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PNAS, 2007, vol.104, no.45, 17891-17896

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Author contributions: B.M. and F.F. contributed equally to this study. B.M. conceived and performed the experiments, R.S., F.F. and B.M. conceived the model, F.F. performed modelling and B.M., F.F. and R.S. wrote the paper.

Abbreviations: PVY, *Potato virus Y*; AAP, acquisition access period; IAP, inoculation access period; VPg, viral protein genome-linked; CP, coat protein; Hc-Pro, helper component-proteinase; λ_a, total number of PVY particles transmitted by an aphid to a plant after *in vitro* acquisition; λ_a*, total number of PVY particles transmitted by an aphid to a plant after *in planta* acquisition; κ, number of aphid probing punctures required to inoculate the acquired virus particles; λ_p, number of PVY particles required to infect a plant.

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The manuscript includes 21 pages, 2 tables and 2 figures. Additional supporting information includes a Methods section and 2 figures. The abstract includes 137 words and the total number of characters is 44,177.

Plant viruses are submitted to narrow population bottlenecks both during infection of their hosts and during horizontal transmission between host individuals. The size of bottlenecks exerted on virus populations during plant invasion has been estimated in a few pathosystems, but is not addressed yet for horizontal transmission. Using competition for aphid transmission between two *Potato virus Y* variants, one of them being non-infectious but equally transmissible, we obtained the first estimates of the size of bottlenecks exerted on an insect-borne virus during its horizontal transmission. We found that an aphid transmitted on average 0.5 to 3.2 virus particles, which is extremely low compared to the census viral population into a plant. Such narrow bottlenecks emphasize the strength of stochastic events acting on virus populations and we illustrate, in modelling virus emergence, why estimating this parameter is important.

Horizontal transmission to switch host individuals, which is a critical step in the life cycle of parasites, is ensured by specific biological vectors for many plant and animal viruses. Two major categories of plant viruses, persistent and nonpersistent, can be distinguished according to their transmission mode. Nonpersistent viruses have very short retention times in their vector, usually less than 12 hours. Acquisition and inoculation access periods (AAP and IAP, respectively) are short for these viruses (less than 5 min). Nonpersistent viruses are not simply transmitted by pure mechanical processes (the vector acting as a "dirty needle") but a high degree of specificity exists between vector and viral components [1]. This kind of virus transmission, largely shared by plant viruses, seems absent among animal viruses. An exception could be *Lumpy skin disease virus* (family *Poxviridae*, genus *Caprifox*), which is transmitted by the mosquito *Aedes aegypti* in a more complex way than by a "dirty needle" [2], but information on the characteristics of the virus - vector interaction is still lacking.

Persistent viruses, in contrast, require longer AAP and IAP, and retention of the virus can last from around 12 hours to the lifetime of the vector. Between acquisition and inoculation, a latent period of one to several days is necessary for persistent viruses to be transmitted, while it is not for nonpersistent ones.

Aphids are the most widespread plant virus vectors and can transmit both persistent and nonpersistent viruses. More than 200 plant virus species are transmitted nonpersistently by aphids. Virus particles inoculated to plants are previously retained either directly or indirectly in the mouthparts of aphids. In the case of indirect interaction, binding of virus particles to the aphid mouthparts is mediated by one or more viral-encoded proteins, the helper component [3]. For example, aphid transmission of viruses from the genus *Potyvirus* involves the helper component-proteinase (HC-Pro) protein, which plays the role of a 'molecular bridge' by its interactions with the virus coat protein (CP) and with the vector's stylet [4]. As a consequence, only mutations in the HC-Pro and CP of potyviruses have been shown to affect their transmissibility by aphids. The aphid receptors allowing retention and inoculation of nonpersistent viruses are unknown, but are presumably localized at the distal tip of the stylet bundle [5,6]. Virus acquisition and inoculation occur during the brief (around 10 s) intracellular punctures (or probes) exerted by aphids into epidermal or mesophyll plant cells to select their hosts.

