Transmission of *Potato virus Y* in Tobacco Plants by *Myzus persicae nicotianae* and *M. persicae* s.str.

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ABSTRACT

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The transmission efficiency of *Potato virus Y*^N (PVY^N) from and to tobacco plants by *Myzus persicae nicotianae*, a tobacco specialist, and *M. persicae* s.str. (Hemiptera: Aphididae), a generalist, was evaluated. In direct transmission tests, 1,004 spring migrants collected as nymphs from peach and 1,434 wingless females from 12 clonal lineages of both taxa were examined. Both the winged and wingless females were starved before being transferred individually for a 3-min acquisition period on infected plants and then moved to healthy plants for a 10-min inoculation access period. Little variation in transmission efficiency was observed. Although not statistically significant, *M. persicae nicotianae* was slightly more effective. This may suggest similar genetic properties (e.g., virion receptors in mouthparts) between the taxa in relation to virus transmission. *M. persicae* s.str. transmitted the virus less efficiently when a shorter inoculation period was given. This may indicate differences in probing behavior during the early phase of host selection. Nevertheless, behavioral differences related to host selection have been found using arena tests with infected and healthy tobacco plants where 180 winged females from each taxon were tested. *M. persicae* s.str. had significantly higher propensity to transmit PVY^N than *M. persicae nicotianae* (31.9 versus 15.3%).

Additional keywords: nonpersistent viruses, tobacco aphid, vector specificity

Potato virus Y (PVY), the type species of the *Potyviridae* family, has a wide host range and is distributed worldwide, causing serious crop losses in solanaceous crops such as potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*), and tomato (*Lycopersicon esculentum*) (9,11,43). PVY is transmitted by more than 50 aphid species in a nonpersistent manner (15,17). Aphid species differ in their ability to transmit the virus (21,26,30,39,44,55); however, the consensus is that *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is the most efficient vector. In this mode of transmission, acquisition and inoculation access periods are very short (seconds to minutes) and the aphids remain viruliferous for a very short period of time (minutes to a few hours) (20,40). Although aphids can acquire PVY during brief probes (5 s) into epidermal

cells, longer acquisition (10 s to 1 min) probes increase transmission efficiency. However, prolonged feeding (more than 5 to 10 min) results in poor transmission or no transmission at all (7,46,57). Aphid fasting prior to acquisition access is another factor affecting the transmission of PVY; starved aphids are more efficient PVY vectors than nonstarved ones (7,46,54,57).

Vector specificity in plant virus transmission is a well-known phenomenon and can be attributed to the genetic properties of the aphid vector or the virus, or to the feeding behavior of the vector (20,40,42). Variation in the transmission efficiency of nonpersistent viruses by different aphid species has been reported frequently (10,14,21,29,41,47,58). This phenomenon also occurs at the intraspecific level and has been recorded in various aphid-virus pathosystems. This can be observed when different laboratory (44,55) and field studies (1,10,26,55) are compared, as well as in standardized laboratory experiments with various clonal lineages of an aphid species (28,33,45,46,49,54). A strong genetic component in the interclonal variation has been observed (45,51,52).

M. persicae, a highly efficient vector of PVY and other nonpersistent viruses, ex-

hibits considerable genetic variation in relation to host-plant use and its reproduction strategy. During the evolutionary history of *M. persicae*, some populations have adapted to tobacco (3) and exhibit unique morphological (3,36) and genetic properties (6; J. T. Margaritopoulos, G. Malarky, J. A. Tsitsipis, and R. L. Blackman, *unpublished data*) compared with the populations colonizing other herbaceous crops such as pepper, potato, and cabbage (*Brassica oleracea*). The tobacco-adapted form is considered to be a distinct subspecies, known as the tobacco aphid *M. persicae nicotianae* (5,34). The two taxa can be found in either the same country (e.g., Greece) or the same locality (e.g., Caserta, southern Italy) (34,36). Recently, it was proved that the winged females of *M. persicae nicotianae* as well as those of the non-tobacco-colonizing taxon *M. persicae* s.str. have developed an improved host-recognition mechanism, enabling them to recognize host from nonhost during the early phase of host-selection behavior (i.e., brief probes of plant tissues; 37). This is especially interesting in regions where PVY is considered to be the most important tobacco virus (e.g., in Greece; 11). The occurrence of tobacco specialist aphid genotypes and those colonizing other crops might be an important factor in the epidemiology of PVY in tobacco crops. Until now, no detailed study has been undertaken providing comparative data on the transmission efficiency of PVY in tobacco by the two taxa.

