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Estimation of vector propensity for *Lettuce mosaic virus* based on viral detection in single aphids

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Abstract

Lettuce mosaic virus (LMV) is transmitted by aphids nonpersistently causing severe disease outbreaks in commercial lettuce crops. New strategies to control plant viruses have arisen based on molecular techniques, which analyze plant-virus-vector interactions. In this work, two PCR-based methods with a previous immunocapture phase, have been developed to detect LMV in single aphids. Detection rates using a RT-nested-PCR method in single aphids and transmission efficiency of *Myzus persicae* (vector species) and *Nasonovia ribisnigri* (nonvector species) were compared. Although the percentage of viruliferous aphids for *N. ribisnigri* (45.8 ± 2.3) was higher than for *M. persicae* (39.2 ± 3.5) after the same acquisition access period, *N. ribisnigri* was unable to transmit the virus while *M. persicae* proved to be an efficient vector (with a transmission rate per single aphid of 10.4 ± 0.8). A method was proposed to estimate vector propensity for nonpersistent viruses based on the relationship between the percentage of viruliferous aphids and their transmission ability. This methodology could be applied to decision-making and implementing control strategies to prevent virus spreading.

Additional key words*:* IC-RT-nested-PCR, lettuce, LMV, *Myzus persicae*, *Nasonovia ribisnigri*, nonpersistent viruses.

Resumen

Estimación de la propensión vectorial para *Lettuce mosaic virus* mediante detección viral en pulgones individualizados

Lettuce mosaic virus (LMV) es transmitido de forma no persistente por pulgones causando epidemias severas en cultivos comerciales de lechuga. En el control de virus vegetales se han desarrollado nuevas estrategias para el análisis de las interacciones planta-virus-vector basadas en técnicas moleculares. En este trabajo se ha llevado a cabo la detección de LMV en pulgones individuales mediante dos métodos basados en la reacción en cadena de la polimerasa (PCR) con una fase de inmunocaptura previa. Se compararon la tasa de detección en un único pulgón obtenida mediante RT-nested-PCR y la eficacia de transmisión de *Myzus persicae* (especie vectora) y *Nasonovia ribisnigri* (especie no vectora). Aunque el porcentaje de pulgones virulíferos para *N. ribisnigri* (45,8 ± 2,3) fue mayor que para *M. persicae* (39,2 ± 3,5) tras el mismo periodo de adquisición, *N. ribisnigri* fue incapaz de transmitir el virus, mientras que *M. persicae* resultó ser un eficiente vector (con una tasa de transmisión de 10,4 ± 0,8 con un único pulgón). Se propone un método para la estimación de la propensión vectorial en el caso de virus no persistentes basado en la relación entre el porcentaje de pulgones virulíferos y su capacidad de transmisión del patógeno. Esta metodología podría ser empleada en la implementación y aplicación de estrategias de control para prevenir la dispersión viral en el cultivo.

Palabras clave adicionales*:* IC-RT-nested-PCR, lechuga, LMV, *Myzus persicae*, *Nasonovia ribisnigri*, virus no persistentes.

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Introduction

Lettuce mosaic virus (LMV) belongs to the genus *Potyvirus* and is the causal agent of lettuce mosaic, the most devastating viral disease affecting lettuce crops (*Lactuca sativa* L.) worldwide. Symptoms caused by LMV can vary considerably depending on the genotype, infective strain or stage of infection and environmental conditions. In addition to the typical symptoms of growth reduction and failure to head, sometimes necrosis and yellowing can appear (Dinant and Lot, 1992). LMV has flexuous particles of 680 to 900 nm long and 11 to 15 nm wide. The genome is positive-sense ssRNA with a VPg at the 5' end and a 3' poly-A tract and it is expressed as a polyprotein that cleaves to functional proteins (López-Moya and García, 1999; Hull, 2002).

