Sucrose in Virus Transmission

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Aided by National Science Foundation Grant No. GB 25122.
Accepted for publication 4 May 1971.

ABSTRACT

Under most routine conditions tested, addition of sucrose to virus inoculum caused only slight or no increase in the mechanical transmission of the viruses of cucumber mosaic (CMV), citrange stunt (CSV), tomato spotted wilt (TSWV), tomato ringspot (TRSV), tobacco ringspot (ToRSV), tobacco mosaic (TMV), tobacco necrosis (TNV), and potato X (PXV), but caused significant increase in the transmission of all these viruses under certain conditions. The greatest increase (average, 55-fold; maximum, 467-fold) in virus transmission due to 5% sucrose (sucrose effect) was in the transmission of CMV from the inner leaves of old, systemically infected sugarbeets when 16% magnesium silicate was added to the inoculum after grinding, and when the young, inoculated cowpea leaves were dried slowly. The presence of $Mg_2Si_3O_8 \cdot 5H_2O$ or caffeine, absence of K_2HPO_4, use of cowpea as an indicator host, and slow drying were the most important variables for inducing a high sucrose effect. Other variables which increased the sucrose effect were: use of upper versus lower leaves of systemically infected cucumber or tobacco as inoculum; use of up to 20% Celite in the inoculum; aging of the inoculum suspension for 10 min to 1 hr; heating the indicator leaves 5-15 sec at 50 C before inoculation; and making inoculations in early morning during the winter months. The addition of sucrose or dextrose increased the longevity of TMV, TSWV, and CMV in liquid suspensions or as drying deposits on leaves. Phytopathology 61:1173-1176.

Additional key word: sugars.

Sucrose was first reported to increase virus transmission by Hansen (6). Since then Brakke (1), Davis & Whitcomb (2), Whitcomb & Sinha (11), Grant & Corbett (5), Desjardins & Wallace (3), and Yarwood & Hecht-Poinar (13) have confirmed the merits of sucrose in virus transmission, but El-Kandelgy & Wilcoxan (4), Lindner et al. (7), Subbarayudu & Wilcoxan (9), and Sukhbir et al. (10) indicate that sucrose and/or other sugars decreased infection. Several other references to the use of sugars in virus inoculation could be given. It is therefore logical to expect that sugars may increase or decrease infection, depending upon inadequately specified conditions. The present report is an attempt to specify under what conditions sugars are most effective.

MATERIALS AND METHODS.—Inoculum was usually prepared by grinding 0.1 or 0.2 g donor leaf tissue plus test supplement and including or excluding sugar, in 1 ml water. Water and Celite were added, and the suspension was wiped over the surface of indicator leaves. Most indicator plants (cucumber, cowpea, bean) had twin leaves; one leaf was inoculated without sugar, and the opposite leaf of the same plant was inoculated with the same suspension plus sugar. Lesions were counted 3-10 days later. The ratio of the number of lesions with sugar to the number of lesions without sugar is the sugar index (see Table 1). In Table 2, the minimum sucrose index is the lowest value for a given treatment in any test, the average is the mean index value for all tests, and the maximum is the highest value for a given treatment in any test. The viruses, donor hosts, and indicator hosts are indicated in Table 2. Numbers of replications within a test varied from zero to six, and the numbers of lesions per leaf ranged from zero to about 1,600. Within a test, replicates usually gave closely similar results, but the same treatment in different trials commonly gave very different results. Therefore, the number of trials is considered of much greater importance in increasing significance than is the number of replications.

Trials of the effect of sugars in the preservation of virus were of two types. In one, the virus suspensions with and without sugar were used as inoculum at different times after preparation. In the other, virus suspensions with and without sugar were sprayed on the indicator leaves, and the leaf surfaces rubbed at various times after inoculation.

Except for trials of time of day or season, all comparisons of a test variable presented in Table 3 were within the same trials.

Trials were progressive in that as different variables were found important in the sucrose effect, these variables were increasingly used as constants in subsequent trials. For example, most trials of the effect of sucrose concentration (Table 3) were performed before the importance of other supplements, of drying of leaves, of position of donor leaves, etc., was realized. Therefore, the values of the sucrose index for different concentrations of sucrose are much lower than if such trials had been made with magnesium silicate ($Mg_2Si_3O_8 \cdot 5H_2O$, here abbreviated as MgSi) or caffeine in the inoculum, without quick drying of leaves, etc.

RESULTS.—Actual results of one representative trial are given in Table 1, and a summary of most trials is given in Tables 2 and 3. Infection of all six viruses tested was increased due to sucrose under some conditions, and infection was decreased by sucrose under other conditions. The greater sucrose indexes with cucumber mosaic virus (CMV) than with other viruses is probably because it was studied at greater length. There was no clear effect of donor host, but average values for CMV from tobacco were the highest secured. Cowpea and bean as indicator hosts gave higher

TABLE 1. Effect of sucrose on transmission of cucumber mosaic virus from cucumber to cowpea in one trial[a]

Supplement to 2% cucumber tissue + 5% Celite	Quick-dried after inoculation	No. lesions on four cowpea leaves		
		Control	5% Sucrose	Sucrose index
0		158	444	2.80
0	+	1,482	1,035	0.70
0.1% Caffeine		20	821	41.00
Before grind	+	807	662	0.82
15% $Mg_2Si_3O_8 \cdot 5H_2O$		17	79	4.70
After dilution	+	811	545	0.67

[a] This trial is atypical in that the sucrose index with caffeine is higher than usual, and the sucrose index with MgSi is lower than usual.

sucrose indexes than did cucumber and *Chenopodium quinoa*.

