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EFFECT OF SOME COMPOUNDS AND BIOLOGICAL PRODUCTS UPON INFECTION BY TOBACCO MOSAIC VIRUS*

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The following work was undertaken to gain further information concerning the mechanism of plant infection when manually inoculated with virus. In such infections it is generally assumed that a mild injury and subsequent healing of the host cells is necessary for the entrance of the virus and establishment of infection. It is also generally considered that plant viruses function only within or upon the protoplasm of living susceptible cells. By the use of a test plant on which a virus causes conspicuous, necrotic, localized lesions at the loci of infection, it is possible to measure the relative infectivity of virus samples. By inoculating opposite halves of the same leaf with a treated and control inoculum, the relative infectivity of the treated virus solution can be determined. This is known as the half-leaf method and was used in this work.

REVIEW OF LITERATURE

A specific review of the literature for this paper seems inappropriate. The paper is primarily intended to report the results obtained from a screening of various substances for their effect upon infection. A general review of some of the aspects of infection and inhibition of infection is available in Chapter 13 of Bawden's "Plant Viruses and Virus Diseases," 3rd ed. (1).

MATERIALS AND METHODS

A tobacco mosaic virus solution, partially purified by ultra-centrifugation, was used throughout the experimentation. The test plants used were *Phaseolus vulgaris* var. Scotia, grown in sterilized soil and pots. When approximately 14 days old they were pinched back to the primary leaves, which were then inoculated. The materials, indicated in table 1, were tested for their effect upon infectivity by mixing them with the virus inoculum. They had a 0.1 percent final concentration, except for the amino acids, which had a 0.05 M final concentration. Hydrogen-ion concentrations in the aqueous inoculum were maintained at 4.5, 6.0, and 8.5 with 0.1 M phosphate buffer. One gram of abrasive, 800-mesh carborundum powder, was added to each 10 ml. of inoculum. Inoculations were made by swabbing the virus solutions onto the upper surfaces of the leaves with sterile cheese-cloth pads of approximately identical size. Each solution at each pH was tested upon five leaves. Immediately after inoculation the leaves were rinsed with tap water to remove any excess buffer or test substance which might have injured the leaf tissues if allowed to remain on the leaves.

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The results are expressed as an infection index, derived as follows:

$$\text{Infection index} = \frac{\text{Number of local lesions on treated half-leaves}}{\text{Number of local lesions on control half-leaves}}$$

An index of 1.0 denotes that a treatment had no effect upon infection; an index of 0.5 shows a 50 percent decrease in infection; and an index of 1.5 shows a 50 percent increase in infection. Since the method of assaying virus infectivity has an inherent variability of approximately 20 percent, differences greater or less than 50 percent were arbitrarily assumed as influencing infection.

Results

The results of the treatments (table 1) have been classified into three categories:

A. Increased infection, infection index of 1.5 or more. Dyes—acridine red and methyl green.

Proteins and protein derivatives—glue.

Amino acids—glycine, l-histidine, lysine, d-l-methionine, and d-l-tryptophan.

Nucleic-acid derivatives—adenosine, adenosine diphosphate, cytidine, cytosine, 2 thio cytosine, protamine nucleinate, d-ribose, uracil, 5 amino uracil, and 6 methyl uracil.

Plant-growth substances—naphthalene acetic acid.

Polypeptides—glycyl glycine, glycyl glycyl glycine, glycyl l tryptophan.

Miscellaneous — glycerophosphate, sodium formate, and sorbitol.

Enzymes—catalase.

B. Decreased infection, infection index of 0.5 or less.

Dyes—acridine yellow, fluorescein, basic fuchsin, iodine green, malachite green, methyl blue, methyl green, orange II, thionin, toluidine blue O, trypan blue, and vita stain.

Proteins and protein derivatives—beef blood serum, beef extract, dried blood, casein, edestin, lactalbumin, malt extract, skim milk, thiotone, and yeast extract.

Amino acids—arginine, asparagine, d-glutamic acid, l-histidine, and lysine.

