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New hosts of *Potato virus Y* (PVY) among common wild plants in Europe

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Abstract The infection capacity of *Potato virus Y* (PVY, genus *Potyvirus*) for wild-living plants, commonly occurring as arable weeds in Europe and native to or naturalised in other continents, was evaluated. In total, 3,712 and 802 seedlings representing 21 weed species were aphid and sap-inoculated with PVY, respectively. Experimentally-inoculated plants of *Erodium cicutarium, Geranium pusillum*, *Lactuca serriola* and *Lamium purpureum* tested positive by ELISA, but they did not display any disease symptoms. The presence of PVY in selected plants of the four species was confirmed by back-inoculations to *Nicotiana tabacum* and by immunocapture-reverse transcription-PCR performed on samples taken from weed and/or tobacco plants. Natural PVY infection in plants of the four wild species collected in the potato-growing area was detected by ELISA and confirmed by back-inoculations to tobacco plants. This study is believed to be the first report of PVY infection in *E. cicutarium, G. pusillum* and *L. purpureum*. Moreover, our findings reveal for the first time PVY infection in *L. serriola* in central Europe.

Keywords Alternative hosts · ELISA · IC-RT-PCR · Inoculation · Natural infection · PVY · Weeds

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Introduction

Potato virus Y (PVY), the type species of the genus *Potyvirus* in family Potyviridae, is a highly destructive virus affecting crops of potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum* spp.) and tomato (*Lycopersicon esculentum*) worldwide. PVY isolates represent three major strain groups: ordinary (PVY^O), tobacco veinal necrosis (PVY^N) and aphid non-transmissible stipple streak (PVY^C). Numerous recombinant as well as non-recombinant forms of PVY have been reported from many parts of the world (Kerlan 2006).

PVY is considered to have a relatively wide host range including mainly solanaceous crops (as listed above) as well as solanaceous and non-solanaceous weeds, and even ornamentals (Kerlan 2006). Susceptibility to PVY of some solanaceous weeds, such as *Physalis floridana*, *Solanum nigrum* and *Solanum dulcamara*, is commonly known, *P. floridana* being used to differentiate PVY strains (Beemster and de Bokx 1987). Arable weeds, especially biennial and perennial ones, can act as natural virus reservoirs for transmission by vectors. In nature, PVY is predominantly spread by aphids, among which *Myzus persicae* is the most effective vector. Aphids transmit the virus in a non-circulative non-persistent manner, which means that virus acquisition from an infected plant and inoculation to a healthy one can be performed during short feeding probes made by aphids in the epidermal tissue to assess plant suitability as a host

(Powell et al. 2006). While searching for a suitable host for settling and reproducing, migrating aphids land on crop and non-crop plants, including weeds growing with crops. Weed plants infected with PVY can thus serve as primary sources of infection.

The objective of these studies was to evaluate the infection capacity of PVY for wild living plants, commonly occurring as arable weeds in Europe, and native to or naturalised in other continents. Moreover, plants of chosen weed species, found in the potato-growing area, were assessed for natural infection with PVY. Identifications of weed species were based on weed atlases (Holm et al. 1979; Skrzypczak et al. 2000; Williams and Morrison 2003) and the Database of IPM Resources (DIR): Internet Resources on Weeds http://ipmnet.org/cicp/gateway/weed.htm and other on-line weed identification guides, e.g. Vegetable MD Online (Zitter 2001), Virginia Tech. Weed Identification Guide http://ipm.ppws.vt.edu/weedindex.htm, ZipcodeZoo.com http://zipcodezoo.com/default.asp, etc.

