Characteristics of Beet Yellows Closterovirus Transmission to Sugar Beets by *Aphis fabae*

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ABSTRACT

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Laboratory studies were conducted on the characteristics of beet yellows closterovirus (BYV) transmission to sugar beets by the bean aphid, *Aphis fabae*. The relative transmission efficiency and the retention, acquisition, and inoculation thresholds were evaluated using clonal aphid colonies. Transmission efficiency was studied between different apterous aphid species (*A. fabae* and *Myzus persicae*), between alate and apterous morphs of *A. fabae* and *M. persicae*, and among five clonal aphid colonies of *A. fabae*. Results indicated, on average, that apterous *M. persicae* transmitted twice as efficiently as apterous *A. fabae* (60 and 34%, respectively), apterous forms of *A. fabae* were equally efficient in transmitting BYV as compared with alate *A. fabae* (28 and 29%, respec-

tively), and the five clonal colonies of *A. fabae* ranged from 28 to 40% transmission efficiency with an average of 34%. The maximum retention time recorded for BYV by *A. fabae* was between 24 and 48 h. After a 1-h acquisition access, a transmission efficiency of 10% was obtained, with maximum transmission efficiency (the highest average percent transmission that is statistically different from all other previous averages) of 39% reached after 6 h of acquisition access. Inoculation thresholds occurred within 1 h, with maximum transmission efficiency (42%) reached after a 6-h inoculation access. Using an electronic monitoring system, a range of 9 to 38 min (20.5 mean) was recorded for the stylets of *A. fabae* to reach the phloem sieve elements. This was consistent with our threshold of a 20-min acquisition access recorded through direct observation.

Additional keyword: semipersistent.

Beet yellows closterovirus (BYV) is an aphid-borne, semipersistently transmitted virus that infects sugar beets, *Beta vulgaris* L. BYV has a flexuous filamentous particle, 1,250 nm in length, containing single-stranded RNA (1). The disease was first reported in Europe in 1935 (2). Although BYV was not in the United States until 1951 (5), it was suspected to have been present there several years earlier. BYV belongs to the yellows virus complex, which, in the United States, also includes beet western yellows luteovirus (BWYV), beet mosaic potyvirus (BtMV), and lettuce infectious yellows closterovirus (LIYV). Of these four viruses, BYV can cause the most severe yield losses in California, reducing tonnage up to 50% (2,7) and sucrose contents from 0.1 to 3.1 percentage points (2). There are 24 aphid species listed as either known or probable vectors of BYV (11,17). Where they have been studied in the field, the two principal vectors are the green peach aphid, *Myzus persicae* Sulzer, and the bean aphid, *Aphis fabae* Scopoli (2,26).

During the last 25 years, a shift in vector populations has occurred from *M. persicae* to *A. fabae*. In 1970 and 1971, *A. fabae* complex made up less than 1% of aphids caught in yellow water pan traps from Yolo County (10,27). During 1988 to 1994, yellow water pan traps have shown *A. fabae* outnumbering *M. persicae* by two- to fivefold (L. D. Godfrey, unpublished data). Recent information on *A. fabae* in California shows that some regional populations are more efficient transmitters than *M. persicae* (13). However, *M. persicae* is still considered to be the primary vector of BYV in England and the United States. This is based on earlier studies showing higher transmission efficiencies in the laboratory (2,8),

peak flights correlating to spread of BYV (18,26), and its greater activity moving plant to plant due to sugar beets being a nonpreferred host. Questions about the relative importance of *A. fabae* and *M. persicae* have been raised because of the population shift between the two vectors and the increases in incidence of BYV.

Currently, there is a lack of information on the transmission characteristics of BYV by *A. fabae*. Additional knowledge of acquisition, inoculation, retention periods, and transmission efficiency would provide a better information base on which to analyze control tactics. The time that a vector needs to acquire a virus and inoculate new plants, in part, determines the rate of virus spread in the field. The time of virus retention by an aphid directly influences the distance the virus can be spread. Most previous work has been performed with *M. persicae* and has indicated that BYV can be acquired, at low levels, in 5 to 60 min, reaching maximum efficiency between 6 to 12 h (2,20,24). BYV can be inoculated to a healthy host plant in 5 to 60 min, reaching maximum efficiency in no more than 6 h (2,20,24), and retention, in some cases, can be as long as 72 h (2,20,24). Minimum acquisition and inoculation access times for *A. fabae* can be obtained indirectly from a study by Watson (24) that showed BYV could be separated from a mixed infection with BtMV by differential infection feeding times and retention periods. Watson's data show an acquisition and inoculation threshold of 10 min and 1 h, respectively, with successful retention between 26 and 50 h (24). There is some evidence that regional populations of *A. fabae* have a differential ability to transmit BYV. Kirk et al. (13) reported a range from 14 to 63% between five colonies of *A. fabae* (nonclonal) collected from sugar beet-growing areas of California. Data on clonal variation could help provide answers as to why some years or some areas have more serious problems with BYV than do others.

