A MOSAIC DISEASE OF RADISH[1]

T. T. KOU[2]

Introduction

Radishes grown in the vicinity of Taipei in autumn of 1957 were infected with a certain mosaic virus, its symptoms consist of a systemic clearing of the veins, followed by general mottling, with little or no distortion of the leaves. This disease was rather serious and caused considerable losses. To determine whether or not this is identical with some of the virue diseases of crucifers already reported from other countries, observation and experiments were made on symptoms, host range, transmission and properties of the virus concerned. The results so far obtained are presented in this paper.

Materials and Methods

The radish mosaic virus used in the present studies was collected from a mature systemically infected radish plants (horticultural variety: Mei Nung Tsao Sheng) grown in the vicinity of Taipei. After the successful establishment of the virus on young radish plants grown in the green house by mechanical inoculation, successive transfers were made to healthy seedlings at frequent intervals in order to provide a constant source of fresh inoculum. Seedlings of *Nicotiana tabacum* var. Turkish were selected as the standard test plant for recovery of virus from infected plants and for property studies.

The plants grown in the green house were frequently sprayed with 1: 1000 Malathion for a complete remove of insects. Inoculation was made by rubbing with the thoroughly agitated virus abrasive suspension, over the surface of one or two lower leaves (Beraha, 1955; Rawlin, 1936).

For aphids transmission trials, adult insects inhabiting the non-viruliferous, mature radish plants were transferred to the stock-virus plant and allowing them to feed for 48 hours or more. The resulting viruliferous aphids were then transferred to the test plant by means of a camel's hair brush. Care has been exercised not to make the brush contact with the test plant. Occasionally a

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(2) Assistant Research Fellow, Institute of Botany, Academia Sinica.

piece of leaf with aphids was detached from the stock plant and placed on a small piece of paper, which then was allowed to rest on a leaf of the test plant until the aphids migrated to the new plant.

For the purpose of studying physical properties of the virus, the affected leaves were first macerated with a pestle in a sterile mortar in the presence of sterile water. The crude extract was filtered through several layers of cheesecloth. For the determination of dilution end point the original extract was diluted to the desired concentrations with N/15 phosphate buffer solution (pH 7). For each dilution a total amount of 100 ml aliquot was drawn for the inoculation, a single pipette being used for each transfer. Inoculation was made first with the highest dilution, followed by successively lower dilutions. For the determination of thermal inactivation point, the expressed juice was first centrifuged at 1500 r. p. m. for 5 minutes, then the supernatant fluids were transferred to each thin-walled glass tubes (2 mm in diameter) which were sealed in a flame at one end and plugged with cotton at the other. The tubes were kept in a bath of desired temperature for a 10-minute period. The temperature was kept constant within ±0.1°C. At the end of the period the sealed end of the tubes was broken and the heated extract was transferred into a test tube, with which the inoculation was made. Determination of longevity *in vitro* was made by storing the centrifuged filterate (5 minute at 1500 r. p. m.) in test tubes plugged with cotton in the incubator kept at 20°C–22°C. A single test tube was removed at a given interval and the contents were used as inoculum without dilution. All glasswares were sterilized before use.

Experimental results

(*A*) *Transmission*

(*1*) *Mechanical transmission*

It has already been confirmed that the radish mosaic virus can be readily transmitted to healthy radish seedlings by means of the carborundum method (Rawlin, 1936) or by rubbing the leaves with cotton dipped in expressed juice of a diseased plant without abrasive. A higher percentage of infection, however, was obtained when powdered carborundum (400 mesh) was used. The incubation period ranged from 12 to 22 days with an average of about 18 days.

(*2*) *Aphids transmission*

Studies on insect transmission of this disease involved the used of the green peach aphid (*Myzus persicae* Sulzer.), false cabbage aphid (*Rhopalosiphum pseudobrassicae* Davis.), and turnip aphid or cabbage aphid (*Brevicoryne brassicae* L.). All of them were collected from radishes and other cultivated cruciferous plants.

