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Broad bean wilt virus: host range, purification, serology, transmission characteristics, and occurrence in faba bean in West Asia and North Africa

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Abstract

A virus affecting faba bean in West Asia and Norht Africa was identified as broad bean wilt virus (BBWV) by host reactions, particle morphology and size, serology and transmission characteristics. An isolate from Syria (SV3-88) and one from Egypt (EV319-86) were found to be serologically identical and of serotype I. In host-range studies, the Syrian isolate infected systemically 59 out of 87 plant species tested. The virus was transmitted non-persistently by four aphid species naturally prevalent in Syria, but most efficiently by *Myzus persicae*. Inoculation of faba bean with SV3-88 14 weeks (pre-flowering) and 6 weeks after sowing (flowering) led to 25.8 and 1.8% yield loss and seed-transmission rates of 0.6 and 0.4%, respectively. The isolate SV3-88 was purified from systemically infected faba bean and yielded 1.5-2 mg of partially purified virus per 100 g of leaves. When samples, with symptoms suggestive of virus infection, were collected during 1985-1989 from a number of countries in West Asia and North Africa and tested by ELISA, the virus was detected in 8 out of 127 samples tested (8/127) from Egypt, 0/44 from Lebanon, 1/23 from Morocco, 38/485 from the Sudan, 38/385 from Syria and 23/138 from Tunisia.

Additional keywords: seed transmission; seed-borne virus; serotype; yield-loss assessment.

Introduction

Faba bean (*Vicia faba* L.) is an important and widely cultivated field crop in West Asia and North Africa (WANA) where it constitutes a cheap source of protein for a large proportion of the population. However the crop is affected by several viruses. In WANA, a field survey for faba bean viruses conducted in 1985-1987 revealed the presence of nine viruses, including broad bean wilt virus (Makkouk et al., 1988).

Broad bean wilt virus (Fabavirus group) was first isolated from broad bean (*Vicia faba* L.) in Victoria, Australia (Stubbs, 1947), and has since been reported from *V. faba* in Europe (Smith, 1949, 1950; Putz and Kuszala, 1973; Russo et al., 1973; Milicic et al., 1976; Schmidt et al., 1977), Asia (Inouye, 1969; Yamamoto and Ohata, 1977; Parvin and Izadpanah, 1978; Yu, 1979; Makkouk et al., 1988; Xu et al., 1988) and North Africa (Eid and Tolba, 1979; Fischer, 1979). The virus can be very damaging on faba bean (Gamal Eldin et al., 1982).

The virus is transmissible by sap inoculation and by several aphid species in the non-persistent manner (Stubbs, 1960) but has so far not been reported to be seed-borne.

This paper describes some of the properties of the BBWV isolate collected from a faba bean field in Lattakia, Syria in 1988, prevalence in a number of countries in West Asia and North Africa and effect on faba bean and some other crops important in the region, and possible seed-transmission in faba bean.

Materials and methods

Field collections and virus isolates. Samples of faba bean leaves with symptoms suggestive of virus infection were collected from farmers' fields and from experimental plots of agricultural research stations in six Arab countries during 1985-1989. Samples were brought to the laboratory at Tel-Hadya, Aleppo, Syria, and each sample was split into two portions; one was desiccated over calcium chloride for later electron microscopy and for virus preservation. The other portion was extracted in 0.2 M phosphate buffer, pH 6.0 using a motorized tissue extractor, and used for testing for the presence of BBWV by ELISA. The antiserum to a BBWV isolate from pea (E229) used in the test was produced at IPO, Wageningen, the Netherlands.

Two BBWV isolates, one from Egypt (EV319-86) and one from Syria (SV3-88), were used for detailed studies. Another isolate PV132 (Uyemoto and Provvidenti, 1974) was used for comparative serology. The three isolates were maintained in faba bean 'Syrian Local'.

Host-range studies. Leaves of faba bean infected with SV3-88 were ground in a mortar and pestle with 0.01 M phosphate buffer, pH 7.2, mixed with celite, and inoculated mechanically to 87 plant species belonging to 11 families. Temperature in the glasshouse ranged from 20 to 30 °C. Four weeks after inoculation, non-inoculated leaves were harvested from all tested plants and assayed for the virus by ELISA.

