Vector activity of three aphid species (Hemiptera: Aphididae) modulated by host plant selection behaviour on potato (Solanales: Solanaceae)

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Summary. Viral diseases non-persistently transmitted by aphids are of great economic importance in several annual crops. Transmission efficiency of these non-persistent phytophuses is dependant on vector efficiency (i.e. vector intrinsic ability to transmit the virus) but also on the vector activity that implies the early steps of aphid host plant selection process (i.e. brief intracellular stylet punctures after landing) and to their interplant movement ability. In Europe, Macrosiphum euphorbiae (Thomas 1878) is considered as one of the most serious virus vectors on potato (Solanum tuberosum L. 1753). Nevertheless, several alate aphid species that do not colonise potato plants are trapped in potato crops. Therefore, we investigated, through laboratory experiments, vector activity of one potato colonising aphid, M. euphorbiae, and two non-colonising potato aphids, the bird cherry-oat aphid Rhopalosiphum padi (L. 1758) and the pea aphid Acyrthosiphon pisum (Harris 1776). A settling experiment was used to evaluate dispersal activity, and the electrical penetration graph (EPG) technique was used to investigate probing activity on potato plants. Results showed that M. euphorbiae exhibited a better vector activity than other two aphid species in terms of landing and probing. By contrast, interplant movements were only recorded on non-colonising aphids, suggesting a better vector activity than M. euphorbiae in terms of locomotive behaviour. These data confirm the involvement of A. pisum and R. padi in the spread of non-persistent viruses.

Résumé. Etude de la modulation de l’activité vectorielle de trois espèces de pucerons (Hemiptera : Aphididae) par le comportement de sélection de la plante hôte sur pomme de terre (Solanales : Solanaceae). Les maladies virales transmises de manière non-persistante par les pucerons sont d’une grande importance économique dans plusieurs cultures annuelles. L’efficacité de transmission de ces phytophuses non-persistants dépend de l’efficacité vectorielle (capacité intrinsèque du vecteur à transmettre le virus), mais aussi à l’activité vectorielle impliquant (1) la capacité pour le puceron vecteur à se déplacer de plantes en plantes (2) les étapes précoces du processus de sélection de la plante hôte par le puceron vecteur (i.e. brèves piqûres intracellulaires après atterrissage). En Europe, Macrosiphum euphorbiae (Thomas 1878) est considéré comme l’un des vecteurs viraux les plus importants sur la pomme de terre (Solanum tuberosum L. 1753). Néanmoins, plusieurs espèces de pucerons ailés qui ne colonisent les plantes de pomme de terre sont piégées dans les cultures de pommes de terre. Par conséquent, nous avons étudié, à travers des expériences de laboratoire, l’activité vectorielle d’un puceron colonisateur, M. euphorbiae, et de deux pucerons non-colonisateurs de la pomme de terre, le puceron du merisier à grappes Rhopalosiphum padi (L. 1758) et le puceron du pois Acyrthosiphon pisum (Harris 1776). Une expérience comportementale d’installation a été utilisée pour évaluer l’activité de dispersion, et la technique d’électropénétrographie (EPG) a été utilisée pour étudier l’activité de piqûre sur la pomme de terre. Les résultats ont montré pour M. euphorbiae une meilleure activité vectorielle par rapport aux deux autres espèces en termes d’attirissage et de piqûre. En revanche, les mouvements entre plantes ont été visualisés uniquement pour les pucerons non-colonisateurs suggérant une meilleure activité vectorielle que celle de M. euphorbiae en termes de comportement locomoteur. Ces données confirment l’implication de A. pisum et R. padi dans la dissémination des virus non-persistants.

Keywords: Macrosiphum euphorbiae; Rhopalosiphum padi; Acyrthosiphon pisum; dispersal behaviour; feeding behaviour; electrical penetration graph (EPG)