In a series of experiments carried out in the years 2007 and 2008, a total of 3,712 and 802 seedlings representing 21 weed species (Table 1) were aphid and sap-inoculated, respectively, with PVY isolates belonging to virus strains PVY^{O} and PVY^{N}, the latter including PVY^{NTN} and PVY^{N}-Wi subgroups. During the summer of 2007, seedlings of all the species were grown in greenhouse conditions from seeds collected in the previous years from plants growing around potato fields of the Plant Breeding and Acclimatization Institute in Młochów. Seedlings, planted individually in 8 cm diam pots, were inoculated with PVY using apterous aphids from a virus-free clone of *Myzus persicae*, reared on *Brassica pekinensis*. The sources of virus inoculum were PVY-infected plants of potato cv. Irga. Aphids were allowed a 10-min acquisition access period (AAP) on infected leaves followed by a 24-h inoculation access period (IAP) on weed seedlings (10–15 aphids/seedling). To assess the capacity of aphids to transmit PVY under the conditions applied, healthy *N. tabacum* var. Samsun assay plants were included. After the IFP, the plants were treated with a systemic insecticide. Sap inoculation trials, in which the same source plants were used for preparing PVY inocula, included seedlings of all the 21 weed species (Table 1).

Both inoculated and non-inoculated (control) plants were tested by DAS-ELISA two or three times, essentially 4 and 5 weeks post-inoculation. A cocktail of monoclonal antibodies (PVY-mono-cock, Bioreba AG, Switzerland) was used. Wells of microtitre Nunc plates (type F) were coated with 1 μg ml^{-1} of γ-globulin for 3 h at 37°C. A sample from each plant consisted of the tissue taken from the top and middle leaves. In the case of sap-inoculated plants, samples from non-inoculated leaves were collected. Leaf sap samples were extracted with a leaf press (Erich Pollähne, MEKU GmbH, Wennigsen, Germany), and 200 μl of sap from each sample was diluted with 400 μl of extraction buffer (PBS-Tween, pH 7.4, containing 20 g l^{-1} of PVP-40). The samples were incubated overnight at 4°C in microtitre plates (200 μl per well). Alkaline phosphatase-conjugated γ-globulin in a 1:1000 dilution was incubated for 4 h at 37°C followed by substrate incubation at room temperature. Absorbance readings at 405 nm (A_{405}) were recorded 1 h and 2 h after adding the substrate, using a Dynex MRX II microplate reader (Dynex Technologies, Inc., Chantilly, VA, USA). A sample was considered positive when its A_{405} value exceeded the mean A_{405} value plus 3×SD of healthy (control) plants.

Only *Chenopodium album* and *Solanum nigrum* showed distinct symptoms of PVY infection, associated with positive reactions in ELISA. Both species are well-known hosts for PVY (Shukla et al. 1994). Also, some of the inoculated plants of *Erodium cicutarium, Geranium pusillum*, *Lamium purpureum* and *Lactuca serriola* tested positive by ELISA following aphid or sap-inoculation (Table 1), but these did not display any disease symptoms. The A_{405} values were generally low (<1), except those for *L. purpureum*, which reached as high as 2.0 after 1 h of substrate incubation. However, non-inoculated plants of this species also gave such high readings in some experiments, suggesting the appearance of false-positive reactions.

Further evaluation for susceptibility to PVY only targeted *E. cicutarium, G. pusillum*, *L. purpureum* and *L. serriola*, the first three species being hitherto unrecorded as hosts for PVY. A series of aphid-inoculation experiments was performed, including mechanical back-inoculations from selected ELISA-positive plants to *N. tabacum* var. Samsun plants. The back-inoculation tests confirmed the presence of PVY in the representative plants. Distinct symptoms of PVY infection were found on tobacco Samsun plants inoculated with sap of *G. pusillum* and *L. serriola.* In some cases, tobacco plants inoculated with sap of *E. cicutarium, G. pusillum* or *L. purpureum* showed mild

Table 1 Experimental and natural infection with PVY of weed and wild plant species

