LEGUME VIRUSES IN CZECHOSLOVAKIA

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The interest in leguminous pulse and forage crops in Czechoslovakia increased mainly in the past two decades in connection with an increasing food consumption and with the need to produce sufficient amounts of protein-rich fodder for cattle production. The yields of leguminous pulse and forage crops are influenced by a number of factors, including virus diseases which in some years may cause considerable losses. Due attention to virus diseases of these crops in Czechoslovakia has been paid since 1960. In the preceding period only a few reports on the incidence of mosaic type diseases and experimental papers were published [13, 83].

After 1960, reports on investigations of the properties of the following viruses in Czechoslovakia were consecutively appearing:

1) pea mosaic virus — PMV [18, 22, 66, 80, 82],
2) pea enation mosaic virus —PEMV [21, 65, 73],
3) pea leaf rolling mosaic virus — PLRMV [36, 48, 53],
4) pea early browning virus — PEBV [86, 86],
5) bean leaf roll virus — BLRV [88],
6) pea green mottle virus — PGMV [92],
7) broad bean stain virus — BBSV [71],
8) white clover mosaic virus — WCMV [49],
9) alfalfa mosaic virus — AMV [62, 76],
10) red clover mottle virus — RCMV [50],
11) red clover necrotic mosaic virus — RCNMV [51, 75],
12) red clover vein mosaic virus — RCVMV [66],
13) clover blotch virus — CBV [70, 74],
14) pea top necrosis virus — PTNV [70],
15) bean yellow mosaic virus — BYMV [66, 72, 75],
16) bean common mosaic virus — BCMV [60, 79, 81],

17) red clover enation mosaic virus — RCEMV [67].

In connection with the identification of the viruses isolated in Czechoslovakia, in addition to the modes of transmission, host range and properties in sap, also other properties (e.g., particle morphology, purification procedures, preparation of antisera, etc.) were studied in selected virus isolates [6, 7, 10, 11, 41, 42, 52, 55, 56, 57].

With some viruses like AMV, RCMV, RCNMV and BYMV including PMV, the course of their multiplication in certain host plants and the effects of temperature and humidity on the establishment of infection and the course of virus accumulation were determined [3, 5, 9, 12, 14, 15, 30, 34, 38, 40, 63, 64].

With RCMV, the enhanced infectivity of combined bottom and middle components [89] was described and the effect of bentonite on infectivity [43] was determined. The degree of serological relationship between some members of the Comovirus group was also studied [90, 91]. In the course of investigations on the factors affecting the establishment of infection, the inhibitory activities of sap from various plant species on the establishment of infection on garden bean (*Phaseolus vulgaris* L.) leaves was determined [1, 2, 8, 58, 61].

For the newly described PLRMV, CBV and PTNV, their transmission by some aphid species was established. The transmission efficiency of BYMV (PMV), PEMV, PLRMV and AMV by some aphid species or by different strains and clones of *Acyrthosiphon pisum* (Harris) was the subject of detailed studies [20, 24-29, 35, 44-47]. The susceptibility and reaction to infection with viruses found in Czechoslovakia of a number of pea, broad bean, garden bean, vetch, lentil, red clover, white clover and lucerne cultivars grown in this country was established [16, 17, 23, 32, 33, 59, 77, 84, 85]. The damage caused by PMV, BYMV, PEMV, RCMV and AMV to certain pea cultivars was determined under greenhouse conditions and in small-scale field experiments [19, 31]. The possibility of rapid diagnosis including biological tests on indicator hosts and appropriate serological tests was also examined [39, 54, 78]. Intensive research on the occurrence of virus infections in legume forage and pulse crops carried out for several years yielded data on the distribution of the individual viruses and their incidence in various crops all over Czechoslovakia [37, 67-69]. Of the 18 viruses identified so far (Table 1), those transmitted by seed, like BCMV in garden bean, SMV in soybean and PLRMV in pea, broad bean, lentil and vetch occur in susceptible cultivars all over Czechoslovakia.