The virus is transmitted by seeds and by several aphid species nonpersistently using the helper component strategy (Dinant and Lot, 1992). Thus, the virus particles have only a transient association with aphid mouthparts and optimal acquisition and inoculation processes are very brief and occur during aphid probing in superficial plant tissues (Ng and Perry, 2004).

To understand the complex phenomenon of virus outbreaks, Irwin and Ruesink (1986) proposed the term «vector intensity» considering two major components: i) the «vector activity», generally quantified by the vector abundance, and ii) the «vector propensity» that defines the probability of a vector transmitting a virus under field conditions. Several authors have developed effective and practical aphid monitoring methods to forecast aphid populations and establish the relationship between their abundance and virus epidemics (Halbert *et al.*, 1981; Sigvald, 1984; Peters *et al.*, 1990; Pérez *et al.*, 1995). Moreover, different mathematical models have been applied in plant virus epidemiology to forecast virus outbreaks such as the ones described by Ruesink and Irwin (1986) or Madden *et al.* (1990). However, none of them have analyzed in depth the relationship between the number of aphids carrying a nonpersistent virus (percentage of viruliferous aphids) and their transmission ability.

To make timely decisions regarding disease control strategies, it is essential to estimate the percentage of viruliferous aphids landing on a particular crop together with an estimation of the relative abundance of the different aphid species. Enzyme-linked immunosorbent assay (ELISA) is often used to detect virus in plant tissues (Hull, 2002). However, detection of viruses transmitted nonpersistently using ELISA in aphid vectors is difficult due to the low concentration of virus in the aphid's body (Carlebach *et al.*, 1982) and is possible only in some cases (Gera *et al.*, 1978; Cambra *et al.*, 1982). The presence of LMV in its host plants has been successfully detected by different methods (Revers *et al.*, 1997); nevertheless, so far, LMV detection in its aphid vectors has not been reported. Nucleic acid amplification and other molecular techniques have been used to detect viruses in plants and insect vectors, including heminested and nested-PCR combined with immunocapture or print/squash-capture (López-Moya *et al.*, 1992; Nolasco *et al.*, 1993; Hadidi *et al.*, 1993; Singh *et al.*, 1995; Olmos *et al.*, 1996, 1999, 2005; Mehta *et al.*, 1997; Revers *et al.*, 1997; Singh, 1998; Nie and Singh, 2001; Wang and Ghabrial, 2002).

In this work we describe the optimization of two methods to detect LMV in lettuce plants and aphids: immunocapture-RT-PCR (IC-RT-PCR) and immunocapture-RT-nested-PCR (IC-RT-nested-PCR) in a single closed tube. The sensitivity and specificity of both techniques are compared, demonstrating that several proteins can be used for the capture phase without requiring virus-specific antibodies. The presence of LMV in a vector and a nonvector species is evaluated and the obtained results for viral detection in individual aphids are compared with their actual ability to transmit the virus under laboratory conditions.

Material and Methods

Virus sources and aphid species

LMV was isolated from seed-infected lettuce plants cv. Valladolid. Viral identification was confirmed by RT-PCR (Revers *et al.*, 1997) and the nucleotide sequence of amplified products of 293 bp was compared with other available LMV isolates using the GenBank data base. The virus was transmitted by aphids (*Myzus persicae* Sulzer) to lettuce test plants 'Cazorla', grown in an aphid-free chamber at 26:20ºC (day:night) and a photoperiod of 16:8 h (light:dark), which served as a virus source for aphids.

M. persicae was selected as an efficient vector, and *Nasonovia ribisnigri* Mosley as a nonvector species (Kennedy *et al*., 1962). *M. persicae* colonies were started from a non-viruliferous single virginiparous female collected at Alcalá de Henares (Madrid) and reared on turnip plants, *Brassica rapa* 'Just Right', in an environmental growth chamber under controlled conditions

[23:16ºC (day:night) and a photoperiod of 16:8 h (light:dark)]. The *N. ribisnigri* clone, collected in Villa del Prado (Madrid), was reared on lettuce plants at a constant temperature of 12°C and a photoperiod of 14:10 (light:dark).

