# Serological and Host Range Evidence for the Occurrence of Beet Western Yellows Virus in Europe

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ABSTRACT

Observations of symptoms on weeds and crop plants in Britain during 1968 and 1969 suggested that beet western yellows virus (BWYV) was present in hosts reported to be immune to beet mild yellowing virus (BMYV). Transmissions from plants showing BWY-like symptoms, using *Myzus persicae* as vector, demonstrated that they were infected with a persistent aphid-transmitted virus resembling BWYV in transmission characteristics and host range. The host range of virus isolates from *Capsella bursa-pastoris* and lettuce growing in eastern England was similar to that of some BWYV strains from California, but differed in several important respects from that of BMYV. The three isolates studied in detail infected and produced symptoms on *Beta macrocarpa, Brassica rapa, Lactuca sativa, Capsella bursa-pastoris, Nicotiana clevelandii,* and *Claytonia perfoliata,* but did not infect *Beta vulgaris, Raphanus sativus, Chenopodium capitatum,* or *Sonchus oleraceus.*

These three English isolates were readily transmitted by *M. persicae,* which had acquired virus by feeding on clarified sap through artificial membranes. In density-gradient columns containing sap from infected plants, the positions of infectious zones corresponded closely with those in gradients containing BWYV. In infectivity-neutralization tests, antisera prepared against seven strains of BWYV neutralized infectivity of the English isolates, and specific antisera against the isolates neutralized infectivity of five BWYV strains as well as the isolates. Antisera prepared against sap from virus-free plants did not affect the infectivity of any virus strain or isolate. The results of these investigations show for the first time that BWYV occurs outside the USA, but do not help to clarify the relationship between BWYV and BMYV. Phytopathology 60:1199-1202.

There are many similarities between beet western yellows virus (BWYV), the most common yellowing virus of sugarbeet in the USA (1), and beet mild yellowing virus (BMYV), which is the most prevalent virus of beet in Europe (6). The viruses are transmitted in essentially the same manner by the green peach aphid, *Myzus persicae* (Sulzer), and they induce similar or identical symptoms in a number of host species, including *Capsella bursa-pastoris* (L.) Medic. (Shepherd's purse), *Senecio vulgaris* L. (groundsel) and *Claytonia perfoliata* Donn. Some isolates of BWYV, however, do not infect sugarbeet, and there is some evidence that the yellowing symptoms of BMYV on sugarbeet in Europe differ from those caused by beet-infecting strains of BWYV in the USA. For example, in Europe the older leaves of BMYV-infected beet in the field usually become orange-colored, unlike those infected with the beet yellows virus (BYV), whereas the symptoms of BWYV and BYV in the USA are usually difficult to distinguish on grounds of color alone. In addition, BMYV has a very restricted host range (23 species in 8 families), as compared with the very wide host range of BWYV (96 species in 21 families).

Field observations in many areas of England and Scotland, including Cambridgeshire, Bedfordshire, and Perthshire, suggested the presence of a virus resembling BWYV in weeds and crop plants, some of which are hosts of BWYV but not of BMYV. Yellowing symptoms were observed on *Malva sylvestris* L., *Brassica* spp., *Sisymbrium officinale* (L.) Scop., *Papaver rhoeas* L., *Geranium dissectum* L., *Echium vulgare* L., and *Lactuca sativa* L., which are not hosts of BMYV, and on *C. bursa-pastoris* and *Senecio vulgaris,* which are hosts of both BMYV and BWYV. This paper describes investigations of the relationships between three BWYV-like virus isolates from *C. bursa-pastoris* and lettuce in England and typical BWYV isolates from California.

