Factors Influencing the Outcome of Barley Yellow Streak Mosaic Virus-Brown Wheat Mite-Barley Interactions

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ABSTRACT

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Barley yellow streak mosaic virus (BaYSMV), the cause of disease in barley, is transmitted in nature only by the brown wheat mite (*Petrobia latens*). Greenhouse and growth chamber experiments were conducted to gain insight into what underlies the reported association between severe BaYSMV-induced disease outbreaks and large mite populations with warm and dry conditions. Experiments were also done to determine the efficiency of the mite as a vector for BaYSMV. The presence of BaYSMV antigen in diseased plants and viruliferous mites was confirmed by enzyme-linked immunosorbent assay (ELISA). Evidence was found for a critical temperature threshold between 21 and 26°C for efficient expression of BaYSMV-induced symptoms in barley. The influence of barley host plants stressed by drought on disease incidence was neutral, positive, and negative at 21, 26, and 30°C, respectively. Applying water periodically to mite egg deposition substrates on the soil surface appeared to reduce the number of eggs deposited. Mite counts were generally higher on BaYSMV-infected barley than on healthy barley. Preadult nonviruliferous mites readily acquired BaYSMV from infected host plants. Adult mites efficiently inoculated the virus into barley plants, and preadults were also able to inoculate the virus. Mite populations were able to expand at temperatures too low to support all but very low incidences of BaYSMV-induced disease in barley. There was also indirect evidence for transovarial passage of BaYSMV to red nondiapausal eggs within the mite vector.

Barley yellow streak mosaic virus (BaYSMV), the causal agent of barley yellow streak mosaic (BaYSM), was discovered in north central Montana in 1982 (29). Particles of BaYSMV are extremely large for a plant virus (64 × 1,000 nm on average), appear to be enveloped, and contain ssRNA (30,31). The virus has not been placed in a currently existing plant virus group or family and, in fact, resembles several animal and insect viruses (8). Barley (*Hordeum vulgare* L.) is the primary host, but wheat (*Triticum aestivum* L.) and several wild annual grasses can also be infected (28). As far as is known, BaYSMV can be transmitted from one plant to another in nature only by feeding activities of the brown wheat mite (*Petrobia latens* (Müller)) (29). This mite is a non-web-spinning spider mite in the family *Tetranychidae* that is generally believed to reproduce parthenogenetically (11,14,21). Arthropods with the feeding style of the brown wheat mite are generally not thought to be well-suited to vector plant viruses (36) and, to date, the brown wheat mite appears to be the only exception to this generalization (24).

The mite deposits eggs on debris in surface soil layers near its host plant (14). Red nondiapausal eggs predominate during the growing season (5,7). Toward the end of the growing season, white diapausal eggs, the stage used by the mite for overwintering, are laid (5,7,20). The brown wheat mite has a broad host range that includes both monocots and dicots (14). Although the mite and barley are distributed worldwide, BaYSMV has been identified to date only in Montana, Idaho, and Alberta, Canada. Previous work on this unusual vector–pathogen system sought to identify and partially characterize the virus, determine the means of virus transmission, and verify the ability of the mite vector to acquire and then inoculate BaYSMV (29,30,31). Initial BaYSMV plant host range data have been obtained (28), and evidence for the transovarial passage of the virus to white diapausal mite eggs has also been reported (29). With the development of an effective BaYSMV purification procedure, it became possible to produce anti-BaYSMV polyclonal antibodies, making possible enzyme-linked immunosorbent assay (ELISA) and other antibody-based detection tests (33). Field observations have associated severe BaYSM disease outbreaks and large brown wheat mite vector populations with warm and dry conditions, particularly in fields where barley has been planted over several successive growing seasons (28). However, the combination of (i) the complexity of interactions among BaYSMV, its mite vector, and the mutual host plant, (ii) the possibility of genetic variability within virus and mite populations, and (iii) continuous environmental fluctuations creates severe difficulties in understanding what drives the development of BaYSM disease in nature. Consequently, many factors suspected of being important in influencing BaYSM incidence and severity have never been studied.

By minimizing variability in components of the system in greenhouse and growth chamber experiments, we sought to gain insight into two key areas. The first concerned the nature of the reported association between severe BaYSM outbreaks and large mite vector populations with warm and dry conditions. The influence of temperature and host plant water status on BaYSM incidence in two genetically dissimilar barley cultivars was examined. In addition, brown wheat mite red nondiapausal egg deposition efficiency was studied on continuously dry versus periodically dampened egg-deposition substrates. Finally, experiments were conducted to compare mite counts over time on healthy versus BaYSMV-infected barley plants. The second area of investigation concerned the efficiency of the brown wheat mite as a vector for BaYSMV. Experiments were conducted to learn how readily the mite transmits the virus and to determine which mite life cycle stages can acquire and transmit BaYSMV. We determined the different effects that cooler temperatures have on BaYSM development and on mite population expansion, and discuss some possible implications of our findings on the likelihood of virus transmission by the mite in nature.

MATERIALS AND METHODS

The barley host. Except where noted, Alpine barley (*H. vulgare* cv. Alpine, CI 9578) (38) served as the host plant. Potted seedlings, grown in Sunshine Mix (Fisons,

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Vancouver, Canada), were bottom-watered by adding a dilute fertilizer (General Purpose 20-20-20, Grace-Sierra, Milpitas, CA) solution (0.6 g of fertilizer per 3.77 liters of water). Barley seedlings were at the one-leaf stage when infested with mites.

