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The use of test tube plantlets for the assessment of *Potato virus Y* transmission by *Myzus persicae* and *Aphis glycines*
Utilisation de vitroplants pour l'évaluation de la transmission du virus Y de la pomme de terre par *Myzus persicae* et *Aphis glycines*

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Résumé de l'article

Des vitroplants ont été utilisés pour évaluer le taux de transmission du virus Y de la pomme de terre (PVY) entre plants de pomme de terre par un puceron inféodé à la pomme de terre, le puceron vert du pêcher (*Myzus persicae*), et un puceron non-inféodé à la pomme de terre, le puceron du soya (*Aphis glycines*). Des taux similaires d'efficacité de transmission du PVY par *M. persicae* ont été observés lorsque des vitroplants ou des plantes en pot étaient utilisés, ce qui démontre la validité de l'utilisation de vitroplants pour l'évaluation du taux de transmission du PVY par les pucerons. La même approche a par la suite été utilisée pour mesurer le taux de transmission du PVY^O et du PVY$^{N:O}$ par *M. persicae* et par *A. glycines* sous deux régimes d'acquisition, soit 5 min de sondage continu et 1 h d'accès. Le taux de transmission de *M. persicae* a été de 24,1 % et de 51,7 % pour PVY^O et PVY$^{N:O}$, respectivement, sous un régime d'acquisition de 5 min, et de 0,0 % et 1,7 % pour PVY^O et PVY$^{N:O}$, respectivement, avec *A. glycines*. Sous un régime d'acquisition d'une heure, aucune infection n'a été observée à l'exception de PVY$^{N:O}$ par *M. persicae* qui a présenté un taux d'infection de 3,4 %.

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The use of test tube plantlets for the assessment of *Potato virus Y* transmission by *Myzus persicae* and *Aphis glycines*

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Test tube plantlets were used to assess the transmission efficiency of *Potato virus Y* (PVY) from and to potato plants by the potato colonizing species green peach aphid (*Myzus persicae*) and the non-potato colonizing species soybean aphid (*Aphis glycines*). Similar levels of transmission of PVY by *M. persicae* were observed in the test tube plantlets and potted plants, demonstrating the reliability of this test for PVY transmission efficiency assay for aphids. The assay was then used to assess the transmission of PVY^O and $PVY^{N:O}$ by *M. persicae* and *A. glycines* with two virus acquisition regimes, one with 5-min continuous probing and the other with 1-h acquisition access. The *M. persicae* mediated-transmission rate was 24.1% and 51.7% for PVY^O and $PVY^{N:O}$, respectively, under the 5-min acquisition regime; under the same acquisition regime, *A. glycines* led to 0.0% and 1.7% infection rates for PVY^O and $PVY^{N:O}$, respectively. Under the 1-h acquisition regime, no infection was observed except for $PVY^{N:O}$ by *M. persicae*, which exhibited an infection rate of 3.4%.

Keywords: *Aphis glycines*, *Myzus persicae*, plantlets, potato, *Potato virus Y*, test tube, transmission.

[Utilisation de vitroplants pour l'évaluation de la transmission du virus Y de la pomme de terre par *Myzus persicae* et *Aphis glycines*]

Des vitroplants ont été utilisés pour évaluer le taux de transmission du virus Y de la pomme de terre (PVY) entre plants de pomme de terre par un puceron inféodé à la pomme de terre, le puceron vert du pêcher (*Myzus persicae*), et un puceron non-inféodé à la pomme de terre, le puceron du soya (*Aphis glycines*). Des taux similaires d'efficacité de transmission du PVY par *M. persicae* ont été observés lorsque des vitroplants ou des plantes en pot étaient utilisés, ce qui démontre la validité de l'utilisation de vitroplants pour l'évaluation du taux de transmission du PVY par les pucerons. La même approche a par la suite été utilisée pour mesurer le taux de transmission du PVY^O et du $PVY^{N:O}$ par *M. persicae* et par *A. glycines* sous deux régimes d'acquisition, soit 5 min de sondage continu et 1 h d'accès. Le taux de transmission de *M. persicae* a été de 24,1 % et de 51,7 % pour PVY^O et $PVY^{N:O}$, respectivement, sous un régime d'acquisition de 5 min, et de 0,0 % et 1,7 % pour PVY^O et $PVY^{N:O}$, respectivement, avec *A. glycines*. Sous un régime d'acquisition d'une heure, aucune infection n'a été observée à l'exception de $PVY^{N:O}$ par *M. persicae* qui a présenté un taux d'infection de 3,4 %.