Non-viruliferous aphids were colonized on healthy radish plants under cages in the green house. When the population increased sufficiently, the detached leaves with aphids were placed on the diseased radish plants under cages to permit natural migration. After the aphids being fed for 24 to 48 hours, transfers in lots of approximately 5 infective aphids each were made to individual, healthy radish seedlings. Radish seedlings infected with noninfective aphids served as control, and also a number of non-inoculated plants free from aphids were used.

Among 89 radish seedlings so far inoculated with viruliferous green peach aphids 37 seedlings showed the typical symptoms within 14 to 23 days. Of 85 plants inoculated with viruliferous turnip aphids, 10 became diseased 16 to 25 days after inoculation and of 78 plants tested for the viruliferous false cabbage aphids 15 became diseased after 16 to 21 day under the green house condition. The virus was recovered from all the infected plants by the carborundum method, while any of the control plants remained healthy in the course of the investigation.

(3) *Seed transmission*

Tests for the possibility of seed transmission by the virus under consideration were made with the use of mature seeds obtained from 15 diseased radish plants grown in the green house. Seeds were planted in a flat pot filled with the autoclaved soil. Plants amounting to 353 were examined 30 days after emergence. It was observed that all of the plants studied remained healthy. It is therefore inferable that the virus under consideration may not be transmitted by seeds, so far as the writer's experiments are concerned.

(*B*) *Varietal susceptibility of radish and Chinese cabbage*

Young seedlings of the following varieties of radish and Chinese cabbage were tested by mechanical inoculation to determine their susceptibility to infection. The experiment was conducted under the green house condition.

Radish varieties: Mei Nung Tsao Sheng, Ta Pai Ih, Ta Mei Hua, Hsiao Mei Hua, Tong Kua Pai.

Chinese cabbage varieties: Ching Keng Pai Tsai, Kuang Tong Iu Tsai, Hsiao Pai Tsai, Ta Pan Chong Sheng, Chin Men Ta Pai Tsai, Uan Yeh Shan Tang Pai Tsai.

A suitable number of plants of each variety was reserved for controls. All varieties proved rather highly susceptible to the virus and the incubation period for each was within the limits of 14 to 31 days.

(*C*) *Symptomatology and host range under the green house conditions*

By means of mechanical inoculation, the radish mosaic virus was transmitted

to the following plants. The symptoms produced by mechanical inoculation on both cruciferous and *Nicotiana* hosts are shown in figures.

Family	Species and common name	Symptoms produced
Cruciferae:	*Raphanus sativus* L. (Radish)	Systemic
	Raphanus sativus L. var. radicula, DC.	Systemic
	Brassica rapa L. (Turnip)	Systemic
	Brassica Juncea Coss. (Leaf-mustard)	Systemic
	Brassica Pe-tsai Bailey (Chinese cabbage)	Systemic
	Brassica compestris L. (Rutabaga)	Systemic
Solanaceae:	*Nicotiana tabacum* var. Turkish	Local
	Nicotiana tabacum var. White Burley	Local
	Nicotiana glutinosa L.	Systemic
	Nicotiana rustica L.	Systemic

With the radish virus in question, no infection was observed on the following plants.

Family	Species and common name
Compositae:	*Lactusa sativa* L. (Lettuce)
	Chrysanthemum coronarium L. (Garland chrysanthemum)
Cruciferae:	*Brassica oleraces* L. var. acephala DC. (Kale)
	B. oleracea L. var. geminifera Zenker (Brussels sprouts)
	B. oleracea L. var. capitata L. (Cabbage)
	B. oleracea L. var. Botrytis L. (Cauliflower)
Cucurbitaceaes:	
	Cucumis sativus L. (Cucumber)
	Citrullus vuglaris Schrader (Watermelon)
Gramineae:	*Zea Mays* L. (Corn)
Leguminosae:	*Phaseolus vulgaris* L. (Kidney bean)
	Pisum sativum L. (Garden pea)
Solanaceae:	*Lycopersicum esculentum* Mill. (Tomato)
	Nicotiana rupenda Lehm
	Solanum melongena L. (Eggplant)
	Capsicum annum L. (Pepper)
Chenopodiaceae:	
	Spinacea oleraces L. (Spinach)

The symptoms produced by the artificial inoculation on these plants will be described below.