Aphid-transmission tests. *Aphis craccivora* and *Aphis fabae* were originally collected from faba bean, *Acyrthosiphon pisum* from pea and *Myzus persicae* from cabbage. These four species were collected from fields near Lattakia, Syria, and reared in screen cages in a glasshouse. In transmission tests the adult insects were fasted for 1 h and then placed in petri-dishes for acquisition feeding on BBWV-infected (SV3-88) faba bean leaves for 4-5 min. The aphids were then transferred to plants of faba bean 'Syrian Local' (five aphids each) for test feeding for 24 h. Plants were thereafter sprayed with 0.5 g/l Pirimor to kill aphids.

Yield-loss assessment and seed-transmission tests. A field experiment was conducted during the growing season 1988-1989 at Tel Hadya, Aleppo, Syria. Eighty faba bean plants were grown in 1.2 × 3.5 m plots with four replicates in a randomized complete block design. Plants were mechanically inoculated with SV3-88 14 weeks (pre-flowering) and 16 weeks after sowing (flowering), respectively. Healthy plots were inoculated with buffer containing celite. After maturation, dry seeds were collected and weighed. The dry seeds were planted in sterile sand in the glasshouse. The resulting seedlings were tested in groups of 10 for BBWV by ELISA. In these tests, each ELISA plate included eight wells with extracts from healthy seedlings. Sample absorbance values (A_{405})

higher than the mean healthy value plus three standard deviations were considered positive.

Virus purification. The purification procedure followed was that of Xu et al. (1988) with minor modifications. The virus was purified from SV3-88-infected faba bean harvested 10-15 days after inoculation. 2% polyvinyl pyrrolidone was added to the extraction buffer and the Clelands reagent in the pellets resuspension buffer was replaced by 0.01 M sodium sulfite.

Electron microscopy. For electron microscopy small pieces of EV319-86-containing leaf, either fresh or dried, were chopped in a few drops of 2% sodium phosphotungstate (PTA), pH 6.5, for negative staining and viewing in the electron microscope. Immunospecific electron microscopy (ISEM) decoration method was conducted by Dr. D.-E. Lesemann, Braunschweig, FRG, on SV3-88 and EV319-86. Antisera used in ISEM for BBWV serotype I and serotype II were those of Drs J.K. Uyemoto and R. Provvidenti, Geneva, New York, USA.

Antiserum production and serology. Five injections of 0.5 mg purified virus (SV3-88) each were administered to a rabbit at weekly intervals. Purified material (middle and bottom components) was emulsified with an equal volume of Freund's complete adjuvant for the first injection and with Freund's incomplete adjuvant for the four subsequent injections. Antiserum was collected eight weeks after the first injection, and weekly thereafter.

Gammaglobulins were fractionated from the antiserum using the caprylic acid method (Steinbuch and Audran, 1969) and the conjugation of gammaglobulin with alkaline phosphatase was according to Clark and Adams (1977). Plates were coated with 5 μg/ml of gammaglobulins and the conjugate dilution used was 1/1000. Absorbance at 405 nm was measured in a Titertek Multiskan MC photometer, 2 h after addition of the substrate.

The gel-diffusion plates were prepared by dissolving 0.9% Noble agar in 0.05 M Tris-HCl buffer, pH 7.2 containing 0.85% sodium chloride (NaCl) and 0.02% sodium azide. The pattern in the agar consisted of six peripheral wells and a central well. BBWV antiserum was placed in the central well and BBWV isolates SV3-88, EV319-88 and PV132 in crude plant extracts were added in the peripheral wells. The antisera AS33 (Type I) and AS45 (Type II) for the gel-diffusion test were from J.K. Uyemoto and R. Provvidenti.

Results

Host range and symptoms. On faba bean 'Syrian Local', the isolate SV3-88 produced on young leaves, two weeks after inoculation, distinct vein clearing of the main and secondary veins, which developed 2-3 weeks later into severe systemic mottle. Leaf size was slightly reduced, but plant height was little affected. On chickpea 'Syrian Local Large', a mild chlorotic mottle developed on the young leaves followed by wilting of the growing point. Plants were significantly stunted when compared with healthy controls.