The effects of horizontal transmission by vectors on the evolution of plant virus populations are largely unknown. Vectors are presumed to impose both selective and bottlenecks effects on virus populations. The selective impact of aphids was clearly demonstrated by relaxing the aphid transmission constraint on plant virus populations, during series of mechanical inoculations in the laboratory [1]. A large number of virus variants issued from these experiments were shown to be poorly or non transmissible by vectors. Differential transmission efficiency of virus variants between different aphid species was also

shown experimentally [7] and could be a major evolutionary constraint on some virus populations [8]. Genetic drift was also demonstrated recently by the stochastic extinction of genotypes in CMV populations after nonpersistent aphid transmission due to narrow bottlenecks during transmission [9]. Besides these qualitative results, estimating the size of the bottlenecks exerted by vectors is crucial to quantify their consequences on virus evolution.

Very few studies have attempted to estimate the size of bottlenecks exerted on plant virus populations, either persistent or nonpersistent, during transmission by vectors. Pirone and Thornbury [10] estimated the number of particles of *Tobacco etch virus* or *Tobacco vein mottling virus* (genus *Potyvirus*) in aphids after acquisition of purified, radioactively-labelled virus. In their experiments, individual aphids that succeeded in infecting a plant after the transmission procedure acquired from 15 to 20,760 potyvirus particles. Given the relatively small number of aphids analysed and given such a huge range of variation, it remains important to get a more precise estimate of the average number of acquired virus particles. In addition, these figures certainly overestimate the number of transmitted virus particles because they include virions that did not contribute to plant infection, *i.e.* (i) those that were ingested, (ii) the stylet-borne virions that will not be inoculated to the plants and (iii) stylet-borne virions that will be inoculated to the plants but that are unviable due to deleterious mutations.

In the present article, we analyse results of competitions for aphid transmission between two *Potato virus Y* (PVY; genus *Potyvirus*) variants to get an estimate of the size of population bottlenecks during transmission. By-products of our analyses are estimates of two other important epidemiological parameters, the number of virus particles required to infect a plant and the number of probes made by aphids to inoculate the acquired virus particles.

Results

Evidence for population bottleneck during PVY transmission by aphids revealed by competition between virulent and avirulent virus variants.

In our experiments, virus acquisition was carried out by allowing aphids to feed on artificial mixtures of two PVY variants (SON41 and SON41xVPgLYE84.2 [11], named in the following Vir and Avir, respectively) in different relative proportions in an *in vitro* device [12]. The two PVY variants differ only by 14 nucleotide substitutions (corresponding to five putative amino acid substitutions) in the VPg (virus protein genome-linked) cistron. These substitutions determine the ability to infect (called virulence in the field of plant pathology [13]) pepper (*Capsicum annuum* L.) plants carrying the $pvr2^1$ recessive resistance allele [11]. The Vir variant is virulent toward $pvr2^1$ while the Avir variant is not (*i.e.* it is avirulent). However, both Vir and Avir are virulent toward the $pvr2^+$ allele. After feeding, aphids were transferred onto pepper plants for inoculation and the number of infected plants was recorded for each Vir:Avir ratio (Table 1, columns I-VIII, XI and XII; Fig. S1).

When a single aphid per plant was used for PVY transmission, the infection frequency was low and decreased rapidly as the proportion of the Vir component decreased. Consequently, the experiment was not much informative and confidence intervals of estimated parameters were excessively large (data not shown). We therefore used two aphids per plant for inoculation in the following experiments. Comparing the infection frequency of 'Yolo Wonder' plants ($pvr2^+/pvr2^+$) inoculated after acquisition from a medium containing only the Vir or the Avir variants with identical concentrations showed no significant differences ($0.35<P\text{-}value<0.69$, depending on the experiments; Fisher's exact test; Table 1, columns XI and XII). We may consequently consider that the two PVY variants share the same aphid transmission efficiency.