Greenhouse and growth chamber conditions. Mite colony maintenance and certain experiments were accomplished in a greenhouse (Montana State University-Bozeman Plant Growth Center) having a target temperature of 26.7°C and with overhead lights (1,000 W metal halide lamps; approximately 50 $\mu E \cdot s^{-1} \cdot m^{-2}$) on between 6 P.M. and 11 A.M. to maximize mite activity. Some experiments were conducted in growth chambers (models E15 and PGR15, Conviron, Winnipeg, Canada) with temperatures set as described below and with lights (cool-white fluorescent tubes and 60 W incandescent bulbs) stepped on at 5, 6, and 7 A.M. (450 $\mu E \cdot s^{-1} \cdot m^{-2}$) and stepped off at 9, 10, and 11 P.M.

BaYSMV and mite sources and mite confinement. To accomplish the research objectives, it was necessary to develop new methods of mite confinement and manipulation (34). Separate nonviruliferous and viruliferous mite colonies were established and maintained. Mites in both colonies were descendants (presumably parthenogenetically derived) of a single nonviruliferous mite selected from a colony of mites originating from Valier, Montana. The BaYSMV isolate originated from a single viruliferous mite also selected from the Valier colony. This viruliferous mite and its progeny were permitted to feed on and infect barley seedlings but were then eliminated. A group of descendants of the initial nonviruliferous progenitor mite acquired BaYSMV by feeding on the infected seedlings, and these mites founded the viruliferous colony used in subsequent experiments. Mite colonies were maintained in the greenhouse on potted barley within specially designed 20.3-cm-diameter × 50.8-cm-tall cylindrical acrylic cages (Interstate Plastics, Boise, ID) with mesh (Stencron imitation silk polyester, Nazdar/KC Western Supply, Garden Grove, CA) windows. For many experiments, the plastic tube method of mite confinement was used. An 8.3-cm-diameter × 30.5-cm-tall acrylic tube (Interstate Plastics, Boise, ID) with four 3.8-cm-diameter mesh windows and mesh top was placed over a barley seedling, after which a 2.5-cm-thick layer of damp Sunshine Mix was packed outside the base of the plastic tube slightly overfilling the pot. In some experiments, containment was achieved using the resealable bag method. A potted barley seedling was sealed in a disposable resealable bag cage made from a 30.5 × 50.8 cm, 4-mil resealable bag (Consolidated Plastics, Twinsburg, OH) having two 20.0 × 20.0 cm mesh windows. To produce virus-inoculated but mite-free plants, the plastic exclusion method was used. A small hole was cut in the center of a 22.9 × 22.9 cm white plastic square cut from a garbage can liner. The square was lowered over a seedling. The opening in the square around the base of the seedling was sealed with putty. A 0.5-cm-deep layer of dry Sunshine Mix was placed over the square around the base of the plant. Further mite confinement was then achieved by either the plastic tube method or the resealable bag method. After permitting mites access to feed on the barley seedling, it was possible to remove completely not only the initially infesting mites but also any eggs laid by the mites, which were deposited within the layer of Sunshine Mix placed on top of the plastic square.

Table 1. Summary of number and percentage of plants becoming symptomatic in growth chamber experiments designed to examine the influence of temperature and host plant water status on barley yellow streak mosaic incidence in the barley cultivars Alpine and Harrington

Temperature[x]	Cultivar[y]	Water status[z]	Symptomatic plants/ total plants tested	Symptomatic (%)
21	A	W	5/44	11.4
21	A	D	5/44	11.4
21	H	W	7/49	14.3
21	H	D	7/46	15.2
26	A	W	29/42	69.0
26	A	D	36/42	85.7
26	H	W	23/43	53.5
26	H	D	30/43	69.8
30	A	W	24/44	54.5
30	A	D	16/44	36.4
30	H	W	30/44	68.2
30	H	D	16/44	36.4

[x] Temperature in °C.
[y] A = Alpine; H = Harrington.
[z] Host plant water status (W = normal-watered; D = drought-stressed).

Table 2. Raw mean proportions and fitted mean proportions of plants becoming symptomatic in growth chamber experiments designed to examine the influence of temperature and host plant water status on barley yellow streak mosaic (BaYSM) incidence in the barley cultivars Alpine and Harrington[u]

Temperature[v]	Water status[w]	Raw data mean proportions[x]	Fitted mean proportions[y]
21	W	0.1290	0.0610 a[z]
21	D	0.1330	0.0685 a
26	W	0.6118	0.6014 b
26	D	0.7765	0.7656 c
30	W	0.6136	0.5922 bc
30	D	0.3636	0.3422 d

[u] Data for Alpine and Harrington have been combined for each temperature–host plant water status combination because analysis of variance indicated no significant differences in BaYSM incidence between the two cultivars for any of the experimental conditions examined.
[v] Temperature in °C.
[w] Host plant water status (W = normal-watered; D = drought-stressed).
[x] Proportions of plants becoming symptomatic based on actual experimental data.
[y] Proportions of plants becoming symptomatic adjusted by the general linear model to a mean level of mite infectivity across the experimental duration.
[z] Numbers followed by the same letter are not significantly different at $P = 0.05$ based on a two-sided test accomplished by the MSUSTAT general linear model procedure, with the exception of the 26°C normal-watered vs. drought-stressed comparison. The P value for that comparison (0.0287) is half the value provided by the general linear model (0.0573) because the research hypothesis was one-sided: at 26°C, drought-stressed plants will become diseased at higher incidence than normal-watered plants.

