PRACE NAUKOWE INSTYTUTU OCHRONY ROŚLIN
Tom XXVIII, zeszyt 1-2 1987

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THE OCCURRENCE OF VIRUSES ON RYEGRASS AND COCKSFOOT CROPS

The occurrence of virus disease induced by mixed infection (filamentous and spherical viruses) on the plantations of ryegrasses (*Lolium*) and cocksfoot (*Dactylis glomerata*) is described in this paper.

On the basis of the obtained results, filamentous virus has been identified as the cocksfoot streak virus, whereas spherical virus has not been identified yet.

I. INTRODUCTION

The present studies were undertaken in connection with the detection of virus infection causing mosaic symptoms on the ryegrass and cocksfoot crops.

According to the literature data these species of grasses may be infected by many viruses, which occur singly or in mixed infections. The most widespread viruses on ryegrass are: the ryegrass mosaic virus (Mulligan, 1960; Hirst, 1974; Salt, 1975; Vacke, Jágrová, Jokeš, Novák, 1976), barley yellow dwarf virus (Catherall, 1963, 1981; Catherall, Wilkins, 1977), brome mosaic virus (Ohmann-Kreutzberg, 1963; Slykhuis, 1967; Bremer, 1964, 1974), Agropyron mosaic virus (Bremer, 1964).

Among viruses occurring on cocksfoot the most frequent are: the cocksfoot streak virus (Slykhuis, 1958; Ohmann-Kreutzberg, 1963; Schumann, 1969; Huth, 1972), cocksfoot mottle virus (Serjeant, 1964, 1967; Rabenstein, Schmidt, 1979) and cocksfoot mild mosaic virus (Huth, Brandes, Paul 1970; Huth 1968, 1972).

Some of the mentioned viruses infect the both grass species.

II. MATERIAL AND METHODS

The work on the occurrence of virus disease included observations and identification studies. The latter took into consideration:

a) different ways of virus transmission (through inoculation with sap, by aphids, mites, through soil and with seeds);
b) determination of physical properties;
c) the range of plant hosts;
d) electron microscopic observations;
e) serological tests;
f) purification of virus material.

The source of virus and a trial plant was Italian ryegrass of the cv. Szelejewski and in some studies (insect transmission tests, examination of physical characters) — also plants of cocksfoot of the cv. Nakielska.

In the tests on virus transmission by mites — *Abacarus hystrix* Nal. (*Eriophyidea, Acarina*) (adult specimens and nymphs) aquisition feeding lasted 24, 48, 72 hours and 14, 21 days. The length of inoculation feeding was 14 days.

In the tests with *Myzus persicae* Sulz., before the transfer to the source of infection, 3-h starvation of virusfree aphids was used. The time of aquisition feeding was: 10 and 20 sec., 2, 5 and 10 min. followed by 1-, 12-and 24-hour-inoculation feeding.

Viruses were purified according the methods described previously (Pospieszny et al., 1983).

Preparations for examination in an electron microscope were directly taken from infected plants according to the dip method (Brenner and Horne 1959) and preparations from purified sap were negatively stained. A contrast agent was phosphorotungistic acid (PTA) at pH = 7.

Observations of the preparations were performed using Siemens — Elmiskop I and Philips EM-201 electron microscopes at magnifications 10 000 and 20 000 x. Electronograms were made on ORWO-EU-2 plates.

III. RESULTS OF STUDIES

Occurrence

The observations on virus disease carried out in 8 voivodeships (Tables 1, 3) showed an almost 23% infection of the total amount of the examined ryegrass crops and a 19% infection of cocksfoot crops.

Marked differences have been found between the annual seed plantations of ryegrass and perennial ryegrass plantations, frequently used as pastures. These differences became evident in the amount of infected

Tabela 1

Occurrence of virus disease with the symptoms of mosaic on ryegrass crops in 1977—1978

Voivodeships	Localities	Totally	Number of Observation stations including crops of					
			Italian ryegrass				perennial ryegrass	
			domestic varieties		foreign varieties			
			1-year plantation	perennial grass plantations	1-year plantations	perennial grass plantations	1-year plantations	perennial grass plantations
Bydgoszcz	0/3*	0/6**	—	—	0/6	—	—	—
Kalisz	10/14	19/66	9/42	7/17	1/5	2/2	—	—
Leszno	1/1	1/2	—	—	—	—	0/1	1/1
Opole	1/7	2/20	0/4	1/1	0/14	1/1	—	—
Poznań	2/4	2/3	—	—	—	—	0/1	2/2
Toruń	1/5	2/15	0/9	1/1	0/4	1/1	—	—
totally	15/34	26/112	9/55	9/19	1/29	4/4	0/2	3/3
percentage	44.1%	23.2%	16.4%	47.4%	3.4%	100%	0%	100%

Explanations:
* in the numerator is the number of localities where virus disease was found
in the denominator is the number of localities where observations were performed
** in the numerator is the number of infected crops
in the denominator is the number of observed crops

Table 2

An analysis of infection of ryegrass crops with virus disease displaying symptoms of mosaics (acc. to the observations in 1977—1978)

