STUDIES ON CUCUMBER GREEN MOTTLE MOSAIC VIRUS IN JAPAN*

INOUYE, T., INOUYE, N., ASATANI, M. and MITSUHATA, K.

I. INTRODUCTION

In the spring of 1966, a virus disease of cucumber new to Japan occurred widely in vinyl-houses in Kinki, Chugoku, Shikoku and Kyushu district. Serious damage was done to most of the vinyl-houses of 'F_1 Kurume-Ochiai H' cucumber suffered from the disease. Especially, in Tokushima Prefecture, it was said that about 60 per. cent of vinyl-houses of cucumber, about 49 ha in area, had suffered from the disease (2). Leaves of cucumber plants infected with the disease exhibited green vein-banding and distinct dark green mosaic symptoms which were different from those induced by cucumber mosaic virus and watermelon mosaic virus. Reversible wilting of infected plants was observed commonly under the vinyl-house conditions. Fruits were severely mottled and deformed by chlorotic spotting and round-shaped swelling dark green in color.

The authors made study on the causal virus isolated from diseased plant samples collected from various localities, and identified the virus with cucumber green mottle mosaic virus (CGMMV) which had first been described by Ainsworth in 1935 in England (1). Further, to achieve the control measure, the authors conducted some experiments on transmission of CGMMV and on inactivation of the virus in infected plant tissues buried in soil by methyl bromide fumigation.

II. IDENTIFICATION OF THE VIRUS

1. *Host Range and Symptoms*

Inoculation tests were conducted in the usual manner using carborundum as an abrasive. To confirm virus infection, back inoculation was made to the test plants such as cucumber, petunia and *Datura stramonium*. For the same purpose, virus particle observation on dip-preparations was made under the electron microscope. Surface of inoculated leaves was sterilized by Na_3PO_4 (10—15 min. in 3 % solution) and washed thoroughly with detergent and running water when back inoculation was made from the inoculated leaves.

In Table 1, host range of CGMMV and symptoms in susceptible plant species are summarized. As seen in the table, CGMMV infects systemically many species of cucurbit plants. It infects locally tobacco, petunia and *Datura stramonium*. Other plant species tested were all found to be not susceptible to CGMMV.

* This article is compiled, based upon the results in the papers written in Japanese in Nogaku Kenkyu, 51, (4) published in 1967 (12, 13, 14). This work was supported by the grant-in-aid for Co-operative Research, Scientific Researches of the Ministry of Education in 1965.

TABLE 1
Host Range of CGMMV

Susceptible plant species	
Benincasa cerifera	Diffuse chlorotic spotting, mottling
Citrullus vulgaris	Leaf yellowing, necrosis, leaf distortion, mosaic, reversible wilting
Cucumis melo	Yellow dots
C. melo var. *conomon*	Yellow dots
C. melo var. *makuwa*	Yellow dots
C. sativus	Vein-banding, severe mottling, reversible wilting, mottled and deformed fruits
Cucurbita maxima	Yellow mosaic, stunting
C. moschata	Faint chlorotic blotch
C. pepo	Yellow mosaic, stunting
Lagenaria leucantha var. *clavata*	Green vein-banding in some plants, others are symptomless
L. leucantha var. *gourda*	Mottling
Luffa cylindrica	Faint mottling in some plants, others are symptomless
Momordica charantia	Veinal chlorosis, mild mottling, stunting
Datura stramonium	Local chlorotic or necrotic spots, no systemic infection
Nicotiana tabacum var. Samsun	Local latent infection
N. tabacum var. White Burley	Local latent infection
Petunia hybrida	Local lesions in various forms, sometimes local latent infection
Nonsusceptible plant species	
Beta vulgaris, Brassica rapa, Callistephus chinensis, Capsicum annuum, Chenopodium amaranticolor, Gomphrena globosa, Lactuca sativa, Lycopersicon esculentum, Nicotiana glutinosa, N. rustica, Phaseolus angularis, P. vulgarls, Raphanus sativus, Soya max, Spinacia oleracea, Tetragonia expansa, Vicia faba, Vinca roseae, Zinnia elegans	

2. *Physical Properties in vitro*

Physical properties of CGMMV in vitro were examined, using cucumber seedlings as the test plant. CGMMV was inactivated at temperatures of 80 to 90°C for 10 minutes exposure. In one experiment, it was still infective at 85°C. It withstood dilution to 10^{-6} and aging for 8 months at 20°C.

3. *Transmission*

CGMMV is easily transmitted by plant juice, inter-plant contact and manual handlings. It is transmitted through infected soil and probably through seeds, but not by *Aphis gosypii* (?) and cucurbit leaf beetle. Results of a series of experiments on transmission of CGMMV are described in the next chapter.

4. *Cross Protection Test with CGMMV and TMV*

Mottled leaves of five Samsun tobaccos infected with TMV were challenge-inoculated with CGMMV. About 20 days after the challenge-inoculation, back inoculations were made to cucumber seedlings from challenge-inoculated leaves of

which surface was sterilized by 3 % Na_3PO_4 solution and washed thoroughly with detergent and running water. All the cucumber seedlings became infected with CGMMV, though the incubation period was prolonged compared with the seedlings inoculated with CGMMV alone. In another test, leaves of five Samsun tobacco plants were heavily inoculated with CGMMV on three successive days. Ten days after the final inoculation, inoculated leaves were challenge-inoculated with TMV. All the challenge-inoculated tobacco plants became infected with TMV, although the appearance of symptoms was delayed 2–5 days compared with the tobacco plants inoculated with TMV alone.

5. *Electron Microscopy*

Numerous TMV-like rod-shaped particles are observed in dip-preparations of infected plants. Modal length of CGMMV particles in dip-preparation mounted in phosphotungustate appeared to be 300 mμ in length and 18 mμ in diameter.

6. *Purification*

CGMMV is easily purified by the following method. Frozen cucumber leaves were disrupted in a blender adding M/10 phosphate buffer and 1 : 1 mixture of chloroform and butanol. The mixture was squeezed with two layers of gauze and centrifuged at 1, 000 *g* for 10 minutes. Aqueous layer was further clarified by a centrifugation for 10 minutes at 10, 000 *g*. After two or three cycles of high and low-speed centrifugations (60 min. at 70, 000 *g* and 10 min. at 1, 000 *g*), purified virus suspension was obtained.

7. *Serology*

Antisera to CGMMV were prepared in rabbits by injecting purified virus suspension intravenously and intramuscularly with the use of Freund's adjuvant.

TABLE 2
Cross reactions among CGMMV, TMV and ORSV in microagglutination test

Antiserum	Antigen	Antiserum diluted (1:)									Saline
		8	16	32	64	128	256	512	1024	2048	
CGMMV	CGMMV	++++	++++	++++	++++	++++	+++	+++	+	−	−
	H	−	−	−	−	−	−	−	−	−	−
	TMV	−	−	−	−	−	−	−	−	−	−
	ORSV	++++	++++	++++	+++	++	±	−	−	−	−
TMV	CGMMV	++++	+++	++	±	−	−	−	−	−	−
	TMV	++++	++++	++++	++++	++++	+++	++	+	−	−
	H	−	−	−	−	−	−	−	−	−	−
	ORSV	++++	++++	+++	++	±	−	−	−	−	−
ORSV	CGMMV	−*	−*	−*	−*	−	−	−	−	−	−
	TMV	++++	+	−	−	−	−	−	−	−	−
	ORSV	++++	++++	++++	++++	++++	+++	+++	+	−	−
	H	−	−	−	−	−	−	−	−	−	−

Source of the antigen: CGMMV (cucumber), TMV (tobacco), ORSV (cattleya).
H: Extracts of healthy cucumber, tobacco, and cattleya, from the top.
* Deubtful reactions were observed

In microagglutination tests, the specific titers of the antisera were 1: 1, 024 and 1: 2, 048. The antisera did not react with the juice of healthy cucumber. Cross reactions in microprecipitin test were conducted, using CGMMV, TMV and ORSV (Odontoglossum ringspot virus) and the respective antisera. Table 2 shows the results.

Anti-CGMMV serum reacted with ORSV, but not with TMV. On the other hand, CGMMV reacted with anti-TMV serum. The reactions between CGMMV and anti-ORSV serun are still inconclusive. Further experiments on the serological relationship among the viruses in this virus group are being continued by the authors.