MATERIALS AND METHODS.—Yellowing virus isolates affecting plants not thought to be hosts of BMYV are common in Britain. During 1968 and 1969, collections of isolates from various weed and crop plants showing yellowing symptoms were made for laboratory and greenhouse tests. Nonviruliferous green peach aphids reared on *Brassica pekinensis* (Lour.) Rupr. were transferred to detached leaves of the field-collected plants and allowed to feed for 48 hr. The aphids were then transferred to seedlings of sugarbeet, shepherd's purse, and groundsel. Control aphids were transferred directly from *B. pekinensis* to indicator plants to ensure that the stock aphid colony was free from contaminant viruses. Three isolates, designated herein as English isolates E-1, E-2, and E-3, were selected for immediate study. Isolate E-1 was obtained from a field *Capsella* plant that showed purple coloration and brittleness of the older leaves, collected at the Plant Breeding Institute, Cambridge. Isolate E-2 was also from *Capsella,* which showed purpling and brittleness and was collected from Everton, Bedfordshire. Isolate E-3, obtained from lettuce (*Lactuca sativa*) from Potton, Bedfordshire, showed marked interveinal yellowing and stunting (8).

Host range studies were carried out by inoculation of at least five seedlings, from a number of different species, with 20-30 viruliferous aphids fed on diseased shepherd's purse for 24 hr. The aphids were permitted to feed on the test plants for 48 hr, and were then

TABLE 1. Results of host range studies with English isolates of Beet western yellows virus

| Spp. inoculated | Results with indicated isolate[a] E-1 | E-2 | E-3 |
|---|---|---|---|
| AIZOACEAE | | | |
| *Tetragonia expansa* Murr. | −(2) | | |
| Boraginaceae | | | |
| *Amsinckia douglasiana* DC | +(2) | | |
| Chenopodiaceae | | | |
| *Beta macrocarpa* Guss. | +(7) | +(4) | +(4) |
| *B. vulgaris* L. | −(>20) | −(>20) | −(>20) |
| *Chenopodium amaranticolor* Coste & Reyn. | −(2) | −(2) | −(2) |
| *C. bonus-henricus* L. | −(3) | | |
| *C. botrys* L. | +(2) | | |
| *C. capitatum* (L.) Asch. | −(4) | −(4) | −(4) |
| COMPOSITAE | | | |
| *Lactuca sativa* L. | +(4) | +(4) | +(4) |
| *Senecio cineraria* DC. | −(3) | | |
| *S. vulgaris* L. | +(8) | +(4) | +(4) |
| *Sonchus oleraceus* L. | −(2) | −(3) | −(3) |
| CRUCIFERACEAE | | | |
| *Brassica carinata* A.Br. | +(1) | −(1) | −(1) |
| *B. juncea* (L.) Coss. | +(3) | +(1) | +(1) |
| *B. kaber* (DC.) L. C. Wheeler | −(3) | +(2) | +(2) |
| *B. marinosa* Bailey | +(1) | −(1) | +(1) |
| *B. napus* L. | −(1) | +(1) | +(1) |
| *B. pekinensis* (Lour.) Rupr. | −(5) | −(2) | −(2) |
| *B. rapa* L. | +(4) | +(4) | +(4) |
| *Capsella bursa-pastoris* (L.) Medic. | +(>20) | +(>20) | +(>20) |
| *Crambe abyssinica* Hochst. | | +(1) | +(1) |
| *Erysimum asperum* DC. | +(3) | −(1) | −(1) |
| *Lepidium nitidum* Nutt. | +(2) | +(2) | +(2) |
| *Lunaria annua* L. | | +(1) | +(1) |
| *Raphanus sativus* L. | −(>20) | −(>20) | −(>20) |
| *Sisymbrium irio* (L.) Britt. | −(2) | | |
| *Thlaspi arvense* L. | +(1) | +(1) | +(1) |
| GERANIACEAE | | | |
| *Geranium dissectum* L. | −(3) | | |
| Leguminosae | | | |
| *Trifolium incarnatum* L. | −(5) | +(1) | +(1) |
| *Vicia faba* L. | −(1) | | |
| LINACEAE | | | |
| *Linum usitatissimum* L. | −(1) | | |
| Malvaceae | | | |
| *Malva parviflora* L. | −(6) | +(2) | +(2) |
| PORTULACACEAE | | | |
| *Claytonia perfoliata* Donn. | +(6) | +(2) | +(2) |
| SOLANACEAE | | | |
| *Datura stramonium* L. | −(2) | | |
| *Hyoscyamus niger* L. | −(2) | | |
| *Nicandra physalodes* (L.) Gaertn. | +(2) | | |
| *Nicotiana clevelandii* Gray | +(5) | +(5) | +(5) |
| *N. megalosiphon* Heurck & Meull. | −(1) | | |
| *Physalis floridana* Rybd. | +(4) | | |
| *P. franchetii* Mast. | −(3) | | |