Species and cultivar of ryegrasses	Total number of infected/ crops	Number of infected/ /observed crops		% of infected plants					
				crops					
				annual			perennial		
		crops		sporadically — 1%	2–10%	15–30%	sporadically — 1%	2–10%	15–30%
		annual	perennial						
Italian ryegrass cv. Szelejewski	17/69	9/51	8/18	4	4	1	2	3	3
Italian ryegrass cv. Skrzeszowicki	1/5	0/4	1/1	0	0	0	0	0	1
Italian ryegrass cv. Lemtal	4/12	1/9	3/3	1	0	0	1	1	1
Italian ryegrass cv. Barmultra	1/14	0/13	1/1	0	0	0	1	0	0
Italian ryegrass cv. Tetila Scempter	0/7	0/7	—	0	0	0	0	0	0
Italian ryegrass cv. Górczański	3/5	0/2	3/3	0	0	0	1	1	1

crops as well as in the intensity of the disease occurrence. The number of infected seed (annual) plantations was not large and amounted to 10 out of 86 observed ones, which constitutes 11.6%. Contrary to them, perennial crops were infected to a larger degree, since 16 out of 26 crops, i.e. 61.5% showed the presence of virus disease (Table 2). The severity of the infection on annual plantations was not large, mostly sporadic, whereas that on perennial plantations often reached 30% (Table 2).

Purification

During electron-microscopic examinations (dip method) the infected plants were observed to have particles of filamentous virus. It has been found that the virus was transmitted mechanically through inoculation with sap.

Purification of sap obtained from artificially infected ryegrasses displayed also the presence of spherical virus in diseased plants. Therefore, infection was caused by a mixture of viruses (Fig. 1).

Table 3

Occurrence of virus disease with the symptoms of mosaic on cocksfoot crops in 1975 and 1977

Voivodeships	Number of			
	Localities	observation stations		
		Totally	including	
			cocksfoot crops	habitats of wild growing cocksfoot
Gorzów	1/4*	1/20**	1/20	—
Kalisz	1/3	1/4	1/4	—
Leszno	2/6	3/12	3/12	—
Poznań	3/4	6/11	6/11	—
Szczecin	1/6	1/16	0/15	1/1
totally	8/23	12/63	11/62	1/1
percentage	34.8%	19.0%	17,7%	100%

Explanations:
* in the numerator is the number of localities where virus disease was found
in the denominator is the number of localities where observations were performed
** in the numerator is the number of infected crops
in the denominator is the number of observed crops

Separation of the virus complex was made by two methods:

a) filamentous virus was separated from the spherical one (Fig. 2) through passage of the virus mixture on *Lagurus ovatus* L. and purification of sap from the infected plants. During purification it was observed that the virus mixture was more stable than the filamentous virus in a homogenic state. It was also manifested in the gradient of saccharose concentration (the complex precipitated in the gradient, the filamentous virus came down to precipitate). It was observed that the filamentous virus easily aggregated.

b) the spherical virus was obtained (Fig. 3) through a series of precipitations at a differentiated concentration of polyethylenoglicol 6000 (PEG-6000) and centrifugations in the gradient of saccharose (Pospieszny et al., 1983) from the virus mixture, previously purified. Plants of ryegrass and *Lagurus ovatus* L., inoculated with purified virus, showed no disease symptoms. However, using an electron microscope, the purified sap of ryegrass was found to have only particles of spherical virus (Fig. 4). It has heen found that the concentration of spherical virus in plants was markedly higher, when it occurred in complex with filamentous virus.

Ryc. 1. Mieszanina wirusów CSV i sferycznego (pow. 230 000×)

Fig. 1. The mixture of CSV and spherical virus (230 000×)

In the so far studies only filamentous virus has been identified, while determination of spherical virus requires further studies.

