J. gen. Virol. (1974), **23**, 11–16
Printed in Great Britain

A Possible Explanation of the Resistance of Virus-infected Tobacco Plants to Second Infection

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(*Accepted* 16 *November* 1973)

SUMMARY

Leaves of tobacco plant cv. Xanthi-nc inoculated or systemically infected with potato virus Y, cucumber mosaic virus, potato virus X, potato aucuba mosaic virus or alfalfa mosaic virus showed varying degrees of resistance to infection with tobacco mosaic virus. The resistance was correlated with the appearance of at least three proteins not present in healthy plants. These were the proteins that appear in leaves injected with polyacrylic acid. Both the resistance to second infection and proteins decreased when the plants were kept for 2 days before inoculation at 32 °C.

INTRODUCTION

Gianinazzi & Kassanis (1974) have shown that when leaves of *Nicotiana tabacum* L. cv Xanthi-nc are injected with polyacrylic acid (of mol. wt. 3500 to 230000), they become completely resistant to infection with tobacco mosaic virus (TMV) and tobacco necrosis virus in about two days after injection. Both viruses cause necrotic local lesions in Xanthi-nc leaves and remain localized in living cells adjacent to the necrotic local lesions (hypersensitive reaction). Polyacrylamide of similar mol. wt. does not cause resistance, suggesting that the polyanionic structure of the polyacrylic acid is the controlling factor. Likewise, yeast nucleic acid, which also has a sequence of negative charges, produces much resistance to infection with TMV when injected in tobacco cv. Samsun NN (Gicherman & Loebenstein, 1968). Furthermore, there seems to be some similarity between local resistance developed in a hypersensitive host after injection with polyacrylic acid and after infection with TMV (Ross, 1961*a*); because in both instances the same three or four new proteins appear, and the resistance and the proteins decrease when the plants are kept at temperatures over 30 °C (Gianinazzi & Kassanis, 1974). If a long molecule with a sequence of negative charges is responsible for resistance then infection with any virus should protect the plant from further virus infection with a virus producing necrotic local lesions. We present results to show that five serologically different viruses, which all cause systemic infections when inoculated to tobacco plants (cv Xanthi-nc), increased the plants' resistance to infection with TMV. When the plants became resistant they contained the same additional proteins as were found after injecting polyacrylic acid or infecting with TMV. Thus our results supplement Thomson's (1958) work on interference between serologically unrelated viruses.

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METHODS

The methods used were as described by Gianinazzi & Kassanis (1974). Local or systemic resistance to infection was assayed in, respectively, the inoculated or systemically infected leaves of tobacco plants cv Xanthi-nc. Plants of uniform appearance were inoculated 6 weeks after sowing to assay systemic resistance or at 7 weeks old to assay local resistance. The five viruses used to induce resistance were cultures, kept at Rothamsted, of potato virus Y, potato virus X, potato aucuba mosaic virus, cucumber mosaic virus and alfalfa mosaic virus. Sap extracts from infected plants were used as inocula. Leaves of an equal number of control plants were rubbed with water. Inoculation with sap from healthy tobacco plants does not cause resistance. In testing for local resistance the inoculated leaves were re-inoculated 10 to 14 days later with purified TMV at a concentration of 1 μg/ml. In testing for systemic resistance leaves of the upper part of the plant, showing systemic mosaic, were inoculated with 10 μg/ml of TMV. Equivalent leaves of the control plants were inoculated with the same concentrations of TMV. In most experiments, half of the infected and control plants were kept at 32 °C for the 2 days before inoculating with TMV. Resistance is expressed as the percentage reduction in lesion number caused by earlier infection with one of the five specific viruses. There were nine leaves for each treatment.

RESULTS

A preliminary experiment to test for local resistance induced after infection with potato virus Y showed that when the interval between the two inoculations was 3, 5, 7 and 10 days there was, respectively, a 0, 46, 86 and 97 % reduction in the number of TMV lesions. The control leaves averaged 148 lesions/leaf. Electrophoresis in acrylamide gels of extracts from inoculated and control leaves showed additional proteins in leaves infected for 7 and 10 days which were not present in control leaves or those infected for 3 and 5 days. There were two additional proteins in plants infected for 7 days and three in those infected for 10 days. In all later experiments concerning local resistance, the plants were re-inoculated after 10 to 14 days. Table 1 shows that the local resistance ranged from 56 % with potato aucuba mosaic virus to 100 % with alfalfa mosaic virus.