Visual, physical and chemical cues are commonly used by phytophagous insects to find and then accept their host plant (Prokopy & Owens 1983; Schoonhoven et al. 2006). In aphids, the host plant selection process can be divided into three behavioural steps: (1) approach and landing on the plant; (2) leaf surface exploration and brief probes; and (3) after assessment of the phloem sap, host acceptance, which leads to sustained sap ingestion (Niemeyer 1990; Caillaud 1999; Powell et al. 2006). Host or non-host plant discrimination implies perception of visual and volatile cues before alighting (Nottingham & Hardie 1993; Powell et al. 1999), but also gustative cues perceived...
during brief plant subepidermal probes (Bernays & Funk 2000; Caillaud & Via 2000; Powell & Hardie 2000; Funk & Bernays 2001) and during phloem sap ingestion (Van Helden & Tjallingii 1993). The relative importance of each of these three steps can be affected by the aphid specialisation with regard to the plant (Bernays & Funk 1999; Funk & Bernays 2001) and according to the aphid species (Tosh, Powell & Hardie 2003). To find a suitable host, alate aphids are confronted by different challenges depending particularly on their host plant range. As only 5% of aphid species are considered as polyphagous (Blackman & Eastop 2000), many of them are highly specialised in their feeding preferences and exploit no more than one or a few closely related plant species (Dixon 1998). Aphids are relatively weak flyers, unable to control both their speed and flight direction, and most of the time they are carried and directed by wind (Dixon 1998). Consequently, only few dispersing aphids reach a suitable host (Ward et al. 1998).

Potato virus Y (PVY) (Potyviridae: Potyvirus) is one of the most economically important viruses affecting potato crops (Solanum tuberosum L. 1753) in the world (Sigvald 1992). This pathogen is mostly transmitted in a non-persistent manner from plant to plant by several alate aphid species, among which are non-colonising of potato species (Kennedy et al. 1962; Van Hoof 1980; Sigvald 1984; Harrington & Gibson 1989; de Boks & Piron 1990; Heimbach et al. 1998). Virus transmission that requires an intrinsic ability to transmit the virus (vector efficiency) is also dependent on the aphid’s ability to move between plants (Irwin & Ruesink 1986) and to perform intracellular punctures within epidermal and mesophyll tissues (Powell et al. 1995; Martin et al. 1997). Such behaviours involved in the spread of non-persistent viruses were defined as vector activity (Irwin & Ruesink 1986).

In Picardy (north of France, 49°53′N, 2°17′E), besides colonising potato aphids such as Macrosiphum euphorbiae (Thomas 1878), non-colonising aphids are abundant in potato fields. The spectrum of the trapped aphid species seems to be related to the crops cultivated near potato plots and is mostly composed of the cereal aphids Sitobion avenae (F. 1794) and Rhopalosiphum padi (L. 1758), the pea aphid Acyrthosiphon pisum (Harris 1776), the black bean aphid Aphis fabae (Scopoli 1763), and the cabbage aphid Brevicoryne brassicae (L. 1758) (Sternorrhyncha: Aphididae) (Boquel 2011). Even though very low densities of potato colonising aphids are trapped in some years, potato crops still show high virus infection rates (Boiteau et al. 1998; Boquel 2011). A previous vector efficiency study showed that all these non-colonising aphid species exhibited a medium to weak ability to transmit PVY (Boquel et al. 2011a). Moreover, their abundance in fields (Difonzo et al. 1997) or their ability to realise numerous interplant movements (Boquel et al. 2012) was suggested to compensate for their low PVY transmission efficiency. Conversely, M. euphorbiae alates do not reach high densities in fields but showed a higher PVY transmission efficiency compared to non-colonising aphids (Boquel et al. 2011a).

Styel penetration and feeding behaviour can be tracked with an electropenetrograph (EPG) device to accurately determine the location of the styels within plant tissues and the insect activity (Tjallingii 1978, 1988). The penetration of aphid styels in plant tissues generates different electrical waveforms which are amplified and acquired on a computer. EPG studies paired with histological sections allowed the different patterns to be linked with different feeding activities such as probing, salivation or ingestion, as well as the position of the styels within plant tissues (Tjallingii 1985). This technique has been extensively used for the last 30 years to characterise aphid behaviour in response to stimuli (Ramirez & Niemeyer 2000; Brunissen et al. 2009, 2010), to host and non-host plants (Powell & Hardie 2000), to plant resistance (Van Helden & Tjallingii 1993; Le Roux et al. 2008, 2010; Pompon et al. 2010a) and to virus transmission (Powell et al. 1995; Collar et al. 1997; Martin et al. 1997; Boquel et al. 2011a).

The aim of this study was to determine the vector activity of the colonising potato aphids M. euphorbiae and two non-colonising potato aphids, R. padi and A. pisum. A settling bioassay was used to investigate the dispersal activity of these aphids (steps 1 and 2 of host plant selection) and electrical penetration graph (EPG) experiments were carried out to assess their probing behaviour (steps 2 and 3 of host plant selection). Such information would be valuable to improve our understanding of the impact of the behaviour of non-colonising aphid species in the spread of PVY in potato fields.

