Relative Transmissibility of Barley Yellow Dwarf Virus from Sources with Differing Virus Contents

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ABSTRACT

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Detached individual leaves of cereal plants with differing virus contents were compared as virus sources for the acquisition and transmission of three barley yellow dwarf virus (BYDV) isolates by specific (i.e., relatively efficient) or nonspecific (i.e., relatively inefficient) vectors. Overall, there was no convincing evidence of significant effects on virus transmission efficiency due to differences in virus content. However, in further experiments in which aphids fed on virus preparations through a Parafilm membrane, correlation between virus concentration and efficiency of acquisition and transmission was evident for both efficient and inefficient vectors. Aphids also acquired virus more efficiently when feeding on virus through a Parafilm membrane than when feeding on leaves containing a similar concentration of virus as assessed by ELISA of extracts. The combined results indicated that overall virus content may not the primary factor limiting the acquisition of BYDV from plants by vectors, and that its effects may be overshadowed by other factors, including uneven distribution of virus within the leaf.

For some viruses that have a nonpersistent virus vector relationship, and are not phloem-restricted, positive correlations have been established between the virus content of infected plants and their efficiency as sources of virus for transmission by aphids (e.g., 18,20,23). But in other work, especially with viruses that have a circulative type of virus vector relationship, such correlations were lacking (3,7,11,12,16), although exceptions to this have been reported, as, for example, with potato leaf roll virus, a circulative, phloem-restricted luteovirus (1).

For barley yellow dwarf luteoviruses (BYDV), which also have a persistent, circulative vector relationship and are phloem-restricted (9,17), "resistance" to symptom production (i.e., tolerance, sensu Cooper and Jones, 2) has been associated in some cases with reduced virus productivity as compared with that in "susceptible" (i.e., sensitive, sensu Cooper and Jones) plants (8,19). Further, it has been suggested that reduced virus titer in tolerant cereals could have an important role in BYDV epidemiology if it was associated with reduced virus transmission (8). Work described in a companion paper (15) clearly indicates wide variation in BYDV content among leaves of the same plant; therefore, tests of virus acquisition from individual detached leaves of the same cultivar offer a simple means to study the influence of host virus content on the acquisition and transmission of BYDV, avoiding genetic variations.

The major objective of this research was to determine if reduced virus titer in the host could be correlated with reduced virus transmission by vectors. The influence of leaf age, and the acquisition and transmission by vectors feeding on virus preparations through Parafilm (M membrane, American Can Co., Greenwich, CT) were also examined.

MATERIALS AND METHODS

Virus isolates and vectors. The three BYDV isolates used were the RPV and MAV isolates of Rochow (17) and the P-PAV isolate (6), which is an Indiana isolate of the PAV type. For MAV and RPV, their respective specific vectors *Sitobion* (= *Macrosiphum*) *avenae* F. and *Rhopalosiphum padi* L. were used. For P-PAV, transmission studies were done with both *R. padi* (a relatively efficient vector) and *S. avenae* (a relatively inefficient vector).

Viruses and viruliferous aphids were maintained on Clintland 64 oats (*Avena sativa* L.) in growth chambers at 20 ± 1 C, with fluorescent and tungsten lighting of approximately 3,000 lx at

a 14-hr photoperiod illumination. Virus-free *R. padi* and *S. avenae* were maintained in separate growth-chambers in the same conditions. Plants on which the two virus-free vector colonies were raised were checked frequently by enzyme-linked immunosorbent assay (ELISA) against the three BYDV isolates. Mixtures of apterous adults and late instar nymphs were used in all experiments.

Virus sources and test plants. For most experiments, Clintland 64 oats were used as sources of leaves for virus acquisition of the three isolates, as this was the cereal cultivar that showed greatest variations in virus content among individual leaves on the same plant (15). Clintland 64 oats were also used as test plants in all experiments.

In experiments on the transmission of P-PAV by *R. padi*, three near-isogenic pairs of barley (*Hordeum vulgare* L. em Boden) with and without the "BYDV resistance" factor Yd2 were also used: Briggs (Yd2−) and Prato (Yd2+); Atlas 57 (Yd2−) and Atlas 68 (Yd2+); California Mariout (Yd2−) and CM67 (Yd2+). For the RPV isolate, Clintland 64 oats were used as sources of virus, together with the wheat (*Triticum aestivum* L. em Thell.) cultivars, Abe and Elmo. For other characterization of the cultivars used, see Pereira and Lister (15).

