Aphid Transmission of Barley Yellow Dwarf Virus: Acquisition Access Periods and Virus Concentration Requirements

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ABSTRACT

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The duration of access periods and the availability of virus in source plants are two factors that influence the transmission of barley yellow dwarf virus (BYDV) by its aphid vectors. This study was conducted to quantify the relationships among acquisition access period (AAP), virus titer in infected oats, and transmission of three isolates of BYDV from New York by two aphid vector species. Thirteen AAPs, ranging from 15 min to 72 hr, were examined, and virus titer was quantified from each virus source leaf by using enzyme-linked immunosorbent assay (ELISA). Two leaves from each plant were used as independent virus sources to test the effect of leaf age, in addition to virus titer, on acquisition efficiency. The older leaf on each source plant almost always contained less virus. The NY isolates of BYDV, RPV and PAV, were acquired by *Rhopalosiphum padi* within a 15-min AAP; however, a 1- to 2-hr or 2- to 3-hr AAP was required for 50% of the aphids to transmit PAV or RPV, respectively. The difference in virus titer among source leaves did not affect the ability of *R. padi* to transmit RPV, but did influence the transmission of PAV. *Sitobion avenae* required a 30-min AAP to acquire the MAV and PAV isolates of BYDV. Fifty percent of the aphids were able to transmit MAV or PAV after a 4- to 6-hr or 10- to 12-hr AAP, respectively. The ability of *S. avenae* to transmit MAV and PAV was significantly lower for older leaves. Analyses of the transmission and titer data revealed that the lower virus content of the older leaves accounted for the significant reduction in virus transmission by *S. avenae*. The transmission efficiency of various BYDV isolates is differentially influenced by several factors including aphid vector, length of acquisition feeding period, and physiological age of source tissue. In addition, our results suggest that virus titer, as it is affected by age and infection stage of the source tissue, can have a strong influence on acquisition and transmission efficiency of aphid vectors.

Additional keywords: luteovirus, persistent transmission.

Barley yellow dwarf virus (BYDV) is a group of related luteoviruses or luteovirus isolates that infect plants in the family Gramineae and are obligately transmitted by one or more specific aphid species in a persistent, nonpropagative manner (23). Several major factors are known to influence the efficiency and specificity of BYDV transmission, including the developmental stage and clonal variation of aphid vectors, virus source, temperature, and heterologous encapsidation or phenotypic mixing of virus components in infections by multiple BYDV isolates (14). Surprisingly, there are limited data to describe the effects of virus titer in source plants or the influence of the duration of acquisition and inoculation access periods on BYDV transmission.

The minimum acquisition access period (AAP) required for BYDV transmission has been reported from several studies (12,22,24). None of these studies, however, were able to evaluate AAP in detail because the investigators had few methods available to quantitate optimal virus concentrations in plants. In addition, the effects of source leaf age on acquisition and transmission efficiency, the relatively few acquisition time-periods evaluated, and low numbers of aphids tested probably contributed to the highly variable estimates of minimal acquisition times, which ranged from 30 min (24) to 24 hr (22).

Gill (3) showed that transmission efficiency from a single source leaf fluctuated cyclically with increasing time after inoculation. He concluded that the fluctuations in transmission were attributable to a difference in virus titer, but he did not rule out the possible effect of leaf age. Foxe and Rochow (1) found a quantitative difference in virus titer among young and old leaves. Transmission efficiency was not affected by leaf age for most virus–vector combinations tested, but leaf age did affect vector specificity.

Pereira et al (10) reported a positive correlation between increasing BYDV content and BYDV transmission efficiency by aphids when purified virus was acquired through parafilm membranes. However, when aphids acquired BYDV from infected plants, BYDV antigen content, measured by enzyme-linked immunosorbent assay (ELISA), was not correlated with BYDV transmission efficiency by aphids. They did not report whether the range of purified virus concentrations used in membrane feeding experiments were representative of actual virus content found in the leaves used as virus sources.

The objectives of our study were to investigate the relationships among acquisition access period, virus concentration, and the probability of transmission of three BYDV isolates by two aphid vectors. A quantitative analysis of the effects of these parameters on BYDV transmission will provide data essential for studies on BYDV epidemiology and studies designed to assess the importance of host plant resistance mechanisms that restrict virus multiplication or accumulation in a plant, or that restrict aphid feeding and settling. The importance of the inoculation access period was the subject of a separate, concurrent study (11).

MATERIALS AND METHODS

Virus isolates and aphid species. The BYDV isolates used in the transmission experiments included the previously characterized NY isolates, PAV, MAV, and RPV (13). PAV is transmitted by *Rhopalosiphum padi* L. and *Sitobion avenae* F. (formerly *Macrosiphum avenae*); MAV is transmitted specifically by *S. avenae*; and RPV is transmitted specifically by *R. padi*. All isolates were maintained in Coast Black oats (*Avena byzantina*

K. Koch) as described by Rochow (13).

The clones of *S. avenae* and *R. padi* used in this study had the same rearing conditions as previously described by Rochow (13).

