Beet Western Yellows Virus—A Major Component of Some Potato Leaf Roll-Affected Plants

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ABSTRACT


Beet western yellows virus (BWYV) has been isolated from potato stocks with typical potato leaf roll (PLR) symptoms. Virus isolates from potato, that induce leaf rolling, interveinal chlorosis, petiole epinasty, and reduction in leaf size in Physalis floridana and interveinal chlorosis on Capsella bursa-pastoris have been shown to be strains of BWYV. Isolates differing in host reaction and serological characteristics have been found in individual potato plants indicating a complex etiology for the PLR syndrome. In addition to potato, and other solanaceous hosts, one isolate has been transmitted to and recovered from species in the Boraginaceae, Chenopodiaceae, Compositae, Cruciferae, Leguminosae, Malvaceae, and the Portulaceae. The BWYV isolates induce primary leaf roll symptoms in PLRV-free and virus-free potato cultivars, indicating that these isolates might easily be confused with "typical" PLRV. Preliminary serological data indicate that there are several serotypes of BWYV in potato that differ from each other and from PLRV in serological reactions. This evidence suggests that serological testing for PLRV occurrence would probably give misleading information. The broad host range of these BWYV isolates raises questions about the reinfection of virus-free potato stocks from infected wild hosts in "isolated" areas.

MATERIALS AND METHODS

Three potato leaf roll-affected potatoes originally were obtained from commercial California potato stocks selected as having PLR symptoms and have been maintained vegetatively in the greenhouse for over 10 yr. Two additional potato leaf roll-affected potatoes were obtained from Oregon. Beet western yellows virus isolates came originally from radish (Raphanus sativus L.) ST-1; broccoli (Brassica oleracea L. var. botrytis L.) ST-7, ST-9, ST-12; malva (Malva parviflora L.) ST-11; and lettuce (Lactuca sativa L.) ST-E. Turnip yellows virus (TuYV) originally came from turnip (Brassica rapa L.) and beet mild yellowing virus (BMYV) came from beet (Beta vulgaris L.). The isolates were maintained in desiccated plant tissue.

For activation of virus strains, desiccated tissue was ground in a food grinder 1:1 (w/v) with 0.05 M phosphate buffer (pH 7.0) containing 0.01 M glycine (1:1, w/w). Extracts were placed directly on sucrose-density-gradients (20–60%) centrifuged 2 hr at 73,450 g in a Beckman SW 50.1 rotor, and (after dilution with buffer to 20%; sucrose) virus zones (18–26 mm from the top of the tubes) were fed to aphids (7).

The handling of BWYV strains, membrane feeding techniques, and antigen and antiserum preparation for BWYV were as reported previously (7,15). Extracts for BWYV antigen preparations, infectivity neutralization, and antigen scanning pattern analyses were prepared from sheep's purse (Capsella bursa-pastoris [L.] Medic.). Frozen plant material was ground in a food grinder 1:1 (w/v) with 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine and then homogenized at 45,000 rpm in a VirTis homogenizer. Crude extracts were heated to 45 C, clarified by low-speed centrifugation (20 min, 12,100 g), and then pelleted by high-speed centrifugation (2 hr, 80,800 g). Pellets were resuspended in 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine.

Gradients were made by layering 4, 7, 7, and 7 ml, respectively, of 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine. Centrifugation was done in a Beckman SW-25 rotor for 4 hr at 58,000 g. Gradients were scanned photometrically with an ISCO Model D density-gradient fractionator operated on the sensitive scale (A254nm= 0.5).

All density-gradient fractions used in feeding extracts were adjusted to 20% sucrose (by dilution with buffer) before being placed on membranes for acquisition test feeding by aphids.

Aphids that had fed through membranes were tested for virus transmission to shepherd's purse and Physalis floridana Rydb. seedlings. Aphids from virus-free colonies, which did not have...
access to virus, were tested on these hosts as controls.

