Serological Relationship Between Beet Western Yellows and Turnip Yellows Viruses

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ABSTRACT

A possible relationship between beet western yellows virus (BWYV) and turnip yellows virus (TuYV) was indicated by the similarities of symptom expression and transmission characteristics of the two viruses, and was further supported by the recent demonstration of the occurrence of BWYV in Europe. A TuYV isolate from turnip in England was transmitted by *Myzus persicae* to *Capsella bursa-pastoris* and studied in regard to host range, transmission, and serology. The reaction on certain key indicator hosts was similar to that of English isolates of BWYV. *Beta vulgaris, Raphanus sativus, Brassica pekinensis, Chenopodium capitatum,* and *Sonchus oleraceus* were immune to TuYV, and *Brassica rapa, Lactuca sativa, Capsella bursa-pastoris, Nicotiana clevelandii, Senecio vulgaris,* and *Claytonia perfoliata* were susceptible. TuYV was 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. Antisera prepared against eight strains of BWYV from America and England neutralized infectivity of TuYV. Antisera prepared against TuYV from England neutralized infectivity of nine BWYV strains from America and England and TuYV isolates from England and Germany. The results of these investigations establish a close serological relationship between BWYV from America and England and TuYV from turnip in England and Germany.

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The importance and widespread distribution of beet western yellows virus (BWYV) is becoming more evident each year. The virus has been reported from North America (3, 5, 20), Europe (9), and Asia (17), and is probably common throughout the world. The virus causes stunting and chlorosis of a wide range of dicotyledonous species, including sugar beet, red beet, spinach, lettuce, broccoli, cauliflower, radish, turnip, pea, and flax.

The interrelationships of beet western yellows virus and yellowing viruses reported on sugar beets and other crops from various parts of the world are important from the standpoint of both disease epidemiology and breeding for resistance.

Turnip yellows virus (TuYV), described by Vanderwalle in 1950 from Belgium (19), was apparently known for some years previous in Europe. The virus induces a red discoloration along the edges of infected turnip leaves, followed by an intense chlorosis of the whole blade, which becomes hard and brittle. Diseased plants remain dwarfed, and have small roots. The disease is widespread in Europe, occurring in Belgium, Germany (1), and England (21), and has been observed in Denmark. The virus, transmitted in a persistent manner by *Myzus persicae* (Sulzer) (1), has a reported host range, mostly in the Cruciferae (11).

Observations in England, Scotland, Denmark, and Germany suggested that BWYV was present in weed and crop hosts in these areas (15). Subsequent serological studies (9) proved the presence of BWYV in Europe. Thus, the occurrence of BWYV in Europe, the similarity of symptom expression on key indicator hosts, and the similarity in transmission characteristics indicated a possible relationship between BWYV and TuYV. This paper describes investigations of the relationships between isolates of BWYV from California and England to isolates of TuYV from England and Germany.

MATERIALS AND METHODS.—TuYV (English isolate) was obtained from Marion Watson (Rothamsted), who described it as "a typical isolate of turnip mild yellowing virus" (21), which is a synonym of turnip yellows virus (2). TuYV (German isolate) was obtained from A. Nagi (Biologische Bundesanstalt für Land-und Forstwirtschaft).

Nonviruliferous green peach aphids reared on *Brassica pekinensis* (Lour.) Rupr. or *Raphanus sativus* L. were transferred to detached leaves of turnip infected with the TuYV isolates and allowed to feed for 48 hr. The aphids were then transferred to seedlings of shepherd's purse (*Capsella bursa-pastoris* [L.] Medic.) and turnip (*Brassica rapa* L.). Control aphids were transferred directly from *B. pekinensis* or *R. sativus* to indicator plants to ensure that the stock aphid colonies were free from contaminant viruses.