Representative dosages of treatment variables for the transmission of CMV from cucumber to cowpea are given in Table 2. Several intermediate values are omitted. Low values of the sucrose index were mostly associated with K_2HPO_4 in the inoculum and quick drying after inoculation. K_2HPO_4 in the inoculum reduced the sucrose index more than 4-fold, and quick drying more than 3-fold. Other treatments which reduced the sucrose index were washing of leaves and/or holding plants in a moist chamber after inoculation. The concentration of sucrose for the highest sucrose index was about 5%. High concentrations of sucrose commonly injured the leaves. High values of the sucrose index were usually associated with high concentrations of Celite as an abrasive, with use of upper

TABLE 2. The effect of sucrose in the inoculum on the transmission of several viruses

Virus[a]	Donor host	Indicator host	Sucrose index				
			Trials	Avg no. lesions on control leaves[b]	Min	Avg	Max
CMV	Cucumber	Cowpea	267	68	0.03	18.0	400.0
CMV	Beet	Cowpea	138	28	0.20	13.0	467.0
CMV	Tobacco	Cowpea	28	75	0.90	22.0	143.0
CMV	Cowpea	Bean	10	51	0.07	5.2	28.0
TRSV	Cucumber	Cucumber	8	30	0.20	1.4	4.0
TRSV	Cucumber	Cowpea	3	33	0.90	5.1	7.8
TRSV	Cucumber	Bean	10	6	0.10	6.4	23.0
TRSV	Bean	Bean	1	12	0.30	3.1	10.0
TRSV	*Baccharis pilularis*	Cowpea	3	15	1.00	2.5	4.7
TRSV	*B. pilularis*	Bean	2	2	2.00	3.0	4.0
TRSV	Cowpea	Cucumber	2	16	0.50	0.8	0.9
ToRSV	Plantain	Cucumber	2	12	1.00	5.5	16.0
ToRSV	Plantain	Bean	4	18	0.50	16.0	37.0
ToRSV	Plantain	*Chenopodium quinoa*	2	70	0.50	0.6	1.1
CSV	Lemon	Cowpea	32	0	0.04	2.8	13.0
CSV	Lemon	Bean	17	8	0.30	2.7	16.0
CSV	Cowpea	Cowpea	15	45	0.20	3.7	14.0
CSV	Cowpea	Bean	3	20	0.80	6.9	23.0
CSV	*Nicotiana clevelandii*	Cowpea	6	65	0.60	4.0	24.0
CSV	*N. clevelandii*	Bean	3	23	0.20	6.7	25.0
TSWV	*Erigeron glaucus*	Cucumber	9	10	0.30	1.2	2.5
TSWV	*E. glaucus*	*C. quinoa*	2	25	0.70	0.9	1.2
TSWV	*E. glaucus*	Cowpea	9	14	0.70	5.0	15.0
TSWV	*E. glaucus*	Bean	5	2	0.20	3.6	12.0
TSWV	*N. rustica*	Cucumber	5	27	0.20	2.4	5.0
TSWV	*N. rustica*	Cowpea	4	14	0.30	13.0	117.0
TSWV	Cowpea	Cucumber	8	12	0.50	1.1	3.0
TSWV	Cowpea	Cowpea	15	11	0.20	8.5	57.0
TSWV	Cowpea	Bean	2	15	6.00	10.0	17.0
TMV	Tobacco	Cowpea	2	60	1.10	3.9	9.0
TMV	Tobacco	Bean	3	220	0.50	1.0	1.5
TMV	Cucumber	Cucumber	3	45	0.70	1.3	2.0
TMV	Cucumber	Cowpea	2	33	1.80	3.1	5.0

[a] CMV = cucumber mosaic virus; TRSV = tomato ringspot virus; ToRSV = tobacco ringspot virus; CSV = citrange stunt virus; TSWV = tomato spotted wilt virus; TMV = tobacco mosaic virus.

[b] These have little significance except to indicate the total number of lesions. Vertical comparison of values is not valid for comparing the relative infectivity of different inocula. Trials were designed to test the effect of sucrose under a variety of conditions, with inoculum without sucrose on one leaf and the same inoculum with sucrose on the opposite twin leaf. For CMV transferred from cucumber to cowpea, the average number of lesions per leaf without sucrose ranged from 0 to 450 in different trials.