Virus assays from the original potato sources and for host range studies were conducted with detached leaves and stems. The samples were washed, surface moisture was removed, and they were placed in petri dishes on moistened filter paper. Approximately 200 nonvuliferous aphids were placed in the dishes with the plant tissue and the dishes were sealed with tape. Aphids were allowed a 24-hr acquisition feeding and were then transferred to test plants for a 48-hr infection feeding interval. They were confined to the test plants by screened sleeve cages.

Nonvuliferous green peach aphids (Myzus persicae [Sulzer]) were reared on radish.

Four antisera prepared against different strains of BWYV originally isolated from species of the Cruciferae, Compositeae, and Malvaceae, antisera against TuYV (12) and BMYV (13) from Europe, and two antisera prepared against legume yellows virus (LYV) (8) were tested against two isolates of BWYV from potato with leaf roll symptoms and a typical BWYV isolate. Virus-antisemixturest were subjected to density-gradient centrifugation, and analyzed photometrically. A positive assay was based on the reduction or elimination of virus antigen in the scanning patterns of density-gradient columns (2,8,11–13).

RESULTS

Virus recovery. The most widely used indicator species utilized in indexing procedures for PLRV has been P. floridana. However, a number of viruses (BWYV, BMYV, Physalis mild chlorosis virus, TuYV, PLRV, etc.) cause similar reactions in this host (10). For this reason, in addition to P. floridana, shepherd’s purse was included as an assay species in attempted recoveries from potato. Shepherd’s purse is a commonly used indicator host for BWYV, but is not known to be a host for PLRV.

Even when using relatively large numbers of aphids per test plant (20–30) recovery of any viruses from the PLR-affected potatoes was erratic and transmission efficiency relatively low.

It soon became apparent, however, that in addition to virus isolates inducing symptoms similar to “normal” PLRV (ie, leaf rolling, interveinal chlorosis, petiole epinasty and reduction in leaf size in P. floridana) yellowing type pathogens that also infected shepherd’s purse occurred in the same plants.

All five potato sources with leaf roll symptoms had naturally occurring isolates that infected shepherd’s purse. Primary recovery (ie, recovery directly from potato) was achieved from all five sources. Transmission directly from potato to shepherd’s purse was very inefficient; a maximum of 5–10% of the test plants became infected. Initial interveinal yellowing symptoms on shepherd’s purse were expressed 5–7 wk after inoculation which is 3–5 wk later than common BWYV strains on that host.

Shepherd’s purse-infecting isolates also were recovered from some P. floridana plants that became infected during primary recovery tests even though the primary indicator shepherd’s purse plants had been negative in the same tests.

In subsequent inoculations of shepherd’s purse-infecting isolates to shepherd’s purse, P. floridana, and potato, differences between the isolates in regard to plant incubation period and symptom severity became apparent. The shepherd’s purse-infecting isolates (designated P-1, P-2, P-3, P-4, P-5, P-6, P-7, P-8, and P-9) were distinct from PLRV in host reactions and serological affinities and therefore are considered to be strains or isolates of BWYV. Although repeated serial transfers of M. persicae on P. floridana test plants have been made, an isolate which does not react with BWYV antiserum and does not infect shepherd’s purse has not been recovered from the five potato sources.

Symptoms. The isolates of BWYV from PLR-affected potatoes differ markedly in the severity of symptom expression on P. floridana and shepherd’s purse. Some isolates induce leaf rolling, interveinal chlorosis, petiole epinasty, and reduction in leaf size in P. floridana that is identical to the reported symptoms of PLRV on this host. Others induce various degrees of interveinal yellowing, accompanied by very slight leaf rolling with little stunting or leaf size reduction. Sometimes, 2–3 wk after initial symptom expression, the most mildly affected plants are very difficult to distinguish from healthy plants.

The isolates also differ in response on shepherd’s purse as to incubation period and stunting. Although shepherd’s purse plants infected directly from potato do not express symptoms for 5–7 wk after inoculation, some isolates have been observed to either change their “affinities” to shepherd’s purse or have become masked by more severe strains by serial passage through this host. These isolates show more severe symptoms on shepherd’s purse and have incubation periods of 2–3 wk. Other isolates apparently have not changed in their “affinities” to shepherd’s purse and have remained mild with long incubation periods. Virus-free Kennebec potatoes and leaf roll-free White Rose potatoes inoculated with two of the BWYV isolates from potato have shown symptoms identical to the original leaf roll plants from which the isolates were obtained and to descriptions of leaf roll symptoms in the literature (ie, an upward rolling of the leaf margins accompanied by interveinal chlorosis and [in some instances] marginal necrosis).