Mots clés : *Aphis glycines*, *Myzus persicae*, vitroplants, virus Y de la pomme de terre, transmission.

Potato virus Y (PVY) is the type species of the genus *Potyvirus*, family *Potyviridae*. It is one of the most economically important viruses of the potato (*Solanum tuberosum* L.) crop worldwide, causing significant yield losses and quality degradations to the crop (Shukla *et al.* 1994). Many strains and substrains of PVY, including the common (ordinary) strain (PVY^O), the tobacco veinal necrosis strain (PVY^N), the potato stipple streak strain (PVY^C), the potato tuber necrosis strain (PVY^{NTN}), and the recombinant N:O strain ($PVY^{N:O}$), have been identified to date (Nie *et al.* 2004, 2011). The emergence of various PVY strains in North America (Baldauf *et al.* 2006; Crosslin *et al.* 2006; Karasev *et al.* 2010; Lorenzen *et al.* 2008; Nie *et al.* 2004, 2011), together with the recent increase in PVY incidence in North American potato crops (Crosslin *et al.* 2006; Nolte 1997; Piche *et al.* 2004; Singh *et al.* 2003), has become a significant concern for the potato industry. PVY is transmitted by a number of aphid species in a non-persistent manner (Radcliffe and Ragsdale 2002). Recent information on the mode by which potyviruses attach to the stylets of aphids (Uzest *et al.* 2010) supports the view that most aphid species can carry potyviruses (Moreno *et al.* 2005). The major factor affecting virus transmission efficacy might reside in the host selection behaviour of the aphid (Pelletier *et al.* 2008).

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All studies on aphid transmission of PVY to date were carried out under controlled conditions with potted plants originating from virus-free *in vitro* plantlets or propagules. This is a time consuming and labour intensive process because the plants not only take a long time to grow before they are ready for the experiment but they also need to be kept free of aphids after the transmission is completed until the virus multiplies sufficiently to be evaluated for its presence. Here, we report the direct use of *in vitro* plantlets, still in the test tube, to study the transmission efficiency of PVY from and to potato plants by aphids. This method was then used to evaluate the transmission efficacy of the soybean aphid (*Aphis glycines* Matsumura).

PVY[N:O] isolate Mb58 (Nie *et al.* 2004) and PVY[O] isolate RB (Nie *et al.* 2011) were used in this study. Potato tubers (cv. Shepody) infected with PVY[N:O]-Mb58 or PVY[O]-RB were planted in the greenhouse at 18-25°C with a 16/8 h (light/dark) cycle. The light intensity was 90 μEm^{-2}s^{-1} and the humidity was 65-75%. When the plants were 3 to 4 wk old, they were used as the virus source for the aphid-mediated transmission assays. *Myzus persicae* Sulzer was obtained and maintained on caged virus-free (VF) potato plants of 'Shepody' as described previously (Pelletier *et al.* 2008). *Aphis glycines* was initially collected from a soybean field in Manitoba and maintained on potted soybean plants produced in the greenhouse. Winged aphids were used for the assays. To select these aphids, all aphids from the roof and sides of the rearing cages were cleared and, 30 min later, the aphids that had flown to the cage ceiling were collected and brought to the laboratory for virus transmission assay (Pelletier *et al.* 2008). The aphids were starved for 30 to 90 min prior to the experiments. All assays were conducted at room temperature (20 ± 2°C). VF test tube plantlets of 'Shepody' at the three-leaf stage were obtained from the Plant Propagation Centre, New Brunswick Department of Agriculture and Aquaculture, Fredericton, New Brunswick, Canada. For the test tube-based PVY transmission assay, the plantlets remained in the test tubes containing MS medium (Murashige and Skoog 1962) and were kept in a growth chamber at 19°C with a 16/8 h (light/dark) cycle, 55% humidity and 70 μEm^{-2}s^{-1} light intensity before and for 3 wk after exposure to an aphid.

