

# Beet Western Yellows Virus in Israel

SHLOMO MARCO, Volcani Center, Agricultural Research Organization, Bet Dagan, Israel 50 250

## ABSTRACT

Marco, S. 1984. Beet western yellows virus in Israel. *Plant Disease* 68:162-163.

Premature yellowing of sugar beet and lettuce has been noticed for many years in Israel. Many of these plants were found to be infected with beet western yellows virus (BWYV), based on aphid transmission to indicator hosts, serology (double-sandwich enzyme-linked immunosorbent assay [ELISA]), and immunosorbent electron microscopy (ISEM), where isometric particles resembling BWYV virions were observed. BWYV was also detected in turnip, radish, mangold, and in some widespread weeds.

Premature yellowing of sugar beet and lettuce, noticed in Israel for many years, has been attributed mainly to deficiency diseases. Attempts were made in 1977 to find a viral cause for yellowing of lettuce (S. Marco, *unpublished*) and sugar beet (M. Bar Joseph, *personal communication*). From both plants, an agent (probably a virus) could be transmitted by aphids to *Claytonia (Montia) perfoliata* that induced reddening of its older leaves, indicating the presence of beet western yellows virus (BWYV) (3). Recently, Duffus (4,5) demonstrated the possible involvement of BWYV with potato leaf roll disease. In Israel, a multiplication scheme for seed potatoes has been initiated. This prompted us to look for the occurrence of BWYV as a potential danger to the potato stock and eventually to other crops in Israel.

## MATERIALS AND METHODS

Aphid transmission tests were conducted according to Duffus (4,5). Detached leaves collected at random were placed on moistened filter paper in petri dishes. Aphids (*Myzus persicae* Sulzer) reared on virus-free Chinese cabbage were placed on the leaves for acquisition feedings of 24–48 hr, then transferred to test plants covered with screened plastic cups. After about 48 hr, the cups were removed and the plants were sprayed with pirimicarb (0.1% Pirimor 50WP) and held in an insect-proof greenhouse for symptom development. Test plants were ground cherry (*Physalis floridana* Rydb.), shepherd's purse (*Capsella bursa-pastoris* (L.) Medic.), red beet and sugar beet (*Beta vulgaris* L.), lettuce (*Lactuca sativa* L.), and *Crambe abyssinica* L. Additional hosts used occasionally were tomato

(*Lycopersicon esculentum* L.), *Datura stramonium* L., *Datura stramonium* L., *Petunia hybrida* Vilm., and various cultivars of potato (*Solanum tuberosum* L.). Five plants of each indicator were used per test. Positive infections were confirmed by serology.

Serological assays were made by double-sandwich enzyme-linked immunosorbent assay (ELISA) using Dynateck polystyrene microplates. The tests were conducted as described by Clark and Adams (2), except the plates were washed with tap water instead of wash buffer, plates coated with gamma-globulin G<sub>1</sub> (IgG) and those containing the plant samples were incubated overnight in a refrigerator, and the conjugated IgG was incubated about 4 hr at 38 C.

The antiserum used was described by Duffus (4,5) as ST-1 prepared against a BWYV strain from radish. This antiserum was cross-absorbed with a preparation from BWYV-free radish. Extracts from healthy radish leaves were clarified with 10% chloroform-butanol (1:1, v/v) and precipitated with 10% polyethylene glycol 6,000 (PEG). The precipitate was suspended in a mixture of 0.1 M NaHCO<sub>3</sub> buffer (pH 8.3) and 0.5 NaCl, coupled to cyanogen bromide-activated sepharose (Pharmacia, Uppsala), and packed on a column. The BWYV antiserum was run through this column according to the manufacturer's instructions, with PBS as the eluting buffer. This antiserum (adjusted to A<sub>405</sub> = 1.4) was used at a dilution of 1:1,000 for coating microplates and 1:750 as enzyme conjugate. The plant samples were diluted 1:5 in PBS-Tween with no additives.