Family[a]	Plant species		Life cycle[b]	Infection capacity of PVY[c]	Inoculation under experimental conditions		Natural infection	
	Scientific name	Common name			Aphids	Sap	Tested	Infected
Amaranthaceae	*Amaranthus retroflexus*	Redroot amaranth, redroot Pigweed	an	-	0/140 (1) [d]	0/27 (1)		
Asteraceae	*Achillea millefolium*	Common yarrow	pe	?	0/75 (1)	0/25 (1)		
	Artemisia vulgaris	Mugwort, common wormwood	pe	-	0/110 (2)	0/25 (1)		
	Cichorium intybus	Chicory (coffee-weed)	pe	+	0/75 (1)	0/25 (1)		
	Conyza canadensis	Horseweed	an	+	0/75 (1)	0/10 (1)		
	Galinsoga parviflora	Smallflower galinsoga	an	?	0/150 (1)	0/25 (1)		
	Lactuca serriola	Prickly lettuce	an/bi	+	59/235 (4)	5/45 (2)	16	2
	Solidago canadensis	Canada goldenrod	pe	?	0/75 (1)	0/10 (1)		
	Taraxacum officinale	Dandelion	pe	+	0/75 (1)	0/10 (1)		
	Tripleurospermum inodorum	Scentless mayweed, scentless Camomile	an	?	0/110 (2)	0/10 (1)		
Brassicaceae	*Capsella bursa-pastoris*	Shepherd's purse	an	+	0/145 (2)	0/45 (2)		
Caryophyllaceae	*Melandrium album*	White campion, white cockle	an/bi/p	-	0/40 (1)	0/10 (1)		
Chenopodiaceae	*Chenopodium album*	Common lambsquarters	an	+	12/215 (2)	4/25 (1)	16	1
Geraniaceae	*Erodium cicutarium*	Redstem filaree	an/pe	-	79/542 (8)	2/111 (3)	110	2
	Geranium pusillum	Small-flowered crane's bill	an/bi	-	30/318 (5)	4/35 (1)	98	3
Lamiaceae	*Lamium purpureum*	Purple deadnettle	an	-	205/445 (7)	19/115 (3)	46	4
Plantaginaceae	*Plantago lanceolata*	Buckhorn plaintain	pe	+	0/25 (1)	0/10 (1)		
	Plantago major	Broadleaf plaintain	pe	-	0/110 (2)	0/25 (1)		
Rubiaceae	*Galium aparine*	Stickywilly, catchweed Bedstraw	an	-	0/75 (1)	0/12 (1)		
Solanaceae	*Solanum dulcamara*	Bittersweet nightshade	pe	+	0/124 (2)	0/62 (2)		
	Solanum nigrum	Black nightshade	an	+	196/553 (2)	133/140 (1)		

[a] Cruciferae = Brassicaceae; Asteraceae = Compositae; Lamiaceae = Labiatae,

[b] an = annual, bi = biennial, pe = perennial,

[c] Based on the compiled data from: Brunt et al. (1996); Chatzivassiliou et al. (2004); Edwardson and Christie (1997); Fletcher (2001); Kazinczi et al. (2004); Kerlan (2006); Shukla et al. (1994); Zitter (2001). The meaning of symbols is as follows: + PVY infection in the plant species was indicated in at least one reference, - the species was reported as not susceptible to PVY, ? no information on the plant species-PVY relation has been hitherto available,

[d] Numerator—the number of ELISA-positive plants; denominator—the number of plants inoculated with PVY. The number of transmission tests is given in parentheses

transient mottling that could indicate infection with PVY, but these gave negative results in ELISA. Despite the negative ELISA, the tobacco plants were kept in the greenhouse for another few weeks but none of them displayed visible symptoms of PVY infection. In order to prolong the life of the senescent tobacco plants, the top part and all leaves were cut off from each plant, including healthy (control) ones, to stimulate the development of new shoots from lateral buds. Flame-sterilised razor blades (a separate one for each plant) were used for trimming to avoid virus contamination. Surprisingly, some of the trimmed tobacco plants, which had been inoculated with sap from PVY-inoculated weed plants, displayed clear-cut symptoms of PVY infection on newly-emerged leaves and scored highly positive on ELISA. The trimming test was then applied several times to enhance the reaction of tobacco plants to infection with PVY back-inoculated

with sap of *E. cicutarium, G. pusillum* and *L. purpureum* plants.