Identification of filamentous virus

Transmission

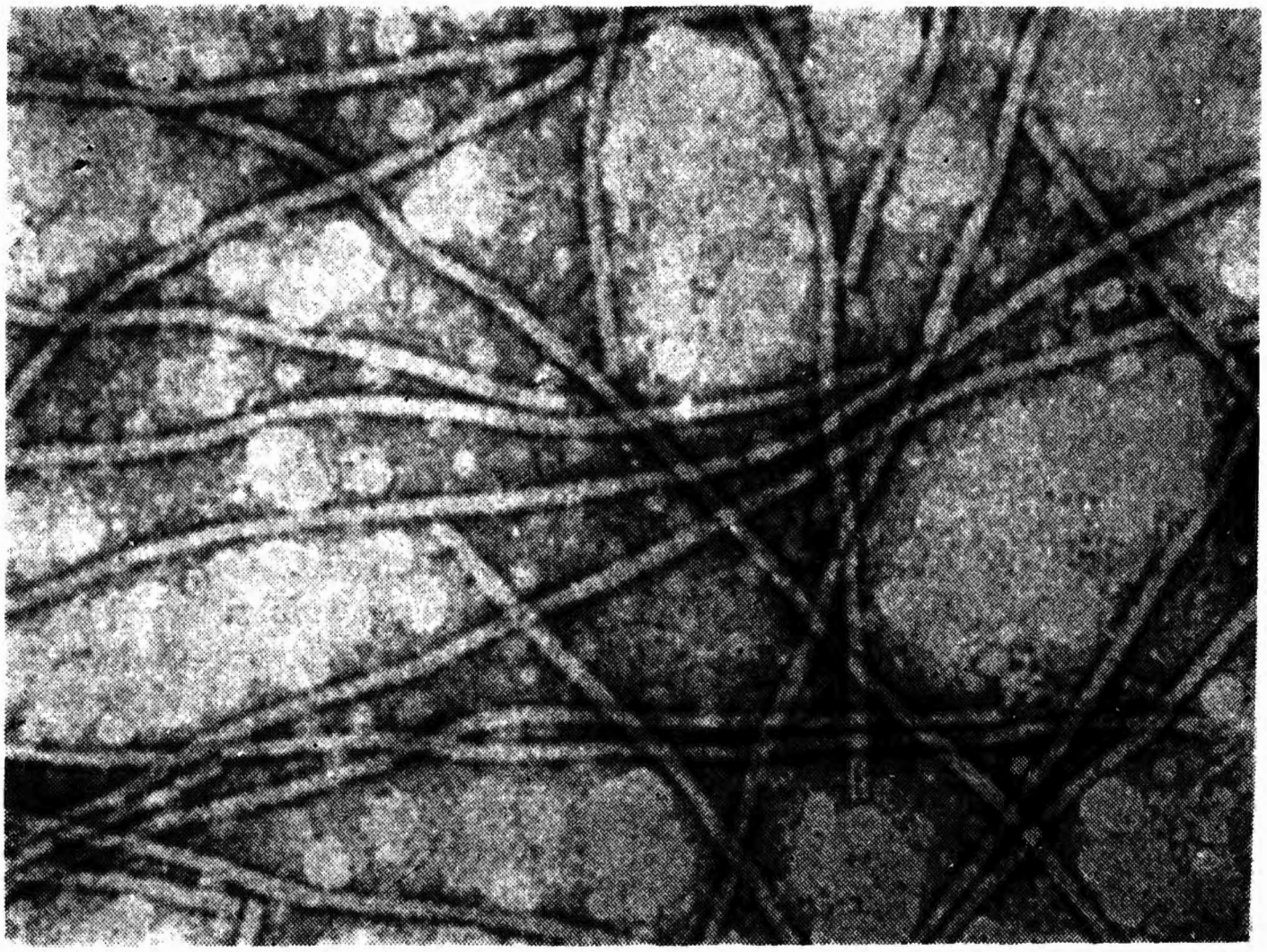
The virus was transmitted mechanically through inoculation with a sap diluted by phosphorus buffer with pH = 7 and pH = 5.9 and by *Myzus persicae* after the application of a 2-min. acquisition feeding and a 1-h inoculation feeding.

The virus wasn't transmitted by mites (*Abacarus hystrix*), through soil and with seeds of diseased plants.

Physical properties

The point of thermal inactivation was within 55-60°C, the dilution end-point was about 1 : 1000 and longevity in vitro lasted for over 7 days (in some experiments — more than 10 days).

An estimate of health condition of plants used in the tests of virus



Ryc. 2. Cząstki CSV w podczyszczonym soku z *Lagurus ovatus* (pow. 210 000×)

Fig. 2. CSV particles in the purified sap from *Lagurus ovatus* (210 000×)

transmission by vectors and in the studies on longevity in vitro was based on the results of macroscopic observations, which were randomly checked under an electron microscope.

Electron-microscopy observations

The virus particles were filamentous and flexuous, about 750 nm long and 13—15 nm thick (Fig. 5).

Serological tests

The serological tests performed by the microprecipitation method under paraffin oil using serum against ryegrass mosaic virus (produced in Czechoslovakia and received from dr. Vacke) showed no reaction.