Virus detection. The presence of BaYSMV antigen in barley plants and mites was determined by double antibody sandwich (DAS)-ELISA using purified rabbit-polyclonal anti-BaYSMV antibodies. ELISA procedures were as described by Skaf (33), with the following modifications. Four replicates per sample were tested in Immulon I polystyrene microtiter wells (Dynatech, Chantilly, VA). Plant tissue was prepared by grinding in general extraction buffer, pH 7.4, (2) at a ratio of 0.1 g of tissue (wet weight) per 1.0 ml of buffer. Between all ELISA steps, five washings with phosphate-buffered saline with Tween 20 (PBS-T), pH 7.4, (2) were done. Wells were read at 410 nm using a Minireader II spectrophotometer (Dynatech, Alexandria, VA) 30 min after adding the substrate for alkaline phosphatase.

Influence of temperature and host plant water status on BaYSM incidence. Experiments were conducted in growth chambers to examine BaYSM incidence over a 25-day observation period in the barley cultivars Alpine (38) and Harrington (10) at 21, 26, and 30°C with both normal-watered and drought-stressed plants

at each temperature. Each of the 12 treatment combinations was repeated twice (except three times for 21°C-grown Harrington plants), totaling approximately 44 plants for each treatment combination. Each seedling was infested at the one-leaf stage with four adult mites from the viruliferous colony. The plastic tube method was used for mite confinement. Normal-watered plants were initiated by supplying 600 ml of water at the time of seed planting and 600 to 900 ml, depending on temperature, in periodic 75-ml bottom-supplied increments over the 25-day post-mite-infestation observation period. Drought-stressed plants were initiated by supplying 400 ml of water at the time seeds were planted and 150 to 225 ml in periodic 75-ml bottom-supplied increments over the 25-day observation period. In normal-watered plants, no wilting ever occurred and growth was vigorous throughout the experimental period. In drought-stressed plants, temporary wilting occurred by the time a 75-ml increment of water was added, and growth was slow in comparison to normal-watered plants (about 50% the number of leaves of normal-watered plants by the end of the experimental period, and leaves were narrower and shorter). Daily observations of symptom appearance and plant growth stage were made. ELISAs were done on examples of plants failing to develop BaYSM symptoms and on plants becoming symptomatic. To define the sensitivity of the test, ELISAs were done on dilutions of symptomatic tissue preparations and on groups of three or four adult mites from the viruliferous colony crushed on 0.2 g of healthy barley leaf tissue, which was then ground in 2 ml of general extraction buffer. Differences in proportions of plants becoming symptomatic under the 12 treatment combinations were analyzed using the general linear model procedure of MSUSTAT (22) to accommodate a nonbalanced blocking variable (mite infectivity over time) and the slight imbalance associated with use of three trials for 21°C-grown Harrington plants and two trials for the others.

Influence of continuously dry versus periodically dampened egg-deposition substrates on mite egg deposition. For each barley plant in each of five repeats over time, a 1.3-cm-deep layer of 50-mil fine white silica sand (Unimin, Emmett, ID) was placed on top of the Sunshine Mix around the base of the potted seedling. Ten white perlite granules, each about 0.25 cm in diameter, from Sunshine Mix were then placed in an area approximately 2.5 cm in diameter on the sand next to the base of each seedling. For half the plants (group I), the granules and underlying sand were initially dampened with 13 ml of tap water. For the other half of the plants (group II), no dampening was done at any time during the experimental period. Each plant was then infested with six adult mites (from the viruliferous colony in some replicates and from the nonviruliferous colony in others). Mites very much prefer perlite granules over fine sand for egg deposition. The perlite granules, therefore, served as egg-deposition "traps." Containment was achieved using the plastic tube method but omitting the layer of damp Sunshine Mix packing. Infested seedlings were maintained at 21°C in a growth chamber. The granules and underlying sand of group I plants were dampened daily with 3 ml of tap water. After 6 days of mite presence, red eggs on white granules from group I versus group II plants were counted using a dissecting microscope, and the numbers were compared statistically using a two-sample t test accomplished by the t paired procedure of MSUSTAT (22).

