The Median Latent Periods for Three Isolates of Barley Yellow Dwarf Virus in Aphid Vectors

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ABSTRACT

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The median latent periods (LP 50) were determined for three isolates of barley yellow dwarf virus in their specific vector or most efficient vector at two temperatures. The latent period per aphid was defined as the time lapse from the start of the acquisition feeding period to the middle of the inoculation day during which transmission of an infectious dose of virus first occurred. At 15 C and 20 C, the median latent periods for *Sitobion avenae* with *S. avenae*-specific isolate 6407 were 65.5 hr and 44.5 hr, for *Rhopalosiphum padi* with *R. padi*-specific isolate 6524 the values were 50.1 hr and 35.0 hr, and for *R. padi* with *R. padi* nonspecific isolate 7410 the values were 62.4 and 35.2 hr, respectively. A decrease equivalent to 50% in the median latent periods for a 10 C increase in temperature was found with the two aphid-specific isolates, but the decrease was more than 50% with the *R. padi*-nonspecific isolate.

Barley yellow dwarf virus (BYDV) is a luteovirus which is transmitted in a circulative manner by its aphid vectors. Currently there is no evidence that this virus multiplies in the aphid (10) and multiplication that occurs in its host plants is limited to phloem cells (3,7,8).

Viruses that are circulative in the vector, are characterized by a latent period which may be defined as the time that elapses between acquisition of the virus and the ability of the vector to first transmit the virus. The virus, therefore, is presumed to progress from the gut to the haemolymph, to the salivary glands and then into the plant via the saliva. Determination of the length of the latent period is important for experimental work and also may be of value in estimating whether the virus multiplies in the vector.

Early estimations of the latent period of BYDV in its aphid vectors produced various results that reflected the different methods used by the investigators. Toko and Bruehl (16) using *Sitobion avenae* (Fabricius) (= *Macrosiphum granarium* [Kirby])-specific and *Rhopalosiphum insertum* (Walker) (= *R. fitchii* [Sand.])- specific isolates reported that after an acquisition feeding period of 24 hr, an inoculation feeding period of 4 hr was necessary before transmission occurred. Aphid nomenclature is according to Eastop and Hille Ris Lambers (2). No transmission resulted following an acquisition feeding period of 16 hr or less, even with an inoculation feeding period of 72 hr. Watson and Mulligan (18) working with a *Rhopalosiphum padi* (L.)-specific and an aphid-nonspecific isolate reported that, of the *R. padi* individuals that eventually transmitted virus, 32 hr of acquisition feeding was required for approximately 50% of the aphids to transmit. Orlob et al (9) reported a minimum latent period of 6 hr for *R. insertum* (= *R. fitchii*) to transmit a Wisconsin strain of BYDV. Finally, Rochow (12) demonstrated that *R. padi* and *S. avenae* (= *M. granarium*) with their specific virus variants required about 5 days to attain maximum transmission ability after acquisition feeding periods of 12 hr or less. These results indicated the presence of at least a short latent period for BYDV in the aphid, but the length of the period for a population of aphids was not well defined.

In 1965, Sylvester (13) published an improved method for determining the latent period of pea enation mosaic virus in its aphid vector, based on an estimation of the time when 50% of the aphids that eventually transmitted virus had achieved a first transmission. These data (hours/percentage cumulative first transmissions) were transformed to log-probits. The resulting curve was rendered linear by a correction factor designed to compensate for the excluded potential first transmissions by aphids that may have transmitted very late. A median latent period (LP 50) was estimated. The method entailed the use of a short acquisition feeding period followed by a series of inoculation feeding periods of equal length on healthy test seedlings. Although empirical in nature, this method appears to be well suited to persistent viruses especially those with rather short latent periods. To enable adequate comparisons to be made between virus isolates, this method was adopted to estimate the LP 50 with three variants of BYDV. The effect of temperature on the latent periods was also examined.

MATERIALS AND METHODS

The aphids used were from one clone each of *S. avenae* and *R. padi*. Each clone was derived from a single adult aphid collected in the field in southern Manitoba in 1964. The aphids were reared on caged barley, *Hordeum vulgare* L. 'Parkland', in separate growth cabinets at 18 ± 1 C with mixed incandescent and cool white fluorescent illumination for a daily 18-hr photoperiod. Aphids from these colonies were tested weekly to ensure that they were free from BYDV.

