

Viruses occurring in red clover (*Trifolium pratense* L.) in some regions of Poland

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Several different viruses have been isolated from red clover (*Trifolium pratense* L.), in different parts of the world. In the countries of West Europe and in North America the problem of virus diseases of red clover has been worked out pretty extensively. The main viruses occurring in red clover in the regions studied have been identified and the ways of spreading and their harmfulness determined. There are also carried out some studies on resistance to viruses in red clover.

In Poland there are but few publications on red clover viruses. In 1967, Kochman and Stachyra [7] reported the appearance of red clover vein mosaic virus. Książek [8, 10] identified red clover vein mosaic virus and bean yellow mosaic virus in red clover and white clover (1963 and 1964). Błaszczak [3] isolated a severe strain of bean yellow mosaic virus from red clover (1966). The occurrence of green petal virus was reported in 1960 by Pielka [12] and in 1963 by Książek [9].

Our investigations taken up in 1965 aimed to quantitative evaluations of occurrence of virus diseases in red clover in 4 districts, identification of sap-transmissible viruses affecting red clover and in the later stage, evaluation of susceptibility to virus diseases of some Polish red clover varieties. Trials were undertaken to find red clover plants resistant to viruses most commonly occurring in this country which might be used in breeding work.

METHODS

FIELD OBSERVATIONS

The observations on the occurrence of virus diseases in red clover plantations were carried out in Poznań, Wrocław, Opole, Kraków, districts and in main red clover breeding centres in Poland in the years 1965-1967 (Fig. 1). They were made chiefly in autumn on plants growing a new after their cutting. The occurrence of plants with virus symptoms was evaluated in about 200 fields. These were multi-hectare and small plantations in private farms as well small plantations and nurseries in Experimental Stations of Variety Evaluation and Plant Breeding Stations. Plants with symptoms of virus diseases were dug out, transported and put into separate