Nucleic-acid derivatives—adenosine triphosphate, adenylic acid, cytidylic acid, desoxyribonucleic acid, 2,6 diamino purine sulfate, guanylic acid, sodium nucleinate, 2,4 dichloro 6 methyl pyrimidine, diazouracil, thiouracil, and hypoxanthine.

Plant-growth substances—indole 3 acetic acid.

Miscellaneous—glycollic acid, orcinol, soybean trypsin inhibitor, tannic acid, and thioglycollate.

Enzymes—alpha amylase, beta amylase, cozymase, beta glucoronidase, hemicellulase, hyaluronidase, lactase, lysozyme, pectinase, rennin, lipase, crystalline trypsin, powdered trypsin, and urease.

C. No response, infection index of 0.51 to 1.49.

Dyes—acid fuchsin, orcein, pyronin B, pyronin 2-G, quinoline yellow, and sudan IV.

Proteins and protein derivatives—egg albumin, gelatin, gelysate, lactalysate, myosate, phytone, polypeptone, and trypticase.

Amino acids—l-threonine.

Nucleic-acid derivatives—d l alanyl d l alanine, adenine, adenosine, isocytosine, guanine, guanosine, 2 amino 4 methyl pyrimidine, 2,4 dichloropyrimidine, 2,6 dichloropyrimidine, thymine, 5 methyl thiouracil, 6 methyl thiouracil, uridine, uridylic acid, xanthine, and xanthosine.

Plant-growth substances—indole butyric acid and 3 indole propionic acid.

Polypeptides — alanyl glycyl glycine, d l leucyl glycine, d l leucyl glycyl glycyl glycine, and glycyl tyrosine.

Miscellaneous—cocoa, glucose 1 phosphate, glucose 6 phosphate, glucosamine HCl, glutathione, Mn glycerophosphate, hexose diphosphate, inulin, melizitose, phloroglucin, phytol, resorcin and salicin.

Enzymes—diastase.

Discussion

From the dyes tested, there was no evidence of a correlation of their effect on infection with their chemical structure as given by Conn (4). However, the basic dyes decreased infection to a greater extent in alkaline solutions than in acid solutions. There was no noticeable injury to the assay plant from the dyes, except with methylene blue and phloxine B, which caused severe injury at the strength used. The reduction of infection from malachite green may have resulted from retarded virus multiplication and not from any effect on infection, as it has been reported that this dye inhibits the multiplication of the virus in tobacco tissues (9).

Among the proteins and protein derivatives, the responses varied. However, three materials, casein, skim milk, and yeast extract, reduced infection appreciably. Milk and an extract from baker's yeast (8) have previously been reported to inhibit infection of tobacco tissues by tobacco mosaic virus (6, 7).

The polypeptides, amino acids, plant-growth substances, and substances classified as miscellaneous were generally without marked influence on infection. However, tannic acid, a protein precipitant, greatly reduced infection at all pH values tested. This confirms a previous report that this substance inhibits infection (10).

Of the nucleic-acid derivatives tested, thiouracil was most effective in reducing infection, whereas substituted thiouracils did not influence infection materially. This reduction by thiouracil may have resulted from the inhibition of virus multiplication in the tissues after infection, as thiouracil has been shown to inhibit the multiplication of tobacco mosaic virus in tobacco tissues (2, 3). If such a mechanism were operative in this experimentation, it must have re-

sulted from that amount of thiouracil introduced into the plant tissues during inoculation, as excess thiouracil and inoculum were rinsed from the leaves immediately following inoculation. Although there appears to be a limited uptake of thiouracil for the test plant as a whole, the concentration in those cells injured for virus entrance may have been sufficient to inhibit virus multiplication. It has been reported that injury to plant foliage by carborundum powder increases the uptake of spray-applied iron solution in sufficient amounts to correct iron deficiency in the coffee plant (5).