Mite counts on healthy versus BaYSMV-infected barley. For each of eight repeats over time of an experiment designed to compare mite counts on healthy versus BaYSMV-infected barley, 10 potted Alpine barley seedlings were each infested with four adult mites from the nonviruliferous colony, and 10 potted seedlings were each infested with four adult mites from the viruliferous colony. Containment was achieved by the plastic tube method, and plants were maintained in the greenhouse. Within a given repeat, all plants were watered equally from the bottom. Timing and amount of watering were designed to be sufficient to promote ongoing plant growth but light enough to keep surface soil layers dry. Beginning at about day 14 after mite infestation, mites on each plant were counted daily for approximately 21 days. Counting was done by raising the containment tube about 2.5 cm and then dislodging mites onto a 6.4-cm-diameter white paper disk surrounding the base of the plant by tapping the plant 30 times with a 25-cm segment of coat hanger, and also by tapping the containment tube eight times by hand. After mites on an individual plant were counted, the paper disk was leaned against the plant, permitting counted mites to move back onto the plant or into the soil layer near the plant, and the tube was replaced over the plant. Therefore, it was possible that individual mites could be counted more than once during the 21-day counting period. The 10 plants infested with mites from the nonviruliferous colony were counted consecutively, as were the 10 plants infested with mites from the viruliferous colony. The group counted first was alternated each day. To minimize potential bias from slightly differing light and temperature conditions in different locations in the greenhouse, each of the eight repeats was conducted in a slightly different location in the greenhouse, and the spatial arrangement of trays on the bench top containing plants was varied. To take into account possible seasonal effects on mite counts, the eight repeats were conducted over approximately a 6-month period (May to October). Statistical comparisons of mite counts on healthy plants and BaYSMV-infected plants were done using the nonparametric sign test (25).

Red egg infestations. Four to 14 red eggs deposited on white perlite granules near the base of plants colonized by viruliferous mites were transferred to the base of each of 24 potted healthy barley seedlings. Containment was achieved using the resealable bag method, and plants were maintained in the greenhouse.

Inoculation of BaYSMV by viruliferous preadults. Five to 15 preadult mites from the viruliferous colony were confined on each of 12 potted healthy barley seedlings. Containment was achieved using the

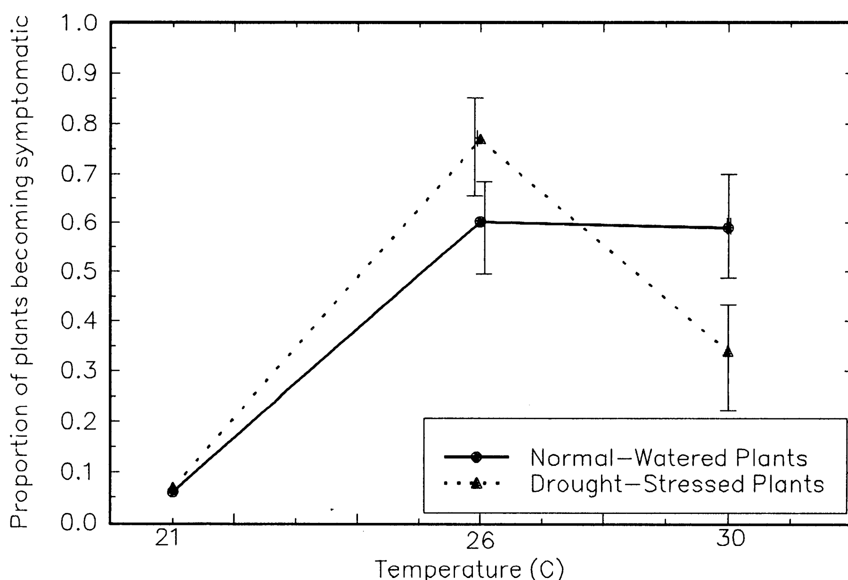

Fig. 1. The influence of temperature and host plant water status on barley yellow streak mosaic incidence. Means at a given temperature with nonoverlapping error bars differ significantly at $P < 0.05$ (LSD) for a two-sided test.

plastic exclusion method combined with the plastic tube method. After a 24- to 48-h inoculation access period, the containment tube was removed, all Sunshine Mix on top of the plastic square was removed, the plastic square and putty seal were removed, and the plant was fumigated with PT1200 (Resmethrin, Whitmire, St. Louis, MO) overnight. Containment was then achieved around each seedling using the resealable bag method, and plants were maintained in the greenhouse.

BaYSMV acquisition by nonviruliferous preadults. Nonviruliferous preadults were confined on BaYSMV-infected, mite-free barley plants for acquisition access periods of 24 to 48 h using the plastic exclusion method in combination with the plastic tube method (using steam-treated tubes). Mites were then tapped from the virus-infected host plant onto the layer of Sunshine Mix at the base of the plant. All Sunshine Mix on top of the plastic square was then placed at the base of a healthy potted barley seedling. Containment was achieved using the resealable bag method. Contained, infested seedlings were maintained in the greenhouse.

Nonviruliferous mite counts at 21 and 30°C. For each of three repeats over time, 20 Alpine barley seedlings were each infested with four adult mites from the nonviruliferous colony. Containment was achieved using the plastic tube method. Ten infested plants were maintained at 21°C and 10 at 30°C in growth chambers. Plants were bottom-watered with 100 ml of water 9, 17, 22, and 26 days after infestation, and mites were counted on each plant 11, 15, 21, 25, and 30 days after infestation, using a procedure similar to that described above for counting mites on healthy versus BaYSMV-infected plants.

RESULTS

Influence of temperature and host plant water status on BaYSM incidence. Data collected from growth chamber experiments intended to elucidate the influence of temperature (21, 26, and 30°C) and host plant water status (normal-watered versus drought-stressed) on BaYSM incidence in the barley cultivars Alpine and Harrington are summarized in Table 1. An analysis of variance showed no statistically significant differences in BaYSM incidence between the two cultivars for any temperature–host plant water status combination tested. However, significant differences in BaYSM incidence at different temperatures ($P < 0.0001$) and a significant temperature × water interaction ($P = 0.0089$) occurred (Table 2, results combined for the two cultivars).