Inoculation of source plants. Viruliferous *R. padi* or *S. avenae*, at eight aphids per plant, were used to infest 6-day-old seedling source plants with the BYDV isolates. Five source plants of each cultivar were used for each virus isolate, acquisition access time, and repetition. Each batch of five source plants provided about 15 individual leaves for testing after optimal virus incubation periods, which were 12, 18, or 22 days for the P-PAV, MAV, and RPV isolates, respectively (19). Aphids were killed with nicotine sulfate after a test feed of 2 days. Plants were grown in growth-chambers at 20 ± 1 C, with a 14-hr photoperiod.

Virus acquisition and transmission from individual source leaves by aphids. After the appropriate virus incubation period, individual leaves were cut under water from each source plant with a razor blade and immediately placed with their cut ends in tap water in small glass tubes. Virus-free *R. padi* or *S. avenae* were then placed on each individual source leaf to acquire virus. Relatively short acquisition access times were used to maximize possible differences in acquisition efficiency. The acquisition access times tested, which were more extensive for P-PAV in Clintland 64 oats than for other virus host combinations, were as follows:

Acquisition of P-PAV by *R. padi*:
from Clintland 64 oats—1, 2, 3, 4, 6, and 18 hr;
from Briggs (Yd2−) and Prato (Yd2+) barleys 1, 2, 3, 4, and 6 hr;
from Atlas 57 (Yd2−), Atlas 68 (Yd2+);
California Mariout (Yd2(−) and CM67 (Yd2+) barleys 3 hr.

Acquisition of P-PAV by *S. avenae*:
from Clintland 64 oats 3, 6, 12, 18, and 48 hr.