A marked decrease of the frequency of PVY-infected 'Yolo Y' ($pvr2^1/pvr2^1$) plants was observed as the relative proportion of the Vir component decreased in the acquisition

medium (Fig. 1). This was not due to the decrease of the absolute concentration of the Vir component in the acquisition medium. Indeed, a strong competition effect is revealed if we compare the frequencies of 'Yolo Y' plants infected from a medium containing 10% of Vir to those obtained from a ten fold dilution of the Vir variant in buffer. The absolute concentration of the Vir component is the same (3.5 ng/µl) in both acquisition media, but the infection frequency is drastically reduced in case of competition for transmission between the two PVY variants (*P-values* < 3×10^{-4}, Fisher's exact test; Table 1, columns II and VII). This is evidence that there is a limited number of binding sites in the aphid stylet and that the number of Vir particles transmitted to the plants is drastically reduced when the Avir component is also present in the acquisition medium, therefore revealing that a bottleneck affected the total PVY population.

Estimation of the size of bottlenecks affecting PVY populations during transmission

A simple stochastic model was developed to estimate the size of bottlenecks affecting PVY populations during aphid transmission from the experiments described above. The model breaks down the virus transmission mechanism into three successive steps, all described by Poisson processes (see Supplementary Information for a detailed presentation of the model): (i) virus acquisition and (ii) virus inoculation by aphids and (iii) plant infection. Each step introduces a single parameter in the model: λ_a, the average number of PVY particles acquired *in vitro* (and further inoculated) by an aphid, κ, the average number of probing punctures required by an aphid to inoculate the acquired viruses and λ_p, the average number of Vir PVY particles sufficient to infect a plant. For the plant infection step, we favoured a "non additive" model, where infection was considered to be the independent issue of each aphid probe. This model corresponds to results obtained previously [14] and is consistent with the fact that two potyvirus variants occupy different sets of cells in a co-inoculated plant [15].

Our results showed that PVY transmission was more efficient when aphids had acquired the virus from infected pepper leaves than from artificial media containing purified virus. Using the Vir variant alone, the percentage of infected 'Yolo Y' plants was always higher when plants were inoculated with one aphid fed on infected leaves than with two aphids fed on artificial medium (Table 1, columns I, II and IX). This could be due either to differences in aphid behaviours between the two virus acquisition protocols, to a damaging effect of the purification step on the virus viability or to a lack of the helper component. As a consequence, estimates of λ_a obtained with PVY acquisition from artificial medium would underestimate the mean number λ_a* of particles transmitted after acquisition from PVY-infected leaves. In contrast, λ_p and κ should be identical for both acquisition protocols within experiments 1, 2 and 3. For a more accurate estimation of κ, we performed an additional experiment (Experiment 4, Table 1) which allowed the comparison of plant infection frequencies by aphids that either were allowed to perform a single probe for inoculation or that were not limited in their number of probes. The results of this experiment were close to those obtained independently by Martín et al. [6] for a similar PVY - *Myzus persicae* – pepper combination with the help of electrical penetration graphs to control the number of aphid probes.

Numerical simulations were used to check that, by combining several subsets of experiments, the four parameters described above were simultaneously identifiable using the maximum-likelihood principle (Fig. S1 in Supplementary information). The different experiments yielded remarkably consistent estimates of λ_a, λ_a*, κ and λ_p (Table 2). Parameters λ_a* and λ_p were found to be very small: λ_a* varied from 0.64 to 3.24 and λ_p varied from 1.65 to 5.13 between experiments. κ was found to vary from 4.37 to 5.25. When combining the results of our four independent experiments, thus incorporating the maximum of information into the model, an average of 1.2 to 1.4 PVY particles was found to be transmitted from plant to plant per aphid (Table 2). Accordingly, 99% of aphids transmitted five virus particles or less to the

plants. Note that the λ_a, λ_a* and λ_p parameter values that could be obtained under an alternative "additive" model for plant infection were very close to the ones obtained with the "non additive" model (data not shown).