At 21°C, BaYSM incidence was quite low and there was no distinguishable difference between normal-watered and drought-stressed plants. At 26°C, disease incidence was approximately 10-fold greater than at 21°C, and drought-stressed plants appeared to become diseased at a slightly higher frequency than did normal-watered plants (Fig. 1). The error bars in Figure 1 and P values provided by the general linear model are derived from two-sided tests. However, the research hypothesis for the 26°C normal-watered versus drought-stressed comparison, based on preliminary observations, was specifically one-sided: at 26°C, the incidence of BaYSM in drought-stressed plants will be greater than in normal-watered plants. Therefore, the P value for the 26°C normal-watered versus drought-stressed comparison (0.0281) is half that provided by the general linear model (0.0573). Normal-watered 30°C-grown plants became diseased at essentially the same frequency as did normal-watered 26°C-grown plants. However, drought-stressed 30°C-grown plants became diseased at about half the frequency of drought-stressed 26°C-grown plants ($P = 0.0014$).

Of 93 ELISA-tested plants that remained nonsymptomatic over the 25-day observation period even though initially infested with adult mites from the viruliferous colony, 84 generated ELISA signals indistinguishable from those of healthy barley controls (Table 3). The remaining nine generated higher signals, and five of the nine became visibly symptomatic 24 to 48 h after ELISA. The other four were not observed after the test. Symptomatic

Table 3. Summary of enzyme-linked immunosorbent assay (ELISA) results

Sample type[x]	Samples tested	Absorbance at 410 nm[y] Mean	Range
Healthy barley	29	0.07	0.03–0.11
Symptomatic barley	29	0.99	0.74–1.11
Dilutions of symptomatic barley[z]			
1/10 dilution	2	1.08	1.07–1.08
1/50 dilution	1	0.96	...
1/100 dilution	4	0.86	0.81–0.95
1/250 dilution	1	0.62	...
1/500 dilution	4	0.44	0.36–0.53
1/1,000 dilution	4	0.34	0.27–0.40
1/2,000 dilution	2	0.19	0.16–0.22
1/4,000 dilution	2	0.13	0.08–0.17
1/5,000 dilution	3	0.13	0.12–0.14
1/10,000 dilution	1	0.05	...
Nonsymptomatic plants from cultivar-temperature-host plant water status growth chamber experiments testing negative for barley yellow streak mosaic virus (BaYSMV)	84	0.08	0.04–0.18
Nonsymptomatic plants from cultivar-temperature-host plant water status growth chamber experiments testing positive for BaYSMV	9	0.50	0.20–0.95
Nonsymptomatic first leaves of otherwise symptomatic plants	6	0.80	0.26–1.03
Barley leaf from nonviruliferous mite colony host plant	2	0.07	0.05–0.10
Batches of three to four mites from the viruliferous colony	9	0.19	0.05–0.33
Batches of three to four mites from the nonviruliferous colony	2	0.06	0.04–0.07
Symptomatic barley resulting from red egg infestations	4	0.92	0.90–0.45
Symptomatic barley inoculated by nonviruliferous preadults that had acquired BaYSMV	4	0.99	0.95–1.10
Symptomatic barley inoculated by viruliferous colony preadults	1	0.98	...

[x] Unless otherwise noted, plants tested are barley.
[y] Readings taken at 30 min.
[z] Dilutions done in general extraction buffer.

Table 4. Red egg counts on periodically dampened versus dry perlite granules[x]

Repeat	Plants with dampened granules	Red egg count[y]	Plants with dry granules	Red egg count[z]
1	4	17	4	121
2	5	11	5	105
3	5	25	5	48
4	5	41	5	233
5	5	49	5	126
Totals	24	143	24	633

[x] All repeats done at 21°C.
[y] Red egg counts are for all plants with dampened granules combined within a given repeat.
[z] Red egg counts are for all plants with dry granules combined within a given repeat.

leaves from 21 plants with clear symptoms were tested with ELISA, and in every case maximum ELISA signals near or slightly above 1.00 were generated (versus average signals of 0.07 generated from healthy controls). ELISAs done on dilutions of the standard symptomatic barley tissue preparation indicated that dilutions down to 1/1,000 consistently generated elevated (average of about five times over healthy controls) ELISA signals. Detectable levels of BaYSMV antigen, as evidenced by ELISA signals of 3.4 to 6 times greater than healthy barley controls, were found in four of the nine batches of three to four mites from the viruliferous colony that were tested. In no instance did any of the negative controls generate elevated ELISA signals.

Influence of continuously dry versus periodically dampened egg-deposition substrates on mite egg deposition. Red egg counts from five repeats of an experiment designed to examine the influence of continuously dry versus periodically dampened egg-laying substrates on the number of red eggs laid by mites are summarized in Table 4. More than four times as many red eggs were deposited on continuously dry perlite granules than on periodically dampened granules. The difference in egg numbers deposited between the two groups is statistically significant at $P = 0.0231$.