Radishes

Symptomatological studies on the radish mosaic virus are mostly limited to those made in the green house. Symptoms do not develop on the inoculated

leaves, but on the leaves which are produced after the systemic spread of the virus. The initial symptoms on Tai Mei Hua radish seedlings consist of vein clearing, small roughly circular to irregular chlorotic lesions which occur indiscriminately between and adjacent to the vein. Fusion of some of these lesions is not uncommon. Within a few days the chlorotic lesions become more numerous and soon replace the normal, dark green tissue, imparting a distinctly chlorotic color and coares mottle in contrast to the normal, healthy condition. After 10 days to 2 weeks the normal dark green tissue appears as irregularly shaped, nonraised island on a conspicuous, yellowish green, chlorotic background. There is little or no leaf distortion, although occasionally raised, dark green islands are observed on radish plants in the green house within a month after inoculation. Necrotic lesion and stunting of infected plants are not known to occur either in the field or in the green house.

Chinese cabbage

The first symptoms of the mosaic disease of Chinese cabbage, Uan Yeh Shan Tong Pai Tsai, consist of a pronounced systemic clearing of veins, which generally commences at or near the base of the leaf and gradually spreads over the entire leaf. After 3 to 4 weeks the early symptoms on the young, inner leaves commence to change gradually into a very conspicuous, coarse type of mottling in marked contrast to healthy leaves. The irregular, light green and dark green areas between the veins, which give rise to the mottled appearance, cause little or no distortion of the leaf surface.

Usually the older, outer leaves of infected plants continue to show clearing of veins without other changes. and this condition persists until they turn yellow and die. The disease causes a mild stunting of the entire plant which may not be apparent, however, if infection occurs later.

Leaf-mustard

Initial symptoms of the disease on leaves of leaf mustard seedlings consist of a conspicuous, coarse, systemic clearing of veins with interveinal mottling; later, irregularly shaped, raised dark green islands are scattered on the markedly chlorotic background.

Turnip and rutabaga

Faint, diffuse, chlorotic spots on young leaves, develop into diffuse mottle of faint chlorotic and light green areas.

***Nicotiana tabacum* var. Turkish**

Small necrotic lesions appear on the inoculated leaves within 5 to 6 days at high temperatures and in 10 to 14 days at low temperatures. The necrotic

area enlarges rapidly up to 10 mm or more in diameter, usually showing a brick red center with concentric rings and a darker band at the edge. The older lesions become dry and die out, and as they coalesce, a large part of the leaf becomes involved, though still meintain their individuality. No systemic invasion occurs.

Nicotiana tabacum var. White Burley

Local chlorotic lesion appears only on the inoculated leaves, which rapidly expand to 5 or up to 10 mm in diameter, then become necrotic with tan center. No systemic symptom is observed.

Nicotiana glutinosa L.

Conspicuous patterns of chlorotic ring and ring spots appear on the inoculated leaves, later these lesions become necrotic. Secondary symptoms appear on the new leaves consisting of distinct mottle, which develop into a severe systemic necrosis and considerably stunted accompanied with distortion of young leaves.

Nicotiana rustica L.

No primary symptom appears on the inoculated leaves. Secondary symptoms appear in about 20 days, consisting of irregular, diffused chlorosis and definitely marked progressive mottling of light and dark green areas. Leaf distortion may follow and sometimes systemic necrosis is observed.