Of the four organic bases normally present in the ribose nucleic acid portion of the virus, uracil gave an infection index of 1.75 at pH 4.5, cytosine an index of 1.53 at pH 8.5. These increases may relate to the multiplication of the virus after virus establishment in the cells.

All the enzymes tested, except catalase and diastase, greatly reduced infection. The marked response from lipase and trypsin suggests that enzymatic action from these substances may have interfered with the formation of a presumable surface film or protective membrane over the wounded cells which may be essential for infection. As many of the enzymes were active against infection, it is difficult to believe that the specific enzymatic action of each enzyme is involved. It would appear that some common property of enzyme protein is influencing infection. As enzymes are thought to conform to the surface of their substrates for enzyme-substrate combination and subsequent reaction, perhaps the property of plasticity of these enzyme proteins is the characteristic by which virus infection is inhibited. If the enzyme proteins could form a film over the surface of injured cells before virus entrance, infection might be prevented by this protein-film barrier to the virus. The surface of noninjured cells apparently is impervious to virus entrance without some vector.

It is conjectured that the test substances affect infectivity in three general ways: (1) by a direct effect solely upon the virus prior to its entrance into host cells, (2) by an action upon the injured cells during and immediately after entrance of virus into the host, and (3) by an influence upon virus multiplication, which presumably involves an interaction between both virus and host. For example, tannic acid, a protein precipitant, appears to act as 1, enzymes as 2, and thiouracil and malachite green as 3.

TABLE 1.—INFECTION INDICES FROM SUBSTANCES TESTED

Substance	pH 4.5	pH 6.0	pH 8.5
Dyes:			
Acridine yellow	.53	.39	.08
Acridine red	1.91	.62	1.14
Fluorescein	.09	.01	.12
Fuchsin, basic	.20	.01	.05
Fuchsin, acid	.94	.89	.71
Iodine green	.93	.70	.49
Malachite green	.01	.18	.29
Methyl blue	.21	.32	.12
Methyl green	1.51	.91	.46
Methylene blue	*	*	*
Orange II	1.18	.48	.63
Orcein	1.44	.94	1.26*
Phloxine B	*	*	*
Pyronin B	.56	.65	.57
Pyronin 2-G	.98	.87	.62
Quinoline yellow	1.05	.83	.93
Sudan IV	1.24	1.00	.78
Thionin	.71	.65	.07
Toluidine blue O	.13	.15	.06
Trypan blue	.25	.33	.77
Vita stain	.44	.44	.30
Proteins and protein derivatives:			
Albumin, egg	.59	1.09	.56
Beef blood serum	.41	.07	.10
Beef extract	1.00	.65	.37
Blood, dried	.49	1.09	.36
Casein	.57	.02	.002
Edestin	.43	.70	.69
Gelatin	.96	1.05	.93
Gelysate	1.34	.76	.64
Glue	1.56	.70	.90
Lactalbumin	.92	.43	.87
Lactalysate	1.07	.75	.97
Malt extract	1.25	.69	.33
Milk, skim	.67	.13	.04
Myosate	1.12	.68	.59
Phytone	.95	.81	.66
Polypeptone	.88	.82	.76
Thiotone	.54	.47	.44
Trypticase	1.10	.85	.64
Yeast extract	.14	.03	.06
Amino acids:			
Arginine	.68	.32	.38
Asparagine	.53	.20	.33
d-Glutamic acid	.19	.10	.94
Glycine	1.47	.67	1.77
l-Histidine	.75	.18	1.53
Lysine	.54	.36	2.11
d-l-Methionine	1.60	.88	.89
l-Threonine	.99	.81	1.09
d-l-Tryptophan	1.65	.71	.51

* Tissue injured.

