Necrotic Lesion Host for Potato Virus Y Useful in Field Epidemiological Studies

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ABSTRACT

Singh, R. P., and Boiteau, G. 1984. Necrotic lesion host for potato virus Y useful in field epidemiological studies. Plant Disease 68: 779-781.

The potato (*Solanum tuberosum*) cultivar Jemseg developed necrotic lesions when inoculated with potato virus Y (PVY). Lesions were produced by both aphid and mechanical inoculation in the field and in the greenhouse. Lesion production was not affected by the presence of potato viruses A, S, or X or potato spindle tuber viroid. The potato-infesting aphids *Aphis nasturtii* and *Myzus persicae* transmitted PVY to Jemseg plants, but the virus was not transmitted to Jemseg by *Aulacorthum solani* or *Macrosiphum euphorbiae*. The transmission of PVY by aphids to Jemseg was similar to that obtained on Russet Burbank potato or *Solanum demissum* plants but differed from that on tobacco plants. In view of this difference, Jemseg may be a more useful "bait" plant than tobacco for field epidemiological studies.

Experimentally, at least 17 aphid species can transmit potato virus Y (PVY) (2,6,12). The relative importance of many of these as vectors has been assessed under laboratory conditions (1,4,12). This ranking, however, is only a measure of the ability of each species to transmit PVY under artificial conditions. Under field conditions, such factors as seasonal variation, abundance, and dispersive ability of each species as well as the attraction of the crop can alter this ranking (1).

The study of field spread of nonpersistent viruses in potatoes is limited by the techniques available, ie, aphid trapping (3) and "bait" plants (7). Water traps give a biased sample of the aphid fauna (10). With the suction trap, aphids are captured alive and can be tested as carriers of viruses. However, because the handling time of the catch and test plant may be longer than the retention period of nonpersistent viruses in their aphid vectors, results may be inconsistent and difficult to interpret (5). In the bait-plant system, tobacco seedlings are planted in the midst of potato plants for different periods, then removed for observation of systemic symptoms of PVY (11).

A comparison of potato with the tobacco bait plants showed that the light green tobacco leaves may well be more attractive to aphids than dark green potato leaves. This broken uniformity of the field with respect to color may affect the flight pattern of aphids (11). To improve the bait-plant system, the tobacco plant should be replaced by a potato cultivar with a capability for differentiating current infection resulting from aphid inoculation at all stages of growth in the field. We describe such a potato cultivar, which develops necrotic lesions when aphid inoculated with PVY.

Accepted for publication 2 March 1984.

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MATERIALS AND METHODS

Virus source and mechanical inoculation. Three common strains of PVY collected from commercial fields were maintained in potato cultivar Saco or in tobacco (*Nicotiana tabacum* 'Samsun'). The potato cultivar Jemseg (13) was obtained from the National Potato Breeding Program at Fredericton Research Station as tubers and grown in the greenhouse and in the field. For mechanical inoculation to test plants, infected leaves were ground in glycine-phosphate buffer (0.05 M glycine + 0.03 M phosphate, pH 9.2). Test plants were dusted lightly with Carborundum (600-mesh) before inoculation.

PVY infection was confirmed in test plants by inoculating tobacco plants and observing systemic veinclearing symptoms, by inoculation to the diagnostic lesion host *Solanum demissum* PI 230579, or by enzyme-linked immunosorbent assay (ELISA) (8).

Aphid transmission. Aphids were reared in a room at 20–24 C with a photoperiod of 18 hr (fluorescent and incandescent lamps) with a light intensity of 3–5 klux. Potato-infesting aphids, ie, *Aphis nasturtii* Kaltenbach, *Aulacorthum solani* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), and *Myzus persicae* (Sulzer), were maintained on potato cultivar Katahdin. Only fourth-instar nymphs and adults were used in the transmission tests.

For the virus transmission tests, aphids were starved for about 90 min, then allowed to probe on virus-infected potato or tobacco leaves for 45–60 sec. They were then transferred one by one to test plants for an incubation feeding of 1.5–2 hr, after which they were removed manually. Test plants were incubated in a greenhouse at 18–25 C with light intensity of 4–6 klux and a 14-hr photoperiod. Control test plants exposed to non-viruliferous aphids and test plants without aphids were included for each test. The tests were performed over a period of 2 yr and the data are summarized in the tables.

Virus transmission in the field. Four plots of two rows 91 cm apart were used. The spacing within the row was 30 cm and each plot was 30 m long. Each plot was separated by six rows of virus-free Russet Burbank potato plants. Two plots were used for the controlled aphid transmission test and the other two plots were used to observe natural virus transmission. In each test, each plot consisted of one row of virus-free Jemseg and an adjacent row of Russet Burbank containing either 0 or 50% PVY.

