Quick Drying Versus Washing in Virus Inoculations

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ABSTRACT

Washing and quick drying of inoculated leaves have separately and in combination increased virus infection under certain conditions, and decreased infection under other conditions, with 15 virus:donor host:indicator host combinations. Increases in infection due to quick drying were up to 3,060-fold in a single test, were greater with cowpea than with any other indicator host, with cucumber mosaic than with any other virus, greater with young than with old cowpeas, greater with concentrated than with dilute inoculum, greater with inoculum from apical than from basal donor cucumber leaves, greater with concentrated, than with dilute Celite, greater if plants were placed in dark rather than in light after inoculation, greater for inoculations made about midday than at other times, greater with inhibitors such as buckeye juice in the inoculum, and greater with inoculum containing caffeine, K_2SO_3, or $Mg_2Si_3O_8$ than with inoculum containing K_2HPO_4 or sucrose. The washing of leaves after inoculation caused up to 600-fold increase in infection, and the conditions favoring high increases in infection due to washing were usually similar to those causing high infection due to quick drying. Washing for more than 10 sec decreased infection. Washing or quick drying reduced plant injury caused by chemicals in the inoculum, and contributed to the ease of counting lesions.
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Holmes (1) showed that when *Nicotiana glutinosa* leaves were washed with water immediately after inoculation with tobacco mosaic virus, the number of infections (lesions) was increased 1.4-fold. This initially surprising result has been adequately confirmed, even though some attempts at confirmation have failed (2), and the practice of the washing of leaves after inoculation of them with viruses has been widely adopted. On the other hand, Yarwood (3), although confirming Holmes' result, also found that if the washing was continued more than 10 sec, reduced infection usually resulted. He also found that quick drying, a treatment in some ways almost the opposite of washing, increased infection up to about 100-fold (4). Actually, washing and quick drying may each greatly increase or decrease infection, depending on a variety of conditions, and these conditions are further explored here.

While this is the third report of the effect of quick drying on infection, it may be justified by the much greater responses than previously reported, the greater variety of variables explored than in previous studies, and by the comparison with washing.

MATERIALS AND METHODS.—Cucumber mosaic virus (CMV) produced in cucumber (*Cucumis sativus* L. 'Ashley') and assayed on cowpea [*Vigna sinensis* (Torner) Savi 'Blackeye'] was the test system primarily used, because with present knowledge, it shows the greatest response to washing and quick drying (QD), but several other virus-host combinations were tested (Table 2). All were well known viruses except the bean rust virus (BRV) which was isolated from rust-infected bean tissue [uredinal stage of *Uromyces phaseoli* (Pers.) Wint. on *Phaseolus vulgaris* L. 'Pinto']. All gave well defined lesions on the indicator hosts except BRV, for which the lesions on bean and cowpea were irregular in outline, variable in necrosis and difficult to count. Typically, the control treatment was on one primary leaf, and the test treatment, washing and/or quick drying, was on the opposite leaf of the same plant.

For preparation of inoculum and inoculations, 0.1 g of systemically infected leaves was typically ground in 1 ml water, 9 ml water and 0.5 g Celite were added, and this mixture was stroked with a finger over the upper surfaces of the indicator leaves.

Washing was performed by holding the inoculated leaf under the slowly flowing water at 14 C from the water tap for about 2 sec immediately after inoculation. Quick drying was performed by moving an air jet from compressed air at 35 psi over the supported inoculated leaf until all free liquid was removed which required about 4 sec. Speed in quick drying is a minimum essential for high QD indexes (4). Lesions were usually counted at 3 days after inoculation.

Numbers of lesions per leaf ranged from 0 to 1,600, and averaged 58 on 124 leaves in three recent trials which are considered typical. Lesions up to about 200/leaf were counted individually, but for values above 200, the number of lesions was usually estimated from the first 100 or 200 counted. The number of lesions on the test leaves (e.g., QD or washed) divided by the number on the control leaves is the treatment index (see Table 1). If the number of the control was 0, the treatment index given is the number of lesions on the treated leaf, which is the same as assuming one lesion on the control.

Replications within a trial were from none to four, and the number of contrasting treatments, other than washing or drying, within a trial varied from 2 to 24. Total trials since the last report (4) were about 1,500. The numbers of trials given in Tables 2 and 3 are for trials where the test variable was varied within the trial, with all other conditions constant within the trial, but not necessarily or even usually between trials.

