ON THE INTERFERENCE OF INACTIVATED VIRUS

II. Potato virus X

By

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I. Introduction

The writers have reported that tobacco mosaic virus (TMV) inactivated by ultraviolet irradiation and heat treatment interfered with active TMV infection. It was further noted that this interference was limited to the inactivated virus with antigenicity.

BAWDEN and KLECZKOWSKI (1953) reported that ultraviolet irradiated TMV was shown to interfere with infection of bushy stunt virus (BSV) and Rothamsted tobacco necrosis virus (RTNV), while the effect of interference was less than that with active TMV. It was also reported that ultraviolet irradiated BSV failed to interfere with the viruses mentioned above.

The present report deals with experiments on interference by potato virus X (PVX) inactivated by ultraviolet irradiation and heat treatment.

II. Materials and methods

Purification of PVX was carried out by BAWDEN and PIRIE's methods (1938). Expressed juice of tomato plants (Marglobe) infected with PVX was frozen, and after thawing it was centrifuged at 3500 rpm for 30 minutes. Ammonium sulfate was added to the supernatant fluid to make one-fourth saturation. The resulting virus precipitate was separated by centrifugation at 3500 rpm for 30 minutes, and was suspended in phosphate buffer solution (1/2–1/10 volume of the original juice). Then the suspension was centrifuged to remove insoluble materials. These procedures were repeated 2 to 3 times and the resulting supernatant was used as PVX sample. As to the purification of tobacco mosaic virus the same method as described in first report was utilized. The juice of tobacco plants (White Burley) infected with TMV was salted out by ammonium sulfate and precipitated at isoelectric point (pH 3.3) which was induced by an addition of diluted HCI.

For ultraviolet irradiation a 15 W MATSUDA's germicidal lamp (wave length

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2537 Å; dose of ultraviolet light was 2,160 $\mu W/cm^2$) was used and in all experiments exposures were made at 10 cm distance from the center of the lamp to virus solution. The virus sample was put in a Petri dish and irradiated, with gentle shaking of the dish in the direction along the long axis of the lamp. For heat treatment the virus sample was put into a thin glass tube with rubber stoppers at both ends, and immersed in hot water of a given temperature for 10 minutes; then the tube was cooled in cold water. Antiserum was prepared from rabbits immunized with repeated virus injection. Antigenicity was verified in the same manner as in first report; the antiserum was mixed with the virus and the resulting precipitation reaction was observed. The degree of infectivity of the virus was determined by the number of local lesions produced by inoculation; PVX sap was inoculated on the leaves of *Gomphrena globosa* L. plants and that of TMV was inoculated on the leaves of *Nicotiana glutinosa* L. plants.

III. Experimental results

A. Interference of PVX inactivated by ultraviolet irradiation with infectivity of active PVX

1. The antigenicity and interference of PVX inactivated by ultraviolet irradiation

As the result of ultraviolet irradiation, the infectivity of PVX was completely destroyed by a 10 minutes irradiation, while the antigenicity showed no changes as compared with non-irradiated control. After 8 hours irradiation it lost the antigenicity. A mixture of irradiated and active PVX was prepared at the ratio of 9 : 1, and the mixture was inoculated on one of opposite leaves of *G. globosa* plants, whilst the other was inoculated with a mixture of phosphate buffer solution and active PVX prepared at an equal ratio as the control. Approximately one week later, the number of local lesions produced on both inoculated leaves was compared. The results are shown in Tables 1 and 2. It was

TABLE 1. Antigenicity of ultraviolet irradiated PVX

Duration of irradiation (hours)	Final dilution of antiserum					Control
	16	32	64	128	256	
5	+	+	+	+	+	−
6	+	+	+	+	+	−
7	+	+	+	±	−	−
8	−	−	−	−	−	−
9	−	−	−	−	−	−

TABLE 2. Interference of ultraviolet irradiated PVX

Duration of irradiation (hours)	Number of local lesions		Percentage against control
	Mixture of act. and inact. PVX	Control	
5	90	159	56.6*
6	328	544	60.3*
7	270	534	50.6*
8	527	560	94.1
9	364	362	100.6

*: The data have a significant difference to population ratio 0.5.

observed that infectivity of PVX was decreased by the increase of irradiated PVX. Thus, it was found that irradiated PVX possesses interference activity. However, it was also ascertained that the interference was shown only in 5 to 7 hours irradiated PVX which still retained its antigenicity, while no interference was observed in 8 and 9 hours irradiated PVX which had by that time lost its antigenicity.