The present study, therefore, aims at examining the efficiency of *M. persicae* s.str. and *M. persicae nicotianae* in transmitting PVY from and to tobacco plants. This was done by conducting two different direct transmission experiments. In the first, the transmission efficiency of PVY was compared between winged spring migrant females (alatae fundatrigeniae) of the two taxa which were collected from peach trees; in the second, the transmission by wingless females (apterae alienicolae) from 12 clonal lineages with known biological and genetic properties was evaluated. In addition, an arena test was performed examining the vector propensity (i.e., a measure of vector importance under

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conditions that allow aphids to move and feed freely; 27) of winged spring migrant females of both taxa.

MATERIALS AND METHODS

Virus isolate, source, and test plants.

A PVY^N isolate obtained from pepper from the Crete Island, Greece was used in all transmission experiments. The Virginia-type tobacco cv. VE9 was used as both a virus source and test plants. Young tobacco plants, inoculated mechanically approximately 1 month before beginning the transmission tests, were used as source plants. All source plants were kept in aphid-proof cages (wooden cages covered with aphid-proof net) in an aphid-free rearing room at 21 to 23°C and 16 h of light/day.

All test plants were grown inside aphid-proof cages in an aphid-free compartment of a greenhouse. The plants used in the transmission tests were approximately 10 cm tall (with at least two true expanded leaves). After transmission, the aphids were removed from the test plants and killed. The plants were sprayed with imidacloprid and kept in the aforementioned cages for approximately 20 days. Then, they were examined for virus infection by enzyme-linked immunosorbent assay (ELISA) according to Clark & Adams (12) using polyclonal antibodies provided by Dr. C. Varveri (Benaki Phytopathological Institute, Athens, Greece). The concentration of γ -globulin (IgG) used was 1 μ g/ml and the conjugate was diluted 1,000 times. ELISA also was used to identify the virus in the source plants. Only plants found positive were used in the experiments.

Transmission efficiency of PVY^N by spring migrants. The spring migrants were collected from peach trees in Meliki (northern Greece) and about 200 km south, in Lehonía (central-eastern Greece). Meliki and other nearby localities in northern Greece are major tobacco-growing regions, whereas tobacco is not cultivated in the Lehonía region. Young shoots of peach infested by aphids were collected and transferred to the laboratory in self-sealing inflated plastic bags, containing a piece of paper towel to reduce moisture, in plastic insulated containers containing ice packs. Morphometric (36,37) and molecular studies (6; J. T. Margaritopoulos, G. Malarky, J. A. Tsitsipis, and R. L. Blackman, *unpublished data*) on *M. persicae* populations from these areas revealed that populations on peach at Meliki are composed of the tobacco-adapted subspecies (*M. persicae nicotianae*); whereas, on peaches in Lehonía, the population consists of only *M. persicae* s.str., which colonizes other crops. Four to five samples were collected from each region, from different peach orchards each time, from late April to early June 2004.

To obtain young post-teneral spring migrants (i.e., winged adult females behav-

iorally inclined to settle on a plant) for the transmission trials, the following method was used. All winged adult aphids were removed from a few heavily infested peach shoots collected from one orchard (from Meliki or Lehonía), which then were placed in pots filled with water and kept in wood-framed cages (40 by 50 by 47 cm). These cages had the two opposite sides and the top covered with glass, a wooden bottom, and the remaining sides covered with fine aphid-proof muslin. They were kept in an insectary (23°C and 16 h of light/day) and inspected three times per day. When winged females appeared, the shoots were transferred to new cages, leaving behind the aphids that had taken off and alighted on the sides and roof of the original cage. The winged females were starved in the empty cages for about 8 to 10 h and then used for the transmission tests.