Modelling the emergence of virus variants

A simulation model was developed to explore how bottlenecks during aphid transmission affect the evolution of plant virus populations (see Supporting Information for a detailed presentation of the simulation model). We considered a virus population composed of Vir and Avir individuals that undergo alternated growth phases within plants and periodical sampling phases during which a random fraction of the virus population is transmitted by aphids to initiate infection of a new plant. We investigated how the size of bottlenecks N during sampling phases, the fitness cost s associated with virulence and the aggregation rate θ of the Vir and Avir particles within plants affected (i) the probability π of extinction of the Vir viruses in the population and (ii) the time τ required to achieve this extinction.

Regarding the probability of extinction, in the absence of any fitness cost of virulence (s=0), π is close to 0.5, whatever N and θ (Fig. 3A). If s>0, the Vir viruses will always get extinct (π=1), except if stochastic effects occur due to bottlenecks or virus aggregation. Depending on s, there is a threshold value N_I of N beyond which extinction of the Vir viruses is nearly certain (Fig. 3A). In the (s,N) plane, the area where the extinction of Vir viruses is nearly certain (let's say higher than 0.95, or equivalently, where the odds of π, $\pi / (1- \pi)$, is higher than 19) defines an upper-right triangle where N_I decreases linearly (in a x-log, y-log scale) with s (Fig. 3B). Importantly, in our range of N estimates (N<10), extinction of Vir viruses is nearly certain only for high fitness costs (s>0.1). Aggregation of Vir and Avir particles counterbalances the effect of s by increasing the strength of stochastic effects, *i.e.* for a given

s, N_1 is increased (Fig. 3A). In the (s,N) plane, the area where the extinction of Vir viruses is nearly certain is consequently reduced (Fig. 3C).

Regarding the time required to achieve extinction of the Vir viruses, the mean of τ ($\bar{\tau}$) increases linearly with N (in a x-log, y-log scale) in the absence of any fitness cost of virulence (s=0). When s increases, the plot of $\bar{\tau}$ *versus* N curves downwards with increasing values of N (Fig 3D). However, as previously, a threshold value N_2 of N appears below which $\bar{\tau}$ depends mainly on N and little on s. There, whatever s, plots of $\bar{\tau}$ *versus* N are all merged. For a given fitness cost s, the ratio $\bar{\tau}(s,N)/\bar{\tau}(0,N)$ is thus close to one when $N<N_2$ (Fig. 3D). In the (s,N) plane, the area where N is the major determinant of $\bar{\tau}$ ($\bar{\tau}(s,N)/\bar{\tau}(0,N)>0.9$) defines a lower left triangle, N_2 decreasing linearly with s (Fig. S2 in Supplementary Information). Importantly, in the range of our estimates of N (N<10), $\bar{\tau}$ is mostly determined by N in a large range of s [10^{-4}, 0.03]. Even moderate rates of aggregation of Vir and Avir particles within plants decreased $\bar{\tau}$, especially for high N values (Fig. 3D). In addition, virus aggregation reduced the effect of s by enlarging the area where N is the major determinant of $\bar{\tau}$ for higher N values [100, 1000], N_2 decreasing only slightly for N>100 (Fig. S2 in Supplementary Information).

Discussion

Are stochastic effects predominant in plant virus evolution?

We obtained the first estimates of the size of the bottleneck acting on plant virus populations during vector transmission. Occurrence of bottlenecks during nonpersistent aphid transmission was recently demonstrated for CMV [9] but the authors did not provide any estimate for the size of bottlenecks. In their experiments, artificial populations of 12 virulent variants of CMV were used in acquisition media for aphid transmission. The decrease of the number of CMV variants detected in inoculated plants evidenced the bottleneck effect of

aphids during virus transmission. It seems however difficult, from these experiments, to untangle the bottleneck effect of aphids during transmission from the bottleneck effect during plant invasion, which was shown to be quite severe [16-18]. In contrast, our analysis of the bottleneck effect exerted by aphids was not based on the loss of richness of virus populations between the inoculum and the inoculated plants, but on the competition between two virus variants, one of them being non-infectious (avirulent). As a consequence, genetic drift potentially acting on viral populations during plant infection did not affect our parameter estimates. Our estimates of the size of bottlenecks during potyvirus transmission by aphids were much smaller than those of Pirone and Thornbury [10], even if the comparison is partial since no average values were given in their study. Reasons of such differences hold certainly for the fact that their estimates included all the virus particles present in the aphid body, while only those retained in the aphid stylet bundle can be efficiently transmitted to the plants. Combined with the high diversity of virus populations owed to their high mutation rates, a consequence of such narrow bottlenecks is certainly an intense genetic drift, since the huge majority of the virus variants will have no progeny following aphid transmission.