Host range studies were carried out by inoculation of at least five seedlings from each of several species, with 20-30 aphids which had fed on diseased shepherd's purse for 24 hr. The aphids were permitted to feed on the test plants for 48 hr, then 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 (6, 10). Extracts for antigen preparation and infectivity neutralization tests were prepared from shepherd's purse plants infected with various strains of BWYV and the TuYV 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*). We prepared gradient columns 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 concentrated about 50 times the concentration 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.*—Previous host range tests with isolates of TuYV indicated an extremely narrow host range for these isolates, mostly in the Cruciferae. Species that were tested and gave negative results were not usually reported in the literature, and it has been difficult to compare the host range of isolates of this virus with that of BWYV isolates. For this reason, one isolate (from England) was selected for extensive studies. The species of plants found susceptible to infection by TuYV in these tests are: BORAGINACEAE: *Amsinckia douglasiana* DC.; CHENOPODIACEAE: *Chenopodium botrys* L.; COMPOSITAE: *Lactuca sativa* L., *Senecio vulgaris* L., *Zinnia elegans* Jacq.; CRUCIFERAE: *Brassica napobrassica* (L.) Mill.; *B. carinato* A. Br.; *B. incana* Tenore; *B. juncea* (L.) Coss; *B. kaber* (DC.) L. C. Wheeler; *B. marinosa* Bailey; *B. napus* L.; *B. oleracea* var. *capitata* L.; *B. oleracea* var. *viridis* L.; *B. rapa* L.; *Capsella bursa-pastoris* (L.) Medic.; *Crambe abyssinica* Hochst. ex R. E. Fries; *Lepidium nitidum* Nutt.; *L. sativum* L.; *Thlaspi arvense* L.; GERANIACEAE: *Geranium dissectum* L.; LEGUMINOSAE: *Pisum sativum* L.; *Trifolium incarnatum* L.; LINACEAE: *Linum usitatissimum* L.; PORTULACACEAE: *Claytonia perfoliata* Donn.; SOLANACEAE: *Nicotiana clevelandii* Gray; *Physalis floridana* Rydb.

Species that showed no symptoms, and from which no virus was recovered, are as follows: AIZOACEAE: *Tetragonia tetragonioides* (Pall.) Ktze.; CHENOPODIACEAE: *Beta macrocarpa* Guss.; *B. vulgaris* L.; *Chenopodium amaranticolor* Coste & Reyn.; *C. capitatum* (L.) Asch.; *C. murale* L.; COMPOSITAE: *Calendula officinalis* L.; *Dahlia variabilis* (Willd.) Desf.; *Picris echioides* L.; *Sonchus oleraceus* L.; CRUCIFERAE: *Brassica pekinensis* (Lour.) Rupr.; *Erysimum asperum* DC.; *Nasturtium officinale* R. Br.; *Raphanus sativus* L.; *Sisymbrium irio* L.; CUCURBITACEAE: *Cucumis sativus* L.; LEGUMINOSAE: *Lathyrus odoratus* L.; *Vicia faba* L.; MALVACEAE: *Malva parviflora* L.; PLANTAGINACEAE: *Plantago lanceolata* L.; SOLANACEAE: *Nicandra physalodes* (L.) Gaertn.; *Nicotiana megalosiphon* Heurck & Muell.-Arg.; UMBELLIFERAE: *Apium leptophyllum* (Pers.) Benth. & Muell.

The English isolate of TuYV studied in these tests produced a reaction on certain key indicator hosts very similar to that reported for English isolates of BWYV (9); *Beta vulgaris* (sugar beet), *R. sativus, B. pekinensis, C. capitatum,* and *S. oleraceus* were immune; and *Brassica rapa, L. sativa, C. bursa-pastoris, N. clevelandii, S. vulgaris,* and *Claytonia perfoliata* were susceptible. Most of the susceptible species produced good diagnostic symptoms when infected, but *S. vulgaris, C. perfoliata,* and *L. sativa*, species commonly used in BWYV indexing tests, showed very mild symptoms, and only after a long incubation period. *Beta macrocarpa* and *Nicandra physalodes*, unlike the English isolates of BWYV (9), were apparently immune to this isolate of TuYV.

Membrane feeding.—The application of a membrane feeding technique to TuYV was necessary to facilitate further characterization of the virus through serological testing by infectivity neutralization (8, 10). The similarity of symptoms and vector relationships of BWYV and TuYV led to membrane feeding studies using techniques shown to be successful for BWYV (6, 7). It was found that the isolates of TuYV could be successfully transmitted to healthy *C. bursa-pastoris* seedlings by green peach aphids feeding through Parafilm (Marathon Products, Neenah, Wisc.) membranes on density-gradient fractions of crude and concentrated 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 by Gold & Duffus (10) that infectivity neutralization could be utilized to determine a serological reaction by feeding aphids through membranes on virus-antiserum reactants prompted an attempt to study the serological relationship of BWYV and the TuYV isolate from Europe. 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 (10). In this case, evidence of serological

reaction was based on the failure to encounter infectivity in the normal virus zone.