III. SOME EXPERIMENTS ON VIRUS TRANSMISSION

1. *Experiments on Seed Transmission of CGMMV*

(1) *Recovery of CGMMV from 'F_1 Kurume-Ochiai H' Seeds Commercially Produced in 1965*

Since the occurrence of CGMMV in cucumber in vinyl-houses in the spring of 1966 was almost exclusively limited to one variety, F_1 Kurume-Ochiai H, the virus-infected seeds in this variety seemed to be within the bound of possibility as the first source of the epidemic. The seeds of the variety commercially produced in 1965, therefore, were tested for the possibility of seed transmission of the virus.

Seeds were divided in 29 groups, each containing 10 seeds. Seed coats and embryos of each seed group were ground in 2 and 4 ml of water, respectively, and the sap extracts were used for electron microscopy and inoculation on the cotyledons of cucumber seedlings. In some experiments the embryos were sown in sterilized soil for testing the seed transmission of the virus, resulting, however, in no infections. In the sap extract of the embryos both virus particles and infectivity could not be demonstrated by electron microscopy and bioassay. In the sap extract preparations from 5 groups of seed coats many rod-shaped particles were detected by electron microscopy and recovery of infectivity was made successfully. Most of the rod-shaped particles were short particles, and only 8.3 per cent. of the measured particles had the length of 300 ± 25 mμ.

As the seed groups containing the rod-shaped particles coincided with the seed groups from which infectivity was recoverd by inoculation, it can be concluded that at least one out of ten seeds in the seed group had been contaminated with infectious virus particles in the seed coat. Thus, it can be inferred that about 1.7 per cent. of the cucumber seeds used in the tests can have carried CGMMV in their seed coats. However, no seedlings produced symptoms when 404 seedlings out of the same batch of the seed samples were grown.

(2) *Infections Caused dy Artificially Contaminated Seeds*

Cucumber seeds contaminated artificially with CGMMV on their surfaces were sown to test whether or not the seedlings grown from them would reveal

any symptom of infection. Healthy seeds were immersed in the sap extract of the infected leaves, dried on a piece of filter paper, and sown in the sterilized soil, 5—6 seeds per 15 cm pot. Half of the seeds were injured on a part of the seed coat before immersing in the sap extract. At the stage when the cotyledons fully expanded, half of the seedlings of each plot were removed from the pots and replanted in the same pots. Infection of cucumber plants was determined finally on the basis of the electron microscopy of dip-preparations from the youngest leaves from 30 to 40 days after sowing.

TABLE 3
Seedling infection through seeds of which surface was contaminated with the sap extract of CGMMV-infected cucumber leaves

Experiment	Uninjured seeds		Injured seeds*	
	A**	B	A	B
I	0/16***	——	1/17	——
II	0/57	1/55	1/56	2/55
III	0/56	1/50	1/53	1/54
IV	1/58	0/57	0/58	0/56
V	0/58	0/57	——	——

* Seeds were injured by cutting a small portion of seed coat. ** Seedlings were not transplanted (A) and transplanted (B). *** Numerator is the number of cucumber plants infected; denominator is the number of plants raised from the treated seeds. Infections occurred as early as at the 1st or 2nd leaf stage. The dates of sowing, transplanting, and final examination are as follows: Exp. I; 1. Jl., ——, 6. Ag.: Exp. II; 25. Jl., 1. Ag., 6. Ag.: Exp. III; 3. Ag., 9. Ag., 30. Ag.: Exp. IV; 1. Sep., 7. Sep., 3. Oct.: Exp. V; 20. Sep., 29. Sep., 30. Oct.

Results are seen in Table 3. Three out of 464 seedlings grown from the uninjured seeds were infected with the virus as early as the second leaf stage. Some of the infected seedlings grown from the injured seeds seemed to have been caused by the infection of embryo due to the deep injury getting to the embryo. After the occurrence of these seedling infection, new occurrence of infection of cucumber plants was not observed until the 6—7 leaf stage. It was inconclusive whether or not transplanting at the cotyledonary stage increased the frequency of infection.

Seeds obtained from artificially infected plants were sown after the storage of about one month. Each seedling was transplanted at the cotyledonary stage in the sterilized soil in a 15 cm pot. Observations were continued until the 6—8 leaf stage. One out of 102 plants was infected on the 35th day after sowing.

2. *Experiments on the Infection with CGMMV through Soil*

(1) *Infectivity of CGMMV Associated with Root Debris in Soil*

Infected cucumber seedlings were raised in 15 cm pots, 7 seedlings per pot. After symptoms appeared on three or four upper leaves, aerial parts of the seedlings were cut off, and then the pots containing the underground parts were buried at the depth of 0 cm (ground level), 25 cm, and 50 cm in soybean field (on the 25th of July). The soil in some other pots was turned over and repacked in the

same pots, which were so buried that the soil surface of the pots was at the same level as that of the field. In another plot the pots were submerged in plastic containers. After about 30, 60, and 70 days the fragments of cucumber roots with adhering soil particles were collected, crushed in mortars in M/100 phosphate buffer at pH 7.0. The sap extracts were rubbed on the cotyledons of cucumber seedlings and tested also electron microscopically.

TABLE 4

Aging of CGMMV in infected cucumber roots buried in soil in soybean field*

Buried at the depth of	Duration of burying					
	1 month		2 months		ca. 70 days	
	A***	B****	A	B	A	B
1 cm**	+	5/5*****	+	1/3	+	0/4
25 cm	− ?	3/5	+	0/3	+	1/3
50 cm	+	4/5	+	0/3	+	2/4
Turned over soil'	+	5/5	+	0/3		
Submerged soil"	+	5/5	+	3/3		

* The aerial parts of the infected cucumber plants raised in 15 cm pots were cut off and these pots were buried in each plot on the 25th of July. ** Depth at the top of the pot buried, measured from the soil surface of the field. *** Electron microscopy of the extract from the root fragments with adhering soil particles. **** Bioassay on cucumber seedlings with the extract used for electron microscopy. ***** Numerator is the number of cucumber plants infected and denominator is the number of plants inoculated. ' The infected soil was loosened and refilled. " Pot filled with the infected soil was immersed in water in plastic container.

As shown in Table 4, the decomposition of the remaining root fragments had not so extremely proceeded after one month burying, and the virus particles and infectivity could be easily demonstrated. Burying for two months or 70 days, however, resulted in almost thorough decomposition of the roots, and it was hard to pick up the segments of roots from the soil in the pots that had been turned over or buried at the surface of the field. On the other hand, in the submerged plot no apparent decomposition of the roots was observed, and a large number of virus particles and strong infectivity were demonstrated. Although the demonstration of a few virus particles from the soil in the pots that had been turned over or buried at the soil surface of the field was made successfully, consistent infectivity could not always be recovered, possibly because the number of cucumber seedlings used was insufficient.

(2) *Infection of Cucumber Roots with CGMMV*

Inoculation of CGMMV to Cucumber Roots Cucumber seedlings raised in sterilized soil were removed, their roots were washed in tapping water, and dipped for one minute in the sap extract of the infected leaves. Then, each of the seedlings was transplanted in a 10 cm pot filled with sterilized soil. As seen in Table 5, the symptoms began to appear as early as 6 days after inoculation in some plants, while it took about one month for some other plants to develop the symptoms. When the experiments were closed, 30–35 per cent. of the plants

TABLE 5
Infection of cucumber plants by artificial inoculation to roots with the sap extract of CGMMV-infected cucumber leaves

Experiment	Growth stage of seedlings inoculated	No. of plants inoculated	No. of plants infected	Incubation period	Electron microscopy and bioassay of symptomless plants*			
					No. of plants examined	R+** L+	R+ L−	R− L−
I	1st leaf	19	14	6—18 d.	5	0	0	5
II	2nd leaf	45	35	11—30 d.	10	5***	0	5

* Specimens of electron microscopy were prepared from the youngest leaves by dip method and from the extract of roots. Finally examined 30 days after inoculation in Exp. I, and 32 days in Exp. II. ** R+ ; roots are infected: R− ; roots are uninfected: L+ ; leaves are infected: L− ; leaves are uninfected. **** Although 3 out of the 5 plants were (R+, L—) as a result of electron microscopy, all the plants were shown to be (R+, L+) by inoculation test.

remained healthy in appearance, out of which 5 plants in experiment II were shown by electron microscopy and bioassay to be virus-infected both in the youngest leaves and roots, but 5 plants in experiment I and 5 plants in experiment II proved to be virus-free.

