SOME PHYSICAL PROPERTIES OF NORTHERN CEREAL MOSAIC VIRUS

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I. Introduction

The northern cereal mosaic disease has been recognized in eastern Hokkaido and the north-eastern portion of the Main Island of Japan. It was reported at first as wheat mosaic disease in the Tokachi district of Hokkaido in 1910 (ITO and FUKUSHI, 1956), and was considered that it was caused by nematodes or by soil acidity. ITO and FUKUSHI (1944, a, b) found the inclusion bodies in the young diseased plant cells of oat and recognized that it was caused by a virus. They also found a planthopper (*Laodelphax striatellus* FALLÉN) as the only vector, because the disease was not transmitted from diseased soil or seeds, or by mechanical inoculation; in addition, the host range of the disease was more extensive than that of the wheat mosaic disease. These characteristics showed that it was different from the wheat mosaic disease so it was named the "northern cereal mosaic disease".

Although the disease has been studied since 1943, no properties of it were known, as the virus could not be transmitted mechanically. STOREY (1933) used a needle to inject the sap of a diseased plant infected with maize streak disease into an inactive race of a virus free vector (*Cicadulina mbila*) of the disease, with the result that healthy vector became viruliferous. Since then it has been possible to learn the nature of insect-borne viruses which are not transmitted mechanically by rubbing.

This paper reports some physical properties of the northern cereal mosaic virus (NCMV) as determined by *in vitro* studies using the injection method.

II. Materials and Methods

The virus source and the nonviruliferous insects (*Laodelphax striatellus* FALLÉN) used in this experiment were kindly offered by Mr. ISHII, member of the Hokkaido National Experiment Station at Kotoni. The healthy planthoppers

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were propagated on rice seedlings, which are immune to this disease, in glass tubes 12 cm in diameter and 20 cm in height. The upper end of the glass tube was covered with cotton cloth and the tubes were kept in a room at 16–27°C. Infected planthoppers were obtained by rearing them on diseased wheat plants for 15 days.

Preliminary tests indicated that barleys and oats are very susceptible to the virus, showing more distinct symptoms than any other host. Among the barleys, the Hokuto Hadaka variety was recognized as a most susceptible one, so it was used as the test plant throughout this experiment.

The diseased plants were obtained by permitting individual viruliferous insects to feed on 3 leaf-stage seedlings in test tubes for 5 days at room temperature. After inoculation, the seedlings were removed from the test tubes and transplanted to the soil in a greenhouse. After 15 days the infected plants showing clear symptoms were harvested and the crushed juice was used as inoculum.

The injection technique was similar to that of KIMURA's method (1960, 1962). A hard-glass tube (5 mm in diameter) was pulled out while warming on a Bunsen burner to make a very fine needle, and then one end of it was again pulled out in the flame of a micro-burner. The top of the needle was cut to make a sharp point for injecting into the insect abdomen; the other end was attached to an injection needle and was coverd with paraffine to avoid leakage of the sap. Thirty healthy adult planthoppers were anesthetized with CO_2 or chloroform, and then insects were set individually in a insect holder on a microscope stage, with the ventral side up. The fine needle with sap was inserted into the abdomen as gently as possible. After injection the insects were put individually on a healthy plant in a test tube, and left there for 20 days. Then the plants were transplanted to the soil in a greenhouse to observe the results.

III. Experimental results

A. Infectivity of NCMV

The plants showing distinct symptoms 15 days after inoculation were cut off, and the leaves (ca. 2 g) were cut into small pieces. After grinding the leaves and adding the phosphate buffer solution (pH 6.98) prepared at 5°C., the juice was squeezed from them. It was then centrifuged at low speed (3,000 rpm) for 15 minutes, and then the supernatant was used as the inoculum to inject into the healthy planthoppers. The juice from healthy plants and the phosphate buffer solution were also injected as the control. It was shown that

injected planthoppers became infective and transmitted the virus to test plants, while the control showed no infectivity (Table 1).