Materials and methods

Insects

The colonies of M. euphorbiae, R. padi and A. pisum were initiated, for each species, from a single alate parthenogenetic female and separately reared in ventilated Plexiglas® cages (360 × 240 × 110 mm) in a growth chamber under controlled conditions (20 ± 2°C, 60 ± 5% relative humidity, and 16L:8D light cycle). The clone of M. euphorbiae (INRA-NSA, Villeurbanne, France, 45°46′N, 4°52′E) was reared on potato (Solanum tuberosum L. var. Désirée), the one of R. padi (UMR-INRA-BIO3P, Rennes, France, 48°06′N, 1°40′W) was reared on wheat (Triticum aestivum var. Mendel) and finally the A. pisum clone (UMR-BGPI, Montpellier, France, 43°36′N, 3°52′E) was reared on broad bean (Vicia faba var. Maya). All bioassays, performed at 20 ± 2°C, 60 ± 5% relative humidity, and 16L:8D light cycle, were run with alates synchronised according to the set-up described by Brunissen et al. (2009).

In vitro plantlets

The potato in vitro line (S. tuberosum L. var. Bintje) was created from a potato tuber germ. In vitro plantlets were micropropagated
by subculturing internode explants on a basal medium according to Murashige & Skoog (1962) (MS) supplemented with sucrose and agar. Each plantlet was grown axenically for 15 days under 20 ± 1°C, 60 ± 5% RH and 16L:8D light cycle in small glass vials (5 ml) placed in a sterile culture glass tube (25 mm × 150 mm). The small vial allowed withdrawal of the plantlet and its use for the experiments.

**Aphid settling and movement activity**

Plant selection and dispersal activity were investigated through a settling bioassay for 48 h. The set-up consisted of a ventilated Plexiglas® chamber (180 × 120 × 75 mm) wherein two in vitro potato plantlets were each set in a small receptacle containing water to prevent aphid colonisation through walking (Boquel et al. 2012). Each synchronised alate aphid was placed on the base of the chamber with a fine paintbrush. At the end of the 48 h experiment, aphid location (i.e. on a plantlet or on the inner wall of the experimental chamber) was recorded. In vitro plantlets were then separately collected and stained with trypan blue to check for the presence of salivary sheaths resulting from aphid stylet insertion within plant tissues to determine, a posteriori, which plantlets were punctured throughout the experiment (Boquel et al. 2012). The trypan blue staining protocol (Koch & Slusarenko 1990) was modified as followed: leaves were boiled 5 min in trypan blue then discoloured for 24 h in a chloral hydrate solution. At the end of the discoloration, plantlets were observed under a microscope (Leica M165C, Wetzler, Germany) and checked for at least 15 min for the presence or absence of aphid salivary sheaths. For each aphid species, 30 individuals were tested.

**Aphid probing behaviour**

Probing behaviour of alate aphids was monitored using a Giga-8 DC-EPG system (EPG-Systems, Wageningen, the Netherlands). A gold wire (diameter 20 µm, 2 cm long) was connected to the EPG amplifier with a copper wire attached to a copper nail. The other end of the gold wire was attached to the dorsum of the aphid with conductive water-based silver glue. The aphid was positioned on the top of a pipette tip connected to a pump. The pump generates a negative pressure that ensures the immobilisation of the aphid during the procedure. The wired aphids were allowed to rest for a period ranging from 10 to 20 min before recording their probing behaviour. Once a set of eight aphids was wired, they were carefully placed on an in vitro plantlet. The plant electrode was inserted into the MS medium to complete the electrical circuit. The recordings started in the morning (typically 9:00 am) and were performed on eight insects at the same time for eight continuous hours. Acquisition and analysis of the EPG waveforms were carried out with PROBE 3.5 software (EPG-Systems) and EPG parameters were calculated using the EPG-Calc 4.9 software (Giordanengo 2014). These parameters were based on five different EPG waveforms described by Tjallingii and Hogen-Esch (1993): C corresponding to stylet pathways in plant tissues except phloem and xylem (Figure 1), E1 to salivation in phloem elements, E2 to passive phloem sap ingestion, G to active xylem sap ingestion, F to derailed stylet mechanics and pd (potential drops) to intracellular stylet punctures. Ten parameters as shown in Table 1 were chosen to describe four behavioural classes analyzed for the whole 8-h recording: the general probing behaviour class (parameters 1 and 2), the pathway phase class (parameters 3–6), the phloem phase class (parameters 7 and 8), and finally the other parameters class (parameters 9 and 10). Only parameters (5 and 6) related to intracellular probing behaviour were analysed during the first hour after the first probe. For each aphid species 19–20 individuals were successfully recorded for 8 h.