Host range. Host range studies of one isolate (P-5) were carried out by inoculation of 10–20 seedlings of a number of different species with M. persicae given 24-hr acquisition feedings on infected shepherd’s purse. Presence or absence of virus in each plant species tested for susceptibility was determined by aphid transfer to C. bursa-pastoris, P. floridana, and Nicotiana clevelandii Gray, 4–6 wk after inoculation.


The virus isolate was not recovered from the following species:

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<td></td>
<td>HSP-5</td>
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<tr>
<td>P-5</td>
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<td>P-7</td>
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<tr>
<td>BWYV ST-1</td>
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*Antiser against selected isolates of BWYV (ST-1-2, ST-9-1, ST-11-1, ST-1-1); turnip yellows virus (TuYV); beet mild yellowing virus (BMVY); legume yellow virus (LYV-1, LYY-3); and controls of healthy shepherd’s purse (HSP-5, HSP-7).

The virus samples were obtained from infected shepherd’s purse, clarified by low-speed centrifugation and pelleted by ultracentrifugation. Pellets were resuspended in buffer to approximately 3.0% of the original volume of sap. The resulting virus samples were mixed with equal volumes of antiserum and incubated 0.5 hr at 37 C. Incubated mixtures were subjected to density-gradient centrifugation (4 hr at 58,000 g), and analyzed photometrically. A positive test was based on the reduction or elimination of virus antigen in the scanning patterns of sucrose density-gradient columns.

Serological tests. Antiserum prepared against the strains of BWYV, TuYV, BMVY, and LYV reacted with the isolates from potato and the typical BWYV isolate. However, two antisera (ST-11 and TMYV) did not react with the P-5 isolate (Table 1).

Antiserum prepared against the P-5 isolate from potato, against typical BWYV (ST-1), and against a Canadian isolate of PLRV supplied by R. Stace-Smith (PLR-Sm), were compared in reactions against five isolates of BWYV, one isolate of TuYV, and the P-5 isolate from potato. All sera reacted with these strains of BWYV. Antiserum against healthy shepherd's purse sap did not react with any of the isolates.

Since the first series of serological tests indicated a differential reaction of two of the BWYV isolates from potato to one antiserum prepared against a BWYV strain and BMVY, further tests were conducted to verify the presence of different BWYV serotypes in potato (Table 2). Four isolates from potato were tested with an antiserum prepared against one of the isolates and another antiserum against a typical BWYV isolate. All of the isolates reacted with BWYV antiserum, but only two of the four isolates reacted with the antiserum prepared against the isolate from potato.

These results and previous work which showed that PLRV did not react with BWYV antiserum in infectivity neutralization (10) and in agar diffusion tests (Duffus and Stace-Smith, unpublished) strongly indicate that these virus isolates from leaf roll-affected potato are indeed strains of BWYV. Further, these isolates from potato differ from each other serologically and in reaction to some isolates of BWYV.

DISCUSSION

The recent discovery of an apparently new luteovirus affecting only solanaceous hosts (Solanum yellows virus) (20), but which is closely related serologically to BWYV, indicated the possibility that PLR was caused by a complex of yellowing agents. A complex etiology would help to explain some of the confusion that has been characteristic of this disease since its discovery. For instance, in early work the known hosts of PLRV were restricted to the Solanaceae. Later, Salaman and Wortley (27) reported that PLRV was transmitted to Campanula, Matthiola, turnip, and Brussels sprouts. However, Nelson and Norris (18) were unsuccessful in transmitting the virus to these species or other crucifers. Nettle et al. (21) inoculated over 150 species in 39 families and found susceptibility in nine species in the Amaranthaceae and one species in the Nolasaceae. MacKinnon reported the susceptibility of spinach in the Chenopodiaceae (19). Thus, attempts to characterize leaf roll isolates by host range characteristics have resulted in conflicting data. The host range of one BWYV isolate capable of inducing leaf roll in potato and infecting hosts in the Boraginaceae, Chenopodiaceae, Compositae, Cruciferae, Leguminosae, Malvaceae, Portulaceae, and Solanaceae reported herein indicates the importance of reevaluating wild species as PLR reservoirs. It seems probable from the few virus isolates tested here, that wild plant species in a number of plant families may serve as reservoirs of leaf roll-inducing agents in potato-producing and/or seed potato isolation areas.

