Differences Among Five Stages of *Schizaphis graminum* in Transmission of a Barley Yellow Dwarf Luteovirus

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ABSTRACT

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The duration of each of four instars of *Schizaphis graminum* varied from 39 to 48 hr at 21 C, intervals that permitted direct comparison among all five stages as vectors of the SGV isolate of barley yellow dwarf virus. When aphids of each stage were allowed a 1-day acquisition feeding at 15 C and a 5-day inoculation test feeding at 21 C, percentages of first, second, third, and fourth instars and adults that transmitted virus were 36, 29, 11, 3, and 2, respectively. Extending inoculation test feedings to 10 days did not change the pattern of stepwise differences in SGV transmission by the five stages. Similar large differences also occurred when aphids acquired virus by feeding through stretched Parafilm membranes on concentrated inoculum, or by being injected with SGV. Use of combinations of temperatures from 15 to 30 C did not increase virus transmission by adults. Studies of virus latent periods in the vector suggested that adults were more likely to transmit SGV if they had acquired virus as nymphs. Combinations of long (5–7 days) acquisition feeding periods with long (10 days) inoculation test feedings also resulted in more than occasional transmission by adults. The incremental pattern of virus transmission differences among the five stages suggests that the *S. graminum*-SGV system could be useful for basic studies of virus-vector interactions.

Among vectors of luteoviruses, *Schizaphis graminum* (Rondani) is probably the most variable. Big differences occur among biotypes of this aphid. Some biotypes are inactive as vectors, others transmit isolates of barley yellow dwarf virus (BYDV) consistently (10). Another major variable in virus transmission is the stage or instar of *S. graminum* that acquires virus. Nymphs are usually better vectors than adults (4–6,16). These variables in virus transmission by *S. graminum* are even pronounced in the transmission of SGV, an isolate of BYDV transmitted specifically by the aphid (3,6,15). As Halstead and Gill (5) have suggested, the apparent scarcity of SGV-like isolates in the field may partly result from the likelihood that such isolates are overlooked in aphid transmission tests because of variations among biotypes and stages of *S. graminum* used as vectors.

In China, *S. graminum* appears to be an important vector of luteoviruses in small grains, especially in low-lying provinces such as Henan, Shaanxi, and Gansu. Because vector transmission patterns are critical in the method for identifying isolates of BYDV that occur in China, we studied factors that influence the ability of different stages of *S. graminum* to transmit SGV.

The purpose of this paper is to describe some details of the role of all five stages of *S. graminum* as vectors of SGV and to evaluate factors that affect virus transmission, especially those that might increase probability of transmission by adults. A preliminary report of this study has appeared (19).

MATERIALS AND METHODS

The aphid species used was the greenbug, *S. graminum*. The biotype was that used in most previous work in New York, a selection from aphids supplied in 1959 by D. C. Arny from Wisconsin (9,12). Aphids were reared on barley (*Hordeum vulgare* L. 'Catskill') in isolated rearing rooms under special precautions to minimize chances of accidental contamination with BYDV or with other aphid species (12). Colonies were grown under continuous fluorescent illumination at 9,360 lux (about 900 ft-c) at about 21 C for 3–4 wk before use. Each colony had been started with 10 viviparae selected from a special colony started 3 wk previously with virus-free newborn nymphs. Some aphids from each colony were always included in each experiment as controls.

The luteovirus used in all tests was SGV, an isolate of BYDV previously described (6,14,15). Young, fully-expanded leaves of symptomatic plants that had been inoculated 2–3 wk previously were usually the virus source. The test plant was *Avena byzantina* Koch 'Coast Black.'

Unless specified otherwise, virus transmission tests were based on 1- or 2-day acquisition feeding on detached leaves at 15 C in the dark, and a 5-day inoculation test feeding on 6-day-old seedlings in a growth chamber illuminated by a mixture of fluorescent and incandescent lights for a 16-hr photoperiod at 21 C. Aphids were removed from test seedlings by fumigation, and plants were placed on benches in a greenhouse under supplemental light for observation during a 3- to 4-wk period (12). Aphids were observed carefully during feeding. Only aphids actually feeding on the detached leaves were transferred to test plants. The presence of circular, chlorotic, injured areas of test seedlings was taken as evidence that the aphids had fed well during inoculation test feedings. Partially purified virus preparations were made as described previously (13). Assays based on feeding aphids through membranes and on injecting inocula were also made by methods described previously (8,12,13).