Batches of four to five winged females were placed on the upper side of a leaf of an infected tobacco plant (two source plants were used in each transmission trial) for a 3-min acquisition period and then transferred individually on the test plants and left for a 3- or 10-min inoculation period. The aphids were allowed to move freely on the plants during both the acquisition and inoculation access periods. All transmission trials (also those with wingless females; see below) were conducted in a room at 25 $\pm$ 2°C. In all, 1,004 tobacco plants were assayed. Due to the different collection dates of the nymphs and their different developmental times, it was not possible to examine batches of aphids from both regions on the same day to conduct a replicated experiment. Thus, the pooled percent transmission efficiency of aphids from Lehonía (286 and 226 winged females for the 10- and 3-min inoculation period, respectively) and Meliki (266 and 226 winged females for the 10- and 3-min inoculation period, respectively), for each inoculation time treatment separately, was compared using the χ^2 test.

Transmission efficiency of PVY^N by wingless females. In this experiment, the transmission of the PVY^N isolate by adult wingless females was compared between five clonal lineages of *M. persicae* s.str. and seven of *M. persicae nicotianae*. The clonal lineages were ascribed to either of the two taxa after their examination by multivariate morphometric analysis (J. T. Margaritopoulos, *unpublished data*). Information about the method is provided by Blackman (4) and Margaritopoulos et al. (36). All clonal lineages had been reared on excised leaves of Chinese cabbage (*Brassica chinensis*) in Blackman boxes (2) at 17°C and 16 h of light/day for about 1 year (2 years for the lineage from Zimbabwe) before the experiments were conducted. The life-cycle category of the aphid lineages was characterized according to Margaritopoulos et al. (35). In addition,

genetic properties of the lineages related to organophosphate and pyrethroid resistance were examined. Their carboxylesterase phenotype was diagnosed by a biochemical assay described by Devonshire et al. (16). The characterization of the susceptible (S) and resistant (R1, R2, and R3) variants to organophosphates was made according to Foster et al. (23). DNA diagnostics were used for the identification of amplified genes encoding carboxylesterases E4 and FE4 (22) and kdr genotypes (those with the mutation in the *para*-type sodium channel gene that confers resistance to pyrethroids and DDT) (25). Details of the biological and genetic properties, the host, and geographical origin of the lineages used in this experiment are shown in Table 1.

Before the transmission trials, the aphids were reared on Chinese cabbage leaves in Blackman boxes at 20°C and 16 h of light/day for four to five generations. Young adults (0 to 1 day old) were obtained from the clonal cultures and starved in glass vials for 30 min. Then, batches of four to five aphids were transferred to the upper side of a leaf of a source plant (two source plants were used in each transmission trial) for a 3-min acquisition access period and then transferred individually onto the test plants and left for a 10-min inoculation access period. The aphids were allowed to move freely on both source and test plants.

In all, 10 to 12 wingless females from each aphid lineage were used and all lineages were examined on the same day. The experiment was conducted in a randomized complete block design with 10 replications over time. In all, 1,434 aphids were examined. The transmission efficiency between the clonal lineages was compared by a two-way analysis of variance (ANOVA) using the arcsine transformed percentages. The analysis was done using the SPSS statistical package (version 10; SPSS Inc., Chicago). Nontransformed mean percentages and standard errors are shown in the text.

Transmission of PVY^N by spring migrants under arena conditions. Vector propensity of the newly emerged adult spring migrants of *M. persicae* s.str. and *M. persicae nicotianae* was evaluated under conditions that allowed aphids to move freely between healthy and infected plants for 24 h. To produce winged females, batches of alate nymphs with external wing buds collected from different peach shoots from Meliki (*M. persicae nicotianae* aphids) and Lehonía (*M. persicae* s.str. aphids) were reared on excised leaves of Chinese cabbage in Blackman boxes at 23°C and a 16-h photoperiod. The nymphs were inspected twice per day until they reached maturity. Each arena consisted of a wooden frame cage (50 by 50 by 40 cm) with the top covered with glass and the remaining sides with aphid-proof net. Two source tobacco plants were placed in the

