Beet Yellow Stunt, a Potentially Destructive Virus Disease of Sugar Beet and Lettuce

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ABSTRACT

Beet yellow stunt, a potentially destructive yellows-type virus disease of sugar beet and lettuce, is recognized as being distinct from other yellowing diseases affecting these crops. Sowthistle is the principal reservoir host of the virus and of the most efficient vector, *Nasonovia lactucae*. The disease is widespread and abundant on this host in California throughout the year. Spread in susceptible crops tends to be marginal; i.e., the disease incidence is high in rows adjacent to areas where sowthistle is prevalent, but becomes progressively less with increased distance from the virus source. Host range of the virus seems limited, but it can induce serious damage to infected sugar beet and lettuce. The virus has not been transmitted mechanically or by seed, but is transmitted in a semipersistent manner by *N. lactucae*, *Myzus persicae*, and *Macrosiphum euphorbiae*.

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Sugar beet production is significantly affected by yellowing diseases in most growing areas of the western United States. A complex of beet yellowing viruses is involved (1, 2, 3, 5). Currently, two components, the beet yellows virus (BYV) and the beet western yellows virus (BWYV), are of the greatest economic importance.

In 1963, a potentially destructive yellows-type virus disease of sugar beet (*Beta vulgaris* L.) and lettuce (*Lactuca sativa* L.) was recognized as being distinct from the other components. An abstract of preliminary results has been presented (4).

Cytological aspects of BYSV infection in *Sonchus* (6) and in *Beta* (7) have depicted the causal entity as a flexuous rod closely resembling the particles of BYV.

This paper reports investigations on the nature and relationships of the causal agent, the beet yellow stunt virus (BYSV).

MATERIALS AND METHODS.—Nonviruliferous green peach aphids (*Myzus persicae* [Sulzer]) were reared on radish (*Raphanus sativus* L.) and tested at frequent intervals for BWYV. Nonviruliferous sowthistle aphids (*Nasonovia lactucae* [L.]) were reared on sowthistle (*Sonchus oleraceus* L.). In routine transmission tests, insects were permitted to feed on the virus source for 24 hr and then for 48 hr on the test plants. Individual aphids were confined in small glass insect cages on seedlings in pots. Host range and recovery tests were carried out in cloth cages large enough to contain ten 6-inch pots.

Isolates of BYSV from sowthistle, sugar beet, and lettuce collected in California were maintained on these same species in the greenhouse.

Antisera against clarified extracts of the BYSV in beet, lettuce, and sowthistle and BYV in beet were prepared by methods commonly used for BYV. Leaves of infected plants grown in the greenhouse were ground in a mortar or ball mill. Crude extracts were clarified by low-speed centrifugation (10 min at 4,000 *g*). Antiserum was prepared against the clarified extracts by six intramuscular injections of rabbits at weekly intervals, using Freund's complete adjuvant.

RESULTS.—*Geographical distribution*.—The beet yellow stunt virus is widespread and of high incidence on sowthistle in the Salinas and San Joaquin Valleys of California at all times of the year. Symptoms on sowthistle identical to those induced by BYSV were observed in beet-growing areas of Oregon and Washington. Similar symptoms were found on sowthistle in England and Scotland. Experimental evidence for the occurrence of BYSV in the Pacific Northwest and in Europe, however, is lacking.

Host range.—Host range studies were carried out by the inoculation of at least five seedlings of a number of different species in an insectary. Nonviruliferous green peach aphids or sowthistle aphids were placed on detached leaves of BYSV-infected sugar beet, lettuce, or sowthistle for 24 hr. The aphids then were transferred to the test plants, where they were permitted to feed for 48 hr. The presence of virus in each plant species tested for susceptibility was determined by aphid transfer to lettuce or sowthistle about 60 days after inoculation.