Responses of the different plant species to inoculation with SV3-88 are summarized in five categories:

(*i*) Local reaction on inoculated leaves followed by systemic symptoms: *Chenopodium album*, *C. amaranticolor*, *C. quinoa*, *Lathyrus annuus*, *L. aphaca*, *L. cicera*, *L. gorgnoi*, *L. marmoratus*, *L. ochrus*, *Nicotiana benthamiana*, *Phaseolus vulgaris* 'Imuna', *Vigna unguiculata* 'California Blackeye No. 5'.
(*ii*) Local reaction without systemic invasion: *Cucumis melo* 'Syrian Local', *Cucumis sativus* 'Chicago Pickling' and 'Syrian Local', *Cucurbita pepo* 'Buttercup' and 'Syrian Local', *Glycine max* 'Altona', 'Bragg' and 'Davis', *Phaseolus vulgaris* 'Redlands Greenleaf B' and 'Redlands Greenleaf C'.
(*iii*) Systemic symptoms without local reaction:
(a) Non-legume species: *Antirrhinum majus*, *Gomphrena globosa*, *Ocimum basilicum* 'Yugoslavia', *Solanum melongena*.
(b) Food-legume species: *Cicer arietinum* land races ILC 6383, ILC 6405, ILC 6410 and Syrian Local, *Lens culinaris* land races ILL 6497, ILL 6529, ILL 6532, ILL 6538 and Syrian Local, *Lupinus albus*, *Pisum sativum* 'Koroza', 'Rondo' and 'Syrian Local', *Vicia faba* 'Kompakta' and 'Syrian Local'.
(c) Forage and wild-legume species: *Cicer pinnatifidum*, *C. reticulatum*, *Lathyrus sativus*, *Lens ervoides*, *L. nigricans*, *L. orientalis*, *Medicago aculeata*, *M. blancheana*, *M. constricta*, *M. orbicularis*, *M. radiata*, *M. rotata*, *M rigidula*, *M. sativa*, *M. truncatula*, *Pisum arvense*, *P. fulvum*, *P. sativum* ssp. *abyssinicum*, *P. sativum* ssp. *arvense*, *P. sativum* ssp. *elateus*, *P. sativum* ssp. *humile*, *P. sativum* ssp. *sativum*, *Scorpiurus muricatus*, *Trifolium pratense*, *Trigonella arabica*, *Vicia benghalensis*, *V. ervilia*, *V. hyaeniscyamus*, *V. hybrida*, *V. johannis*, *V. lutea* var. *hirta*, *V. mollis*, *V. monantha*, *V. narbonensis*, *V. pannonica*, *V. peregrina*, *V. sativa* ssp. *nigra*, *V. villosa*.
(*iv*) Symptomless infection: *Phaseolus vulgaris* 'Amanda'.
(*v*) No infection:
(a) Non-legume species: *Beta vulgaris*, *Beta vulgaris* ssp. *cicla*, *Brassica oleracea* var. *botrytis*, *Brassica oleracea* var. *capitata*, *Carthamus* sp., *Datura stramonium*, *Daucus carota* var. *sativa*, *Helianthus annuus*, *Lactuca sativa* 'Syrian Local', *Lycopersicon esculentum* 'Marglobe S-16' and 'Syrian Local', *Ocimum basilicum* 'Japan', *Raphanus sativus*, *Solanum nigrum*, *Spinacia oleracea* 'Bloom Long Standing' and 'Syrian Local', *Tetragonia expansa*.
(b) Food-legume species: *Cicer arietinum* 'ILC 6437', *Phaseolus vulgaris* cvs Black Turtle Soup, Bountiful, Canadian Wonder, Dubbele Witte, Great Northern 31, Great Northern 1140, Jubila, Michelite, Monroe, Pinto 14, Prelude, Red Mexican 34, Red Mexican 35, Sanilac, Stringless Green Refugee, Sutter Pink, Topcrop, Widusa, *Vigna unguiculata* 'Syrian Local'.
(c) Forage and wild-legumes species: *Cicer judaicum*, *Lathyrus inconspicuus*, *Medicago littoralis* var. *inermis*, *M. polymorpha*, *M. polymorpha* var. *brevispina*, *Trigonella monospeliaca*, *Vicia bithynica*, *V. palaestina*, *V. sativa*, *V. sativa* ssp. *sativa*.

Aphid transmission. BBWV symptoms appeared on faba bean plants 10-15 days after aphid inoculation. Results were recorded based on characteristic symptoms produced and were confirmed by serology (ELISA) when necessary. Results obtained indicated that 20 plants became infected out of 25 inoculated (20/25) with *Myzus persicae*, 29/72 with *Acyrthosiphum pisum*, 24/78 with *Aphis craccivora* and 4/59 with *A. fabae*. Accordingly, efficiency of transmission expressed as a percent of successful transmission was 80.0, 40.3, 30.8 and 6.8% for the above aphid species, respectively.