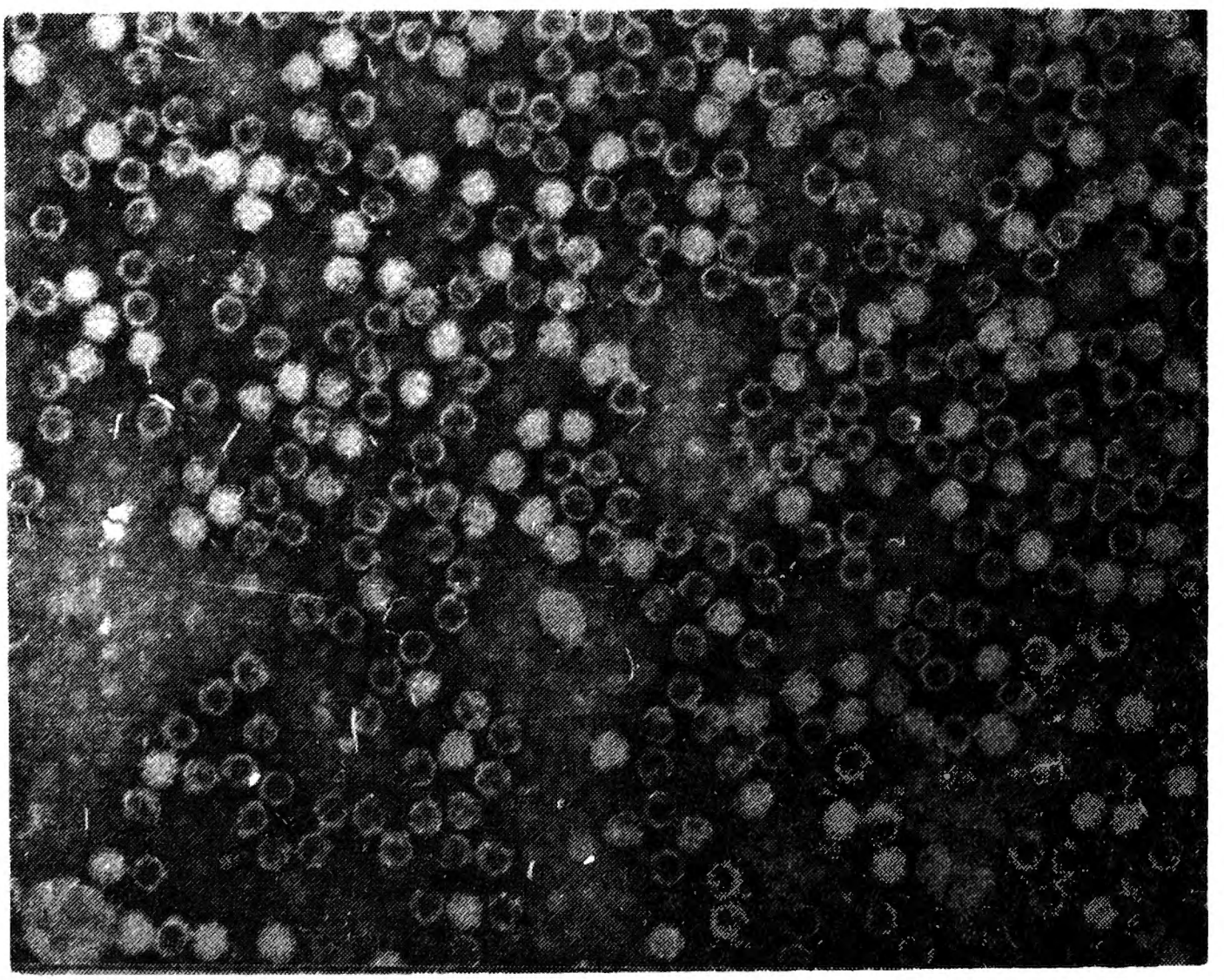
The range of host plants

The studies were conducted on 35 species of monocotyledonous plants from the family *Gramineae* and on 19 species of dicotyledonous plants from 6 families: *Chenopodiaceae, Cucurbitaceae, Solanaceae, Papilionaceae, Plantaginaceae* and *Apocynaceae.*

Virus infected only monocotyledonous plants. Among the tested plants, virus infected the following 10 grass species: *Dactylis glomerata* L., *Lolium multiflorum* Lam., *L. perenne* L., *L. temulentum* L., *L. remotum* Schrk., *Lamarckia aurea* L., *Bromus mollis* L., *Lagurus ovatus* L., *Avena sativa* L. and *A. fatua* L.

The time of virus disease incubation ranged from about 10 to about 20 days, depending on the environmental conditions. Infected plants always showed systemic symptoms.

On the leaves of infected plants of cocksfoot (*Dactylis glomerata*) there initially occurred distinct light-green or yellowish streaks (necrotized in some places). They were the most distinct on young leaves (Fig. 6a). On older leaves the symptoms of streak virus were less pronounced and resembled rather those of green-yellow mosaics (Fig. 6b). Disease symptoms on the remaining species proceeded and developed in a similar way (Fig. 7). Mosaics was also visible on the leaf sheaths and on the stems. The ears of plants infected by virus complex (filamentous and spherical viruses) were noticeably degenerated (Fig. 8).