Mite counts on healthy versus BaYSMV-infected barley. Mite counts on healthy versus BaYSMV-infected barley for each of the eight repeats are summarized in Table 5. In each repeat, mites were counted daily over the counting period; daily counts have been summed for week 1, week 2, week 3, and totaled for the 21-day counting period. For the eight repeats combined, 28,973 mites were counted on infected group plants (plants initially infested with mites from the viruliferous colony) and 20,577 mites were counted on healthy group plants (plants initially infested with mites from the nonviruliferous colony), giving a composite I/H ratio (mite counts for infected group plants/mite counts for healthy group plants) of 28,973/20,577 = 1.41/1. Approximately 75% of the 79 total infected group plants exhibited BaYSM symptoms by the end of the 21-day counting period. The timing of symptom appearance in plants infested with mites from the viruliferous colony was highly variable, ranging from 7 days after mite infestation at the earliest to approximately 30 days after infestation at the latest. Of 79 total healthy group plants, five showed symptoms by the end of the 21-day counting period, indicating the inadvertent transfer of a viruliferous mite, probably early in the counting period. The 21-day I/H ratio was greater than one for each of the eight repeats, indicating a statistically significant difference in mite counts between the infected group plants and healthy group plants with $P < 0.002$. The 21-day I/H ratio varied in individual repeats from 1.04/1 (repeat seven, in which three of the five inadvertent contaminations leading to symptomatic healthy group plants occurred) to 2.39/1 (repeat four). In repeats two, six, seven, and eight, week 1 mite counts for healthy group plants were greater than for infected group plants, while the reverse was true for the other four repeats. However, counts were greater for infected group plants than for healthy group plants in all eight repeats for week 2 and for seven of eight repeats for week 3 (the exception being repeat seven). The eight repeat average I/H ratio was lowest (1.24/1) for week 1, highest (1.58/1) for week 2, and intermediate (1.41/1) for week 3. Of 164 total counting days in all eight repeats combined, mite counts for infected group plants were greater than for healthy group plants on 129 days (79%). Counts for infected group plants were greater than for healthy group plants on 33 of 56 (59%) week 1 counting days, 53 of 56 (95%) week 2 days, and 43 of 52 (83%) week 3 days.

Efficiency of acquisition and inoculation of BaYSMV by the mite. Of 24 plants, each infested with four to 14 red eggs from the viruliferous colony, four became symptomatic. Approximately 75% of barley seedlings became BaYSMV-infected under conditions favorable for disease development (26°C with host plants drought-stressed) when infested at the one-leaf stage with four adult mites from the viruliferous colony. In greenhouse trials, some infestations with only one adult mite resulted in symptomatic barley seedlings, sometimes with inoculation access periods as brief as 20 to 120 min. Of 12 plants, each infested with five to 15 preadults from the viruliferous colony, one became symptomatic. In one experiment that tested the ability specifically of six-legged larvae to acquire BaYSMV leading eventually to viruliferous mites, acquisition occurred and the assay plant used as a mite host became symptomatic. Of the five experiments that tested the ability of preadults in general (without distinguishing between larvae

Table 5. Mite counts on healthy versus infected barley plants over a 21-day counting period beginning approximately 14 days after infestation of each plant with four adult mites[u]

Repeat	Group[v]	Week 1 counts[w]	Week 1 I/H[x]	Week 2 counts[w]	Week 2 I/H[x]	Week 3 counts[w,y]	Week 3 I/H[x]	Total counts[w]	Total I/H[x]
1	I	2,992	1.67	2,995	1.67	1,579	1.59	7,566	1.65
	H	1,792		1,795		990		4,577	
2	I	396	0.62	1,345	1.13	1,347	1.87	3,088	1.21
	H	639		1,188		720		2,547	
3	I	1,176	1.09	1,511	1.29	255	1.14	2,942	1.19
	H	1,077		1,174		224		2,475	
4	I	1,587	1.94	688	3.13	321	6.42	2,596	2.39
	H	816		220		50		1,086	
5	I	1,211	1.35	1,583	1.92	1,090	1.47	3,884	1.57
	H	900		826		743		2,469	
6	I	1,049	0.89	1,393	1.52	1,043	1.90	3,485	1.32
	H	1,174		915		550		2,639	
7	I	613	0.74	1,837	1.61	894	0.72	3,344	1.04
	H	824		1,140		1,248		3,212	
8	I	258	0.88	1,097	1.71	713	1.12	2,068	1.32
	H	293		640		639		1,572	
Total[z]	I	9,282	1.24	12,449	1.58	7,242	1.40	28,973	1.41
	H	7,515		7,898		5,164		20,577	

[u] Counts are for all 10 plants in a group (repeat 4 had only nine plants in each of the two groups).
[v] I = Infected group of plants (plants infested with mites from the viruliferous colony). H = Healthy group of plants (plants infested with mites from the nonviruliferous colony).
[w] Counts are for all 7 days combined within the week indicated.
[x] Quotient of number of mites counted on infected group plants/number of mites counted on healthy group plants for the period indicated.
[y] Only 3 days of counting during week 3 for repeat 3.
[z] Composite data from all eight repeats combined.

and nymphs) to acquire BaYSMV, successful acquisition occurred in three, as evidenced by the development of symptoms in assay plants. All ELISA-tested symptomatic plants showed positive reactions (Table 3).

