Mechanical Transmission of Viruses from Sweet Potato

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ABSTRACT

Distilled water was compared to 0.1 M concentrations of ascorbic acid, cysteine hydrochloride, sodium sulfite, thioglycolic acid, phosphate buffer, and diethyl-dithiocarbamic acid as additives to virus-infected leaf sap of sweet potatoes in mechanical inoculations. Week-old seedlings of *Ipomoea batatas, I. nil, I. purpurea*, and *I. violacea* became infected when their cotyledons were mechanically inoculated with leaf sap from sweet potato cultivar Sunny Side with feathery mottle symptoms, and P.I. No. 320448 with purple ring symptoms. No infection was detected when the first true leaves of sweet potato seedlings were inoculated. The use of distilled water as an additive resulted in appreciably greater percent infection over the other additives, except diethyl-dithiocarbamic acid. No consistent difference was detected in the symptoms observed in plants inoculated with either inoculum source or additive. The use of cysteine hydrochloride did not result in greater stability of the pathogen in infected sap. Pinwheel inclusions were observed in tissues of Sunny Side with feathery mottle symptoms, and long flexuous rods (average length, 750 nm) were observed in infected sap of cultivar P.I. No. 320448 and of the seedlings inoculated with both inoculum sources. Observations suggest similar infective pathogens and high variability of symptom expression due to cultivar differences.

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Additional key words: antioxidants, virus instability, host variability, host susceptibility, flexuous rod virus particles.

Some pathogens that cause viruslike symptoms in sweet potato are apparently unstable in infected sap. This and other related problems have precluded their purification and subsequent characterization. Their mechanical transmission to sweet potato has failed in many cases, but has been achieved in other species of *Ipomoea* and a few other genera (4, 8, 13, 17, 18). Some of these studies have been made with the aid of antioxidants (5, 15).

This study compares distilled water, the antioxidants ascorbic acid, cysteine hydrochloride, sodium sulfite, thioglycolic acid, phosphate buffer, and the phenoloxidase enzyme inhibitor diethyl-dithiocarbamic acid (sodium salt) as additives to virus-infected leaves of sweet potato in mechanical inoculations. It compares the susceptibility to infection of cotyledons and first true leaves of sweet potato seedlings in mechanical inoculations. It also compares viruslike particles observed in the inoculum sources and inoculated plants of sweet potato.

MATERIALS AND METHODS.—*Test species.*—One-week-old seedlings of *Ipomoea batatas, I. nil* 'Scarlet O'Hara', *I. purpurea* 'Annual Morning Glory', and *I. violacea* 'Pearly Gates' were used as indicator plants. Healthy cuttings of *I. batatas* from sources already described (2) were also used as inoculum source for the controls and as test plants in some inoculations.

Inocula.—Leaves of sweet potato cultivar Sunny Side with feathery mottle symptoms, and P.I. No. 320448 with purple ring symptoms, were used as sources of infected sap for the inoculations.

Additives.—All additives except distilled water were added to the infected sap as 0.1 M distilled water solutions at ca. 5 ml/g of leaf tissue. The pH of the additives were 6.8 for distilled water, 3.0 for ascorbic acid, 2.7 for cysteine hydrochloride, 9.6 for sodium sulfite, 2.9 for thioglycolic acid, 6.8 for phosphate buffer, and 10.2 for diethyl-dithiocarbamic acid (sodium salt).

Mechanical inoculations and growing conditions.—Leaf tissues with the additives were ground in mortars, and the expressed sap was applied to the cotyledons of the test species. In sweet potato cuttings the young leaves were inoculated, and in some trials the first true leaves of sweet potato seedlings were inoculated. Carborundum dust applied to the cotyledons or leaves was used as abrasive.

The test species were planted as seeds in polyethylene bags with sterilized soil (15 seeds/bag) in a greenhouse with moderate shade (1,500 ft-c) and temperatures (25-35 C). Observations were made regularly for 1 month in each of three trials.

Observations with the electron microscope.—Infected leaf specimens were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer, pH 6.8, and postfixed in 1% osmium tetroxide. The tissues then were dehydrated in a series of ethanol, propanol, and butanol, and embedded in epoxy resin. Thin-sections of this material were observed with an electron microscopy by R. H. Lawson. Infected leaf sap was observed directly on grids after negative staining with 2% phosphotungstic acid. R. H. Lawson kindly made measurements of the viruslike flexuous rods observed in the preparations.