RESULTS

Differentiation among nymphal instars. The duration of each instar was determined to provide a basis for use of specific instars in transmission experiments. Adult apterous aphids were placed in

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plastic dishes on healthy barley leaves in the growth chamber at 21 C. Every other hour, newborn nymphs were transferred to another leaf in a separate dish, one nymph per dish. When about 25 nymphs had been selected, they were observed at 3-hr intervals to record the time of each molt. The experiment was repeated four times during a period of 6 wk. The mean duration of first, second, third, and fourth instars was found to be 44, 42, 39, and 48 hr, respectively. Since each instar lasted nearly 2 days, this duration was found to be convenient in future experiments because 1-day acquisition feedings could be completed before a molt occurred. These data are in general agreement with results of a similar study in Kansas (7).

Comparison of five aphid stages as vectors. In one series of experiments, aphids were selected after birth or molting to permit comparison of all five stages. Aphids of each instar were allowed a 1-day acquisition at 15 C. They were then transferred singly to test seedlings for a 5-day inoculation test feeding. Similar results were obtained in each of four experiments (Table 1). Percentages of aphids that transmitted virus were 36, 29, 11, 3, and 2%, for those that had acquired virus at the first, second, third, or fourth instars, or adult stage, respectively.

Adequacy of the 5-day inoculation test feeding period was evaluated in an experiment in which aphids of each of the five stages were given a 1-day acquisition feeding before being placed singly on seedlings for inoculation test feeding. Every day, 12–16 plants from each group were fumigated to provide inoculation test feeding periods ranging from 1–10 days. More first-instar nymphs again transmitted than any of the other stages; maximum transmission (88%) occurred after 3 days of inoculation test feeding. Transmission by second and third instars was not increased by extending inoculation test feedings to 7 days. Only one transmission occurred by aphids that acquired virus as fourth instars or adults, even when the inoculation test feeding period was extended to 10 days. None of 20 plants infested as controls in this experiment became infected.

Transmission of SGV from virus preparation. The ability of nymphs and adults to transmit SGV was also compared following injection of virus into the hemocoel, or following virus acquisition by aphids fed through stretched Parafilm membranes. In the membrane-feeding experiments, aphids fed on partially purified preparations of SGV, either mixed with sucrose (to provide 20% final concentration), or from virus-containing zones in sucrose gradient tubes. First-instar nymphs, third instars, and adults were allowed to feed through stretched Parafilm on virus inoculum for 16–18 hr at 15 C. Aphids were transferred from the membranes to test seedlings (three aphids per plant) for a 5-day inoculation test feeding period. As in experiments when acquisition had been from infected tissue, aphids that acquired the virus as first-instar nymphs transmitted more often than did those that acquired it at the third-instar stage (Table 2). Of 224 plants infested with first instars, 35% became infected; only 2% of those infested with third instars became infected. None of 633 adults transmitted virus in these experiments (Table 2).

When SGV preparations were injected into the hemolymph of *S. graminum*, nymphs also transmitted virus more often than did adults. Since first-instar nymphs were too small for consistent injection, second or third instars were used in experiments in comparison with adults. Three injected aphids were placed on each of 40 test seedlings in two separate experiments. Twenty-four of the plants infested with injected nymphs became infected, but only four plants infested with injected adults became infected. None of 80 control aphids transmitted virus.