2. Pre-inoculation with irradiated PVX

Experiments were carried out in order to determine whether pre-inoculation with irradiated PVX on the leaves of *G. globosa* plant may produce the interference phenomenon or not, when the re-inoculation was undertaken by active PVX on the same leaves. Twenty leaves of *G. globosa* plants were at first inoculated with the inactivated PVX irradiated by ultraviolet light, and the other opposite 20 leaves were rubbed with phosphate buffer solution as the control. After this inoculation, the active PVX diluted one to ten with phosphate buffer solution was inoculated on each surface of the leaves at 1, 3, and 5 days interval. The total number of local lesions produced on the leaves pre-inoculated with irradiated PVX was compared with that on the control. As shown in Tables 3 and 4, it was found that the total number of local lesions on the leaves inoculated previously with irradiated PVX was less than

TABLE 3. Antigenicity of ultraviolet irradiated PVX

Duration of irradiation (hours)	Final dilution of antiserum					Control
	16	32	64	128	256	
5	+	+	+	+	+	−
6	+	+	+	+	+	−
7	+	±	−	−	−	−
8	−	−	−	−	−	−

TABLE 4. Interference of ultraviolet irradiated PVX

Intervals after inoculation of irradiated PVX (days)	Duration of irradiation	Number of local lesions		Percentage against control
		Pre-inoculation by irradiated PVX	Control	
1	5	30	47	63.8*
	6	35	49	71.4*
	7	47	54	87.0*
	8	45	43	104.7
3	5	51	56	91.1
	6	51	50	102.0
	7	50	46	108.7
	8	59	60	98.3
5	5	45	47	95.7
	6	39	40	97.5
	7	39	39	100.0
	8	44	46	95.7

that of the control, when the leaves were pre-inoculated with 5 and 6 hours irradiated PVX at one day interval. However, such interference was not observed in 8 and 9 hours irradiated PVX even when the interval was one day. Furthermore, interference was not recognized in 5 and 6 hours irradiated PVX when the interval was 3 or 5 days. Further it was noted that the interference activity was considerably decreased when the leaves were pre-inoculated with 7 hours irradiated PVX accompanied by decrease of antigenicity and when the interval was one day.

B. Interference of heat treatment

1. Antigenicity and interference of heat-treated PVX

PVX treated by heating at 68°C and over for 10 minutes completely lost infectivity and antigenicity, but when it was heated at 64° and 66°C for 10 minutes it still had a low infectivity and a weak precipitation reaction.
These heat-treated samples of PVX were used in order to ascertain whether or not they possessed the interference activity by the same means as in the case of ultraviolet irradiated PVX. A mixture of active and treated PVX was made in the same ratio as mentioned before. As PVX heated at 64° and 66°C showed a low infectivity, treated PVX was inoculated on the leaves of *G. globosa* plants in order to ascertain its infectivity. The number of local lesions was deducted from that produced by the mixture of active and treated PVX, and then the

remainder was compared with that of control. As shown by the results exhibited in Tables 5 and 6, PVX subjected to temperatures of 68°C and over had almost lost its antigenicity and also interference activity. However, PVX heated at 64° and 66°C did not completely lose infectivity, but showed the interference phenomenon.

TABLE 5. Antigenicity of heat-treated PVX

Temperature of treatment	Final dilution of antiserum					Control
	16	32	64	128	256	
not treated	++	++	+	+	+	−
64°C	+	+	+	+	+	−
66	+	±	−	−	−	−
68	−	−	−	−	−	−
70	−	−	−	−	−	−
72	−	−	−	−	−	−
74	−	−	−	−	−	−

TABLE 6. Interference of heat-treated PVX

Temperature of treatment	Number of local lesions				Percentage against control
	Mixture of act. and treated PVX (A)	Heat-treated PVX (B)	A−B	Control	
64°C	61	14	47	99	47.5*
66	61	2	59		59.6*
68	99	0	99		100.0
70	95	0	95		96.0
72	89	0	89		89.9
74	97	0	97		98.0

2. Pre-inoculation with heat-treated PVX

Experiments were carried out in order to know whether or not the interference phenomenon was observable when the leaves of *G. globosa* plants were pre-inoculated with heat-treated PVX and then they were inoculated by active PVX as in the case of ultraviolet irradiated PVX. As the results exhibited in Table 7 show, PVX heated at 64° and 66°C retained its antigenicity, and showed interference activity when the leaves were re-inoculated with active PVX at one day interval. However, the interference activity was not recognized in all cases of the intervals when pre-inoculation was made with PVX heated at 68°C or over which had already lost its antigenicity.

TABLE 7. Interference by pre-inoculation of heat-treated PVX

Intervals after inoculation of heat-treated PVX	Temperature of treatment	Number of local lesions				Percentage of A–B/control
		Pre-inoculation with heat-treated PVX (A)	Heat-treated PVX (B)	A—B	Control	
1 day	64°C	93	23	70	164	42.7*
	66	98	3	85		51.8*
	68	148	0	148		90.2
	70	151	0	151		92.1
	72	174	0	174		106.1
	74	161	0	161		98.2
3 days	64	63	8	55	55	100.0
	66	53	1	52		94.6
	68	49	0	49		89.1
	70	58	0	58		105.5
	72	50	0	50		90.9
	74	54	0	54		98.2

C. Interference by different virus

1. Interference with active TMV by ultraviolet-irradiated PVX

Experiments were conducted in order to know whether or not irradiated PVX interferes with the infectivity of TMV. The irradiated PVX was mixed with active TMV at the ratio of irradiated PVX 99 to active TMV 1, and phosphate buffer solution was mixed with active TMV at the same ratio as control. Both mixtures were inoculated on the leaves of *N. glutinosa* plants by half-leaf method in order to compare the infectivity. As the results exhibited in Tables 8 and 9 demonstrate, a definite interference with TMV infectivity was shown in the cases of 15 minutes and 2 hours irradiated PVX. However,