Yield-loss evaluation. In experimental plots (1.2 × 3.5 m) yield of faba bean 'Syrian Local' inoculated with BBWV at 14 weeks (pre-flowering) and 16 weeks after sowing (flowering) was 190 and 252 g per plot (average of four replications), and induced 25.8 and 1.8% yield loss, respectievely, when compared with the healthy control (256 g).

Seed-transmission. When embryo axes of 990 and 1060 germinated seeds from plants inoculated with BBWV at the stage of pre-flowering and flowering, respectively, were tested in groups of 10 by ELISA, virus was detected in 6 out of 99 groups and 4 out of 106 groups of seeds obtained from plants inoculated early and late, respectively.

Using the formula $P = [1 - (H/N)^{1/n} \times 100$ where: P = percentage of infection, H = number of groups free of virus, N = number of groups tested, n = number of seeds per group, seed transmission rates were 0.6% for early inoculation and 0.4% for late inoculation. Extracts from some embryo axes found to be BBWV-positive by ELISA were also found infectious on faba bean, and the infectious agent was BBWV.

Virus purification. Faba bean was an adequate source for BBWV purification. Inoculated plants of faba bean were tested by ELISA 4, 6, 9, 12, 14 and 16 days after inoculation. Highest virus concentration was detected 12 days after inoculation as measured by ELISA. Thereafter, virus concentration declined. Accordingly, infected tissue for virus purification was harvested 12-14 days after inoculation.

Following the procedure described above, three well defined opalescent bands were obtained in the sucrose gradient tube (Fig. 1). Presence of the virus in the three bands was confirmed by ELISA. Virus was recovered from the sucrose bands by high-speed

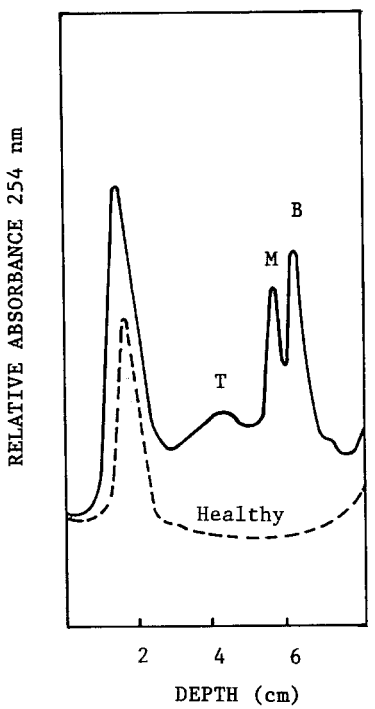

Fig. 1 Rate-zonal sucrose density gradient centrifugation analysis of purified broad bean wilt virus. Preparations from faba bean infected and healthy tissue were centrifuged for 2 h at 38 000 rpm in a 10-40% sucrose gradient in a Beckman SW 41 rotor. Tubes were scanned through an ISCO density gradient fractionator with a UV monitor.

centrifugation. Virus pellets of the different bands resuspended in 0.01 M phosphate buffer, pH 7.5, had a UV 260/280 absorbance ratio of 1.3 (top), 1.5 (middle) and 1.6 (bottom). Values were not corrected for light scattering. Assuming that the extinction coefficient for BBWV is 8.0 (Xu et al., 1988), the yield of purified virus was calculated to be 15-20 mg/kg infected tissue.

Electron microscopy. Faba bean tissue infected with isolates EV319-86 and SV3-88 contained isometric virus particles ca. 25 nm in diameter typical of BBWV. They were detected in field samples desiccated over calcium chloride and in faba bean plants inoculated with the virus and maintained in the glasshouse. In ISEM (decoration) tests both isolates reacted strongly with antiserum to serotype I of BBWV and weakly with antiserum to serotype II.

Serology. With AS33 antiserum (serotype I) a single precipitin line was produced in gel-diffusion tests against isolates SV3-88 and EV319-88 in sap from inoculated plants of faba bean. It fused without spur formation with the precipitin line of the antigen PV132 (BBWV, serotype I) with its homologous antiserum. No reaction in gel diffusion was obtained between either isolate and antiserum AS45 to BBWV serotype II. In ELISA, the antiserum to SV3-88 produced in this study gave a reading of A_{405} = 0.43 with tissue infected with SV3-88 and 0.18 for healthy tissue as compared to the Dutch E299 antiserum which gave A405 = 0.54 with infected faba bean and 0.10 for healthy tissue.