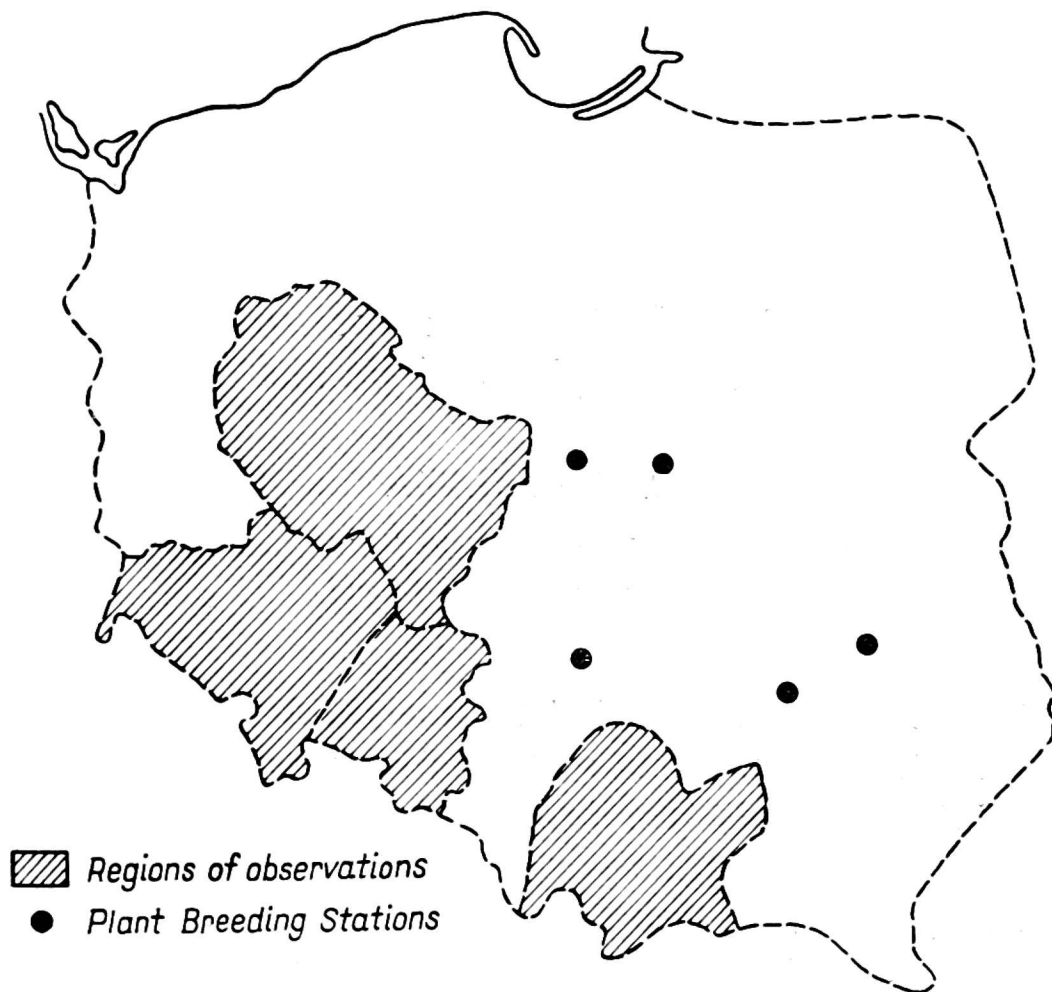


Fig. 1. Regions and Plant Breeding Stations where the occurrence of virus disease of red clover were observed (1965-1967).

pots in glasshouses, being sprayed beforehand with an insecticide. Sometimes only the leaves of virus affected plants were collected for immediate isolation. As a whole, over 400 specimens of plant material were collected.

GLASSHOUSE WORK

The biological test was applied. For the first isolations, *Phaseolus vulgaris*, *Pisum sativum* and *Vicia faba* were used as test plants. Test plants were inoculated and kept in the glasshouse for 4-6 weeks for the symptoms to develop. Reisolations were performed on a similar set of plants in order to separate possible complex infections. This was repeated twice, including *Pisum sativum* L. var "Cud Ameryki", *Nicotiana tabacum* L., *Chenopodium amaranticolor* Coste and Reyn, *Lupinus albus* L., some species of *Trifolium*, *Cucumis sativus* L. as test plants. Different dilutions and even isolations from single spots were used. Some plants with distinct persistent disease symptoms, at which the isolation failed, were sent to the Institute of Plant Protection in Poznań for further investigations. After carrying out a number of comparative test and separating complex infections, 6 groups representing separate kinds of viruses were obtained. Their physical properties (6-12 isolates from each group), resistance to ageing, thermal inactivation point and dilution end-point were determined.

At present the susceptibility of seedlings and cuttings of 4 native red clover

varieties to 5 isolates have been examined. Moreover, the effect of virus infection on the growth of plants, flowering, yielding and transmission of viruses by seeds have been studied.

RESULTS

TYPES OF VIRUS DISEASES OCCURRING IN RED CLOVER IN THE AREAS UNDER OBSERVATION

On the basis of field observations 7 types of disease symptoms caused by viruses were distinguished:

- (1) chlorotic veinbanding,
- (2) mosaic,
- (3) mottling with chlorotic interveinal spots,
- (4) yellow mottling with slight leaf distortion and crinkling,
- (5) mottling, necrotic spots, distortion and malformation of leaf blades,
- (6) severe chlorosis and dwarfing of leaves,
- (7) distinct commalike white spots and leaf malformation, dwarfing.

