Unique Type of Curly Top Virus and Its Relationship with Horseradish Brittle Root

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ABSTRACT

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A curly top virus-like entity transmitted by *Circulifer tenellus* was isolated from horseradish plants (*Armoracia rusticana*) affected by brittle root and also from plants not diseased with brittle root in the same fields in Illinois. Inoculation of healthy horseradish with the virus did not result in brittle root under greenhouse and field conditions in these tests. The virus from horseradish was serologically related to beet curly top virus and was transmitted in a similar manner by the leafhopper vector, but it was unique from all known curly top types in its very limited host range of cruciferous species.

Brittle root of horseradish, *Armoracia rusticana* Gaertn., Mey. & Scherb., is considered the most destructive disease of this perennial crucifer. Aboveground symptoms include a general chlorosis of the foliage and collapse of plants. Roots of diseased plants develop vascular discoloration and become turgid and brittle.

Most of the commercial horseradish production in the United States is located in the Mississippi River Valley near St. Louis, MO, and Eau Claire, WI (3). Brittle root occurs sporadically in Illinois and caused extensive damage in 1935–1936, 1953–1954, 1975, and 1979. Thornberry and Takeshita (14) cited circumstantial evidence that indicates that beet curly top virus (BCTV) may be responsible for at least a portion of the brittle root syndrome.

The disease was suspected to be curly top when it was first reported and described by Kadow and Anderson in 1936 (7) because it was essentially identical to curly top on horseradish as described by Severin (12). Although Severin reported that BCTV was isolated from "diseased" field horseradish in California, he indicated that this was very rare.

The symptoms described by Severin (12) for horseradish diseased with curly top were from "naturally infected" plants adjacent to a badly diseased beet field in the Sacramento Valley of California. Attempts to isolate BCTV from horseradish diseased with brittle root in Illinois (14) have apparently been unsuccessful.

The object of this study was to reexamine the role of BCTV and beet leafhoppers in the brittle root syndrome of horseradish in Illinois.

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MATERIALS AND METHODS

Isolates of BCTV used in these studies were maintained in beet leafhoppers, *Circulifer tenellus* (Baker), reared on diseased sugar beet (*Beta vulgaris* L.) plants. Young adult leafhoppers of similar age reared on healthy plants were used in membrane feeding tests. Nonviruliferous leafhoppers were obtained from eggs from about 200 nonviruliferous adults kept on individual beet plants for 2 days.

Membrane feeding tests were conducted, using feeding cages as previously described (4). Groups of 26 nonviruliferous leafhoppers were placed in these cages. Approximately 0.5 ml of the liquid extract to be tested was placed on the membrane and covered with another thin membrane of Parafilm. Extracts were adjusted to 15% sucrose by the addition of sucrose or dilution with buffer. Cages with leafhoppers and liquid extract were placed membrane-side down on white paper in a controlled temperature chamber maintained at 37 C. The leafhoppers were allowed to feed 4 hr on the extracts and then caged singly on seedling shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.) or sugar beet.

Extracts for antigen preparation and infectivity neutralization tests were derived from infected plant tissue partially purified by an alcohol precipitation technique described by Bennett (1).

Healthy shepherd's purse antigen was prepared by clarifying crude extracts by low-speed centrifugation (10 min at 4,200 *g*) followed by ultracentrifugation (2 hr at 80,800 *g*). The pellets were resuspended in 1/150 of the original volume in 0.05 M phosphate buffer, pH 7.0, containing 0.01 M glycine.

Sera were prepared from rabbits after six intramuscular injections at weekly intervals, using Freund's complete adjuvant (Difco-Bacto).

RESULTS

Attempts to isolate BCTV from field-collected brittle root. Horseradish plants with typical brittle root symptoms collected periodically in Illinois from 1975 to 1980 were sent to California for testing with the beet leafhopper for presence of BCTV. Acquisition access periods ranging from 1 day to several months (rearing nonviruliferous leafhoppers on the diseased plants) were utilized in attempts to recover the virus. No evidence of typical BCTV was obtained from 22 plants diseased with brittle root that were exhaustively tested using more than 700 susceptible beet indicator plants.

Attempts to determine susceptibility of horseradish to common isolates of BCTV. Because the association of brittle root and BCTV in midwestern horseradish plantings was based on circumstantial evidence, and because of the failure to isolate the virus from plants diseased with brittle root, attempts were made to determine whether common isolates of the virus could induce brittle root under greenhouse conditions.

Inoculation of horseradish seedlings. Twelve seedling horseradish plants were inoculated with either BCTV strain 11 (collected in the 1950s from California [6]) or Fresno II strain (collected in the 1970s from California [8]). After 4–8 wk, when all control beet plants were showing severe curly top symptoms, attempts were made to recover the virus from inoculated plants. In no instance in 224 attempts was BCTV isolated from the inoculated horseradish seedlings. Control insects placed on the beets diseased with curly top transmitted the virus to more than 98% of the indicator beet seedlings.