Infection through Cucumber Roots in the Soil Permeated with the Sap Extract of CGMMV-Infected Cucumber Leaves Cucumber seedlings were raised in sterilized soil in 10 cm pots, 3 seedlings per pot, and at three different stages of growth, 10 ml of 1/10 dilution of infected plant juice was dripped into each pot by a syringe so as to avoid the contact with the aerial part of the seedlings. Half of the plants in each plot were injured at their roots by pricking the soil with a pincette before dripping the infected juice.

TABLE 6
Infection of cucumber seedlings with CGMMV through roots in the soil permeated with the sap extract of infected cucumber leaves

Growth stage of seedlings at treatment*	Roots were	
	Injured	Uninjured
Cotyledon	0/30**	0/27
1st leaf	0/30	1/30
2nd leaf	1/18	0/21

* The soil of each plot was permeated with the 1/10 dilution of the extract of infected cucumber leaves on the 4th of October, and injuries were given to roots with pincette on the previous day. ** Numerator is the number of cucumber plants infected and denominator is the number of plants treated.

As shown in Table 6, a small percentage of infection could be obtained. Also, in another set of experiment, 2 out of 30 plants were infected when the infected juice was dripped into the soil before the pricking. It was not clear whether or not the ratio of infection could be increased when the roots were injured, because the percentage of infection was not high enough to discuss.

Infection of Cucumber in the Soil Contaminated with CGMMV-Infected Cucumber Root Fragments. The aerial parts of the infected cucumber plants raised in pots were cut off, and the soil containing the live roots was piled on a sheet of slate and turned over thoroughly, then repacked in pots, which was used as the 'infected soil'.

In one experiment, germinating cucumber seeds were sown on the day when the 'infected soil' was prepared. Three out of 126 seedlings grown were infected as early as at their primary leaf stage of growth, but the additional new infections did not occur until the 5th or 6th leaf stage of growth when the experiment was closed. In another experiment, the 'infected soil' was covered with about 1 cm layer of sterilized soil to avoid the direct contact between 'infected soil' and seeds sown. Just after the preparation of these pots containing the 'infected soil' coated with sterilized soil, 228 cucumber seeds were sown on the layer of sterilized soil, and 130 germinating seeds were sown on the day next. There was, however, no early infection in both of the plots.

Also, 86 seedlings were transplanted into the same infected soil 8 days after the preparation, but no infections were observed in the early stages of growth.

3. *Experiments on the Transmission of CGMMV by Plant-to-plant Contact and Manual Handlings*

(1) *Infection Caused by Plant-to-plant Contact*

Five cucumber seedlings were raised in a 15 cm pot, 4 healthy seedling were at the rim and one diseased at the center, the latter of which was inoculated by rubbing with an infected leaf at the primary leaf stage, to see whether or not infections could be caused by natural contact either between leaves or roots. To avoid contact either between aerial parts or roots, infected plant was isolated from the other healthy plants, using a vinyl tube. Experiments were carried out under the glasshouse conditions.

Seven out of 20 plants were infected by contact between the aerial parts of the plants, while no infection was obtained by contact between the roots. On the other hand, infections took place by contact between roots when cucumber plants were raised in hydroponics in plastic containers. However, it is possible that some injuries may have been given to the roots at the renewals of the nutrient solution, which may affect the result.

(2) *Artificial Inoculation by Plant Contact and Manual Handlings*

Some sorts of artificial inoculation by contact and manual handlings as shown in Table 7 were made on cucumber seedlings. The virus was easily transmitted by all the methods listed, suggesting high frequency of transmission of the virus by manual handlings during cropping.

(3) *Disinfecting Effects of Some Chemicals against CGMMV*

Infected leaves were rubbed with fingers, and the fingers were dipped for 5

TABLE 7
Transmission of CGMMV by various ways of contact and handling

Ways of contact and handling	Infection
1. Cotyledons of healthy seedlings were rubbed with the cut end of infected leaf	10/10*
2. Cotyledons of healthy seedlings were rubbed gently with infected leaf	10/10
3. Infected leaves were rubbed with fingers, and then cotyledons of healthy seedlings were rubbed gently with them	12/12
4. Infected leaves were cut with scissors, and then cotyledons of healthy seedlings were cut with them	3/20
5. Sap extract of infected leaves was dropped on the surfaces of cotyledons of healthy seedlings	1/12

* Numerator is the number of cucumber plants infected and denominator is the number of plants inoculated.

seconds in water, 1% Lipon F (an anionic detergent), 10% Teepol, or 3% trisodium orthophoshate (tsop), washed for a moment in tapping water, and then rubbed the primary leaves of cucumber seedlings. Inoculation by rubbing was also applied on seedlings by the fingers that were contaminated with the virus but cleaned by soap and running water. In another experiment each of 3% tsop and 10% Teepol solutions was mixed in various proportions with the sap extract of the infected leaves, and the resultant solutions were used for the bioassay on cucumber seedlings and electron microscopy.

TABLE 8
Disinfection of CGMMV-contaminated hands and infectious cucumber sap by chemicals

Experiment	Untreated	Virus-contaminated hand was immersed in*					Nine volumes of sap extract were mixed with one volume of		
		Water	1% Lipon F	10% Teepol	3% Na_3PO_4	Soap**	3% Na_3PO_4		10% Teepol
I	16/16***	16/16	12/16	10/16	0/16	0/16	1/16′		——
II	10/10	9/10	——	0/10	0/10	0/10	0/10′	0/10″	8/10

* Fingers that rubbed infected leaves were dipped for 5 seconds in each of the solutions, washed for a moment in tapping water, and then the first leaves of healthy cucumber seedlings were rubbed by them on their surfaces. ** Fingers were sufficiently cleaned with soap and tapping water. *** Numerator is the number of cucumber plants infected and the denominator is the number of plants inoculated. ′ Treated for 5 minutes. ″ Treated for 1 hour.

Results are presented in Table 8. Water and 1% Lipon F solution could not eliminate the virus particles effectively from the fingers. The effect of 10% Teepol solution was not always consistent, in one experiment giving a positive result but in another little, if any, effect. Both 3% tsop solution and washing with soap proved to be effective for the disinfection of the virus in both experiments repeated. When 3% tsop and 10% Teepol were poured into the 1/10 dilution of the extract of the infected leaves and reacted for 5 minutes, the former gave a considerable effect but the latter little effect.

As 3% tsop solution was mixed in various proportions into the 1/20 dilution of the infected leaves and reacted for 5 minutes, a considerable inactivating effect was observed in the preparation containing 20 vol. per cent. of 3% tsop solution, but no noticeable effect in the preparations containing less than 20 vol. per cent. of the solution. However, mixing of 10 vol. per cent. of 3% tsop solution and reaction for 30 minutes brought about a complete inactivation of the virus.