Controls for different trials of the same variable, and controls for different variables, were not necessarily the same. Tests of a given variable were designed to show the effect of that variable, and if these conditions were not properly chosen, the effect of the test variable could be weakly expressed. For

example, K_2HPO_4 and sucrose usually reduced the effect of quick drying so, except when they were being specifically studied, K_2HPO_4 and sucrose were omitted. As knowledge of the effect of different variables became better understood, these variables were either omitted or included as constants throughout the test, depending on the purpose of the test. The variation in the values for the controls for different test variables is largely because the constants within the test (time of day of inoculation, concentration of tissue, concentration of Celite, etc.) were changed according to the state of knowledge of conditions for lesion formation as learned over the 9 years of these tests.

RESULTS.—Partial results of a typical trial are given in Table 1, and a summary of most trials is given in Tables 2 and 3. A discussion of these results and some added information follows. Quick drying increased the transmission of all viruses tested and on all indicator hosts, but there were important differences. The higher values with CMV than with any other virus are considered significant, but are partly because CMV was more tested than any other virus. The lowest QD values were with tobacco necrosis virus (TNV) and BRV, and these viruses are considered significantly different in this regard from the other viruses tested. Donor host was apparently not important, and the low values for inoculum from tobacco are likely because it was so little tested, and these few tests were mostly before most of the critical variables were discovered.

Indicator host is clearly more important than donor host, and cowpea has given greater QD values than bean or cucumber, especially in trials where the same virus (e.g., TMV, TSWV) was compared simultaneously on all three hosts. *Chenopodium quinoa* and *C. amaranticolor,* which are good indicator hosts for many viruses, gave QD index values of about 1,

TABLE 1. Effect of washing and quick drying on the transmission of cucumber mosaic virus from cucumber to cowpea. Partial results of a typical recent trial

Supplement to 4% cucumber inoculum	Test treatment	Lesions on one plant		Treatment index
		Control leaf	Treated leaf	
1% Celite	Washed	11	120	11
1% Celite	Quick-dried	6	238	40
1% Celite	Washed + quick-dried	6	108	18
20% Celite	Washed	0	244	244
20% Celite	Quick-dried	7	700	100
20% Celite	Washed + quick-dried	5	800	160
0.1% caffeine + 20% Celite	Washed	0	86	86
0.1% caffeine + 20% Celite	Quick-dried	0	252	252
0.1% caffeine + 20% Celite	Washed + quick-dried	0	600	600

TABLE 2. Quick drying in virus inoculations

Virus	Donor host	Indicator host	Number of trials	Quick drying index	
				Minimum[a]	Maximum[a]
Cucumber mosaic (CMV)	Beet	Cowpea	25	0.190	700.0
	Cowpea	Bean	10	0.400	150.0
	Cucumber	Cowpea	1,100 (est.)	0.100	3,060.0
	Tobacco	Cowpea	3	0.300	25.0
Tobacco mosaic (TMV)	Tobacco	Bean	85	0.300	26.0
Tobacco necrosis (TNV)	Cowpea	Cowpea	22	0.520	86.0
	Cowpea	Cucumber	8	0.200	2.8
	Cucumber	Cucumber	8	0.450	4.0
Tomato ringspot (TRSV)	Erigeron	Cowpea	5	0.500	55.0
Tomato spotted wilt	Erigeron	Cowpea	10	0.080	167.0
(TSWV)	Erigeron	Cucumber	9	0.250	7.0
	Cowpea	Cucumber	12	0.250	15.0
Artichoke latent (ALV)	Artichoke	Bean	12	0.500	19.0
Citrange stunt (CSV)	Lemon	Cowpea	15	0.320	54.0
Bean rust[b] (BRV)	*C. quinoa*[c]	Cowpea	4	0.048	6.4

[a]The minimum is the quick-dried (QD) value for the least effective treatment; the maximum is the QD value for the most effective treatment. Examples of treatments are given in Table 3.

[b]Most inoculations of bean rust virus were with K_2HPO_4.

[c]*Chenopodium quinoa*.

TABLE 3. The quick drying effect in the transmission of cucumber mosaic virus from cucumber to cowpea