Given the conditions over the past 20 years of increasing BYV in California and a concurrent shift in aphid species composition,

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there is a strong need for a study on the characteristics of BYV transmission by *A. fabae*. The objectives of this research were to quantify the characteristics of BYV transmission by *A. fabae* in the Sacramento Valley of California and compare the relative transmission efficiencies between different aphid species (*A. fabae* and *M. persicae*), between apterous and alate *A. fabae* and *M. persicae*, and among five clonal colonies of *A. fabae*.

MATERIALS AND METHODS

Plants and soil mix. All seedlings were grown in 6×8 -cm-square pots (190 ml) and maintained in a greenhouse at $25 \pm 2^\circ\text{C}$ and ambient relative humidity. Metal halide lamps (1,000 W) provided additional light, as needed, to produce a photoperiod of 15 h and a light intensity of $20.59 \pm 2.94 \text{ W/m}^2$. Plants were inspected biweekly for aphid infestation and fumigated with nicotine sulfate on alternate weeks. Healthy plants and BYV-infected plants were grown and maintained in separate greenhouses at the same temperature and photoperiod. Fluorescent, cool white, 75-W bulbs maintained a light intensity of 2.35 to 2.94 W/m^2 after dark. Plants were fertilized weekly using Stern's Miracle-Gro (Stern's Miracle-Gro Products, Port Washington, NY), 15-30-15 (N-P-K), 4.0 g/liter of water. These greenhouses were monitored for aphids with yellow sticky cards and fumigated bimonthly with Vapona (dichlorvos [DDVP]). All plants used throughout this experiment were grown in a soil mix containing 40% sand, 20% peat moss, 20% redwood-treated compost, and 20% pumice. Additional components included 2.6 kg/m^3 of dolomite lime, 0.9 kg/m^3 of oyster-shell lime, 0.9 kg/m^3 of superphosphate (0-20-0), 140 ml/m^3 of potassium nitrate, and 90 ml/m^3 of potassium sulfate.

Isolation of BYV. A sugar beet leaf sample infected with BYV was obtained from a sugar beet field located south of Davis in Solano County, CA, in July 1991. BYV infection was initially verified by the use of an indirect enzyme-linked immunosorbent assay (ELISA). BYV was then transferred to alternate hosts to further verify the identification of the virus. For this procedure, *M. persicae* was allowed to feed for 24 h on an infected sugar beet plant and transferred to a New Zealand spinach plant (*Tetragonia expansa* Thunb. ex J.A. Murray) and allowed to feed for 24 h. Once symptoms developed on New Zealand spinach plants, aphids were fed on the infected leaves and transferred to *Chenopodium capitatum* (L.) Asch. The symptoms of this isolate included vein clearing in young leaves of recently inoculated sugar beet, a lethal effect on *C. capitatum*, and a tendency to produce pinpoint lesions on older beet leaves. Based on observations made by Bennett (2), which grouped BYV isolates into one of two groups and six strains, these symptoms indicated it to be one of the more virulent strains (group 2, strain 5).

Virus source plants. BYV source plants were established by inoculating 6-week-old, noninfected, healthy sugar beet plants grown in 14×11 -cm (diameter $\times$ depth)-round pots using 10 to 20 apterous *A. fabae*. Two weeks after inoculation, a high BYV titer was recorded by indirect ELISA in all new leaves. These plants were transplanted into 25×21 -cm (diameter $\times$ depth)-round pots and maintained for 3 to 5 months as BYV sources for all transmission experiments. When BYV titer began to decline, new source plants were established and maintained in the greenhouse following the procedures described above. The sixth to eighth newest leaf was used as a virus source for each experiment.