TABLE 1.—(Continued)

Substance	pH 4.5	pH 6.0	pH 8.5
Nucleic acid derivatives:			
d l alanyl d l alanine	.94	.74	.98
Adenine	.83	.87	.79
Adenosine	1.27	.92	1.58
Adenosine diphosphate	1.09	1.62	1.17
Adenosine triphosphate	.50	.64	.58
Adenylic acid	.47	.69	.81
Cytidine	1.65	1.01	.82
Cytosine	.75	.80	1.53
Isocytosine	.86	.85	.74
2 thio cytosine	1.97	.90	.89
Cytidylic acid	.42	.60	.92
Desoxyribonucleic acid	.51	.39	.44
2, 6 diamino purine sulfate	1.19	.48	.97
Guanine	.80	1.10	.90
Guanosine	1.14	1.10	1.05
Guanylic acid	.85	.39	1.02
Protamine nucleinate	2.53	1.26	.62
Sodium nucleinate	1.22	.43	.79
2 amino 4 methyl pyrimidine	.94	.85	.84
2, 4 dichloro 6 methyl pyrimidine	.72	.43	.99
2, 4 dichloropyrimidine	.72	.69	.94
2, 6 dichloropyrimidine	.87	1.06	1.09
d-ribose	1.73	1.07	1.16
Thymine	1.03	.79	1.26
Uracil	1.75	.92	1.01
5 amino uracil	1.72	1.13	1.07
Diazouracil	.41	.56	.56
6 methyl uracil	1.10	1.70	.69
5 methyl thiouracil	.99	.80	.71
6 methyl thiouracil	1.46	.84	.74
Thiouracil	.07	.28	.09
Uridine	1.05	.93	.77
Uridylic acid	.77	.83	.75
Xanthine	1.41	1.17	1.06
Hypoxanthine	.46	.83	.93
Xanthosine	.92	.84	1.15
Plant growth substances:			
Indole 3 acetic acid	.39	.47	.80
Indole butyric acid	.64	.75	1.27
3 indole propionic acid	.76	.55	.83
Naphthalene acetic acid	.86	1.24	1.68
Polypeptides:			
Alanyl glycyl glycine	.74	.65	.76
d l leucyl glycine	.73	.74	1.30
d l leucyl glycyl glycine	1.26	.85	.91
Glycyl glycine	1.63	.93	1.34
Glycyl glycyl glycine	1.69	1.08	1.10
Glycyl l tryptophan	1.95	2.01	1.43
Glycyl tyrosine	1.00	.98	.83
Miscellaneous:			
Barbituric acid	.50	.55	.96
Cocoa	1.12	.72	.81
Glucose 1 phosphate	1.20	.61	1.31
Glucose 6 phosphate	.85	.76	1.15

TABLE 1.—(Continued)

Substance	pH 4.5	pH 6.0	pH 8.5
Glucosamine HCl	.85	1.16	1.10
Glutathione	.68	.95	.65
Glycerophosphate	.83	1.45	1.55
Glycerophosphate (Mn)	1.26	.62	.75
Glycollic acid	.36	.70	.91
Hexose diphosphate	.83	1.03	1.14
Inulin	.90	.99	.65
Melizitose	1.00	.68	.93
Orcinol	.75	.62	.08
Phloroglucin	.97	.94	.71
Phytol	.85	.70	.63
Resorcin	1.08	.76	.97
Salicin	.86	.72	.91
Sodium formate	1.14	.84	1.55
Sorbitol	1.97	.68	1.12
Soybean trypsin inhibitor	.68	.97	.42
Tannic acid	.02	.13	.01
Thioglycollate	1.15	.23	.41
Enzymes:			
Amylase, alpha	.27	.008	.006
Amylase, beta	.77	.32	.26
Catalase	1.73	.75	1.13
Cozymase	.48	.60	.99
Diastase	.90	.53	.79
Glucoronidase, beta	.13	.06	.12
Hemicellulase	.24	.11	.07
Hyaluronidase	.045	.004	.004
Lactase	.28	.11	.53
Lysozyme	.17	1.06	1.22
Pectinase	.095	.09	.05
Rennin	.10	.18	.42
Lipase	.00	.00	.00
Trypsin (crystallized)	.12	.12	.07
Trypsin (powdered)	.03	.01	.01
Urease	.74	.50	.42

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