A leaflet from a plant emerged from a PVY[O]- or PVY[N:O]-infected tuber was detached, and its petiole was placed into a 25 mL vial containing water covered with a parafilm membrane. The aphid was considered to be probing when the rostrum was touching the substrate and the antennae were pointing backward. Two different acquisition periods were used in this study, one with a 5-min continuous probing and the other with a 1-h acquisition access period. For the 5-min acquisition regime, one alate aphid was placed on the PVY-infected leaflet, covered with a cage made of a Plexiglas cylinder (15.3 cm in diam and 30 cm high, with the top covered with fine screening), and the probing time was recorded. Aphids that probed continuously for 5 min within a 20-min period were used. Immediately after probing continuously for 5 min, the aphid was carefully picked by the wings with forceps and placed on a plantlet in a test tube. For the 1-h acquisition, five to ten aphids were placed on the detached PVY-infected leaf covered with a Plexiglas cylinder (15.3 cm in diam and 30 cm high, with the top covered with fine screening). The aphids were monitored periodically to ensure the probing was taking place during the acquisition time period. After 1 h, aphids were transferred singly to a plantlet in a test tube. The test tube was re-capped immediately after the aphid was placed on the plantlet. The aphid was allowed to probe the plantlet for 1 h and was then removed from the test tube with a disinfected paintbrush. The tube was capped and sealed with tape, and the plantlet was returned to and kept in the growth chamber for 3 wk. The inoculated test tube plantlets were then subjected to a PVY infection test. The validity of using test tube plantlets directly was evaluated by comparing the infection rate by *Myzus persicae* to results obtained 1 yr earlier using the same protocol, aphid colony and facilities, but by growing the tissue culture plantlets in pots prior to using them (Pelletier *et al.* 2008). Reverse transcription polymerase chain reaction (RT-PCR) was used for detection of PVY from potato plants as described previously (Nie and Singh 2001a, 2001b; Pelletier *et al.* 2008). Total RNA was extracted from potato leaves using an RNeasy plant mini kit (QIAGEN Sciences, Germantown, MD, USA) according to the manufacturer's instructions. The resulting RNA was quantified using a ND-1000 spectrophotometer (NanoDrop) and used for RT-PCR assay. Duplex RT-PCR was carried out to detect PVY and the potato cytochrome c oxidase subunit I gene (*cox1*) using target specific primers (PVY: forward, 5' ACGTCCAAAATGAGAATGCC 3'; reverse, 5' TGGTGTTCGTGATGTGACCT 3'; *cox1*: forward, 5' GGTCGGACATACCCTGAAAC 3'; reverse, 5' CAAAAGTATGAAAAGCTGGAG 3'). The size of the target amplicons was 480 bp for PVY (Nie and Singh 2001a) and 332 bp for *cox1* (Nie and Singh 2001b). *cox1* served as an internal control to indicate the successfulness of the RT-PCR assay (Nie and Singh 2001b). The comparison between two transmission efficacy proportions was done using a *Z*-test with Systat ver. 11 (Systat Software Inc., Chicago, IL, USA).

Potted plants originating from virus-free *in vitro* plantlets have been used previously as clean plants for the evaluation of PVY transmission efficacy of aphids (Pelletier *et al.* 2008). It takes approximately 2 to 4 wk after transplanting for the plantlets to be large enough and strong enough for the assay. Moreover, the potted plants need to be protected from aphids before and after the test. This requires the use of a cage during the transmission period to restrict the aphid to the plants. Direct usage of test tube plantlets, using the test tube as a cage, becomes appealing for conducting the assay. To test the possible impact of using tissue culture plantlets on aphid transmission efficacy measurement, single *M. persicae* that had probed continuously for 5 min on PVY[N:O]-infected plants were used immediately for the transmission test, following the protocol used with potted plants as described previously (Pelletier *et al.* 2008). PVY infection was tested 3 wk after aphid inoculation using RT-PCR. The plantlets were also visually inspected for obvious symptoms and abnormalities. All plantlets survived well during the complete course of experiments, indicating that the medium was rich enough

to sustain the plant growth and/or vigor essential for the test. However, the symptoms, mainly mosaic, were not severe enough to warrant a clear-cut diagnosis at least partially due to the mild symptom expression of PVY infection in 'Shepody' (Hane and Hamm 1999). Of the 105 test tube plantlets inoculated by single aphids, 35 became PVY-positive, giving a transmission efficiency of 33.3%. This rate was not statistically different ($Z = 0.561$, $P = 0.575$) from that in the potted plants reported previously in a parallel study (Pelletier *et al.* 2008), in which 30 out of 101 (29.4%) plants became PVY-positive. These results demonstrate that the test tube-based aphid transmission efficiency analysis is reliable and can thus be used as an effective alternative for the assessment of aphid-mediated virus transmission and other applications such as the assessment of resistance to viruses in early breeding lines and potato germplasms.