Plants susceptible to BYSV are:

CHENOPODIACEAE: *Beta macrocarpa* Guss., *B. vulgaris* L., *Chenopodium capitatum* (L.) Asch. COMPOSITAE: *Lactuca sativa* L., *L. serriola* L., *Senecio vulgaris* L., *Sonchus oleraceus* L., *Zinnia elegans* Jacq. GERANIACEAE: *Geranium dissectum* L. PORTULACACEAE: *Claytonia perfoliata* Donn. SOLANACEAE: *Nicotiana clevelandii* Gray.

Plants showing no indication of infection include: AIZOACEAE: *Tetragonia expansa* Murr. AMARANTHACEAE: *Gomphrena globosa* L. CHENOPODIACEAE: *Beta patula* Ait., *Spinacia oleracea* L. COMPOSITAE: *Callistephus chinensis* (L.) Nees, *Helianthus annuus* L., *Taraxacum officinale* Weber. CRUCIFERAE: *Capsella bursa-pastoris* (L.) Medik., *Raphanus sativus* L. CUCURBITACEAE: *Cucumis melo* L. LEGUMINOSAE: *Medicago sativa* L., *Pisum sativum* L. LINACEAE: *Linum grandiflorum* Desf., *L. usitatissimum* L. MALVACEAE: *Gossypium hirsutum* L., *Malva parviflora* L. SOLANACEAE:

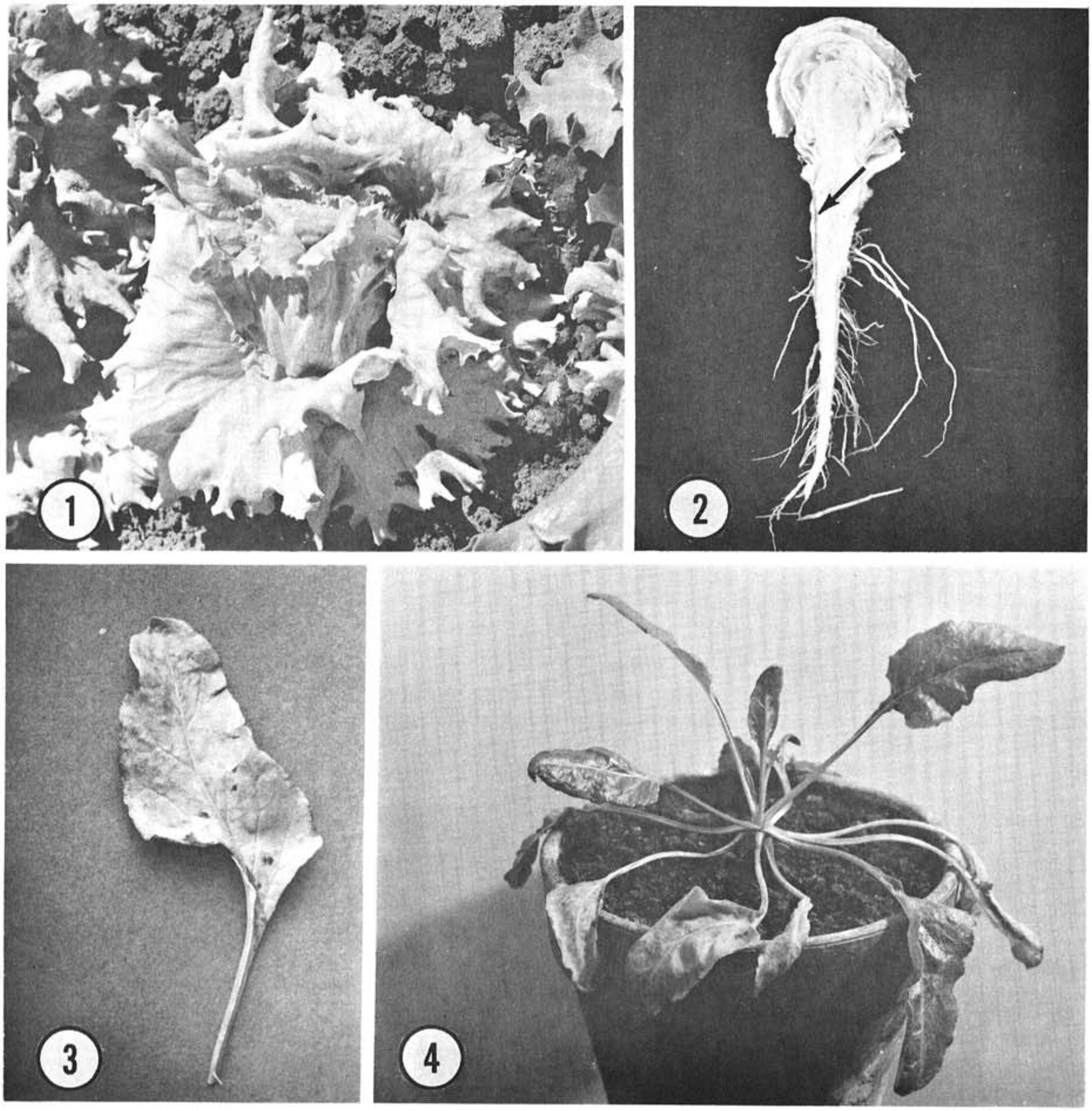

Fig. 1. Symptoms induced by the beet yellow stunt virus. **1)** Field lettuce plant showing complete chlorosis. **2)** Field lettuce plant cut longitudinally showing veinal necrosis (arrow). **3)** Detached sugar beet leaf showing twisting and interveinal chlorosis. **4)** Greenhouse sugar beet plant showing stunting, distortion, and twisting.

Lycopersicon esculentum Mill., *N. glutinosa* L., *Petunia hybrida* Vilm., *Physalis wrightii* Gray. UMBELLIFERAE: *Daucus carota* L. URTICACEAE: *Urtica californica* Greene.

Symptoms.–Species infected by BYSV showed, in general, yellowing of the lower and intermediate leaves (Fig. 1). On some species, the symptoms were mild and barely discernible, whereas on others, especially on sugar beet and lettuce, they were extremely severe, with stunting, necrosis, and sometimes death of the plants. Symptoms on a selected group of host plants are as follows:

1) *Sonchus oleraceus.*–Infected plants in the greenhouse initially showed veinclearing on the older and middle leaves. These leaves later developed interveinal reddening and yellowing. The leaves were markedly thickened and brittle, and had a tendency to twist. Healthy *Sonchus*, of the line commonly used in these greenhouse studies, has a tendency to produce diffused red spots, even when healthy. BYSV-infected plants showed intensified red spotting at a much earlier age than did healthy plants. Infected plants usually recovered from the acute stage of infection, and often were difficult to distinguish from healthy plants.

In the field, symptoms on sowthistle are quite variable. Most plants showed brilliant red interveinal coloration; however, others showed interveinal yellowing.

2) Beta vulgaris.–Initial symptoms on sugar beet infected with the BYSV were characterized by severe twisting, cupping, and epinasty of one or two intermediate-aged leaves. Petioles were shortened, and the leaves became mottled and yellow. Young leaves were dwarfed, malformed, twisted, and slightly mottled. As the leaves aged, the mottle became more intense and the leaf sometimes became completely chlorotic. The plants were severely stunted and often collapsed and died.

3) *Lactuca sativa.*–Initial symptoms on rosette-stage lettuce included chlorosis and folding back of the older leaves. As the disease progressed, the affected plant was severely stunted, and developed an extreme yellowing. The older leaves collapsed prematurely and turned necrotic. Plants infected at a young stage sometimes collapsed and died before head formation. The disease, at this stage, resembled *Sclerotinia* rot and has been termed "drop without the fungus" by farmers.

The disease may be rapidly diagnosed in the field by pulling affected plants and cutting the stem and crown tissue longitudinally. The phloem tissue, in BYSV-infected plants, is severely necrotic, and shows distinct brown zones extending into the crown tissue.

4) *Senecio vulgaris.*–Initial symptoms induced by BYSV on groundsel are irregular purple spots on the interveinal areas of older leaves. As the disease progresses, the spots coalesce until the leaves are almost completely purple, except for isolated green areas near the midrib. Symptoms of beet western yellows on this host are similar in coloration to those induced by BYSV; however, the distribution of the purpling is confined to the margins of mature leaves in the former disease.