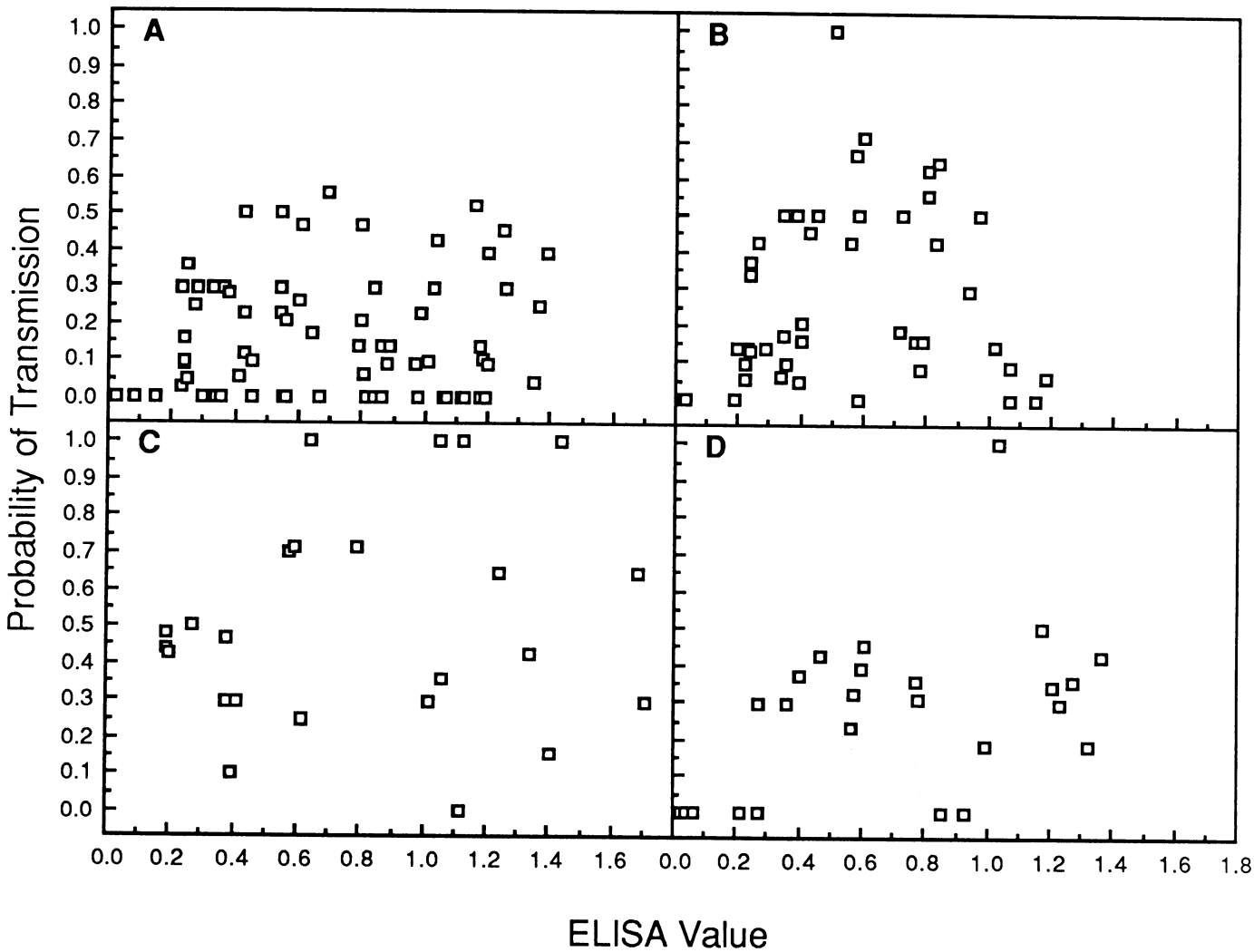

Fig. 1. Relationship of the probability of transmission of three isolates of barley yellow dwarf virus by single aphids fed on individual leaves of Clintland 64 oats, to the virus content of the leaf as assessed by enzyme-linked immunosorbent assay (ELISA). Each point is the average for individual leaves grouped in 0.200 increments of ELISA values (except for values below 0.100 see text). **A,** Values for P-PAV isolate transmitted by *Rhopalosiphum padi*; **B,** values for P-PAV isolate transmitted by *Sitobion avenae*; **C,** values for MAV isolate transmitted by *S. avenae*; **D,** values for RPV isolate transmitted by *R. padi*.

Acquisition of MAV by *S. avenae*:
from Clintland 64 oats 3 and 6 hr.
Acquisition of RPV by *R. padi*:
from Clintland 64 oats 3 and 6 hr;
from Abe and Elmo wheats 6 hr.

After acquisition access, aphids that had settled were selected and transferred to 6-day-old Clintland 64 test plants (two aphids per plant). Usually, four test plants were used for each source leaf, but sometimes only enough aphids were recovered for three or, rarely, two test plants to be used per source leaf. The aphid test feeds were for 2 days, at room temperature, with a low light intensity to encourage settling. The test plants were then sprayed with an insecticide containing 0.2% pyrethrins, and kept in a greenhouse for 12–15 days to allow virus to multiply and accumulate. Control plants were kept in the same conditions.

ELISA of plant extracts and statistical analysis. After use, all individual source leaves were tested quantitatively (i.e., to estimate comparative virus contents) by double antibody sandwich DAS-ELISA (10,15). Each test plant was tested qualitatively by DAS-ELISA, to determine the percentage of infections.

The probability of BYDV transmission by single aphids (P_t) was calculated by the maximum likelihood estimator (5,21) of: $P_t = 1 - (1 - S/N)^{1/k}$, in which N = total number of test plants used for each source leaf; S = number of test plants that became infected; and k = number of aphids per test plant (i.e., $k = 2$ in these experiments).

The relationship between P_t and virus content (i.e., ELISA value, 15) for each source leaf was determined for each cereal cultivar and each acquisition access time as one treatment. Correlation required that $P \leq 0.05$, but consideration was also given to the r-value (correlation coefficient) and the sample size (df). Because the numbers of test plants used for each source leaf varied (i.e., $N = 4$, 3, or 2), each treatment was also analyzed after grouping ELISA values for the individual leaves in increments of 0.200, except for values below 0.100, which were grouped as Group 1. Group 1 therefore included all individual leaves with very low or no detectable virus (ELISA value ≤ 0.099), Group 2 included ELISA values between 0.100 and 0.300, Group 3 included ELISA values between 0.301 and 0.500, etc. Correlations between P_t and the ELISA values of each group of individual leaves were then examined.