Genetic drift seems to affect frequently and severely plant virus populations. Indeed, recent studies have demonstrated that drift occurs intensely during plant invasion by viruses. Only around ten virus particles contributed to the systemic infection of plants after mechanical inoculation [16,18] although several hundreds initiated an infection [18]. We show here that, in addition to genetic drift experienced by viral populations within plant hosts, extremely narrow bottlenecks also shape plant virus populations during nonpersistent aphid transmission. These repeated and narrow bottlenecks during virus epidemics may have several important consequences on virus evolution, such as decreases of fitness [19,20], of (broad-sense) virulence [21], of the capacity to respond to natural selection [22] and could also contribute to the differentiation of plant virus populations at the field or regional scales. Also,

the quasi-species model of viruses, which assumes an absence of genetic drift during the evolution of virus populations, could be irrelevant for some plant viruses due to the drastic effect of bottlenecks [23].

From an applied perspective, deriving estimates of parameters central to virus evolution is of major interest for modelling disease emergence. This is an important issue in agricultural production as illustrated by the breakdown of resistance genes by virulent virus isolates [24]. Mathematical models are key tools here in allowing to describe the interplay between evolutionary and epidemiological processes acting on virus population both at the within-host and at the plant population scales [25,26]. This is not classically done in plant virus epidemiology, where models are most often focused on the spatio-temporal dynamics of epidemics at the field scale [25]. Our simulation model illustrates nicely that bridging evolutionary and epidemiological processes which can operate at different scales is required to understand the emergence of virulent virus variants. It explores how describing the within-host dynamics of virus population (fitness cost s of virulence and heterogeneity θ of distribution of virus variants) and the between-host transmission (number N of virus transmitted by aphids), allow to predict the emergence of a virulent virus. Interestingly, we found that, in the range of our estimated N values, emergence of a virulent virus variant, while highly dependent on N, was rather insensitive to s, highlighting the poor responsiveness of the virus population to selection mentioned above. The insensitivity to s was still enhanced by increasing values of θ. θ is an important parameter to take into account for plant viruses, since compartmentalization of virus variants within plants was frequently observed [15,16,27] and may be the consequence of genetic drift that occurred at the plant tissue level during plant infection.

As a conclusion, we showed that estimating the number of virus particles involved in horizontal transmission in crucial for predicting the emergence of virus variants and we

provided such estimates for an aphid-transmitted nonpersistent plant virus. Our experimental approach based on the competition between a virus and a non-infectious but equally-transmissible variant, as well as our stochastic model, could be used for other kinds of viruses, provided the availability of an efficient *in vitro* acquisition protocol and of a suitable host resistance system.

Methods

PVY transmissions. The two PVY variants Vir and Avir (called SON41 and SON41xVPgLYE84.2, respectively, in Moury et al. [11]) were purified separately from infected tobacco (*Nicotiana tabacum* cv. Xanthi) plants using a modified version of method 1 of Moghal and Francki [28], where chloroform and carbon tetrachloride in the extraction buffer were replaced by 1,1,2-trichlorotrifluoro-ethane. Their concentrations were estimated with DAS-ELISA (double-antibody sandwich enzyme-linked immunosorbent assay) dilution – absorbance curves as in Moury et al. [11] by comparison to a reference purified stock of the Vir variant of known concentration. A virus-free fraction of Xanthi plants infected by the Vir variant containing 20% (w/v) sucrose was obtained as in Thornbury et al. [29] and added to the acquisition medium. This fraction contained the PVY HC-Pro, a protein required for aphid transmission.

