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Transmission Efficiency of the Strain PVY^{NTN} by Commonly Captured Aphids in Tunisian Potato Fields

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Abstract: In the context of Potato virus Y epidemiological study, fourteen aphid species were selected to investigate their relative transmission efficiency in laboratory using tobacco plant tests *Nicotiana tabacum* var. Xanthi. These aphid species were the ones most often trapped in Yellow Water Traps (YWTs). Transmission efficiency was evaluated in both winged and wingless individuals in cages under controlled conditions. The transmission efficiencies obtained varied from 3 to 95%. Besides *Myzus persicae* (Sulzer), a highly efficient vector, 13 other aphid species were screened for their capability of transmitting PVY^{NTN}. Three aphid species, *Aphis spiraecola* Patch, *A. gossypii* Glover and *Brachycaudus helichrysi* (Kaltenbach), appeared to propagate PVY greatly, with transmission efficiencies of 73, 71 and 68%, respectively. Even though *Aphis fabae* Scopoli was less efficient, with only a moderate efficiency of 43%, it is also suspected of being implicated in PVY dissemination. In 60% of the cases, results obtained from wingless and winged forms were very close. Consequently, five aphid species seem to represent a real risk for the spread of viruses given their abundance in traps.

Key words: Aphids, virus, vectors, efficiency, plant tests, epidemiology

INTRODUCTION

Some of the most damaging diseases in an economically important crop such as potatoes are caused by viruses particularly PVY (Valkonen, 2007) spread by aphid vectors (Buchen-Osmond, 2002). It is more difficult to control non-persistent viruses than persistent ones, since insecticides act too slowly to kill the aphids before they transmit a non-persistent virus because of both the short acquisition access period and inoculation access period (Matthews, 1991). During the aphid's dispersal phase in search of new hosts, both potato-colonizing and non-potato-colonizing aphid species can transmit Potato virus Y (PVY). The availability of vectors and alternative hosts in potato regions explain the PVY propagation (Bostan and Halilogl u, 2004).

PVY is a non-persistent aphid-transmitted virus and a very harmful pathogen in the potato crop. More than 70 additional aphid species are able to transmit PVY (Varveri, 2000; Halbert *et al*., 2003; Kerlan, 2006; Ragsdale *et al*., 2001; Robert *et al*., 2000). The green peach aphid, *Myzus persicae* (Sulzer), is by far the most efficient vector of PVY in potato crops worldwide (Ragsdale *et al*., 2001; Muthomi *et al*., 2009). Other aphid species are relatively less efficient (Sigvald, 1984; Van Harten, 1983). However, aphid species colonizing potato plants, like *Aphis nasturtii* Kaltenbach, *Macrosiphum euphorbiae* (Thomas) and *Aulacorthum solani* (Kaltenbach), should not be excluded from vector lists, despite their low transmission efficiency, since they too can be responsible for the transmission of PVY within a field. Non-colonizing species such as *Brachycaudus helichrysi* (Kaltenbach), *Phorodon humuli* (Schrank) (Rolot, 2005), *Rhopalosiphum padi* (Linnaeus) (Sigvald, 1989) and *Acyrthosiphon pisum* (Harris) (Halbert *et al*., 2003) are also reported to be involved in PVY epidemiology. Given that virus spread into and within a field depends on vector activity and virus sources, knowing aphid efficiency in transmitting PVY is a prerequisite to successfully controlling it.

Tunisian seed potato suffer of PVY contamination and every year we loss 12% of seed potato production (Groupement Inter-professionnel des Légumes (GIL, 2003) which exceed 5% of infected plants with viruses tolerated by the legislation from certified Elite seed 'Spunta' (2% infection) imported from France and Netherland. An