Acquisition and transmission of P-PAV by vectors feeding on virus preparations through Parafilm membrane. All membrane feeding experiments were done with the P-PAV isolate and the two aphid vectors *R. padi* and *S. avenae*. Purified virus preparations, which were stored at −80 C until use, consisted of the virus fractions collected directly from density gradients used for the final steps of purification as described elsewhere (6), and were in approximately 20% sucrose in 0.1 M phosphate buffer, at pH 7.0. For membrane feeding experiments (6), they were thawed and used directly or after further dilution in buffered 20% sucrose solution. Glass vials 18 mm in diameter × 60 mm high were used as feeding cages. Thirty to 40 virus-free *R. padi* or *S. avenae* were enclosed in each vial by stretched Parafilm that covered the top. Then 0.2 ml of virus preparation in 20% sucrose was placed on the top of each membrane and covered with another stretched Parafilm membrane to produce a small sachet containing the virus. These feeding cages were placed under light, at room temperature, for a 16–18-hr acquisition feeding, after which the membranes were removed and aphids that had settled were transferred to 6-day-old Clintland 64 test plants (two aphids per plant), for virus transmission. Aphids were allowed to feed on the test plants for 2 days at room temperature, with low light intensity. Test plants were then sprayed with 0.2% pyrethrins, transferred to the greenhouse for 10–12 days, and tested for virus by DAS-ELISA. Control plants were kept separately but in the same conditions. Several aphids from each feeding cage were also used directly for DAS-ELISA.

ELISA of aphid extracts. After acquisition access on purified virus preparations, aphids were tested by DAS-ELISA. Each antigen sample consisted of two aphids homogenized in 0.25 ml of 0.1 M potassium phosphate buffer, pH 7.0. Extracts were incubated at 37 C for 1 hr, centrifuged for 10 min at 8,000 rpm, and the supernatants were used in DAS-ELISA. Incubation of aphid extracts yielded lower ELISA values for extracts of virus-free aphids (i.e., reduced "background"), without decreasing values for extracts of viruliferous aphids (22). Control virus-free aphids were processed in the same way as aphids from the feeding cages. These procedures were based on preliminary experiments, in which the influence of extracts of virus-free aphids on the sensitivity of DAS-ELISA for BYDV detection was found to be negligible, and the ELISA reliably detected virus in extracts from pairs of aphids raised on virus-infected plants (14).

TABLE 1. Correlation analyses relating the probability (P_t)[a] of transmission of isolates of barley yellow dwarf virus by single aphids fed on individual leaves of several cereal cultivars, to the virus content of the leaf as assessed by enzyme-linked immunosorbent assay (ELISA)[b]

Virus isolate	Vector	Cultivar	AAT[c] (hr)	df[d]	r	p
P-PAV	*R. padi*	Clintland 64	1	9	0.008	0.982
			2	9	0.566	0.070
			3	10	0.245	0.444
			4	10	0.471	0.122
			6	9	0.561	0.073
			18	11	0.208	0.495
			Total	68	0.101	0.406
		Briggs (Yd2−)	1	2	0.015	0.985
			2	1	0.986	0.106
			3	6	0.762*[e]	0.028
			4	1	0.999*	0.023
			6	1	0.999*	0.034
			Total	19	0.585**	0.005
		Prato (Yd2+)	1	2	0.334	0.667
			2	0	...	...
			3	4	0.456	0.364
			4	1	0.893	0.298
			6	1	0.091	0.942
			Total	16	0.239	0.340
		Atlas 57 (Yd2−)	3	3	0.231	0.709
		Atlas 68 (Yd2+)	3	1	0.737	0.473
		California Mariout (Yd2−)	3	2	0.599	0.401
		CM 67 (Yd2+)	3	1	0.999*	0.032
P-PAV	*S. avenae*	Clintland 64	3	8	0.046	0.899
			6	5	0.300	0.513
			12	7	0.211	0.586
			18	7	0.908**	0.001
			48	7	0.133	0.734
			Total	42	0.114	0.461
MAV	*S. avenae*	Clintland 64	3	10	0.284	0.371
			6	10	0.586*	0.045
			Total	22	0.133	0.537
RPV	*R. padi*	Clintland 64	3	12	0.525	0.054
			6	9	0.319	0.342
			Total	23	0.448*	0.025
		Abe	6	4	0.302	0.561
		Elmo	6	4	0.104	0.845

[a] P_t = Probability of transmission by single aphids; estimated by $1 - (1-S/N)^{1/k}$, where S = number of virus-infected test plants, N = number of test plants used, and k = number of aphids per test plant.
[b] Aphids were fed on individual leaves, then data were grouped in 0.200 increments by ELISA value of individual leaves (except for values below 0.100).
[c] Acquisition access time.
[d] Degrees of freedom.
[e] * Denotes statistical significance, $P \leq 0.05$; ** denotes significance, $P \leq 0.01$.