BYMV (including PMV) is also frequent in forage and pulse crops on the whole Czechoslovak territory. Similarly, PTNV, PEMV, RCVMV and WCMV have been found in various parts of the country without

Table 1

Survey of legume viruses reported from Czechoslovakia

Virus	*Medicago sativa*	*Trifolium*	*Pisum*	*Faba*	*Vicia*	*Phaseolus*	*Soja*	*Lupinus*
BCMV						+		
SMV							+	
PLRMV			+	+	+			
BYMV		+	+	+	+	+	+	+
PMV		+	+	+	+			
RCVMV		+	+		+			
WCMV		+						
AMV	+	+	+	+	+	+	+	+
RCNMV		+						
RCMV		+						
BBSV				+	+			
PTNV		+						
CBV		+						
CMV								+
PEMV		(+)	+	+	+			
BLRV	+		+	+				
RCEMV		+						
PEBV				(+)				
PGMV						(+)		

+ Reaction positive. + reaction probably.

an apparent connection with the character of the geographic region, i.e. they occur in both the lowlands and submontaneous regions. On the other hand, the distribution of AMV is limited to regions where lucerne is grown. The distribution of RCMV is limited to hilly regions of South Slovakia and to central Bohemia. By contrast, RCNMV occurs in submontaneous and montaneous regions of North Slovakia and montaneous regions of Bohemia and Moravia. The remaining viruses have been found so far only in a few localities so that no definite conclusions are possible concerning their distribution being limited to certain geographic areas.

Recently, a further member of the Comovirus group, namely BBSV, was found in vetch plants (*Vicia sativa* L.). This virus is serologically related to RCMV. Antisera to both viruses in addition to species-specific antibody contain also antibody common to both viruses (Fig. 1). Due to the latter antibody, both antisera react with homologous and heterologous antigens. By absorption of antisera with the respective heterologous antigen the common antibody is removed, but the species-spe-

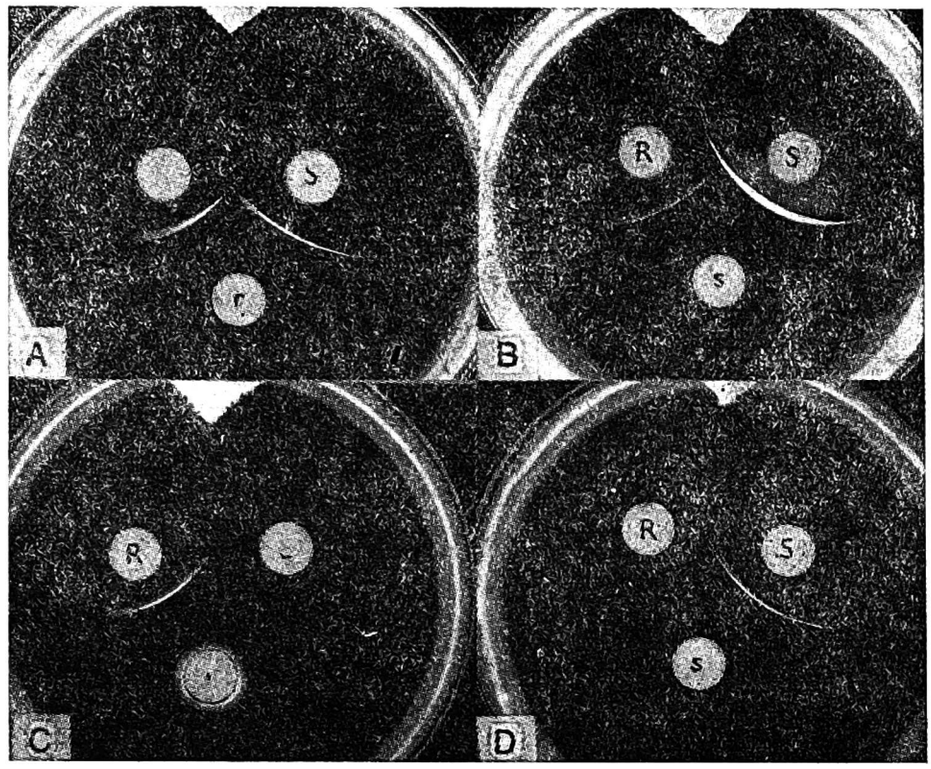

Fig. 1. Immunodiffusion reactions unabsorbed (A, B) and absorbed (C, D) antisera (r, s) with BBSV (S) and RCMV (R) antigens