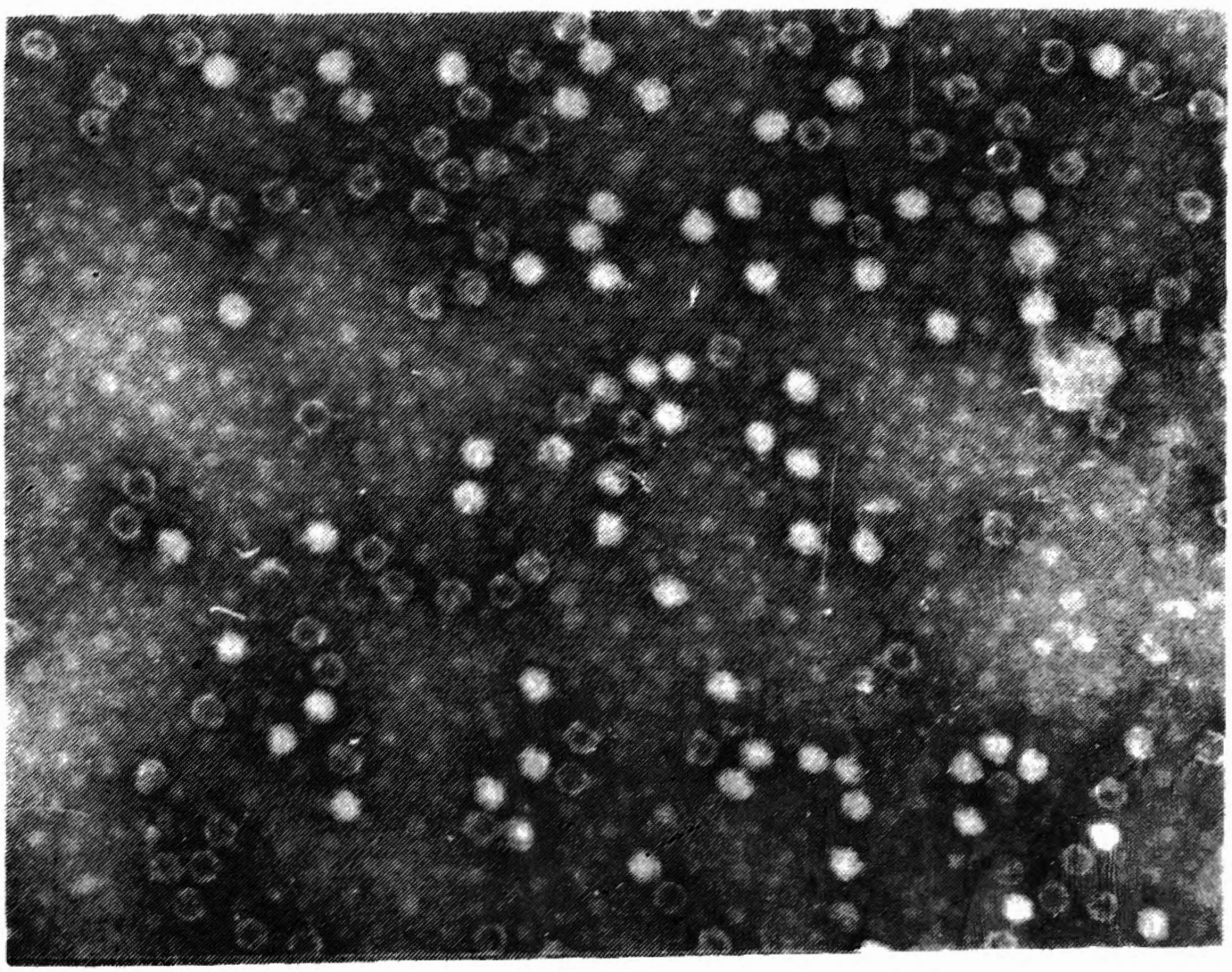
Ryc. 3. Cząstki oczyszczonego wirusa sferycznego (pow. 140 000×)

Fig. 3. Particles of purified spherical virus (140 000×)

IV. DISCUSSION AND CONCLUSIONS

According to the literature data, similar disease symptoms on cocksfoot and ryegrass plants are ascribed mostly to two filamentous viruses, i.e. to the ryegrass mosaic virus (RMV) or to the cocksfoot streak virus (CSV). The both viruses have certain common properties (mechanical transmission through sap, similar measurements of particles, some common host plants). However, they also display differences concerning transmission by vectors (some RMV strains are transmitted by mites, and CSV is transmitted by aphids), longevity in vitro (RMV remains infectious for 24—48 hours and CSV — up to 16 days) and antigenic properties (Catherall 1971; Slykhuis, Paliwal 1972).

The morphology and measurements of the studied filamentous virus were in agreement with the data of Brandes (1959, 1964) for the particles of the cocksfoot streak virus (CSV). Many authors described the ways of



Ryc. 4. Cząstki wirusa sferycznego w podczyszczonym soku z *Lolium multiflorum*; brak CSV (pow. 140 000×)

Fig. 4. Particles of spherical virus in the purified sap from *Lolium multiflorum*; the absense of CSV (140 000×)

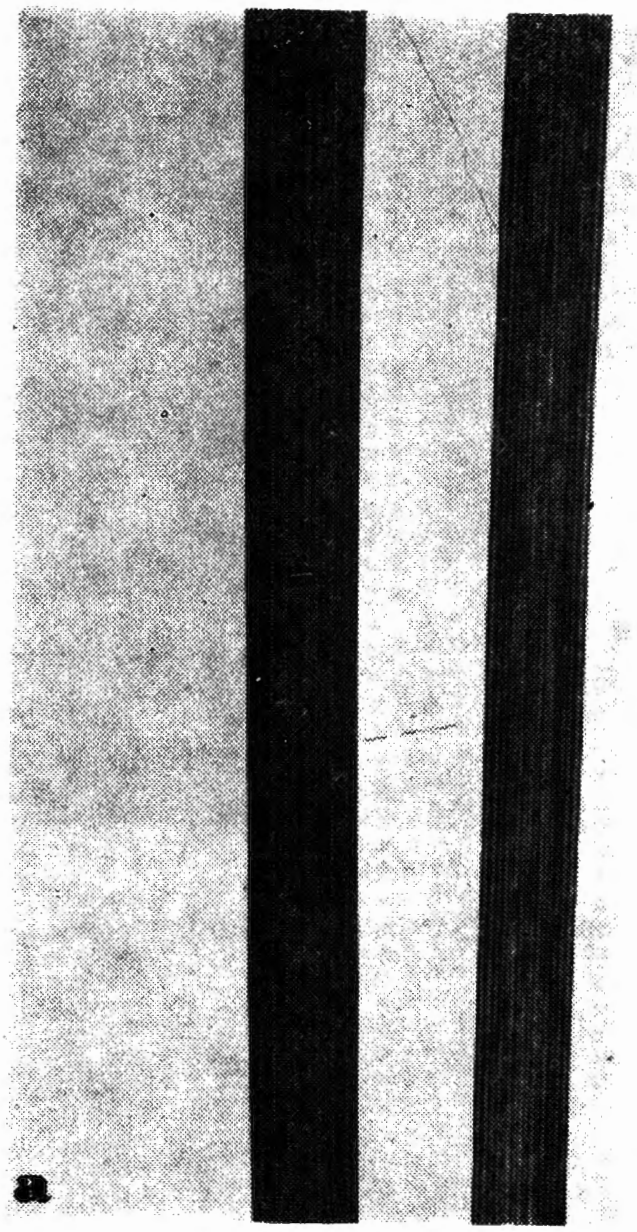
Ryc. 5. Cząstki CSV uzyskane dip method (pow. 44 000×)