These symptoms chiefly appear on the youngest leaves, especially on shoots after cutting. On flowering plants they are often masked. Sometimes, when the symptoms are severe, the growth is stunted and the plants become dwarfed. Infected plants most commonly found were those with symptoms of typical mosaic mottling on their young leaves. with chlorotic or yellow spotting (Fig. 2), and in sub-

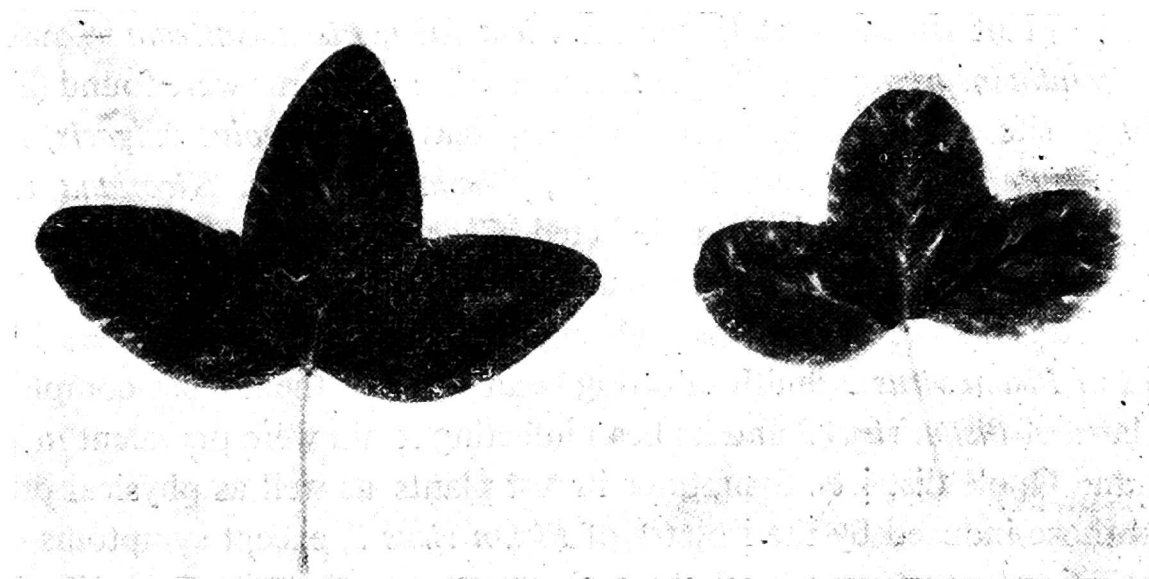


Fig. 2. Most commonly occurring virus symptoms on red clover leaves.

montane regions the plants with mottling, spot necrosis and distortion of leaf blades. At a broad spacing infected plants had badly narrowed leaf blades (resembling alfalfa leaves) and very distinct changes in the plant shape, dwarfing, leaf and stem colour deviations and occasionally green petals.

INCIDENCE OF VIRUS DISEASES IN THE YEARS 1965-1967

In the first growing year among the red clover plants hardly any with mosaic symptoms were found. It was only in small fields, in autumn after cutting the clover, especially in the vicinity with other legume plants as pea, alfalfa and two

year clover crop, that plants with mosaic symptoms were found on the field edges. In Plant Breeding Stations, as early as in the first year crop, the virus infection of breeding material at broad spacing was very high, amounting to 30 and more per cent. At narrow spacing, plants with symptoms of virus infection were found rather sporadically.

In the second growing year, in late autumn, after the second cutting, both in small and multihectare plantations a relatively high percentage of infected plants was observed. Most mosaic infected were fields at private farms, particularly in submontaneous regions. Three year plantations of pasture type, encountered here and there, showed 100% mosaic infection.

The number of infected plants first of all depended on the age and size of the plantations and their environment, whereas the symptom appearance of virus diseases was dependent on the developmental stage of clover and season of the year.

IDENTIFICATION OF VIRUSES

Out of over 400 collected samples about 200 positive isolations were obtained, and after separating about 30 complex infections, over 200 isolates were collected. On the basis of the hitherto investigations the following was found:

1. About 50 isolates were identified as *Pisum virus 2 Smith-12* of them from complex infections. This virus could be found in clover fields in all the regions under study and in Plant Breeding Stations. On *Vicia faba*, *Pisum sativum* — susceptible variety, *Trifolium incarnatum* and *T. pratense*, mosaic symptoms were found (Table 1). *Lupinus albus* showed mosaic and necrotic top leaves. *Phaseolus vulgaris*, resistant variety of *Pisum sativum* "Cud Ameryki", *Cucumis sativus*, *Nicotiana tabacum*, remained uninfected. Some isolates infected *Chenopodium amaranticolor* systemically. Physical properties of 8 isolates examined varied as shown in Table 1.