Factors that affect virus transmission. In one kind of experiment, inoculation test feeding was extended to 27 days for individual aphids given access to SGV as first-instar nymphs. Newly emerged nymphs were allowed a 1-day acquisition feeding. Each of 24 selected nymphs was then given a 1-day inoculation test feeding on each of 27 successive plants. All aphids had become adults by day 7. Twenty-two of the 27 aphids transmitted virus at least once. The numbers of plants that became infected for the first seven intervals in the series were 5, 11, 12, 8, 7, 2, and 2, respectively. Although most transmissions were by first and second instars, five of the viruliferous aphids did transmit SGV as adults. In one of these cases the transmission occurred only on day 12. This experiment confirmed the persistence of SGV in the vector (6) and suggested that adults were more likely to transmit virus if they had acquired it as nymphs, an observation previously made on similar aphid-virus systems (18).

We obtained more than occasional transmission by adults by extending both acquisition and inoculation test feeding periods. Adults were allowed acquisition feedings of 1, 3, 5, or 7 days on detached leaves in one experiment. They were then transferred daily to new test plants for 8–14 days. Following acquisitions of 1, 3, 5, or 7 days, 1, 1, 7, and 3 of 20 aphids, respectively, transmitted virus. In contrast to the tests described above (where adults had acquired virus as nymphs), none of these 12 adult aphids transmitted SGV to more than one of the 780 plants involved in the series. In another experiment, adults were allowed acquisition feedings of 1, 3, or 5 days on intact plants in the growth chamber at 21 C, instead of on detached leaves at 15 C. The numbers of adults (of 64) from each group that transmitted SGV were 2, 1, and 3, respectively, following inoculation test feedings for 5 days. We were not able to find conditions under which adults tansmitted SGV as well as nymphs.

Next, we focused on the question of a latent period in the transmission of SGV by *S. graminum*. Nymphs 6-hr old were allowed acquisition feedings of 3, 6, 12, 24, or 48 hr on detached leaves. Single aphids from each group were transferred successively to a seedling for inoculation test feedings of 3, 6, 12, 24, 24, and 120 hr. In general, transmission increased as the acquisition period increased from six to 48 hr (Fig. 1). Following acquisition feedings of 48 hr, 42 of 60 aphids transmitted SGV. A clear delay in virus transmission occurred when acquisition periods were 3 or 6 hr long. Only two of 63 aphids transmitted virus following the 3-hr acquisition period; these occurred following the long inoculation

TABLE 1. Transmission of the SGV isolate of barley yellow dwarf virus acquired by five stages of *Schizaphis graminum*

	Transmission by stage shown[a]				
Test	First instar	Second instar	Third instar	Fourth instar	Adult
1	17/40	9/40	3/40	2/40	0/60
2	9/30	8/30	3/30	1/30	1/60
3	9/45	8/45	6/45	1/60	1/60
4	21/40	20/40	3/18	0/12	1/33
Total	56/155	45/155	15/133	4/142	3/213
Percentage	36	29	11	3	2

[a] Numerator is number of plants that became infected; denominator is number infested with single aphids that had a 1-day acquisition feeding period at 15 C and a 5-day inoculation test feeding period at 21 C. None of 104 plants infested as controls became infected.

TABLE 2. Transmission of the SGV isolate of barley yellow dwarf virus by three stages of *Schizaphis graminum* allowed to acquire virus by feeding through membranes on partially purified preparations

Estimated SGV concentration (μg/ml)[a]	Transmission after acquisition by stage shown[b]		
	First instar	Third instar	Adult
20	22/28	2/29	0/27
20	17/28	1/28	0/28
28	30/60	1/60	0/60
28	7/80	0/80	0/80
14	3/28	1/12	0/16

[a] Concentration fed on by aphids was half that shown in 20% sucrose.
[b] Numerator is number of plants that became infected; denominator is number infested with three aphids that had 18-hr acquisition feeding through membranes at 15 C and 5-day inoculation test feeding at 21 C. None of 60 plants infested as controls became infected.