middle of the cage and 12 healthy tobacco plants were set in a circular pattern along the edge of the cage. Tobacco plants were planted individually in small pots and there was enough space between pots to avoid plant-to-plant contact. Thirty newly emerged winged females from both taxa were placed in a petri dish located between the two source tobacco plants and remained inside the cages for 24 h. Two cages, one with aphids of *M. persicae nicotianae* and the other with those of *M. persicae* s.str., were set up simultaneously in controlled conditions (23°C and a 16-h photoperiod) and the experiment was replicated six times. The transmission rates between the two taxa were compared by two-way ANOVA of the arcsine transformed percentages using the SPSS statistical package (version 10; SPSS Inc., Chicago). Nontransformed mean percentages and standard errors are shown in the text.

RESULTS

Transmission efficiency of PVY^N by spring migrants. Winged females of both taxa transmitted the PVY^N isolate with similar efficiency when a 10-min inoculation access period was used (Table 2). Winged females of *M. persicae nicotianae* were more efficient than those of *M. persicae* s.str. when the inoculation access period was 3 min; however, these differences were significant only at a 0.10 *P* level. The inoculation access period did not affect transmission efficiency of *M. persicae nicotianae* aphids. By contrast, winged females of *M. persicae* s.str. transmitted the virus more efficiently when they were allowed access for a 10-min than for a 3-min inoculation access period.

Transmission efficiency of PVY^N by wingless females. Transmission efficiency of PVY^N by wingless females of seven clonal lineages of *M. persicae nicotianae* and five of *M. persicae* s.str. is shown in Table 3. Some variation in the transmission efficiency between the clonal lineages was observed, with a twofold difference be-

tween the least (*M. persicae* s.str. lineage) and most (*M. persicae nicotianae* lineage) effective aphid lineage. The lowest transmission efficiency was observed in two lineages of *M. persicae* s.str., whereas lineages from both taxa were ranked among the most efficient ones. The two-way analysis, however, showed that the differences in transmission efficiency between the lineages, irrespective of their biological and genetic properties or their geographical origin, were not significant. By contrast, the analysis revealed signifi-

cant variation between replications ($F_{9,99} = 6.4$, $P < 0.001$).

Comparisons also were performed between wingless females and winged spring migrant females, subjected to the same inoculation access period, from each taxon separately. In this analysis, the pooled data of the clonal lineages from each taxon were used. In both cases, the spring migrants were more efficient vectors than the wingless females, 37.6 versus 28.8% ($\chi^2_1 = 7.4$, $P < 0.001$) and 35.7 versus 23.8% ($\chi^2_1 = 13.5$, $P < 0.001$) in *M. persicae*

Table 2. Percent transmission efficiency of *Potato virus Y^N* from and to tobacco plants by spring migrants of *Myzus persicae* s.str. and *M. persicae nicotianae*^a

Taxa (collection region)	Percentage (%) of infected plants		
	Inoculation period		χ^2_1
	10 min	3 min	
<i>M. persicae nicotianae</i> (northern Greece)	37.6 (<i>n</i> = 266)	33.2 (<i>n</i> = 226)	1.04 (<i>P</i> < 0.31)
<i>M. persicae</i> s.str. (central eastern Greece)	35.7 (<i>n</i> = 286)	26.1 (<i>n</i> = 226)	5.35 (<i>P</i> < 0.02)
χ^2_1	0.22 (<i>P</i> < 0.64)	2.72 (<i>P</i> < 0.099)	...

^a Aphids were starved for 8 to 10 h before being subjected to a 3-min acquisition and a 10- or 3-min inoculation period; *n* = number of plants tested; one aphid per plant was used.