**Data analysis**

Statistica 8.0 software (StatSoft 2013) was used for all statistical analyses. A contingency table involving a chi-squared test was done to compare (1) the aphid’s location after 48 h of the settling period (final choice); and (2) the distribution of salivary sheaths in one plantlet and (3) in both plantlets. Because EPG data were not normally distributed, a Kruskal–Wallis one-way analysis of variance (H) was done between aphid species followed by a pairwise comparison with a Mann–Whitney U-test. Differences were considered as significant when p < 0.05.

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**Figure 1.** Typical waveforms seen in an electropenetrograph.

Results

Aphid settling and movement activity

Location of the aphids at the end of the 48-h settling bioassay (Figure 2) showed significant differences between the tested species ($\chi^2 = 46.67, df = 2, p < 0.01$). All *M. euphorbiae* were located on plantlets, while significantly lower proportions of non-colonising potato aphids were observed on plantlets (*R. padi*: 33%, $\chi^2 = 27.08, df = 1, p < 0.01$; *A. pisum*: 17%, $\chi^2 = 39.50, df = 1, p < 0.01$). No significant difference was observed between the two non-colonising aphids ($\chi^2 = 1.42, df = 1, p = 0.23$).

The occurrence of salivary sheaths (Figure 3) in one plantlet showed significant differences between the tested species ($\chi^2 = 25.99, df = 2, p < 0.01$). All *M. euphorbiae* probed in only one plantlet, whereas a significantly lower proportion of *R. padi* (40%, $\chi^2 = 22.94, df = 1, p < 0.01$) and *A. pisum* (53%, $\chi^2 = 15.75, df = 1, p < 0.01$) was observed. Furthermore, these two latter species did not exhibit a significant difference in the proportion of salivary sheaths found in one plantlet ($\chi^2 = 1.42, df = 1, p = 0.23$).

The occurrence of salivary sheaths (Figure 3) in both plantlets also showed significant differences between the tested species ($\chi^2 = 19.30, df = 2, p < 0.01$). *Macrosiphum euphorbiae* probed in only one of the two plantlets while a significant higher proportion of *R. padi* (50%, $\chi^2 = 19.84, df = 1, p < 0.01$) and *A. pisum* (37%, $\chi^2 = 12.64, df = 1, p < 0.01$) probed in the two plantlets. No significant difference was noticed among the non-colonising aphid species ($\chi^2 = 0.67, df = 1, p = 0.41$).

Aphid probing behaviour

The time to first probe, the total duration of probing, the number of pathway phases, the number of potential drops, the number of phloem phases and the total duration of