Field survey. A survey of faba bean viruses in West Asia and North Africa was made during the last five years. Testing 1201 faba bean samples with virus-like symptoms collected from different locations in Egypt, Lebanon, Morocco, Sudan, Syria and Tunisia, BBWV was detected in ELISA with antiserum to E229 in 6.3, 0.0, 4.3, 7.8, 9.9 and 16.8% of the samples tested from the above countries, respectively. The figures were 8/127 (8 out of 127 samples tested) from Egypt, 0/44 from Lebanon, 1/23 from Morocco, 38/485 from Sudan, 38/385 from Syria and 23/137 from Tunisia. The number of samples tested from Lebanon and Morocco was small; further sampling and testing is required for more reliable reporting on the relative occurrence of BBWV in these countries. Other viruses were also detected in these samples but were reported previously (Makkouk et al., 1988).

Discussion

The morphological, serological and host-range properties of the isolates studied indicated a close resemblance to BBWV serotype I described earlier (Stubbs, 1947; Uyemoto and Provvidenti, 1974). Serotype I of BBWV has been reported previously on faba bean in Jordan (Al-Musa and Mansour, 1984).

In the experimental host-range studies the reactions obtained in many species were similar to those reported earlier (Stubbs, 1947; Kim and Hagedorn, 1959; Schmelzer, 1960; Frowd and Tomlinson 1970; Boccardo and Conti, 1973; Russo and Rana, 1978; Vega et al., 1980; Provvidenti, 1983). However, an additional 40 leguminous species are now reported as susceptible and eight as non-susceptible to BBWV.

Spinacia oleracea was reported to be susceptible to BBWV infection (Stubbs, 1947;

Yamamoto and Ohata, 1977; Lockhart and Betzold, 1982), whereas in this study the cvs Bloom Long Standing and Syrian Local were found to be immune. BBWV was reported to infect *Lactuca sativa* (Schmelzer, 1974), *Cucumis sativus* (Russo et al., 1973), *Cucurbita pepo* (Rosciglione and Connizzaro, 1977), *Datura stramonium* (Vega et al., 1980; Lockhart and Betzold, 1982), *Solanum nigrum* (Schmelzer, 1960), *Lycopersicon esculentum* and *Vicia sativa* (Stubbs, 1947), *Vicia bithynica* (Schmelzer and Stahl, 1977), whereas the cultivars of these species tested in this study were found not systemically susceptible to infection.

Similar to the dogwood (*Cornus florida*) strain (BBWV-DW) (Scott and Barnett, 1984), isolate SV3-88 did not produce local lesions on *Datura stramonium*, a useful local-lesion and assay host to most BBWV strains (Taylor and Stubbs, 1972). However, BBWV-DW is serologically closer to serotype II, whereas isolate SV3-88 is closer to serotype I.

When reviewing the host reactions reported for the BBWV serotypes I and II, it is not easy to correlate host reactions with serotyping. In fact, there is no set of hosts that can sharply differentiate the two serotypes. *Lactuca sativa*, *Lycopersicon esculentum* and *Beta vulgaris* were reported not to be infected by isolates of serotype I, as was the case with the Syrian isolate, but could produce local lesions after inoculation with isolates of serotype II (Vega et al., 1980; Lockhart and Betzold, 1982; Scott and Barnett, 1984; Xu et al., 1988). But the variation in host reactions reported for isolates of the same serotype further prevents conclusive statements.

The majority of the chickpea cultivars (*Cicer arietinum*) tested were found to be sensitive to BBWV infection as reported earlier (Kim and Hagedorn, 1959) except ILC 6437, which was apparently resistant.

Myzus persicae has been reported to be the most efficient vector of BBWV (Stubbs, 1960; Vega et al., 1980; Lockhart and Betzold, 1982) which is in agreement with the results obtained in this study. Yield losses of faba bean induced by BBWV infection reported here were less than those reported in Egypt (Gamal-Edlin et al., 1982), but this may be due to the differences in environmental conditions and cultivars used.

Seed transmission of BBWV was not mentioned by Taylor and Stubbs (1972) and was mentioned as an observation without experimentation to be low in faba bean by Putz and Kuszala (1973). Results obtained in this work proved seed-transmission in faba bean at a rate of 0.4-0.6%. BBWV seed-transmission in different faba bean cultivars, and its possible occurrence in other leguminous crops, requires further investigation.