Plant-extract preparation and RNA isolation from lettuce plants

Extracts from infected and healthy plant samples were prepared by grinding plant material 1/20 (w/v) in extraction buffer (PBS buffer pH 7.2, supplemented with 2% (w/v) polyvinylpyrrolidone (PVP-10) and 0.2% (w/v) sodium diethyl dithiocarbamate).

RNA purification was performed using the RNeasy Plant Minikit, according to the manufacturer's protocol (Qiagen). Serial dilutions of plants extracts and purified LMV-RNA samples (10^{-1} to 10^{-7}) were prepared in extraction buffer and water, respectively, for sensitivity analysis.

Aphid preparation and squashing procedure

Groups of 25-30 individuals of *M. persicae* were collected, starved for one hour and placed, for a 5-min acquisition period, on LMV infected plants, which had been inoculated 3 to 4 weeks previously. Negative controls were managed similarly using healthy plants. Aphids fed on lettuce plants were squashed individually and in groups of five aphids on Whatman 3MM paper using the round bottom of an Eppendorf tube. RNA was extracted from the squashed aphids with 200 ml of 0.5% Triton X-100 (Olmos *et al.*, 1996, 1997).

Primer design

Primers were designed according to Olmos *et al.* (2005). Briefly, sequenced regions of LMV were recovered using the Nucleotide Sequence Search program located in the Entrez Browser program provided by the National Centre for Biotechnology Information (NCBI; (http://www.ncbi.nlm.nih.gov/Entrez; Bethesda, MD, USA). Conserved regions for the virus were studied using the Similarity Search tool Advanced BLAST 2.0, with the BLASTN program designed to support nucleotide analysis (http:/www.ncbi.nlm.nih.gov/blast/; Altschul *et al.*, 1997). The alignment view was performed as master slave with identities, to analyse significant nucleotide homologies in the molecular data retrieved from NCBI's integrated databases, GenBank, EMBL and DDBJ. The capsid protein gene region was selected and a pair of specific primers with similar annealing temperatures based on the OLIGO program (http:/www.lifescience-software.com/oligo.htm) (LRS, Long Lake, MN, USA) was designed for LMV. These primers were P7 (5'GACGGCTACGAGGCTTGAC3') and P8 (5'GAAGAGAACACGGAGAGGC3'). Primers P5 (5'ACAAGAAGAAACCGTATATGCC3') and P6 (5'CACTAAAGGCGTGTGTTGGC3') described by Revers *et al.* (1997) were also used. Primers P7 and P8 were used as internal primers in the RT- nested-PCR. According to the LMV sequence presented by Revers *et al.* (1997), the nucleotide position of P5 is equivalent to position 9588, the nucleotide position of P6 is equivalent to position 9867, the nucleotide position of P7 is equivalent to position 9619 and the nucleotide position of P8 is equivalent to position 9792.

RT-PCR optimization

To optimize RT-PCR, different parameters were analyzed such as primer concentration (from 0.1 to 2 µM), annealing temperature (50, 55 and 60ºC) and other reagent concentrations ($MgCl_2$, 0.1 to 0.4 µM; DMSO, 2 to 7%; Triton-X100, 0.1 to 0.5%). In addition, two different pairs of primers (P5, P6) and (P7, P8) were tested. RT-PCR was performed in a final volume of 50 µl containing 10 mM Tris-HCl pH 8.8, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.3% Triton X-100 (w/v), 1 µM of each primer, 2 µl of purified RNA, 2 units of TaqDNA Polymerase (Promega) and 4 units of AMV-RT (Promega). The following parameters were used: one cycle at 42ºC for 45 min; one cycle at 94ºC for 2 min; 40 cycles at 94ºC for 30 s, 50ºC for 30s and 72ºC for 1 min; followed by one cycle at 72ºC for 10 min. PCR products (10 µl) were analyzed by electrophoresis in 1.5% agarose gels and stained by ethidium bromide.