(*D*) *Properties of the virus*

In the study of the properties of the radish mesaic virus, expressed juice of diseased radish plants treated in the manner already described was used to inoculated tabacco plant (*Nicotiana tabacum* var. Turkish). In the determination of longevity *in vitro,* tolerance to dilution, and the point of thermal inactivation the local lesion method (Bawden 1950) was applied. The average number of local lesions was based on a total of 5 to 10 half leaves.

The longevity *in vitro* at about 20°C to 22°C was found to be between 48 and 72 hours. The virus has an inactivation temperature at or near 55°C for a 10-minute treatment and dilution tolerance is about 1:3000 to 1:4000.

Table 1. Properties of radish mosaic virus in vitro

Longevity in vitro				Thermal inactivation			Tolerance to dilution			
Period of exposure at 20°-22°C (hours)	Presence or absence of symptoms on the test plant			Temperature at 10 minute exposure (°C)	Average number of lesions on 5-10 half leaves		Dilution	Average number of lesion on 5-10 half leaves		
	1	2	3		1	2		1	2	3
0	+	+	+	Untreated	24.6	20.3	0	32.2	28.2	25.0
2	+	+	+	35	24.8	17.6	1:10	34.1	28.0	30.0
4	+	+	+	40	22.1	15.7	1:100	26.1	28.0	19.1
8	+	+	+	45	5.4	7.2	1:200	14.2	23.7	26.8
14	+	+	+	50	0.3	1.2	1:500	13.0	19.2	19.1
24	+	+	+	55	0	0	1:1000	17.1	12.0	7.1
48	+	+	+	60	0	0	1:2000	7.6	1.3	3.0
72	−	−	−				1:3000	2.1	0	0
94	−	−	−				1:4000	0	0	0

Table 2. A list of data indicating the physical properties of viruses hitherto reported on the cruciferous hosts

Virus	Aging *in vitro* at 20-22°C	Inactivation temperature (10 minute)°C	Dilution end point
Cauliflower mosaic (Tompkins 1938)	336-360	75	1:2,000
Radish mosaic (Tompkins 1939)	336-384	68	1:14,000 1:15,000
Mild stock mosaic (Tompkins 1939)	144	60	1:5,000
Chinese cabbage mosaic (Tompkins 1938)	96	75	1:6,000
Cabbage mosaic (Larson & Walker 1950)	48-72	55	1:2,000
Cabbage ring necrosis (Larson & Walker 1941)	36-48	50	1:600
Japanese radish stunt (Isiyama & Misawa 1947)	552	70	1:15,000
Cabbage virus A (Walker 1949)	144-168	59-60	1:4,000 1:10,000
Cabbage virus B (Walker 1949)	144	74-76	1:500 1:1,500
Radish mosaic virus	48-72	55	1:3,000 1:4,000

Table 3. Comparison of the host range of our radish mosaic virus with that of other viruses on cruciferous plants

Host \ Virus	Cabbage virus A (Walker, 1949)	Cabbage virus B (Walker, 1949)	Radish mosaic (Tompkins, 1939)	Mild stock mosaic (Tompkins, 1939)	Cauliflower mosaic (Tompkins, 1937)	Chineses cabbage mosaic (Tompkins, 1938)	Japanese radish (Isiyama, 1947)	Cabbage ring necrosis (Larson, 1941)	Cabbage mosaic (Larson, 1950)	Radish mosaic
Turnip	+	+	+	+	+	+	+	+	+	+
Cabbage	+	+	+	−	+	+	−	+	+	−
Rutabaga	+		−	−	+			+	+	+
Cauliflower	+	+	+	−	+	+		+	+	−
Kale	+	+	+	−	+			+		−
Rape	+	+	−	−	+	+	−	+	+	
Leaf-mustard	+	+	+	+	+	−	+	+	+	+
Radish	+	+	+	+	+	+	+	+	+	+
Chinese cabbage	+	+	+	+	+	+	+	+	+	+
Tobacco	L	−	L	L	−	L	−	L		L
Nicotiana glutinosa	+	−	L	L	−	L	−	+	+	+
N. rustica	+	−		−	−			+	+	+
N. repanda	+	−		−	−			+	+	−
Spinach	+	−	+		−	−		+		−