Moreover, samples were picked 4–5 weeks post-inoculation from the middle leaves of selected ELISA-positive *L. purpureum, E. cicutarium, G. pusillum* and *L. serriola* plants as well as from non-inoculated leaves of the back-inoculated tobacco plants to further confirm the presence of PVY by immunocapture-reverse transcription-PCR (IC-RT-PCR). Samples from PVY-infected tobacco leaves were always added as a positive control. The IC-RT-PCR tests were performed essentially as described by Weidemann & Maiss (1996), with minor modifications. Antibodies to PVY, the buffer and a general procedure used to coat 0.2 ml PCR tubes were as described above for the DAS-ELISA. Leaf sap samples were extracted with a leaf press and homogenised with PBS-T. The homogenised samples were placed in the pre-coated PCR tubes (100 µl per tube) and incubated overnight at 4°C. After the final washing and drying, the tubes were filled with 5–10 µl of deionised water each and heated at 70°C for 15 min. The oligonucleotide sequences of primers reported by Schubert et al. (2007) were applied. The PCR assay was done using a pair of primers specific to isolates of all PVY strains: PVY5-420F (**5′** CGATACA AGACTGATGYCCA GAT **3′**) and PVY3-1200R (**5′** GGCAAGYTGGCA TAYTGTTGRGC **3′**) yielding a PCR product of 820 bp. The primers are localised in viral sequences corresponding to the region of P1 protease and HC-Pro genes according to the position in the PVY isolate Jakab, SA110, Adgen-C and Nicola; Genbank accession numbers were X97895, AJ585195, AJ890348 and AJ890346, respectively. To perform the reaction of cDNA synthesis, the 3′1500 primer (**5′** CATTCAY GAGCTGTATYTTCYTTTCC **3′),** kindly provided by Dr. J. Schubert, was used. Reverse transcriptase (M-MLV RT; Invitrogen) assay was performed as recommended by the manufacturer. The RT mixture was added to the tubes prepared previously in the IC step. Final volume of 20 µl contained 1× first-strand buffer, 0.5 mM of each dNTP, 1 µM of specific primer or 5 µM oligo $(dT)_{20}$, 0.01M DTT, 40 U RiboLock RNase Inhibitor (Fermentas), 200 U of M-MLV RT and deionised water. The tubes were first incubated at 65°C for 5 min and then at 37°C for 1 h. Finally, the inactivation of M-MLV RT was performed by heating at 70°C for 15 min. The prepared cDNA was directly used as a template for PCR. The reaction of PCR was performed with *Taq* DNA Polymerase (Fermentas), according to the manufacturer's instruction. For PCR, 3 µl of template cDNA was amplified in a 50-µl total volume containing 1× reaction buffer, 0.3 mM of each dNTP, 2.5 mM $MgCl_2$, 0.4 µM of each primer, 1.25 U of *Taq* DNA polymerase and nuclease-free water. The amplification programme included: 3 min denaturation at 95°C followed by 35 cycles at 96°C for 30 s, at 62°C for 30 s, and at 72°C for 1 min, ending with the final extension at 72°C for 5 min. PCR products of amplification were visualised in 1.4% agarose gels with TBE buffer and ethidium bromide (0.5 mg ml^{-1}).

For PVY, the 820-base pair (bp) products were expected in the IC-RT-PCR. Such products were found in the samples collected from PVY-inoculated plants of *L. serriola* and *E. cicutarium* (Fig. 1). As regards the samples from *L. purpureum,* the PCR amplification only yielded a non-specific DNA band of >300 bp, of unclear origin. However, a product of the expected size of 820 bp was obtained with samples taken from symptomatic Samsun tobacco plants, back-inoculated with sap from ELISA-positive plants of *L. purpureum* (Fig. 1). As seen, the same product was also obtained with samples from tobacco plants inoculated with sap from *G. pusillum* and *E. cicutarium*.

The infection of PVY-inoculated plants of *L. serriola* and *E. cicutarium* was additionally confirmed by aphid-transmission tests performed using *N. tabacum* assay plants. From two ELISA-positive *L. serriola* plants *M. persicae* transmitted PVY altogether to six out of 20 assay plants, and from three *E. cicutarium* plants the virus was transmitted to five out of 30 tobacco plants.

Moreover, a total of 286 plants of *E. cicutarium, G. pusillum*, *L. purpureum* and *L. serriola,* as well as of *C. album*, growing on the edges of potato fields of the Plant Breeding and Acclimatization Institute in Młochów, were sampled during 2008 to be assayed for natural infection with PVY (Table 1). The sample plants were chosen on the basis of suspicious symptoms they exhibited, for example leaf reddening in *G. pusillum* or chlorotic spots in *L. purpureum*. The presence of PVY was detected by ELISA in some of the samples taken from all the five weed species (Table 1). The virus presence in ELISA-positive samples was further confirmed by mechanical back-inoculations to Samsun tobacco plants. However, the symptoms of PVY infection were no sooner expressed than the assay plants had been trimmed, as described above.