Transmission assays. Transmission assays were done for four aphid–isolate combinations: *R. padi* transmitting RPV; *R. padi* transmitting PAV; *S. avenae* transmitting PAV; and *S. avenae* transmitting MAV. For each aphid–isolate combination, 26 Coast Black oat seedlings (one-leaf stage) were inoculated and grown in the greenhouse for 4 wk before use. The two youngest, fully expanded leaves on each plant (usually the fourth and fifth leaves) were used as the virus source. Adult aphids were starved overnight and then 12–15 were confined on each leaf in a clip cage (2 cm diameter) and allowed one of 13 AAPs: 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, or 72 hr. In the first two experiments investigating the transmission of PAV by *S. avenae*, a 16-hr AAP was used instead of the 0.25-hr AAP. Two plants, i.e., four individual leaves, were used for each AAP treatment. After the AAP, 10 aphids from a clip cage attached to each leaf were individually transferred to 10 Coast Black oat seedlings for an inoculation access period of 5–7 days in a growth chamber maintained at 21 C. Aphids were killed by fumigation with DDVP (O,O-dimethyl O-[2,2-dichlorovinyl] phosphate) in a closed chamber, and test plants were placed in a greenhouse and observed for symptom expression for 4–5 wk. The percentage of the 10 plants that became infected was regarded as the transmission efficiency. Each transmission experiment with the series of 13 AAPs and each aphid–isolate combination was repeated three times.

Virus titer determination. Double antibody sandwich-ELISA was used to measure virus titer in source leaves and was done as described by Rochow (15) with the following modifications. A 0.5-g section of leaf, including the area enclosed by the clip cage, was excised, cut into small pieces, and frozen at −80 C until the assay was done. All assays were completed within 2 wk of freezing the tissue. For ELISA, the frozen tissue was diluted 1:4 (w/v) in phosphate-buffered saline (pH 7.4) (PBS). Two volumes of chloroform were added, the suspension was homogenized with a Polytron (Brinkman Instruments, Westbury, NY) and then centrifuged (5,000 × *g*/5 min). Clarified sap (200 μl/well) was added to duplicate wells of a microtiter plate (Immulon 1, Dynatech, Rockville, MD) previously coated with immunoglobin (2.0 μg/ml, 2 hr at 37 C) prepared against the homologous purified virus and incubated overnight at 4 C. The production and specificity of the antibodies have been described elsewhere (16). Unbound antigen was removed by washing the plates with PBS + 0.5% Tween 20 (PBST). Alkaline phosphatase-conjugated immunoglobulin homologous to the coating antibody was incubated in the wells for 2–3 hr at 37 C. Unbound conjugate was removed by washing with PBST. Alkaline phosphatase reactions were measured at A_{405nm} with a microtiter plate reader (Dynatech MR-580 for PAV assays or Biotek 312 for MAV and RPV assays) (Biotek, Winooski, VT). Known concentrations of purified virus, diluted in healthy plant sap, were included on each plate as controls and to permit the direct comparison of absorbance readings from samples on different plates. The three isolates of BYDV were purified as described by Hammond et al (4). Virus was stored at −80 C in small volumes to avoid repeated freeze-thaw cycles. Concentration (estimated spectrophotometrically) was periodically checked and adjusted to correct for the degradation of virus during storage.

Each plate used to assay PAV-infected samples included duplicate wells containing 50, 100, and 200 ng of purified PAV in addition to healthy sap and buffer controls. The absorbance values (A_{405nm}) of those wells were monitored during the alkaline phosphatase-mediated color development, and the entire plate was read when the absorbance value for wells containing 100 ng of virus was about 0.25. Virus titer data for experiments involving the PAV isolate were presented as absorbance values (A_{405nm}) read directly from the ELISA plates. The set of three purified PAV standards (50, 100, and 200 ng), included on each PAV-ELISA plate, allowed for direct comparison of absorbance values among plates, but did not provide a standard curve that would allow absorbance values to be converted to virus concentration.

Each plate used to assay MAV- or RPV-infected samples included duplicate wells containing a twofold dilution series of purified virus from 160 to 2.5 or from 40 to 2.5 ng/well, respectively, and healthy sap controls. Absorbance values obtained from the dilution series were used to calculate a standard curve for each ELISA plate and to determine the concentration of virus in the source plants used for the transmission tests. Virus titer data were presented as mean nanograms of virus in the 200-μl sample of clarified sap loaded into two ELISA plate wells. All calculations were done with the KinetiCalc software (version 1.11) (Biotek) developed for use with the Biotek 312 microtiter plate reader. The use of a standard curve generated for each ELISA plate relieved us of monitoring the development of plates to standardize the absorbance values for the purified virus standards. However, because of the various development times, absorbance values could not be directly compared among plates as was possible with the PAV-ELISA.

Data analysis. Two types of data were analyzed with analysis of variance (ANOVA): the percentage of aphids that successfully transmitted the virus in each AAP from each source leaf; and the virus titer in source leaves (estimated using absorbance values for the PAV isolate). All analyses were done with the MANOVA program of SPSS (5).