RT-nested-PCR optimization

The RT-nested-PCR in a single closed tube method was performed according to Olmos *et al.* (1999). The cocktail for reverse transcription and external amplification was a mixture of 30 ml containing 10 mM Tris-

HCl pH 8.8, 3 mM $MgCl_2$, 0.25 mM dNTPs, 0.3% Triton X-100 (w/v), 0. 2 mM of external primers (P5, P6), 5 ml of purified RNA, 2 units of TaqDNA Polymerase (Promega) and 4 units of AMV-RT (Promega). For the second amplification the cocktail was a mixture of 10 ml containing 10 mM Tris-HCl, pH 8.8 and 2 mM of internal primers (P7, P8). The conditions were one cycle at 42ºC for 45 min; one cycle at 94ºC for 2 min and 25 cycles of amplification (94ºC for 30 s; 50ºC for 30 s and 72ºC for 1 min). After RT-PCR, tubes were vortexed and centrifuged (6,000 × g for 2 s). Nested-PCR began with a denaturation phase of 2 min at 94ºC followed by 40 cycles of amplification 94ºC for 30 s; 50ºC for 30 s and 72ºC for 1 min and 10 min at 72ºC. PCR products (10 µl) were analyzed as above.

Evaluation of different substrates in the immunocapture phase

Plant or aphid extracts (100 ml) were subjected to an immunocapture phase directly in the tubes used for RT-PCR or RT-nested-PCR, precoated with different proteins. The immunocapture tubes were coated in carbonate buffer using: i) specific monoclonal antibodies for LMV (Agdia. Elkhart, Indiana, USA) (1:200); ii) specific monoclonal antibodies for *Plum pox virus* (PPV) (5B-IVIA) (Durviz, Valencia, Spain) (1 mg ml^{-1}); iii) polyclonal antibodies against *Potyvirus* genus (Agdia) (1:1,000); and iv) 5% bovine serum albumin (BSA fraction V) (Boehringer Mannheim, Germany). After incubation of extracts (4ºC; overnight), the tubes were washed 3-4 times with PBS-Tween. The amplification techniques were performed as previously indicated.

Transmission assays and detection in single aphids

Transmission tests of LMV were essentially performed as previously described by Fereres *et al.* (1993) using *M. persicae* and *N. ribisnigri* as vectors. After a 1-h preacquisition starving period, groups of 25 to 30 young-adult apterae aphids were released on the upper side of an infected leaf for virus acquisition. After a 5-min acquisition access period, aphids were transferred in groups of 5 onto 15-day-old lettuce seedlings 'Cazorla' for at least a 2-h inoculation period. Lettuce test plants were finally sprayed with imidacloprid (Confidor, Bayer) and transferred to an aphid-free climatic growth chamber, where they were checked regularly for the appearance of LMV symptoms during a period of 3 to 5 weeks. Six replicates of 28 plants each were used for each aphid species ($6 \times 28 = 68$ tested plants). A 28-rack tray of lettuce seedlings was used as an uninoculated control in each transmission experiment. Leaf samples from all test plants were checked for LMV using a DAS-ELISA kit (Agdia), with a LMV-specific monoclonal antibody 5 weeks after inoculation. Simultaneous to the transmission test, LMV was detected in single aphids by IC-RT-nested-PCR (6 replicates of 20 aphids for each transmission experiments were conducted = 120 analyzed aphids). The Gibbs and Gower formula (1960) was used to calculate the probability of transmission by a single aphid when groups of aphids were used to determine transmission efficiency. The transmission rates obtained with both species were compared. Data on transmission rate (number of infected plants divided by number of test plants) and detection level were subjected to variance analysis (Abacus Concepts, 1989) after using the transformation: $X = \arcsin \sqrt{(x+1/100)}$. Multiple mean comparisons were made between the transmission rates obtained for each aphid species using the Fisher's protected LSD test (Abacus Concepts, 1989).