ELISA. An indirect ELISA was used to detect BYV in sugar beet leaves using antiserum described by Reed and Falk (15). Leaf tissue was macerated using the Dayton leaf roller (Piedmont Tool and Die, Six Mile, SC) from a 0.2- to 0.4-g piece of new leaf tissue. Sap pressed from the tissue was diluted 1:10 (wt/vol) with sodium carbonate buffer (pH 9.6) and $100 \mu\text{l}$ was transferred into individual wells of Immulon-2 flat-bottom-well microtiter ELISA plates (Dynatech Laboratories Inc., Chantilly, VA). Duplicate wells were used for each sample. One noninfected and one known BYV-

infected sugar beet plant were included in three different positions on each plate, as healthy and infected controls, respectively. Absorbancies were measured at 405 nm with a V_{max} kinetic microplate reader (Dynatech Laboratories Inc.). The plants were recorded as infected if the absorbance values were greater than three times the mean of the healthy check.

Aphid colonies. Collections of apterous virginoparae *A. fabae* were made from commercial sugar beet fields during March to May 1993 in the Central Valley of California. A sample was taken from each of the following five areas: Elk Grove (south Sacramento County), Davis and Woodland (south and central Yolo County, respectively), Dixon (Solano County), and Willows (Glenn County). These sites were representative of major sugar beet-growing areas in California's Central Valley, where bean aphids are a pest. Different geographic sites were chosen to insure the aphids were of separate parental lines, so that variability in virus transmission characteristics could be studied. Aphids were initially reared on faba bean, *Vicia faba* L. var. Lohse, a nonhost for BYV, for 1 week to insure they were not viruliferous. Following this elimination of the virus, single aphid colonies were established from each site by transferring individual insects to separate two- to four-leaf-stage sugar beet seedlings, var. SS-501 (Spreckels Sugar Company, Pleasanton, CA). Aphids were later maintained on six- to eight-leaf-stage plants. A single aphid colony of *M. persicae*, collected from cheeseweed (*Malva parviflora* L.) near Elk Grove, was established using the same procedure as above, except radish (*Raphanus sativus* L. var. White Icicle) was used to eliminate the virus and maintain the colony for use throughout the studies. This plant was chosen because it is a preferred host for *M. persicae*.

Colony maintenance and aphid transfer procedures. Aphid colonies were maintained at a 15:9 (day/night) photoperiod and 22°C in the laboratory. Under these conditions, alate aphid production was minimized (2) and *A. fabae* was found to complete a life cycle in a minimum of 8 days. To maximize the number of young adults in each new colony, 50 apterous adults were initially transferred to the plants. After 8 to 10 days, the majority of colony adults were newly emerged (2 to 3 days old). Recently emerged adult apterous aphids were used throughout the experiment, unless otherwise stated. Aphids were transferred using standard procedures described by Watson (25). Subsequent to transferring aphids to a virus source leaf for the acquisition access period, the leaf was placed in a 1-liter, wide-mouth mason jar containing 4-cm depth of sand that was saturated with water. The leaf petiole was submerged into the sand, and the jar was covered with a moistened piece of plastic wrap, secured with a rubber band, and placed into a Percival model 1-35LVL (Percival Manufacturing Co., Boone, IA) growth chamber for 24 h. The jars were partially covered with a cloth to reduce light stimulus to the aphids and encourage probing or feeding on the plants. After access to a virus source leaf, aphids were directly transferred to individual two-leaf-stage sugar beet seedlings. Each seedling was covered by a whole plant cage made from 40-dram (70.88 g) crystal plastic vials (Thornton Plastics, Salt Lake City, UT) that was sealed at the top with fine organdy cloth and pushed into the soil to a depth of 1 to 2 cm. The caged aphid(s) and plant were placed in a growth chamber for the assigned inoculation access periods. The growth chamber was maintained at a photoperiod and temperature similar to the greenhouse: 15:9-h (day/night) photoperiod and $22:19^\circ\text{C}$ (day/night) temperature. The lighting system included a combination of 20-W, cool white fluorescent bulbs from the top and 40-W, cool white bulbs from the sides. Light intensity was measured at 4.41 W/m^2 . At the end of each treatment, all test plants were sprayed with diazinon, and then incubated in a greenhouse for a minimum of 14 days. After this time, the most recent growth was assayed for BYV using indirect ELISA.