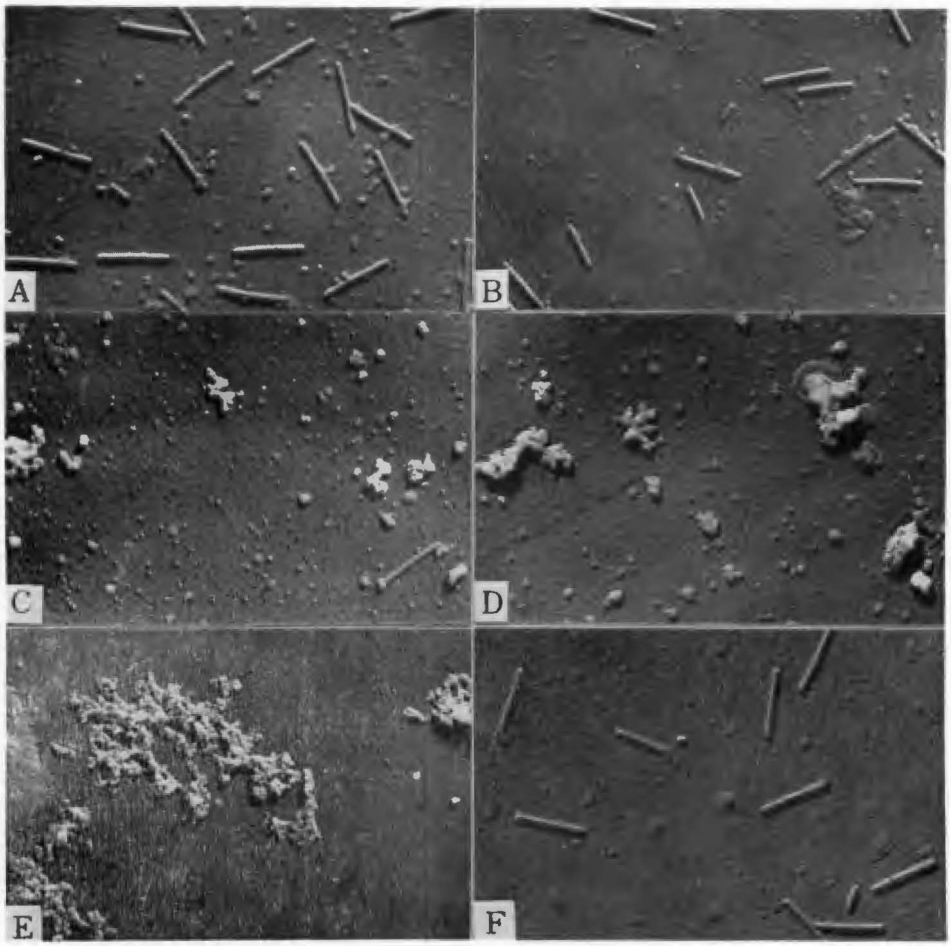


Fig. 1. Disintegration of CGMMV particles in the sap extract of infected leaves mixed in various proportions with trisodium orthophosphate. 3% solution of tsop was mixed into 1/20 dilution of the sap extract of infected cucumber leaves in the proportions of 0 (A), 2.5 (B), 5.0 (C), 10.0 (D), and 20% (E). 10% solution of Teepol was mixed in the proportion of 20% for comparison (F).

Figure 1 shows the effect of tsop on CGMMV particles. Most of the virus particles in the preparations containing 5 vol. per cent. of 3% tsop solution were broken down, and on mixing more than 10 vol. per cent. of the solution no virus particles could be demonstrated by electron microscopy. With N/10 NaOH in

comparison with tsop, pHs of the sap extracts of the infected leaves were adjusted to 9.0, 10.0, 11.0, and 11.5, and virus particles in the sap extracts were examined electron microscopically. At pH 11.0 and 11.5 considerable and complete collapse of the virus particles occurred, respectively. From the results, therefore, it may be inferred that the active principle of tsop against the virus consists in its high values of pH.

IV. INACTIVATION OF CGMMV IN INFECTED PLANT TISSUES BURIED IN SOIL BY METHYL BROMIDE

Since, as mentioned in the previous chapter, CGMMV persists for a long period of time in infected cucumber tissues and it is impossible, in practice, to remove the refuses of infected plants entirely from the soil, there may be the risk of causing the second epidemic in the following season when cucumber is planted again in the fields, vinyl-houses, or the equipments for gravel culture that have been attacked by this virus. Effective chemical agents for the practical purpose to eradicate CGMMV are not known hitherto. However, a few chemicals, such as methyl bromide (15, 31) and ethylene oxide (25), have so far been reported to be effective against TMV. Also, Saito et al. (22) reported that methyl bromide was found to be an excellent chemical against soil-borne wheat mosaic virus. But, on the other hand, Broadbent et al. (9) reported that methyl bromide was not effective against TMV in tomato under the glasshouse conditions in winter.

The authors made a series of experiments to see whether or not methyl bromide could inactivate CGMMV in plant tissues in soil.

1. *Materials and Methods*

Methyl bromide, packed in ampoules or cans, was supplied from Kunoshima Chemical Industry Co., Ltd. Experiments were carried out during from July to October in 1966.

Two series of experiments were made. In the first series, 20 *l* plastic buckets were used as containers for the fumigation of methyl bromide, and, in the second series, experiments were done with semi-field scale.

One gram of green, or air-dried leaves, stems, and roots of CGMMV-infected cucumber was wrapped in cotton gauze and buried in soil in both series of experiments. Closed plastic containers were placed in the airy shade out of doors. Materials were buried at the depth of 20 cm from the soil surface in a plastic container and an ampoule with a given dosage of methyl bromide was placed on the surface. The container was covered with a vinyl sheet and sealed tightly with vinyl tape. Ampoule was then broken with pincers under the vinyl sheet. Five hundred grams of chloropicrin per m^3 of soil were applied as a control chemical, injecting into soil by a syringe. In one experiment, TMV-infected green, or dry tobacco leaves were also buried in the plastic container together with CGMMV-infected cucumber tissues, and treated with methyl bromide.

In field tests, virus materials were buried at three different depths, 1, 20,

and 30 cm, in each of the plots to be treated, and in the untreated control plots. Each of the plot was covered with a 5 m^2 vinyl sheet, and the center space under the sheet was set between 15 and 30 cm in hight. The edges of the sheet were tightly covered with soil. A given dosage of methyl bromide was introduced into, and evaporated in, the space under the vinyl sheet by a rubber tube connected with a can from the outside of the plot.

Materials were treated for 24 or 48 hours in the tests of container series, and 48 hours in the field series. The infected plant tissues treated with methyl bromide were ground in a mortar in 10 ml of M/100 pH 7.0 phosphate buffer, and the sap extracts were used for inoculation on cucumber, *Datura stramonium*, or petunia seedlings for CGMMV, and *N. glutinosa* for TMV.

2. *Effect of Methyl Bromide on the Infectivity of CGMMV in Infected Cucumber Tissues Buried in the Soil in Tightly Sealed Plastic Container*

In Table 9 the results of the treatment with methyl bromide of CGMMV-infected cucumber tissues as well as TMV-infected tobacco tissues buried in the soil in closed plastic containers are shown.

TABLE 9
Inactivating effect of methyl bromide against CGMMV and TMV in infected cucumber and tobacco tissues buried in soil in sealed plastic container (1)

Time of treatment (hr.)	24				48				
Dosage (g/m^3)	0	160	320	640	0	160	320	640	Chloropicrin
CGMMV in cucumber									
Green leaves	10*	0	0	0	10	0	0	0	10
Dry leaves	10	0	0	0	10	0	0	0	10
Live stems	10	1	0	0	10	0	0	0	10
TMV in tobacco									
Green leaves	>800**	99	61	1	>800	13	6	0	>750
Dry leaves	>750	147	101	0	>800	44	25	0	>600

* Number of infected cucumber plants out of 10 plants inoculated. ** Total number of local lesions on 3 leaves of *N. glutinosa*. Tested on the 25—26. July. Maximum temperature; 33.7°C: Minimum temperature; 23.7°C: Average temperatures; 29.1 and 27.9°C.

Methyl bromide was found to be effective on the inactivation of both CGMMV and TMV, while chloropicrin could not inactivate the viruses.

TMV was completely inactivated when treated at the dosage of 640 g/m^3 (ca. 4 $lb/100\ ft^3$) of methyl bromide for 48 hours. This result is in consistence with that of Johnson et al. (15). Local lesions produced on the leaves of *N. glutinosa* on inoculation with TMV treated at different dosages are shown in Fig. 2.

CGMMV, on the other hand, was completely inactivated by methyl bromide at the dosages of more than 160 g/m^3 (ca. 1 $lb/100\ ft^3$) for 24 or 48 hours in all the plant tissues, except for the incomplete effect in the tissues of live stems treated for 24 hours.

To know whether or not CGMMV could be inactivated with smaller dosages of methyl bromide than 160 g/m^3, CGMMV-infected green leaves and roots were

Fig. 2. Inactivation by methyl bromide of CGMMV (A) and TMV (B) in plant tissues buried in soil (cf. Table 9).

(A) Left : *D. stramonium* leaf inocnlated with CGMMV fumigated at the dosage of 55 g/m^3 for 48 hours.