Nonviruliferous mite counts at 21 and 30°C. At 30°C, mite populations did not increase; whereas at 21°C, mite populations increased substantially over the 30-day observation period (Table 6).

DISCUSSION

BaYSM incidences in the barley cultivars Alpine and Harrington did not differ significantly for any of the six temperature–host plant water status combinations imposed in spite of the substantial genetic differences between these two cultivars (10,38). Alpine was the laboratory host for BaYSMV and its mite vector in previous work (28,29,30,31), and Harrington is currently the most important malting barley cultivar in Montana. There appears to be an important temperature threshold for BaYSM expression between 21 and 26°C. At 21°C, little disease occurs but at 26°C, BaYSM occurs readily. The influence of the drought-stressing of barley host plants on BaYSM incidence was neutral, positive, and negative at 21, 26, and 30°C, respectively. Normal-watered 26°C-grown and 30°C-grown plants became diseased at similarly high levels. In contrast, the combination of drought stress and a temperature of 30°C led to a significantly reduced BaYSM incidence. A stress-induced downregulation of host cell protein synthesis capacity, which might inhibit virus replication and thus disease incidence, may have occurred (1,6,13,23,32). Under highly stressful conditions, virus-induced disease expression in susceptible plants may indicate indirectly the status of the host tissue's capacity to synthesize protein.

In some virus–plant interactions, a host plant may be tolerant of the presence and replication of the inoculated virus, sometimes expressing few or no symptoms and often suffering little in terms of growth and yield (24). However, in this system, our data strongly suggest that ELISA-detectable levels of BaYSMV antigen are generally absent when BaYSM symptoms are absent. Two specific exceptions to this generalization were identified. High ELISA signals were generated from nonsymptomatic leaves of plants in which symptoms first began to appear within 24 to 48 h after ELISA and from nonsymptomatic first leaves (the only leaves present at the time of mite infestation) of otherwise symptomatic plants.

In total, these findings do not support the notion that drought stress in the barley host is an important factor in promoting BaYSMV-induced disease under the experimental conditions imposed. In contrast to most arthropod vectors of plant viruses, the brown wheat mite is unusually dependent on the use of surface soil layers to complete its life cycle (5,14). Eggs are deposited on suitable substrates within surface layers of soil, and inactive chrysalids between each of the four active mite stages reside within that layer. This dependence suggests that the most important influence of dryness may be in promoting development and survival of the mite vector. Rainfall amounts influence brown wheat mite population sizes in the field (5), and this mite has long been considered primarily a dryland pest (7). Mite eggs and active stages appear capable of surviving the presence of water; in preliminary experiments, some red eggs remained viable even after 13 days of total submersion. The possibility that soil moisture may interfere with a critical aspect of mite behavior, egg-deposition, rather than with mite survival per se, was therefore investigated. We found that mites deposit significantly fewer eggs on periodically dampened substrates than on continuously dry substrates (143 versus 633 red eggs over 6 days). Edaphic and climatic characteristics favoring the maintenance of dry surface soil layers where preferred mite egg-deposition substrates are located may therefore tend to maximize mite egg-deposition, mite population expansion, and thus successful BaYSMV inoculation events. Such conditions may include intense sun, wind, lack of frequent natural precipitation, low relative humidity, soil with poor moisture retaining characteristics, and an open plant canopy. It may be significant that this list of characteristics very much describes the conditions that prevail in early to midseason barley crops of north central Montana, the region where BaYSM was first discovered and where disease outbreaks have tended to be most severe. It should be noted that many spider mites that do not use soil for egg deposition are also favored by hot, dry weather (14). Therefore, other factors in addition to maintenance of dry surface soil layers may be important in brown wheat mite population expansions in the field under dry conditions. Influences of virus-infected host plants on vectoring arthropods, first described by Kennedy (16,17), have been reported in other virus-vector systems, the most thoroughly studied being the barley yellow dwarf virus-aphid vector system (26). Our data suggest that mite counts became higher on BaYSMV-infected barley plants than on healthy plants. The composite infected plant group mite count/healthy plant group mite count ratio was approximately 1.4/1. It appears that the first week of counting still tended to reflect initial differences in reproductive capacity of the infesting mites, week 2 appeared to reflect maximum influences of host plant physiology on mite counts, and week 3 began to reflect a diminution of those influences. These findings may, in part, underlie the reported association of severe BaYSM outbreaks and large mite vector populations with warm and dry conditions. However, data collected in these eight repeats represent only a 21-day "snapshot," conducted under artificial conditions, of a phenomenon that occurs over more than 21 days in the field and in which migration, predation, and many other factors may influence the overall process. The results should, therefore, be viewed with these limitations in mind.