RESULTS

Relative transmissibility of BYDV from individual leaves with differing virus contents. When individual leaves with different virus contents were compared as virus sources for the acquisition and transmission of three BYDV isolates by efficient or inefficient vectors, there was, overall, no convincing evidence of differences in virus acquisition efficiency due to differences in virus content of the leaves. Figure 1 presents the relationship of the probability of transmission of three BYDV isolates by single aphids fed on individual leaves of Clintland 64 oats, to the virus content of the leaf as assessed by ELISA. Similar results were obtained for the P-PAV isolate in the three pairs of barleys, for the MAV isolate in Clintland 64 oats, and for the RPV isolate in Clintland 64 oats and the wheats Abe and Elmo. Statistically significant correlations between virus content and probability of transmission by single aphids occurred erratically among the experiments (Table 1). In a total of 31 experiments testing various combinations of host, virus, vector, and acquisition access time, 23 showed no correlation, seven showed correlations significant at the 5% level, and one showed correlation significant at the 1% level. The latter was with respect to the transmission of the P-PAV isolate by its inefficient vector *S. avenae*, allowed an 18-hr acquisition access, while the remaining correlations occurred in transmissions of isolates by efficient vectors (Table 1).

Relative transmissibility of BYDV from individual old or young leaves. Because differences in acquisition efficiency could result from different feeding behavior of the aphids on old versus young leaves, the percentages of test plants infected were also calculated in relation to source-leaf age. Overall, acquisition efficiencies for all three BYDV isolates by aphids fed on individual leaves of different ages were similar (Table 2).

Efficiency of acquisition of P-PAV by vectors fed through membranes on purified virus preparations. The proportion of samples of *R. padi* that acquired ELISA-detectable P-PAV by feeding through a membrane increased as the virus concentrations used increased, and was 100% for those fed on 70 μg/ml of virus for 18 hr (Table 3). No virus was acquired by either vector species when the virus concentration was 5 μg/ml. Linear regression analysis for *R. padi* ($y = -5.31 + 1.64x$, $r^2 = 0.891$) and *S. avenae* ($y = -8.32 + 1.54x$, $r^2 = 0.769$), revealed a significant response of acquisition efficiency to virus concentration, but indicated that the regressions for the two vectors differed neither in position nor slope. Thus, we conclude that both species acquired P-PAV equally efficiently through the membrane.

Efficiency in transmission to test plants of P-PAV by vectors fed through membranes on purified virus preparations. When *R. padi* and *S. avenae* fed through membranes on samples of the same virus preparations, transmission of P-PAV to test plants by *R. padi* was always higher than transmission by *S. avenae* (Table 4). Neither species transmitted virus when fed on 5 μg/ml of purified preparation, but with an increased concentration of virus, both species transmitted virus to Clintland 64 oats. At concentrations of 18 μg/ml and above, *R. padi* consistently transmitted P-PAV to more plants than did *S. avenae*. Regression analysis of transmission efficiency as a function of virus concentration for *R. padi* ($y = -0.98 + 0.70x$, $r^2 = 0.902$) and *S. avenae* ($y = -4.98 + 0.51x$, $r^2 = 0.953$), revealed a significant ($P = 0.05$) response of transmission efficiency to virus concentration and indicated that the regression for the two aphid species differed in position but not in slope.

Comparative virus acquisition by *R. padi* fed on individual oat or on virus preparations through membranes. For the same acquisition time and under the same conditions, *R. padi* acquired P-PAV more readily from a purified virus preparation estimated by ELISA to contain 60 μg/ml of virus than from individual leaves of Clintland 64 oats, extracts from which were similarly estimated to contain the same amount of virus (Table 5).