(+)=infection; (−)=no infection; (L)=local lesion

Comparison of our radish mosaic virus with some other crucifer viruses

Studies on the virus disease of cruciferous crops have been made by different investigators. A summary of the data so far reported was briefly tabulated in Tables 2-3 for the purpose of comparison. As will be seen in these tables, some questionable points still exist as to the number and identity of these viruses. The radish mosaic virus reported herein appears to be very closely related to the cabbage virus A described by Walker (1949), mild stock mosaic virus described by Tompkins (1939), cabbage mosaic virus (Larson, 1950), cabbage ring necrosis virus of Larson and Walker (1941). While, cauliflower mosaic virus (Tompkins, 1937), radish mosaic virus described by Tompkins (1939), japanese radish stunt virus by Isiyama and Misawa (1947) and cabbage virus B of Walker (1949) are considered apparently different from the radish mosaic virus in question.

Cauliflower mosaic virus of Tompkins differs from the writer's in the failure of infecting such plants as, *Nicotiana tabacum* L. and *Nicotiana glutinosa* L., and the ability to infect cauliflower, cabbage, rape and kale, its greater longevity *in vitro* and higher thermal inactivation point. Radish mosaic virus of Tompkins differs from the present virus in unsuccessful transmission of the virus by some aphids, such as cabbage aphid, false cabbage aphid and green peach aphid as well as in systemic infection of *Nicotiana tabacum* L. var. Turkish, *Nicotiana tabacum* var. White Barley and spinach. Japanese redish virus of Isiyama and Misawa also differs in the failure of infecting *Nicotiana tabacum* var. White Burley, *Nicotiana tabacum* var. Turkish and *Nicotiana glutinosa*, and in the formation of marked stunting, malformation and enation in contrast to the vein clearing and general mottling induced by our virus. Cabbage virus B of Walker differs from the radish mosaic virus in the failure of infecting *Nicotiana tabacum*, *Nicotiana rustica* and *Nicotiana glutinosa*, in the ability of affecting cabbage cauliflower and kale, in its higher thermal inactivation point, longer longevity *in vitro*, and lower dilution tolerance. Chinese cabbage mosaic virus of Tompkins can be differentiated from the present radish mosaic virus in difference of hosts, such as cabbage, cauliflower, leaf mustard and *Nicotiana glutinosa*, and also by its higher thermal inactivation point. Cabbage mosaic virus of Larson and Walker shows systemic infection on cabbage and cauliflower, and local lesion on *Nicotiana rupanda* in 3 to 4 days. This virus is thus considered to be quite distinct from ours. Cabbage ring necrosis of Larson and Walker differs from the writer's in the fact that it produces systemic symptoms on cabbage, cauliflower, spinach and *Nicotiana rupanda*. Mild stock mosaic virus of Tompkins is unable to infect rutabaga and *Nicotiana rustica*, and has greater longevity *in vitro*. Cabbage virus A of Walker can infect cabbage, cauliflower, and spinach.

From the above comparison the radish mosaic disease in question appeared

to differ considerably from other mosaic disease of crucifers. Although virus may be identified by method of transmission, comparative symptomology, differential hosts and physical characteristics, but cross protection test and serological method are also the best method to show that the virus are probably the same or related, which should be further investigated. The course of this experiment did not prove the purification of this virus is much to be regretted.