Wingless aphids (*Myzus persicae* Sulzer) reared on *C. annuum* 'Yolo Wonder' plants in a growth chamber were starved during three to four hours prior acquisition. They were allowed to feed during five to six minutes in a medium containing the purified PVY variants in various relative proportions and half volume of the solution containing HC-Pro through a stretched Parafilm membrane in a similar way as in Govier et al. [12]. This AAP was shown to be optimal for transmission (not significantly different from a one-to-two minute AAP), while transmission efficiency decreased significantly for a ten-to-eleven or fifteen-to-sixteen minute

AAP (data not shown). The total (Vir plus Avir) PVY concentration was identical for all the artificial acquisition media (35 ng/µl), except one series of inoculations performed after acquisition from a medium containing 3.5 ng/µl PVY (Table 1, column II). Feeded aphids were transferred with a paintbrush to *C. annuum* 'Yolo Y' (*pvr2*^{1}/*pvr2*^{1}) or 'Yolo Wonder' (*pvr2*$^{+}$/*pvr2*$^{+}$) seedlings at the cotyledon or one-expanded-leaf stage (18 or 25 days after sowing, respectively), depending on the experiment. 'Yolo Wonder' and 'Yolo Y' are nearly isogenic inbred lines with different alleles at the *pvr2* locus [30]. Inoculations were performed with one or two aphids per plant, depending on the experiment, which were maintained on a single cotyledon of the plants overnight and treated with an insecticide. Additional experiments were performed using apical leaves of 'Yolo Wonder' plants infected with the Vir variant for acquisition. In one of these latter experiments, aphids were allowed to perform only one probe during the inoculation access period as assessed by the observation of their behaviour under the microscope (immobilization of aphids during a short period of about 10 seconds with typical posture of legs and antennae). Afterwards, they were immediately withdrawn from the plants with a paint brush. After the IAP, the plants were maintained in greenhouse conditions for one month. Their infection status was then checked by symptom reading and DAS-ELISA performed on apical, uninoculated leaves.

We thank Joël Chadœuf for his help in modelling, Benny Raccah for sharing his experience in PVY acquisition *in vitro*, Vincent Simon, Julien Petit-Pierre and Bérenger Janzac for their help in the transmission experiments, Catherine Rys for raising the aphids, and Hervé Lecoq and two anonymous reviewers for their stimulating comments on the manuscript. Funding by the ECOGER action of the National Programme ANR « Ecosphère continentale: processus, modélisation et risques environnementaux » is also acknowledged.

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Figure legends

Fig. 1. Competition for aphid transmission between PVY variants affects plant infection. Proportion of PVY-infected pepper plants (*Capsicum annuum* cv. 'Yolo Y', *pvr2*^{1}/*pvr2*^{1}) after inoculation by aphids that acquired the virus from an artificial mixture of the two variants Vir and Avir with different relative proportions. Only the Vir variant is able to infect (*i.e.* is virulent to) these plants. The results were obtained in three independent experiments (Table 1) with inoculations performed by two aphids per plant. Curves represent the results expected with the maximum-likelihood estimates under the "non additive" model for plant infection (see the "Methods" section). In experiment 3, inoculated plants were older.

Fig. 2. Effect of the size of population bottlenecks during aphid transmission on the emergence of virus variants.

The effect of (i) the size N of population bottleneck during aphid transmission, (ii) the fitness cost s of virulence and (iii) the rate θ of aggregation of Vir and Avir particles within plants is illustrated on both the probability π of extinction of the Vir variant in the viral population (A, B and C) and on the mean time $\bar{\tau}$ to achieve this extinction (D). (A) Plots of π versus N, without (θ=0 – black lines) and with (θ=0.026 – red lines) virus aggregation within plants, for three values of s. (B, C) Plots of the odds in favour of Vir extinction (*i.e.* $\pi/(1-\pi)$) in the (N, s) plane for θ=0 (B) and θ=0.026 (C). (D) Plots of $\bar{\tau}$ versus N, without (θ=0 – black lines) and with (θ=0.026 – red lines) virus aggregation within plants, for three values of s. Estimations of π and $\bar{\tau}$ are based on 25,000 simulations per (N, s, θ) parameter set. The value θ=0.026 describes a situation of moderate aggregation of Vir and Avir particles within plants; for N=5, the variance of the beta-binomial distribution used to describe the number of Vir viruses

transmitted between plants is 10% higher than the variance of the corresponding binomial distribution (see Supporting Information).