cific antibody is preserved. Antisera thus absorbed can be used for specific diagnosis. Absorption of antisera by heterologous antigen can be by-passed by using an appropriate serum dilution above the limit for group-specific antibody. In such diluted antiserum the concentration of species-specific antibody is sufficient for use in the gel double diffusion precipitation reaction. This method of antiserum dilution, however, is only applicable to antisera in which the titre of species-specific antibody is at least 10-fold higher than that of the common group-specific antibody.

Detailed studies on the antigenic and serological properties of CBV revealed its serological relationship with CMV (common strain from *Cucumis sativus*). Antiserum to CBV contained two groups of antibody, similarly to the antiserum against CMV (Fig. 2). In both antisera, homologous antibody occurred in a higher titre than heterologous antibody. Homologous antibody was not absorbed by heterologous antigen (Table 2). These results suggest that the antigenic differences between the two viruses are greater than the antibody response they elicit in immunized rabbits. In purified virus preparations, only homologous antigen without a substantial presence of the second antigen bound to the same virus particles could be demonstrated. On immunization of rabbits, homologous antibody appeared in the blood immediately after the 1st immunization cycle, while antibody of the second (heterologous) group appeared only after the 2nd immunization cycle. The occurrence of

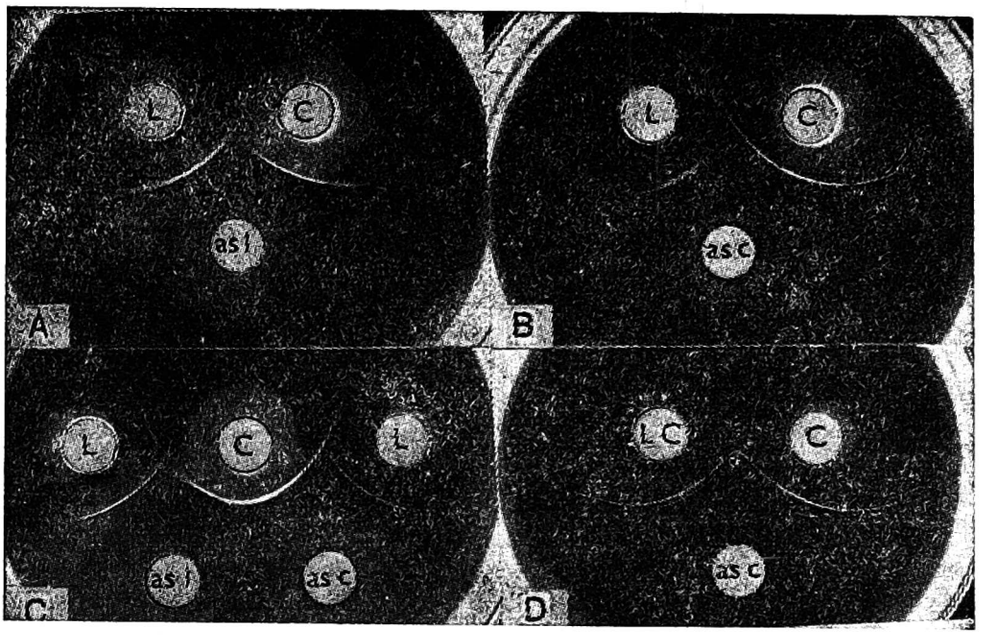

Fig. 2. Immunodiffusion reactions with CBV (L) and CMV (C) antigens; as L: antiserum CBV, as C: antiserum CMV

two or more antibody groups in antisera against members of the Cucumovirus group is characteristic of these viruses [4]. This fact makes difficult an evaluation of the serological differences, namely as to whether they represent a serological relationship of two distinct virus species or only a serological difference between strains of a single virus.