Summary

1. A mosaic disease of radish prevalent in the vicinity of Taipei during the fall of 1957 was studied.
2. The symptoms of the disease consist of a systemic clearing of veins, followed by general mottling, with little or no distortion of the leaves.
3. Different cruciferous plants so far tested were found to be susceptible to the virus in question. The suscept includes those belong to radish, Chinese cabbage, leaf mustard, rutabaga, turnip. Besides, *Nicotiana tabacum* var. White Burley, *Nicotiana tabacum* var. Turkish, *Nicotiana glutinosa* L. and *Nicotiana rustica* L. were found to be susceptible to the virus.
4. In the green house, transmission of the virus was made by mechanical inoculation with carborundum and also by means of the cabbage aphid (*Brevicoryne brassicae* L.), green peach aphid (*Myzus persicae* Sulzer.) and false cabbage aphid (*Rhopolosiphum pseudobrassicae* Davis). Seed transmission tests gave negative results.
5. In longevity tests, the virus was active at the end of 48 hours, but inactivated after aging for 72 hours at 20°–22°C. It has an inactivation temperature at or near 55°C for a 10 minute exposure and a dilution tolerance of about 1:3000 or 1:4000.

蘿蔔之嵌紋病

郭 宗 德

1. 一九五七年秋天於臺北近郊之蔬菜園圃發現一種毒素病，普遍爲害十字花科之蔬菜，尤以蘿蔔爲甚。筆者自表現典型初期病徵之蘿蔔病株上摘取其心葉，磨汁以其汁液接種於健全之蘿蔔上，發病後以此爲接種原繼續接種保存於溫室中，備以研究其寄主範圍，傳染方法，耐熱性，耐稀釋性及生存期間之用。
2. 在蘿蔔上之病徵初爲葉脈之透明化，其後全葉呈現綠色及淡綠色之嵌紋病斑，被害株之葉並無變形或扭曲，唯於自幼期就被害之病株生長稍呈阻滯。
3. 寄主範圍於溫室中以金剛砂接種法接種結果，在十字花科之蘿蔔，白菜，芥菜，蕪菁，瑞典蕪菁等呈現系統性病徵。在烟草之 *Nicotiana tabacum* 上呈現局部性病徵 *Nico-*

tiana glutinosa, Nicotiana rustica 上呈現系統性病徵，而對甘藍，花椰菜，牛蒡，胡瓜，西瓜，萵苣等得陰性結果。

4. 在溫室中除可藉金剛砂接種法傳染外，又可藉僞菜蚜，菜蚜，桃蚜等蚜蟲傳佈。種子傳佈之實驗得陰性結果。
5. 本病毒汁保存於 20°-22°C 之溫度下其生存期間介于48-72小時之間。其耐熱性在55°C下經十分鐘之處理後失去其致病性。其耐稀釋性在1：3000至1：4000之間。（摘要）

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Explanation of Plates

Plate I.

Fig. 1. Symptoms produced by the radish mosaic virus on radish leaf.
Fig. 2. Symptoms produced by the radish mosaic virus on Chinese cabbage leaf.
Fig. 3. Symptoms produced by the radish mosaic virus on leaf-mustard leaf.
Fig. 4. Turnip inoculated with the radish mosaic virus.
Left: Uninoculated control.
Right: Inoculated plant showing conspicuous mottling.
Fig. 5. *Nicotiana glutinosa* inoculated with the radish mosaic virus:
Left: Uninoculated control.
Right: Inoculated plants showing distinct mottling on the new leaves which develop into severe systemic necrosis.