For the immunosorbent electron microscopy (ISEM) tests, leaves of *P. floridana* suspected of being BWYV-infected were frozen by means of liquid nitrogen, and the virus was partially purified from extracts as described previously. PEG precipitate was resuspended in 0.1 M (pH 7.0) phosphate buffer and concentrated by one cycle of differential centrifugations. Formvar- and carbon-coated grids were floated on

drops of BWYV antiserum at various concentrations overnight at room temperature. The grids were washed with phosphate buffer and floated again for about 4 hr on virus preparation or on similar preparations from virus-free *Physalis*. The grids were stained with 2% uranyl acetate and examined with a JEM 7A electron microscope operating at 100 kV.

## RESULTS AND DISCUSSION

Using aphids and/or ELISA, BWYV was detected in the following plants: red beet (8/28), mangold (*Beta vulgaris* var. *circla* (L.) Aell) (3/12), lettuce (3/18), turnip (4/8), radish (11/18), shepherd's purse (18/31), *Brassica kaber* var. *pinnatifida* (Stokes) L. C. Wheeler (4/28), *Raphanus raphanistrum* L. (8/25), and *Senecio vulgaris* L. (1/12) (numerator = number of plants infected, denominator = number of plants tested).

BWYV was found in samples from the Coastal Plain, Northern Negev, and Jordan Valley but not from the Golan Heights—the area where seed potatoes are grown.

*C. abyssinica* appeared to be the best indicator. BWYV infection induced obvious reddening of the older and middle leaves. This agrees with the results of Ashby et al (1). Results with shepherd's purse were less obvious; in many cases, plants were infected (as determined by ELISA) but did not express clear symptoms. Differences in symptoms also were related to different sources of shepherd's purse seeds. Thus, BWYV-infected plants from seeds collected in Canada mainly expressed reddening of older leaves, whereas some *C. bursa-pastoris* from Israel expressed mild chlorosis and undulation of leaves after infection with the same BWYV source. Red beet and sugar beet expressed very mild symptoms that appeared after 6–8 wk only in older leaves. *P. floridana* expressed symptoms earlier, but in most cases, they were much milder than those induced by potato leaf roll virus in this host. In lettuce, infection was mostly symptomless, and only in very old plants was some interveinal chlorosis of lower leaves perceptible. Isolates of BWYV from Israel were not able to infect potato, *D. stramonium*, *D. stramonium* L., tomato (*P. hybrida*), or pepper (*Capsicum annuum* L. var. Maor). Double-sandwich ELISA indicated a higher rate of infection than symptoms did. Only in *C. abyssinica* was there a high correlation between symptoms and positive serological reaction. Isometric particles

Accepted for publication 25 September 1983.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

©1984 The American Phytopathological Society

resembling BWYV virions (6) were observed in extracts from infected *P. floridana*. Similar particles were not found on the control grids.

The present work demonstrated for the first time the occurrence of BWYV in Israel, and our results show that the virus seems to be widespread in several cultivated crops and weeds.

#### ACKNOWLEDGMENTS

I wish to thank J. E. Duffus, USDA, ARS, Salinas,

CA, for a gift of BWYV antiserum; M. Bar Joseph, ARO, Volcani Center, Bet Dagan, Israel, for information on his work; and Sara Hen and Natasha Pisarev for technical assistance.

#### LITERATURE CITED

1. Ashby, J. W., Bos, L., and Huijberts, N. 1979. Yellows of lettuce and some other vegetable crops in the Netherlands caused by beet western yellows virus. *Neth. J. Plant Pathol.* 85:99-111.
2. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* 34:475-483.
3. Duffus, J. E. 1964. Host relationships of beet western yellows virus strains. *Phytopathology* 54:736-738.
4. Duffus, J. E. 1981. Beet western yellows virus—A major component of some potato leaf roll-affected plants. *Phytopathology* 71:193-196.
5. Duffus, J. E. 1981. Distribution of beet western yellows virus in potatoes affected by potato leaf roll. *Plant Dis.* 65:819-820.
6. Smith, K. M. 1972. *A Textbook of Plant Virus Diseases*. 3rd ed. Longman Group Ltd. 684 pp.