None of the inoculated horseradish plants exhibited symptoms of curly top as described by Severin (12) or of brittle root.

Inoculation of commercial horseradish. A healthy horseradish plant (type 'Common') collected in a field that also

contained plants diseased with brittle root was colonized with more than 500 beet leafhoppers viruliferous with the Logan strain, the most virulent BCTV strain known (10). Leafhoppers were transferred from this plant to indicator beet seedlings daily for 1 mo to verify that the colonizing leafhoppers were viruliferous; 116 of 120 (96.7%) indicator plants thus inoculated became infected. At the end of this period, the remaining adults were removed. Nymphs developing on the horseradish plant from eggs laid by the viruliferous leafhoppers were transferred to indicator beet seedlings to determine whether they could acquire virus from the inoculated horseradish. In no instance in 40 separate recovery attempts was BCTV transmitted by these nymphs.

These same procedures were followed in the inoculation of 10 additional horseradish plants. Colonies of more than 500 leafhoppers viruliferous with different BCTV strains (strain 1, strain 11, strain 22, Logan, Paso Robles, Los Banos, Fresno I, and Fresno II) were established on cultivars of field-collected horseradish that included six healthy Common plants, one Common with brittle root, one Swiss with brittle root, one healthy Japanese, and one healthy Canadian. In all instances, the inoculating leafhoppers transmitted BCTV to a high percentage (about 98%) of indicator beets. However, nymphs from these adults on the horseradish plants did not transmit the virus to indicator plants in more than 300 separate tests.

Recovery attempts from site of inoculation. In an effort to explain the recovery of BCTV from horseradish by Severin in 1925 (12), the possibility was investigated that even if the plants were immune (as our tests indicated), nonviruliferous leafhoppers might be able to acquire virus from the immune plants if tested soon after inoculation. BCTV can induce symptoms and be recovered from immune hosts, such as dodder (*Cuscuta* sp.) and *Nicotiana glauca* (2). Leaf cages, each containing four leafhoppers viruliferous with the Logan strain of BCTV, were placed in 20 separate locations on a Common horseradish plant. After a 4-day inoculation access period, the cages with the viruliferous leafhoppers were removed and cages of four nonviruliferous leafhoppers were placed on the same leaf locations. These leafhoppers were allowed an acquisition access period of 24 hr and were then transferred to indicator sugar beets for 7 days. Recovery attempts were conducted every 24 hr for 3 additional days. Recovery attempts were also made from 20 other locations on the plant over the 4-day post inoculation period. In no instance was BCTV recovered from the inoculated plants.

Recovery of another agent from plants diseased with brittle root. The complete failure to recover typical BCTV isolates from plants diseased with brittle root, coupled with the evidence that horseradish does not appear to be a host of the virus, suggested that the beet leafhopper, which has had a long circumstantial association with brittle root, might be the vector of other agents that may be involved in the brittle root syndrome.

Inoculations of hosts other than BCTV-susceptible sugar beet were attempted using beet leafhoppers that had fed on horseradish plants diseased with brittle root. In the first several attempts, an entity that induced curly top-like symptoms on shepherd's purse was transmitted. Subsequent assays indicated that 25 of 63 (39.7%) horseradish plants diseased with brittle root and 10 of 57 (14.9%) normal-appearing horseradish plants from the same fields contained the entity that infected shepherd's purse.

Characterization of the leafhopper-transmitted entity from horseradish. The entity from horseradish was acquired by nonviruliferous beet leafhoppers during a 5-min acquisition access period and was transmitted after a 5-min inoculation access period. A latent period of between 12 and 24 hr was demonstrated by various acquisition and inoculation access periods with individual beet leafhoppers. These properties were very similar to the insect transmission properties of BCTV in parallel tests and to those previously reported for BCTV (2).

More than 40 species and 27 cultivars in 11 families that were susceptible to BCTV in other tests were inoculated with one of the isolates from horseradish. These species included early American and European cultivars of sugar beet known to be highly susceptible to BCTV. Each inoculated plant was back-tested to shepherd's purse. Only horseradish, shepherd's purse, and pennycress (*Thlaspi arvense* L.) were susceptible to the horseradish isolate.

Symptoms on shepherd's purse and pennycress infected with the entity from horseradish were identical to those induced by common BCTV strains on these hosts, and included stunting of plants, curling of leaves and stems, swelling of leaf veins and flower parts, and production of phloem exudates. Horseradish seedlings and commercial cultivars that were inoculated with the agent from horseradish showed no symptoms under greenhouse or field conditions; however, the agent was recovered from inoculated plants.

Partially purified preparations contained paired (geminate) viruslike particles (Fig. 1) measuring about 20 × 35 nm that were similar to particles from purified BCTV preparations (9).