[a] + = Infection; − = no infection. Figures in parentheses refer to number of experiments on which evidence is based.

killed with a nicotine sulfate spray. Recovery attempts from all inoculated plants were carried out to verify susceptibility.

The handling of aphids, strains of BWYV, membrane-feeding technique, and antigen and antiserum preparation were as previously reported (2, 5). Extracts for antigen preparation and infectivity neutralization tests were prepared from shepherd's purse plants infected with various strains of BWYV and the English isolates. Fresh plant material was ground in a food grinder 1:1 with 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine. Crude extracts were clarified by low speed centrifugation (10 min at 6,000 rpm, 4,220 *g*) in a Sorvall SS-1 rotor. Clarified juice was centrifuged for 2 hr at 35,000 rpm (80,800 *g*) in the No. 40 rotor of a Spinco Model L ultracentrifuge. Pellets were resuspended in 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine.

Density-gradient centrifugation was done in a SW-39 rotor for 2 hr at 30,000 rpm (73,450 *g*). Gradient columns were prepared by layering 0.9 ml each of 20, 30, 40, 50, and 60% sucrose dissolved in 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine. Samples were removed from the zone 18-26 mm from the top of the tubes by means of a j-shaped hypodermic needle.

All density-gradient fractions used in feeding extracts were adjusted to 20% sucrose (by dilution with buffer) before they were placed on the membranes. This dilution prepared the samples for membrane feeding and resulted in preparations concd about 50 times the concn of the original sap.

Aphids from each colony used in membrane feeding experiments were tested on shepherd's purse simultaneously with each membrane feeding test. In no instances, during the course of these studies, were viruliferous aphids found in the stock aphid colonies.

RESULTS.—*Host range.*—Numerous host range tests with isolates of BMYV from sugarbeet in Britain have indicated a narrow host range for these isolates (7). It became apparent, in preliminary tests, that virus isolates collected from weeds and crop plants, other than sugarbeet in Britain, differed significantly in host range from common isolates of BMYV.

The results obtained in host range studies with three isolates selected for immediate study are presented in Table 1. There was considerable variation in the number of tests in which different plant species were included, and the failure to infect a species in only one or two tests is obviously not conclusive.

The English isolates produced a common reaction on certain key indicator hosts. *Beta vulgaris* (sugarbeet and red table beet), *R. sativus, B. pekinensis, C. capitatum,* and *S. oleraceus* were immune to these isolates. *Beta macrocarpa, Brassica rapa, L. sativa, C. bursa-pastoris, N. clevelandii,* and *Claytonia perfoliata,* on the other hand, were all susceptible to the three isolates and produced good diagnostic symptoms when infected. A total of 23 species of 40 tested were found to be susceptible to the isolates. These belong to eight families. No susceptible species were recorded in three families.

Fifty-eight different BMYV isolates from sugarbeet fields throughout eastern England were tested during 1969; none of these have infected *L. sativa.* New collections of yellowing virus isolates were made from lettuce in Bedfordshire during 1969; these infected lettuce, *C. bursa-pastoris, C. perfoliata,* and *S. vulgaris,* but not sugarbeet.

*Membrane feeding.*—The similarity of symptoms and vector relationships of BWYV and the English virus isolates from *C. bursa-pastoris* and *L. sativa* and the availability of an effective tool, infectivity neutralization (4, 5), to detect BWYV strains led to studies on membrane feeding of the isolates and the application of this technique to serological studies.