TABLE 1. The infectivity of NCMV

Injecting source	No. of injected insects	Survivors after 10 days	No. of infected insects	Percent infected
Diseased plant juice	53	26	15	57.7
Healthy plant juice	40	30	0	0
Phosphate buffer solution	40	31	0	0

B. Longevity *in vitro* of the virus

In order to know the longevity of the virus in the partially purified juice of diseased plants, the juice was divided into two parts. One was stored at 5°C, while the other was kept at room temperature. The juices were injected into healthy planthoppers every day, and the control juice was also injected in the same manner. In the first experiment the juice which was stored for 2 days at 5°C retained its infectivity, but no insects survived the injection

TABLE 2. Longevity *in vitro* of NCMV

Injecting source (5°C)	No. of injected insects	Survivors after 10 days	No. of infected insects	Percent infected
Control	35	16	7	43.8
1 (day)	40	21	4	19.0
2	40	22	4	18.2

TABLE 3. Longevity *in vitro* of NCMV

Injecting source (5°C)	No. of infected insects	Survivors after 10 days	No. of infected insects	Percent infected
Control	35	25	9	36.0
1 (day)	30	17	4	23.5
2	30	5	1	20.0
3	30	16	2	12.5
4	35	16	1	6.3
5	35	20	0	0
6	35	17	0	0

with juice stored at room temperature (Table 2). In the second experiment the juices were stored for 6 days at 5°C, it was shown that one of the injected insects became infectious by injecting the juice stored at 5°C for 4 days (Table 3), but the insects injected with juice stored at room temperature, few survived and none showed infectivity.

C. Thermal inactivation point

The supernatant of diseased plant juice centrifuged at low speed was put in small glass tubes (0.7 cm in diameter) plugged at both ends with rubber stoppers and heated at 30, 50, and 70°C for ten minutes in hot water, and then cooled immediately in running tap water. The heated juices were injected into healthy planthoppers. Results are shown in Table 4. In this experiment the juices heated at 30 and 50°C, still showed infection, but no transmission was found at 70°C. In a second experiment, the supernatant of diseased plant juices were heated with the same manner at 50, 55, and 60°C. Results are shown in Table 5.

TABLE 4. Thermal inactivation point of NCMV

Injecting source	No. of injected insects	Survivors after 10 days	No. of infected insects	Percent infected
Control	35	26	10	38.5
30 (°C)	40	12	5	41.7
50	40	21	1	14.0
70	40	36	0	0

TABLE 5. Thermal inactivation point of NCMV

Injected source	No. of injected insects	Survivors after 10 days	No. of infected insects	Percent infected
Control	35	22	10	45.5
50 (°C)	40	21	0	0
55	35	19	0	0
60	35	11	0	0

D. Dilution end point

The experiment conducted in order to determine the dilution end point of NCMV was carried out using both diseased plants and viruliferous planthoppers. About third-stage planthoppers were fed on diseased plants for 5 days and then removed to healthy plants on which they were kept for 20 days

to pass over the latent period. The insects, the total weight of which was about 0.2 g, were ground adding 1/150 M phosphate buffer 10 times the insect weight, and then the juice was clarified by low speed centrifugation. Both diseased plant juice and infected insect juice were diluted with the same phosphate buffer solution (pH 6.98) at 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. Those juices were injected into the healthy vectors in order to assay their infectivity. The results indicated that the dilution end point of this virus was between 10^{-2} and 10^{-3} in the case of diseased plants, whereas the juice of viruliferous insects had a somewhat higher dilution end point.

TABLE 6 Dilution end point of NCMV

Injecting source		No. of injected insects	Survivors after 10 days	No. of infected insects	Percent infected
1 : 10^{-1}	plant	35	21	6	28.6
	insect	30	0	—	—
1 : 10^{-2}	plant	35	16	4	25.0
	insect	30	7	3	42.9
1 : 10^{-3}	plant	35	13	0	0
	insect	30	15	2	13.3
1 : 10^{-4}	plant	35	16	0	0
	insect	30	24	0	0

E. Infectivity of stored plant tissues frozen at −35°C

In order to determine whether diseased plant tissues still retained their infectivity after freezing at −35°C for 1 month or 1 year, the diseased plant tissues after freezing were ground in a mortar and partially clarified by low speed centrifugation, and then the supernatant fluids were injected into the insects. The results indicated that the diseased plant tissues frozen at −35°C retained their infectivity for 1 year.

TABLE 7. Infectivity of stored plant tissues frozen at −35°C

Diseased plant tissues stored at −35°C	No. of injected insects	Survivors after 10 days	No. of infected insects	Percent infected
1 month	30	16	4	25.0
1 year	30	16	5	31.3

IV. Discussion and conclusion

The northern cereal mosaic virus (NCMV) was neither transmitted to plants by diseased juice nor transmitted from diseased seed or soil, but was only transmitted by a planthopper (*Laodelphax striatellus* FALLÉN) (ITO and FUKUSHI, 1944 a). According to a recent report (ISHII, 1966), *Delphacodes albifascia* (MATSUMURA) also can transmit NCMV in the greenhouse, but till now no viruliferous insects were found in the field.