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epidemiology study was important to be conducted for a better sanitary control. This study is subsequent to the work which previously reported diversity of aphid species in seed potato production in Tunisia using yellow water traps and one suction trap (Boukhris-Bouhachem *et al*., 2007), 103 aphid species were identified and fourteen of them are numerous and known as PVY vectors. A work was also conducted about PVY strains; the prevalent type was PVY^{N} (Boukhris-Bouhachem *et al*., 2004). It focuses on the strain PVY^{NTN}, belonging to the PVY^{N} group (Rolland *et al*., 2008), newly detected in Tunisia (Boukhris-Bouhachem *et al*., 2008). Aphid transmission of PVY^{NTN} has never been studied before under these conditions and is rarely mentioned by Davis *et al*. (2005) especially when the changing of their virulence properties is recently reported by Volkov *et al*. (2009) and Chikh Ali *et al*. (2010). The objective of this research was to assess PVY^{NTN} transmission efficiency under laboratory conditions of the prevalent aphid species, both colonizing and non-colonizing of potato plants, captured with traps in Tunisian certified seed potatoes in order to know how to control the main vectors and the spread of PVY^{NTN}.

MATERIALS AND METHODS

Aphid species: Those aphid species were selected that are known for their capability to transmit PVY, are abundant in yellow water traps (Boukhris-Bouhachem *et al*., 2007) and are present on potato foliage (Boukhris-Bouhachem *et al*., 2004). Four water pan traps were placed in four seed potato production field (Batan, Bousalem, Sidi Mahmoud, Douala) from 2001 to 2006. The traps were weekly collected and aphids identified and counted under stereomicroscope using identification keys. During 2002 and 2003, every two weeks from marsh to may, one hundred potato leaves were collected in each potato field visited to identify colonizing aphids. All potato aphids were from the Cap Bon region (northeastern Tunisia) except *Aphis gossypii* Glover that was from the northern Manouba region. Non-colonizing aphids were collected from their respective host plants (Table 2) near a potato field in Cap Bon, except for *R. padi* that was collected from Jendouba in the northwest.

The aphids were reared since 2003, on their known host plants in hermetic closed cages at INRAT, under controlled conditions (temperature 23°C, photoperiod L: D 16: 8 h). All aphids rearing were started from a single parthenogenetic female, except for *Aphis spiraecola* Patch, *Brachycaudus cardui* (Linnaeus), *B. helichrysi* and *Hyperomyzus lactucae* (Linnaeus), which were reared from mixed populations.

Virus source: PVY^{NTN} is the prevalent strain in Tunisia on potato var. "Spunta" and "Nicola," characterized on the basis of test plant reactions (*N. tabacum* var. Xanthi). It is confirmed by RT-PCR protocols with specific primers based on the polymorphism in the P1 genes (Glais *et al*., 2001) and the three recombinant sites specific to PVY^{NTN} isolates RJ1 and RJ2 (Nie and Singh, 2003) RJ3 (Glais *et al*., 2005). Among these isolates, only the PVY^{NTN}C1-3 isolated from potato cv. 'Spunta' and cultivated at Cap Bon was used for all of the transmission tests. This virus is maintained in the laboratory, on *Nicotiana tabacum* var. Xanthi and transmitted mechanically from tobacco plant to tobacco plant. Tobacco seedlings at the 4-leaf stage were inoculated with plant sap. Two weeks after inoculation, the plants developed vein necrosis and leaf distortion, characteristic symptoms of isolates belonging to the PVY^{N} strain group.

Transmission of PVY^{NTN} by aphids: Transmission tests were conducted between 2004 and 2007 both with wingless and winged aphids from tobacco plant to tobacco plant (*N. tabacum* var. Xanthi) for practical reasons, as was done by many other authors (Basky and Almasi, 2005; Boiteau *et al*., 1998; Derron and Goy, 1990; Harrington *et al*., 1986; Kanavaki *et al*., 2006; Van Hoof, 1980; Volkov *et al*., 2009). After rearing, 100 wingless individuals were collected in Petri dishes and starved for 2 h. The aphids were then allowed to probe on PVY-infected tobacco leaves for 2 min, called the Acquisition Access Period (AAP). The aphids inoculated twenty tobacco plants. Five aphids were placed on each plant with four true leaves over a 24 h period, called the Inoculation Access Period (IAP). After 24 h, the aphids were killed with an insecticide, Imidacloprid (Confidor, Bayer CropScience), following the manufacturer's instructions. Three separate repetitions of 20 tobacco plants were carried out for each aphid species.