Table 3. Mean percent ($\pm$ standard error) transmission efficiency of *Potato virus Y^N* from and to tobacco plants by wingless adult females of clonal lineages of *Myzus persicae* s.str. and *M. persicae nicotianae*^a

Clonal lineage	<i>n</i>	Transmission efficiency (%) ^b
<i>M. persicae nicotianae</i>		
Mpn 1	118	21.7 $\pm$ 3.9
Mpn 2	116	29.3 $\pm$ 5.8
Mpn 3	126	31.8 $\pm$ 7.6
Mpn 4	123	25.0 $\pm$ 5.8
Mpn 5	133	33.8 $\pm$ 7.7
Mpn 6	135	26.0 $\pm$ 5.1
Mpn 11	111	27.1 $\pm$ 6.8
<i>M. persicae</i> s.str.		
Mp 7	118	30.1 $\pm$ 4.0
Mp 8	117	32.6 $\pm$ 4.5
Mp 9	129	24.7 $\pm$ 8.0
Mp 10	111	19.7 $\pm$ 7.6
Mp 12	127	17.0 $\pm$ 5.6

^a Aphids were starved for 30 min before being subjected to a 3-min acquisition and a 10-min inoculation access period; *n* = no. plants tested by each lineage; one aphid per plant was used and 10 replications were performed.

^b Effect of aphid lineage on transmission efficiency: $F_{11,99} = 1.19$, $P < 0.31$.

Table 1. Clonal lineages of *Myzus persicae nicotianae* (Mpn1–6, 11) and *M. persicae* s.str. (Mp7–10, 12) used in the direct transmission tests

Code	Region	Collection date	Host ^a	Color	Life cycle ^b	CP ^c	E4/EF4 genes ^d	Para-type sodium channel gene ^e
Mpn1	Northern Greece	June 2003	T	Red	Hol	R2	E4, FE4	RR
Mpn2	Zimbabwe	2002	T	Red	An	R2	FE4	RR
Mpn3	Northern Greece	May 2003	P	Green	Hol	R3	FE4	RS
Mpn4	Northern Greece	May 2003	P	Green	Hol	R2	FE4	RR
Mpn5	Northern Greece	May 2003	P	Green	Hol	R2	FE4	RS
Mpn6	Northern Greece	May 2003	P	Green	Hol	R2	FE4	RS
Mpn11	Northern Greece	May 2003	P	Green	Hol	R2	FE4	RS
Mp7	Central-eastern Greece	May 2003	P	Green	Hol	R2	FE4	RR
Mp8	Central-eastern Greece	May 2003	P	Green	Hol	R2	FE4	RR
Mp9	Central-eastern Greece	May 2003	P	Green	And	R3	FE4	RS
Mp10	Central-eastern Greece	May 2003	P	Green	Hol	R2	FE4	RR
Mp12	Central-eastern Greece	May 2003	P	Green	Hol	R2	FE4	RS

^a T = tobacco and P = peach.

^b Hol = holocyclic, An = anholocyclic, and And = androcyclic life cycle categories.

^c Susceptible (S) and resistant variants (R1, R2, and R3) of carboxylesterase phenotypes.

^d Amplified genes encoding carboxylesterases E4 and FE4.

^e kdr genotypes; R = resistant allele of *para*-type sodium channel gene encompassing the kdr mutation and S = susceptible allele without mutation.

nicotianae and *M. persicae* s.str., respectively.

Transmission of PVY^N by spring migrants under arena conditions. The results of this assay showed significant differences ($F_{1,5} = 6.8$, $P < 0.05$) in the transmission rates between the two taxa (Fig. 1). The winged females of *M. persicae* s.str. infected twice as many tobacco plants as those of *M. persicae nicotianae* (transmission rates: 32.0 and 15.3%, respectively). The analysis did not reveal any significant variation between replications ($F_{1,5} = 0.5$, $P < 0.78$).

