in

R and S melon plants (Figure 7). Within the EM group, subgroup EM1 was present every year and, except in 2014, was the most frequent (Figure 7). Subgroup EM3 was observed in 2014 both in R and S melon plants, and EM4 was detected at a low frequency every year except in 2012 (Figure 7). Ten plants displayed mixed infections between EM1 and one of the minor groups or subgroups present in the same year, and five samples also presented mixed infections with two haplotypes from the same subgroup and differing by 1 to 3 mutations. There was no evidence for recombination in the sequenced fragment. The major haplotypes were the same in R and S melon plants, and the differences in frequencies were not significant ($p=0.625$, paired t-test). No mutations were observed in or close to the 'DAG' triplet in the N-terminal variable part of the coat protein, involved in aphid transmission (Lopez-Moya *et al.*, 1999).

4. Discussion

4.1. Virus epidemics

Eight field experiments were conducted in one of the main melon production areas in France. In the course of the study (2011-2015), CABYV, CMV and WMV were observed every year, whereas ZYMV was observed only in 2012, in agreement with the sporadic pattern described for this virus (Lecoq *et al.*, 2009, 2014). Regardless of the planting date (late April or late May) CABYV and CMV were always detected first, generally 2-3 weeks after planting, leading to epidemics with mean incidence mostly intermediate to severe for CABYV and severe to extreme for CMV. WMV was always detected later, generally after 7 weeks of cropping, leading to epidemics mostly mild to intermediate. Since its first description in 1992 in France, CABYV has been detected in an ever-increasing number of countries and recent surveys indicate that CABYV is becoming one of the most common cucurbit viruses in a wide variety of areas (Kassem *et al.*, 2007; Lecoq and Desbiez, 2012; Juarez *et al.*, 2013).

There was a complete decoupling between the progress curves of the different viruses during a cropping season and between different seasons for a given virus, suggesting that biotic and/or abiotic factors involved in the epidemiology of these viruses are different.

Virus progress curves were successfully described by the Gompertz model in all cases except one. This model commonly used to characterize the temporal dynamics of plant viruses (Nutter, 1997) was in particular reported as the most appropriate to compare epidemics of aphid-transmitted viruses in melon crops in Spain (Alonso-Prados *et al.*, 2003). Unlike the latter study where the linearized form of the model was used, nonlinear model fitting was applied to incidence data in our study, allowing the estimation of the three parameters of the model (α , β ,

γ). The effect of *Vat* on the model parameter estimates and on the mean incidence (AUDPC divided by the epidemic duration) was investigated for each virus.

4.2. Effect of *Vat* on CABYV

Vat had a marked impact on CABYV epidemics. The mean incidence, calculated over the total duration of the epidemic, was significantly and consistently reduced. An average reduction of 37% was observed over the eight trials. *Vat* impacted the temporal progress of CABYV epidemics mostly by slowing down the infection rate as shown by the significant reduction of parameter α of the Gompertz model. *Vat* allowed to delay disease onset by 6-14 days in half of the trials.

These results suggest that a part of CABYV primary and secondary infections comes from *A. gossypii* clones that are controlled by *Vat*. The effect of *Vat* on CABYV has not been entirely characterized in the laboratory yet. Nevertheless, it was observed that *A. gossypii* has far less access to phloem on *Vat*-carrying plants than on susceptible plants (Chen *et al.*, 1997; Klinger *et al.*, 1998) and colonize *Vat*-carrying plants less than susceptible plants (Boissot *et al.*, 2016a). This may explain the low transmission rate of CABYV by *A. gossypii* on *Vat*-carrying plants as suggested by preliminary results (Lecoq, 1999; unpublished data).

Although marked, the effect of *Vat* on CABYV epidemics was not total. Other aphid species such as *Myzus persicae* and *Macrosiphum euphorbiae* are known to be efficient vectors of CABYV (Lecoq and Desbiez, 2012), but they probably play a limited role in the epidemiology of CABYV in southeastern France. First, the relative abundance of these species within the winged aphid population visiting the melon crops was low (Schoeny, unpublished data) and second, they have never been observed colonizing melon crops in southeastern France (Lecoq, personal communication). Accordingly, using real-time quantitative RT-PCR, Kassem *et al.*

(2013) found that *A. gossypii* was the species with the highest number of individuals carrying CABYV suggesting that it was the main aphid species involved in the spread of CABYV in melon crops in southeastern Spain. It is more plausible that the incomplete control of CABYV by *Vat*-carrying plants is related to transmission of the virus by *A. gossypii* clones able to colonize *Vat*-carrying plants. Such adapted clones were collected in southeastern France (Boissot *et al.*, 2016b). They may explain why CABYV development was not totally controlled in R plots.