Table 2

Antibody titres of nonabsorbed and absorbed CBV and CMV antisera

Antiserum	Antigens	
	CBV	CMV
CBV	512	128
CMV	512	1024
CBV absorbed with CBV	<2	<2
CBV absorbed with CMV	256	<2
CMV absorbed with CMV	<2	>2
CMV absorbed with CBV	<2	256

Among representatives of RCNMV we found another type of serological difference. We distinguished two serotypes represented by isolates 34 and 48, respectively (Fig. 3A). Representatives of the two serotypes differ antigenically from one another so much that the isolate 34 induced the formation of antibody only against the homologous antigen. By contrast, isolate 48, representing the second serotype, induced the formation of two groups of antibody: one group, characteristic

of isolate 48, regularly was present in a higher titre than the second group which was identical with antibody against the first serotype (Fig. 3B). The reaction of these antigens with antisera against isolate 50, which represented a mixture of both serotypes, confirmed the antigenic diversity of representatives of the two serotypes (Fig. 3C-E). Characteristic crossings of the precipitation lines clearly indicate that representatives of the two serotypes are antigenically distinct. Cross-absorption tests confirmed this conclusion [52]. Recent surveys showed that, in Czechoslovakia, the isolates represented by 48 are prevalent. This serotype approaches by its antigenic and serological properties isolates of RCNMV from Sweden and England.

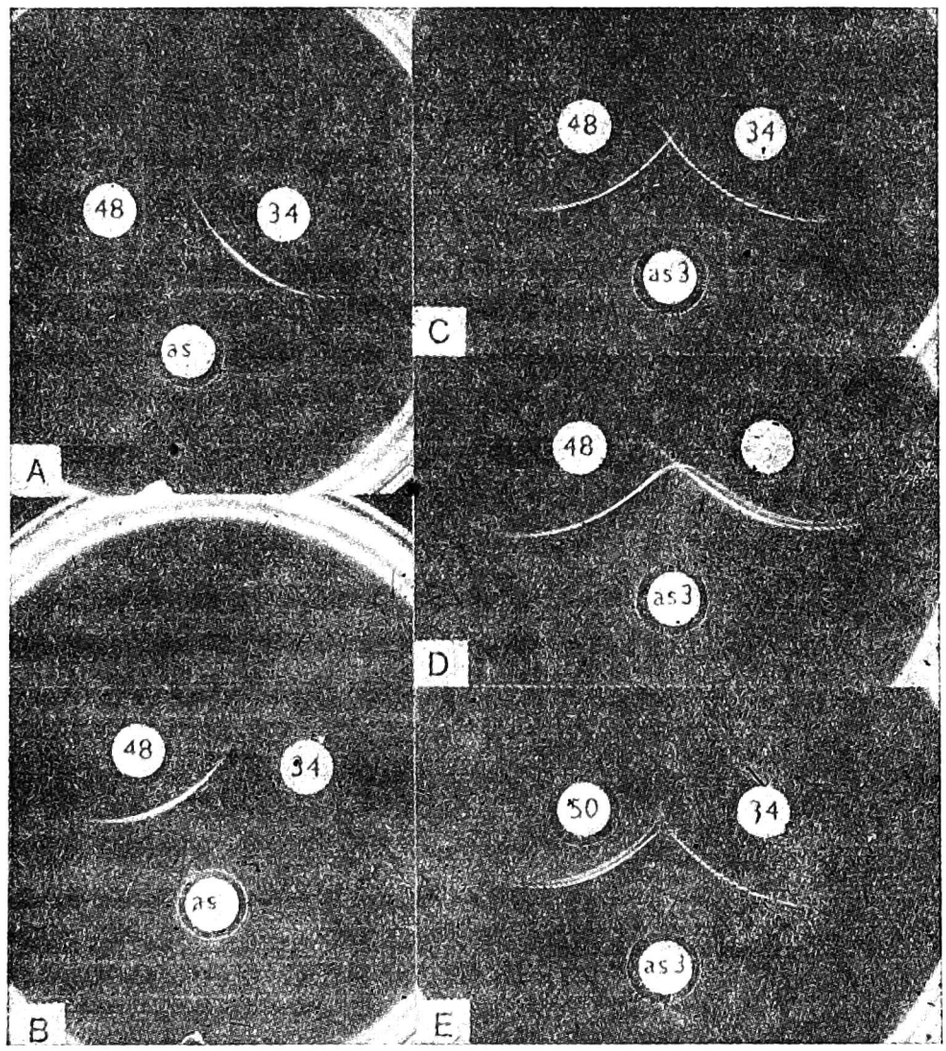

Fig. 3. Immunodiffusion reactions with RCNMV isolates (34, 48, 50); as 1, as 2, as 3: antisera against 34, 48 and 50 respectively