2. Over 60 isolates collected from all regions under observation were identified as a strain of *Pisum virus 2 Smith* infecting bean — 17 of them from complex infections. Isolates of *Pisum virus 2* and its bean infecting strain were prevalent in Poznań, Wrocław and Opole districts. Symptoms in test plants as well as physical properties resembled those induced by the isolates of *Pisum virus 2*, except symptoms on bean (Table 1). This plant showed local chlorotic spots of various intensity, blurred and later disappearing. On bean variety Saxa no systemic infection was found. A few isolates infected Pinto variety systemically (Fig. 3). Physical properties of the 6 examined isolates varied as shown in Table 1. The examined varieties of red clover proved to be susceptible to both strains of *Pisum virus 2*, being rather hard transmissible mechanically and inducing mosaic and mottling of various kind in clover plants.

3. About 20 isolates — 8 of them from complex infections were identified as white clover mosaic virus — Brandes and Quantz. This virus occurred in all the districts under observation. All isolates infected broad bean locally and systemically (Table 1). *Phaseolus vulgaris* showed local vein necrosis and chlorotic irregular spotting. Systemic infection was found only in summer in the form of asteroid spots. Under winter glasshouse conditions, pea showed local necrotic lesions,

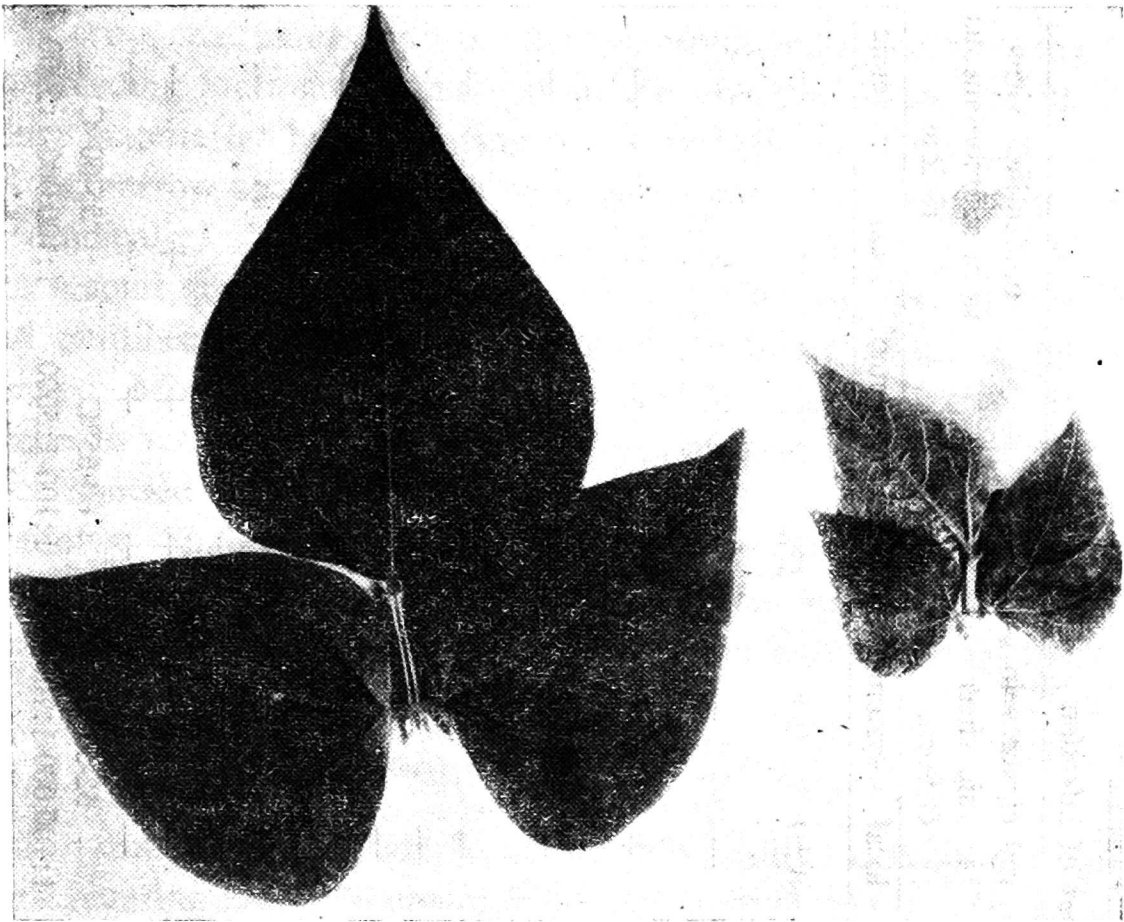