Right : *D. stramonium* leaf inoculated with CGMMV fumigated at the dosage of 480 g/m^3 for 48 hours.

Right half-leaves were inoculated with untreated control.

(B) From the left to the right, N. glutinosa leaf inoculated with TMV fumigated at the dosage of 0, 160, 320, and 640 g/m^3, respectively.

treated at four dosages, 55, 110, 160, and 480 g/m^3, for 24 or 48 hours. Table 10 shows the results obtained. All the dosages were sufficient to inactivate CGMMV after 48 hours as bioassayed on cucumber seedlings. On 24 hours treatment, however, complete inactivation could not be obtained at the minimum dosage of 55 g/m^3. Although three doubtful local lesions were produced on 3 half-leaves of *Datura stramonium* on inoculation with the sap extract of the infected green leaves treated at 160 g/m^3 for 48 hours, they might have little, if

TABLE 10
Inactivating effect of methyl bromide against CGMMV in infected cucumber tissues buried in soil in sealed plastic container (2)

Time of treatment (hr.)		24			48				
Dosage (g/m³)		55	110	160	55	110	160	480	0
Material	Bioassayed on								
Green leaves	Cucumber	1*	0	0	0	0	0	0	10
	Datura				3/>900**	0/>4100	0/>3100	0/>1150	
Live roots	Cucumber	0	0	0	0	0	0	0	10
	Datura				0/>1000	0/>900	0/>1200	0/>3200	

* Number of infected cucumber plants out of 10 plants inoculated. ** Numerator is the sum of the number of local lesions on 3 half-leaves of *Datura stramonium* inoculated with the fumigated material; denominator is the sum of the number of local lesions on 3 half-leaves inoculated with untreated material. Tested on the 8—9. August. Maximum temperature; 34.5°C: Minimum temperature; 24.6°C: Average temperatures; 28.7 and 29.4°C.

any, effect on the result because *Datura stramonium* as test plant was shown to be more diverse and lower in susceptibility to CGMMV than cucumber.

It must be noted that all the results described above were obtained under high temperature conditions during summer. Under the cooler conditions in autumn, however, the minimum dosage of methyl bromide for the complete inactivation of CGMMV increased to 160 g/m³ on 48 hours treatment, which is about three times as much dosage as obtained in summer (Table 11). Also, TMV could not be any longer inactivated at 640 g/m³ for 48 hours.

TABLE 11
Inactivating effect of methyl bromide against CGMMV and TMV in infected cucumber and tobacco tissues buried in soil in sealed plastic container (3)

Time of treatment (hr.)	48				
Dosage (g/m³)	0	160	320	480	640
CGMMV in cucumber leaves	15*	1	0	0	0
TMV in tobacco leaves	1501**	570	325	28	41

* Number of infected cucumber plants out of 15 plants inoculated. ** Total number of local lesions on 4 leaves of *N. glutinosa*. Tested on the 18—19. October. Maximum temperature; 21.8°C: Minimum temperature; 8.7°C: Average temperatures; 14.1 and 16.0°C.

3. *Effect of Methyl Bromide on the Infectivity of CGMMV in Infected Cucumber Tissues Buried in Soil in a Field*

As, in the former experiments made by using the closed containers, methyl bromide proved to inactivate CGMMV, two experiments were carried out under the field conditions in summer. In the first experiment infected cucumber leaves were buried at three depths of 1, 20, and 30 cm from the ground level, and treated separately at the dosages of 18, 36, and 54 g/m², for 48 hours. CGMMV in all the infected cucumber leaves buried in soil at any depth was completely inactivated at the minimum dosage of methyl bromide, 18 g/m².

TABLE 12
Inactivating effect of methyl bromide against CGMMV in infected cucumber tissues buried in soil in soybean field in summer

Location of the treating material		Dosage g/m²			
Distance from the nozzle	Buried at the depth of	0	18	36	54
0.3 m	0.2 m	—	0*	0	0
	0.3 m	—	0	0	0
0.6 m	0.01m	10	0	0	0
	0.2 m	10	0	0	0
	0.3 m	10	0	0	0

* Number of infected cucumber plants out of 10 plants inoculated. Fumigated for 48 hours. Tested on the 18–19. August. Maximum temperature ; 31.3°C: Minimum temperature; 24.9°C: Average temperatures; 28.9 and 28.0°C.

In the second experiment, to examine the distribution of the fumigant in soil, infected cucumber leaves were buried at the distance of 30 and 60 cm from the nozzle for fumigation, but the other conditions such as the burying depths, dosages, and time of fumigation were the same as the former experiment. As shown in Table 12, CGMMV in soil was inactivated at the minimum dosage of 18 g/m³, regardless of the burying spots and depths.

4. *Morphology and Antigenicity of CGMMV Particles Treated with Methyl Bromide*

From the materials treated with methyl bromide for 48 hours in closed plastic containers, 1/500 dilutions of the extracts were prepared and examined electron microscopically. The ratio of the short particles seemed to increase after the treatment, though complete collapse of the virus particles was not observed. The ratio of the virus particles 300 ± 25 mμ in length to all the virus particles measured were 37, 57, 50, and 36% in materials treated at 55, 110, 160, and 480 g/m³, respectively, whereas 67% in the untreated materials. Inactivated virus particles, after the treatment with methyl bromide, did not lose their antigenicity and reacted with anti-CGMMV serum in the microagglutination test.

V. DISCUSSION

On the basis of the host range of the virus that is nearly limited to *Cucurbitaceae*, the strong stableness, and the morphological resemblance to TMV with modal length of 300 mμ and the width of 18 mμ, the virus under discussion is concluded to be identical with cucumber green mottle mosaic virus. CGMMV was first described by Ainsworth and the type strain of this virus was named as cucumber virus 3 in 1935, and thenceforth the virus has been reported from many countries in Europe (1, 3, 11, 16, 18, 21, 23, 24, 26, 32). Other strains of this virus are known hitherto, such as cucumber aucuba mosaic virus (=cucumber virus 4, *Cucumis* virus 2A) in Europe (1, 3, 11, 27, 28) and *Cucumis* virus 2c and its variants in India (19, 29, 30).

The virus reported in this article, however, has several points that are inconsistent with the nature of the virus originally described by Ainsworth as well as of the strains of CGMMV reported. This virus is infective to *Cucurbita pepo* and solanaceous plants including *N. tabacum*, whereas Ainsworth's CV 3 is not. Furthermore, as the symptoms on cucumber leaves induced by this virus are distinct from the aucuba mosaic, this virus cannot be identified as CV 4. Although CV 4 produces clear necrotic local lesions on the leaves of *N. tabacum* var. Samsun according to Brčák et al. (3), this virus infects locally the same host without any visible symptom. The virus seems to be somewhat similar in the host range to *Cucumis* virus 2c in India, but the former produces apparent symptoms in *Datura stramonium*, watermelon, and loofah, which are infected symptomlessly by the latter virus.

There was once a controversy with respect to the relation between CGMMV and TMV in some aspects. Rochow (20) concluded as a result of cross protection test that CV 3 was one of the special strains of TMV, whereas Fulton (10) and Brčák et al. (3) asserted that CV 3 was a virus distinct from, and independent of, TMV. There are some similarities, however, between the two viruses in their morphology and physical properties, and a weak, but positive, serological reaction between the two viruses was observed (a strong reaction was also noticed by Kristensen (18)). For these reasons, some workers regarded CGMMV as a strain of TMV. Nevertheless, Knight (17) showed that CGMMV was a distinct virus because CGMMV had different chemical components as compared with those of TMV. The results of the cross protection test between the two viruses in this study are essentially in agreement with those of the previous workers, no clear cross protection effect being observed.

As the results of the serological reactions including CGMMV, TMV, and ORSV, CGMMV, in so far as the results of experiments obtained by the authors are concerned, seems to be related serologically distantly with ORSV and TMV. Further experiments on the serological relationship among CGMMV, ORSV, and TMV are under investigation, and the detailed results about the problem will be reported in another paper.