<table>
<thead>
<tr>
<th>EPG classes</th>
<th><em>M. euphorbiae</em></th>
<th><em>R. padi</em></th>
<th><em>A. pisum</em></th>
<th>$H(p)^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>General probing behaviour</td>
<td></td>
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<tr>
<td>1. Time to first probe (min)</td>
<td>$14.6 \pm 2.2$ b</td>
<td>$79.5 \pm 20.9$ a</td>
<td>$25.6 \pm 7.4$ b</td>
<td>$13.5 (p &lt; 0.01)$</td>
</tr>
<tr>
<td>2. Total duration of probing (min)</td>
<td>$441.4 \pm 5.3$ a</td>
<td>$172.0 \pm 22.6$ b</td>
<td>$202.2 \pm 17.8$ b</td>
<td>$38.7 (p &lt; 0.01)$</td>
</tr>
<tr>
<td>Pathway phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Number of pathway phases</td>
<td>$13.4 \pm 1.8$ b</td>
<td>$9.5 \pm 1.5$ b</td>
<td>$28.0 \pm 2.9$ a</td>
<td>$23.7 (p &lt; 0.01)$</td>
</tr>
<tr>
<td>4. Total duration of pathway phases (min)</td>
<td>$158.9 \pm 17.5$ a</td>
<td>$113.0 \pm 17.2$ b</td>
<td>$135.2 \pm 14.8$ a</td>
<td>$5.06 (p = 0.08)$</td>
</tr>
<tr>
<td>5. Number of potential drops</td>
<td>$35.9 \pm 4.2$ a</td>
<td>$14.4 \pm 2.4$ a</td>
<td>$24.4 \pm 2.9$ b</td>
<td>$16.7 (p &lt; 0.01)$</td>
</tr>
<tr>
<td>6. Mean duration of potential drops (s)</td>
<td>$5.2 \pm 0.2$</td>
<td>$4.8 \pm 0.4$</td>
<td>$4.8 \pm 0.2$</td>
<td>$4.7 (p = 0.09)$</td>
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<tr>
<td>Phloem phase</td>
<td></td>
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<tr>
<td>7. Number of phloem phases</td>
<td>$1.7 \pm 0.4$ a</td>
<td>$0.2 \pm 0.1$ b</td>
<td>$0.0 \pm 0.0$ b</td>
<td>$40.2 (p &lt; 0.01)$</td>
</tr>
<tr>
<td>8. Total duration of phloem phases (min)</td>
<td>$233.6 \pm 27.3$ a</td>
<td>$0.7 \pm 0.6$ b</td>
<td>$0.0 \pm 0.0$ b</td>
<td>$43.9 (p &lt; 0.01)$</td>
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<tr>
<td>Other parameters</td>
<td></td>
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<tr>
<td>9. Total duration of xylem ingestion (min)</td>
<td>$42.1 \pm 10.5$ a</td>
<td>$57.0 \pm 12.1$ b</td>
<td>$48.6 \pm 5.4$ a</td>
<td>$0.8 (p = 0.68)$</td>
</tr>
<tr>
<td>10. Total duration of stylet derailment (min)</td>
<td>$6.4 \pm 6.0$</td>
<td>$0.5 \pm 0.5$</td>
<td>$10.2 \pm 4.4$</td>
<td>$3.8 (p = 0.15)$</td>
</tr>
</tbody>
</table>

a All parameters were calculated for the whole 8-h recording except parameters 5 and 6, which were calculated for the first hour after the first probe. n corresponds to the number of individuals tested.

b H (p): Kruskal–Wallis value with its probability within brackets. Different letters indicate significant differences between species for each parameter (Mann–Whitney U-test, $p < 0.05$).
phloem phases significantly differed among the three species (Table 1). In the general probing behaviour class (parameters 1 and 2), *M. euphorbiae* exhibited the shortest time from the start of the recording to the first probe. Twice as much time was necessary for *A. pisum* and more than 5 times as much time for *R. padi* to probe the plant. *Macrosiphum euphorbiae* exhibited the longest total durations of probing compared to non-colonising potato species. Despite the lack of difference observed between species in the total duration of pathway phases, the number of pathway phases was higher in *A. pisum* compared to *M. euphorbiae* and *R. padi*. For parameters related to intracellular punctures (parameters 5 and 6), the number of potential drops was higher in *M. euphorbiae* than in *R. padi*, and *A. pisum* exhibited an intermediate value. Mean duration of potential drops was the longest in *M. euphorbiae* even though there was no significant difference with *R. padi* and *A. pisum*.

In the phloem phase class (parameters 7 and 8), the highest numbers and total duration of phloem phases were observed in *M. euphorbiae*. Few individuals of *R. padi* exhibited brief phloem phases, explaining the very low values. Meanwhile, *A. pisum* never reached phloem vessels. All aphid species showed xylem ingestion phases (parameter 9) without any differences between species.

**Discussion**

Aphid vector activity is the most important cause of the spread of phytovirus (Irwin & Ruesink 1986). An efficient transmission thus essentially relies on vector probing activity and dispersal activity.