The isolates of BWYV recovered from leaf roll-affected potatoes reacted serologically the same as other BWYV isolates from beets, crucifers, and composites. All of the isolates reacted with BWYV antiserum, thus distinguishing the entities from a California PLRV isolate tested in the 1960s (10), and the Canadian PLRV isolate of Stace-Smith (Duffus and Stace-Smith, unpublished). Antiserum to PLRV reacted with all BWYV strains tested, but the reciprocal reaction (BWYV antiserum against PLRV) has been negative in all tests except the recent distant relationship demonstrated by Roberts et al (24) with an extremely sensitive electron microscope technique.

The host range of the one isolate tested here is similar to other isolates of BWYV, but it seems to have affinities to the Solanaceae and much less affinity to the Brassicas than BWYV strains tested previously.

It is very difficult to separate PLRV isolates from BWYV isolates in the same potato. Both can induce identical symptoms on the P. floridana indicator hosts. Serial transfers with continued indexing back to shepherd's purse thus far have failed in our tests. The inability to easily separate PLRV from the complex has serious implications for previous studies on "PLRV" in regard to properties, insect transmission, and serology.

The report of Wright and MacCarthy (31) of the recovery of PLRV from potatoes that did not develop diagnostic symptoms of leaf roll in the field or greenhouse was criticized (4) because these isolates produced only mild symptoms on P. floridana and apparently did not cross protect against severe isolates of PLRV. Previous work with BWYV indicates a lack of cross protection between strains of this virus (10). Also, the work reported herein indicates the recovery of several serologically distinguishable and host specific entities from one potato. These results indicate the possibility that Wright and MacCarthy (31) and later Wright and Cole (30) in their reports of mild aphidborne potato virus isolates from various sources in North America were dealing with isolates of BWYV. Certainly their techniques would not distinguish between PLRV and BWYV.

Efforts to rid potato stocks of PLRV by indexing procedures and isolation is a slow and costly process. Recently rapid and economical serological methods (5,6,16) have been suggested as an important alternative. The results of serological studies reported herein, indicate that there are several BWYV serotypes that induce leaf roll. These isolates differ among themselves, from PLRV, and from some isolates of BWYV. The occurrence of these BWYV isolates in potato stocks raises serious questions as to the validity of any currently used serological indexing procedures.

The present studies answer only a few of the important questions concerning the PLR syndrome. However, the results have significant impact on current virus-free potato production and indexing procedures, and on the interpretation of relationship

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<td>P-2*</td>
<td>HSP-5: +, ST-1: -</td>
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<td>P-3</td>
<td>HSP-5: +, ST-1: -</td>
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<tr>
<td>P-4</td>
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<tr>
<td>P-5</td>
<td>HSP-5: +, ST-1: -</td>
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<tr>
<td>BWYV ST-1</td>
<td>HSP-5: +, ST-1: -</td>
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*Antisera against healthy shepherd’s purse (HSP-5), beet western yellows virus (ST-1), and an isolate of BWYV from potato (P-5).

The virus samples were obtained from infected shepherd’s purse, clarified and in agar diffusion tests (Duffus and Stace-Smith, unpublished) strongly indicate that these virus isolates from leaf roll-affected potato are indeed strains of BWYV. Further, these isolates from potato differ from each other serologically and in reaction to some isolates of BWYV.
studies.
Studies are continuing on the distribution of BWYV isolates in potato stocks, the economic impact of these isolates, and the occurrence, distribution, and impact of different serotypes on indexing procedures.

LITERATURE CITED