Supporting Information

SI Methods.

A stochastic virus transmission and plant infection model.

A simulation model to explore the effect of population bottlenecks in the emergence of plant virus variants.

SI Fig. S1. Theoretical and practical identifiability of parameters λ_a, λ_a*, κ, and λ_p.

A: The four parameters are theoretically identifiable if the structure of the model used for their estimations ensures the existence of a unique value for $\Phi = (\lambda_a, \lambda_a^*, \lambda_p, \kappa)$, for an infinite size dataset. This property was checked using numerical simulations as follows. First, 60 sets of Φ were drawn uniformly in intervals ($\lambda_a \in]0, 100]$, $\lambda_p \in]0, 100]$, $\lambda_a^* \in]\lambda_a, 2\times\lambda_a]$, $\kappa \in]0, 50]$). Then, datasets were simulated, each including three kinds of experiments: (i) virus acquisition from artificial medium with 100 Vir:Avir ratios and a 16 h inoculation access period (IAP) on 5,000 plants per ratio, (ii) virus acquisition from leaves infected with the Vir variant of PVY and a 16 h IAP on 10^5 plants and (iii) virus acquisition from leaves infected with the Vir variant of PVY and a single probe for inoculation on 10^5 plants. Then, for each dataset, parameters were jointly re-estimated using the maximum-likelihood method. The

correlation between true and re-estimated parameters indicates that Φ is theoretically identifiable.

B: Even when a model is theoretically identifiable, it may not be possible to retrieve efficient estimates of the parameters, due to insufficient informative data. The practical identifiability of the parameters was evaluated as previously in a context similar to our experimental design (*i.e.* five Vir:Avir ratios (1, 0.75, 0.5, 0.25, 0.1) for experiment (i) and 200 plants per ratio for all kinds of experiments) by randomly drawing Φ in intervals ($\lambda_a \in]0, 20]$, $\lambda_p \in]0, 20]$, $\lambda_a^* \in]\lambda_a, 2\times\lambda_a]$, $\kappa \in]0, 10]$). The correlation between true and re-estimated parameters indicates a good practical identifiably.

SI Fig. S2. Relative effect of the size *N* of population bottleneck during aphid transmission and of the fitness cost *s* of virulence on the mean time $\bar{\tau}$ to the extinction of the virulent virus variant, Vir.

The relative effect of *N* and *s* on $\bar{\tau}$ was estimated by the ratio $\bar{\tau}(s,N)/\bar{\tau}(0,N)$. A ratio close to 1 (red-orange colours) indicates (*N, s*) values where $\bar{\tau}$ is essentially determined by *N* (and only slightly sensitive to *s*). A ratio close to 0 (blue colours) indicates (*N, s*) values where $\bar{\tau}$ is essentially determined by *s* (and only slightly sensitive to *N*). (A) Ratio $\bar{\tau}(s,N)/\bar{\tau}(0,N)$ without aggregation of Vir and Avir particles in plants (θ=0). (B) Ratio $\bar{\tau}(s,N)/\bar{\tau}(0,N)$ with a moderate level of aggregation of Vir and Avir particles in plants (θ=0.026). Estimations of $\bar{\tau}$ are based on 25,000 simulations per (*N, s*, θ) parameter set.

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Moury, B., Fabre, F., Sénoussi, R. (2007). Estimation of the number of virus particles transmitted by an insect vector. Proceedings of the National Academy of Sciences of the United States of America, 104 (45), 17891-17896. , DOI : 10.1073/pnas.0702739104