Sodium Azide as a Protectant of Serological Activity and Infectivity of Plant Viruses

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ABSTRACT

Antigenicity of tobacco mosaic virus (TMV), potato virus Y (PVY), tobacco etch virus (TEV), potato virus X (PVX), alfalfa mosaic virus (AMV), and cucumber mosaic virus (CMV) was preserved in tobacco leaf tissue stored in 1% sodium azide (NaN_3) at 25 C for 4 weeks. TMV, PVY, and TEV were infectious after 4 weeks, and PVX after 1 week's storage at 25 C in NaN_3. Neither AMV nor CMV were infectious after 1 week at 25 C in NaN_3. Phytopathology 61:943-944.

Additional key words: quarantine, culture storage.

This study was initiated to determine if sodium azide (NaN_3), at biocidal concentrations, would protect the antigenicity of certain viruses in plant tissue. The premise was that such a system would permit serological identification of viruses from foreign areas without the danger of the introduction of pathogens. Sodium azide was selected because of its biocidal properties and because it is nondetrimental to certain antigens (1).

The first test was designed to determine the effect of NaN_3 concentration on biocidal effectiveness, serological activity, and infectivity of tobacco mosaic virus (TMV) and potato virus Y (PVY). Thirty-two TMV-infected leaf samples of McNair 12 (*Nicotiana tabacum* L.) weighing 0.25 g each were placed in 200 ml of aqueous solutions containing 0, 0.01, 0.1, and 1.0% NaN_3 (w/v), respectively, and stored at 25 C. At weekly intervals, two samples were assayed serologically and two for infectious virus. Serological assays were conducted using the agar-gel double-diffusion technique in a gel containing 0.8% Difco purified agar and 1% NaN_3. Infectivity was determined by grinding the samples in 1 ml of 0.01 M Na_2HPO_4-KH_2PO_4 buffer (pH 7.2) and assaying on Carborundum-dusted *Nicotiana glutinosa* L. leaves. To test biocidal activity, four samples were washed for 15 min in three 100-ml changes of distilled H_2O to reduce residual NaN_3, two samples plated on acidified potato-dextrose agar, and two placed in nutrient broth. The test was repeated once, so a total of four samples was exposed to each treatment. Potato virus Y (PVY) in McNair 12-infected leaf tissue was subjected to the same test except that the technique of Gooding & Bing (2) was used for serological analysis and infectivity was determined on tobacco cultivar Burley 21.

Serological activity and infectivity of TMV were maintained in the water check and at all concentrations of NaN_3 for all time periods. The effect of NaN_3 on PVY is summarized in Table 1, and its biocidal effect in Table 2.

The preservation of serological activity of TMV and PVY by NaN_3 was as expected. However, protection of infectivity of PVY for 1 month at 25 C was somewhat unexpected, as this virus has an aging in vitro inactivation time between 24 and 48 hr (3). A second test was conducted to determine the effect of 1% NaN_3, the optimum concentration tested for biocidal activity that also protected serological activity, on four other viruses; i.e., tobacco etch virus (TEV), potato virus X (PVX), alfalfa mosaic virus (AMV), and cucumber mosaic virus (CMV). Experimental procedures were essentially as described for test 1. Serological activity was determined in gels as follows: (i) TEV and PVX, same as PVY; (ii) AMV, same as TMV; and (iii) CMV, 0.8% agarose containing 0.02% NaN_3. Results are summarized in Table 3.

In these tests, a 1% solution of NaN_3 (25 ml of solution/g of tissue) inactivated microorganisms culturable on PDA or in nutrient broth from tobacco tissue while protecting the serological activity of TMV,

TABLE 1. Effect of sodium azide concentration on serological activity and infectivity of potato virus Y over a period of 4 weeks at 25 C

Concentration of NaN_3, %	Weeks of storage							
	1		2		3		4	
	S[a]	I	S	I	S	I	S	I
0.00	+[b]	+	0	+	0	0	0	+
0.01	+	+	+	0	+	0	+	0
0.10	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+

[a] S = Serological activity; I = infectivity.
[b] + = Virus serologically active or infectious from at least one of four pieces of tissue; 0 = no activity.

TABLE 2. Bacteriacidal and fungicidal activity of different concentrations of sodium azide over a period of 4 weeks at 25 C

Concentration of NaN_3, %	Weeks of storage							
	1		2		3		4	
	B[a]	F	B	F	B	F	B	F
0.00	+[b]	+	+	+	+	+	+	+
0.01	0	0	+	+	0	+	0	+
0.10	+	0	+	+	0	0	0	+
1.00	0	0	0	0	0	0	0	0

[a] B = Bacteria; F = fungus.
[b] + = Bacterial or fungal growth from at least one of four pieces of tissue; 0 = no growth.

TABLE 3. Effect of sodium azide (1%) on serological activity and infectivity of tobacco etch virus (TEV); potato virus X (PXV); alfalfa mosaic virus (AMV); and cucumber mosaic virus (CMV) over a period of 4 weeks at 25 C

Virus	Concentration of NaN_3, %	Weeks of storage							
		1		2		3		4	
		S[a]	I	S	I	S	I	S	I
TEV	0	+[b]	+	+	+	+	0	+	+
	1	+	+	+	+	+	+	+	+
PVX	0	+	+	+	0	+	0	0	0
	1	+	+	+	0	+	0	+	0
AMV	0	+	0	+	0	+	0	+	0
	1	+	0	+	0	+	0	+	0
CMV	0	+	+	0	+	0	+	0	0
	1	+	0	+	0	+	0	+	0

[a] S = Serological activity; I = infectivity.
[b] + = Virus serologically active or infectious from at least one of four pieces of tissue; 0 = no activity.

PVY, TEV, PVX, AMV, and CMV for 1 month at 25 C. The term "inactivated" is used because tests were not conducted to discern between biocidal and biostatic effect. The infectivity of TMV, PVY, and TEV in tobacco tissue was protected for 1 month and PVX for 1 week by 1% NaN_3. AMV and CMV were not infectious after 1 week in NaN_3.

The mode of action of NaN_3 as a protectant of serological activity and infectivity was not determined in these tests. However, there was a general correlation between tissue destruction by microorganisms and loss of activity. Therefore, protection against degradation by microorganisms was probably a prime factor affecting preservation of serological activity and infectivity.

Some situations where information reported in this paper may be of value are (i) in movement of TMV, PVY, TEV, and perhaps PVX, in leaf tissue through quarantines directed toward nonvirus pathogens; (ii) movement of AMV- or CMV-infected tissue through quarantines for serological identification; and (iii) storage of a large number of samples prior to virus assay.

LITERATURE CITED

1. FEINBERG, J. G. 1957. Identification, discrimination and quantification in Ouchterlony gel plates. Int. Arch. Allergy 11:129-152.
2. GOODING, G. V., JR., & W. W. BING. 1970. Serological identification of potato virus Y and tobacco etch virus using immunodiffusion plates containing sodium dodecyl sulfate. Phytopathology 60:1293 (Abstr.).
3. SMITH, K. M. 1957. A textbook of plant virus diseases. J & A Churchill, Ltd., London. 652 p.