Influence of Aliphatic Organic Acids and Metal Ions on Numbers of Local Lesions Produced by a Tobacco Necrosis Virus

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SUMMARY: Many aliphatic organic acids, when sprayed on the leaves of bean plants, decreased the numbers of local lesions produced following inoculation with a tobacco necrosis virus. Citric and succinic acids were effective only when applied before or during the period of virus establishment. The inhibitory effect of these acids could be annulled by certain metal nitrates.

The susceptibility of a species of host plant to a virus may vary greatly, as judged by the numbers of local lesions produced following inoculation with a standard preparation of virus. Age, nutrition, temperature, illumination and water supply are all known to affect susceptibility. Some of these factors alter the mechanical condition of the leaf, thus affecting the ease with which wounds necessary for virus entry are produced. Others are considered to alter 'host plant metabolism' but the chemical basis for such changes is unknown.

In an attempt to eliminate the daily cycle of variation in susceptibility to tobacco necrosis virus (Matthews, 1953) bean plants were grown under continuous illumination for several days before inoculation. Such plants were found to have a greatly decreased susceptibility to the virus compared with plants grown under natural illumination at about the same temperature. In most experiments no local lesions were produced at all, and no virus was detected in the inoculated leaves 9 days after inoculation. Preliminary analyses of such resistant leaves by chromatographic methods showed no major changes in sugar and amino acid content as compared with normally grown plants. However, there appeared to be a fairly marked increase in the amount of certain aliphatic organic acids. Subsequent tests described here showed that a variety of such acids, when sprayed on the leaves, decreased the number of lesions produced, an effect which was annulled by certain metal nitrates. The results suggest that metal ions may be important for the successful establishment of plant viruses and that one of the factors influencing plant susceptibility may be the balance between metal ions and organic acids in the leaf at the time of inoculation.

METHODS

The host plant, *Phaseolus vulgaris* var. Black Prince, and the tobacco necrosis virus were grown and inoculated as described previously (Matthews, 1953). Lesion counts were made 3-5 days after inoculation. Statistical analyses were made on transformed lesion numbers using the transformation $\log_{10} (x+1)$, where x = the number of local lesions per leaf.

RESULTS

The effect of treatment with organic acids on numbers of local lesions

Preliminary tests showed that when bean plants were sprayed with a solution of citric acid at *c.* 0.01N the numbers of local lesions produced by a standard inoculum were greatly decreased. Various organic acids (22 in all) were then tested by a standard procedure. Each acid at a concentration of 0.02N was sprayed on the two primary leaves of 12 plants. Four sprays were given—one on each of the 2 days before and after inoculation. Local lesions were counted after 5 days and the numbers compared with appropriate control

Table 1. *Effect of organic acids on numbers of local lesions produced by a tobacco necrosis virus in beans*

Acid	Decrease in number of lesions: <u>treated</u>	Significance of decrease (<i>t</i> test)	Leaf damage
	control	(%)	
Monocarboxylic			
Glyoxalic	0.60	5.0	Nil
Pyruvic	0.54	5.0	Slight
Glycollic	0.24	1.0	Nil
Lactic	1.80	5.0	Nil
α -Ketobutyric	0.75	Not significant	Nil
Dicarboxylic			
Oxalic	0.15	1.0	Marked
Malonic	0.32	1.0	Nil
Succinic	0.06	0.1	Very slight
Glutaric	0.14	1.0	Marked
Adipic	0.03	0.1	Marked
Oxaloacetic	0.15	0.1	Nil
α -Ketoglutaric	0.36	5.0	Nil
Malic	0.09	0.1	Nil
Fumaric	0.03	0.1	Moderate
Maleic	0.09	0.1	Nil
Mesaconic	0.08	0.1	Moderate
Itaconic	0.10	0.1	Heavy
Citraconic	0.03	0.1	Moderate
Tricarboxylic			
Tricarballic	0.14	1.0	Nil
Citric	0.21	1.0	Slight
Isocitric	0.13	0.1	Nil
Aconitic	0.06	0.1	Moderate

plants. The mean number of lesions/leaf on 192 control leaves was 46. The pH values of the solutions used for spraying ranged from 2.7 to 3.6. The results are summarized in Table 1. Some acids caused necrotic damage on the leaves. This was readily distinguished from the virus lesions. No correlation was apparent between the pH value of the solution sprayed, plant damage, and decrease in number of local lesions. The decrease in number of local lesions might have been due to some effect on the virus of the acid which remained on the surface of the leaf. This possibility was eliminated, at least for citric acid, by the following experiment. Bean plants were cut just above soil level and

the stems placed immediately in various concentrations of citric acid. After 24 hr. plants were inoculated with tobacco necrosis virus. Numbers of local lesions produced were (mean of 20 leaves): distilled water, 152; 0.00016N citric acid, 106; 0.00048N citric acid, 75; 0.0016N citric acid, 47. There was a progressive decrease in the numbers of local lesions.