Variable	Dosage of variable	Number of trials	Average lesions per leaf on controls	Average quick drying index
Concentration of inoculum	0.1% donor tissue	6	97.00	2.0
	1.0% donor tissue	6	112.00	24.0
	10.0% donor tissue	6	5.60	53.0
Position of donor leaves	Cotyledon (basal)	6	140.00	13.0
	First true leaf	8	77.00	9.5
	Second true leaf	8	75.00	12.0
	Third true leaf	8	68.00	16.0
	Fourth true leaf	8	35.00	40.0
	Fifth true leaf, terminal	8	8.00	70.0
Time of day of inoculation	0400-0700	11	89.00	12.0
	0700-1000	5	44.00	21.0
	1000-1300	10	52.00	71.0
	1300-1600	4	47.00	44.0
	1600-1900	7	52.00	12.0
	1900-2200	5	78.00	13.0
Age of indicator cowpeas, days from seeding until inoculation	7	22	45.00	40.0
	10	34	56.00	32.0
	13	36	42.00	11.0
	16	10	22.00	5.0
	19	2	7.00	1.2
Period in dark chamber after inoculation	0	7	30.00	30.0
	24 hr	7	1.90	72.0
Duration of immersion in water after inoculation	0	8	14.00	13.0
	1 sec	8	38.00	2.9
	10 sec	8	12.00	1.9
	60 sec	8	3.00	1.0
Heat to indicator host before inoculation	0	10	44.00	27.0
	5 sec at 50 C	10	61.00	27.0
	10 sec at 50 C	8	55.00	26.0
	20 sec at 50 C	2	25.00	13.0
Time from grinding until inoculation	3 sec	11	38.00	24.0
	3 min	5	24.00	18.0
	30 min	12	10.00	11.0
Concentration of Celite, added after dilution	0	9	0.66	2.1
	1%	9	51.00	4.3
	5%	9	80.00	15.0
	20%	9	11.00	48.0
Concentration of charcoal, added after dilution	0	6	19.00	20.0
	0.5%	6	25.00	45.0
	0.3%	6	31.00	14.0
	1.0%	4	15.00	9.0
Concentration of K_2HPO_4, added before grinding	0	6	29.00	29.0
	0.1%	6	32.00	38.0
	1.0%	6	70.00	4.0
	2.0%	6	15.00	4.5
Concentration of caffeine, added before grinding	0	45	13.00	27.0
	0.1%	37	4.50	82.0
	0.2%	18	1.90	156.0
	0.4%	12	0.60	194.0
	0.8%	4	0.14	761.0
	1.0%	1	0.00	2,000.0
Concentration of K_2SO_3, added before grinding	0	10	36.00	2.7
	0.1%	10	31.00	18.0
	0.3%	10	18.00	33.0
	0.8%	2	10.00	48.0
Concentration of sucrose, added before grinding	0	12	20.00	39.0
	5%	12	52.00	13.0

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TABLE 3. The quick drying effect in the transmission of cucumber mosaic virus from cucumber to cowpea, *(Continued)*

Variable	Dosage of variable	Number of trials	Average lesions per leaf on controls	Average quick drying index
Concentration of $Mg_2Si_3O_8$, added before grinding	0	53	83.00	25.0
	10%	13	11.00	65.0
Concentration of bentonite, added before grinding	0	8	38.00	15.0
	0.1%	8	7.70	24.0
Concentration of healthy buckeye (*Aesculus californica*) juice	0	2	88.00	4.8
	0.1%	2	38.00	5.0
	0.3%	2	21.00	11.0
	1.0%	2	7.00	16.0
	3.0%	2	0.50	150.0

and this heavy infection without quick drying (data not in table) may be one reason why they are such good indicator hosts.

Large increases in infection due to quick drying resulted under several conditions which were not realized in the earlier trials (3, 4). With CMV, the QD effect increased rapidly with increasing concentration of inoculum (Table 3). This was apparently not true with TMV (3) or with BRV (this study), so generalization is not safe. With CMV from cucumber (Table 3) and tobacco (data not in table), the QD index increased rapidly as inoculum was taken from an increasingly higher (or more apical) position on the donor host. Data is given for cucumber plants with five true leaves, but the phenomenon was also observed with plants having up to 20 consecutive leaves. Also, if healthy tissue was added to inoculum, the healthy distal tissue caused a greater increase in the QD index than did healthy proximal tissue.

Time of day yielded erratic results not adequately indicated by the data in Table 3, but in six trials, the highest QD index was about 11 AM. Charcoal at 0.05% increased the QD index, but further increases in charcoal reduced the QD effect. K_2HPO_4 at 0.05% and 0.1% increased the QD effect slightly, 0.5 and 1% K_2HPO_4 reduced the QD effect, and 2 and 3% K_2HPO_4 increased the QD effect. Caffeine gave the largest increases in QD index of any chemical tested.

Concentrations of chemicals, or treatments, which caused low infection and/or considerable host injury without QD usually caused high QD indexes. This was true with caffeine, bentonite, K_2SO_3, K_2HPO_4, and extracts of healthy oak, cucumber, buckeye, *C. amaranticolor,* carnation, and rusted bean.