Vat had no impact on CABYV population structure. All CABYV isolates from the trials were closely related molecularly based on comparison of capsid gene sequences and showed also a high degree of similarity with the first CABYV isolate sequenced, originating from southwestern France (Lecoq *et al.*, 1992). There was no evidence for recombination in the sequenced region, but longer sequences should be analyzed to confirm the absence of recombinants, because recombination has been described for other parts of the CABYV genome (Knierim *et al.*, 2010). To date, the phylogeny of CABYV populations shows that Asian isolates differ from those collected in the Mediterranean region (Kassem *et al.*, 2013). The French CABYV isolates all belong to the same molecular group, defined as ‘Iib’ based on Spanish isolates (Kassem *et al.*, 2013) (Figure 5). All haplotypes defined in this study clustered together, with the exception of the divergent haplotype G observed 3 times during the assay in 2011, 2013 and 2015 in R and S melon plants. Its sequence was identical or very similar to some isolates from southeastern Spain that constitute a well-supported subgroup within group Iib. The lack of strong phylogenetic differentiation of CABYV populations in the Mediterranean basin is probably an indication of frequent long-distance exchanges, either through viruliferous aphids or *via* human transfers of infected plant material. Alternatively, the presence of the same haplotypes over multiple years in the assay could be due to maintenance of the virus in local reservoir host plants that occur frequently around the experimental plots (Lecoq *et al.*, 1992;

Lecoq and Desbiez, 2012). Although the *Vat* gene significantly reduced CABYV epidemics, the structure of virus populations was not significantly different in R and S crops, showing that the selective pressure exerted by *Vat* is weak, or that it applies to a few target sites that may not be located in the CP.

4.3. Effect of *Vat* on CMV

Vat significantly reduced CMV mean incidence. The intensity of the reduction was highly variable (5-83%) and inversely proportional to mean incidence. The reduction became smaller with increasing incidence. None of the Gompertz model parameter estimates was significantly affected by *Vat*, although it occasionally induced a delay of 7-8 days in disease onset and/or a reduced incidence at harvest. So, the effect of *Vat* on the temporal progress of CMV epidemics was trial-dependent suggesting that biotic and/or abiotic factors involved in the epidemiology of this virus were variable among trials.

The partial effect of *Vat* on CMV epidemics in the field can be explained by different elements. First, the resistance to CMV triggered by *A. gossypii* is clone specific (Boissot *et al.*, 2016b). All *A. gossypii* clones collected to date from Cucurbits in southeastern France have been found to trigger resistance to CMV in the laboratory. These clones belong to the Cucurbit host race, and numerous clones belonging to other host races also visit melon crops in the spring in southeastern France (Thomas *et al.*, 2012). Clones not belonging to the Cucurbit host race may be viruliferous but may not elicit resistance to CMV in *Vat*-carrying plants. Second, many aphids in addition to *A. gossypii* species visit the melon crops (Schoeny, unpublished data) and do not trigger the resistance to CMV, as previously shown (Lecoq *et al.*, 1979, 1980). Interestingly, the *Vat* resistance to CMV appears to be highly durable as suggested by the absence of CMV evolution toward overcoming the gene (Boissot *et al.*, 2016b). Moreover the

low variability of CMV observed in this study (data not shown) also suggests that in fields, *Vat* resistance did not lead to selection of particular CMV isolates.

4.4. Effect of *Vat* on WMV

Vat had no favorable impact on WMV epidemics except in one trial for which *Vat*-carrying plants exhibited delayed disease onset by 8 days and reduced disease progress. Preliminary field studies already indicated that *Vat* had a very limited effect on WMV (Gray *et al.*, 1986; Lecoq and Pitrat, 1989). As with CMV, the effect of *Vat* on WMV was characterized at the laboratory, but fewer *A. gossypii* clones were tested. Nevertheless, if we consider that clones that trigger resistance to CMV also trigger resistance to WMV, then the effect of *Vat* on CMV and WMV is expected to be similar in the field if both viruses are transmitted by the same aphid clones and/or species. Our results clearly showed that it was not the case and suggested that WMV was less frequently transmitted by *A. gossypii* than CMV. Spatial analyses also suggested that different aphid species could be the main vectors of CMV and WMV in melon crops in Spain (Alonso-Prados *et al.*, 2003).

Vat had no impact on WMV population structure. Four of the molecular groups and subgroups already known to be present in southeastern France have been observed in the assay. This includes a ‘classic’ (CL) group that has been present in France for at least 40 years, as well as an ‘emerging’ group (EM) probably of Asian origin, observed in France since 2000, that is currently spreading in the Mediterranean basin and is locally replacing CL isolates (Desbiez *et al.*, 2009; Lecoq and Desbiez, 2012). Subgroup EM1 was the most frequently observed over the past 15 years in an experimental plot 4 km away from where these assays were conducted (Lecoq *et al.*, 2014). In this region, CL isolates were entirely replaced by EM1 isolates within a few years, possibly in relation to fitness differences in crops or reservoirs (Fabre *et al.*, 2010;

Lecoq *et al.*, 2011, 2014), whereas CL and EM isolates seem to have reached an equilibrium in southeastern France (Desbiez *et al.*, 2009; Fabre *et al.*, 2010). No recombination was detected in the sequenced region in the assay. However, even though recombinants have been described in the CP coding region of WMV, recombination hotspots are located in other parts of the genome (Desbiez and Lecoq, 2008; Desbiez *et al.*, 2011). The same molecular groups and subgroups were present in R and S melon plots, and the frequencies of the major haplotypes were not significantly different, indicating that *Vat* has no strong effect on the population structure of the non-persistently transmitted WMV. Accordingly, we showed that in most cases *Vat* had no effect on the development of WMV epidemics, and it was shown that WMV, like CMV, could not adapt to *Vat*-mediated resistance under experimental conditions (Boissot *et al.*, 2016b).