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Samenvatting

Tuinboneverwelkingsvirus: waardplantenreeks, zuivering, serologie, overbrenging, en vóórkomen in veldboon in West Azië en Noord-Afrika

Een virus uit veldboon of faba boon (*Vicia faba*) in West Azië en Noord-Afrika werd als tuinboneverwelkingsvirus (Fabavirus-groep) herkend aan zijn waardplantreacties, deeltjesvorm en grootte, serologie en wijze van overdracht. Een isolaat uit Syrië (SV3-88) en één uit Egypte (EV319-86) bleken serologisch identiek te zijn en te behoren tot serotype I van het virus. Met het Syrische isolaat kon in 59 van de 87 getoetste plantesoorten systemische infectie worden verkregen. Met vier veel in Syrië voorkomende bladluissoorten kon het virus worden overgebracht, maar met *Myzus persicae* naar de meeste plantesoorten. Inoculatie van veldboon met SV3-88 vóór de bloei (14 weken na het zaaien) en tijdens de bloei (16 weken na het zaaien) gaf aanleiding tot respectievelijk 25,8 en 1,8% opbrengstreductie en tot 0,6 en 0,4% zaadoverdracht. Bij zuivering van isolaat SV3-88 uit systemisch geïnfecteerde fababoon was de opbrengst tot 1,5 à 2 mg gedeeltelijk gezuiverd virus per 100 g blad. Bij ELISA-toetsing in 1985-1989 van een groot aantal monsters afkomstig uit een aantal landen in West-Azië en Noord-Afrika werd het virus aangetoond in 8 van de 127 (8/127) monsters uit Egypte, 0/44 uit Libanon, 1/23 uit Marokko, 38/485 uit Soedan, 38/385 uit Syrië en 23/138 uit Tunesië.

References

Al-Musa, A.M. & Mansour, A.N., 1984. Broad bean wilt virus on broad bean in Jordan. Plant Disease 68: 537.

Boccardo, G. & Conti, M., 1973. Purification and properties of nasturtium ringspot virus. Phytopathologische Zeitschrift 78: 12-24.

Clark, M.F. & Adams, A.N., 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34: 475-483.

Clark, M.F. & Lister, R.M., 1971. The application of polyethylene glycol solubility-concentration gradients in plant virus research. Virology 43: 338-351.

Eid, S.A. & Tolba, M.A., 1979. Studies of some viruses isolated from broad bean (*Vicia faba*) in Egypt. FABIS, No. 1, p. 26.

Fischer, H.U., 1979. Agents viraux isolés des cultures de Fève. Leur détermination et differenciation. Al Awamia 57: 41-72.

Frowd, J.A. & Tomlinson, J.A., 1970. Nasturtium ringspot virus in parsley crops in Britain. Plant Disease Reporter 54: 734-735.

Frowd, J.A. & Tomlinson, J.A., 1972. Relationships between a parsley virus, nasturtium ringspot virus and broad bean wilt virus. Annals of Applied Biology 72: 189-195.

Gamal-Edlin, A.S., El Amrety, A.A., Mazyad, H.M. & Rizkallah, L.R., 1982. Effect of bean yellow mosaic and broad bean wilt viruses on broad bean yield. Agricultural Research Review (Egypt) 60: 195-204.

Inouye, T., 1969. The legume viruses of Japan. Review of Plant Protection Research 2: 42-51.

Kim, W.S. & Hagedorn, D.J., 1959. Streak-inciting viruses of canning pea. Phytopathology 49: 656-664.

Lockhart, B.E.L. & Betzold, J.A., 1982. Broad bean wilt virus in begonia in Minnesota. Plant Disease 66: 72-73.

Makkouk, K.M., Bos, L., Azzam, O.I., Kumari, S. & Rizkallah, A., 1988. Survey of viruses af-