Additional species tested.—Seedlings of *Nicotiana glutinosa, N. tabacum* (Burley), *Lycopersicon esculentum* (H24), *Gomphrena globosa*, and *Petunia hybrida* were used as additional indicator species. These plants were grown under conditions similar to those of the *Ipomoea* species, and inoculated with the same inocula with either distilled water or

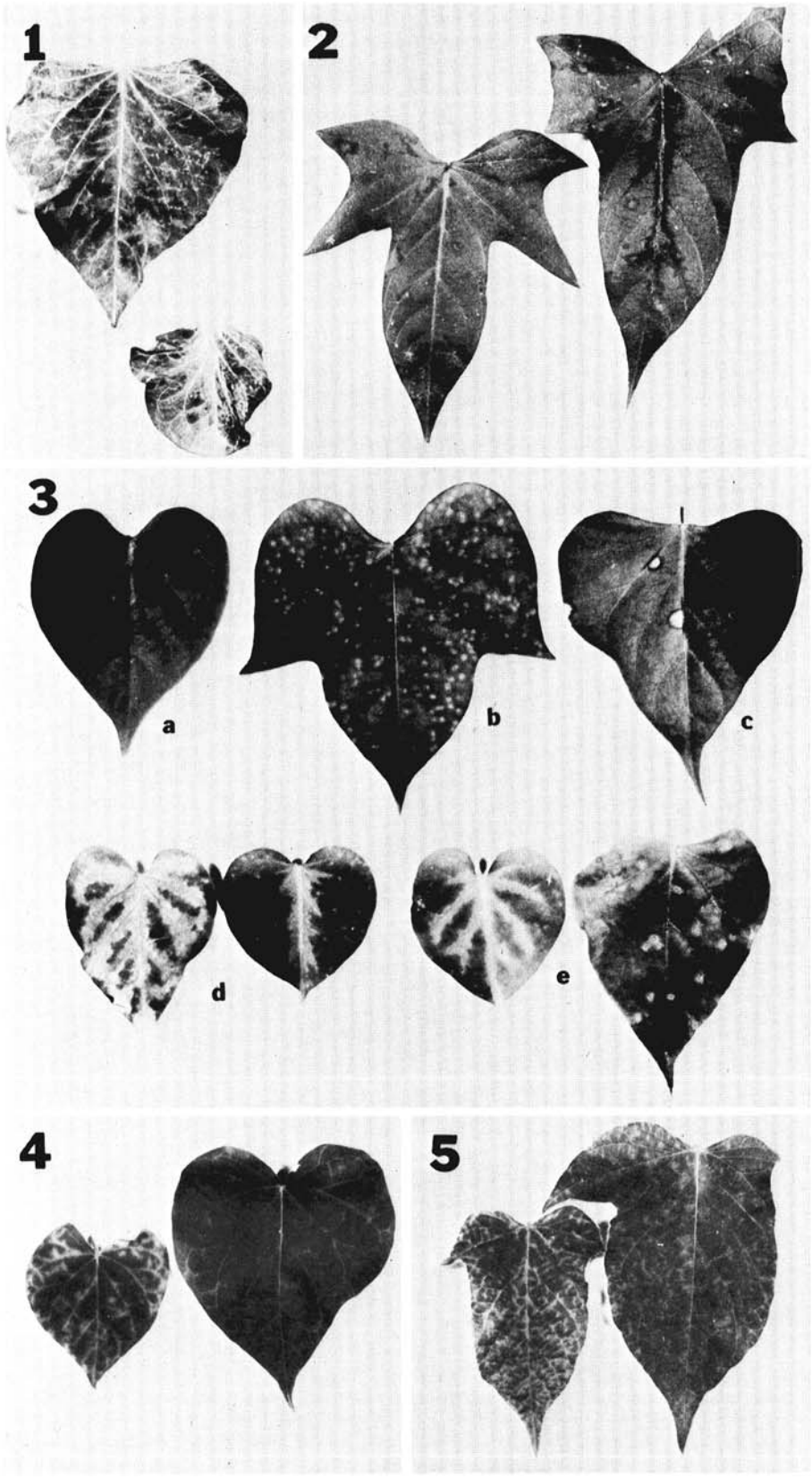
1
2
3
a
b
c
d
e
4
5

TABLE 1. Percent infection of *Ipomoea* spp. after mechanical inoculations of their cotyledons with virus-infected leaf sap with distilled water, phosphate buffer, antioxidants, and diethyl-dithiocarbamic acid

Additives	Test species							
	I. batatas		*I. nil*		*I. purpurea*		*I. violacea*	
	1[a]	2[a]	1	2	1	2	1	2
Water	19	71	59	63	31	6	74	67
Ascorbic acid	10	0	0	0	11	4	31	11
Cysteine hydrochloride	6	10	3	10	3	9	10	13
Sodium sulfite	17	8	0	1	27	1	7	3
Phosphate buffer	12	17	5	29				
Diethyl-dithiocarbamic acid	24	44	81	59	51	36	5	19

[a] 1 = feathery mottle inoculum source. 2 = P.I. 320448 inoculum source. Controls were without symptoms throughout the experimental period. There were usually more than 100 seedlings/treatment.

diethyl-dithiocarbamic acid. These plants were observed for 1 month after inoculation.