4.5. How to reinforce the efficiency of *Vat*?

Because *Vat* does not reduce virus epidemics as expected by farmers for a resistance, *Vat* should be combined with other genes conferring resistance to the specific viruses. The seminal work by Lecoq and Pitrat (1983, 1989) based on PI 161375 melon line that carried both *Vat* and the oligogenic and partial recessive resistance to CMV ‘common’ strains (Guiu-Aragones *et al.*, 2014) illustrates the fact that combining both types of resistance is of value. A recent work by Boissot *et al.* (2015) based on field data and modeling also suggested that combining *Vat* with an oligogenic resistance to CABYV (Dogimont *et al.*, 1997; Kassem *et al.*, 2015) may increase the efficiency of CABYV resistance on the long term.

Resistant cultivars should be at the heart of virus disease management strategies whenever available. Complementary practices could be associated to ensure an efficient and durable virus control. In particular, cultural practices intended to delay or reduce virus spread such as weeding

to remove virus reservoirs and the use of plastic mulches acting as aphid repellent have been shown to be partially efficient (Lecoq and Pitrat, 1989). An appropriate manipulation of field margins could also contribute to regulate the populations of aphids and/or their virus load. For example, flower strips can participate in aphid biological control by favoring natural enemies (Pfiffner and Wyss, 2004) and strips of non-host plants can protect crops from non-persistently aphid-transmitted viruses by allowing aphids to probe on healthy plants and thus to lose their virus load before reaching the crops (Hooks and Fereres, 2006). So, combining *Vat* resistance with sown flower strips could possibly enhance virus control in melon crops. Such innovative cropping systems remain to be tested.

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Table 1: Melon planting and virus sampling details for field trials conducted in Avignon between 2011 and 2015

Trial code	Date of planting	Date of first sampling	Number of sampling dates	Number of plants per modality	Number of plants sampled per modality per date (sampling effort %)
V11	09/05/11*	16/05/11	10	120	24 (20%)
V12	11/05/12*	21/05/12	8	150	24 (16%)
V13	06/05/13*	13/05/13	11	150	24 (16%)
P11	24/05/11	31/05/11	9	208	40 (19%)
P12	31/05/12	06/06/12	8	240	40 (17%)
P13	24/05/13	19/06/13	7	240	32 (13%)
P14	27/05/14	04/06/14	8	240	40 (17%)
P15	28/05/15	05/06/15	8	240	40 (17%)

*Agryl P17 fleece removal; fleece optimizes plant growth by increasing both air and soil

temperatures and reducing wind damage

Table 2: Number of CABYV and WMV isolates sequenced from different trials and years. Isolates were obtained from epidemics on melons crops with (R) or without (S) the *Vat* gene in eight field trials conducted in Avignon between 2011 and 2015.

Year (corresponding trials)	CABYV		WMV	
	R	S	R	S
2011 (P11+V11)	20	24	28	22
2012 (P12+V12)	11	17	34	19
2013 (P13+V13)	23	23	33	39
2014 (P14)	9	15	24	29
2015 (P15)	16	35	34	37
Total	79	114	153	146

Table 3: Parameter estimates of virus disease progress curves and mean incidence on melons crops with (R) or without (S) the *Vat* gene in eight field trials conducted in Avignon between 2011 and 2015. The p-value of the sign test performed between R and S values is given in bold.

Virus	Trial	Parameter ^x						Mean incidence (%) ^y	
		α		β		γ		R	S
		R	S	R	S	R	S		
CABYV	V11	0.0071	0.0237	8	176	100	100	38.3	55.7
	V12	0.0041	0.0052	7	4	100	92	11.6	27.1
	V13	0.0129	0.0187	58	135	96	96	24.8	33.5
	P11	0.0159	0.0204	17	16	100	100	58.7	66.8
	P12	0.0097	0.0119	7	8	65	87	33.6	49.2
	P13	0.0074	0.0111	13	21	87	89	19.3	28.8
	P14	0.0039	0.0073	8	10	100	81	14.5	29.6
	P15	0.0091	0.0103	21	13	61	100	23.3	51.4
p-value		0.008		>0.10		>0.10		0.008	
CMV	V11	0.0142	0.0095	51	17	9	37	3.7	13.6
	V12	0.0214	0.0368	91	1065	88	100	41.4	54.4
	V13	0.0116	0.0147	79	199	81	98	16.3	21.0
	P11	nd ^z	0.0044	nd	21	nd	100	0.7	4.2
	P12	0.0176	0.0155	24	10	100	100	59.0	63.3
	P13	0.0226	0.0375	116	569	100	98	48.4	60.7
	P14	0.0183	0.0181	55	33	100	100	58.7	62.0
	P15	0.0182	0.0196	111	69	94	99	49.7	58.9
p-value		>0.10		>0.10		>0.10		0.008	
WMV	V11	0.0062	0.0046	20	9	100	97	16.8	16.8
	V12	0.0070	0.0046	10	7	56	12	13.2	1.9
	V13	0.0180	0.0303	5835	1.1e6	78	84	7.8	10.6
	P11	0.0155	0.0106	1077	285	66	100	13.0	10.3
	P12	0.0124	0.0151	28	112	77	88	32.8	33.3
	P13	0.0363	0.0254	9.5e5	21794	100	100	22.6	20.1
	P14	0.0141	0.0165	228	142	100	98	29.8	43.4
	P15	0.0339	0.0257	50078	3933	99	99	45.1	45.7
p-value		>0.10		>0.10		>0.10		>0.10	

^x Parameters were estimated by fitting the Gompertz model to observed data:

$$y_t = \gamma e^{-\beta e^{-\alpha t}}$$

in which y_t is the incidence expressed as a percentage and t is the thermal time expressed in degree-days (basis 12°C); α is linked to disease progress: when α increases, the epidemic is more rapid; β is linked to disease onset: when β increases, the epidemic is delayed; γ is the asymptote, i.e., the carrying capacity: when γ increases, maximum incidence is raised.