Results

Detection of LMV RNA in plants: RT-PCR and RT-nested-PCR

RT-PCR using (P5, P6) and (P7, P8) pairs of primers successfully amplified 297 and 193 bp fragments, respectively, from 10^{-1} to 10^{-3}-fold dilution of LMV extracts. Inhibition of amplification reactions was observed when non diluted purified RNA was used as the template.

RT-nested-PCR was able to detect LMV targets from 10^{-1} to 10^{-4} dilutions. No amplification products were obtained with either healthy or negative controls.

Immunocapture phase. Sensitivity analysis

The immunocapture phase was successful with all proteins tested. Amplified products were obtained from all infected plant materials analyzed. IC-RT-PCR with LMV-specific polyclonal antibodies and monoclonal antibodies against *Potyvirus* genus were able to detect

Table 1. Sensitivity afforded by different PCR-based methods using several proteins in the immunocapture phase (BSA; LMV, PPV or potyvirus antibodies). The sensitivity is shown as the infected plant extract dilution endpoint with positive signal

Detection method	Conventional PCR	BSA[1]	LMV[2]	PPV[3]	*Potyvirus*
RT-PCR	10^{-3}	—	—	—	—
RT-nested-PCR	10^{-4}	—	—	—	—
IC-RT-PCR	—	10^{-2}	10^{-3}	10^{-2}	10^{-3}
IC-RT-nested-PCR	—	10^{-6}	10^{-7}	10^{-6}	10^{-6}

[1] BSA: bovine serum albumine. [2] LMV: *Lettuce mosaic virus.* [3] PPV: *Plum pox virus.*

the virus up to a dilution of 10^{-3}, which was 10 times more sensitive than using BSA or PPV-specific monoclonal antibodies in the capture phase.

Results obtained by IC-RT-nested-PCR showed that detection was 10^3 to 10^4 fold more sensitive than IC-RT-PCR depending on the antibodies used in the immunocapture phase. The best results were obtained with LMV specific antibodies, detecting the virus up to a 10^{-7} dilution (Table 1, Fig. 1). To discard the non-specific virus trapping onto the tube walls, the virus detection was conducted directly onto microfuge tubes. No amplification products were obtained by placing virus extracts in coating buffer in the tubes without previous protein capture (data non shown).

Detection of LMV in aphids using IC-RT-nested-PCR

Given the increase in sensitivity afforded by IC-RT-nested-PCR using LMV specific antibodies, this method was used routinely to detect LMV in aphids that had previously been squashed on paper. IC-RT-nested-PCR amplification products of 193 pb were observed for groups of five and single squashed aphids (Fig. 1). No amplification was obtained from control aphids fed on healthy plants.

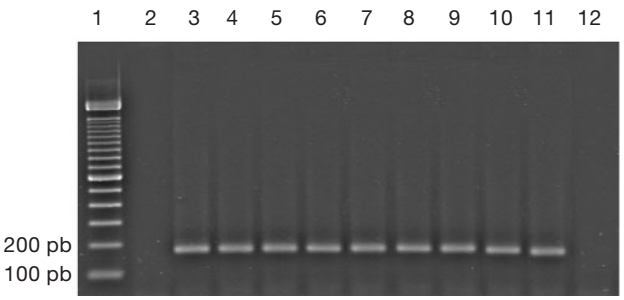

Figure 1. IC-RT-nested-PCR amplification products using LMV specific monoclonal antibodies in the immunocapture phase from serial dilutions (10^0-10^{-7}) of samples from LMV infected lettuce plants (lanes 2-9) or from groups of five (lane 10) and individual LMV-carrying aphids (lane 11). Lane 12, non infected plant control. Lane 1, DNA molecular weight marker (100 bp Gibco BRL).

Estimation of LMV transmission efficiency and comparison with virus detection in aphids

Transmission and LMV detection experiments are shown in Table 2. Data show that the LMV strain tested is transmissible by *M. persicae* (10.4% transmission rate by a single aphid). However, *N. ribisnigri* was unable to transmit LMV. IC-RT-nested-PCR was successfully used to detect LMV both in vector (39.2 %) and non-vector (45.8 %) species without significant differences (Table 2).