The protocol for the winged aphids was different. One hundred winged aphids were transferred onto 4 infected tobacco plants, which were put into a cage with 20 healthy tobacco plantlets with 4 true leaves during a 24 h IAP.

Two weeks later, all inoculated plants were tested for the presence of PVY with DAS-ELISA according to Clark and Adams (Clark and Adams, 1977), using a commercial diagnosis kit (PVY^{N}, Bioreba, AG, Reinach, Switzerland) as followed in Boonham *et al*. (2002). After incubation at room temperature, the absorbance of the samples was read at 405 nm using an automatic microplate reader (Multiscan Ascent Labsystems, Waltham, MA, USA). Tobacco samples with an extinction value exceeding twice the average value of the healthy control were considered PVY positive.

Statistical analyses: All statistical analyses were done using SPSS software. Aphid transmission rates obtained were compared using Duncan's test. Transmission rates between wingless and winged forms were then analyzed with analyses of variation (ANOVA). The test was significant when p = 0.05.

RESULTS AND DISCUSSION

Five aphid species were identified on potato leaves, *Aphis fabae*, *A. gossypii*, *Aulacorthum solani*, *M. persicae* and *Macrosiphum euphorbiae*. The most abundant species was *A. gossypii* while the least one was *A. solani* (Table 1).

Major aphid species captured in yellow pan traps were listed in Table 2. Among potato aphids, *A. fabae*, *A. gossypii*, *M. persicae* were the numerous ones compared to *M. euphorbiae* and *A. solani*. *A. spiraecola* and *Aphis* sp. were the most abundant non colonizing potato aphids especially in Batan and Bousalem. However, high levels of *Aphis* sp. were observed in Douala. *A. pisum* was caught with important numbers in three regions: Bousalem, Sidi Mahmoud and Douala. Results indicate that many aphid species were present on potato fields and they may play a role in PVY dissemination.

Except *Aphis* sp. these aphid species were all used on transmission tests. All of the tested aphid species were vectors of the PVY^{NTN}C1-3 isolate (Table 3). Duncan's test, applied to transmission efficiency results for wingless aphid species, showed five significantly distinct classes of vectors. As expected, *M. persicae* was found to be the most efficient vector with a transmission efficiency of 95%; it was therefore grouped into class 1. Following that came *A. spiraecola*, *A. gossypii* and *B. helichrysi* with a transmission efficiency ranging between 68 and 73% which were grouped into class 2. *Rhopalosiphum maidis* (Fitch), *Aphis fabae* Scopoli and *H. lactucae*, with a virus transmission of almost 50%, were grouped into class 3. *B. cardui*, *R. padi* and *Lipaphis erysimi* (Kaltenbach) transmitted PVY at a lower level, ranging between 25 and 34% and were grouped into class 4. Finally, *Hyalopterus pruni* (Geoffroy), *A. pisum*, *Sitobion avenae* (Fabricius) and *M. euphorbiae*, with a transmission efficiency of less than 10% were the poorest vectors and were grouped into class 5.

The results obtained with winged aphids showed three significant classes using Duncan's test. No differences were observed between *M. persicae*, *A. spiraecola* and *A. gossypii* (class 1), *R. maidis*, *A. fabae* and *H. lactucae* (class 2) and *R. padi*, *H. pruni*, *A. pisum* and *M. euphorbiae* (class 3).