Utilizing the techniques previously described (2, 3), it was found that the English isolates could be successfully transmitted to healthy *C. bursa-pastoris* seedlings by green peach aphids feeding through Parafilm (Marathon Products, Neenah, Wisconsin) membranes on density-gradient fractions of crude and concd sap from infected *C. bursa-pastoris.* The infectious fractions in the density-gradient columns appeared to be in one zone 18-26 mm from the top of SW-39 tubes. This is the same location in the density-gradient columns from which BWYV has been repeatedly recovered.

*Serological relationships.*—The demonstration that infectivity neutralization could be utilized effectively to determine serological relationships of BWYV strains prompted an attempt to study the serological relationships of the English yellowing isolates and BWYV. Since previous work with green peach aphids had indicated poor feeding when the insects were fed directly on the virus-antiserum reactants, the reactants were subjected to density-gradient centrifugation prior to the feeding of the insects (5). In this case, evidence of serological reaction was based on the failure to encounter infectivity in the normal virus zone.

Eight antisera prepared from seven different strains of BWYV were tested against the English isolates (Table 2). Antisera prepared against two of the English isolates were tested against five BWYV strains and the three English virus isolates (Table 3). Antiserum against healthy shepherd's purse juice did not affect infectivity of BWYV or the English isolates.

Antisera against all the BWYV strains tested effectively neutralized the infectivity of the three English yellowing isolates. In several instances, an occasional infected plant occurred from samples incubated with certain sera. These isolates that survived incubation were subsequently tested with the same and other sera and were found to have escaped neutralization because of a low titer of the sera.

TABLE 2. Serological interactions of American BWYV antiserum with English BWYV isolates

| Sample tested | Infectivity of virus zone after incubation with the indicated sera | | |
|---|---|---|---|
| | Isolate E-1[a] | Isolate E-2 | Isolate E-3 |
| ASHSP[b] + virus[c] | 73[d] | 132 | 117 |
| | 80 | 140 | 140 |
| ASST1-1 + virus | 0 | 0 | 0 |
| | 20 | 40 | 40 |
| ASST3-1 + virus | | 1 | 1 |
| | | 40 | 40 |
| ASST7-2 + virus | | 1 | 1 |
| | | 40 | 40 |
| ASST7-3 + virus | | 0 | 2 |
| | | 20 | 40 |
| ASST8-1 + virus | | 0 | 0 |
| | | 20 | 20 |
| ASST9-1 + virus | 0 | 0 | 0 |
| | 60 | 20 | 20 |
| ASST10-1 + virus | | 1 | 0 |
| | | 40 | 40 |
| ASST11-1 + virus | 0 | 0 | 1 |
| | 20 | 40 | 40 |

[a] English isolates of BWYV.

[b] Antiserum to healthy shepherd's purse (ASHSP); antiserum to strain 1 BWYV (ASST1-1); antiserum to strain 3 BWYV (ASST3-1); etc.

[c] The virus samples were obtained from infected shepherd's purse, cleared by low-speed centrifugation, and pelleted by ultracentrifugation. Pellets were resuspended in buffer to approximately 1/50 of the original volume of sap. The virus sample was mixed with an equal volume of serum and incubated for 2 hr at 37 C. Incubated mixtures were subjected to density-gradient centrifugation, and samples for infectivity assays were removed from the zone 18-26 mm from the top of SW 39 tubes.

[d] The numerator indicates the number of plants infected and the denominator the number of plants inoculated by 10 green peach aphids fed through membranes on each sample.

Antisera against English isolates E-1 and E-3 completely neutralized all infectivity of the BWYV strains tested and also the three English isolates tested.