Relative transmission efficiency. In determining the relative transmission efficiency of BYV by alate *A. fabae*, comparisons were made between morphs of different aphid species (*A. fabae*

and *M. persicae*), between apterous and alate *A. fabae*, and among five clonal colonies of apterous *A. fabae* collected from the Sacramento Valley (Elk Grove, Davis, Dixon, Woodland, and Willows). For the comparison between apterous *A. fabae* and *M. persicae*, 100 *A. fabae* and 100 *M. persicae* were used per replication. Fifty *A. fabae* and 50 *M. persicae* were transferred to each of two virus source leaves for the 24-h acquisition access period, followed by a 24-h inoculation access period on the healthy seedlings. The entire procedure was repeated four times. The comparison between different morphs was performed with alate and apterous forms of *A. fabae* and alate forms of *M. persicae*, both colonies from the Elk Grove area. Alates were obtained by permitting the colonies to reach high population densities until winged adults occurred. Approximately 30 alates of each species and 30 apterae of *A. fabae* were transferred to separate virus source leaves using identical transfer procedures. Three replications were performed for each aphid morph except alate *M. persicae*, in which only one was conducted. In comparing the five regional clonal colonies of *A. fabae*, 20 to 35 aphids from each colony were transferred to separate virus source leaves for a 24-h acquisition access period, followed by a 24-h inoculation access period on healthy sugar beet seedlings. Three replications were performed for each colony.

Retention time. The length of time that *A. fabae* and *M. persicae* remained capable of transmitting BYV (retention time) was determined. For each species, aphids were given access to virus source leaves for 24 h. After acquisition, *A. fabae* was split into two retention treatments, either on nonvirus host faba bean plants (2 weeks old) or in glass petri dishes with moistened cellophane. *M. persicae* retention time was tested using only a nonvirus host radish. All aphids were then held in the growth chamber under the same conditions as virus acquisition. The time that the aphids spent on the faba beans, radish, or petri dishes functioned as isolation from the virus inoculum source. For the *A. fabae*-faba bean treatment, 0, 1.5, 3, 6, 12, 19, 24, and 48 h were used as the time periods away from the virus source, and then 60 aphids were transferred to 20 sugar beet seedlings per time period (three aphids per plant). The *A. fabae*-petri dish treatment tested 0, 3, 6, 9, and 12 h with 10 sugar beet seedlings per time period and three aphids per plant, while the *M. persicae*-radish treatments tested 0, 6, 12, 24,

and 48 h with 10 seedlings per time period and three aphids per plant. After the transfer to the seedlings, a 24-h inoculation access period was given. The faba bean treatment was repeated seven times for the Elk Grove *A. fabae* colony and two times each for the Dixon *A. fabae* and Elk Grove *M. persicae* colonies. The petri dish treatment was repeated three times for the Elk Grove *A. fabae* colony only.

Acquisition access period. The acquisition access period needed for *A. fabae* to acquire sufficient BYV from infected plants to inoculate new plants was examined. For this, nonviruliferous aphids from both the Elk Grove and Dixon *A. fabae* colonies were placed on separate virus source leaves for 0, 0.17, 0.33, 1, 2, 4, 6, 12, 24, or 48 h. At the completion of each feeding period, two adult apterous aphids for each colony were transferred directly to each of 20 healthy sugar beet seedlings and allowed to feed for 24 h. The point of maximum transmission efficiency, which is defined as the highest average percent transmission that is statistically different from all previous averages (least significant difference [LSD] test). The entire test was replicated three times for each colony.

Inoculation access period. The relationship between duration of inoculation access by viruliferous aphids and BYV infection in sugar beets was tested. For this, aphids from the Elk Grove *A. fabae* colony were given a 24-h acquisition access period on virus source leaves, and then two aphids were transferred to each of 20 sugar beet seedlings for the indicated feeding times: 0, 0.17, 0.33, 1, 2, 6, 12, 24, or 48 h. At the end of the inoculation access period, aphids were removed manually or by spraying with 1 tbsp/gal (3.785 liters) of diazinon (Chevron Chemical Co., San Ramon, CA). Similar to the acquisition access period, the maximum transmission efficiency is determined for the inoculation access times. This experiment was repeated three times.