Fig. 3. Systemic symptoms produced by a strain of *Pisum virus 2* on trifoliate leaves of the Pinto bean.

mottled and wilted top leaves and the plants died. In summer the infected pea demonstrated only severe systemic mosaic. The pea variety "Cud Ameryki", resistant to *Pisum virus 2*, also became infected. Some isolates infected *Chenopodium amaranticolor* and white lupin locally. Tobacco and cucumber showed no symptoms. Red clover varieties proved to be highly susceptible to white clover mosaic virus. Physical properties of the 10 isolates varied as shown in Table 1.

4. Six isolates from complex infections were identified as red clover vein mosaic virus-Osborn. This virus was found in all 4 districts under observation, but its occurrence was rather sporadic. Pea reacted with a severe systemic infection (Table 1) in the form of chlorosis, severe veinbanding and crinkling. Plants were badly dwarfed and died soon. The pea variety "Cud Ameryki" was symptomless. Crimson clover reacted strongly and showed veinbanding with chlorotic spotting and sometimes lesions and leaf malformations (Fig. 4). Plants displayed severe stunting and usually

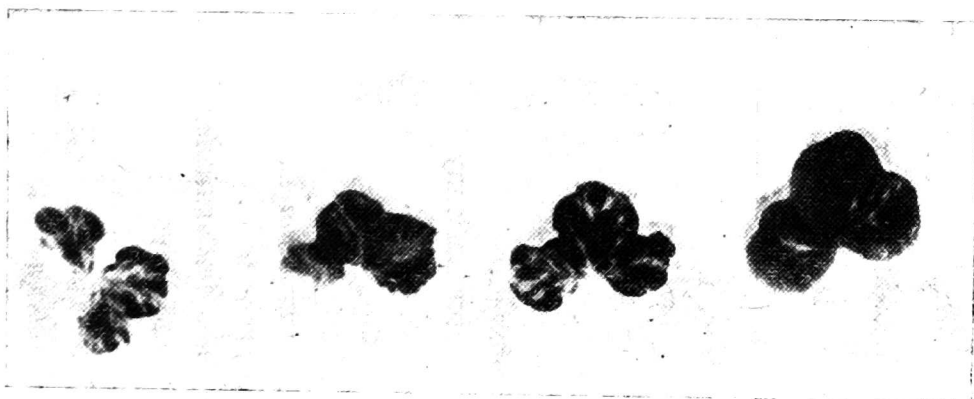


Fig. 4. Systemic symptoms produced by red clover vein mosaic virus on *Trifolium incarnatum* leaves.

died. Broad bean showed local chlorotic lesions and systemic infection. On *Gomphrena globosa* distinct local spots were found. Tobacco and cucumber were symptomless. Physical properties of one isolate are shown in Table 1.