^y The mean incidence over the epidemic was approximated by the area under the disease progress curve (AUDPC) standardized by the total duration of the monitoring in each trial. On the basis of their mean incidence, viral epidemics were categorized as mild [0-20%], intermediate [21-40%], severe [41-60%] or extreme [61-100%].

^z nd=not determined (procedure NLIN failed to converge)

Table 4: P-values for the effect of *Vat* on the probability of infection of one melon plant by a given virus in eight field trials conducted in Avignon between 2011 and 2015. P-values in bold represent a significant effect of *Vat*. Unless otherwise noted, *Vat* reduces the probability of infection.

Trial	Virus		
	CABYV	CMV	WMV
V11	0.0005	0.0197	>0.10
V12	0.0230	0.0088	0.0058*
V13	0.0530	0.0387	>0.10
P11	0.0013	0.0203	>0.10
P12	0.0077	>0.10	>0.10
P13	0.0028	0.0007	0.0966
P14	0.0083	>0.10	0.0007
P15	<0.0001	0.0063	>0.10

* (R>S)

Figure legends

Figure 1: CABYV disease progress in melon crops with (R) or without (S) the *Vat* gene in eight field trials conducted in Avignon between 2011 and 2015. Symbols represent observed mean incidences for each treatment. Lines are fitted curves (Gompertz model).

Figure 2: CMV disease progress in melon crops with (R) or without (S) the *Vat* gene in eight field trials conducted in Avignon between 2011 and 2015. Symbols represent observed mean incidences for each treatment. Lines are fitted curves (Gompertz model).

Figure 3: WMV disease progress in melon crops with (R) or without (S) the *Vat* gene in eight field trials conducted in Avignon between 2011 and 2015. Symbols represent observed mean incidences for each treatment. Lines are fitted curves (Gompertz model).

Figure 4: (a) Haplotypic network based on 193 partial CABYV sequences obtained from field trials conducted in Avignon between 2011 and 2015. Each pattern corresponds to a different haplotype. Branch lengths are proportional to the number of mutations between haplotypes; the scale bar represents one mutation. Node sizes are related to the number of isolates sharing the same haplotype. (b) Frequency of the different CABYV haplotypes in melon plants with (R) or without (S) the *Vat* gene

Figure 5: Distance tree based on partial CP/MP coding nucleotide sequences for the 13 CABYV haplotypes observed on melon plants with (R) or without (S) the *Vat* gene in field trials conducted in Avignon between 2011 and 2015. Genbank accession numbers and geographic origin of the isolates are indicated on the tree. Bootstrap values (n=500 bootstraps) above 50%

are indicated for each node. The asterisk indicates the « Spanish » subgroup containing haplotype G.

Figure 6: Distance tree based on partial Nib-CP coding nucleotide sequences for the 27 WMV haplotypes observed on melon plants with (R) or without (S) the *Vat* gene in field trials conducted in Avignon between 2011 and 2015. Bootstrap values (n=500 bootstraps) above 80% are indicated for each node. Reference isolates are framed for each subgroup.

Figure 7: Frequency of the different WMV molecular groups observed in melon plants with (R) or without (S) the *Vat* gene in field trials conducted in Avignon between 2011 and 2015