Plate I

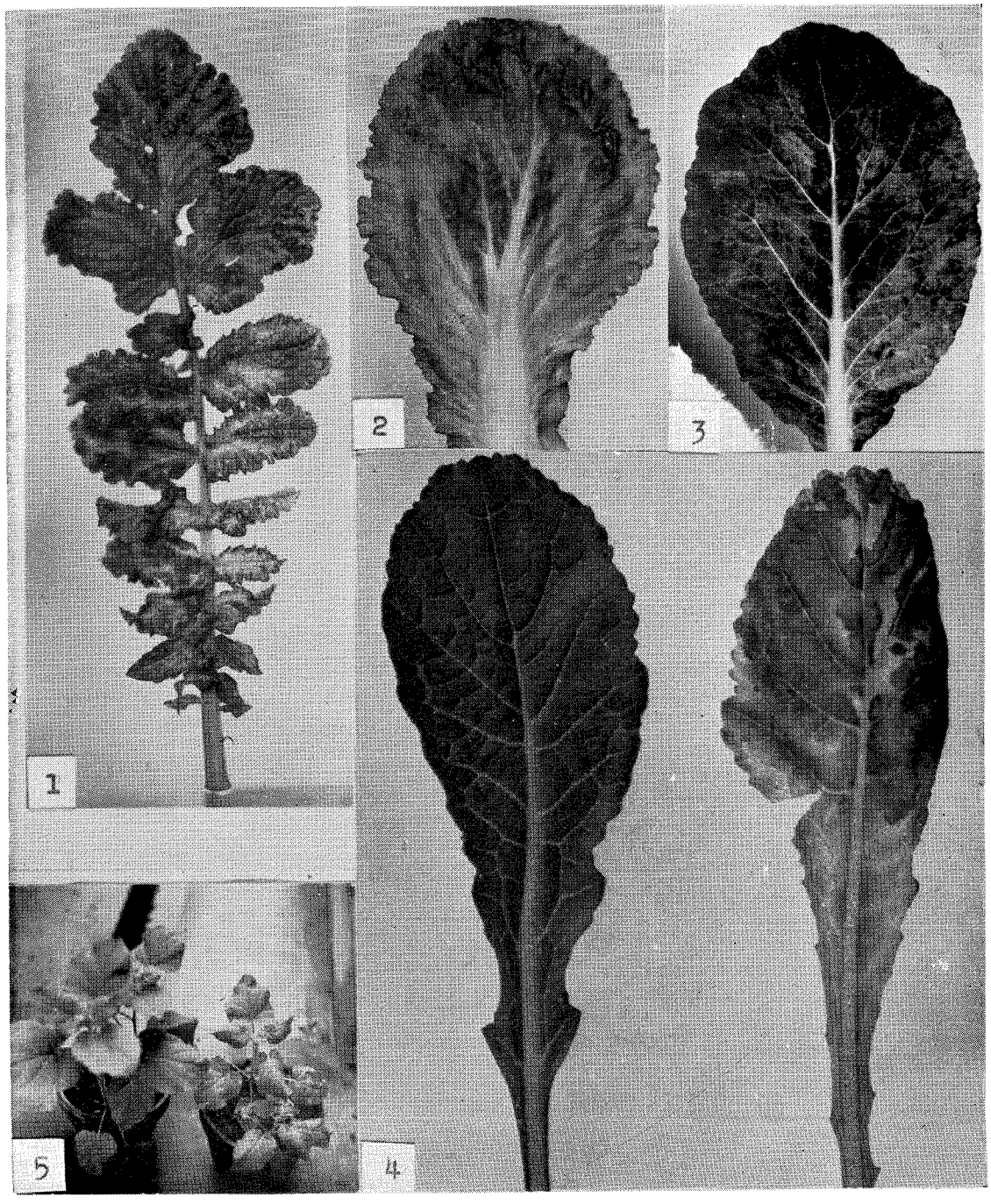

Plate II.

Fig. 6 .Chinese cabbage inoculated with the radish mosaic virus showing vein clearing and chlorosis.

Fig. 7. Local chlorotic lesions on the inoculated leaf of *Nicotiana tabacum* var. White Burley, which later become necrotic with tan centers.

Fig. 8. Local necrotic lesions on the leaf of *Nicotiana tabacum* var. Turkish showing a brick red center with concentric rings and darker band at the edge.

Fig. 9. *Nicotiana rustica* infected by radish mosaic virus showing chlorosis and stunting, while old leaves show necrosis.

Plate II