The BYDV isolates were 6407, an isolate transmitted specifically by *S. avenae* (4); 6524, transmitted specifically by *R. padi*; and 7410, an aphid-nonspecific isolate transmitted most efficiently by *R. padi* (6), called *R. padi*-nonspecific. These virus isolates were maintained in oats, *Avena byzantina* C. Koch 'Coast Black', in a growth cabinet at 17 ± 1 C. Individuals of *S. avenae* were used as vectors with isolate 6407, and individuals of *R. padi* were used with isolates 6524 and 7410.

The method used for determining the median latent periods was that of Sylvester (13) but with some modifications. Infected Coast Black oat seedlings were prepared as follows for use as virus source plants. Leaves were detached from oat plants in which virus isolates were maintained, and placed with the basal end in moist sand in a petri dish. Virus-free aphids were placed in the dish and allowed an acquisition feeding period of 2 days at 15 ± 0.5 C in the dark. Then 10 feeding aphids were transferred in the morning to each of several individually caged Coast Black oat seedlings at the one-leaf stage. After a 30 hr inoculation feeding period the plants were sprayed with insecticide and were placed in a growth cabinet at 18 ± 1 C. These plants served as virus source plants 7 days after the start of the inoculation period.

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Each trial to determine the latent period involved the daily transfer of individual aphids to successive test seedlings. Test aphid nymphs were derived by placing virus-free alatae on washed, detached, virus-free Parkland barley leaves in a petri dish with moist sand. After 24 hr at 18 C in a growth cabinet, nymphs deposited by the alatae were transferred to freshly detached barley leaves in a second petri dish and kept at 15 C for 2.5 days. Then in the evening, the middle portion of the second leaf on the virus source plant was excised and placed in a petri dish. The test nymphs were transferred to these infected leaf pieces for an acquisition feeding period of 12 hr in a plant growth cabinet operated at either 15 ± 1 C or 20 ± 1 C. Nymphs were then taken from the dish and one nymph was placed on each of 20 individually caged Coast Black oats at the one- or two-leaf stage. These oat seedlings had been transplanted into 7.6-cm-diameter peat pots, one seedling per pot, a day in advance. At 24-hr intervals each aphid was transferred to another seedling at the one- to two-leaf stage. After feeding on the sixth seedling, the aphid was either removed or was transferred to a seventh seedling for a 6-day inoculation feeding period. All seedlings were sprayed with insecticide when the inoculation feeding period was ended and were maintained in a greenhouse for about 6 wk at 16–19 C when final inspections were made for plants with symptoms. Greenhouses were sprayed twice weekly with insecticide, and cool, white fluorescent lamps prolonged the daily photoperiod to 16 hr with a minimum light intensity of ~ 13,000 lux at soil level.

The latent period for each aphid that transmitted virus was defined as the time lapse from the start of the acquisition feeding period to the middle of the 24-hr inoculation feeding period on which the first transmission occurred. To estimate the median latent period, the cumulative totals of first transmissions in all trials were derived for each 24-hr period. The cumulative value for the sixth feeding period was assumed to be 100%. The probits of the cumulative percentages of the first transmissions were then plotted against the logarithm of the time from the beginning of the acquisition feed. The probit scale was the ordinate and the log scale was the abscissa. Linear regression analysis was applied to the data for goodness of fit to the plotted points and to estimate the median latent period as the time period required for 50% of the aphids to attain a first transmission.

RESULTS

Three trials were performed with *S. avenae*-specific isolate 6407 at 15 C and four at 20 C. Five trials were performed with *R. padi*-specific isolate 6524 and four with *R. padi*-nonspecific isolate 7410 at each temperature. Twenty aphids were used in each trial and the percentage that completed six daily serial transfers at 15 C and 20 C was 83 and 94, respectively, with isolate 6407, 84, and 79 with isolates 6524, and 76 and 73 with isolate 7410. However, of the total of 93 aphids in all trials that did not complete six serial transfers, 26 transmitted virus at least once.