Figure 1

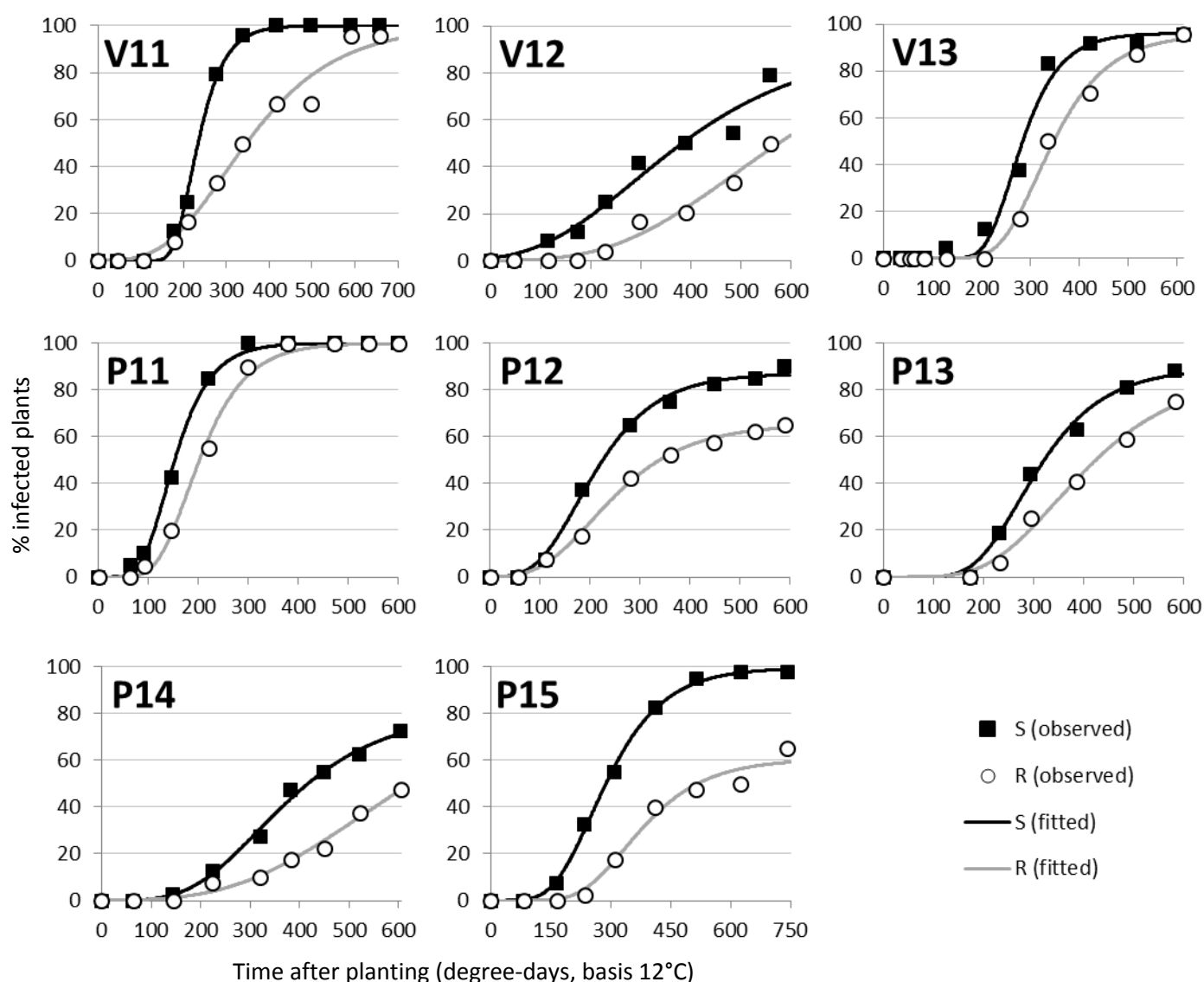


Figure 2