Since the first outbreak of CGMMV in cucumber in Japan in the spring of 1966 was almost exclusively limited to one variety, F_1 Kurume-Ochiai H, the seed-borne or contaminated virus in this variety was suspected to be the first source of the epidemic. Except that the affirmation of the infection through seeds produced in 1965 could not be obtained, the possibility of the seed transmission of the virus cannot be denied because virus particles were dectected from the seed coats. Also, a limited number of seedlings out of the seeds that had been artificially contaminated with the virus on their surfaces developed the symptoms of infection.

According to Van Koot et al. (28) and Yakovleva (32), the ratio of seed transmission of CGMMV is fairly high when seeds are tested after the short period of storage, e. g., within a year. In this study, however, only one out of 102

seedlings grown from the seeds obtaind from the artificially infected cucumbers was found to be infected. This result is contradictory to those of Van Koot et al. and of Yakovleva, and it is still inconclusive whether or not the difference was caused by the difference of the virus strains or of the cucumber varieties used.

Broadbent, in his series of reports on the epidemiology of tomato mosaic (5, 6, 8), showed that TMV infections occurred by the seed-transmitted virus only when tomato seedlings were transplanted, whereas no clear correlation was established in this study between the ratio of seed transmission and transplanting in CGMMV.

As in the case of TMV in tomato, soil transmission in CGMMV is suspected to be another important source of the primary infection, though no detailed works as to this point have appeared. Rydèn (21) reported that irrigated water contaminated with CGMMV could be a source of the occurrence, and that the virus particles could pass through the layer of soil at least 80 cm. Although experiments on the soil transmission were insufficient in this study and many must be done in the future, the following facts were noticed. The remaining roots decayed much more rapidly in the field conditions in summer than in the submerged conditions, and the nearer the surface the roots were buried in soil, the more rapidly proceeded the decaying. In well turned over soil decomposition was fairly rapid. Nevertheless, it would take a much longer period of time for the virus to disappear completely from the soil. In practice, the root reminants buried in deeper soil than tested in this study may become an impotant problem in the future.

Cucumber roots seem to be a little lower in susceptibility to CGMMV than the aerial parts of the plants, because all the plants inoculated at roots were not always infected in spite of 100 per cent. infection as inoculated on leaves, and it takes more days for some plants to produce the symptoms as inoculated at roots than the plants inoculated on the leaves (the delay in symptom appearance is not so long as TMV in tomato).

Spread of the disease was implied to be caused by natural contact of cucumber leaves and manual handlings during cucumber growing as in the case of tomato mosaic. (4, 7) To reduce the chances of touching the plants with contaminated hands or implements, trisodium orthophosphate proved to be as effective against CGMMV as against TMV (4), while Teepol did not give consistent results. The high values of pH of tsop solution seem to be the immediate cause of the inactivating effect, and this chemical may be recommended as a disinfectant against CGMMV.

According to Broadbent et al. (9), TMV in tomato buried in soil was not inactivated by methyl bromide at 2 lb/100 ft^3 (ca. 97 g/m^3) for 120 hours under glasshouse conditions in winter. However, Wiggs et al. (31) get positive results and showed that fumigation at 3 lb/100 ft^3 of soil for 48 hours was sufficient to inactivate TMV buried in soil in closed containers. Also, Johnson. et al. (15) reported that from 3 to 4 lb of methyl bromide per 100 sq. ft were required for the complete inactivation of TMV on 48 hours treatment.

The results obtained by the authors were that fumigation at 640 g/m³ (ca. 4 lb/100 ft³) for 48 hours was needed to inactivate TMV buried in soil in closed plastic containers. This is in agreement with those of Wiggs et al. and Johnson et al. At cooler temperatures, as in autumn, however, larger dosages of the chemical were required for the inactivation of TMV, which also coincides with the result of Wiggs et al.

Furthermore, it became evident from the results obtained in this series of tests that CGMMV could be more easily inactivated with methyl bromide than TMV. For instance, methyl bromide is used in Japan at ca. 33 g/m³ against Phytophthora rot of cucumber in vinyl-house, and at ca. 18 g/m³ against the same disease in the field. If used at high temperatures as in summer, there may be some prospects of the practical application of methyl bromide against CGMMV, for, according to the authors' results, 18 g/m³ for 48 hours inactivated the virus in the field tests in summer.

However, as fumigations were made only for the infected cucumber tissues buried at the maximum 30 cm in soil and it is not clear whether or not methyl bromide can be also effective to inactivate CGMMV in infected roots remained deeper in soil, and as the mechanisms of the virus transmission in soil and the permeability of methyl bromide through soil are still unsolved, it will be too early to say that methyl bromide can be used in practice. At least implements in vinyl-house and equipment for gravel culture may be fumigated effectively with this chemical under high temperature conditions.

According to Saito et al. (22), purified soil-borne wheat mosaic virus particles collapsed when treated with methyl bromide. Nevertheless, CGMMV particles retained larger portions of their morphology after treatment, except that cutting of the virus particles seemed to occur and the number of segments of the particles were observed to increase somewhat.

Antigenicity of the inactivated CGMMV remained unaffected after treatment. To give a solution to the mechanisms of inactivation by methyl bromide of the virus, and to find a reason why TMV is stabler against methyl bromide than CGMMV, are the future problems to be solved.

SUMMARY

In the spring of 1966, a virus disease of cucumber occurred widely and caused a serious damage in vinyl-houses at many localities in the west part of Japan. The causal virus was identified with cucumber green mottle mosaic virus (CGMMV) new to Japan in this paper. Vein-banding and distinct green mottling of leaves, reversible wilting of infected plants, as well as severe mottling of deformed fruits are the characteristic symptoms in cucumber. The causal virus infects various species of cucurbit plants systemically and produced distinct symptoms in many of the susceptible plants. It forms local lesions in petunia and *Datura stramonium*. In Samsun and White Burley tobacco, infection is local without visible symptoms. The virus in infected plant juice is inactivated at 80—

90°C for ten minutes exposure. It withstands dilution to 10^{-6} and aging for 8 months at 20°C. Particles of the virus appear as TMV-like straight rods 300 mμ in length and 18 mμ in diameter. No apparent cross protection was observed between the virus and TMV when tested in Samsun tobacco. Purified virus was obtained from the infected cucumber leaves, clarified by chloroform-butanol mixture and following differential centrifugations. Antiserum with titer of 1 : 2, 048 was prepared from rabbits, injecting the purified virus intravenously and intramuscularly. In the microprecipitin test, CGMMV reacted with anti-TMV serum. On the other hand, anti-CGMMV serum was observed to react with ORSV, but not with TMV.

CGMMV was detected in the extract of seed coats of F_1 Kurume-Ochiai H cucumber seeds produced commercially in 1965, which might suggest the possibilities of seed transmission of the virus. However, no seedling infection was obtained out of about 400 seedlings raised from the same batch of the seeds of the variety. Seedling infection was confirmed through seeds which had been artificially contaminated with the virus, immersing cucumber seeds into the infected leaf juice just before the sowing. Only one instance of seed transmission was observed out of 102 seedlings grown from the seeds obtained from the infected cucumber plants.

CGMMV associated with root debris of infected cucumber was recovered after at least 4 months' burying in soil under the field conditions during the period of summer and autumn, although a considerable decrease of virus particles and infectivities were noticed in infected soil samples buried near the surface of the ground and in those of loosened soil. Under the submerged conditions, on the other hand, a great deal of virus was recovered without appreciable decomposition. A small ratio of seedling infection through soil occurred when diseased plant juice was dripped into the soil in which cucumber seedlings were grown, and also when cucumber seedlings were transplanted into the soil containing roots of infected plants. So far as tested, however, no seedling infection was observed when cucumber seeds were sown in the soil containing the roots of infected plants.

CGMMV was easily transmitted by inter-plant contact at their aerial parts and virus contaminated tools and hands. For the purpose of disinfection of virus contaminated hands, clean washing with soap, dipping in Teepol or Na_3PO_4 solution were proved to be effective. Above all, Na_3PO_4 was demonstrated to be very effective in breaking down the virus particles, which was probably due to the high values of pH of the solution. In the tests of insect transmission, both *Aphis gossypii* (?) and cucurbit leaf beetle did not transmit the virus.