Fig. 5. CSV particles obtained by the dip method (44 000×)

virus transmission for CSV, some of them mentioning besides *M. persicae*, still other aphid species — CSV vectors (Smith, 1952; Watson, Mulligan, 1959; Ohmann-Kreutzenberg 1963; Chamberlain, Catherall, 1976). The obtained in our studies virus transmission by *M. persicae* after 2-min. acquisition feeding is in agreement with the data of Watson and Mulligan (1959) and differs from the data of Ohmann-Kreutzberg (1963), according to which the author obtained CSV transmission only after 10 sec. of acquisition feeding.

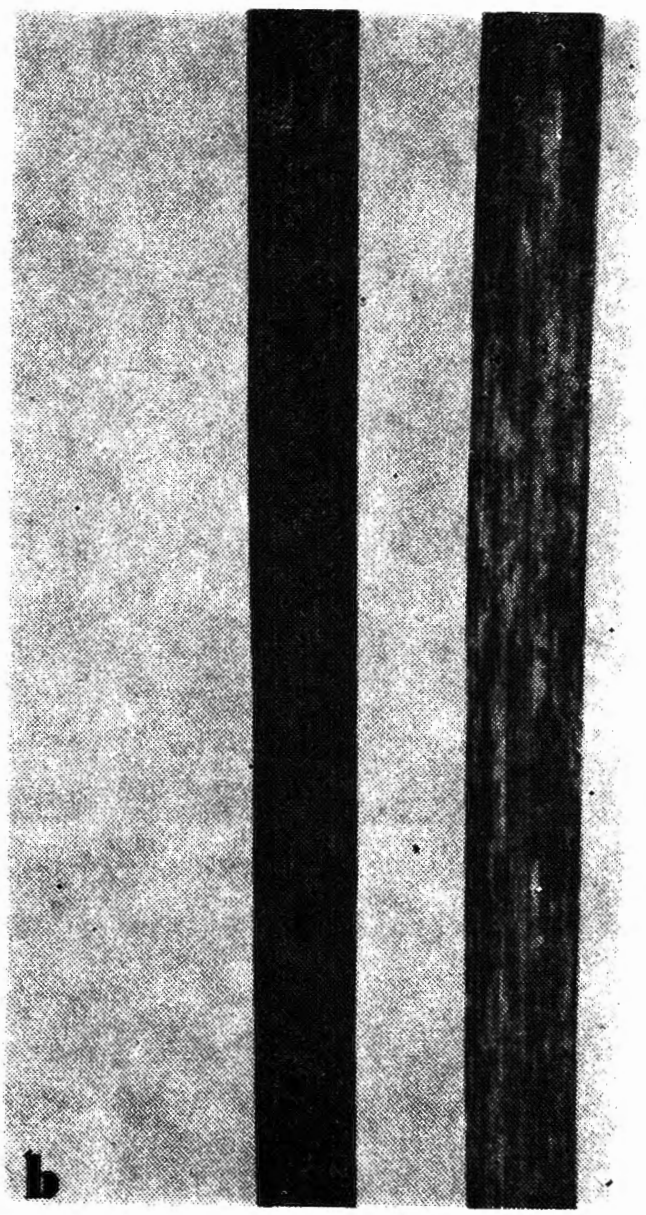
Physical properties of the studied virus were similar to CSV properties described in the literature. The thermal inactivation point (55—60°C) and longevity in vitro (over 10 days) were similar to thermal inactivation point (55°C) and longevity in vitro (up to 16 days) of the CSV studied by Ohmann-Kreutzberg (1963), whereas the dilution end-point (1 : 1000) differed markedly from dilution end — point reported for CSV (2.5 and $3 \cdot 10^{-3}$) by the mentioned author.

The range of host plants covered a smaller number of infected species than it was found in the studies of Ohmann-Kreutzberg (1963) and Schu-



Ryc. 6a. Liście *Dactylis glomerata* — rośliny zdrowej i porażonej przez CSV (objawy początkowe)

Fig. 6a Leaves of *Dyctylis glomerata* — from a healthy plant and from a CSV-infected plant (with initial symptoms)