DISCUSSION

Because they vary in virus content as assessed by ELISA (15), detached individual leaves of the same plant provided a convenient set of virus sources, uniform in other respects, for experiments relating the probability of BYDV transmission by single aphids to the virus content of leaves. From our experiments testing various combinations of host, virus, vector, and acquisition access time, there was no convincing evidence of differences in virus transmission due to differences in virus content of the sources. In similar work, but assessing virus content by purification and not using detached leaves, Foxe and Rochow (4) showed that efficient vectors of the PAV, MAV, or RPV isolates transmitted

TABLE 2. Relative acquisition of barley yellow dwarf virus by aphids fed on individual old or young leaves of several cereal cultivars with relatively high (H) or low (L) overall virus content[a]

Virus	Vector	Cultivar (relative virus content)	Percentage of test plants infected[b] (number used)		
			Position (relative age) of virus source-leaf		
			1 (old)	2	3 (young)
P-PAV	*Rhopalosiphum padi*	Clintland 64 −H	28 (223)	25 (229)	36 (254)
		Briggs (Yd2−) −H	19 (78)	30 (124)	20 (162)
		Atlas 57 (Yd2−) −H	14 (7)	45 (11)	20 (59)
		California Mariout (Yd2−) −H	6 (18)	29 (21)	21 (58)
		Total	24 (326)	24 (385)	30 (533)
		Prato (Yd2+) −L	28 (29)	38 (97)	29 (143)
		Atlas 68 (Yd2+) −L	0 (3)	28 (18)	39 (49)
		CM 67 (Yd2+) −L	0 (5)	22 (27)	16 (62)
		Total	22 (37)	34 (142)	30 (254)
		Grand total	24 (363)	26 (527)	27 (787)
P-PAV	*Sitobion avenae*	Clintland 64 −H	42 (158)	36 (184)	45 (123)
MAV	*S. avenae*	Clintland 64 −H	66 (77)	70 (76)	69 (72)
RPV	*R. padi*	Clintland 64 −H	68 (58)	48 (67)	64 (58)
		Abe −H	38 (16)	30 (20)	50 (18)
		Total	46 (74)	44 (87)	61 (76)
		Elmo −L	7 (14)	12 (17)	29 (17)
		Grand total	40 (88)	38 (104)	55 (93)

[a] Overall virus contents of the plants were compared by enzyme-linked immunosorbent assay (15).

[b] Infections assessed by enzyme-linked immunosorbent assay as described in the text. Same individual leaves as used in Table 1 but data grouped by age of the leaf instead of virus content.

these equally well from old or young leaves, even though the latter contained approximately three times more virus than old leaves. However, they found that transmission of the PAV isolate by the inefficient vector *S. avenae* was correlated with leaf age, and therefore, presumably, with virus content. This correlation was not detected in our experiments with the P-PAV isolate and *S. avenae*.

Even cultivars that had a relatively low virus content overall were good sources of virus for vectors in our experiments. Reduced virus content is sometimes correlated with tolerance to BYDV in cereals (19), but overall, whether the leaves tested were from cultivars assessed as tolerant or sensitive to BYDV did not affect the occurrence of correlations. Correlations were observed between virus content and virus transmission for leaves from both Briggs (Yd2−) and CM67 (Yd2+) barleys, but they were low, and the fact that no correlations were seen with the other barleys tested suggests that even these were probably due to chance. There was therefore no convincing evidence of intrinsic differences in virus acquisition efficiency due to differences in cultivar tolerance or sensitivity.

It appears likely that an overriding limiting factor in virus acquisition in vivo is the availability of virus in the phloem from which ingestion is taking place. Electron microscopy indicates that virus is erratically distributed within the phloem of barley (9), and, therefore, this may also apply with other cereals. Thus, even where ELISA indicates a relatively high virus content within the leaf, the constraint on virus acquisition is whether aphids actually probe phloem cells that contain virus. Conversely, the fact that relatively high acquisition efficiencies were sometimes obtained with source leaves whose extracts gave relatively low ELISA values, suggests that whether vectors fed in phloem tissue containing virus was more crucial for virus acquisition than the overall virus content of the leaf.