BYMV is one of the most frequent agents causing mosaic type diseases of pulse and legume forage crops (with the exception of lucerne, in which no BYMV infection was proved so far) in Czechoslovakia. Based on the reaction of pea plants, the Czechoslovak isolates of BYMV can be divided into two groups: mosaic-type isolates and necrotic-type isolates.

Both groups are also serologically distinct. Antisera against mosaic-type isolates contain two groups of antibody, one of which is characteristic of this group of isolates, while the other group of antibody is common to the second group of isolates. The situation with antisera against necrotic-type isolates is similar, although in this case the common group of antibody occurs in lower titres or may be absent. In this connection it should be mentioned that, by its biological properties, clover yellow vein virus — CYVV approaches the group of necrotic-type isolates. But the latter differ serologically from type CYVV (as examined with antiserum to type CYVV from M. Hollings) as well as from mosaic-type BYMV isolates. Therefore we consider the group of isolates of the necrotic-type as a strain group of BYMV.

PLRMV occurs every year in pea, broad bean, lentil and vetch crops. In most cultivars, the infection is manifested by characteristic leaf rolling. Only in some cultivars (especially the early ones) there is no leaf rolling, but the apical leaves are reduced and chlorotic. Because the virus is seed-transmitted, we tested the transmission rates by pea, broad bean and lentil seeds (Table 3). We found that the seed trans-

Table 3

Transmission rates of PLRMV by seed of *Pisum sativum* L., *Lens culinaris* Med. and *Faba vulgaris* Moench.

Cultivar	% of infected seeds		
	A	B	C
Pisum sativum L.			
Juwell	10,1	7,3	1,6
Meteor	36,8	16,0	9,2
Pyram	6,2	1,5	4,6
Raman	22,9	22,8	2,3
Vesna	6,9	11,2	6,5
Zázrak z Kelvedonu	1,8	0,8	1,0
Lens culinaris Med.			
Hrotowická	1,0	0	0
Lenka	0,3	0	0
Okula	1,0	0,7	0
Slovenská modrá	3,3	0,3	0
Trebišovská	0,7	0	0
Faba vulgaris Moench			
Považský	3,0	—	—
Inovec	2,0	—	—

A — Seed from plants experimentally infected before flowering
B — Seed from plants experimentally infected during flowering
C — Seed from plants naturally infected in field.

mission rate of PLRMV depends on the cultivar, time of the onset of infection, degree of seed maturity and external temperature.

The rate of seed infestation with PLRMV can be determined by a greenhouse propagation test which proved to be more sensitive than serological testing. Similarly, in determining the infestation rate of garden bean seed by BCMV, the former test proved to be superior to the assay on hypersensitive bean cultivars (e.g. Top crop or Monroe — Table 4). The greenhouse propagation test proved to be the most sensitive also in determining the infestation rate of soybean seeds by SMV.

Table 4

Seed transmission of BCMV in garden bean cultivars Beka and Saxa as detected by various assay methods

Mode of assay	% of seedlings infected	
	cv. Beka	cv. Saxa
Symptoms on primary leaves	26 - 31 - 29*	28 - 26 - 38*
Symptoms on trifoliate leaves	29 - 21 - 27*	31 - 28 - 22*
Infectivity test on cv. Topcrop	16* - 11**	17* - 16**
Infectivity test on cv. Monroe	17* - 30**	28* - 31**

* Evaluations by three persons.
\+ Sap from primary leaves.
++ Sap from the first trifoliate leaves.

This survey of recent investigations on legume viruses in Czechoslovakia shows that we are attempting to approach the problem from a broad aspect, paying attention also to the requirements of breeders and growers. In this respect, however, many questions still remain open.