RESULTS.–Symptoms shown in Fig. 1 and 2 by leaves of Sunny Side and P.I. 320448, respectively, were similar to those leaves used as inoculum sources. The experiments were made from June 1971 to June 1972. During this time, the leaves used as inoculum source varied in their intensity of symptom expression, and probably in the concentration of virus particles. In some trials, the use of inoculum from Sunny Side resulted in greater infection of the test plants; in other trials, P.I. 320448 was the best source of inoculum. Symptom expression of the infected test species was essentially similar whatever the inoculum source, however. The greatest variation in symptoms was observed in seedlings of sweet potato (Fig. 3). Plants with purple pigments in many cases showed purple rings in their foliage. In a few cases, necrotic lesions, such as those noted in the russet crack-affected sweet potatoes (R. H. Lawson, *personal communication*), were observed (Fig. 3-c).

Table 1 illustrates the effect of mechanical inoculation of the test species with the different additives. The observations shown in this table are of one trial, and are representative of those in the other two trials. Antioxidants and phosphate buffer were comparatively ineffective as additives. Thioglycolic acid at the concentration used caused death of the inoculated plants. Damage to the inoculated cotyledons was observed when cysteine hydrochloride and sodium sulfite were used, but to a lesser extent. Percent infection with water as additive compared well with that observed when diethyl-dithiocarbamic acid was used. In some cases, the acid appeared to be better as an additive, but in others the reverse was true.

I. purpurea (Fig. 4), *I. nil* (Fig. 5), and *I. violacea* showed vein clearing and yellow spots when inoculated with either inoculum source. Vein-clearing and small yellow spots were the first symptoms of infection observed. These could be seen in all species tested 1 week after inoculation. Other symptoms, such as purple ring and necrosis in *I. batatas*, were usually observed later.

Healthy sweet potato cuttings apparently were not susceptible to mechanical inoculation. Distilled water and diethyl-dithiocarbamic acid were used as additives in these trials. The same additives were used in trials of mechanical inoculation of the first true leaves of sweet potato seedlings and of other genera. No symptoms of infection were observed in these trials, and no symptoms were observed in seedlings of Scarlet O'Hara inoculated with leaf sap of the inoculated plants with no symptoms.

Cysteine hydrochloride, diethyl-dithiocarbamic acid, and distilled water did not appear to preserve the infectivity of the pathogen beyond 3 hr in expressed leaf sap at ambient temperature (29 C), and percent infection decreased markedly after 2 hr.

Pinwheel inclusions were observed in infected leaf tissues of Sunny Side under the electron microscope. Long flexuous rods averaging 750 nm in length were observed in infected sap of Sunny Side, P.I. No. 320448 and inoculated sweet potato seedlings. No other viruslike particles were observed in any of the preparations.

DISCUSSION.–Students of sweet potato viruses have noted many symptom expressions of infection in sweet potato (3, 4, 6, 7, 9, 10, 11, 12, 13, 14, 16, 19). Interpretations of the relationship between pathogens and host response, however, must remain tentative until purified infective virus particles are obtained and used in inoculations. The variability of sweet potato is high. This variability must be

Fig. 1-5. 1) Leaves of cultivar Sunny Side with symptoms of feathery mottle. 2) Leaves of P.I. No. 320448 with purple ring and purple vein symptoms. 3) Leaves of *Ipomoea batatas* seedlings after mechanical inoculation with symptoms showing light yellow spots (a); yellow spots with purple edges (b); necrotic lesions (c); veinbanding and yellow spots (d); veinbanding and yellow spots with necrotic centers (e). 4) Leaves of *I. purpurea* with veinbanding and yellow spots after mechanical inoculation. 5) Leaves of *I. nil* with veinbanding and yellow spots after mechanical inoculation.

important in the response of each clone to virus infection. Until one is assured of the purity of the inoculum, and is also aware of the high variability in sweet potato clones or seedlings, the names given to the pathogens based on symptom expression must remain tentative.

In this study, Sunny Side expressed symptoms similar to those reported caused by the feathery mottle virus (18). Whatever the cause of these symptoms, they could not be reproduced in any of the sweet potato seedlings that became infected upon inoculation. Sunny Side leaves contained pinwheel inclusions associated with infection of the potato virus Y group. Sweet potato seedlings infected with this inoculum source, as well as with the inoculum from infected leaves with purple rings, contained long, flexuous rods associated with that virus group also. Many preparations were made, and no other viruslike particle was observed.

It is possible that one or both inocula contained a mixture of viruses, and that only one type was infective under the test conditions. This possibility could be checked when purified preparations are obtained of the pathogens involved, and perhaps when other genera are found to be susceptible. The possibility that there may be several strains of a few or one pathogen involved in the United States, Africa, and other countries in this crop, will also remain to be determined until we know more about the pathogens.

The data in this study suggest that cotyledons of sweet potato are susceptible to mechanical inoculation, but not the true leaves. This apparent difference may account for previous failures in some cases to infect sweet potato by this method (1, 11, 15, 18). Although the sap preparations did not remain infective for longer than 3 hr, it is apparent that the inactivation time is not as short as had been previously reported (5). Stability and percent infection may be improved by other additives than those tested, but in this study distilled water was more effective than any other additive except diethyl-dithiocarbamic acid.

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