5) *Claytonia perfoliata.*–The first symptoms are yellowing, with very small red flecks on the spade-shaped lower leaves and later on the circular perfoliate leaves below the inflorescences. Later, the spots enlarge and red streaks appear along the veins. The absence of red-rimmed necrotic spots readily distinguishes the symptoms of BYSV from typical beet yellows virus symptoms on this host.

6) *Chenopodium capitatum.* – Infected plants showed symptoms similar to those induced by mild isolates of the beet yellows virus. Interveinal reddening of the older leaves, with no veinclearing or vein etching, are characteristic of symptoms on this host.

Transmission tests.–1) *Mechanical.*–Numerous attempts were made to transmit BYSV mechanically by the use of abrasives, phosphate buffer, liquid nitrogen, and sodium sulfite. Virus sources included sugar beet, *B. macrocarpa*, lettuce, and sowthistle. The plants inoculated included these and several other species, some of which were found to be susceptible when inoculated by the aphid vectors. The results were negative in all tests.

2) *Dodder.–Cuscuta californica* Choisy was established on diseased plants of sugar beet, lettuce, *C. capitatum*, and sowthistle. Stems of the parasite were trained to the same species of test plant. At least 15 healthy plants of each species were parasitized by the dodder, but none developed symptoms of the disease.

3) *Insects.*–Preliminary studies indicated that BYSV was transmitted by the green peach aphid. At first, the virus isolate was thought to be a strain of BWYV, and early studies were designed to test possible transmission differences between the isolate from sowthistle and common BWYV strains. It became apparent that BYSV differed from BWYV in transmission characteristics. Tests to determine whether some of the common aphid species found on sowthistle were vectors of BYSV were carried out with lettuce, sowthistle, and sugar beet as virus source and test plants. Nonviruliferous aphids of the species tested were placed on the source plants for 24 hr, and then about 25 individuals were transferred to each of at least 20 test plants for about 48 hr. Under these conditions, BYSV was transmitted by *M. persicae*, *N. lactucae*, and *Macrosiphum euphorbiae* (Thos.).

Virus-vector relationships.–Relative efficiency of aphid species as vectors.–Early studies showed marked differences in transmitting ability among the aphid vectors of BYSV. *Myzus persicae*, found abundantly on sugar beet and lettuce, was a relatively inefficient vector. Individual aphids in over 800 tests rarely transmitted BYSV.

Groups of ca. 25 individuals were used in attempts to distinguish factors involved in green peach aphid vector inefficiency. Virus sources, species of test plant, and the age of infection had significant effects on transmission efficiency. The highest infection rate with the green peach aphid resulted when *C. capitatum* was used as both source and test plant. Following, in decreased order of efficiency, were *S. vulgaris*, *L. sativa*, and *B. vulgaris*. In tests with *B. vulgaris*, detached leaves were shown to be poor virus sources, as were old infected plants (45 days after inoculation). The severe degenerative changes in phloem parenchyma cells in older infections of BYSV in beet (7) may play an important role in the ability of the vector to acquire virus from such plants. The highest infection percentage observed in any test with beet, using the green peach aphid, was 26.3%. This was with groups of aphids (20-25) fed on plants showing initial symptoms of BYS (ca. 26 days after inoculation).

In other tests, *M. euphorbiae*, which feeds readily on sugar beet in the greenhouse, but is rarely found on this host in the field, was about as efficient a vector as the green peach aphid.

Nasonovia lactucae, commonly found on sowthistle, feeds only transiently on lettuce, and probably rarely on sugar beet. In tests to determine vector efficiency with sowthistle as source and test plants, single insects were capable of transmitting the virus to 56% of the test plants.