From the symptoms on the leaves of infected plants, the disease is considered to be very close to the mosaic disease of oats (Zakuklivanie), which occurs in Siberia (SUKHOV, 1938 ; MOURASHKINSKY, 1955), the wheat striate mosaic disease of North America (TIMIAN, 1960 ; SLYKHUIS, 1963), the European wheat striate mosaic disease (WATSON and SINHA, 1959 ; SLYKHUIS, 1963), and the Australian wheat striate mosaic disease (SLYKHUIS, 1963). Both northern cereal mosaic disease and mosaic disease of oats are transmitted by the same planthopper, and their host range is quite similar (ITO and FUKUSHI, 1944 a, b). The vectors of the North American and the Australian wheat striate mosaic viruses are leafhoppers, *Endria inimica* and *Nesocluta obscura*, respectively, both species belonging to the superfamily *Cicadoideae*. However, the European wheat striate mosaic virus is transmitted by a planthopper, *Delphacodes pellucida*. According to SLYKHUIS (1963), the North American wheat striate mosaic virus's vector, *Endria inimica*, does not transmit the European virus. The two leafhopper-borne viruses are not transmitted through the eggs of their vectors. Among the three planthopper-borne viruses ; European wheat striate mosaic virus, mosaic disease of oats and NCMV, the former is transovarial to its offspring (WATSON and SINHA, 1959). Although these 5 diseases appear quite similar in their symptoms, it is not known whether they are caused by the same virus or not.

NCMV was transmitted by the injection method into the planthopper, as are some leafhopper-borne viruses such as the aster yellows virus (BLACK, 1940, 1941 ; MARAMOROSCH, 1951 a, 1952), wound-tumor virus (MARAMOROSCH, BRAKKE and BLACK, 1949 ; BLACK and BRAKKE, 1952), corn stunt virus (MARAMOROSCH, 1951, b), sugar beet curly-top virus (MARAMOROSCH, 1955), rice dwarf virus (FUKUSHI and KIMURA 1960, 1962), and wheat striate mosaic virus (LEE, 1963, a, b ; LEE and BELL, 1963), and the aphid-borne virus, causing potato leafroll (HEINZE, 1955 ; DAY, 1955 ; STEGWEE and PONSEN, 1958 ; and MURAYAMA and KOJIMA, 1965). In the North American wheat striate moaic virus (LEE, 1963, b), the supernatant fluid from low speed centrifugation showed a higher infectivity than the partially purified ones, in the case of diseased plants when determined by the injecting method. The solution containing 0.01 M

$MgCl_2$ and 0.1 M glycine in 0.01 M phosphate buffer (pH 7) containing 0.85% NaCl showed higher infectivity than the 0.01 M phosphate buffer containing 0.85% NaCl. However, in the northern cereal mosaic virus a 1/30 M phosphate buffer solution (pH 6.98) containing 0.01 M $MgCl_2$, 0.1 M glycine and 0.85% NaCl, when compared with a 1/30 M phosphate buffer (pH 6.98), did not show much differences of infectivity, so we used 1/30 M phosphate buffer solution throughout the experiment.

According to our recent experiment, the infectivity obained by injecting method carried out at 5°C showed as high as, or nearly so, as that of vectors fed on diseased plants.

Abstract

NCMV was found in eastern Hokkaido and in the north-eastern portion of the Main Island of Japan. It was identified as a virus disease in 1943. As it is not transmitted by mechanical inoculation with crude sap, and is transmitted only by a planthopper (*Laodelphax striatellus* FALLÉN), few of the physical properties of this virus are known. The injecting method into insects was used in order to determine some properties of this virus. From this experiment it was known that the virus in the crude sap of the diseased plants and in viruliferous insects was transmitted to healthy planthoppers. The longevity *in vitro* of the virus was between 4 and 5 days at 5°C, but at room temperature there was no insect survival after 10 days. The thermal inactivation point of the virus was 50 to 55°C for 10 minutes. The dilution end point was between 10^{-2} and 10^{-3} in diseased plant juices, and 10^{-3} and 10^{-4} in viruliferous insects. Diseased plant tissues frozen for 1 month and 1 year under −35°C still retained their infectivity.

The mosaic disease of oats (Zakuklivanie) in Siberia is quite similar to the northern cereal mosaic disease, and both are transmitted by the same vector. The wheat striate mosaic disease that has been found in North America, Europe, and Australia had different vectors, but their symptoms were all similar, and some of the hosts were the same as those of the northern cereal mosaic disease. It is not clear whether they are caused by a same virus or not.

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