5. Three isolates from two Poznań district plantations situated near an alfalfa field were identified as alfalfa mosaic virus — *Marmor medicaginis* — Holmes. All the test plants used, became infected (Table 1). In pea varieties local necrotic lesions, necrosis in stem and severe systemic infection were found. *Chenopodium amaranticolor* reacted distinctly with local and systemic infection. Tobacco showed etching, symptoms of oak-leaf pattern and mild disappearing symptoms of systemic infection. Typical alfalfa mosaic virus symptoms also appeared in broad beans, *Gomphrena globosa* and *Datura stramonium*. French bean gave systemic and white lupin local infections. The isolates were readily transmitted to red clover by rubbing. Physical properties of 3 examined isolates varied as shown in Table 1.

6. Over 60 isolates in about 20 cases from complex infections were obtained from submontane regions. The isolates displayed characteristic symptoms in test plants (Table 1). Symptoms of local infection in the form of distinct necrotic spots could be observed in pea, broad, bean, *Chenopodium amaranticolor* and tobacco. No systemic infection was observed in those plants. White lupin and french bean

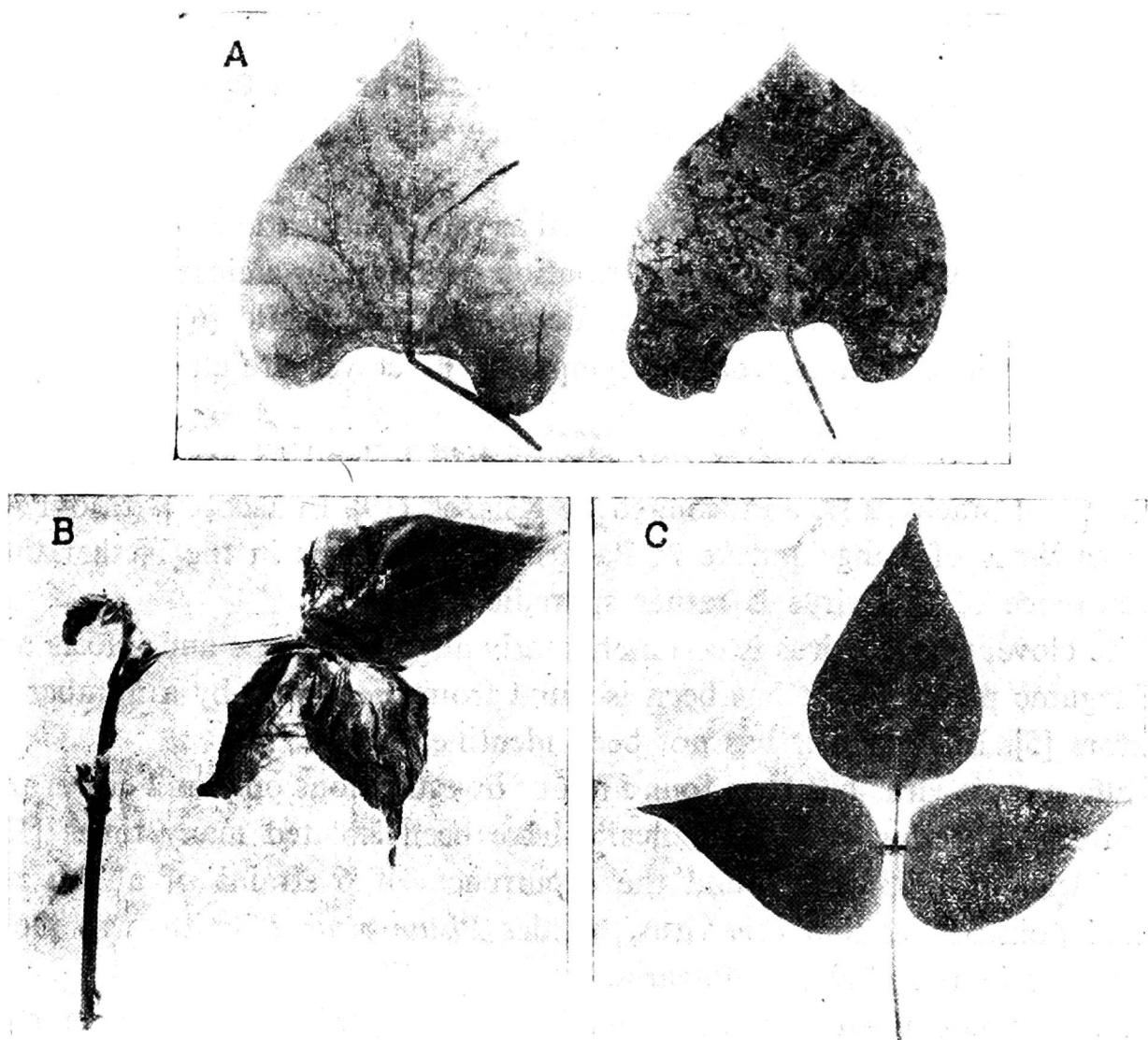


Fig. 5. Symptoms produced on the Pinto bean by a virus strain in submontaneous region. A — local necrotic lesions, B — stem streak and top necrosis, C — vein necrosis on trifoliate leaves after shock reaction.

reacted with a very severe local infection in the form of necrotic spots, necrosis in veins, petioles as well as in stems and tops (Fig. 5 A, B, C). The shock reaction was followed by death or, in very few cases, recovery of plants. Some *Trifolium* species reacted with a local and systemic infection in the form of necrotic mosaic and leaf deformation. In *Nicotiana clevelandi* only systemic infection occurred. Physical properties of the 3 examined isolates varied as shown in Table 1.

DISCUSSION AND CONCLUSIONS

Observations and investigations carried out hitherto in 4 districts lead to the conclusion that virus infection in large multihectare red clover plantations is no important problem at present, as two year red clover crops are a common practice in Poland. Langelüddeke [11] in Bavaria came to the same conclusion. But the high percentage of plants with virus disease symptoms in small two and three year plantations, especially at private farms, may not only bear an influence on the amount and quality of crops, but above all constitute a dangerous source of virus disease infection for other legume crops. The high virus infection of clover plants at Plant Breeding Stations is quite a problem even now.