The time interval chosen for mite counting, starting approximately 14 days after mite infestation and then continuing for 21 consecutive days, was selected for several reasons. Mites used in infestations apparently varied in their reproductive capacities at the time of infestation, as evidenced by the fact that week 1 I/H ratios were greater than one in four of the repeats and less than one in the other four repeats. Waiting 14 days after infestation before beginning to count was intended to diminish the influence of initial reproductive capacities of infesting mites on mite counts obtained over the counting period. In addition, waiting 14 days before beginning to count allowed a significant proportion of plants infested with viruliferous mites to become BaYSMV-infected and thus to affect mites feeding on those

Table 6. Nonviruliferous mite counts on barley plants at 21 and 30°C[w]

		Ten plant mite counts[z]				
Temperature[x]	**Repeat[y]**	**11 days**	**15 days**	**21 days**	**25 days**	**30 days**
21	1	33	46	72	142	532
21	2	55	85	122	234	554
21	3	4	9	28	50	182
30	1	45	57	39	50	60
30	2	17	8	9	9	37
30	3	2	0	12	31	8

[w] Each plant was initially infested with four adult nonviruliferous mites.

[x] Temperature in °C.

[y] Repeat number; 21 and 30°C repeats bearing the same number were conducted simultaneously. During the interval in which repeat 3 was conducted, mite population expansion in general and in individual experiments was low. This appeared to be due, at least in part, to a high proportion of mites laying diapausal white eggs.

[z] Within each repeat, mite counts are for the day indicated after infestation for all 10 plants combined.

plants. Finally, to avoid unwanted microclimatic influences within containment tubes on mite population development or on mite behavior, it was necessary that counting be completed before host plants began to fill up the space within containment tubes (12). The counting period was thus limited to 21 days by the volume available within containment tubes for host plant growth.

Data have intentionally been described in terms of "mite counts" rather than "mite numbers" or "mite populations." At any one time, a portion of the population of mites infesting a plant was on the plant or on the inside walls of the containment tube, while the remainder of the population, that portion uncountable by this approach, was within surface layers of soil surrounding the plant. In addition, there are many possible explanations for the finding that mite counts tended to be higher on infected group plants than on healthy group plants (3,15,19). Mite populations may not have been larger on infected plants in spite of the higher counts. Rather, individual mites may have fed longer or more frequently, perhaps because of phagostimulants produced in BaYSMV-infected plants (35), or because nutrition is more difficult to obtain per unit feeding time on diseased plants (3), or because somewhat drier diseased plant tissue creates problems in maintaining water balance in feeding mites (3). Mites may have required more time to go through each active life cycle stage because of nutritional limitations imposed by diseased host plants. Systemic virus disease may inhibit plant defenses that would normally be deployed in response to mite feeding (but see 18). Therefore, mite numbers and/or life spans may not have been enhanced on plants sustaining a systemic virus infection relative to healthy plants, but rather they may have been decreased less because of virus disease–impaired antimite defenses. Alternatively, BaYSMV-infected plants may have provided enhanced nutrition, perhaps in the form of essential amino acids (37), leading to more rapid mite growth, greater numbers of individuals surviving to the egg-laying adult stage, enhanced reproductive output per individual either per unit time or because of an extended egg-laying lifetime, or some combination of these, culminating in accelerated expansion of mite populations. Jones (15) has very logically described, using vector diagrams, the possible outcomes of the effects of pathogen-infected host plants on arthropods feeding on those plants; and in seven out of 10 possible outcomes, a net positive effect of pathogen-infected host plants on feeding arthropods is not predicted to occur. Fritzsche and Thiele (9) reported opposite influences of virus-infected host plants on reproduction of a nonvector spider mite (*Tetranychus urticae* Koch) in two different host plant/virus–mite combinations. Castle and Berger (4) studied the impact of host plants infected with three different viruses on aphid performance (growth, reproduction, and survival) and found that host plants infected with viruses increasingly dependent on vector transmission had increasingly positive effects on vector performance. The influence of virus-infected host plants on phytophagous arthropods (including vectors of pathogens) may also vary with the stage of disease development in the plant (3) and may differ in arthropods having different feeding styles (3,19,27). Finally, it is possible that the influence of BaYSMV infection leading to greater mite counts on infected plants was actually an effect of the virus directly on the mite rather than a result of virus-induced disease changes in the host plant affecting the mite.

The brown wheat mite appears to be an efficient vector for BaYSMV. Acquisition of BaYSMV by nonviruliferous mites appears to occur readily, definitely by preadults and possibly by adults. Developing embryos apparently are able to acquire the virus while still within the body of the maternal mite prior to egg deposition, as evidenced by the production of diseased plants following infestation with red eggs. This is in agreement with previously reported indirect evidence for transovarial passage of BaYSMV to white diapausal eggs (29). Adult viruliferous mites efficiently inoculate barley plants with BaYSMV, and preadults also appear to be able to inoculate.

Data suggesting that mite populations expand vigorously at 21°C are consistent with the work of Cox and Lieberman (5). However, the finding that a temperature of 21°C is capable of supporting only very low incidences of BaYSM suggests an aspect of the BaYSMV–brown wheat mite relationship that may reduce the likelihood that the mite vectors BaYSMV in nature. When viruliferous mites were permitted to infest a cucurbit (a mite host but a virus nonhost), the expanding mite colony was apparently purged of BaYSMV over time, presumably because of lack of ongoing access to BaYSMV within the plant cells fed on, resulting eventually in a nonviruliferous colony (Brumfield, *unpublished data*). At temperatures too low to support virus-induced disease, barley becomes, in effect, a virus nonhost. Viruliferous mite populations that expand at temperatures too low to support virus-induced disease in barley may similarly become purged of BaYSMV over time, again because of lack of ongoing access to BaYSMV within the plant cells fed on.

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