Table 2 summarizes the effects of the same five viruses on systemic resistance to TMV 3 to 4 weeks after inoculation. The resistance ranged from 32 % with alfalfa mosaic virus to 100 % with cucumber mosaic virus. Resistance did not develop when the interval between inoculations was less than 3 weeks. When plants, first inoculated with potato virus Y, were reinoculated with TMV at the vein clearing stage (14 days after the first inoculation) there was hardly any resistance, whereas plants re-inoculated when mosaic symptoms showed (23 days after the first inoculation) had 80 % resistance.

All infected leaves showing local or systemic resistance contained three and sometimes four proteins not present in healthy leaves (Fig. 1). The amount of the new proteins, judged by the size of the bands formed in polyacrylamide gels, varied between experiments but did not seem related to the degree of resistance. The proteins seem identical to those which developed in plants after injection with polyacrylic acid (Fig. 1).

Kassanis (1952) showed that plants kept above 30 °C for some days were more susceptible to virus infection than others kept at 20 to 24 °C. When we kept healthy and either locally or systemically infected plants for 2 days at 32 °C before inoculating them with TMV, they produced more lesions than plants kept at 20 to 24 °C, but the increase in the number of lesions was greater in infected plants than in healthy plants so the percent resistance de-

Table 1. *Resistance of leaves, previously inoculated with other viruses, to infection by TMV*

First virus	Days between the two inoculations	20 to 24 °C			32 °C		
		Uninfected	Infected	Reduction (%)	Uninfected	Infected	Reduction (%)
Potato virus Y	11	110*	22	80	128	66	48
Cucumber mosaic virus	14	142	36	75	—	—	—
Potato virus X	10	240	86	64	278	206	26
Potato aucuba mosaic virus	14	105	46	56	—	—	—
Alfalfa mosaic virus	13	33	0	100	—	—	—

* Average number of TMV lesions per leaf inoculated with 1 μg/ml of virus.

Table 2. *Resistance of leaves, systemically infected with other viruses, to infection by TMV*

First virus	Days between the two inoculations	20 to 24 °C			32 °C		
		Uninfected	Infected	Reduction (%)	Uninfected	Infected	Reduction (%)
Potato virus Y (vein clearing stage)	14	142*	126	11	—	—	—
Potato virus Y (mosaic stage)	23	110	22	80	128	66	48
Cucumber mosaic virus	28	60	0	100	80	24	70
Potato virus X	21	144	35	76	201	78	61
Potato aucuba mosaic virus	21	28	18	36	46	70	0
Alfalfa mosaic virus	22	50	34	32	32	28	12

* Average number of TMV lesions per leaf inoculated with 10 μg/ml of virus.

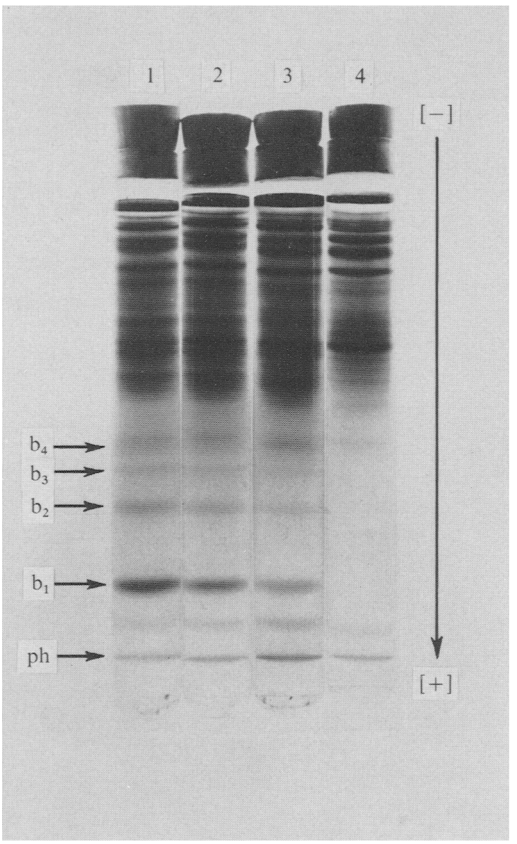

Fig. 1. Electrophoretic gels of centrifuged extract from tobacco cv. Xanthi-nc after infection with TMV (1), potato virus Y (2) (local infection), injection with polyacrylic acid 3500 mol. wt. (3) or untreated control (4). Electrophoresis was conducted at 4 °C for 15 min at 2 mA per tube and subsequently for 90 min at 4 mA per tube in 10 % acrylamide gels. b_1 to b_4, bands of new proteins; ph, the plant phenol band.

creased (Tables 1, 2). This decrease in resistance was accompanied by a decrease or total disappearance of the additional proteins.