The inoculated *Capsella bursa-pastoris* plants displayed vague symptoms which could suggest infection

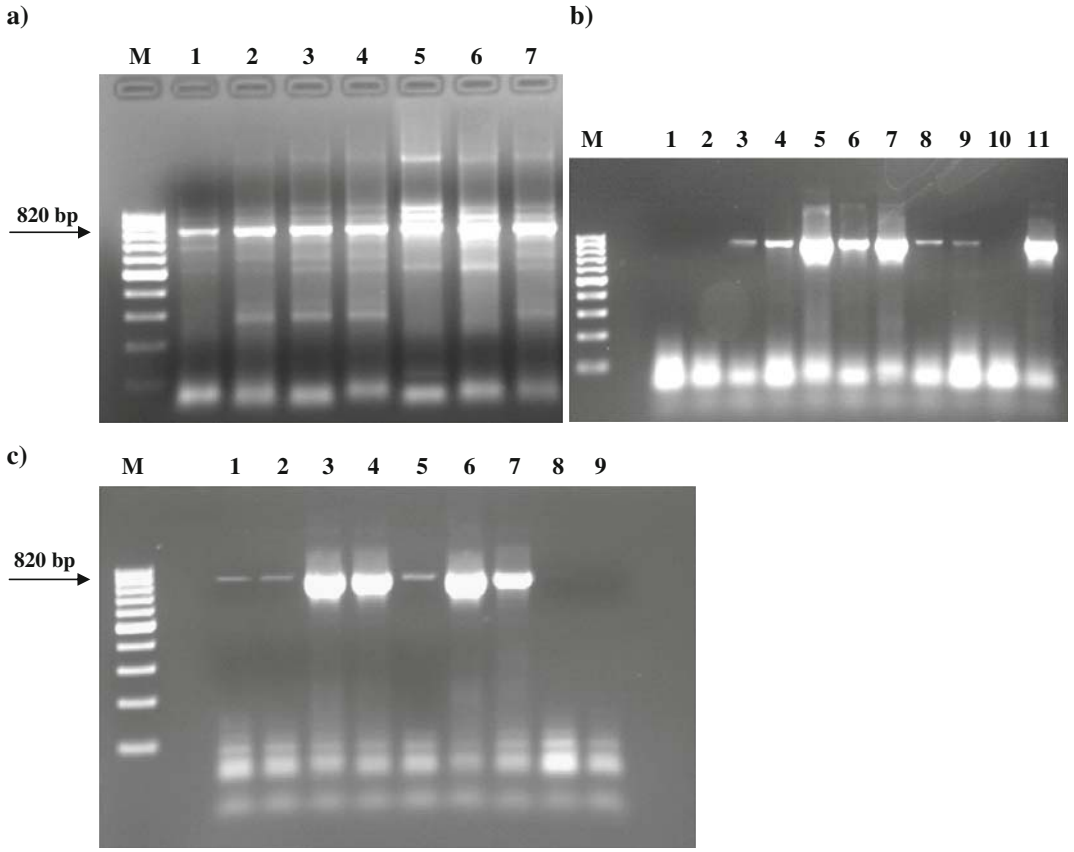

Fig. 1 The IC-RT-PCR products of PVY. **a** M—100 bp ladder, 1–5: *Lactuca serriola*, 6–7: PVY—positive control; **b** M—100 bp ladder, 1–6: *Nicotiana tabacum* inoculated with sap from *Erodium cicutarium,* 7–9: *N. tabacum* inoculated with sap from *Geranium pusillum,* 10: negative control, 11: positive control; **c** M—100 bp ladder, 1 and 2: *E. cicutarium,* 3–4: *N. tabacum* inoculated with sap from *L. purpureum,* 5–6: *N. tabacum* inoculated with sap from *E. cicutarium* (5) and *G. pusillum* (6), 7: positive control, 8: negative control, 9: RT control