TABLE 3. Some variables affecting sucrose in the transmission of cucumber mosaic virus from cucumber to cowpea

Variable	Treatment	Trials	Avg no. lesions per leaf on control leaves	Avg sucrose index
Concentration of sucrose	0.5% Sucrose	19	42	2.2
	2% Sucrose	19	42	4.2
	8% Sucrose	19	42	4.6
Environment	Normal	5	40	12.0
	Moist chamber for 24 hrs	5	10	3.8
Drying of inoculated leaves	Control	48	35	9.0
	Quick-dried	48	92	2.5
Washing of inoculated leaves	Control	5	47	8.4
	Washed	5	122	2.7
Position of donor leaves	Lower (basal)	15	42	9.0
	Central	14	110	13.0
	Upper (terminal)	20	20	17.0
Age of indicator plants	7-11 Days from seeding	17	34	18.0
	12-16 Days from seeding	12	45	7.2
Time of day of inoculation	0400-0800	96	37	23.0
	1400-1800	35	21	12.0
Aging of inoculum suspension	Fresh	16	52	13.0
	Aged 10-40 min	8	40	20.0
Heat to indicator leaves before inoculation	0	20	14	8.9
	10 Sec at 50 C	13	18	20.0
	20 Sec at 50 C	7	4	7.0
Concentration of K_2HPO_4	0	34	36	9.5
	0.5% K_2HPO_4	34	254	2.1
Concentration of $Mg_2Si_3O_8 \cdot 5H_2O$	0	25	20	6.6
	2%	14	18	17.0
	6%	19	23	22.0
	15%	11	3	55.0
Concentration of caffeine	0	9	97	6.7
	0.1%	9	59	12.0
Concentration of Celite	0.1	8	9	3.4
	1.0	16	82	6.8
	10.0	24	96	18.0
	20.0	6	26	28.0
Type of abrasive	15% Celite	2	42	15.0
	15% MgSi	2	3	27.0
	15% Corundum	1	11	2.2
	15% Carborundum	1	11	2.0
Time of adding sucrose	Before grinding	8	33	16.0
	After dilution	8	33	9.8
Time of adding MgSi	Before grinding	8	38	11.0
	After dilution	8	38	26.0

donor leaves, with the use of young indicator plants, with making inoculations in the early morning, and with high concentrations of MgSi added after dilution of the inoculum. High concentrations of Celite and/or MgSi delay the natural drying of inoculated leaves, and their effect may therefore be related to the effect of quick drying of leaves with or without these supplements.

Sucrose was the sugar used unless otherwise indicated, but lactose, mannose, and dextrose were used in seven trials, with results not clearly distinguished from those with sucrose.

Of 762 trials over 7 years, the number of trials and the average maximum sucrose index per trial per month, respectively (data not in tables), were as follows: January, 76 and 77; February, 55 and 203; March, 32 and 32; April, 55 and 33; May, 56 and 47; June, 58 and 46; July, 63 and 90; August, 64 and 73; September, 73 and 105; October, 66 and 61; November, 84 and 92; and December, 80 and 53. Since the average sucrose index was 111 for the winter months, 37 for spring months, 69 for summer months, and 86 for fall months, there is support here for Whitcomb & Sinha's report (11) that the sucrose effect is greatest in the winter months. More clear than seasonal differences are the progressive annual differences. As trials progressed, each trial was based on knowledge secured in previous trials, and the average maximum sucrose index per trial was 7.6 in 1963 and 133 in 1970.

A clear relation of concentration of inoculum to the sucrose effect was not established. In 38 trials, the average sucrose index was 11 for 0.25% tissue concentration, 8.9 for 0.5%, 6.4 for 1%, 7.6 for 2%, 8.3 for 4%, and 5.7 for 8%.

Virus was inactivated more slowly in sucrose suspensions than in water. In eight trials, the average half-life (8) of a suspension of tomato spotted wilt virus (TSWV) in water was 21 min; in 5% sucrose, 41 min. In four trials, the average half-life of CMV in water was 91 min; in 5% sucrose, 303 min. In six trials, the average half-life for CMV suspensions sprayed on leaves and allowed to dry was 30 min in water and 190 min in 5% sucrose. In two trials, the average half-life of tobacco mosaic virus (TMV) suspensions sprayed on leaves was 104 min for water and 420 min for sucrose. In trials with CMV only, dextrose appeared to behave similarly to sucrose in the preservation of virus infectivity.

DISCUSSION.—As sucrose may cause such great increases in virus transmission, situations might be expected where sucrose would be a major aid in virus transmission. No such case is apparent from this study, nor was this study properly designed to reveal such. It was once believed (5) that sucrose and/or charcoal were uniquely useful in the transmission of a virus from citrus to herbaceous indicator plants, but recent studies with charcoal (12) and the present studies with sucrose do not support this. While sucrose and charcoal separately or in combination increase transmission of citrange stunt virus to cowpea, they do not give greater transmission than can be secured with other supplementary treatments such as K_2HPO_4 and quick drying, without sucrose or charcoal (data not presented here).

The objective of this study was to find the conditions under which sucrose would produce the greatest increases (or decreases) in virus transmission. We found that the conditions which resulted in the highest sucrose indexes (slow drying, absence of K_2HPO_4,

high MgSi) are conditions which produce low infection; and the reversal of these conditions (quick drying, presence of K_2HPO_4, low MgSi) will produce greater infection than any addition of sucrose.

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