Discussion

In the host selection process, the feeding behaviour of aphids is a very important factor affecting viral transmission. Nonpersistent virus epidemics often occur when noncolonising species land and probe on the crop in large numbers (Raccah *et al.*, 1985; Pérez *et al.*, 1995). For this reason, in any epidemiologic study it is important to consider both vector propensity (rate of transmission under field conditions) and vector activity (number of aphids landing on the crop) to determine the major aphid species involved in spreading viruses.

Viral targets are detected by molecular procedures with higher sensitivity and reliability than other methods (Olmos *et al.*, 2005). Consequently, the prospect of detecting plant viruses by nucleic acid amplification

Table 2. *Lettuce mosaic virus* transmission efficacy of *Myzus persicae* and *Nasonovia ribisnigri* and comparison with the proportion of virus detection in both species.

Aphid species	Transmission rate (%) Mean ± SE (n = 6)		Detection (%) Mean ± SE (n = 8)
	5 aphids	1 aphid[1]	1 aphid
M. persicae	42.1 ± 2.1	10.4 ± 0.8	39.2 ± 3.5 a[2]
N. ribisnigri	0 ± 0	0 ± 0	45.8 ± 2.3 a

[1] Calculated values of the transmission efficacy using the Gibbs and Gower (1960) formula. [2] Means followed by different letters are significantly different according to Fisher's protected LSD test.

has increased, especially for viruses occurring in very low concentrations in plants or vectors. Previous works have shown that different nucleic acid amplification techniques are available to detect stylet-borne viruses with different sensitivity ranges and also, that a number of proteins can be used for the capture phase, thus obviating the need for virus-specific antibodies (Olmos *et al.*, 1996). In this study, we have shown the feasibility of detecting LMV from infected plants or single LMV-viruliferous aphids by IC-RT-PCR and IC-RT-nested-PCR using nonspecific proteins for the capture phase, although the best results were obtained using specific immunoglobulins. Sensitivity of LMV detection by IC-RT-nested-PCR was higher than IC-RT-PCR being sufficient to detect LMV in the 10^{-7} dilution of the pure plant extract. Gel electrophoretic analysis shows that the intensity of the amplified fragments was similar in all dilution tested except for the crude extract sample. These results suggest that the detection limit was not reached. It is known that the extracts of plants are frequently rich in polyphenolics and polysaccharides that can inhibit the RT or PCR reactions (Singh *et al.*, 1998). These data could explain why no amplification was obtained in non-diluted samples from infected plants.

The IC-RT-nested-PCR procedure described in our work proved to be 10-fold more sensitive than conventional RT-PCR. Differences of sensitivity and specificity between several detection methods have been previously described (Wetzel *et al.,* 1992; Olmos *et al.,* 1999). Therefore, this procedure could be a useful tool for epidemiological studies of LMV and other viruses transmitted nonpersistently and might also help to elucidate virus-vector interactions.

LMV has been detected in the two aphids species selected without significance differences, suggesting that the virus accumulates the same in both species. However, only *M. persicae* was able to transmit efficiently the virus. These results show that the lack of LMV transmissibility by a nonvector species is not related to either the viral acquisition phase or the capacity to retain virus particles inside the insect's body. Instead, the lack of transmissibility is most probably associated with the ability of virus particles to be retained on the distal joint duct of the aphid's stylets. Another possible explanation might be the vector's inability to inoculate the plants with the virus during the intracellular salivation phase, which is most probably involved in the inoculation process of noncirculative viruses by aphids (Martín and Fereres, 2003; Moreno *et al.*, 2005; Powell, 2005). Our data agree with those reported by other authors who detected other noncirculative viruses in nonvector species (Mehta *et al.*, 1997; Cambra *et al.*, 1982; Olmos *et al.*, 2005).