The relationship between AAP and transmission was examined for each isolate–aphid combination individually, using a split-split plot. Each transmission experiment with the series of 13 AAPs was treated as a replication. The whole plots were replicates, with whole-plot treatment AAP, applied in a randomized block design. Accordingly, AAP was tested against the AAP*replicate sum of squares. The subplot was plant with no treatment. This yielded plant as an appropriate error term for replicate. The sub-subplot was source leaf, with sub-subplot factor as leaf age, which was tested against leaf*replicate. Finally, leaf age*AAP was tested against the leaf*AAP*replicate interaction to test whether the effect of leaf age differed among different AAPs. This model was run both with and without virus titer or absorbance values as a covariate to determine whether the effect of leaf age on transmission depended on virus titer. The same ANOVA model was used to test for differences among virus titer or absorbance values themselves, with respect to period, source plant, and age of source leaf. Because of the variability in source plant virus titer among replicate assays, data on virus titer were also analyzed separately for each replicate assay. This is a standard split-plot design, with whole-plot plant and subplot leaf. The whole-plot factor, AAP, was tested against plant (within AAP). The subplot factors, age of source leaf and AAP*age, were tested against the leaf*plant interaction.

A three-level nested ANOVA model was used to compare the transmission patterns of the two aphids for different isolates. Three analyses were done: (1) for *R. padi*, we compared the transmission of PAV and RPV; (2) for *S. avenae*, we compared the transmission of PAV and MAV; and (3) for PAV, we compared transmission by *R. padi* and *S. avenae*.

For comparisons 1 and 2, experiment replicates were nested under isolate and the effect of isolate was tested by using the variance between replicate assays as the error term. The effect of AAP and the interaction between AAP and isolate were tested with the interaction between AAP and replicate as the error term. The effect of the age of source leaf and the interaction between leaf age and isolate were tested by using the interaction between leaf age and replicate as the error term. The interaction between leaf age and AAP and the interaction between leaf age, AAP, and isolate were tested by using the interaction between AAP, isolate, and replicate as the error term. Finally, the interaction between AAP, isolate, and replicate was tested with the interaction between leaf and plant as the error term.

This nested ANOVA addressed various questions about the relationship between vector, isolate, and age of source leaf. The test of isolate alone indicated whether there was an overall differ-

TABLE 1. Effects of virus source tissue, differing in age and virus titer, on the transmission efficiency of three New York isolates of barley yellow dwarf virus by their specific aphid vector(s)

Virus isolate	Aphid species	Virus source[a]	Transmission efficiency[b]	Virus titer[c]	
				Absorbance	Concentration
RPV	*Rhopalosiphum padi*	Older leaf	56.4 ± 2.7	...	10.7 ± 12.9
		Younger leaf	55.3 ± 2.7	...	17.1 ± 12.9
			$P = 0.301$ (0.180)		$P = 0.216$
PAV	*R. padi*	Older leaf	58.0 ± 5.5	0.180 ± 0.114	...
		Younger leaf	64.6 ± 5.5	0.271 ± 0.114	...
			$P = 0.049$ (0.413)	$P = 0.105$	
MAV	*Sitobion avenae*	Older leaf	44.6 ± 3.4	...	34.2 ± 49.0
		Younger leaf	57.7 ± 3.4	...	73.5 ± 49.0
			$P = 0.007$ (0.157)		$P = 0.129$
PAV	*S. avenae*	Older leaf	22.2 ± 8.1	0.150 ± 0.031	...
		Younger leaf	40.9 ± 8.1	0.252 ± 0.031	...
			$P = 0.015$ (0.327)	$P = 0.008$	

[a] Coast Black oat seedlings were inoculated 4 wk before use as source plants. The two youngest, fully expanded leaves, usually the fourth (older) and fifth (younger), were used as the virus source.

[b] Aphids were given various acquisition access periods, ranging from 0.25 hr to 72 hr, on the virus source before being transferred to Coast Black oat seedlings (one aphid per seedling) for a 5- to 7-day inoculation access period. The transmission efficiency represents the mean percentage ± standard error ($n = 78$) of infected indicator plants for three experiments of 13 individual acquisition access periods repeated over time. Ten indicators were used to determine the transmission efficiency for each AAP. P values indicate the significance level of the difference between the transmission efficiencies from the older and younger leaves. The P value in parentheses was calculated with virus titer data as a covariate in the analysis.

[c] Values are the mean ± standard error of virus titers of old and young source leaves used for three replicates of 13 AAPs ($n = 78$ for each leaf-type). The P value indicates the significance level of the difference between the two titer values. Titer data for PAV are presented as mean absorbance data (A_{405nm}) read directly from ELISA plates. Titer data for MAV and RPV represent the concentration of virus (ng) in a 200-μl sample of infected plant sap.

ence in the probability of transmission of the two isolates. A significant interaction between AAP and isolate would have indicated that the temporal pattern of transmission differed for the two isolates. To address the question of whether the effect of leaf age was constant over all AAPs, the interaction between AAP and leaf age was examined. The interaction between isolate and leaf age was tested to determine whether the effect of leaf age was similar for both isolates.

This three-level nested model was also used to compare the transmission of PAV by *R. padi* and *S. avenae* (comparison 3 above) by substituting "aphid species" for "isolate" in the model description. For comparison 3 only, this model was run both with and without virus titer (or absorbance values) as a covariate to determine whether the effect of AAP and/or leaf age on transmission depended on virus titer.