The ANOVA showed no significant difference between winged and wingless forms of the same species in six of the ten cases compared. *M. persicae* was the most efficient PVY vector in both winged and wingless

Table 1: Total potato aphids per 100 leaves related to sites and years during March to May

	Batan		Bousalem		Sidi Mahmoud		Douala	
Species	2002	2003	2002	2003	2002	2003	2002	2003
Aphis gossypii	33111	595	25047	4115	287	49	108	423
Macrosiphum euphorbiae	149	6	44	583	18	104	385	2203
Myzus persicae	763	114	1197	23	132	88	246	136
Aulacorthum solani	86	2	146	0	3	2	173	47
Aphis fabae	44	7	0	22	2	16	14	99

Table 2: Mean aphid species per yellow water traps during 2002-2006

Aphids	Species	Batan	Bousalem	Sidi Mahmoud	Douala
Potato aphids	*A. fabae*	19	21	16	48
	A. gossypii	25	46	13	14
	M. persicae	16	13	17	12
	M. euphorbiae	1	2	3	8
Non colonizing	*A. spiraecola*	521	163	31	117
potato aphids	*Aphis* spp.	111	37	32	162
	A. pisum	5	46	54	63
	L. erysimi	50	75	50	4
	H. lactucae	20	22	40	11
	B. cardui	10	3	1	13
	H. pruni	9	3	10	4
	B. helichrysi	5	4	4	9
	R. maidis	-	8	-	1
	R. padi	2	6	1	1
	S. avenae	-	3	-	-

Table 3: Transmission efficiency of PVY^{NTN} by different aphid species in percentage

			Transmission efficiency (%)			
Aphid species	Host plants sampled	Host plants for rearing	Wingless mean±SE	Class	Winged mean±SE	Class
Myzus persicae	Potato	Pepper	95^{a}±8.66^{A}	1	86^{a}±2.89^{A}	1
*Aphis spiraecola**	Citrus	Citrus	73^{b}±5.77^{A}	2	81^{a}±2.89^{B}	
Brachycaudus helichrysi *	Artichoke	Artichoke	71^{b}±9.06		-	
Aphis gossypii	Potato	Potato	68^{b}±10.41^{A}		82^{a}±0.00^{A}	
Rhopalosiphum maidis	Barley	Barley	47^{c}±17.02^{A}	3	42^{b}±2.89^{A}	2
Aphis fabae	Potato	Faba bean	43^{c}±2.89^{A}		42^{b}±2.89^{A}	
*Hyperomyzus lactucae**	Sowthistle	Sowthistle	42^{c}±2.51^{A}		53^{b}±27.50^{B}	
*Brachycaudus cardui**	Artichoke	Artichoke	34^{d}±4.08	4	-	3
Rhopalosiphum padi	Barley	Barley	28^{d}±10.41^{A}		12^{c}±2.89^{B}	
Lipaphis erysimi	Turnip	Turnip	25^{d}±5.00		-	
Hyalopterus pruni	Almond	Almond	10^{e}±5.00^{A}	5	7^{c}±0.00^{A}	
Acyrthosiphon pisum	Faba bean	Faba bean	7^{e}±2.74^{A}		13^{c}±2.96^{B}	
Sitobion avenae	Barley	Barley	5^{e}±0.00		-	
Macrosiphum euphorbiae	Potato	Potato	3^{e}±2.89^{A}		5^{c}±5.00^{A}	

The aphids were starved for two hours before being subjected to a two-minute AAP and a 24 h IAP; five aphids per plant were used and three replications of 20 tobacco plants (four true leaves) were performed; (*) population origin; (-) not tested; values of aphid transmission efficiency with different lower case letters are significantly different (p = 0.05, Duncan's test); ANOVA was used t o study significant differences in wingless and winged forms (capital letters)

forms with no significant difference. However, the winged forms of *A. spiraecola*, *H. lactucae* and *A. pisum* were significantly more efficient than the wingless forms. In contrast, the winged *R. padi* was a significantly less efficient vector.

This assay indicates that *M. persicae*, *A. spiraecola*, *B. helichrysi* and *A. gossypii* were the most important vectors under controlled conditions.

Of the 14 aphid species tested, it was demonstrated that all of them transmitted PVY^{NTN} whether or not they colonized potato plants. PVY^{NTN} is a new prevalent strain in potato fields in Tunisia. Not much data is currently available about the transmission efficiency of PVY^{NTN} by other aphid vectors, as it is for *Aphis glycines* Matsumura (Davis *et al*., 2005).