At 15 C and 20 C, the median latent periods for *S. avenae* with *S. avenae*-specific isolate 6407 were 65.5 hr and 44.5 hr, for *R. padi* with *R. padi*-specific isolate 6524 the values were 50.1 hr and 35.0 hr, and for *R. padi* with *R. padi*-nonspecific isolate 7410 the values were 62.4 and 35.2 hr, respectively. Graphs comparing the regression lines for each isolate at 15 C and 20 C are shown (Fig. 1). The percentages of aphids that transmitted virus during the six daily transfers at 15 C and 20 C, respectively, were 36.7 and 46.3% for isolate 6407, 57.0 and 52.0% for isolate 6524, and 40.0 and 45.0% for isolate 7410. In 17 of the 25 trials, individual aphids that survived the six daily transfers, were allowed a final 6-day feeding period on one extra seedling. Six percent of the 177 aphids that transmitted virus in these 17 trials did so only during the final 6 days.

During two of the trials with *R. padi* and isolate 6524 at each temperature, observations were made on the developmental stages of the aphids. Most nymphs were second instars when transferred to the first in the series of the six plants used for daily transfers. In trials at 20 C, all aphids were mature apterae at the end of the sixth daily feeding period (aphid age 9–10 days) and some had one or more nymphs. All except two of the 36 survivors passed through the final moult during the sixth daily feeding period. At 15 C, all aphids were in the fourth instar at the end of the sixth daily feeding period, and only two of 40 were alatoid (having wingpads). There was no evidence of undue aphid mortality on plants infected with any of the isolates at either temperature.

DISCUSSION

As with Sylvester's method (13) the acquisition feeding period was included in the latent period because only a short part of about 10–30 min of this feeding period is required for aphids to penetrate with their stylets to the phloem (11). Also, the end of the inoculation feeding period was timed to the middle rather than to the end of the relevant 24-hr period, thereby employing a mean value for the time span during which a first transmission might occur. The median latent periods were determined from equations derived by linear regression analysis since the plotted points on the graphs fitted a straight line with a high degree of correlation. Thus, no correction factor was used in our work.

Because of the relatively short latent periods found with the three virus isolates, there was no clear evidence that these isolates were propagative in their vectors. Although the latent periods for the *R. padi*-specific and *R. padi*-nonspecific isolates were similar at 20 C with *R. padi* as the common vector, the latent periods at 15 C differed appreciably, which suggested a differential effect of temperature on the translocation of these two virus isolates in the aphid.

The median latent period may be affected by several variables such as the vector species clone used, insect stadium, length of acquisition feed, virus strain, and host plant. Recently it was shown that the use of short inoculation feeding periods could influence the median latent period considerably if the virus is capable of multiplying in the mesophyll (17). Another possible variable, the concentration of virus in the source plant, was reduced in our work by timing the acquisition feed to coincide with the first peak of virus infectivity in the second leaf (5), and by using only the middle portion of the leaf.

Temperature also may exert considerable influence on the latent period. For example, Duffus (1) demonstrated with sowthistle yellow vein virus and the sowthistle aphid, that the average latent period was halved for each 10 C rise in the 5–25 C range. Although the median latent period found by Sylvester and Richardson (15) for pea enation mosaic virus (PEMV) in *Acyrthosiphon pisum* (Harris) decreased at higher temperatures, the latent periods at 10, 20, and 30 C did not fit the pattern reported by Duffus (1). These latent period values may have been affected by the fact that PEMV multiples in the mesophyll (17). On the other hand, Sylvester and Osler (14) found that the median latent period for filaree red leaf virus in *Acyrthosiphon pelargonii zerozalphum* (Knowlton) was the same at 20 C and 25 C.

To determine whether the median latent period in our work with BYDV was halved for a rise of 10 C, the expected latent period values at 20 C were calculated from those determined at 15 C by multiplying with the factor $\sqrt{0.5}$. The calculated latent periods at 20 C were 46.3, 35.4, and 44.1 hr, respectively, for the *S. avenae*-specific, *R. padi*-specific, and *R. padi*-nonspecific isolates, compared with experimental values of 44.5, 35.0 and 35.2 hr. Therefore, with the two aphid-specific isolates, the decrease in the latent periods with a rise of 5 C, represented the equivalent of half the value at 15 C for a rise of 10 C. This, however, was not true for the *R. padi*-nonspecific isolate 7410.