Persistence.–Retention of the virus by single sowthistle aphids was determined by daily serial transfers to healthy sowthistle seedlings. Viruliferous aphids lost their transmitting ability in 4 days or less

TABLE 1. Sowthistle seedlings infected (+) and noninfected (–) in daily serial transfers, using single sowthistle aphids fed 24 hr on a beet yellow stunt source plant

Aphid no.	Successive daily transfers[a] 1	2	3	4	5
1	+	+	–	–	–
2	+	+	–	–	–
3	+	–	–	–	–
4	+	+	–	–	–
5	+	+	–	–	–
6	–	+	–	–	–
7	+	+	+	–	–
8	+	+	–	–	–
9	+	+	+	+	–
10	+	+	–	–	–
11	+	+	–	–	–
12	+	–	–	–	–
13	+	–	–	–	–
14	+	+	–	–	–
15	+	–	–	–	–
16	+	–	–	–	–
17	+	+	+	–	–
18	+	–	–	–	–
19	+	–	–	–	–
20	+	–	–	–	–

[a] No seedlings were infected by the aphids in daily transfers from the 6th to 10th day, inclusive.

(Table 1). Most insects were infective only on the 1st day, or the first 2 days after virus acquisition.

In a group of experiments, single insects were given a 24-hr acquisition feeding, then transferred in daily serial transfers to healthy sowthistle seedlings for 5 days. The acquisition and serial transfers were repeated in the same sequence for the life of the insects. Single aphids, in some instances, were capable of acquiring the virus and losing it three successive times (Table 2).

Serial transfers of viruliferous sowthistle aphids every hour for 8 hr resulted in eight successive transmissions in some tests.

Throughout the tests on retention of BYSV by the sowthistle aphid, careful attention was paid to insect molting; in no instance did the aphids retain inoculativity through a molting period.

Similar trials on persistence were conducted with groups of green peach aphids. These tests indicated that maximum persistence of BYSV in green peach aphids was 2 days.

The data of all the retention studies indicate that BYSV is transmitted in a semipersistent manner, similar to the transmission of the BYV.

Epidemiology.—The beet yellow stunt disease was recognized in 1963 as being distinct from other yellowing diseases of sugar beet. At that time, the incidence of the disease in sugar beet and lettuce in the Salinas Valley was not known. Subsequently, visual surveys and plant indexing have indicated a wide distribution but a relatively low (2-3%) incidence of the disease in beet and lettuce fields during most seasons.

In June and July 1967, an epidemic occurrence of the beet yellow stunt disease was recognized in lettuce fields in an area several miles in diameter southeast of Salinas, Calif. Crop loss was estimated at between 50 and 85% in the affected lettuce fields. Observations indicated a high weed population in nearby areas, including neglected farm lands and roadways. Heavy, late spring rains contributed to the abundant weed growth.

Sowthistle is the principal source of the virus, although wild lettuce, *L. serriola*, is commonly found infected. Sowthistle is also the principal naturally occurring host of the most efficient vector, *N. lactucae*. There is no evidence that the virus is seed-borne in beet or lettuce.

Spread in the crop tends to be marginal; i.e., the disease incidence is high in rows adjacent to areas where sowthistle is prevalent, but becomes progressively less with increased distance from the virus source. Scattered infection centers are also characteristic.

Sowthistle is commonly found infected with BYSV at all times of the year. The vector transmits the virus readily to lettuce, but very inefficiently to beet. The sowthistle aphid apparently does not

TABLE 2. Sowthistle seedlings infected (+) and noninfected (–) in daily serial transfers using single sowthistle aphids fed at 6-day intervals on beet yellow stunt source plants

Aphid no.	Successive daily transfers BYS[a]	1	2	3	4	5	BYS	1	2	3	4	5	BYS	1	2	3	4	5
1		–	–	–	–	–		–	–	d[b]								
2		+	+	–	–	–		+	–	–	–	–		+	–	–	–	–
3		+	–	–	–	–		+	+	+	–	–		d				
4		–	–	–	–	–		–	–	–	–	–		–	–	d		
5		–	–	–	–	–		+	+	+	+	–		–	d			
6		+	–	–	–	–		–	–	–	–	–		+	+	+	–	–
7		–	–	–	–	–		–	–	–	–	–		+	+	+	+	–
8		+	–	–	–	–		–	–	–	–	–		d				
9		+	+	–	–	–		+	–	–	–	–		+	+	–	–	–
10		+	+	–	–	–		+	d									

[a] Aphids fed 24 hr on beet yellow stunt source plants.
[b] d indicates "aphid died".

reproduce on beet or lettuce. The green peach aphid, although capable of reproducing on beet and lettuce, is a relatively poor vector of the virus. For these reasons and other observational evidence, it appears that the disease is unlikely to reach serious proportions, except where large concentrations of sowthistle are found in neglected areas.

