

The Occurrence of Beet Mild Yellowing Virus in Czechoslovakia

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Abstract. The occurrence of beet mild yellowing virus (BMV) on feeding- and sugar-beet in Czechoslovakia has been proved. The virus was transmitted by aphid *Myzus persicae* (SULZ.) on indicator plants *Sinapis alba* L., *Capsella bursa-pastoris* MEDIK. and *Claytonia perfoliata* DONN. and from these plants back to sugar-beet cv. 'Dobrovická A.' A weed plant *Raphanus raphanistrum* L. was identified as a new natural host plant of BMV. The virus was identified in ten of twelve biologically examined samples of beet with BMV-like symptoms, which were collected at various places in Czechoslovakia.

Additional index words: beet yellows viruses; identification; indicator and host plants.

Beet mild yellowing virus (BMV) in England was found different from the beet yellows virus (BYV) by RUSSELL (1958, 1960), who identified it as a separate virus which is not related to BYV. Shortly afterwards BMV was differentiated from BYV in Sweden (BJÖRLING 1961) and its occurrence was reported in some other West-European countries (TAHON 1962) including the F.R.G. (THIELEMAN 1965) and in the G.D.R. (WIESNER 1967). Recently it has also been identified in Poland (KUBACKA 1969). There are no reports on its occurrence in other East-European countries. PRABKHA (1969) did not find it in 44 samples of yellowing leaves of beet from different regions of the U.S.S.R.

BMV is transmitted persistently by aphid *Myzus persicae* (SULZ.) but not by aphid *Aphis fabae* (SCOP.) (RUSSELL 1962, BJÖRLING and NILSSON 1966). The minimum acquisition feeding period of the virus by *M. persicae* (SULZ.) is 2 h. The maximum efficiency of transmission is, however, after 48 h of acquisition feeding. When the acquisition lasted long enough the virus can be transmitted by inoculation suction lasting less than half an hour. The latent period of BMV in the vector is at least 24 h. From this it follows that the total minimum period of time required for transmission of the virus by *M. persicae* (SULZ.) is from 24 to 32 h. The virus probably does not multiply itself in the insect. Larvae of the aphids are able to transmit BMV even after moulting. BMV is not transmitted from the vector to a viviparitive progeny.

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Host-range of the virus was described by RUSSELL (1965a) and by BJÖRLING and NILSSON (1966). RUSSELL (1963, 1965a) recommended for the differentiation of BYV and BMVY plant species *Capsella bursa-pastoris* MEDIK. which is sensitive to BMVY but not to BYV, *Chenopodium foliosum* (MOENCH) ASCH. sensitive to BYV but not to BMVY and *Claytonia perfoliata* DONN. sensitive to both viruses. BJÖRLING and NILSSON (1966) found that *C. bursa-pastoris* MEDIK. is also sensitive to BYV but the symptoms are not conspicuous. According to these authors *C. foliosum* (MOENCH) ASCH. and *Tetragonia expansa* MURR. are also sensitive to BMVY. With respect to the very typical symptoms they do not recommend any changes in differential host plants for both viruses.

Most authors (RUSSELL 1963, THIELEMANN 1965, NAGI and THIELEMANN 1970, WIESNER 1967, KUBACKA 1969) reported that BMVY prevails under field conditions over BYV. On the other hand KERSHAW (1965) found differences in the occurrence of both viruses in England in various years. He explains the higher expansion of BYV in 1963 by the increased occurrence of aphid *A. fabae* (SCOP.) on beet. According to RUSSELL (1965b) hibernating weeds, hosts of BMVY, such as *C. bursa-pastoris* MEDIK., *Senecio vulgaris* L. and *Stellaria media* CYR. have an important role in spreading of BMVY.