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ВИРУСЫ МОТЫЛЬКОВЫХ РАСТЕНИЙ В ЧЕХОСЛОВАКИИ

Резюме

Представлены результаты исследований по болезням клевера люцерны и бобовых растений в Чехословакии. вызванные вирусами мозаики люцерны, некротической мозаики клевера красного, мозаичности красного клевера, крапчатости конского боба, верхушечного некроза гороха, деформирующей мозаики гороха, крапчатости клевера, огуречной мозаики, мозаики белого клевера, жилковатой мозаики красного клевера, желтой мозаики фасоли, мозаики гороха, мозаики скручивания листьев гороха, мозаики сои, мозаики фасоли и деформирующей мозаики красного клевера. Кроме того представлены результаты исследований по свойствам некоторых вышеуказанных вирусов.

Сравнивались биологические свойства вирусов мозаичности красного клевера и крапчатости конского боба, а также крапчатости клевера с вирусом огуречной мозаики: констатированы биологические и серологические разницы между изолятами некротической мозаики красного клевера и желтой мозаики фасоли, а также рассмотрены возможности родства „некротических типов" изолятов желтой мозаики фасоли и желтой мозаики жилок клевера. Проведены также исследования по патогенности некоторых вирусов бобовых растений и сортов клевера и люцерны, возделываемых в Чехословакии. Опытным путем доказано перенесение вируса мозаики скручивания листьев с семенами гороха, конских бобов и чечевицы, а также установлено влияние некоторых факторов на степень поражения семян.

Предлагается применение диагностических методов для выявления вирусов в семенах бобовых растений, а именно: вируса мозаики скручивания листьев, вируса мозаики фасоли в семенах фасоли и вируса мозаики сои в семенах сои. Прорастающие семена поддавались биологическим тестам — размножения и серологическим.

Miloš Musil

WIRUSY ROŚLIN MOTYLKOWATYCH W CZECHOSŁOWACJI

Streszczenie

Przedstawiono wyniki badań nad chorobami koniczyny, lucerny i roślin strączkowych w Czechosłowacji wywołanymi przez wirusy mozaiki lucerny, nekrotycznej mozaiki koniczyny czerwonej, mozaikowatości czerwonej koniczyny, plamistości bobiku, wierzchołkowej nekrozy grochu, ostrej mozaiki grochu, plamistości koniczyny, mozaiki ogórka, mozaiki białej koniczyny, mozaiki nerwów koniczyny czerwonej, żółtej mozaiki fasoli, mozaiki grochu, mozaiki liściozwojowej grochu, mozaiki soi, zwykłej mozaiki fasoli i ostrej mozaiki koniczyny czerwonej. Ponadto przedstawiono wyniki badań nad własnościami niektórych wirusów.

Porównano własności biologiczne wirusów mozaikowatości koniczyny czerwonej i plamistości bobiku oraz plamistości koniczyny z wirusem mozaiki ogórka; stwierdzono biologiczne i serologiczne różnice między izolatami nekrotycznej mozaiki koniczyny czerwonej i żółtej mozaiki fasoli oraz rozpatrzono możliwości pokrewieństwa „nekrotycznych typów" izolatów żółtej mozaiki fasoli i żółtej mozaiki nerwów koniczyny. Przeprowadzono również badania nad patogenicznością niektórych wirusów roślin strączkowych oraz odmian koniczyny i lucerny uprawianych w Czechosłowacji. Wykazano doświadczalnie przenoszenie się wirusa mozaiki liściozwojowej z nasionami grochu, bobiku i soczewicy oraz stwierdzono wpływ pewnych czynników na stopień porażenia nasion.

Proponuje się zastosowanie metod diagnostycznych dla wykrywania wirusów w nasionach roślin strączkowych, a mianowicie: wirusa mozaiki liściozwojowej; wirusa zwykłej mozaiki fasoli w nasionach fasoli i wirusa mozaiki soi w nasionach soi. Kiełkujące nasiona poddawano testom biologicznym — rozmnożeniowym i serologicznym.

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