Effect of treatment with metal nitrates on numbers of lesions

Metal ions are essential for infection by some bacterial viruses. Specific cations may be required, either for adsorption of phage on to sensitive bacteria (e.g. Puck, 1953) or for multiplication steps after phage attachment has taken place (Adams, 1949; Fildes, Kay & Joklik, 1951); no such evidence is available

Table 2. *Effect of succinic acid, magnesium nitrate and calcium nitrate on number of local lesions produced by tobacco necrosis virus in beans*

Spraying solution	No. of local lesions (mean of 16 leaves)	Significance of difference from	
		Control (%)	Sprayed with acid only (%)
Unsprayed control	19	—	0.1
Mg(NO ₃) ₂ , 0.001 M	27	1.0	0.1
Mg(NO ₃) ₂ , 0.01 M	36	0.1	0.1
Succinic acid, 0.01 N	8	0.1	—
Mg(NO ₃) ₂ , 0.001 M + succinic acid, 0.01 N	13	5.0	0.1
Mg(NO ₃) ₂ , 0.01 M + succinic acid, 0.01 N	24	None	0.1
Unsprayed control	84	—	0.1
Ca(NO ₃) ₂ , 0.001 M	77	None	0.1
Ca(NO ₃) ₂ , 0.01 M	70	None	0.1
Succinic acid, 0.01 N	20	0.1	—
Ca(NO ₃) ₂ , 0.001 M + succinic acid, 0.01 N	37	1.0	0.1
Ca(NO ₃) ₂ , 0.01 M + succinic acid, 0.01 N	49	1.0	0.1

for plant viruses. However, many organic acids are known to chelate metal ions. This suggested that if metal ions were essential for plant virus establishment organic acids might act, at least in part, by sequestering such ions. To test the effect of metal ions on the production of local lesions, plants were sprayed in the 4th and 2nd day before inoculation with an organic acid, and on the 3rd day and the day before inoculation with a metal nitrate. The results of one such experiment are summarized in Table 2. Both calcium and magnesium nitrates partially or completely annulled the effect of succinic acid. Magnesium nitrate alone at 0.01 M increased the number of local lesions about twofold. Various other nitrates were tested in experiments like that recorded in Table 2. Potassium and ammonium nitrates had no effects. In some experiments sodium nitrate significantly reduced the effect of succinic and citric acids. Caesium, barium and aluminium nitrates applied alone significantly increased the number of local lesions and also reduced the inhibitory effect of succinic acid. Zinc nitrate alone caused a striking reduction in number of lesions, as has been found previously for tobacco mosaic virus (Weintraube, Gilpatrick & Willison, 1952).

The extent to which numbers of local lesions were decreased by citric and succinic acids varied in different experiments, as did the annulling effect of metal ions. Until the factors affecting this variation are understood it is not possible to give firm comparative figures for the effects of different metals.

Age of plant does not appear to affect the activity of succinic acid. Plants in four age groups, ranging from those in which the leaves were not fully expanded to plants approaching senescence, gave the following figures for the ratio of number of local lesions on succinic acid treated plants: control, namely 0.15, 0.13, 0.14, 0.13 in order of increasing age of plant.

The effect of time of treatment in relation to time of inoculation

Preliminary experiments showed that a single treatment with an organic acid was effective when applied 1 or 2 days before inoculation, but ineffective when applied 1 day after inoculation. The effect of single treatments at times close to the time of inoculation was then examined. Since spraying with water alone just before inoculation caused a marked decrease in numbers of local lesions, a control set of plants was sprayed with water for each time tested. The results of one experiment are summarized in Table 3.

Table 3. *Effect of 0.02N succinic acid applied at various times before and after inoculation*

Time of spraying in relation to time of inoculation	Ratio of number of local lesions: acid sprayed water sprayed
15 min. before	0.14
Immediately before	0.28
Immediately after	0.50
15 min. after	0.56
30 min. after	0.56
45 min. after	0.67
60 min. after	0.55
1.5 hr. after	0.71
2 hr. after	0.82

A treatment immediately before inoculation decreased the number of lesions more than a treatment immediately after inoculation. In various experiments treatments with succinic or citric acids after about 2–4 hr. were ineffective.

DISCUSSION

The fact that the decrease in numbers of lesions by organic acids was annulled by metal ions does not necessarily imply a close connexion between action of these two types of compound. However, some organic acids are known to sequester metal ions through the formation of chelate compounds. The simplest explanation for the effects described here is to assume that the tobacco necrosis virus requires certain metal ions for successful establishment and that the organic acids inhibit this process by decreasing the concentration of

available metal ion. However, in preliminary tests the well-known chelating agent, ethylenediamine tetra-acetic acid caused no greater decrease in lesion number than did citric and succinic acids.

With bacterial viruses it is possible to control the concentration of compounds in the medium in which virus establishment occurs. No such control is possible with plant leaves. The variation in the magnitude of the effects we have observed may well be due to natural variation in the state of the plant with respect to metal ions and organic acids or other chelating compounds at the time of inoculation. Some of the acids on entering the leaf are probably rapidly metabolized. In addition, there is no reason to suppose that all the acids listed in Table 2 exert their inhibitory effect in the way suggested above. For these reasons no correlation can be expected between data such as are given in Table 1 and the chelate stability constants for the various acids with calcium and magnesium (Martell & Calvin, 1952). It may be of interest from the point of view of virus control that a class of compound which must be universally present in susceptible leaves can in increased concentration inhibit virus establishment.

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