The test tube-based PVY transmission efficiency assay was employed to investigate the transmission rate of PVY^O and PVY$^{N:O}$ by *M. persicae* and *A. glycines*. The latter is an exotic species that has been found in over 21 states in the USA and three provinces in Canada (Ragsdale *et al.* 2011), and it has been associated with the transmission of PVY in the potato crop (Davis and Radcliffe 2008; Davis *et al.* 2005). Two acquisition regimes, one with a 5-min continuous probe and the other with the 1-h acquisition access on leaves infected with PVY^O or PVY$^{N:O}$, were carried out prior to test tube plantlet inoculation. As shown in Table 1, under the 5-min acquisition regime, *M. persicae* led to infection rates of 24.1% for PVY^O and 51.7% for PVY$^{N:O}$. The latter was significantly higher than the former ($Z = 2.201$, $P = 0.028$). Under the same acquisition regime, the infection rates by *A. glycines* were 0.0% and 1.7% for PVY^O and PVY$^{N:O}$, respectively, and there was no statistical difference between the two rates ($Z = 1.418$, $P = 0.156$). The infection rates by *M. persicae* were significantly higher than those by *A. glycines* for both PVY^O ($Z = 4.516$, $P = 0.000$) and PVY$^{N:O}$ ($Z = 5.900$, $P = 0.000$). Under the 1-h acquisition regime, all infection rates were low, with infection rates by *M. persicae* at 0.0% and 3.4% for PVY^O and PVY$^{N:O}$, respectively, and those by *A. glycines* at 0.0% for both PVY^O and PVY$^{N:O}$. No statisti-

cal differences were observed between PVY^O and PVY$^{N:O}$ or between *M. persicae* and *A. glycines* (*Z*-test, $P > 0.05$). PVY transmission rates of 14-75% and 100% were reported by Davis *et al.* (2005) with a 10-min acquisition regime for *A. glycines* and *M. persicae*, respectively. These rates are significantly higher than the ones obtained in this study. Most reports on *M. persicae* have demonstrated a PVY transmission rate ranging from 20% to 70% (Basky and Almási 2005; Gibson *et al.* 1988; Pelletier *et al.* 2008; Verbeek *et al.* 2009), which is comparable to the rates obtained in this study and is lower than that reported by Davis *et al.* (2005). It is also noteworthy that different PVY strains and isolates have been demonstrated to show different levels of transmissibility by *M. persicae* (Basky and Almási 2005; Gibson *et al.* 1988).

Various studies have demonstrated that acquisition of PVY occurs after probes lasting seconds, and the infectivity decreases rather than increases with sustained feeding on PVY-infected plants (Bradley 1954; Bradley and Rideout 1953; Katis and Gibson 1985; Kotzampigikis *et al.* 2009; Pelletier *et al.* 2008). The low transmission rates exhibited in the 1-h acquisition regime by the aphids, especially by *M. persicae*, are in agreement with previously published studies. Various factors such as aphid probing behaviour and aphid activity between the last cell puncture during the acquisition phase and the first cell puncture at the inoculation phase have been suggested to contribute to the decreased PVY transmission (Pelletier *et al.* 2008). Since limited study has been carried out on soybean aphid-mediated PVY transmission, more research is needed to clarify its role in PVY spread, especially its spread in the fields. The higher transmission rate exhibited by PVY$^{N:O}$, which possesses a recombinant genome resulting from the natural recombination of PVY^N and PVY^O (Nie *et al.* 2004), might be associated with its predominance over other isolates/strains in the potato crop in Manitoba, Canada, and the neighbouring states in the USA (Singh *et al.* 2003).

In conclusion, this study demonstrates that *in vitro* plantlets in test tubes can be used directly to study the transmission efficiency of PVY from and to potato plants by aphids.

Table 1. Transmission of *Potato virus Y* strain N:O (PVY$^{N:O}$) and strain O (PVY^O) by green peach aphid (*M. persicae*) and soybean aphid (*A. glycines*) after a 5-min probing or a 1-h acquisition access on a detached leaf from a PVY^O- or PVY$^{N:O}$-infected plant to test tube plantlets

Aphid species	Acquisition time	PVY strain	No. of infected/ no. of tested plantlets	Transmission (%)
M. persicae	5 min	O	7/29	24.1%*
		N:O	15/29	51.7%*
	1 h	O	0/31	0.0%
		N:O	1/29	3.4%
A. glycines	5 min	O	0/58	0.0%
		N:O	1/58	1.7%
	1 h	O	0/60	0.0%
		N:O	0/60	0.0%

* Under the 5-min acquisition regime, *M. persicae* led to statistically different (*Z*-test at $\alpha = 0.05$) transmission values.

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