Electronic monitoring system (EMS). The EMS greatly facilitates the study of feeding and probing by piercing and sucking insects. It helps determine when an aphid's stylets reach the phloem tissue and when ingestion begins. This is done by detecting changes in electrical impedance between the aphid and the leaf substrate while the aphid is probing and feeding. The system developed by McLean and Kinsey (14) and modified by Kimsey and McLean (12) was used. A 15- μ m-diameter gold wire (Sigmund Cohn Corp., Mt. Vernon, NY) was attached to the dorsum of *A. fabae* with silver conducting paint. The opposite end of the wire was attached to an alligator clip that was wired to the EMS for recording. A voltage probe from the EMS was inserted into the soil of a potted BYV-infected source plant, providing a current to the plant leaves. Probing patterns and times were recorded for periods leading up to phloem ingestion. The general feeding activities of an individual aphid have been broken into four recognizable wave patterns by McLean and Kinsey (14): (i) S wave, salivation; (ii) X wave, when the stylets reach the sieve elements; (iii) Y wave, recorded just prior to sieve element ingestion; and (iv) I wave, sieve element ingestion. Recent findings (23) in the feeding activities during sieve element ingestion using the electrical penetration graph (EPG) technique (22) have shown that the above I wave pattern, labeled as ingestion, actually consists of both ingestion and salivation in the sieve elements. For this reason, we used the terminology suggested by Reese et al. (16) and labeled ingestion as phloem position.

Statistical analysis. All statistical analyses were performed using SAS version 6.03 (SAS Institute Inc., Cary, NC). Independent *t* tests were performed to detect differences in transmission efficiencies between aphid species and between *A. fabae* morphs. A LSD test was used to compare transmission efficiencies of clonal aphid colonies and determine the time to reach maximum transmission efficiency for inoculation and acquisition access periods. To detect differences among aphid colonies in retention time, the curve responses were analyzed using an analysis of variance (ANOVA) procedure.

Fig. 1. Retention time of beet yellows closterovirus by aphids from two clonal colonies of *Aphis fabae* and one colony of *Myzus persicae*, Sacramento Valley, CA, spring 1993. Percent transmission based on an average of seven replications for the Elk Grove colony and two replications for both the Dixon and *M. persicae* colonies. Each replication consisted of 20 plants per time with three aphids per plant.

RESULTS

Relative transmission efficiency. Apterous *M. persicae* were twice as efficient in transmitting BYV as apterous *A. fabae* (60 and 34%, respectively). Transmission efficiency by *M. persicae* ranged from 51 to 73% (three replications, one colony), while the range by *A. fabae*, averaged for each of the five colonies (Elk Grove 28%, Willows 30%, Davis 34%, Woodland 37%, and Dixon 40%), was 28 to 40% (three replications, five colonies). These differences between aphid species were statistically significant ($P < 0.01$; t test). In comparing the transmission efficiencies between each of the five clonal *A. fabae* colonies, no statistically significant differences were found based on a LSD test ($F = 0.52$; $df = 4$ and 10 ; $P = 0.72$). In testing different morphs of *A. fabae*, alates transmitted BYV at an average of 29% (range of 27 to 33%), while apterous aphids showed an average of 28% (range of 23 to 33%). No statistically significant differences were found ($P > 0.05$; t test). Out of 30 alate *M. persicae* tested, none transmitted BYV to the sugar beet seedlings.

Retention time. Aphids from both the Elk Grove and Dixon colonies of *A. fabae* that had fed on the nonvirus host faba beans showed a rapid decrease in virus retention during the first 3 h; efficiency decreased by greater than 50% (Fig. 1). The decrease was more gradual from 3 to 24 h. The retention threshold occurred at some point between 24 and 48 h. Based on a comparison of curve response using ANOVA, no statistically significant difference was detected between the Elk Grove and Dixon *A. fabae* colonies ($F = 0.24$, $df = 6$ and 49 ; $P = 0.96$). *M. persicae*, which was placed on radish, retained BYV at a significantly higher level for the first 24 h than did *A. fabae* from the Elk Grove colony ($F = 5.79$, $df = 4$ and 35 ; $P = 0.001$). BYV was transmitted by *M. persicae* at a low level after 24 h, but no transmission occurred after 48 h. *A. fabae* in petri dishes with cellophane (no feeding) showed a loss of about 80% of its transmission efficiency in the first 3 h, going from 52.5 to 10.3%. At 6 h, the transmission percentage decreased to 3.3% and no transmission occurred after 12 h.