A. gossypii, the cotton aphid with high population levels on potato plants, was an efficient vector under experimental conditions and transmitted PVY^{NTN} with more efficiency than was reported by many authors who obtained a 12 to 31% transmission rate with PVY^{N} (Fereres *et al*., 1993; Raccah *et al*., 1985).

The green citrus aphid, *A. spireacola*, seemed to be a very good vector of PVY^{NTN} under laboratory conditions, in contrast with the transmission rate of 6.2% with PVY^{N} (Raccah *et al*., 1985).

The leaf-curling plum aphid, *B. helichrysi*, mentioned by Harrington and Gibson (1989), Piron (1986) and Powell *et al*. (1992) as a PVY vector with low transmission efficiency (4.8 to 12.5%) was found here to be a good vector (71%). Moreover, it is also considered an important vector in Belgium (Rolot, 2005).

With a moderate efficiency of virus transmission in Tunisia, the black bean aphid, *A. fabae* (43%) and the currant-sowthistle aphid, *H. lactucae* (42%), were reported as poor vectors, with a transmission efficiency of 7.6 to 24% and 0.4 to 17.4%, respectively (Harrington and Gibson, 1989; Piron, 1986). The cereal aphid, *R. maidis* (47%), had not been tested until now (Ragsdale *et al*., 2001).

The bird-cherry aphid, *R. padi*, with a transmission rate of 28%, was more efficient in this study than reported before where it varied from 0.5 to 11.5% (Harrington and Gibson, 1989; Harrington *et al*., 1986; Piron, 1986; Van Hoof, 1980) and less than 40% (Sigvald, 1984). In the same class 4, *L. erysimi*, the turnip aphid, had a transmission efficiency of 25%, percentage higher than 10% previously mentioned by Ragsdale *et al*. (2001).

Class 5 aphid species were poor vectors. *H. pruni* was found to have a transmission efficiency of 8.5%, less efficient than the rate of 13.9% reported by Perez *et al*. (1995). The transmission efficiency of *A. pisum* was between 7 and 13%, quite similar to the results (3.8-14%) mentioned by Fereres *et al*. (1993), Harrington and Gibson (1989), Harrington *et al*. (1986), Piron (1986) and Raccah *et al*. (1985). *S. avenae* also proved to be a poor vector both in this study and in those done by many other authors (Harrington and Gibson, 1989; Perez *et al*., 1995; Piron, 1986; Sigvald, 1984). *S. avenae* was therefore not considered a vector. The colonizing potato aphid, *M. euphorbiae*, transmitted PVY^{NTN} under the conditions here with an efficiency of 4%. This was the same result as that obtained by Kostiw (1980) but different from other authors who found a transmission efficiency of 17 to 29% (De Bokx and Van der Want, 1987).

Potato aphids were present in important numbers on potato fields exceeding the threshold, 3-10 aphids per 100 leaves, recommended for seed potato production (Muthomi *et al*., 2009; Capinera, 2001). Non-colonizing winged aphids increased opportunity for movement of viruliferous aphids around the crop (Harrington *et al*., 2003) and are then implicated in PVY epidemiology.

In comparing wingless and winged forms, no significant difference was noted between the two forms of the efficient vectors *M. persicae*, *A. gossypii*, *A. fabae* and *R. maidis*, except for *A. spiraecola*. This may be due to similar genetic properties (e.g., virion receptors in the mouthparts) between the two forms of the same species in relation to virus transmission. Nevertheless, transmission efficiency by the winged form was slightly superior in all of the species. This heterogeneity could be attributed to the methodology used, the experimental conditions or the age and activity of winged aphids that increase transmission probability because of flight. In fact, it is unknown if flight behavior is similar for all species or if winged aphids touched on all plants during the IAP. Another factor involved in transmission rates was virus concentration.