TABLE 8. Antigenicity of ultraviolet-irradiated PVX

Duration of irradiation	Final dilution of antiserum					Control
	16	32	64	128	256	
15 min.	+	+	+	+	+	−
2 hours	+	+	+	+	+	−
4	+	+	+	+	+	−
6	+	+	+	+	+	−
8	±	−	−	−	−	−
10	−	−	−	−	−	−

TABLE 9. Interference of ultraviolet-irradiated PVX against TMV

Duration of irradiation	Number of local lesions		Percentage against control
	Mixture of act. TMV and inact. PVX	Control	
15 min.	960	1444	66.5*
2 hours	403	690	58.4*
4	379	466	81.3*
6	392	482	81.0*
8	437	506	86.4*
10	168	166	101.2

in the case of 4 and 6 hours irradiated PVX a slight decrease in the number of local lesions was observed. No interference was recognized against TMV infectivity in the case of 10 hours irradiated PVX in which antigenicity was completely lost. Thus, as indicated above, it was shown that ultraviolet-irradiated PVX not only interfered with active PVX infectivity but also with active TMV infectivity.

IV. Discussion and conclusion

That the infectivity of PVX exposed to ultraviolet light is destroyed without the loss of antigenicity was reported by BAWDEN and PIRIE (1938), BAWDEN (1950), and MURAYAMA (1959). In the present experiments it was found that PVX was caused to lose infectivity by 10 minutes irradiation and the antigenicity was lost after 8 hours or over of irradiation. When mixture of irradiated and active PVX was inoculated on *G. globosa* plants, it was recognized that the inactivated PVX interfered with active PVX infectivity. However, the interference, as in the case of TMV, was recognized only in irradiated PVX which retained antigenicity, while the interference was not recognized in irradiated PVX which had lost its antigenicity. Furthermore, the interference was shown when the leaves of *G. globosa* plants were pre-inoculated with irradiated PVX which still retained antigenicity, and then active PVX was inoculated after one day. However, no interference was shown when the leaves were re-inoculated with active PVX at 3 days or more intervals or when they were pre-inoculated with the irradiated PVX which had lost antigenicity. In addition, it was found that the irradiated PVX which retained antigenicity interfered with the infectivity of TMV on *N. glutinosa* plants, but irradiated PVX which had lost antigenicity did not. It is considered that interference phenomenon is not specific to related viruses. That infectivity and antigenicity of PVX are destroyed at approximately

the same time by heat treatment was reported by BAWDEN (1935, 1950), CHESTER (1935), and BAWDEN and PIRIE (1938). MURAYAMA (1959) reported that antigenicty of PVX was destroyed by heat treatment at 66° to 70°C for 10 minutes. In the present experiments, it was also found that infectivity of PVX is lost as a result of heat treatment at 68°C or over for 10 minutes, and that in the inactivated PVX which had already lost the antigenicity no interference activity was recognized. However, the inactivation was incomplete when PVX was treated at 64° and 66°C for 10 minutes; further, the interference was observed in this treated PVX. The interference was also shown when the leaves of *G. globosa* plants were pre-inoculated with PVX subjected to 64° or 66°C at one day interval, while no interference was shown in 68°C or over heat-treated PVX even when the interval was only one day. When the interval was 3 or more days, interference was not shown in all cases of the heat-treated PVX. As in the results mentioned above, the interference was recognized only in the inactivated PVX which retained itsantigenicity.

It is considered that, as observed by electron microscopic study of TMV subjected to ultraviolet irradiation (SHIKATA, 1953) and heat treatment (SHIKATA, 1953; HIDAKA and KIRIYAMA, 1953), PVX particles would be broken into small pieces by ultraviolet irradiation and heat treatment and finally they would undergo a complete morphological change and a complete destruction of antigenicity.

It is further considered that these completely denaturated viruses lost not only the antigenic property, but also the interfering activity. The mechanism of this interference is not clear, so further studies must be done along this line.

Summary

The infectivity of potato virus X (PVX) was destroyed by 10 minutes exposure to ultraviolet irradiation, and the antigenicity was completely destroyed by 8 hours irradiation. Inactivated PVX interfered with the infectivity of PVX when the mixture of inactivated and active virus suspension was inoculated on the leaves of *Gomphrena globosa* plants. Interference was recognized when *G. globosa* plants were re-inoculated with active PVX one day after inoculation with the inactivated PVX, but no interaction occurred when re-inoculation with active PVX was performed 3 days later. The inactivated PVX also interfered with the infectivity of TMV when both viruses were simultaneouly introduced into *Nicotiana glutinosa* plants. Interference was recognized between the active PVX and the inactivated one having antigenicity, whereas the completely destroyed PVX showed no antagonistic reactions.

Furthermore, PVX previously subjected to temperatures beyond 68°C (10

min.) lost both pathogenicity and ability to interfere with active PVX. However, PVX that was partially inactivated at 64° and 66°C (10 min.) interfered with the infectivity of PVX.

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