Other properties of BMVY are not known but increasing attention is paid to the determination of its characters and to its relation to other viruses. HARTLEB (1974) prepared concentrated extracts from beet plants infected with BMVY and succeeded to transfer the virus by aphid *M. persicae* (SULZ.) which sucked these extracts through an artificial membrane. The concentration of the virus in beet plants is very low and its suction in a sufficient amount by aphids through an artificial membrane is not yet possible without previous concentration of the extract. At present he studies the host plants of the virus and tries to separate and to purify the virus from plants containing it in high concentration and to prepare an antiserum against BMVY. According to recent information (THIELEMANN and NAGI 1973), it seems to be necessary to re-examine the disability of *A. fabae* (SCOP.) to transmit the BMVY. DUFFUS (1973) in his review assumes that the BMVY is a strain of the beet western yellows virus (BWYV) group. He also classifies the turnip yellows virus (TYV) and the malva yellows virus (MYV) as the members of this group. He proves their relations by antiserum against BWYV using test of neutralization of infectibility. With respect to the low virus concentration in plants and to the failure to obtain at least partially purified virus preparation, this evidence seems to us very uncertain. If the theory of DUFFUS is correct than the BMVY is thermolabile with a low level of final dilution (up to 10^{-1}) and its particles are isometric in shape with diameter approximately 26 nm.

We studied the natural occurrence of BMVY on beet in the G.D.R. Dr. Hartleb from the Institute of Phytopathology in Aschersleben provided us with the isolate of the virus which helped us to identify the BMVY on beet in Czechoslovakia.

Material and Methods

Leaves of beet plants with typical symptoms of BMVY, i.e. with yellow-orange tinge of leaves, with exception of green veins (Fig. 1), were selected from plants grown under natural field conditions and used for isolation and identification of BMVY. Each sample consisted of three leaves

TABLE 1

DIFFERENTIATION OF PRESENCE OF BMV AND BYV IN BEET SAMPLES

Beet sample Locality	P.B.S. Ostrov-Veltrusy			Kostelec u Křížku			B.R.I. Semčice		Miletín			Dymo- kury
	I	II	III	II	III	IV	II	IV	I	II	III	I
<i>Sinapis alba</i> -BMV	—	+	+	+	+	+	+	+	—	+	—	+
<i>C. bursa-pastoris</i> -BMV	+	+	+	0	0	0	0	0	—	+	—	+
<i>T. expansa</i> -BYV	+	+	+	—	—	—	—	+	+	+	+	—
<i>C. perfoliata</i> — BYV BMV	+	+	+	—	0	0	0	+	0	0	0	0
	—	—	—	+				+				
Antiserum BYV	+	+	+	—	—	—	—	+	+	+	+	—

+ = positive transmission of the virus on indicator plant

— = the virus was not transmitted; neither was it identified at back transmission

0 = was not investigated

taken from one plant. The leaves were transported to laboratory wrapped in moistened cotton-wool. Sap for serological tests using antiserum against BYV was collected from one leaf. The remaining two leaves were used for 24 h acquisition feeding of aphids *M. persicae* (SULZ.). Aphids were then transferred to indicator plants for 48 or 72 h of inoculation feeding and then they were killed by an insecticide. Five aphids were always fed on one plant. As indicator plants were employed *Sinapis alba* L. and *Capsella bursa-pastoris* MEDIK. for BMV, *Tetragonia expansa* MURR. for BYV and in some cases we also used *Claytonia perfoliata* DONN. which reacts typically to BYV and BMV (RUSSELL 1965a). *Sinapis alba* L. reacts with distinct symptoms to BMV and is not sensitive to BYV (RUSSELL 1965, BJÖRLING and NILSSON 1966). *C. bursa-pastoris* MEDIK. reacts significantly and typically to BMV infection. *T. expansa* MURR. responds similarly to BYV regardless of BJÖRLING and NILSSON's (1966) finding that both these plants can be symptom-less hosts of the second virus.