RESULTS

Virus content. The amount of RPV and MAV in source tissue ranged from 1 to 42 (13.9 ± 6.3; mean ± standard error (SE), $n = 156$) and from 3 to 395 (50.3 ± 46.6; mean ± SE, $n = 152$) nanograms per ELISA sample, respectively. The absorbance values of the PAV-infected source tissue samples ranged from 0.041 to 0.420 (0.201 ± 0.065; mean ± SE, $n = 159$) and from 0.059 to 0.472 (0.226 ± 0.063; mean ± SE, $n = 156$) for transmission tests with *S. avenae* and *R. padi*, respectively. The mean absorbance values for the PAV standards were 0.16, 0.24, and 0.39 for 50, 100, and 200 ng, respectively. Mean virus titer was lower in the older of the two leaves of source plants for all three virus isolates; although, the difference was not significant when data from all three experiments for each aphid–isolate combination were analyzed together (Table 1). A large variation in mean titer among plants used as virus sources in experiments resulted in large standard error terms. When the titer data were analyzed separately for each experiment the titers in the older leaves were consistently and significantly lower than in the younger leaves in all but one case (Table 2).

Virus transmission. A small percentage of the aphids of *R. padi* tested were able to acquire the RPV or PAV isolates of BYDV within 15 min (6 and 2.5% for RPV and PAV, respectively). Fifty percent of the aphids were able to transmit after a 1- to 2-hr AAP on PAV-infected source tissue or a 2- to 3-hr AAP on RPV-infected source tissue. Maximum transmission efficiency for the AAPs tested occurred at 12 and 24 hr for RPV and PAV, respectively (Fig. 1B).

TABLE 2. Mean virus titer in older and younger source leaves[a] used to determine aphid transmission efficiency of three New York isolates of barley yellow dwarf virus by their specific aphid vector(s)

Aphid species–virus isolate combination	Older leaf	Younger leaf	P value[c]
Rhopalosiphum padi – RPV			
Experiment 1	9.31 ± 1.57[b]	22.38 ± 1.57	0.000
Experiment 2	17.23 ± 1.18	18.12 ± 1.18	0.605
Experiment 3	5.62 ± 0.40	10.77 ± 0.40	0.000
R. padi – PAV			
Experiment 1	0.236 ± 0.011	0.312 ± 0.011	0.000
Experiment 2	0.135 ± 0.010	0.285 ± 0.010	0.000
Experiment 3	0.171 ± 0.008	0.215 ± 0.008	0.002
Sitobion avenae – PAV			
Experiment 1	0.111 ± 0.008	0.229 ± 0.008	0.000
Experiment 2	0.150 ± 0.009	0.252 ± 0.009	0.000
Experiment 3	0.185 ± 0.010	0.272 ± 0.010	0.000
S. avenae – MAV			
Experiment 1	8.04 ± 0.53	15.05 ± 0.53	0.000
Experiment 2	65.46 ± 13.03	127.27 ± 13.03	0.005
Experiment 3	29.15 ± 13.23	69.19 ± 13.23	0.052

[a] Coast Black oat seedlings were inoculated 4 wk before use as source plants. The two youngest, fully expanded leaves, usually the fourth (older) and fifth (younger), were used as the virus source.

[b] Values are the mean ± standard error of virus titers of old and young source leaves used for each experiment of 13 AAPs ($n = 26$ for each leaf-type). Titer data for PAV are presented as mean absorbance data (A_{405nm}) read directly from ELISA plates. Titer data for MAV and RPV represent the amount of virus (ng) in a 200-μl sample of infected plant sap.

[c] Indicates the significance level of the difference between the two titer values.

The mean titer of RPV in the older leaf of the source plants was 37% lower than in the younger leaf (Table 1), but transmission efficiency by aphids of *R. padi* that fed on these leaves was not significantly different with ($P = 0.180$) or without ($P = 0.301$) virus titer as a covariate (Table 1; Fig. 2A).

The mean titer of PAV and the mean transmission by *R. padi* was lower in the older of the two source leaves and there was a significantly higher mean transmission by *R. padi* from the younger source leaves ($P = 0.049$; Table 1). When the effect of

virus titer was removed by using it as a covariate in the analysis, the difference in transmission between older and younger leaves was not significant ($P = 0.413$; Table 1). This indicated that virus titer was responsible for the significant difference in transmission from leaves of different ages.

When the transmissions of PAV and RPV by *R. padi* were compared (Table 3), there was no significant difference ($P = 0.574$) in the overall probability of transmission regardless of AAP, e.g., 61 and 56% for PAV and RPV, respectively. The length of the AAP significantly affected transmission efficiency ($P < 0.000$), but the ability of *R. padi* to acquire and transmit RPV or PAV was similar for any individual AAP, as indicated by the non-significant ($P = 0.710$) isolate by the AAP interaction term. That is, there were no significant differences in the overall shape of the two transmission curves presented in Figure 1B.

S. avenae was unable to transmit MAV or PAV given a 15-min AAP. The increase in the probability of transmission with increasing length of AAPs was less rapid than for RPV and PAV transmitted by *R. padi* (Fig. 1). Fifty percent of *S. avenae* transmitted MAV or PAV after a 4- to 6-hr or 10- to 12-hr AAP, respectively, but maximum transmission efficiency for the AAPs tested was not achieved until 72 hr for both isolates (Fig. 1A).