Several combinations of the variables listed in Table 3 have consistently given larger QD indexes than have the variables studied separately. The inoculum which has been used several times and which has consistently given a QD index of about 500 with little leaf injury was 5% tissue of upper leaves of systemically infected cucumber + 0.2% caffeine + 20% Celite applied at about 11 AM to primary leaves of cowpea at 7 to 10 days after seeding.

Other things being equal, high QD indexes indicate high levels of infection, but because of the association of high QD indexes with low levels of infection in the controls, this is not necessarily so. For example, the QD index of 150 for 3% buckeye juice added to CMV inoculum (Table 3) indicates a total of 150 X 0.5 = 75 lesions/leaf, whereas the QD index for the controls without buckeye juice indicates 4.8 X 88 = 422 lesions/leaf.

Quick drying gave potentially infinite increases in several situations, and there were 48 pairs of leaves in the last 10 routine trials (153 pairs of leaves of which one was quick-dried) in which the number of lesions on the control was 0 and the number on the quick-dried was 50 or more. A high QD index depends in large part on few infections on the controls, but there were many treatments such as low concentration of inoculum, resistant hosts, and unfavorable environment (data not presented here) which resulted in low infection but did not result in high QD indexes.

The lowest QD indexes resulted when K_2HPO_4 was added to the inoculum, but even with K_2HPO_4, QD indexes were usually above 1. Only with K_2HPO_4 added to inoculum of TSWV or BRV were QD indexes commonly below 1. With TSWV, the average QD index was 1.4 for 0.2% K_2HPO_4 (14 trials), 0.86 for 0.5% K_2HPO_4 (72 trials, 8,454 lesions), and 1.4 for 1% K_2HPO_4 (9 trials). Of the 72 trials with 0.5% K_2HPO_4, 43 trials gave QD indexes of 1 or below and 14 of these were below 0.5. In four trials (24 paired leaves, 2,740 lesions) with BRV, the minimum QD index for 1% tissue + 0.5% K_2HPO_4 was 0.05, the average was 0.60, and the maximum was 1.7.

Washing of leaves after inoculation was much less tested than quick drying, but in 28 trials in which they were directly compared using the same inoculum, washing was consistently less effective in increasing infection than was quick drying. In these tests, including CMV, TSWV, and TMV, the average increase in infection from washing was 13-fold, and from quick drying was 36-fold. The combined effect of washing followed by quick drying of the same leaf usually gave greater infection than washing only, but not clearly more than quick drying only. Drying

followed by washing usually gave lower infection than quick drying only, and approximately the same as washing only.

The greatest increase in infection due to washing in a single trial was with 2% inoculum from an upper cucumber leaf + 0.2% caffeine + 16% Celite, in which there were no lesions on the two control leaves and an estimated 600 lesions total on the two treated leaves.

Increases in infection due to washing were greater when the inoculum contained caffeine and/or high concentration of Celite than when these chemicals were low in concentration or absent. When the inoculum contained K_2HPO_4 or sucrose, the washing index was commonly less than 1. In these regards, the effect of washing was like the effect of quick drying. Quick drying and washing were distinctly different in the response of infection to dosage. If quick drying was continued after the 4 sec usually necessary to remove visible liquid, there was little additional effect on infection, but if washing was continued longer than the 2 sec or so necessary to remove the visible inoculum, infection was sharply reduced.

Concentrations of caffeine, sucrose, K_2SO_3, and K_2HPO_4 which may increase virus infection when added to the inoculum may cause severe leaf injury. Washing and quick drying were about equally high effective in reducing this chemical or molar injury without reducing infection, except with K_2HPO_4, where washing reduced both injury and infection. Supplements such as Celite, Carborundum, $Mg_2Si_3O_8$, and charcoal added to inoculum commonly obscure the virus lesions which later appear. Washing and quick drying both reduce these obscuring deposits, but washing was more effective than quick drying for this purpose.

DISCUSSION.—What is probably the fastest manifestation of the infection process recorded for plant viruses (increase in infection due to time and duration of a treatment) is the response of inoculated leaves to quick drying and washing as reported here and earlier. Quick drying is believed to be the only treatment, which applied within 4 sec after completion of a normal inoculation, may increase infection up to 1,000-fold or more over the untreated. At present, this high magnitude of increase has been shown to apply to only a relatively few situations, but further study and understanding may increase the applicability of the method to general virology, and should be an aid in increasing understanding of the infection process. The mode of action of quick drying in increasing infection is not understood, but the suggestion made earlier (3) that it is due to removal of inhibitors at the infection site seems a promising interpretation.

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