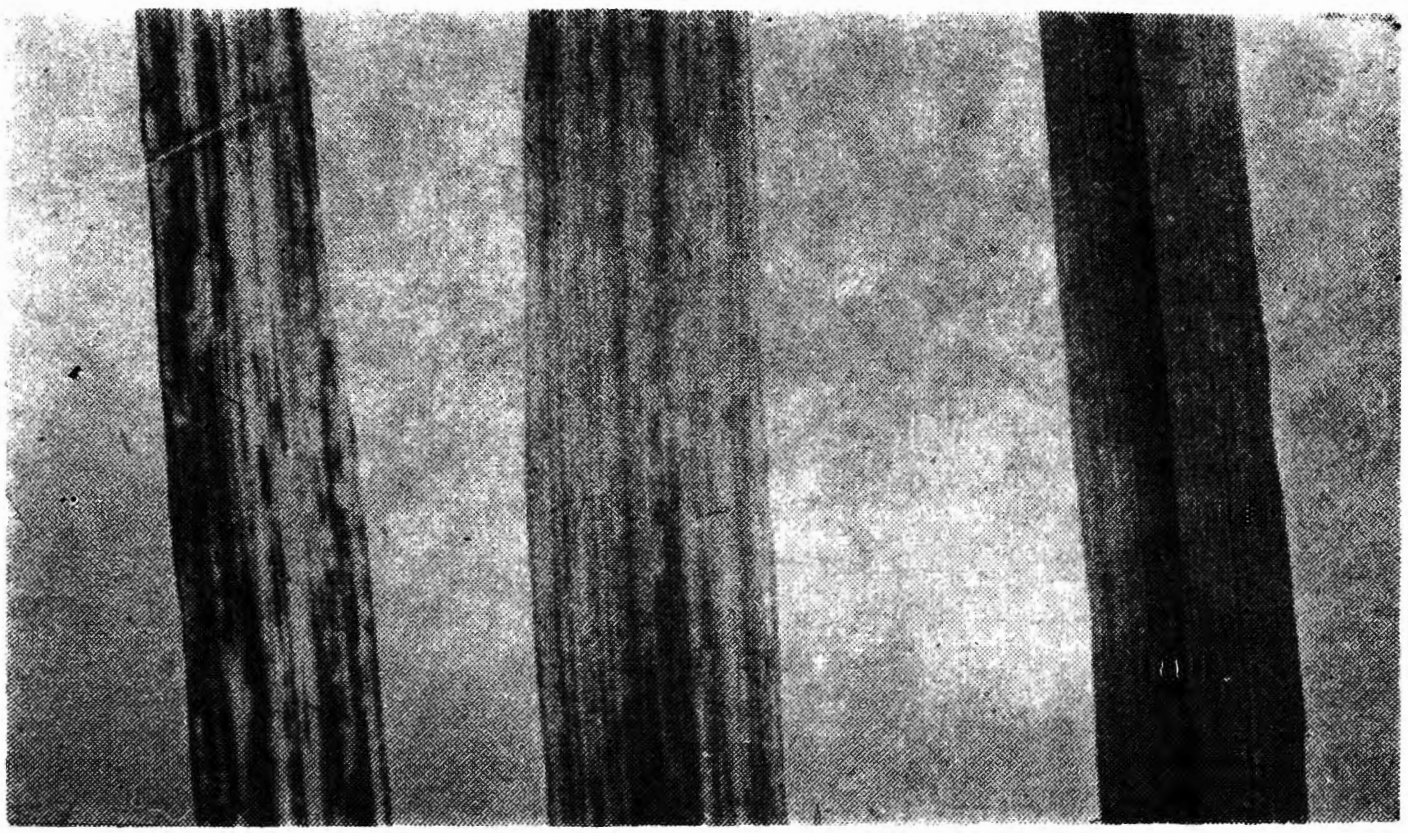
Ryc. 6b. Liście *Dactylis glomerata* — rośliny zdrowej i porażonej przez CSV (objawy późniejsze)

Fig. 6b. Leaves of *Dactylis glomerata* — from a healthy plant and from a CSV-infected plants (with later symptoms)

mann (1969). Besides that, some species given by Ohmann-Kreutzberg (1963) as symptomless CSV carriers (*Lagurus ovatus, Bromus mollis*), in our studies were observed to have disease symptoms.

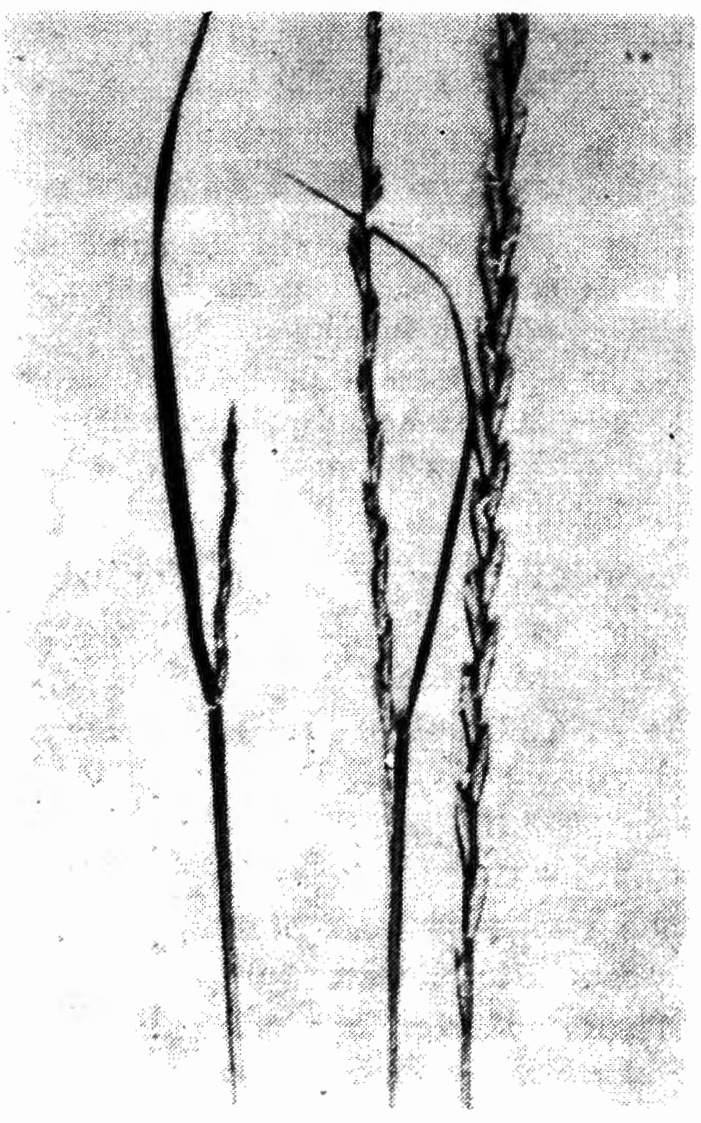
A negative result of serological tests pointed out to the lack of serological relationship between the studied virus considered to be CSV and RMV, which agrees with the literature data (Mulligan 1960).