Six plants of *C. bursa-pastoris* MEDIK., *Sinapis alba* L., *Tetragonia expansa* MURR. and in some cases of *Claytonia perfoliata* DONN., respectively, were employed in each transmission experiment. When the transmission of BMV on *Sinapis alba* L. or on *Capsella bursa-pastoris* MEDIK. gave positive results, the virus was transmitted back to five plants of *Beta vulgaris* L. cv. 'Dobrovická A' by aphid *M. persicae* (SULZ.). In these back-transmissions 10 aphids were put on each beet plant for inoculation feeding. The presence of BYV in analyzed samples was tested serologically using microprecipitin assay with antiserum against BYV prepared by our method (POLÁK 1972, 1974) and diluted 1 : 50. The following samples of beet were employed in experiments with BMV transmission: 3 samples of feeding-beet cv. 'Kostecká Barres' from the Plant Breeding Station in Kostelec u Křížku, district Prague-East, 3 samples of feeding-beet from Plant Breeding Station Ostrov-Veltrusy, 2 samples of sugar-beet from Beet Research Institute in Semčice, 3 samples of sugar-beet from a locality in Miletín, district Jičín, and 1 sample of sugar-beet from a locality in Dymokury, district Nymburk. In addition to these samples 19 samples of feeding-beet cv. 'Kostecká Barres' from the Plant Breeding Station in Kostelec u Křížku, which showed typical BMV symptoms, were tested serologically on the presence of BYV.

The presence of BMV was also tested by the same methods in weed plants *Raphanus raphanistrum* L. which were collected on the periphery of a feeding-beet field (Kostelec u Křížku) and which showed yellowing symptoms and were solid and rigid and did not lose their turgor after cutting off the plants (Fig. 2).

Results and Discussion

The occurrence of BMV on feeding- and sugar-beet in Czechoslovakia was proved by transmission of this virus on indicator plants *Sinapis alba* L. (Fig. 3) and *Capsella bursa-pastoris* MEDIK. (Fig. 4) and by its back-trans-

mission on *Beta vulgaris* L. 'Dobrovická A' (Fig. 5). Ten BMV isolates were obtained from 12 samples of beet collected according the BMV symptoms (Table 1). The BMV only was found in five samples, both BMV and BYV were detected in another five samples and two samples contained only BYV.

The isolates obtained are characterized by different intensity of symptoms on indicator plants. The isolates which induced very distinct symptoms (samples from the Plant Breeding Station in Ostrov-Veltrusy) correspond to Dr. Hartleb's isolate from Aschersleben. The remaining preparations induced less expressive symptoms on indicator plants. Symptoms of BMV and BYV on beet are very similar and their distinction is difficult. BMV does not induce necrosis and lesions (Fig. 6) and no clearing of veins, no netting and no skin-etching was observed on the lower side of *Beta vulgaris* L. leaves as in the case of leaves infected with BYV. On the other hand, leaves infected with BMV uniformly become from the tip to the base orange-yellow with the exception of the tissue nearby veins which remains green (Fig. 1). The incubation period of BMV is four weeks or longer, i.e. longer than that of BYV. The symptoms of BMV are much less distinct under greenhouse than under field conditions. In agreement with findings of WIESNER (1967) it has been observed that leaves infected with BMV are easily secondary attacked by fungus *Alternaria* sp. (Fig. 7). *Sinapis alba* L. reacts on BMV by yellowing of the tissue among veins on the upper side of leaves and by formation of violet spots on their lower side (Fig. 3). Yellowing and the appearance of violet colour was observed on leaves of *Capsella bursa-pastoris* MEDIK. after BMV infection. Leaves of both these indicator plants infected with BMV are very rigid and symptoms of the infection appear after a shorter incubation period i.e. after 2 to 4 weeks. *Claytonia perfoliata* DONN. reacts on BMV by yellowing of leaf tips and by red-violet colouring of leaves (Fig. 8).