DISCUSSION

Both *M. persicae nicotianae* and *M. persicae* s.str. transmitted the PVY^N isolate from and to tobacco plants. The transmission efficiency ranged from 17.0 to 37.6% according to the aphid morph and genotype used. Even though some differences in transmission efficiency were observed between the two taxa when either spring migrants or wingless females were used, with *M. persicae nicotianae* showing a slightly higher transmission, the differences were not significant. It should be mentioned here that the data obtained in the present study were derived from different aphid genotypes. The spring migrants were progeny of stem mothers (fundatrix) after the hatch of the overwintering egg, the outcome of the sexual reproduction. It is not possible to estimate the number of genotypes used due to the experimental procedure. Also, each of the aphid lineages that were used in the experiments with the wingless females represent a different genotype. Most of them were holocyclic genotypes (i.e., genotypes that are able to reproduce sexually and come from the overwintering egg) collected from different peach trees and, therefore, they were progeny of different stem mothers. The two nonholocyclic lineages represent a different genotype in respect to insecticide resistance (16,25). Thus, the finding of the

similar transmission efficiency by the two taxa is based on a sufficient number of different aphid genotypes, so that increased genetic variability in respect to aphid-vectors was included in the experiments.

Interestingly, winged aphids *M. persicae* s.str. transmitted the virus less efficiently when a 3-min than when a 10-min inoculation access period was used. In contrast, no differences were found in *M. persicae nicotianae* aphids. In addition, the difference between the two taxa, when aphids were given a 3-min inoculation access period, was significant at the $P < 0.10$ level. These results may indicate differences in probing behavior between the winged females of the two taxa during the early phase of host selection. A recent study with the electrical penetration graph technique (37) showed that more winged spring and summer migrants of *M. persicae nicotianae* (70 to 86%) punctured epidermal cells of tobacco leaves during the first 2 min of recording than those of *M. persicae* s.str. (42 to 48%). In that period, spring and summer migrants of *M. persicae nicotianae* also performed a higher mean number of cell punctures (1.1 to 1.6) than those of *M. persicae* s.str. (both morphs 0.6).

The small variation in the transmission efficiency found in the present study (two-fold difference and 1.1 to 1.3 when wingless females and spring migrants were used, respectively) contradicts the results of Upreti and Nagaich (54) and Singh et al. (46). These authors obtained a four- to fivefold difference between the clonal lineages of *M. persicae* examined and they attributed this variation to genetic rather than environmental factors. However, their results cannot be directly compared with ours because they used a different plant species, *Datura metel*, as source and test plant. It is known that transmission efficiency of a given virus is affected by the plant species used for both virus acquisition

and inoculation (39). In the aforementioned studies, no information is provided on the number of aphid genotypes used and few details on the host plant from which the aphid lineages were collected although, in one study, aphids were collected from tobacco. In cases where there is uncertainty about the number of genotypes represented by the aphid lineages (especially when a loss of sexuality is involved) used in an assay, it is difficult to attribute intraspecific variation in transmission efficiency exclusively to genetic factors. In a large, well-documented study, Terradot et al. (50) reported large-scale intraspecific variation in virus transmission efficiency in *M. persicae*, although their system involved the circulative *Potato leaf roll virus*. Generally, there is little data in the literature involving the tobacco aphid or a similar system (i.e., tobacco source and test plants) for comparison. Similar transmission rates (27 to 39%) were obtained by Fereres et al. (21) using a similar system (but with five aphids per test plant) with a *M. persicae* clonal culture collected from pepper (presumably this belongs to the non-tobacco-colonizing taxon).

Significant variation between replications observed in the transmission tests by wingless females may have been due to variation in the virus source plants. It is probable that, if more than two source plants had been used in each replication, the variation related to source plants would have been limited. Nevertheless, variation related to the test plants used in each replication also may be responsible because the plants were grown at different times, although a standard protocol was followed. This phenomenon also has been observed in other related studies. For instance, Lupoli et al. (33) reported a significant variation between trials in the transmission efficiency of *Papaya ringspot virus* T-strain by different clonal lineages of *Aphis gossypii* Glover (Hemiptera: Aphididae).