Acquisition access period. No transmission was recorded after 0.17 h of access, and only low transmission levels resulted from 0.33 to 1 h (Fig. 2). A moderate transmission efficiency of 20 and 28% for aphids from the Elk Grove and Dixon colonies, respec-

Fig. 2. Acquisition access period of beet yellows closterovirus by two clonal colonies of *Aphis fabae*, Sacramento Valley, CA, spring 1993. Percent transmission based on an average of three replications per time period per colony. Each replication consisted of 20 plants per time point with two aphids per plant.

tively, was obtained after 2 h of access. In determining the maximum transmission efficiency, there was no significant difference between 6, 12, and 24 h as determined by a LSD test. For this reason, we can only conclude that the maximum transmission efficiency occurred after 6 h of feeding. It was not possible to statistically determine the maximum efficiency point for aphids from the Dixon colony because of variation between replications, however, the curve indicated results similar to the Elk Grove colony.

Inoculation access period. An inoculation access time of 0.17 h resulted in no transmission of BYV by *A. fabae* to sugar beet seedlings. The inoculation threshold occurred at 0.33 h, but transmission efficiencies remained low up to 1 h (Fig. 3). An average transmission efficiency of 17% was reached after 2 h of inoculation access. Because of the large variability within the 6-, 12-, and 24-h access times, the maximum transmission efficiency was statistically reached after 6 h of feeding (LSD test).

EMS. A total of 27 wave patterns (S, X, Y, and I) were recorded. The mean time for *A. fabae* to reach the sieve elements was 20.5 ± 1.5 h ($\pm$ standard error).

DISCUSSION

Most current information on transmission characteristics of BYV is derived from research that has been performed with *M. persicae*, because it is generally considered the most important vector. Other than studies on transmission efficiency, no work has been conducted on the transmission characteristics of BYV by *A. fabae* in the United States. Watson (24) performed a study in England, which is the only one to date having information on the acquisition and inoculation times of BYV by *A. fabae*. Our results showed that *A. fabae* requires amounts of time similar to *M. persicae* for BYV acquisition and inoculation. We recorded initial BYV transmission at 0.33 h, moderate transmission efficiency levels after 1 to 2 h, and maximum transmission efficiency following 6 h for both acquisition and inoculation. Since it was not the nature of Watson's 1946 experiment (24) to look at the transmission characteristics of BYV by *A. fabae*, few time periods were tested and we cannot make a complete comparison with her work. Watson's results showed a minimum transmission efficiency after 1 h of acquisition and a moderate transmission effi-

Fig. 3. Inoculation access period of beet yellows closterovirus by *Aphis fabae*, Elk Grove, Sacramento Valley, CA, spring 1993. Percent transmission based on an average of three replicates. Each replication consisted of 20 plants per time point with two aphids per plant.

ciency after 2 h of inoculation, but no other times were tested between 2 and 24 h. Three previous studies on *M. persicae* have shown maximum acquisition of BYV after 6 h (2), 12 h (20), and between 10 to 20 h (24). For the inoculation access period, maximum efficiency by *M. persicae* was reported after 6 h of access (2,20,24). These rates of acquisition and inoculation directly affect the rate of spread of BYV within a field. Based only on these two parameters, similar times would indicate equal potential for BYV spread by *A. fabae* and *M. persicae*.

Comparison of retention times of BYV for both aphid species that were given access to nonvirus host plants showed some distinct differences. Under feeding conditions, our study recorded minimal transmission for both *A. fabae* and *M. persicae* colonies at 24 h and no transmission at 48 h. Previous studies with *M. persicae* and *A. fabae* reported retention of up to 72 h (2,20,24) and 48 h (24), respectively. In this study, greater differences were seen in the rate at which retention decreased. The ability of *A. fabae* from both the Elk Grove and Dixon colonies to transmit BYV decreased by 50% in 3 h, while the *M. persicae* colony decreased 50% between 12 and 24 h. In the starvation treatment (*A. fabae* in petri dishes), we found an even greater decrease in retention. The transmission efficiency of *A. fabae* decreased by 80% after being in the petri dish for 3 h. The total retention time of BYV by the starved aphids was also considerably lower than those that were allowed to feed. Aphids in the petri dishes did not transmit BYV at 12 h, while aphids on the faba bean plants transmitted at 24 h. Other studies supporting these findings include those by Watson (24), who found retention to decrease more rapidly when *M. persicae* individuals fasted than when they fed. It took between 10 to 20 h of feeding for *M. persicae* to decrease by 50%, while it took only 5 h when *M. persicae* fasted. Sylvester (20) found that, when *M. persicae* fasted, its retention dropped 50% in 2.55 h (half-life), but, under feeding conditions, there was an 8-h half-life. Sylvester (21) conducted a follow-up experiment and concluded that fasting aphids maintained their inoculativity over 12 h at a level equal or superior to those having access to a nonvirus host plant. If this lower retention of BYV by starved aphids does occur in the field, then these results more accurately depict what is happening to a migrating aphid. A decrease in retention would mean a reduction in the distance aphids could migrate and remain viruliferous.