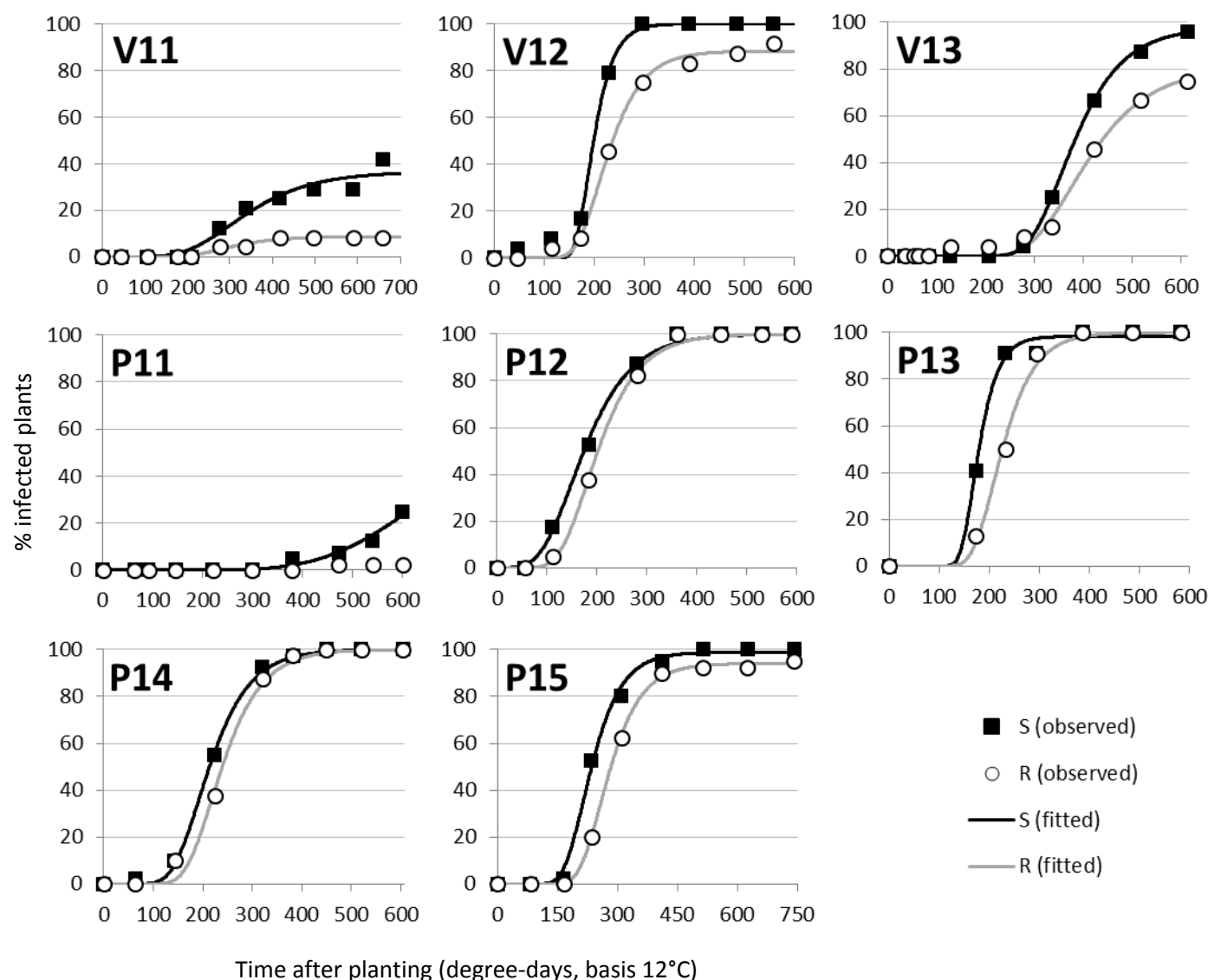


Figure 3

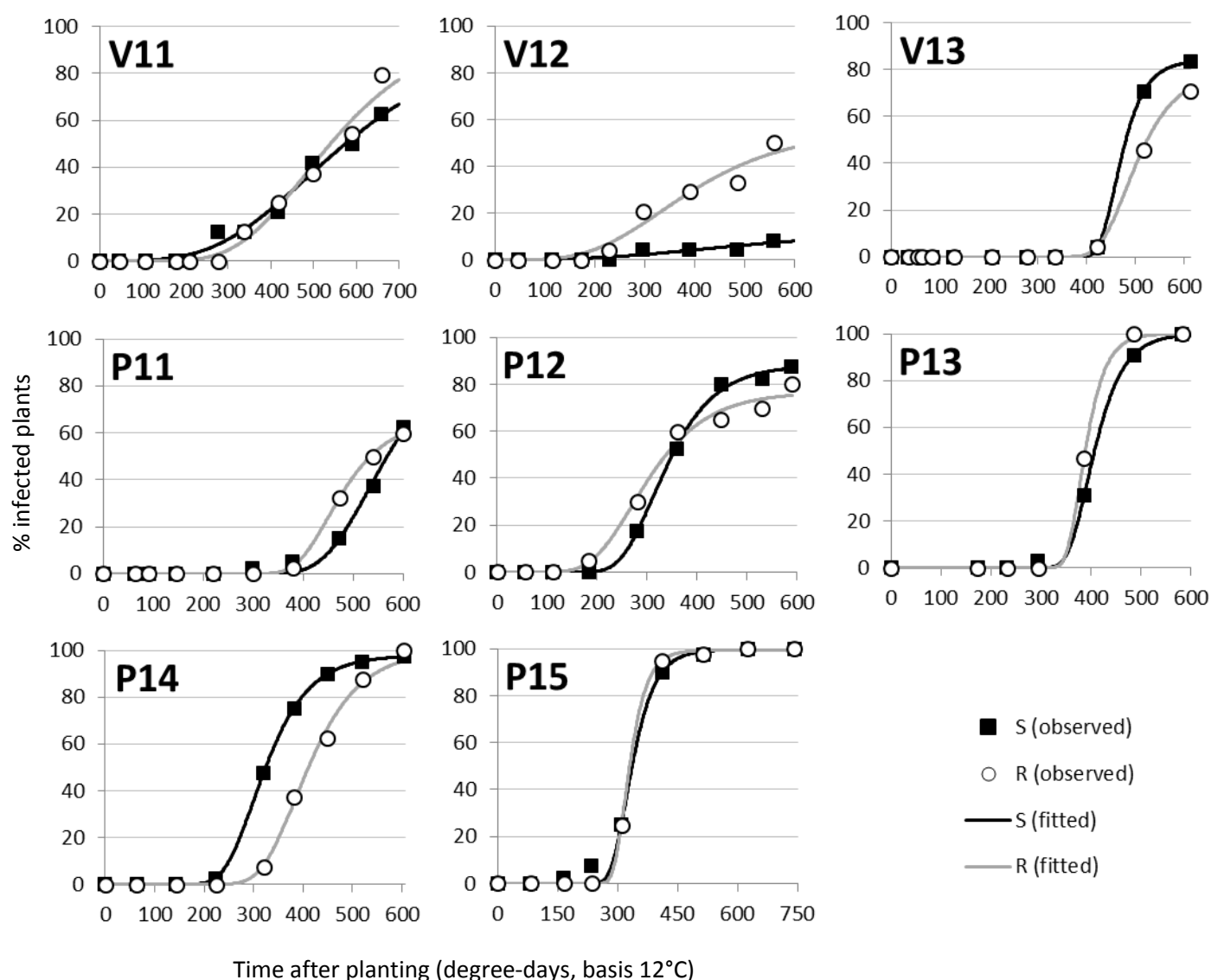