On the basis of diagnostic investigations of over 400 samples collected from about 200 plantations, the strains of *Pisum virus 2* Smith were found as the main incitants of virus diseases in red clover. In Poland, many a time different strains of bean yellow mosaic virus and *Pisum virus 2* have been already identified in legume plantations [2, 3, 8, 10].

The results of those investigations as well as our identification of *Pisum virus 2*—infecting bean may be still another contribution speaking for a close strain relationship between the two viruses. According to Bercks [1], Goodchild [6], Bos [4], Gibbs [5], and other investigators, mosaic symptoms in clover are all caused by *Pisum virus 2*.

Red clover vein mosaic virus was observed in Poland as early as in 1957 by Kochman and Stachyra [7] and isolated by Książek [10]. In accord with our results as well as those of Langelüddeke in Bavaria [11] and Bos in the Netherlands [4] the occurrence of this virus is rather sporadic.

White clover mosaic virus is common nearly all over Europe and infects a number of legume plants [4]. It has been isolated from red clover by a number of investigators [5]. In Poland it has not been identified as yet.

Alfalfa mosaic virus has been found in our investigations only in 2 clover plantations. Strains infecting bean systemically have been isolated many times [13, 14]. Langelüddeke in 1963 [11] found the occurrence of 6 strains of alfalfa mosaic virus and pointed out that this virus, besides *Pisum virus 2* Smith, was the most common one in red clover in Bavaria.

The virus unidentified by us as yet and infecting clover in submontaneous regions, has been probably isolated in Slovakia. Some physical properties of this virus suggest that it should be a strain of alfalfa mosaic virus, but as many test plants display a tendency to recover from it may be equally well a strain of *Nicotiana virus 12*.

Five red clover varieties in this country were examined and they all proved to be susceptible to the above mentioned viruses.

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