Transmission efficiencies of MAV and PAV by *S. avenae* were significantly lower ($P = 0.007$, $P = 0.015$; Table 1) than the older of the two source leaves of each plant used in the respective experiments (Fig. 2C,D). Mean virus titers of both viruses were also lower in the older leaves (Tables 1 and 2), and when titer was used as a covariate in the analyses, the differences in transmission efficiencies between older and younger leaves were no longer significant ($P = 0.157$, $P = 0.327$; Table 1). This indicated that differences in virus titer between leaves of different ages did account for the significant differences in transmission efficiency.

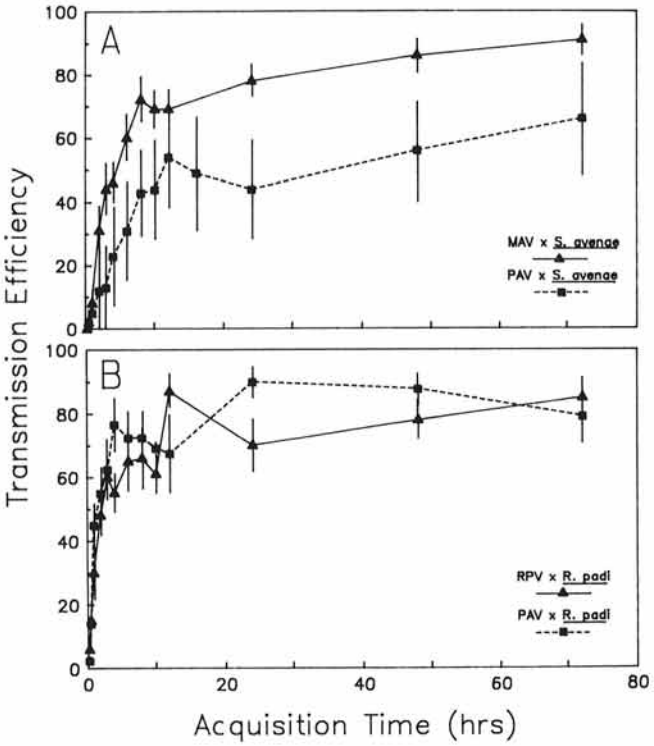

Fig. 1. The mean transmission efficiency and associated standard errors of three isolates of barley yellow dwarf virus (BYDV) by two aphid species allowed various acquisition access periods (AAP) on infected oat leaves. The actual AAPs used were 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, and 72 hr. A 16-hr AAP was also used in one aphid–isolate combination. Source plants were inoculated 3–4 wk before use. The two youngest, fully expanded leaves were used as a virus source. Two plants, i.e., four leaves, were used for each AAP treatment. After the AAP, 10 aphids were individually transferred from each source leaf, 40 total, to 10 Coast Black oat seedlings for an innoculation access period of 5–7 days. Each transmission experiment with the series of 13 AAPs and each aphid–isolate combination was repeated three times over time. Therefore, each data point represents the percentage of 120 test plants that were infected with BYDV after inoculation by a single aphid.

When the transmissions of PAV and MAV were compared for *S. avenae* (Table 3), the overall transmission efficiency, regardless of AAP, was significantly higher ($P = 0.01$) for MAV (50%) than PAV (32%). A weakly significant isolate by AAP interaction ($P = 0.051$) indicated there was also a difference in the length of AAP necessary for optimal acquisition and transmission of MAV and PAV by *S. avenae*, i.e., there was a significant difference in the overall shape of the two transmission curves presented in Figure 1A.

There was a significant difference ($P = 0.029$) between *R. padi* and *S. avenae* in their overall ability to transmit PAV, independent of AAP; however, if the data were adjusted for virus titer the difference was only weakly significant ($P = 0.073$) (Table 4). A weakly significant interaction between aphid species and AAP ($P = 0.062$) indicated some difference in the ability of the two aphids to acquire and transmit PAV given similar AAPs. In addition, a significant leaf age ($P = 0.001$) and leaf age by aphid interaction ($P = 0.016$) indicated that the ability of the two aphids to acquire and transmit PAV was differentially affected by leaf age.

DISCUSSION

Our findings demonstrated that transmission efficiency and the factors that influence transmission efficiency differ for each BYDV isolate and aphid vector combination tested. The length of the AAP was of major importance in determining the overall transmission efficiency of a population of vectors. Persistently transmitted viruses can be acquired in a very brief period of time, i.e., <30 min (6,20,24), presumably limited only by the time it takes for the vector to locate and begin feeding on phloem tissue. But, on average, efficient transmission clearly requires a more extended AAP.

In our study, *R. padi* was equally efficient at transmitting both the RPV and PAV isolates of BYDV for the various AAPs investigated. The ability of this aphid to transmit RPV was not influenced by virus titer; however, the range of virus titers in RPV source tissues was narrow, relative to the range of PAV titers. Therefore, our ability to detect a relationship between virus titer and RPV transmission efficiency may have been reduced. A reduction in PAV titer did reduce the ability of *R. padi* to transmit PAV. Similarly, the ability of *S. avenae* to transmit both MAV and PAV was also significantly influenced by titer. These data contrast with the finding of Pereira et al (10) that virus titer did not affect the probability of transmission. The design of our experiments and the methods used to analyze the data allowed for a statistical comparison of transmission efficiencies among aphids and virus isolates. The use of two-leaf categories, i.e., older and younger leaves, with significantly different virus titers allowed for a more accurate determination of titer effects than simple regression analysis of titer and transmission used in previous studies (10). In addition, the differences in cultivars, aphid clones, virus isolates, and environmental conditions may have contributed to the discrepancies between the results of our two studies.