DISCUSSION.—The results of these experiments establish a serological relationship between BWYV and yellowing virus isolates from weeds and lettuce in England. They show for the first time that BWYV occurs outside the USA. In the USA, strains of BWYV can induce serious diseases of sugarbeet, lettuce, radish, turnip, broccoli, cauliflower, and flax, and many strains have a very wide range of weed hosts which can significantly affect the epidemiology of the diseases.

It is interesting that the English isolates studied thus far have a somewhat restricted host range, and

TABLE 3. Serological interactions of antiserum to English BWYV isolates with American BWYV

| Sample tested | Infectivity of virus zone after incubation with the indicated sera | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ST-1[a] | ST-2 | ST-7 | ST-9 | ST-10 | E-1 | E-2 | E-3 |
| ASE-1[b] + | 0[d] | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | — | — | — | — | — | — | — | — |
| virus[c] | 40 | 20 | 20 | 20 | 40 | 60 | 20 | 40 |
| ASE-3 + | 0 | 1 | 0 | | | 0 | 0 | 0 |
| | — | — | — | | | — | — | — |
| virus | 20 | 20 | 40 | | | 20 | 20 | 40 |
| ASHSP + | 38 | 16 | 34 | 20 | 37 | 54 | 39 | 33 |
| | — | — | — | — | — | — | — | — |
| virus | 40 | 20 | 40 | 20 | 40 | 60 | 40 | 40 |

[a] American strains of BWYV: (ST-1), (ST-2), (ST-7), (ST-9), (ST-10); English isolates of BWYV: (E-1), (E-2), (E-3).

[b] Antiserum to English isolate 1 (ASE-1); antiserum to English isolate 3 (ASE-3); antiserum to healthy shepherd's purse (ASHSP).

[c] The virus samples were obtained from infected shepherd's purse, cleared by low-speed centrifugation, and pelleted by ultracentrifugation. Pellets were resuspended in buffer to approximately 1/50 of the original volume of sap. The virus sample was mixed with an equal volume of serum and incubated for 2 hr at 37 C. Incubated mixtures were subjected to density-gradient centrifugation, and samples for infectivity assays were removed from the zone 18-26 mm from the top of SW 39 tubes.

[d] The numerator indicates the number of plants infected and the denominator the number of plants inoculated by 10 green peach aphids fed through a membrane on each sample.

that none has infected sugarbeet or radish. Also, all isolates of BMYV collected from sugarbeet fields in eastern England have failed to infect lettuce, radish, or chinese cabbage. According to the available evidence, therefore, BWYV isolates from lettuce do not constitute a threat to the sugarbeet crop in England.

The severe chlorosis and stunting of infected lettuce plants in Bedfordshire in 1968 and 1969 (8) suggests that BWYV may be of considerable economic importance in lettuce crops in Britain. Lettuce cultivars of the Butterhead type are more susceptible to damage by isolates of BWYV than are cultivars of the Crisphead type. The chief symptoms, interveinal yellowing and stunting, are especially important on cultivars of the Butterhead type which tend to have loose heads and a more open appearance. Even late symptoms give the plant an unsightly appearance and reduce its market value.

The greater tolerance to symptoms exhibited by lettuce cultivars of the Great Lakes type to BWYV may be partially responsible for shifts from the Butterhead types to Crisphead types in the Salinas Valley of California.

The present investigations have not helped to clarify the interrelationships of BMYV and BWYV, but further serological and host-range studies of several isolates of the two viruses are planned to provide more information on this subject. Such information could be important in programs of breeding for resistance to virus yellows of sugarbeet. For example, if BWYV and BMYV are shown to be unrelated or only distantly related to each other, it is unlikely that plants resistant to one virus will necessarily be resistant to the other. Under these circumstances, both BMYV and beet-infecting strains of BWYV would be a threat to sugarbeet production in America and Europe; and it would be necessary to test simultaneously for resistance to BWYV, BMYV, and BYV in both continents. If, on the other hand, BWYV and BMYV are closely related, resistance to one virus would probably be associated with resistance to the other, and the present programs of breeding for resistance to virus yellows in Europe and America are probably adequate.

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