Methyl bromide was confirmed to be effective to inactivate CGMMV in green and dried plant tissues of infected cucumber. The minimum dosages of the chemical for the complete inactivation of CGMMV in plant tissues buried in soil in tightly sealed 20 *l* containers were found to be 55 g/m^3 for 48 hr. and 110 g/m^3 for 24 hr. of fumigation in summer, although it was 320 g/m^3 for 48 hr. in cool season. On the other hand, TMV was hardly inactivated by methyl bromide at the dosage of 640 g/m^3 for 48 hr. of fumigation in summer. Chloropicrin showed

no effect on the inactivation of the viruses. In the field tests conducted in mid-summer, CGMMV buried in soil at the depths of 1—30 cm from the ground level was inactivated completely by methyl bromide at the dosage of 18 g/m^3 for 48 hr. of fumigation. Accompanying a little increase in the ratio of short particles, modal length of the particles of CGMMV inactivated by methyl bromide fumigation did not altered compared with the untreated virus, and the strong antigenicity of the inactivated virus was also demonstrated.

Acknowledgement The authors express their sincere thanks to Mr. N. Sasaki, Mr. T. Yamamoto and Mr. Y. Kashiwagi of Tokushima Agr. Exp. Sta., and Dr. H. Ishii of Tokushima Agr. High School for the collection of plant samples and the survey of the disease occurrence, and also to Dr. Y. Komuro of Institute for Plant Virus Research, and Mr. K. Tomaru of Hatano Tobacco Exp. Sta. for the varuable advices on virus identification and virus transmission.

LITERATURE CITED

1. Ainsworth, G. C. 1935. Mosaic diseases of the cucumber. Ann. appl. Biol. 22 : 55—67.
2. Annual Meeting of Plant Quarantine in Chugoku-Shikoku Region in 1966.
3. Brčák, J., Ulrychová, M. and Čech, M. 1962. Infection of tobacco and some Chenopodium species by the Cucumber Virus 4 (3) and by its nucleic acid. Virology 16 : 105—114.
4. Broadbent, L. 1963. The epidemiology of tomato mosaic. III. Cleaning virus from hands and tools. Ann. appl. Biol. 52 : 225—232.
5. Broadbent, L. 1965a. The epidemiology of tomato mosaic. VIII. Virus infection through tomato roots. Ann. appl. Biol. 55 : 57—66.
6. Broadbent, L. 1965b. The epidemiology of tomato mosaic. XI. Seed transmission of TMV. Ann. appl. Biol. 56 : 177—205.
7. Broadbent, L. and Fletcher, J. T. 1963. The epidemiology of tomato mosaic. IV. Persistence of virus in clothing and glasshouse structure. Ann. appl. Biol 52 : 233—241.
8. Broadbent, L. and Fletcher, J. T. 1966. The epidemiology of tomato mosaic. XII. Sources of TMV in commercial tomato crops under glass. Ann. appl. Biol. 57 : 113—120.
9. Broadbent, L., Read, W. H. and Last, F. T. 1965. The epidemiology of tomato mosaic. X. Persistence of TMV-infected debris in soil, and tbe effects of soil partial sterilization. Ann. appl. Biol. 55 : 471—483.
10. Fulton, R. W. 1950. Cross protection tests with cucumber viruses 3 and 4 and tobacco mosaic virus. Phytopath. 40 : 219—220.
11. Ges', D. K. 1965. Mozaichnye bolezni ogurtsov zakrytogogrunta. Dokl. Akad. Nauk belorussk. SSR 9 : 555—557. (R. A. M. 45 : 56, 1966)
12. Inouye, T., Inouye, N., Asatani, M. and Mitsuhata, K. 1967. Studies on cucumber green mottle mosaic virus in Japan. I. Identification of the virus. Nogaku Kenkyu 51 : 175—186. (in Japanese)
13. Inouye, T., Inouye, N., Asatani, M. and Mitsuhata, K. 1967. Studies on cucumber green mottle mosaic virus in Japan. II. Some experiments on virus transmission. Nogaku Kenkyu 51 : 187—197. (in Japanese)
14. Inouye, T., Inouye, N., Asatani, M. and Mitsuhata, K. 1967. Studies on cucumber green mottle mosaic virus in Japan. III. Inactivation of CGMMV in infected plant tissue buried in soil by methyl bromide. Nogaku Kenkyu 51 : 199—207. (in Japanese)
15. Johnson, E. M. and Chapman, R. A. 1663. Chemical inactivation of tobacco mosaic virus in tomato roots. Pl. Dis. Reptr. 47 : 389—391.
16. Klinkowski, M. and Uschdraweit, H. A. 1960. Die Virosen der Gemüsepflanzen. (Pflanzliche Virologie Bd. II. Klinkowski, M.) Akademie-Verlag, Berlin.
17. Knight, C. A. 1955. Are cucumber viruses 3 and 4 strains of tobacco mosaic virus? A review

of the problem. Virology 1 : 261—267.
18. Kristensen, H. R. 1956. Virussygdomme hos Agurker i Danmark. Horticultura 10 : 161—172. R. A. M. 36 : 165, 1957.
19. Report of the Division of Mycology and Plant Pathology. 1954. Sci. Rep. Agr. Res. Inst. N. Delhi 1951—1952 : 75—87. (R. A. M. 34 : 348—350, 1955)
20. Rochow, W. F. 1956. Interference with tobacco mosaic virus infection by cucumber viruses 3 and 4. Phytopath. 46 : 133—137.
21. Rydèn, K. 1966. Investigations on the spread of cucumber green mottle mosaic virus (*Cucumis* virus 2 Smith) by watering. Viruses of Plants (Edited by Beemster, A. B. R. and Dijkstra, J. 7 pp. 317—319. North-Holland Bub. Co., Amsterdam.
22. Saito, Y., Takanashi, K., Iwata, Y. and Okamoto, H. 1964. Studies on the soil-borne virus diseases of wheat and barley. III. Influence of chemicals on the infected soils and the viruses. Bull. Nat. Inst. Agric. Sic., Series C, 17 : 41—59.
23. Smirnova, Mme V. A. and Shteĭn-Margolina, Mme V. A. 1962. Issiwdovanie virusa Ogurechnoĭ mosaiki (*Cucumis virus 2*) v èlektronnom mikroskope. C. R. Acad. Sci. U. S. S. R. 144 : 1384—1386. (R. A. M. 42 : 69, 1963)
24. Smith, K. M. 1949. Viruses and virus diseases. J. R. Hort. Soc. 74 : 482—491. R. A. M. 29 : 198.
25. Tomaru, K. and Nishida, K. 1966. Inactivation of tobacco mosaic virus by ethylene oxide. (Abstract) Ann. Phytopath. Soc. Japan, 32 : 94.
26. Uschdraweit, H. A. 1955. Das Grünscheckmosaik der Gurke. NachrBl. Dtsch. PflSchDienst 7 : 150—151. (R. A. M. 35 : 269, 1956)
27. Valentin, H. 1958. Das Gurkengelbmosaik (*Cucumis* virus 2A, Smith). NachrBl. Dtsch. PflSchDienst. 10 : 93—94.
28. Van Koot, Y. and Van Dorst, H. J. M. 1959. Virusziekten van de Komkomer in Nederland. Tijdschr. Plziekt. 65 : 257—271. (R. A. M. 39 : 527—528, 1960)
29. Vasdeva, R. S., Raychaudhuri, S. P. and Singh, J. 1949. A new strain of *Cucumis* virus 2. Ind. Phytopath. 2 : 180—185. (R. A. M. 30 : 259—260, 1951)
30. Vasdeva, R. S. and Nariani, T. K. 1952. Host range of bottle-gourd mosaic virus and its inactivation by plant extracts. Phytopath. 42 : 149—152.
31. Wiggs, D. N. and Lucas, G. B. 1962. Inactivation of tobacco mosaic virus by volatile chemicals. Phytopath. 52 : 983—985.
32. Yakovleva, N. 1965. Bor'ba s zelenoĭ mozaikoĭ ogurtsov. (Control of green mosaic of cucumber.) Zashch. Rast. Vredit. Bolez. 10 : 50—51. (R. A. M. 45 : 234, 1966)

EXPLANATION OF PLATE

Plate I. Symptoms of CGMMV in F_1 Kurume-Ochiai H cucumber (A, B, and C), watermelon (D), and *Cucurbita pepo* (E).