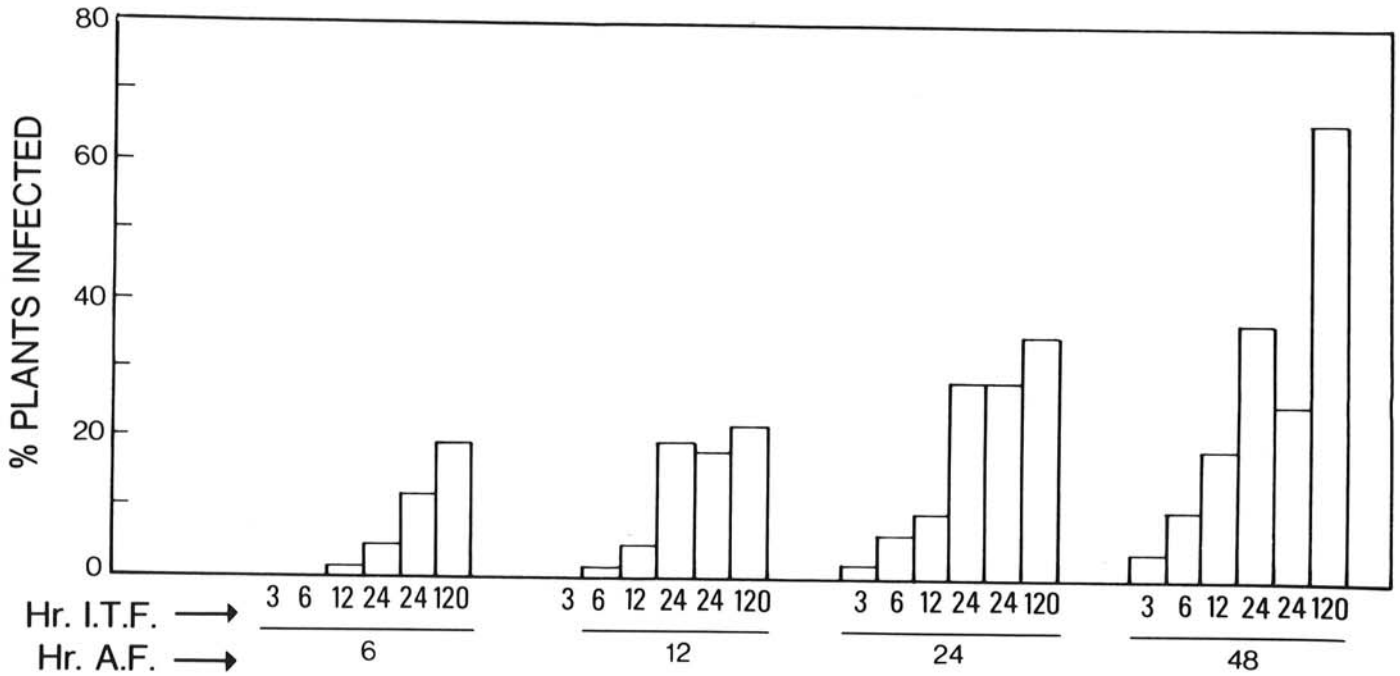

Fig. 1. Percentages of plants infected by the SGV isolate of barley yellow dwarf virus transmitted by single *Schizaphis graminum* that acquired virus as first instar nymphs during acquisition feeding (A.F.) for 6, 12, 24, or 48 hr. Sixty aphids from each of the four groups were allowed inoculation test feeding (I.T.F.) periods on successive plants for 3, 6, 12, 24, and 24 hr, followed by a final feeding for 5 days (120 hr) on a sixth test plant. None of 120 aphids tested as controls transmitted virus.

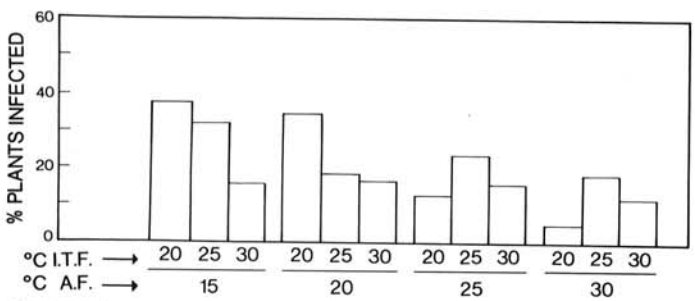

Fig. 2. Influence of temperature on transmission of the SGV isolate of barley yellow dwarf virus by first instars of *Schizaphis graminum* allowed a 2-day acquisition feeding (A.F.) at one of four temperatures, followed by a five-day inoculation test feeding (I.T.F.) period at one of three temperatures. Percentages, based on a total of 45–60 single aphids for each treatment, represent combined results of three experiments. None of 360 aphids tested as controls transmitted virus.