On the basis of the obtained results of the studies concerning the ways of transmission, physical properties, the range of host plants, electron-microscopic observations, serological tests and disease symptoms, it is considered that the filamentous virus isolated from the complex is the cocksfoot streak virus.



Ryc. 7. Liście *Lagurus ovatus* — rośliny zdrowej i porażonej przez CSV

Fig. 7. Leaves of *Lagurus ovatus* — from a healthy and CSV-infected plants



Ryc. 8. Kłosy *Lolium multiflorum* odm. Szelejewski — rośliny zdrowej i porażonej (infekcja mieszana)

Fig. 8. Ears of *Lolium multiflorum* of the cv. Szelejewski — from a healthy and infected (mixed infection) plants

ACKNOWLEDGMENT

We are thankful to Docent dr. hab. W. Chmielewski for cooperation in performing tests of virus transmission by mites — *Abacarus hystrix* and to dr J. Vacke from Czechoslovakia for sending us the anti-RMV serum.

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WYSTĘPOWANIE WIRUSÓW NA UPRAWACH RAJGRASÓW I KUPKÓWKI POSPOLITEJ

STRESZCZENIE

W 7 województwach Polski zaobserwowano schorzenie wirusowe na plantacjach rajgrasów (*Lolium*) i kupkówki pospolitej (*Dactylis glomerata*). Zainfekowane rośliny wykazywały objawy mozaiki na liściach, pochwach liściowych i łodygach oraz częściową płonność kłosów.

W zainfekowanych roślinach występowały, w mieszaninie, dwa wirusy: nitkowaty — zidentyfikowany jako wirus smugowatości kupkówki (cocksfoot streak virus) i sferyczny — na razie nie oznaczony.

Wirus smugowatości kupkówki przenosił się na drodze mechanicznej (przez inokulację) i przez mszyce *Myzus persicae*. Wirus nie przenosił się przez roztocze (*Abacarus hystrix*), przez ziemię i z nasionami chorych roślin.

W badaniach nad zakresem roślin gospodarzy, spośród 54 testowanych gatunków roślin wirus zainfekował 10 gatunków traw. Były to: *Dactylis glomerata* L., *Lolium multiflorum* Lam., *L. perenne* L., *L. temulentum* L., *L. remotum Schrk.*, *Lamarckia aurea* L., *Bromus mollis* L., *Lagurus ovatus* L., *Avena sativa* L. i *A. fatua* L.

Cząstki wirusa miały kształt giętkich nitek. Długość cząstek wynosiła 750 nm a średnica — 13—15 nm.

Fizyczne właściwości wirusa, oznaczane w soku, były następujące: punkt inaktywacji termicznej mieścił się w granicach 55—60°C, graniczny punkt rozcieńczenia wynosił około 1 : 1 000, a trwałość in vitro w pokojowej temperaturze — ponad 7 dni.

Ванда Хоппе, Войцех Каневски, Хенрик Поспешны, Мария Вечорек

ПОЯВЛЕНИЕ ВИРУСОВ НА РАЙГРАСЕ И ЕЖЕ СБОРНОЙ

РЕЗЮМЕ

В 7 воеводствах Польши на плантациях райграсов (*Lolium*) и ежи сборной (*Dactylis glomerata*) появился вироз. Зараженные растения имели следующие симптомы болезни: мозаика на листьях, листовых влагалищах и стеблях и частичную пустозёрницу.

В зараженных растениях обнаружено два вируса выступающие в смеси: нитевидный — идентифицирован как вирус полосатости ежи сборной (cocksfoot streak virus) и изомерический не идентифицирован.

Вирус полосатости ежи сборной переносился механически и через *Myzus persicae*. Вирус не переносился клещами (*Abacarus hystrix*), почвой и семенами.

Среди 54 видов тестированных растений 10 видов трав подвергалось заражению. Вирус имел нитевидные частицы, гибкие о размерах 750×13—15 нм. Физические свойства вируса в соке были следующие: термическая инактивация в 55—60°С, граничная точка растворения около 1 : 1000, прочность ин витро в соке — в комнатной температуре свыше 7 дней.