fecting faba bean in six Arab countries. Arab Journal of Plant protection 6: 53-61.
Milicic, D., Juretic, N., Plese, N. & Wrischer, M., 1976. Some data on cell inclusions and natural hosts of broad bean wilt virus. Acta Botanica Croatica 35: 17-24.
Parvin, S. & Izadpanah, K., 1978. Broad bean wilt virus – identification, host range and distribution in the Fars province of Iran. Iranian Journal of Agricultural Research 6: 81-90.
Provvidenti, R., 1983. Reaction of bean cultivars to broad bean wilt virus. Annual Report of the Bean Improvement Cooperative (USA) No. 26: 69-90.
Putz, C. & Kuszala, M., 1973. Deux nouveaux virus attaquant la féverole (*Vicia faba* L.) en France. I. Essais d'identification et d'évaluation de leur importance économique. Annales de Phytopathologie 5: 447-460.
Rosciglione, B. & Cannizzaro, G., 1977. Reperimento del virus dell' avvizzimento della Fava nell' isola di linosa, Phytopathologia mediterranea 16: 140-142.
Russo, M., Martelli, G.P. & Vovlas, C., 1973. Le virosi delle piante ortensi in Puglia. XIV. Il virus dell' avvizzimento della fafa su fava Peperone. Phytopathologia Mediterranea 12: 61-66.
Russo, M. & Rana, G.L., 1978. Occurrence of two legume viruses in artichoke. Phytopathologia Mediterranea 17: 212-216.
Schmelzer, K., 1960. Untersuchungen über das Ringmosaik-Virus der Kapuzinerkresse. Zeitschrift für Pflanzenkrankheiten (Pflanzenpathologie) und Pflanzenschutz 67: 193-210.
Schmelzer, K., 1974. Untersuchungen an Viren der Zier- und Wildgeholze.8.Mitteilung: Neue Befunde an *Forsythia, Hydrangea und Philadelphus* sowie Viren und Virosen an *Rhamnus, Centaurea, Galvezia, Cistus, Forestiera, Abeliophyllum, Celastrus, Staphylea* und *Crambe.* Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene II, 129: 139-169.
Schmelzer, K. & Stahl, P., 1977. Spontane Wirtspflanzen des Ackerbohnenwelke-Virus. 2. Mitteilung. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene II, 132: 123-134.
Schmidt, H.E., Dubnik, H., Karl, E., Schmidt, H.B. & Kamann, H., 1977. Verminderung von Virusinfektionen der Ackerbohne (*Vicia faba* L.) im Rahmen der Blattlausbekämpfung auf Grossflächen. Nachrichtenblatt für Pflanzenschutz DDR 31: 247-250.
Scott, S.W. & Barnett, O.W., 1984. Some properties of an isolate of broad bean wilt virus from dogwood (*Cornus florida*). Plant Disease 68: 983-985.
Smith, K.M., 1949. Viruses and virus diseases. Masters Memorial Lectures, 1949. Journal of the Royal Horticultural Society 74: 521-528.
Smith, K.M., 1950. Some new virus diseases of ornamental plants. Journal of the Royal Horticultural Society 75: 350-353.
Steinbuch, M. & Audran, R., 1969. The isolation of IgG from mammalian sera with the aid of caprylic acid. Archives for Biochemistry and Biophysics 134: 279-284.
Stubbs, L.L., 1947. A destructive vascular wilt virus disease of broad bean (*Vicia faba* L.) in Victoria. Journal of the Department of Agriculture, Victoria 46: 323-332.
Stubbs, L.L., 1960. Aphid transmission of broad bean wilt virus and comparative transmission efficiency of three vector species. Australian Journal of Agricultural Research 11: 734-741.
Taylor, R.H. & Stubbs, L.L., 1972. Broad bean wilt virus. CMI/AAB Descriptions of Plant Viruses, No. 81: 4 pp.
Taylor, R.H., Smith, P.R., Reinganum, C. & Gibbs, A.J., 1968. Purification and properties of broad bean wilt virus. Australian Journal of Biological Sciences 21: 929-935.
Vega, J., Gracia, O. & Feldman, J.M., 1980. Characteristics of argentine broad bean wilt virus: cytopathological aspects. Phtopathologische Zetschrift 99: 242-250.
Uyemoto, J.K. & Provvidenti, R., 1974. Isolation and identification of two serotypes of broad bean wilt virus. Phytopathology 64: 1547-1548.
Xu, Z.G., Cockbain, A.J., Woods, R.D. & Govier, D.A., 1988. The serological relationships and some other properties of isolates of broad bean wilt virus from faba bean and pea in China.

Annals of Applied Biology 113: 287-296.
Yamamoto, T. & Ohata, K., 1977. Purification and electron microscopy of broad bean wilt virus. Bulletin of the Shikoku Agricultural Experimental Station No. 30: 115-127.
Yu, T.F., 1979. *Vicia faba* diseases. Scientific Press. Peking, 168 pp (in Chinese).