Obtained results indicate that after short acquisition periods, the transmission and detection rates of LMV are not equal, in disagreement with the results reported by Wang and Ghabrial (2002) for *Soybean mosaic virus* (SMV). This discordance between transmission and detection rates seems to be a more realistic result that could easily be explained by the fact that not all RNA targets detected in aphids can be transmitted to the plant.

The acquisition of transmissible and nontransmissible virus isolates by aphids has been reported in the past (López-Moya *et al.*, 1992; Olmos *et al.*, 1999), suggesting that the presence of virus in a given vector species is not a good indicator when determining the potential for virus transmission. The inescapable conclusion which emerges from all this is that aphid behaviour and virus-vector interaction determines the transmission efficiency of a given aphid species in the field.

Related to this, several works have shown the compatibility of the techniques described in this paper with epidemiological field studies (Singh *et al.*, 1995; Olmos *et al.*, 1996).

Thus, several studies conducted under field and laboratory conditions have tried to establish relationships between the presence of an aphid species in the field and the spread of a virus. However, none of these studies consider the number of virus-carrying insects with respect to how many of them can actually transmit the virus. For example, *M. persicae* is one of the most abundant aphid species present in lettuce crops in central Spain during the autumn season when LMV epidemics are usually most severe (Moreno *et al.*, 2004; Nebreda *et al.*, 2004). The vector activity data and the results obtained in the transmission tests confirmed that *M. persicae* is the most important LMV vector and could be the main responsible for spreading the virus in lettuce.

Here, a method is proposed to forecast the spreading risk of a given nonpersistent virus in the field based on the level of virus detection in single aphids. It is possible to calculate the number of aphids carrying the virus (= viruliferous aphids) and how many of them are able to transmit the virus to test plants (through transmission tests). By relating these data, it is possible to calculate the value of a constant *K*, which will be specific for each aphid species (Number of aphids able to transmit a virus = $K \times$ Number of viruliferous aphids; where $K \leq 1$). For example, we could use the data given in Table 2 to calculate the theoretical *K* value for *M. persicae* by dividing the virus transmission rate (0.104) by the virus detection rate (0.392) which gives a value of 0.26. An estimation of K constant values could be applied to studies such as those described by Halbert *et al.* (1981) or Raccah (1986) to estimate the risk of a virus spreading. Such studies estimate the vector propensity by trapping aphids periodically with the help of a net trap located in the field where the virus occurs. Then, the captured aphids are transferred individually to healthy test plants to determine which specimens account for the transmission observed. The massive capture of vector insects during an epidemic could be a feasible way to check the number of individuals belonging to different species and thus determine the proportion of aphids carrying the virus and their vector propensity (i.e. their actual ability to transmit the virus under field conditions). When the value *K* is determined for each species, the number of aphids transmitting the virus can be estimated according to the virus detection data obtained for single aphid samples collected in the field. In this way, results could be obtained much more quickly than in conventional transmission experiments, because diagnosis is faster and a lot of samples can be analyzed in a short time. However, it would still be necessary to identify the aphid species caught in the field traps in order to check for the virus only in those species known to be capable of transmitting it. The real risk of virus transmission under field conditions would be calculated as the final product of vector propensity and vector activity, which gives vector intensity (*sensu* Irwin and Ruesink, 1986).

Obviously, to use this methodology to estimate vector propensity in field conditions, one must analyze a great many captured aphids over several years in order to obtain a good estimate of the relationship between real transmitters and the level of virus detection in single aphids. Through such a study, a representative sample of different vector aphid biotypes and virus isolates present in a specific area could be obtained and this information could be used to forecast the risk of outbreaks and thus implement timely control measures to prevent LMV epidemics.

In summary, our work suggests that using IC-RT-nested-PCR to detect LMV or any other nonpersistent virus in vectors would enable the transmission mechanisms to be examined at the virus-vector interaction level. Such studies are necessary to improve existing preventive models used in plant virus epidemiology, thus developing new epidemiological approaches aimed at preventing or reduce the spread of viral diseases.

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