The RPV titer was numerically greater in the younger of the two leaves from 65 of 78 source plants, and the mean titer was significantly greater in two of three experimental replicates (Table 2). This finding is in contrast with the finding of Pereira and Lister (9) that RPV titer was higher in older leaves of infected spring oats, but this difference may be cultivar-specific. Foxe and Rochow (1) also reported minor differences in RPV titer among infected spring oat plants, but generally found the titer in young leaves higher than in older leaves. In addition, they found that transmission efficiency of RPV by *R. padi* was unaffected by virus concentration, but that a reduced concentration of PAV lowered the transmission efficiency.

The low transmission efficiency of PAV by *S. avenae*, relative

to *R. padi*, was due in part to differences in virus titer in the source tissue. Use of virus titer as a covariate removed the effect of virus titer, but the difference in transmission was still weakly significant (Table 4). The differences in the acquisition efficiency of PAV and MAV by *S. avenae* may have been attributable to a difference in feeding behavior and the efficiency of circulative virus transport through the aphid vector. Shukle et al (18) found that the period of phloem ingestion for *R. padi* feeding on oats was twice that for *S. avenae*. Phloem contact is required for the acquisition of any luteovirus by an aphid (17), and the longer the AAP the greater the amount of virus detected in the aphid (8,21). Acquisition efficiency may depend more on the length of time the aphid spends acquiring virus than on the amount of virus in the infected sap. Virus must be transported from the