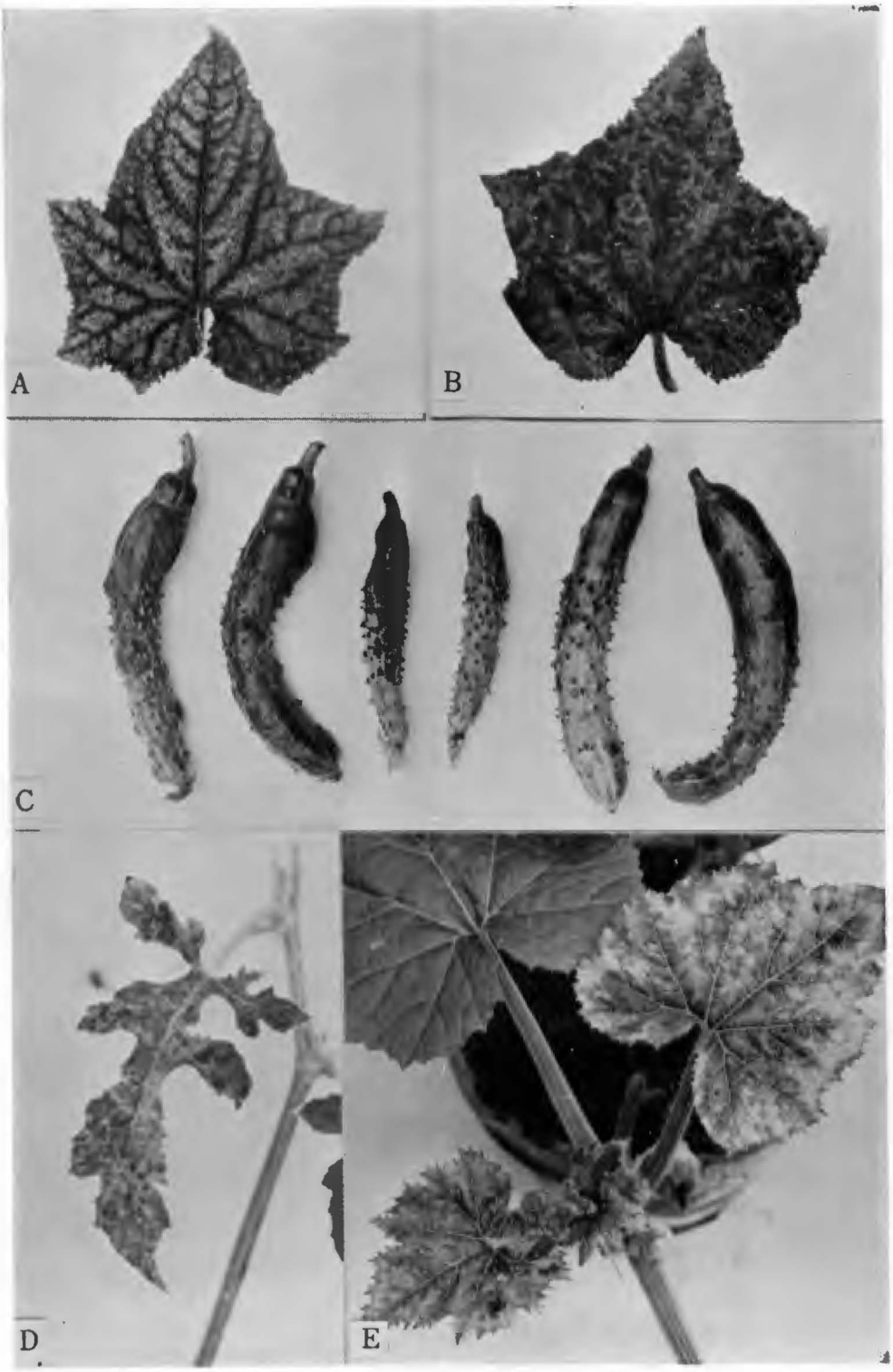
Plate II. Systemic symptoms of CGMMV in *Cucumis melo* var. *conomon* (A), and *Lagenaria leucantha* (B).

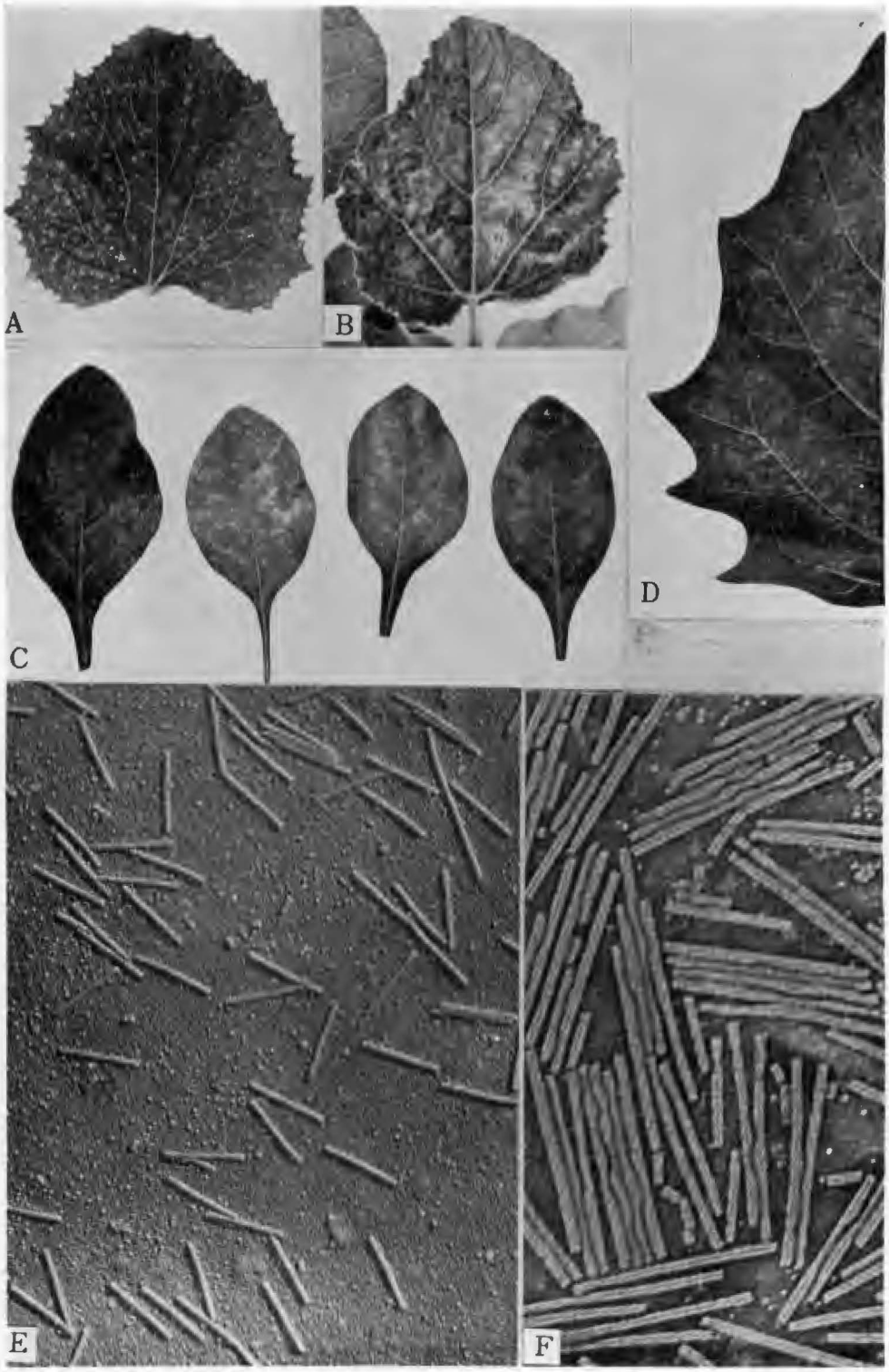
C Various forms of local lesions formed in petunia.

D Local lesions in *Datura stramonium*.

E Particles of CGMMV in dip-preparation (Cr-shadowing, × 40, 000).

F Negatively stained particles of CGMMV in dip-peparation mounted in 1% phosphotungstate (× 100, 000).


A
B
C
D
E


A
B
C
D
E
F