In controlled aphid transmission, 50 winged adults of *M. persicae* were given PVY access by allowing them to probe (45–60 sec) individually in the field on PVY-infected leaves, then the aphids were transferred to a terminal leaflet of the Jemseg plant to permit virus transmission. Only one aphid was caged on each plant and tests were done on 20 July and 10 August.

RESULTS

Jemseg as a necrotic lesion host for PVY. During a study of the reactions of 16 potato cultivars to three isolates of PVY, we observed that inoculated plants of the potato cultivar Jemseg consistently developed circular necrotic local lesions 8–10 days after mechanical inoculation (Fig. 1A) followed by necrotic secondary lesions on uninoculated leaves. The presence of PVY in the lesions was confirmed by positive reaction with PVY-antiserum in ELISA. Leaf extracts from several lesions induced typical PVY symptoms in tobacco plants.

Presence of other potato viruses. To study the effect of virus mixture on transmission efficiency, PVY inoculum was mixed equally with a 1/10 dilution of sap containing potato virus A (PVA) and a 1/20 dilution of potato virus S (PVS), potato virus X (PVX), or potato spindle tuber viroid (PSTV) and inoculated to Jemseg leaves. Necrotic lesions of PVY were produced regardless of virus mixture, and with the exception of PSTV, no other virus showed symptoms on Jemseg plants. Upon reinoculation to test plants no PVA, PVS, or PVX was recovered.

Field susceptibility of Jemseg. To determine the field susceptibility of Jemseg to PVY, 20 randomly distributed leaves on each of five plants were mechanically inoculated on 12, 19, and 26 July and on 3, 11, and 18 August 1982. Lesion counts 10 days later averaged 209, 83, 84, 21, 25, and 24 lesions per leaf, respectively, indicating a season-long susceptibility. There was no significant difference in numbers of lesions produced when inocula were prepared from field-grown or greenhouse-grown infected leaves.

Development of necrotic lesions after aphid feeding. The four potato-infesting aphid species were tested for their ability to induce necrotic lesions on test plants while viruliferous. Single aphids were caged on terminal Jemseg leaflets after one probe of 45–60 sec on infected leaves. The general area where each aphid fed was marked. After 7–10 days, lesions developed either as a necrotic spot (Fig. 1B) or as a necrotic streak on a vein. With time, the lesions increased in size and eventually spread to midveins of the leaflets (Fig. 1C). Necrotic lesions or veinal necrosis symptoms also appeared on new leaves (secondary lesions), indicating systemic spread of the virus within the plant. Only one lesion was produced on each leaflet by a single

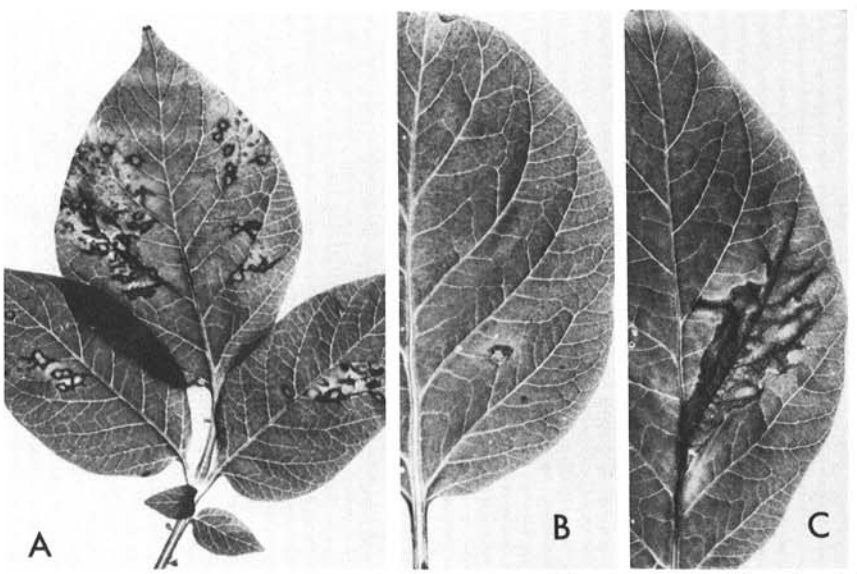

Fig. 1. Necrotic lesions caused by potato virus Y on potato cultivar Jemseg **(A)** with mechanical inoculation, **(B)** with aphid inoculation at initial stage, and **(C)** with aphid inoculation after veinal spread.

Table 1. Development of potato virus Y local lesions on leaves of potato cultivar Jemseg inoculated with viruliferous, wingless aphids

Aphid species[a]	Total no. of lesions	No. infected/ no. tested	Percent transmission
Myzus persicae	41[b]	38/98	39
Aphis nasturtii	10	10/60	17
Macrosiphum euphorbiae	0	0/64	0
Aulacorthum solani	0	0/67	0

[a] Fifteen nonviruliferous aphids of each species tested did not cause any symptoms.
[b] Usually one potato virus Y local lesion developed on each leaflet; however, in three cases, two lesions were observed on each leaflet, with only one aphid per leaflet.