Figure 4

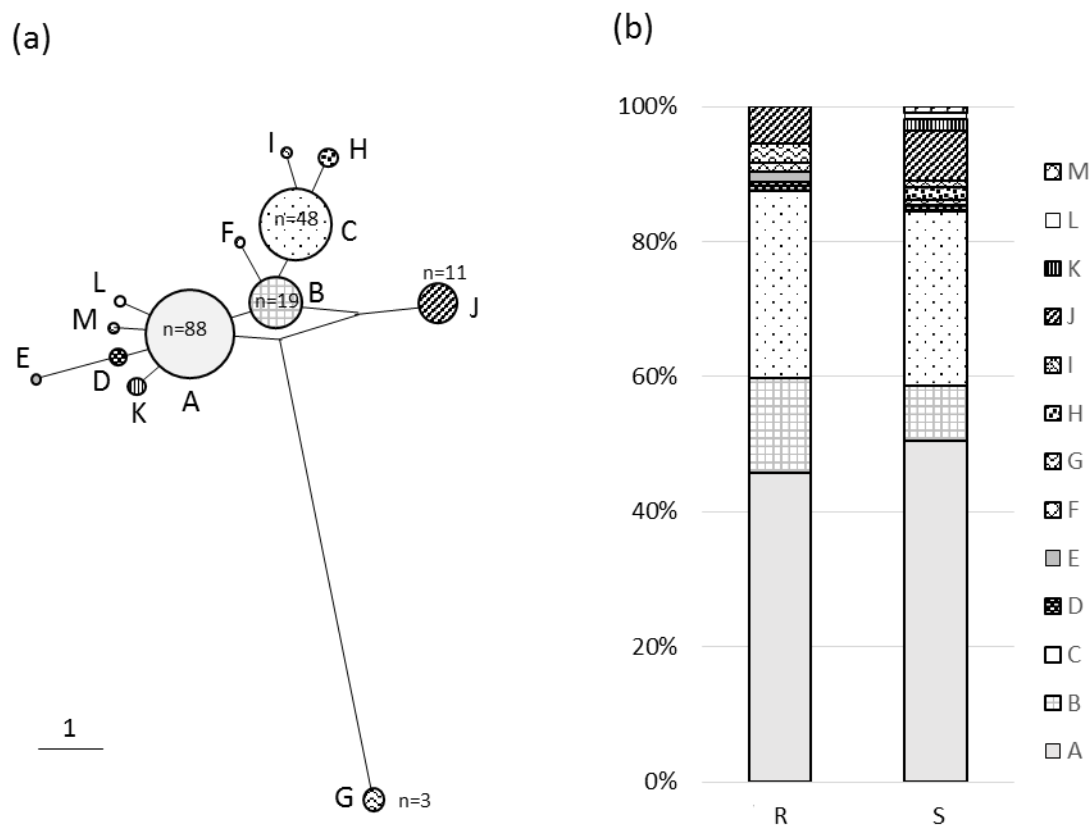


Figure 5

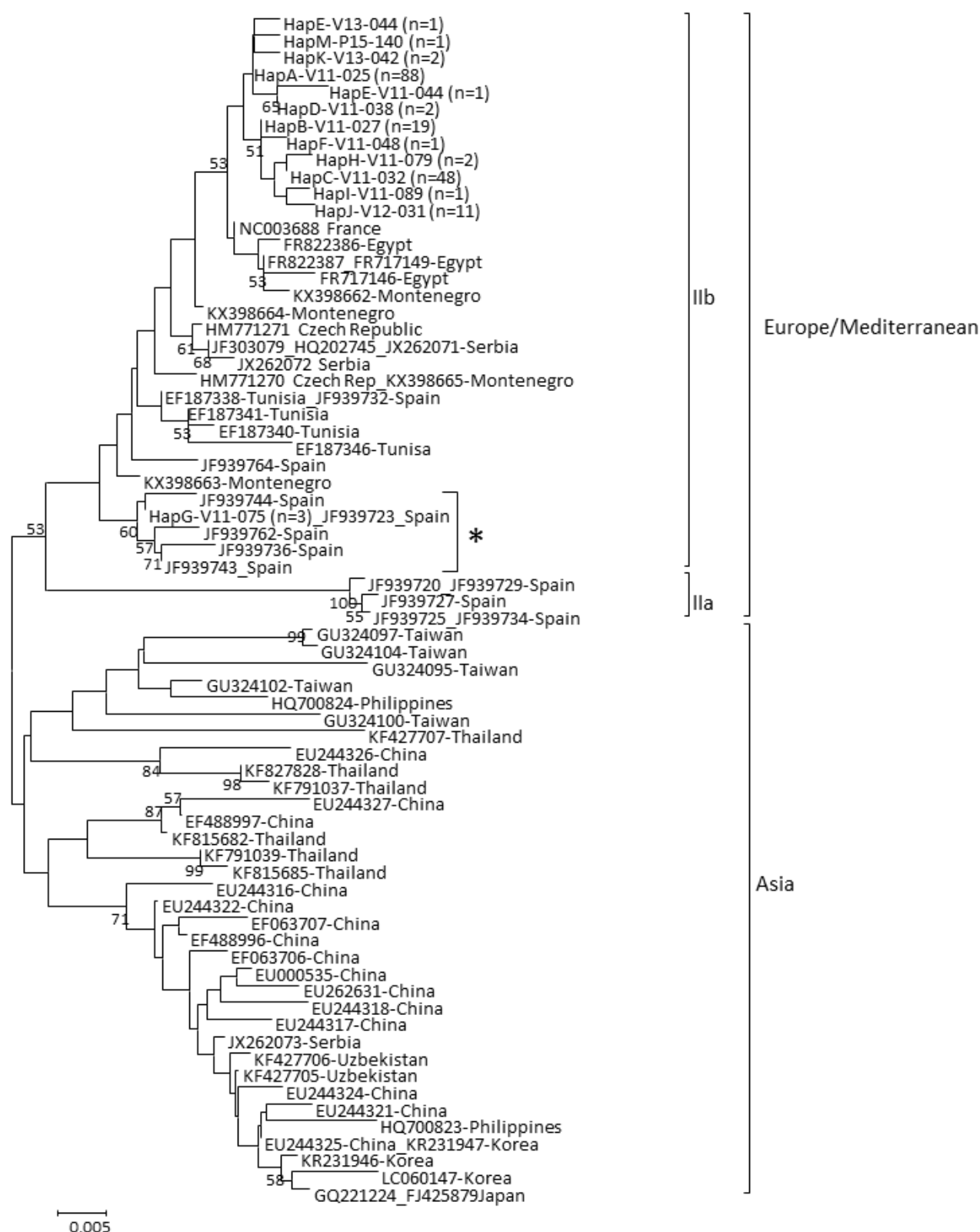