For comparison with our results, the data obtained by Rochow (12) for the 12-hr acquisition feeding period was transposed to log-probits. For this conversion it was assumed that once an aphid transmitted, it would continue to do so to the remaining test plants during the series of daily transfers. These trials were performed in a greenhouse at variable temperatures. The calculated median latent periods were 46.0 hr for MGV, an *S. avenae* (= *M. granarium*)-specific isolate, and 56.6 hr for RPV, a *R. padi*-specific isolate. The derived values are in the general range of our results for isolate 6407 at 20 C and for isolate 6524 at 15 C.

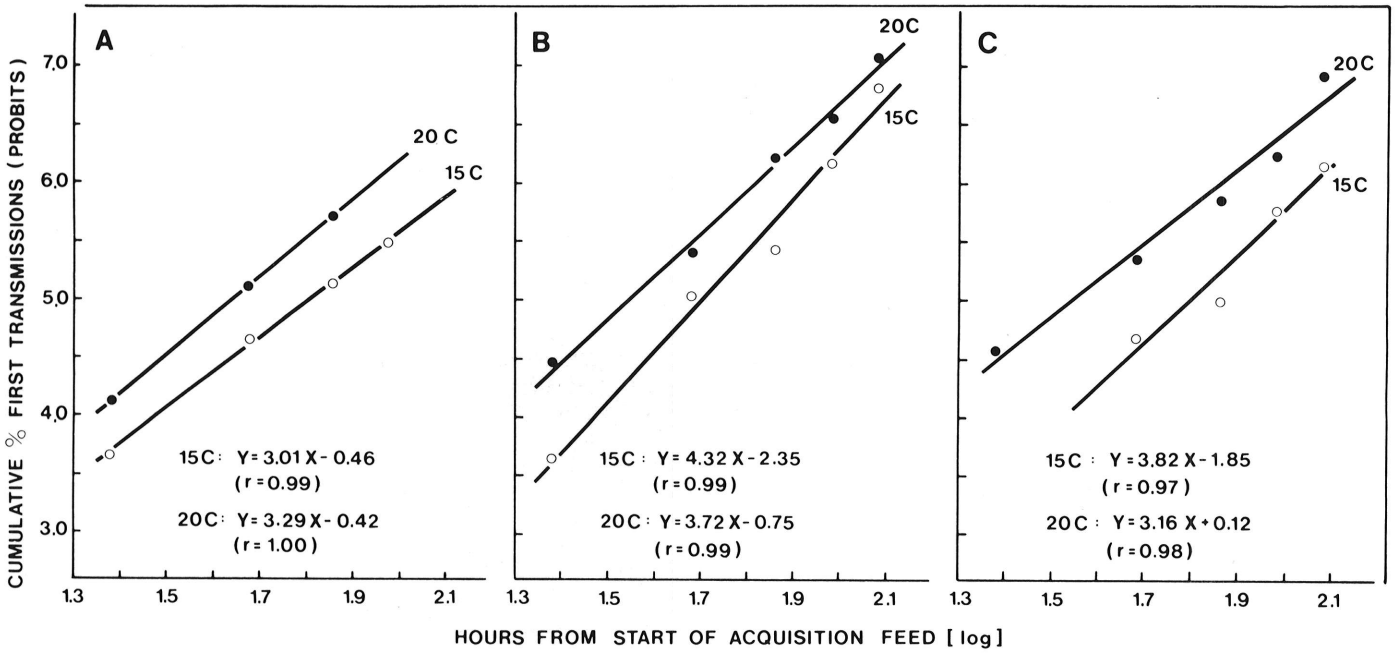

Fig. 1. Regression lines showing relationship between the cumulative percentages of first transmissions and time from the start of the acquisition feed for: **A,** a *S. avenae*-specific isolate of barley yellow dwarf virus with *S. avenae*; **B,** a *R. padi*-specific isolate with *R. padi*, and **C,** a *R. padi*-nonspecific isolate with *R. padi*, at 15 C (o) and 20 C (•).

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