The transmission efficiencies of the five clonal colonies of *A. fabae* were relatively high at 28 to 40% (average 34%) when compared with the previous work by Bennett (2), showing only a 13% average transmission (6 to 33% range), and Heathcote and Cockbain (8), reporting 10% transmission by apterous *A. fabae*. This agrees with the approximate range of efficiencies, 14 to 63% (33% average), recently reported by Kirk et al. (13) for *A. fabae* from different regions of California. While *M. persicae* appears to be at least twice as efficient as *A. fabae* (60 versus 34%, respectively) in transmitting BYV, these numbers suggest that, during high flight densities, *A. fabae* would be efficient enough to contribute to virus spread.

The EMS provided detailed information for the feeding times needed for *A. fabae* to reach the sieve elements of a current variety of sugar beets. Since BYV is concentrated in the phloem (6), it is important to determine the time between stylet penetration of the leaf surface and initial ingestion from the phloem sieve elements to more accurately estimate acquisition time. It is not possible to determine this by visual observation, because it is not known when the aphids stylets reach the sieve elements and begin ingestion. Our results showed that, out of 27 aphids tested, an average of 20.5 min was required to reach the sieve elements or phloem position. Hennig (9) reported 30 to 60 min, on average, for *A. fabae* to reach the phloem of *V. faba* using histological techniques of leaf sectioning. Chang (3), using sugar beet varieties susceptible to BYV, found a mean time of 14.12 min (range of 4.92 to 56.83 min) for *M. persicae* to reach the sieve elements. Our low percent transmission obtained for acquisition times at 20 min and

less through visual observation may be explained by the sieve elements not generally being reached during that time period.

Previous studies have compared transmission of BYV with alate and apterous aphids in the laboratory (4,8). Because of behavioral variation upon forming wings, different methods have been described for handling different species. In our study, alate *A. fabae* transmitted BYV as efficiently as apterous aphids of the same colony (29 and 28%, respectively), while alate *M. persicae* showed no BYV transmission. The majority of alate *M. persicae* were observed walking around and many died after 24 h on the virus source leaves, while *A. fabae* appeared to readily settle down and start feeding. Swenson (19) suggested that there might be a temporary inhibition of feeding until some species have flown. He also noted that the inhibition is not always absolute. In the case of *M. persicae* and *A. fabae* reared on plants infected with the nonpersistently transmitted BtMV, 16% of *M. persicae* transmitted BtMV after they migrated, but no *A. fabae* transmitted BtMV (4). Cockbain and Heathcote (4) associated this difference with a difference in probing behavior at adult maturation rather than with an intrinsic ability of the two species to transmit BtMV. On the other hand, there is evidence that some winged *A. fabae* do not fly at all, but feed and reproduce on the plants on which they developed (19). This information, in addition to our observations of *M. persicae* and *A. fabae* behavior, indicates that there probably were differences between the two aphid species in preflight feeding behavior that effected BYV transmission. It is important to understand these issues when studying differences in transmission efficiency between alate and apterous aphids. Alate aphids not only have the ability to transmit viruses efficiently, but also carry them long distances.

This study addressed a current need for information on the transmission characteristics of BYV by *A. fabae*. This information will allow us to better compare the vector status of *A. fabae* with that of *M. persicae*. It is important to integrate these results into a pest management program that addresses multiple factors of BYV spread, such as vector population size, behavior, and periods of peak flights. These parameters help determine the vector status of an aphid species. The recent high flight densities of *A. fabae* suggest that *A. fabae* may be contributing to the current breakdown in BYV control. Our data on transmission characteristics further implicate *A. fabae* as an important vector of BYV. Given our current lack of field information on the spread of BYV in sugar beets by *A. fabae*, our situation in California opens opportunities to study whether other factors, such as flight times or behavioral activities, are contributing to the increase in incidence of BYV. If *A. fabae* is a major vector, as our results suggest, it may be contributing to the spread of BYV in ways we are not yet aware.

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