Our results with aphids feeding on sucrose solutions containing virus were consistent with these conclusions. Virus acquisition efficiency was a function of concentration of virus present, for both *R. padi* and *S. avenae* feeding through membranes. In an earlier study with a BYDV isolate and its vector *R. padi*, a linear relationship was also found between the percentage of plants infected and the log of the virus dilution used in membrane feeding experiments (13). Our results are also in agreement with previous work indicating that *R. padi* was a more efficient vector for P-PAV than *S. avenae* in such experiments (4). Higher proportions of *R. padi* than of *S. avenae* were able to transmit virus to test plants when fed on a range of concentrations of the P-PAV isolate, from 18 μg/ml to 70 μg/ml. However, while 100% of samples of *R. padi* contained ELISA-detectable virus after feeding on 70 μg/ml of the P-PAV isolate, only 50% of samples were able to infect test plants, confirming that the detection of virus in aphids by ELISA need not mean that they are able to transmit it, as demonstrated also for other virus vector combinations (3,16). A similar conclusion can also be derived from the results with *S. avenae*. Conversely, when *R. padi* were fed on leaves of Clintland 64 oats, extracts of which contained an average of 60 μg/ml of the P-PAV isolate, a lower proportion of aphids acquired ELISA-detectable virus than when aphids fed through a membrane on preparations of the same concentration.

Although tolerance to BYDV is a useful attribute in cereal breeding, our results indicate that its association with reduced levels of virus may not be advantageous in relation to reducing spread. Whether virus is readily available for acquisition by vectors will depend on the virus content of the specific phloem vessels probed, and, therefore, on the dynamics of virus movement, which may not necessarily reflect the overall virus content of the leaf or the plant as a whole. Vector behavior will also play a part. This may also to be true of other phloem-restricted viruses, for although Barker and Harrison (1) obtained indications of correlations between virus content and virus transmissibility in potatoes infected with potato leaf roll virus, there were exceptions to this relationship.

TABLE 3. Comparative efficiency of acquisition of the P-PAV isolate of barley yellow dwarf virus by *Rhopalosiphum padi* and *Sitobion avenae* fed through Parafilm membranes on four purified virus preparations

Virus concentration (μg/ml)[a]	Number of samples tested for each species[b]	Percentage of aphid samples containing detectable virus (ELISA values)[c]	
		R. padi	*S. avenae*
5	21	0 (0.058 ± 0.021)	0 (0.038 ± 0.011)
18	24	17 (0.112 ± 0.007)	4 (0.108 ± 0.023)
41	16	81 (0.147 ± 0.047)	81 (0.162 ± 0.065)
70	8	100 (0.274 ± 0.052)	88 (0.149 ± 0.057)

[a] Preparations were in 20% sucrose in 0.1 M phosphate buffer, pH 7.0.
[b] Each sample consisted of two aphids ground in 0.25 ml of buffer. The acquisition feeding on virus concentrates was for 18 hr at 21 C. Mean enzyme-linked immunosorbent assay (ELISA) values for untreated aphids were 0.05 for *R. padi* and 0.04 for *S. avenae*.
[c] Mean values ± standard deviations are presented for the positive samples.

TABLE 4. Comparative efficiency in transmission to test plants of the P-PAV isolate of barley yellow dwarf virus by *Rhopalosiphum padi* and *Sitobion avenae* fed through Parafilm membranes on four purified virus preparations[a]

P-PAV concentration (μg/ml)[b]	Percentages of test plants infected (number used)	
	R. padi	*S. avenae*
5	0 (28)	0 (30)
18	18 (28)	3 (34)
41	22 (23)	13 (23)
70	50 (12)	33 (12)

[a] Test plants were Clintland 64 infested with two aphids each for a 2-day inoculation test feeding at 21 C. Acquisition access on virus concentrates was for 18 hr at 21 C. Infections were determined by enzyme-linked immunosorbent assay.
[b] Preparations were in 20% sucrose in 0.1 M phosphate buffer, pH 7.0.

TABLE 5. Detection, by enzyme-linked immunosorbent assay (ELISA), of the P-PAV isolate of barley yellow dwarf virus in samples of *Rhopalosiphum padi* fed on infected Clintland 64 oats or by feeding through Parafilm on a virus preparation

Source and mode of acquisition	Virus concentration (μg/ml)	No. of aphids in sample	
		2	5
Purified virus through Parafilm[a]	60	9/12[b]	8/8
Feeding on Clintland 64 oat leaf[c]	60	2/10	2/5

[a] Purified virus preparation was in 20% sucrose in 0.1 M phosphate buffer, pH 7.0.
[b] Numerator is number of ELISA-positive aphid samples; denominator is total number of aphid samples tested, after an acquisition access of 18 hr at 21 C.
[c] Individual leaf of Clintland 64 oat plant, 17 days old and infected for 11 days.

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