Leaves injected with polyacrylic acid are less resistant to potato virus X than to TMV (Gianinazzi & Kassanis, 1974). Previous inoculation with potato virus Y had the same effect. Leaves were inoculated with potato virus Y or water and 10 days later half of each leaf was inoculated with TMV at 1 μg/ml and the other half with undiluted sap from plants infected with potato virus X. In one experiment, when the lesions of potato virus X were distinct enough to be countable, they averaged 232 lesions per half-leaf on the water-inoculated leaves and 143 on the virus-inoculated leaves, while there were 94 and 2 lesions of TMV respectively for the two types of leaves. In other experiments potato virus X lesions were not sufficiently distinct to be counted but when the sap was extracted and the virus estimated serologically, the concentration of potato virus X was 8 times greater in leaves inoculated with water than in leaves previously infected with potato virus Y, whereas there was complete resistance with TMV.

We have shown that viruses causing systemic infection produce resistance in the entire plant. However, Ross (1961*b*) showed that even viruses that are localized can stimulate

resistance in leaves above the inoculated ones and suggested that this was because substances were transported there from the sites of infection. We have confirmed this using tobacco cv. Xanthi-nc infected with TMV, and found also that resistant leaves contained the same additional proteins as the inoculated leaves. We previously neglected to test whether the resistance induced by injecting polyacrylic acid spreads beyond the injected leaves (Gianinazzi & Kassanis, 1974), but we have now found that, unlike virus infection, injection with polyacrylic acid causes only local resistance.

DISCUSSION

Infection of a plant with a virus usually prevents or interferes with subsequent infection by another strain of the same virus and such 'cross-protection' is a criterion of virus relationship. The most likely explanation for this phenomenon is that strains compete for the same multiplication sites or the same enzymes (Kassanis, 1963). Interference between serologically unrelated viruses, as in the present examples, is less likely to result from such competition. We suggest that virus-infected plants develop an inhibitory system which prevents or interferes with the multiplication of a second virus. This acquired resistance seems identical to that described in plants infected with viruses that cause only necrotic local lesions (Yarwood, 1960; Ross, 1961*a*, *b*; Batra & Kuhn, 1973). Probably it is this inhibitory system which is stimulated when plants are injected with polyacrylic acid (Gianinazzi & Kassanis, 1974) or yeast nucleic acid (Gicherman & Loebenstein, 1968). The resistance induced by infection with a virus or injection with a polyanionic substance seems to be closely correlated with the presence of three or four additional proteins, because both resistance and protein amounts decreased when the plants were kept at 32 °C. Also, in leaves inoculated with potato virus Y, the proteins appeared and resistance developed 7 to 10 days after inoculation. By contrast, interference between strains occurs much sooner after the first inoculation. For example, leaves inoculated with TMV showed 90 % resistance after 3 days against aucuba mosaic virus strain of TMV which causes necrotic local lesions (Sadasivan, 1940). This suggests that the mechanisms of interference between strains and serologically unrelated viruses are not the same.

However, the resistance induced by virus infection in a hypersensitive species and injection with polyacrylic acid seem to differ in that the former acts at some distance from the sites of infection, whereas polyacrylic acid confers resistance only in the injected leaf. Perhaps some small mol. wt. substance moves from the virus-inoculated leaf to other leaves where it induces the production of the additional proteins and resistance to virus infection, whereas this substance is either not produced after injecting polyacrylic acid or, more likely, too little is produced for it to be effective at a distance.

The proteins themselves might be responsible for the resistance but when sap containing the proteins was extracted from tobacco cv. Xanthi-nc having TMV lesions and injected to leaves of healthy plants these showed no resistance against TMV. The sap was extracted in a solution of ascorbic acid to prevent oxidation because it was found that such extracts caused less harm when injected. Electrophoresis showed that the extracts contained all four proteins.

The acquired resistance is not specific to the virus that induced it but seemed greater against TMV that causes localized infection than against potato virus X that causes systemic infection; this resembles the action of injected polyacrylic acid (Gianinazzi & Kassanis, 1974).

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(*Received* 17 *October* 1973)