Table 2. Relative transmission of potato virus Y by potato-infesting aphids to systemic and necrotic lesion hosts

Aphid species[a]	Tobacco to tobacco[b]				Potato to potato[c]		Tobacco to *Solanum demissum*[d]	
	Wingless		Winged					
	No. infected/ tested	Percent transmission	No. infected/ tested	Percent transmission	No. infected/ tested	Percent transmission	No. infected/ tested	Percent transmission
Myzus persicae	14/30	47	14/22	64	9/16	56	35/86	41
Aphis nasturtii	8/30	27	5/22	23	3/16	19	15/63	24
Macrosiphum euphorbiae	9/30	30	5/22	23	0/16	0	0/36	0
Aulacorthum solani	0/30	0	0/22	0	...[e]		...[e]	

[a] Nonviruliferous aphids of each species tested did not cause any symptoms, local or systemic.
[b] Aphids fed on potato virus Y-infected Samsun tobacco leaves, then transferred to tobacco test plants.
[c] Aphids (wingless and winged) fed on virus Y-infected Russet Burbank potato leaves, then transferred to young Russet Burbank potato plants.
[d] Aphids (wingless) fed on potato virus Y-infected tobacco leaves, then transferred to diagnostic lesion host *S. demissum* PI 230579.
[e] Not tested.

aphid, and this appeared in the area where the aphids had fed. No local lesions or necrotic streaks developed on leaflets on which nonviruliferous aphids were placed. The presence of PVY in necrotic lesions was confirmed by ELISA and by subtransferring to tobacco where typical symptoms of PVY were produced.

M. persicae transmitted PVY to 39% and *A. nasturtii* to 17% of the inoculated plants. *M. euphorbiae* and *A. solani* did not transmit PVY to Jemseg (Table 1). Because our observation with *M. euphorbiae* differed from previous reports (1,9,12), aphid transmission tests were repeated using the systemic hosts tobacco and potato and a diagnostic lesion host *S. demissum*. Results (Table 2) show that *M. euphorbiae* transmitted PVY only when the virus source and the test plant were tobacco but not when potato or *S. demissum* was used as the test plant. Both wingless and winged forms of *M. euphorbiae* transmitted PVY to tobacco, whereas a mixture of both forms on potato or only the wingless form on *S. demissum* failed to transmit PVY (Table 1).

Field transmisson of PVY by aphids to Jemseg plants. In a controlled test when viruliferous *M. persicae* were caged on Jemseg plants in the field in July, positive transmissions were obtained in 22% of the plants. In the second test (in August), PVY was transmitted to 18% of the plants. The symptoms on infected plants were first observed on the leaves on which aphids were caged followed by the appearance of secondary lesions and veinal necrosis on other leaves. The symptoms observed in the field were similar to those obtained in the greenhouse. All plants inoculated with aphids were tested by ELISA, and only those with symptoms were found positive for PVY.

In plots with natural spread of PVY, Jemseg plants were periodically observed for PVY symptoms (secondary lesions and necrotic streaks). At the end of August, 15 and 75% of the Jemseg plants, planted next to 0 or 50% PVY-infected rows, respectively, were infected with PVY. The PVY infections were confirmed by ELISA test of symptomatic leaves.

DISCUSSION

Because Jemseg is immune or highly resistant to PVX, PVS (13), and PVA (R. P. Singh, *unpublished*), it is a particularly useful indicator host for epidemiological studies of PVY in the field. It developed necrotic lesions caused by PVY in both the greenhouse and in the field, with mechanical inoculation as well as with aphid inoculation. After the formation of primary lesions within 8–10 days, necrotic veinal streaks and spots developed on new leaves. These symptoms persisted throughout the growing season, thus facilitating the diagnosis of PVY-infected plants in the field. The usefulness of Jemseg is further increased by its susceptibility to PVY at varying light intensity (4–10 klux) and at a temperature range of 15–27 C (R. P. Singh, *unpublished*).

In tests on the relative transmission of PVY by different species of potato-infesting aphids, results obtained with Jemseg as the indicator plant were closer to results obtained with Russet Burbank or *S. demissum* than with tobacco. *M. persicae* and *A. nasturtii* transmitted PVY to all indicator plants but *M. euphorbiae* did not (Table 2). This species can transmit PVY to tobacco but not to *S. demissum* or *S. tuberosum* 'Jemseg' or 'Russet Burbank' (Tables 1 and 2). This observation raises an important question regarding the actual role of *M. euphorbiae* in the transmission of PVY in potato fields. The different responses of an aphid species to tobacco and potato indicate that the use of tobacco as bait plant for studies on the spread of PVY in potato fields may not be appropriate and should be replaced by a *Solanum* species like the cultivar Jemseg.

ACKNOWLEDGMENTS

We wish to thank M. E. Drew, T. H. Somerville, and G. E. Pitteollo for technical assistance.

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