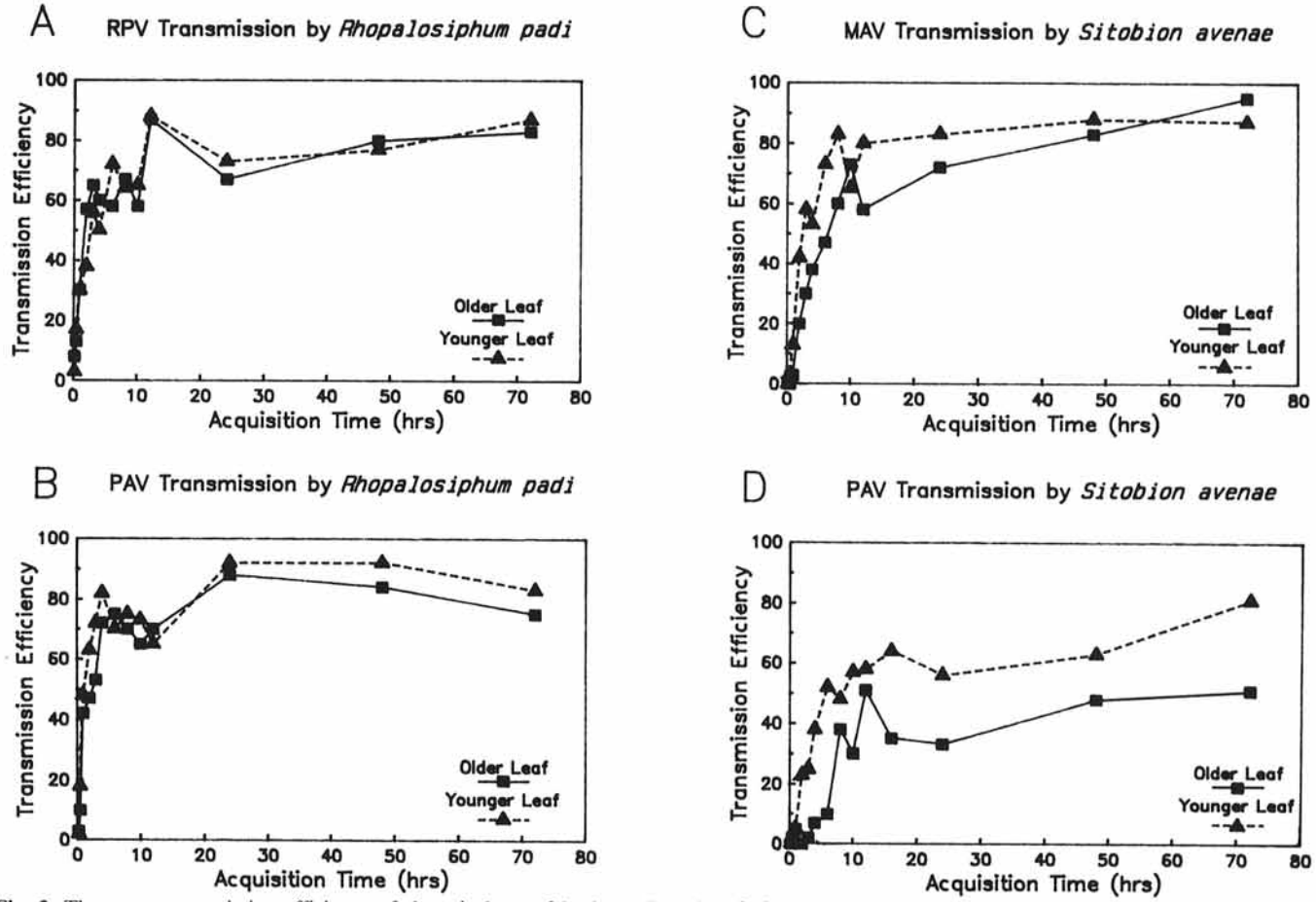

Fig. 2. The mean transmission efficiency of three isolates of barley yellow dwarf virus (BYDV) by two aphid species when two different ages of leaves (usually leaves 4 and 5 of Coast Black oat plants inoculated 3–4 wk before use) were used as a virus source. The actual acquisition access periods (AAP) used were 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, and 72 hr. A 16-hr AAP was also used in one aphid–isolate combination. Two leaves of the same age were used for each AAP treatment. After the AAP, 10 aphids were individually transferred from each source leaf, 20 total, to 10 Coast Black oat seedlings for an inoculation access period of 5–7 days. Each transmission experiment with the series of 13 AAPs and each aphid–isolate combination was repeated three times over time. Therefore, each data point represents the percentage of 60 test plants that became infected with BYDV after inoculation by a single aphid.

TABLE 3. Analysis of variance of the transmission efficiency of New York isolates PAV and RPV of barley yellow dwarf virus (BYDV) by *Rhopalosiphum padi* and of New York isolates PAV and MAV of BYDV by *Sitobion avenae*

		R. padi RPV vs. PAV			*S. avenae* MAV vs. PAV		
Source	df	MS	*F*	*P*	MS	*F*	*P*
Isolate	1	0.27	0.37	0.574	3.13	21.75	0.010
error 1[a]	4	0.73			0.14		
AAP[b]	11	0.95	9.09	0.000	1.50	42.57	0.000
Isolate*AAP	11	0.08	0.72	0.710	0.07	2.01	0.051
error 2	44	0.10			0.04	151.59	
Leaf age	1	0.08	13.26	0.002	1.77		0.000
	4					6.10	
Leaf age*isolate	1	0.12	19.57	0.011	0.07		0.069
error 3	4	0.01			0.01	1.56	
Leaf age*AAP	11	0.01	0.26	0.991	0.05	1.40	0.144
Leaf age*isolate*AAP	11	0.03	1.30	0.259	0.05		0.207
error 4	44	0.02			0.03		
Residual error	144						

[a] Error 1 = replicate within isolate; error 2 = AAP*replicate within isolate; error 3 = leaf age*replicate within isolate; error 4 = leaf age*AAP*replicate within isolate.
[b] Acquisition access period.

TABLE 4. Analysis of variance of the transmission efficiency of New York isolate PAV of barley yellow dwarf virus (BYDV) by *Rhopalosiphum padi* and *Sitobion avenae* with or without enzyme-linked immunosorbent assay (ELISA) absorbance values used as a covariate

	No covariate					ELISA as covariate			
Source	df	MS	*F*	*P*	Source	df	MS	*F*	*P*
Aphid	1	7.60	11.03	0.029	Aphid	1	4.78	7.34	0.073
error 1[a]	4	0.69			ELISA	1	0.80	1.23	0.349
AAP[b]	11	0.92	13.85	0.000	error 1	3	0.65		
Aphid*AAP	11	0.13	1.92	0.062	AAP	11	0.92	14.11	0.000
error 2	44	0.07			Aphid*AAP	11	0.12	1.85	0.075
Leaf age	1	1.23	81.96	0.001	ELISA	1	0.13	2.00	0.165
Aphid*leaf age	1	0.24	15.94	0.016	error 2	43	0.06		
error 3	4	0.02			Leaf age	1	0.13	6.84	0.079
AAP*leaf age	11	0.03	1.05	0.425	Aphid*leaf age	1	0.24	12.06	0.040
Aphid*AAP*leaf age	11	0.04	1.09	0.393	ELISA	1	0.00	0.05	0.854
error 4	44	0.03			error 3	3	0.02		
					AAP*leaf age	11	0.03	1.02	0.445
					Aphid*AAP*leaf age	11	0.04	1.06	0.412
					ELISA	1	0.00	0.00	0.966
					error 4	43	0.03		
					Residual error	144			

[a] Error 1 = replicate within aphid species; error 2 = AAP*replicate within aphid species; error 3 = leaf age*replicate within aphid species; error 4 = leaf age*AAP*replicate within aphid species.
[b] Acquisition access period.

ingested sap across the hindgut membrane into the aphid hemocoel by receptor-mediated endocytosis (2). A majority of the acquired virus is voided in the aphid honeydew (21) suggesting that one limiting factor is the rate at which virus can be transported across the hindgut membrane. Increasing the concentration of virus would not necessarily increase the efficiency of membrane transport if the limiting factor was the availability of virus receptors on the aphid hindgut. Increasing the length of time that the virus moves through the hindgut by increasing the phloem feeding period would provide a continuous supply of virus to be transported across the hindgut membrane as receptors become available to accept and initiate transport of virus particles. This hypothesis is supported in part by our data, which showed a diminishing effect of virus titer as AAP increases. The greatest difference in the probability of transmission from the higher titer young leaves and lower titer older leaves occured at the shortest AAPs. The difference between transmission efficiency from the younger and older source leaves was reduced for all isolate–aphid combinations at the longer AAPs (Fig. 2).