with PVY. However, these plants tested negative by ELISA (Table 1), but were further evaluated for the presence of virus in back-inoculation to Samsun tobacco plants. None of the tobacco plants showed any symptoms of virus infection. No PVY infection was also detected in experimentally-inoculated plants of the other weed species, some of them having been reported by Chatzivassiliou et al. (2004) and Fletcher (2001) to be the hosts of PVY. Surprisingly, *Solanum dulcamara* plants inoculated with PVY by aphids or sap did not become infected under the conditions of our experiments (Table 1). The reason of this failure is unclear, as this weed is considered to be one of the major natural reservoirs of PVY in some parts of the world (Kerlan 2006; Shukla et al. 1994). Perhaps, the line of *S. dulcamara* tested in our studies was not susceptible to infection with PVY.

Taken together, the presented results prove the infection capacity of PVY for *L. serriola, E. cicutarium, G. pusillum* and *L. purpureum* plants. No obvious relationships between weed species and PVY isolates used for inoculation were found. The presence of PVY in ELISA-positive *L. serriola* and *E. cicutarium* was confirmed by the IC-RT-PCR performed on samples taken from the weed plants, whereas the infection of *G. pusillum* and *L. purpureum* plants was confirmed by the IC-RT-PCR done on samples picked from the back-inoculated tobacco plants. The IC-RT-PCR failed to detect PVY in samples taken directly from ELISA-positive plants of the latter two species. A similar discrepancy between ELISA and RT-PCR results was recently found by Sampangi et al. (2007) in detecting *Iris yellow spot virus* (genus *Tospovirus*, family Bunyaviridae) in some weed species. The discrepancy was suggested to be due to non-specific reactions in

ELISA or difficulty associated with obtaining RT-PCR-quality templates for amplification. The appearance of non-specific ELISA reactions could explain the divergent ELISA and IC-RT-PCR results obtained with *L. purpureum* in our investigations, as the ELISA-positive samples assessed by back-inoculations to Samsun tobacco plants gave mostly negative results. Similar problems with (most probably) non-specific reactions in ELISA were reported by Chatzivassiliou et al. (2004) for several weed species suspected of being natural hosts for important tobacco viruses, including PVY. However, unlike those examined by these authors, some samples from ELISA-positive plants of *L. purpureum* tested positive in the back-inoculation experiments, and the PVY presence in Samsun tobacco plants was confirmed by typical symptoms of infection, positive ELISA readings and the results obtained with the IC-RT-PCR.

To the best of our knowledge this is the first report of infection of *E. cicutarium, G. pusillum* and *L. purpureum* by PVY (Brunt et al. 1996; Chatzivassiliou et al. 2004; Edwardson and Christie 1997; Fletcher 2001; Kazinczi et al. 2004; Kerlan 2006; Shukla et al. 1994; Zitter 2001). Moreover, our findings confirm that *L. serriola* is a host for PVY, as recently reported from Greece by Chatzivassiliou et al. (2004), and reveal for the first time PVY infection in this species in central Europe.

PVY is transmitted by at least 50 aphid species (Robert and Bourdin 2001), but only some of them share the same plant host species. *Erodium cicutarium* and *G. pusillum*, both belonging to the family Geraniaceae, are the principal hosts of *Acyrthosiphon malvae* (based upon: Ecological Flora of the British Isles: http://www.ecoflora.co.uk/index.php). Interestingly, this aphid has been recognised as a vector of PVY (according to Boukhris-Bouhachem et al. 2007). Although *A. malvae* does not reproduce on solanaceous crop plants, it can certainly visit and probe them, thus spreading PVY. In turn, we found that *M. persicae* (the principal vector of PVY), while shortly probing *E. cicutarium* or *L. serriola,* was able to acquire and transmit PVY to solanaceous (tobacco) plants. Moreover, some of the wild-sampled plants of *E. cicutarium, G. pusillum, L. serriola* and *L. purpureum* appeared to be naturally infected with PVY. Altogether, these findings indicate that the new weed hosts of PVY reported in this paper can act as reservoirs of the virus and play a role as primary sources of infection. These findings also imply the need for further investigations in order to assess the impact of weed hosts of PVY upon the virus epidemiology.

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