Winged spring migrant females transmitted the virus more efficiently than wingless females in both taxa. There have been contradictory reports regarding the relative effectiveness of the two morphs in various aphid–host–virus systems (8,13,19, 46,48,54). The restless behavior of the winged aphids (13,19) might be responsible for this discrepancy, although factors related to the virus species also may be involved (13). The 2-h starvation period used by some authors (19,46,54) possibly is not enough to induce a mood for probing and settling on the plants in winged females. The 8- to 10-h starvation period of the winged females, used in the present study, seems to be adequate to induce the aphids to exhibit the typical host selection behavior. Thus, the transmission efficiency differences between the two morphs may be explained by the different starvation periods used, which can lead to differences in probing behavior during the penetration

Fig. 1. Vector propensity of wingless newly emerged adult females of *Myzus persicae* s.str. and *M. persicae nicotianae* in PVY^N transmission to tobacco plants. Numbers in brackets denote mean percentages. Bars denote $\pm$ standard error.

of superficial plant tissues (13,14). However, the possibility that this difference is related to the fact that spring migrants and laboratory-reared lineages represented different genotypes or to an innate ability of winged females cannot be ruled out. Nevertheless, the results suggest that both morphs have the intrinsic ability to transmit the PVY^N isolate adequately in tobacco plants.

Contrary to the situation observed in some circulative viruses (e.g., correlation between transmission of viruses causing the barley yellow dwarf disease and host adaptation within *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae); 24), the examination of the system used in the present study (tobacco-specialist, generalist taxa/PVY^N/tobacco) did not reveal any correlation between virus transmission and vector host adaptation. A link between aphid–host adaptation and virus transmission seems rational for circulative viruses which may need the evolution of vector host-adapted genotypes in order to capture novel hosts (24). This probably is not the case for the nonpersistent viruses that often are transmitted by noncolonizing aphid species. It is worthwhile, however, to examine virus transmission in relation to vector host adaptation in other aphid–virus systems, because this insect group is characterized by a substantial intraspecific genetic variation in host-plant use (18). An interesting finding was that geographically separated lineages (Zimbabwe versus Greece), which probably have evolved independently, transmitted the virus with similar efficiency. This may suggest similarities in the virion receptors in the epicuticular lining of aphid mouthparts (i.e., the binding site of helper component protein [HC]–virion complex). Wang et al. (56) provided convincing evidence of the important role of HC in aphid–vector specificity by interacting differentially to specific binding sites in the food canal or to constituents of saliva of different aphid species. This similarity in transmission efficiency between the two taxa found in the present study reflects the evolutionarily recent adaptation of *M. persicae nicotianae* to tobacco (3). *M. persicae* s.l. has come into contact with PVY before the aphid tobacco-adapted genotypes arose, and it seems probable that they retained the genetic properties required for virus transmission after the capture of the new host.

The similar transmission efficiency between *M. persicae nicotianae* and *M. persicae* s.str. is an important factor affecting the epidemiology of PVY in tobacco crops. In tobacco-growing regions where the two taxa coexist (e.g., southern Italy; 34), the contribution of the winged females of *M. persicae* s.str. to virus spread may be higher. The higher relative abundance of tobacco plants compared with other herbaceous plants and the fact that winged fe-

males of both taxa recognize tobacco plants as host or nonhost during brief probes of superficial plant tissues should be taken into account (37,53). Also, it should be considered that the optimum strategy for winged migrants of both taxa is to seek the appropriate host (37,38). Given the typical behavior of winged aphids when they select a plant during their attack flight (i.e., differential rate of retake-off from host and nonhost and take-off even from hosts; 31,32), those of *M. persicae* s.str. may sample and reject many tobacco plants until they find a suitable host, but they probably transmit the virus. In contrast, winged females of *M. persicae nicotianae* will visit only one or at least fewer tobacco plants than those of *M. persicae* s.str. This hypothesis has been well verified by the arena tests performed in the present study that revealed the higher propensity of *M. persicae* s.str. than of *M. persicae nicotianae*. When winged females of *M. persicae* s.str. were allowed to move freely between tobacco plants, they transmitted PVY^N twice as frequently as those of *M. persicae nicotianae*. The results of the arena test are in agreement with the growing evidence reported by studies using this method (19,58), which revealed that noncolonizing species may contribute more to the spread of a nonpersistent virus in the field than colonizing species. Nevertheless, from an epidemiological point of view, the relative abundance of each aphid taxon also should be taken into account.

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