PVY transmission variability between the aphid's species was confirmed by the results obtained here and also reported by Kennedy *et al.* (1962), Sigvald (1984), Van Harten (1983) and Mirmomeni *et al.* (2008). Biotype variability of the same species (Verbeek *et al.*, 2010) was also confirmed. Moreover, a difference in transmission efficiency was reported for several other strains of PVY. It has been demonstrated that *M. persicae* transmit PVY^{N} with more efficiency than PVY^{O} (Basky and Almasi, 2005; Sigvald, 1984; Van Hoof, 1980).

Furthermore, it was clear that *M. persicae* was the best vector of PVY^{NTN}, with a very high average of transmission efficiency, nearly 100% under laboratory conditions. In general, according to previous studies, PVY^{NTN} is transmitted with more efficiency than PVY^{N}, thus increasing the risk of the spread of PVY^{NTN} in potato crops and making sanitary control more complex.

Four species (Table 1) were very efficient vectors in this experiment (with a transmission rate of >68%). However, a greater number of inefficient vectors may be more important than a fewer number of efficient vectors in the epidemiology of virus diseases (DiFonzo *et al.*, 1997). Their flight behavior and/or high numbers could compensate low transmission efficiencies of some species during the potato-growing season (Sigvald, 1990). Such is the case for the non-colonizing aphids, *A. pisum* and *R. padi*, which may be involved in PVY epidemics (Halbert *et al.*, 2003; Sigvald, 1984). These species were trapped in high numbers in the YWTs in Tunisia during the late season.

Colonizing aphid species such as *M. persicae* and *A. gossypii* probably play a role in the epidemiology of PVY since they transmit PVY rather efficiently. *A. fabae*, a moderately efficient vector, is frequently captured in YWTs (Boukhris-Bouhachem *et al.*, 2007) and is likely to contribute to the dissemination of PVY^{NTN} as well.

Non-colonizing winged aphid species, such as *A. pisum*, *A. spiraecola*, *B. helichrysi*, *B. cardui*, *Dysaphis* sp., *H. lactucae*, *H. pruni*, *L. erysimi*, *R. padi* and *S. avenae*, were also found on potato plants during the growing season. Among these, *A. pisum*, *A. spiraecola*, *B. cardui* and *H. lactucae* were commonly captured in YWTs (Boukhris-Bouhachem *et al.*, 2007). All of these species have been found to transmit PVY and seem to contribute greatly to the PVY epidemic during the season compared to their number. In fact, *A. pisum*, *A. spiraecola*, *B. helichrysi*, *H. lactucae, L. erysimi* and *R. padi* are often caught in large numbers in YWTs, meaning that these non-colonizing aphid species can be held responsible for the epidemic spread of PVY^{NTN} (DiFonzo *et al.*, 1997; Halbert *et al.*, 1981).

Simulating the situation occurring in the field with winged aphids in cages gave better practical results than the laboratory experiments with wingless individuals obligated to feed on tobacco which gave transmission efficiencies that were too high for those aphid species that do not colonize potato plants. By taking the vector efficiency of a particular aphid species into account together with its abundance in the traps, the potential risk of infection can be evaluated for potato crops (Sigvald, 1986). The use of tobacco in laboratory transmission tests gives an indication of the vector's behavior and its capacity to transmit PVY^{NTN} under controlled conditions. Based on these results (Table 3) and aphid abundance (Boukhris-Bouhachem *et al.*, 2007), it can be assumed that *M. persicae*, *A. spiraecola*, *A. gossypii*, *A. fabae* and *A. pisum* are the key vectors of PVY^{NTN} in potato crops in Tunisia.

Transmission efficiencies for PVY^{NTN} strains seem to be higher within the others PVY strain and seem determined by aphid species, by clone used and virus concentration in the source leaf. These results will lead to a better understanding of PVY^{NTN} epidemiology and to a better control of aphid vectors in order to improve seed potato sanitary conditions. However, the transmission method must be improved by using potato plants and it must be standardized in order to use it as a routine technique to evaluate the vector capacity of aphids in transmitting PVY^{NTN} in the field.

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