The typical symptoms of BMV occur very rarely on beet in Bohemia. One growth of feeding-beet with prevailing symptoms of BMV, however, was found in Plant Breeding Station in Kostelec u Křížku. The presence of BMV, on these plants was proved by the transmission of BMV on indicator plants. From 19 serologically examined samples of beet from this locality, which showed very distinct yellowing, only 3 samples gave positive reaction with antiserum against BYV.

There has been found a new natural host plant of BMV, *Raphanus raphanistrum* L. BMV was successfully transmitted by aphid *M. persicae* (SULZ.) from *R. raphanistrum* L. plants with symptoms of heavy yellowing and orangeing of leaves on the indicator plant *Capsella bursa-pastoris* MEDIK.

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J. POLÁK, J. CHOD, (Praha): Výskyt viru mírné žloutenky řepy v Československu. — Biol. Plant. **17** : 304—308, 1975.

V Československu byl experimentálně prokázán výskyt viru mírné žloutenky řepy (BMVY) na krmné a cukrové řepě. Virus byl přenesen mšicí *Myzus persicae* (SULZ.) na indikátorové rostliny *Sinapis alba* L., *Capsella bursa-pastoris* MEDIK. a *Claytonia perfoliata* DONN. Z indikátorových rostlin byl virus mšicemi přenesen zpět na řepu cukrovou cv. 'Dobrovická A'. Jako nový přirozený hostitel BMVY byla zjištěna plevelná rostlina *Raphanus raphanistrum* L., ze které byl virus přenesen na indikátorovou rostlinu *Capsella bursa-pastoris* MEDIK. a z ní na řepu cukrovou. Biologicky bylo zkoumáno 12 vzorků řepy s příznaky BMVY z různých míst v Čechách, v deseti z nich byl prokázán BMVY.

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Fig. 3. Leaves of *Sinapis alba* L. with BMV symptoms. Yellowing of tissue between veins on the upper side (right) and violet spots on the lower side of the leaf (left).



Fig. 5. Leaves of sugar-beet cv. 'Dobrovická A' with BMV symptoms 6 weeks after inoculation with aphid *M. persicae* (SULZ.). Chlorotic thickened leaves with yellowing on the tissue between veins (right) and leaf from a healthy plant (left).

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Fig. 6. Leaf of sugar-beet cv. 'Dobrovická A' with BYV symptoms: necrosis of the apical part, spotting and yellowing.

Fig. 7. Leaf of sugar-beet infected with BMV. The yellowing of the tissue among veins, and necrosis caused by fungus *Alternaria* sp.

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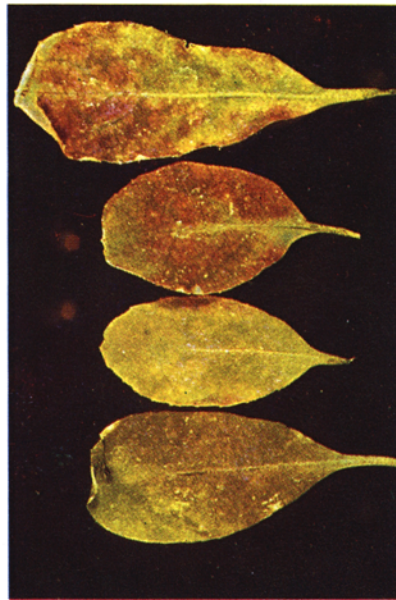
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Fig. 1. Leaf of sugar-beet infected with BMVY. The tissue near-by veins remains green while the rest of the leaf become yellow.
Fig. 2. *Raphanus raphanistrum* leaves with BMVY symptoms: Yellowing and thickening of leaves. Wilted leaf of a healthy plant (left).
Fig. 4. Leaves of *Capsella bursa-pastoris* with BMVY symptoms. Yellowing leaves with violet spots (right) and a healthy leaf (left).
Fig. 8. Leaves of *Claytonia perfoliata* infected with BMVY. Yellowing and violeting of the apical part of leaves (right) and a healthy leaf (left).