Nine antisera prepared from eight different strains of BWYV from America and England and beet yellows virus (BYV) from America were tested against the English isolate of TuYV (Table 1). Antisera prepared against the English isolate of TuYV and an English isolate of BWYV (9) were tested against nine BWYV strains from America and England and the TuYV isolates from England and Germany (Table 2). Antiserum against healthy shepherd's purse, saline or antiserum against the beet yellows virus did not affect infectivity of TuYV. Antisera against all the BWYV strains tested effectively neutralized the infectivity of TuYV. Antiserum against the English isolate of TuYV completely neutralized all infectivity of the BWYV strains tested and the TuYV isolates from England and Germany.

TABLE 1. Serological interactions of beet western yellows virus (BWYV) antiserum with English turnip yellows virus (TuYV)

Sample tested	Infectivity of virus zone after incubation with the indicated sera
ASHSP[a] + TuYV[b]	110[c] / 120
Saline + TuYV	113 / 120
ASBYV + TuYV	37 / 40
ASST1-1 + TuYV	0 / 40
ASST3-1 + TuYV	0 / 80
ASST7-2 + TuYV	0 / 40
ASST7-3 + TuYV	0 / 40
ASST8-1 + TuYV	2 / 40
ASST9-1 + TuYV	0 / 40
ASST11-1 + TuYV	1 / 40
ASE1-1 + TuYV	0 / 40
ASE3-1 + TuYV	0 / 40

[a]Antiserum to healthy shepherd's purse (ASHSP); antiserum to beet yellows virus (ASBYV); antiserum to strain 1 BWYV (ASST1-1); antiserum to strain 3 BWYV (ASST3-1); etc.

[b]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 2.0% 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.

[c]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.

DISCUSSION.—The results of these experiments establish a close serological relationship between BWYV from America and England and TuYV from turnips in England and Germany. Furthermore, they establish a relationship between TuYV (turnip mild yellowing virus) from England and from Germany. The results indicate that TuYV isolates from Europe have a wider host range than was previously recognized; a host range which may affect crop plants in families other than the Cruciferae.

The relationship of TuYV isolates and other BWYV variants to yellowing diseases of flax (13), pea (14), broad bean (18), and turnip (12) reported from other parts of the world have not been studied. The possibility exists that BWYV is also involved in several of these yellowing diseases. Evidence is accumulating which indicates that BWYV is extremely widespread in the world, occurring naturally on a large number of wild and cultivated species, and occurring in a number of strains or variants with distinctive host ranges.

Little information is available concerning the economic significance of BWYV isolates except those occurring on sugar beet and lettuce (4, 5, 15, 16, 22), but the damage induced by TuYV isolates on turnip in Europe is well documented (1, 19). The effects of these isolates on other Cruciferous crops, however, has been given little attention. In the coastal areas of California, Cruciferous crops (broccoli, cauliflower, cabbage, and mustards) have a very high incidence of BWYV, but little is known of the effects of the virus on yield or uniformity on these crops.

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TABLE 2. Serological interactions of turnip yellows virus antiserum (ASTuYV) with beet western yellow virus (BWYV) isolates

Sample tested	Infectivity of virus zone after incubation with the indicated sera										
	TuYV-E[a]	TuYV-G	ST-1	ST-2	ST-3	ST-7	ST-8	ST-10	ST-11	E-1	E-3
ASHSP[b] + virus[c]	39[d]/40	38/40	37/40	18/20	18/20	40/40	19/20	18/20	37/40	36/40	40/40
ASE1 + virus	0/40	0/40	1/40	0/20	0/20	1/40	0/20	0/20	0/40	0/40	0/40
ASTuYV + virus	0/40	0/40	0/40	0/20	0/20	0/40	0/20	0/20	0/40	0/40	0/40

[a]TuYV-E (turnip yellows virus-England); TuYV-G (turnip yellows virus-Germany); ST-1, ST-2, ST-3, ST-7, ST-8, ST-10, ST-11 (American strains of BWYV); E-1, E-3 (English strains of BWYV).

[b]Antiserum to healthy shepherd's purse (ASHSP); antiserum to English isolate 1 BWYV (ASE1); antiserum to English isolate TuYV (ASTuYV).

[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 2.0% 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.

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