test feedings. Relationships between length of acquisition and test feeding periods are similar to those observed for other isolates of BYDV (11) and suggest the presence of a latent period in the transmission of SGV by *S. graminum*. The data again suggest that adults are likely to transmit SGV only if they have acquired virus as nymphs.

Since temperature has pronounced effects on transmission of some luteoviruses by certain aphid species (12), three experiments were carried out to study the role of temperature in transmission of SGV by different stages of *S. graminum*. These experiments were done in three reach-in growth chambers that provided a 14-hr photoperiod at 8,320 lux (about 800 ft-c) and temperatures of 20, 25, or 30 C. First-instar nymphs and adults were given two-day acquisition feedings on detached leaves at 15 C in an incubator, as well as at 20, 25, or 30 C in growth chambers. Single aphids were then transferred from each acquisition temperature to individual test seedlings and kept in one of the three growth chambers for a 5-day inoculation test feeding period. Results of each of three experiments were similar. Only 3 of 670 adults transmitted SGV. These few transmissions occurred only following acquisitions at 15 or 20 C. Thus, increased temperatures clearly did not increase the ability of adults to transmit SGV. Aphids that acquired virus as first instars also transmitted virus more often following acquisitions at 15 or 20 C, but some virus transmissions occurred at all of the various combinations (Fig. 2). These results with SGV are similar to previous studies with *S. graminum* in the transmission of other isolates of BYDV at the same temperatures (6,12).

DISCUSSION

Nymphs have long been known to be better vectors than adults for several aphid-virus systems, including the one studied here (1,4,6,16–18). This work shows that such differences in transmission ability decrease incrementally for each instar of *S. graminum*. Each time the aphid molts it is less likely to acquire and transmit SGV. Even when aphids acquired virus by feeding through membranes on concentrated SGV preparations, or by being injected with virus, large differences between nymphs and adults still occurred. Attempts to make adults become consistent vectors by changing experimental conditions were unsuccessful. Only when adults had acquisition feeding periods of 5–7 days, or when they had acquired virus as nymphs, did more than occasional transmissions occur. In contrast, first-instar nymphs were efficient vectors in all kinds of tests.

The stepwise nature of the transmission differences among the five aphid stages suggests that the *S. graminum*-SGV system could be used to study the mechanism controlling aphid transmission of luteoviruses. Differences between nymphs and adults are large; they occur consistently among experiments done in a variety of ways. Such differences seem more pronounced for the *S. graminum*-SGV system than for others. Differences between nymphs and adults of *S. graminum* in the transmission of several other isolates of BYDV were not consistent (6).

Many possible explanations about how nymphs can be better vectors of virus than adults are based on physiological differences between young and old aphids. Suggestions have included more rapid accumulation of virus in salivary glands of nymphs than of adults, a shorter latent period in nymphs than adults, and a faster feeding rate by immature aphids than by mature ones (1,4–6,17). Since the limiting factor in circulation of luteoviruses through aphids appears to be movement through salivary glands (2),

perhaps such movement is more likely to occur in young glands than in older ones, or perhaps the rate of virus movement is faster in young ones. For *S. graminum*, another possible explanation is the role of the potent salivary toxin secreted during feeding. The toxin not only injures plant tissue at feeding sites, but it also might interfere more directly with infectivity of virus particles. In all our experiments, the visible injury of plant tissue by toxin was consistently more extensive following feeding by adults than following feeding by younger instars. Future studies on the identification and purification of the salivary toxin might be a useful approach to study mechanisms that permit nymphs of *S. graminum* to be better vectors of SGV than are adults.

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