Serological neutralization of infectivity was tested by feeding beet leafhoppers directly on virus-antiserum reactants. Antigens derived from partially purified preparations were mixed with equal volumes of buffer or of antisera (1:5 dilution) to the juice of healthy shepherd's purse, BCTV, and the entity from horseradish. After incubation for 0.5 hr at 37 C, the mixtures were fed to nonviruliferous beet leafhoppers through membranes, and the leafhoppers were caged individually on shepherd's purse seedlings. Infectivity of the entity that infected shepherd's purse from horseradish and BCTV from beet was completely neutralized by antisera to BCTV and the horseradish entity but was not affected by antiserum against healthy shepherd's purse juice (Table 1).

DISCUSSION

A curly-top type virus was isolated from Illinois horseradish plants with brittle root and from plants showing no

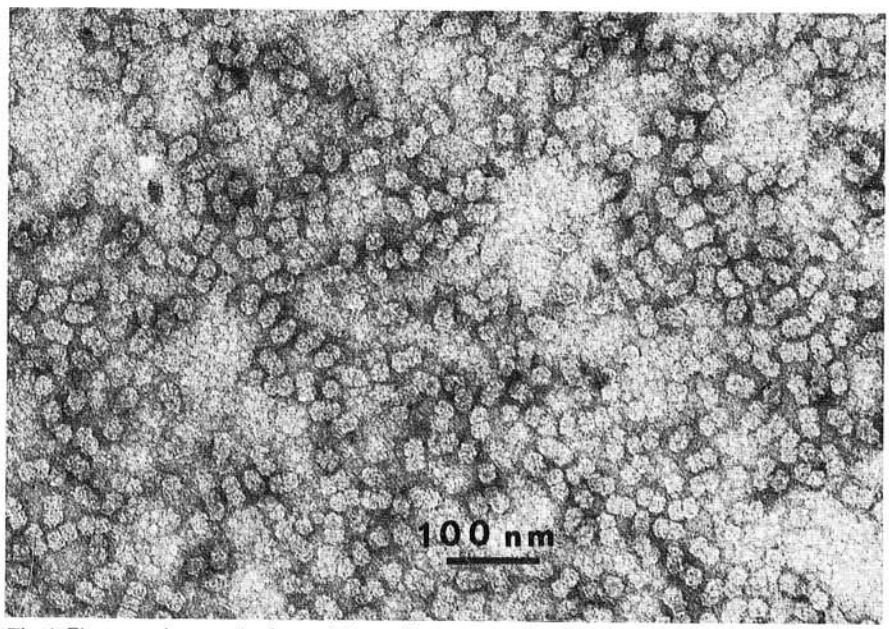

Fig. 1. Electron micrograph of a partially purified preparation of the curly top virus-like entity from horseradish showing paired (geminate) virus particles stained with 2% uranyl acetate.

Table 1. Infectivity neutralization of beet curly top isolates from horseradish and beet

Treatment[a]	Infectivity[b] of isolate[c] after incubation with the indicated diluent	
	HR-1	BCTV
Buffer + virus	79	42
ASHSP + virus	82	39
ASCT-1 + virus	0	0
ASHR-1 + virus	0	0

[a] Antisera to healthy shepherd's purse (ASHSP), BCTV strain 11 (ASCT-1), and curly top-like isolate from horseradish (ASHR-1).

[b] Number of plants infected out of 200 infested with individual beet leafhoppers fed through a membrane on each sample.

[c] Virus samples were partially purified from infected plant tissue by an alcohol precipitation technique; HR-1 = isolate from horseradish, BCTV = beet curly top virus strain 11. Virus samples were adjusted to 15% sucrose, mixed with an equal volume of the indicated diluent, and incubated 0.5 hr at 37 C.

symptoms of the disease. No evidence of damage or symptom production on horseradish was associated with this curly top virus-like entity.

Other infectious entities, including turnip mosaic and cauliflower mosaic viruses, fungal pathogens (Duffus, *unpublished*), and *Spiroplasma citri* (5,11), have been isolated from field horseradish showing brittle root symptoms. Whether one, several, all of these entities, or even others may be involved in the field syndrome of brittle root is not clear.

Isolates of common BCTV collected and tested over the last 40 yr do not infect modern horseradish cultivars. It is difficult to reconcile this fact with the indications of Severin (12) over 50 yr ago that typical BCTV caused a serious disease on the crop. Possibly, some selections or importations of horseradish used in California in the 1920s were susceptible to the virus.

This report constitutes the first record of a curly top virus-like entity, transmitted by *Circulifer* sp., with a narrow host range. The reported host range of BCTV comprises some 300 species and 44 plant families (2). Little is known about BCTV with diverse host ranges, probably because sugar beets have always been used as the bioassay host.

There have been numerous occasions in the past in which curly top epiphytotics have been severe on one host but insignificant on others. For instance, in 1966 curly top devastated sugar beets in California, but tomatoes were not seriously affected; in 1977, the disease was severe on tomatoes and did little damage to sugar beets. These differences were blamed on leafhopper flight times, cropping sequences, etc.; possibly, host-specific strains played a role in these and previous epiphytotics.

Whether the horseradish curly top isolate represents a whole new complex of host-specific BCTV-"like" entities or whether or how these might be related to curly top virus-like entities from other parts of the world (2,13) will require much more basic information on the viruses.

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