The findings of this study are also important to the design and implementation of BYDV control strategies that are concerned with reducing the spread of the virus as well as improving yields of the affected crop. A reduction in virus titer has been suggested as an indicator of BYDV resistance in cereal crops (7,19). Our findings suggest that, in addition to reducing the viral impacts on growth and yield, a reduced titer can reduce the transmission of the virus from infected plants to other healthy plants or nearby alternative crop hosts. However, this may be effective only if the aphids feed on infected plants for relatively short periods of time, or may be completely ineffective as was the case with *R. padi* and RPV. A more effective disease control strategy, or one to be used in conjunction with reduced virus titer, would be to reduce the ability or desire of the aphid to feed on the plant for sustained periods of time. Reducing the acquisition access time will reduce the level of virus in the aphid vector and the probability that the aphid will transmit virus to additional host plants.

LITERATURE CITED

1. Foxe, M. J., and Rochow, W. F. 1975. Importance of virus source leaves in vector specificity of barley yellow dwarf virus. Phytopathology 65:1124-1129.
2. Gildow, F. E. 1987. Virus-membrane interactions involved in circulative transmission of luteoviruses by aphids. Curr. Top. Vector Res. 4:93-120.
3. Gill, C. C. 1969. Cyclical transmissibility of barley yellow dwarf virus from oats with increasing age of infection. Phytopathology 59:23-28.
4. Hammond, J., Lister, R. M., and Foster, J. E. 1983. Purification, identity and some properties of an isolate of barley yellow dwarf virus from Indiana. J. Gen. Virol. 64:667-676.
5. Hull, C. H., and Nie, N. H. 1981. SPSS Update 7-9. McGraw-Hill Publishing Co., New York.
6. Leonard, S. H., and Holbrook, F. R. 1978. Minimum acquisition and transmission times for potato leaf roll virus by the green peach aphid. Ann. Entomol. Soc. Am. 71:493-495.
7. Lorens, G. F., Falk, B. W., Qualset, C. O. 1989. Inheritance of resistance to barley yellow dwarf virus detected by northern blot analysis. Crop Sci. 29:1076-1081.
8. Paliwal, Y. C., and Sinha, R. C. 1970. On the mechanisms of persistence and distribution of barley yellow dwarf virus in an aphid vector. Virology 42:668-680.
9. Pereira, A. -M., and Lister, R. M. 1989. Variations in virus content among individual leaves of cereal plants infected with barley yellow dwarf virus. Phytopathology 79:1348-1353.
10. Pereira, A. -M., Lister, R. M., Barbara, P. J., and Shaner, G. E. 1989. Relative transmissibility of barley yellow dwarf virus from sources with differing virus contents. Phytopathology 79:1353-1358.
11. Power, A. G., Seaman, A. J., and Gray, S. M. 1991. Aphid transmission of barley yellow dwarf virus: Inoculation access periods and epidemiological implications. Phytopathology 81:545-548.
12. Rochow, W. F. 1963. Latent periods in the aphid transmission of barley yellow dwarf virus. Phytopathology 53:355-356.
13. Rochow, W. F. 1969. Biological properties of four isolates of barley yellow dwarf virus. Phytopathology 59:1580-1589.
14. Rochow, W. F. 1974. Vectors and variations. Pages 75-83 in: Third International Symposium on Virus Diseases of Ornamental Plants. R. H. Lawson and M. K. Corbett, eds. Tech. Commun. No. 36. Int. Soc. Hortic. Sci., The Hague, Netherlands.
15. Rochow, W. F. 1986. Barley yellow dwarf viruses. Methods Enzym. Anal. 11:420-430.
16. Rochow, W. F., and Carmichael, L. E. 1979. Specificity among barley yellow dwarf viruses in enzyme immunosorbent assays. Virology 95:415-420.
17. Scheller, H. V., and Shukle, R. H. 1986. Feeding behavior and transmission of barley yellow dwarf virus by *Sitobion avenae* on oats. Entomol. Exp. Appl. 40:189-195.
18. Shukle, R. H., Lampe, D. J., Lister, R. M., and Foster, J. E. 1987. Aphid feeding behavior: Relationship to barley yellow dwarf virus resistance in *Agropyron* species. Phytopathology 77:725-729.
19. Skaria, M., Lister, R. M., Foster, J. E., and Shaner, G. 1985. Virus content as an index of symptomatic resistance to barley yellow dwarf virus in cereals. Phytopathology 75:212-216.
20. Tamada, T. 1970. Aphid transmission and host range of soybean dwarf virus. Ann. Phytopathol. Soc. Jpn. 36:266-274.
21. Tamada, T., and Harrison, B. D. 1981. Quantitative studies on the uptake and retention of potato leafroll virus by aphids in laboratory and field conditions. Ann. Appl. Biol. 98:261-276.

22. Toko, H. V., and Bruehl, G. W. 1959. Some host and vector relationships of strains of the barley yellow dwarf virus. Phytopathology 49:343-347.
23. Waterhouse, P. M., Gildow, F. E., and Johnstone, G. R. 1987. The luteovirus group. No. 339 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 9 pp.
24. Watson, M. A., and Mulligan, T. 1960. The manner of transmission of some barley yellow dwarf viruses by different aphid species. Ann. Appl. Biol. 48:711-720.