Figure 6

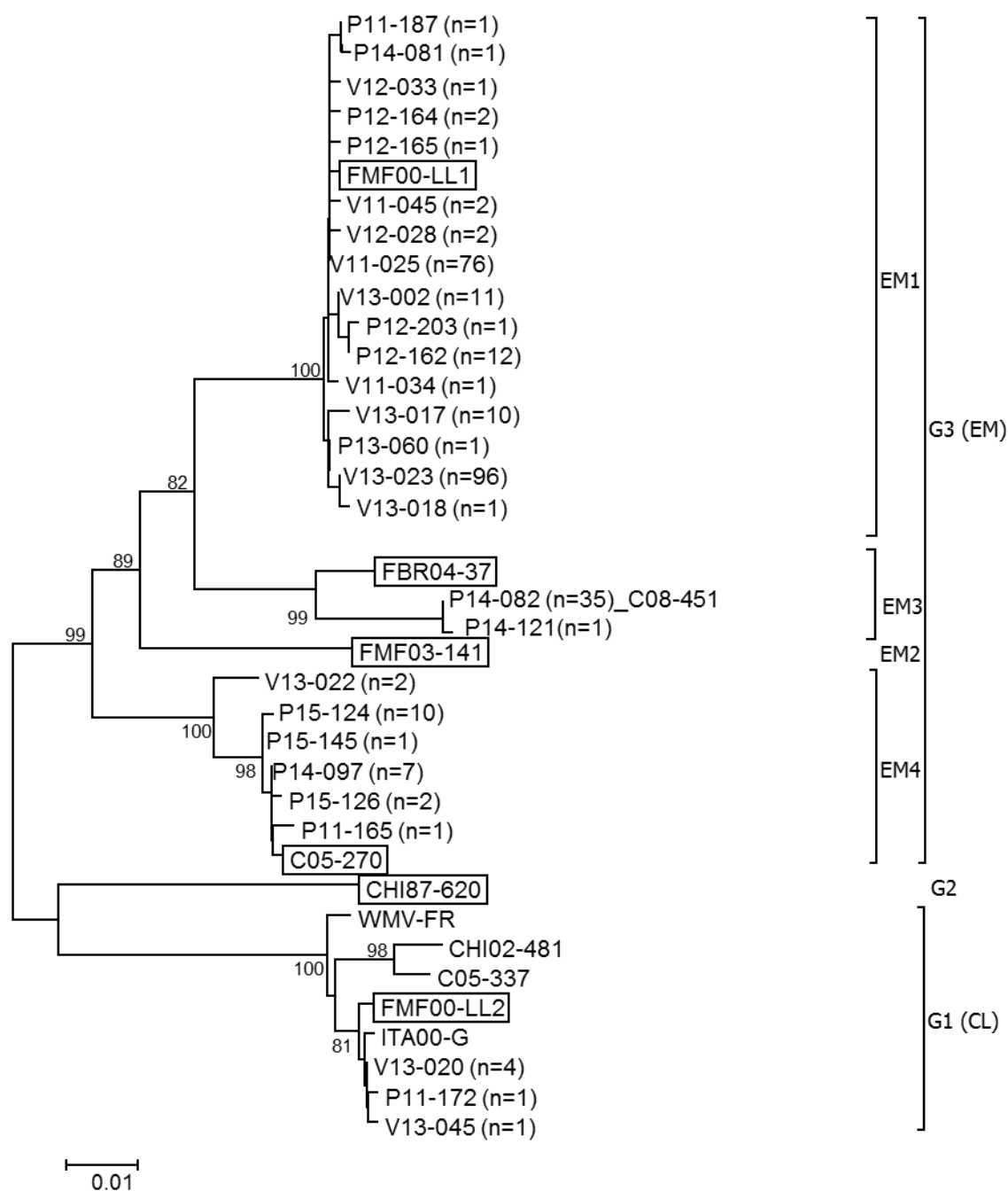


Figure 7