The Salinas Valley of California, which grows large acreages of lettuce every year, also uses selective herbicides on much of this acreage. Changes in the weed population of the area have been noticeable over the last several years, with a marked increase in weeds of the Compositae (James M. Thompson, Soil Serv. Inc., and Harry Agamalian, Farm Advisor, Monterey County, Calif., *personal communications*). Sowthistle has been especially abundant, and its occurrence is directly correlated with increased incidence of BYSV the last several years.

Serological tests.–Precipitin tests were conducted with juice clarified by centrifugation. Precipitin tests were positive with BYV antiserum against plants infected with this virus. No precipitate was formed with sap from healthy or BYSV-infected beet, lettuce, or sowthistle. Precipitin tests were positive with BYSV antiserum (prepared from beet) against sap from BYSV-infected lettuce and sowthistle. No precipitate was formed with sap from healthy beet, lettuce, or sowthistle, or from beet infected with BYV or BYSV.

Cross absorption tests were conducted with the BYV antiserum and the BYSV antiserum. Crude juice from BYV and BYSV-infected plants was incubated for 2 hr at 37 C (4 parts juice:1 part serum). The absorbed serum was centrifuged for 10 min at 4,000 *g*, then used in precipitin tests. Precipitin tests were positive with BYV antiserum absorbed with BYSV. No precipitate was formed when the BYV antiserum was absorbed with the BYV. No precipitate was formed with the BYSV antiserum in these tests.

DISCUSSION.–Early experimental evidence on host range and vector relationships easily separated BYSV from the other yellowing viruses of sugar beet. The virus is semipersistent in its aphid vectors, and is readily distinguished from beet pseudo yellows, malva yellows, and beet western yellows viruses on this basis. Retention of the virus by aphid vectors is of the same order as retention of BYV, but host reactions, especially in regard to beet, lettuce, and sowthistle differentiate the two viruses. The evidence of the occurrence of flexuous rods in leaves of sugar beet infected with BYSV has resulted in some confusion about the relationships between these two viruses. Purified preparations of BYSV have not yet been obtained, and proof of the identity of the flexuous rods described from BYSV-infected sugar beets as the infectious agent of BYS is lacking. The flexuous rods observed in thin sections of both diseases are remarkably similar, however.

Serological tests reported are of the negative type. Although a positive reaction may be accepted as proof of the presence of the beet yellows virus, a negative tests does not necessarily exclude it (8). Thus, it is not possible at this time to definitely establish that the two viruses are unrelated. However, the data indicates that, if they are related, the relationship is more distant than commonly excepted strains of the beet yellows virus.

Of the viruses in the aphid-transmitted semipersistent group that have been characterized as to particle shape, BYV, citrus tristeza virus, and BYSV appear to be long flexuous rods. The parsnip yellow fleck virus, which is transmitted as a semipersistent virus only when associated with a second "helper" virus (anthriscus yellows) (9), has isometric particles.

BYSV and BYV have several points in common. Both are transmitted in a semipersistent manner by the green peach aphid, and both apparently have long, flexuous rod-shaped particles. The viruses induce yellowing diseases of sugar beet and *C. capitatum*, a major indicator host of the BYV.

The diseases differ markedly, however, in host range reactions, especially in the Compositae. Sowthistle is a widespread, natural reservoir of the BYSV. There is good evidence that BYV can be effectively controlled by eliminating beets at any time during the year. The distribution of BYSV in wild *Sonchus* in California is so extensive that it seems unlikely that cropping changes, except those that affect the weed populations, would have any effect on the virus.

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