The settling experiment showed that the potato colonising aphid *M. euphorbiae* settled on plantlets after 48 h. They were also able to probe the plantlets as demonstrated by the stained salivary sheaths. The long association of *M. euphorbiae* with *Solanum* plants could have played a major role in its preference for Solanaceae (Flanders et al. 1992; Blackman & Eastop 2000) through the perception of phagostimulant stimuli (Tosh, Powell, Holmes, et al. 2003) promoting such sedentary behaviour. EPG study also underlined such behaviour. Indeed, *M. euphorbiae* spent a long time probing the plant and especially ingesting phloem. By contrast, a lower number of non-colonising aphids settled on potato plantlets. Occurrence of salivary sheaths on both plantlets leads to the conclusion that most of them landed on plantlets, probed, and then flew away, leading to interplant movements. These non-colonising potato aphids are oligophagous species that develop on a few closely related plant species within the Poaceae family for *R. padi* and Fabaceae for *A. pisum* (Blackman & Eastop 2000). Moreover, a previous study highlighted that colonising aphids (Myzus persicae Sulzer 1776) spent more time on potato plants in comparison to non-colonising ones (*S. avenae, A. fabae* and *B. brassicae*) and they did not discriminate between healthy and PVY-infected plants (Boquel et al. 2012). Together, our results suggest that visual and olfactory cues are weakly involved in the early steps of the plant selection process by aphids.
suggesting a later discrimination of the plant (Kring 1972). Such prevalence of gustative cues perceived during brief probes was reported for *R. padi* (Olivares-Donoso & Niemeyer 2005). Taken together, the low proportion of non-colonising aphids observed on a potato plantlet at the end of the experiment and the presence of salivary sheaths in both plantlets seem to support the above hypothesis, and plant suitability was assessed through gustatory cues perceived during probing. EPG study strengthened the prevalence of such gustatory cues in plant selection: non-colonising aphids spent less time probing into plant tissues and most of them did not reach phloem vessels. Host plant acceptance is considered when phloem contact is approximately longer than 10 min (Powell et al. 2006). As expected, non-colonising species did not exhibit potato plant acceptance. Secondary metabolites are known to negatively affect insect metabolism (i.e. antibiosis) but also to modulate their behavioural response (i.e. antixenosis). Perception of such compounds, like potato glycoalkaloids considered as deterrent for *M. euphorbiae* (Güntner et al. 1997, 2000), could provide enough information for plant rejection and induction of aphid flight. A high level of xylem ingestion was observed for the two non-colonising aphids and for *M. euphorbiae* as well. The lack of phloem sap ingestion in non-colonising aphids corresponds to a fasting period reported to lead to rehydration in xylem vessels (Spiller et al. 1990; Ramirez & Niemeyer 2000). In *M. euphorbiae*, it could rather result from a dilution of phloem diet to replenish their water balance (Powell & Hardie 2002), thought to play a role in the osmoregulation of haemolymph (Pompon et al. 2010b).

Some parameters related to probing activity allowed an estimation of the main behavioural components involved in the spread of non-persistent viruses. Since all aphid species performed intracellular punctures they are all able to transmit non-persistent viruses (Pelletier et al. 2008; Boquel 2011a). Nevertheless, as the binding of viruses in the stylet tip would last not more than about two hours (Van Hoof 1980), the time elapsed from the last intracellular puncture into an infected plant to the first intracellular puncture into a healthy plant is a critical step (Pelletier et al. 2008). Therefore, the delayed stylet insertion in plant tissue observed in *R. padi* could affect virus transmission; this species has been previously reported as a poor (Pelletier et al. 2008) or an inefficient vector (Boquel 2011a). Likewise, other studies reported medium transmission rates for *M. euphorbiae* (Boquel et al. 2011b) and a weak or absent ability to transmit PVY for *R. padi* and *A. pisum* (Van Hoof 1980; Harrington & Gibson 1989; Boquel 2011a). The low number of intracellular punctures observed for these latter species could partly explain these results, as intracellular punctures and PVY transmission efficiency are positively correlated (Collar et al. 1997).

In summary, from a vector activity point of view, discrepancies were reported between species in terms of probing activity and dispersal activity according to their ability to exploit the potato plant. As expected, potato colonising species and in a lesser way non-colonising ones exhibited adapted probing activity. Contrary to non-colonising aphids, no dispersal activity was observed for the potato colonising aphid *M. euphorbiae*. Nevertheless, final plant acceptance leading to settlement then reproduction (Powell et al. 2006) could enhance the impact of this aphid by the numerous apterous progeny produced, favouring a secondary short-range spread of the virus through walking.

Considering that the spread of virus is closely linked to the vector dispersal activity (Irwin & Ruesink 1986) and more particularly to interplant movements, our data suggest an involvement of non-colonising aphids in the spread of PVY. In addition to their high abundance in potato fields, their vector activity, which fits to a rapid spread of the virus, suggests that the impact of non-colonising potato aphids in PVY epidemics has probably been underestimated.

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**References**


