Factors influencing the outcome of barley yellow streak mosaic virus-Petrobia latens-barley interactions
by Eric Daniel Smidansky

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology
Montana State University
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Abstract:
Barley yellow streak mosaic virus (BaYSMV), the cause of disease in barley, is transmitted in nature only by the brown wheat mite (Petrobia latens Muller). Many aspects of the ecology of the BaYSMV-brown wheat mite-barley host plant system have never been studied. Therefore, greenhouse and growth chamber experiments were conducted to test specific hypotheses that may help explain field reports associating severe BaYSMV-induced disease outbreaks and large mite populations with warm and dry conditions. In addition, experiments were undertaken to define the efficiency of the mite as a vector for BaYSMV. The presence of BaYSMV antigen in experimental plants and mites was confirmed by ELISA.

A critical temperature threshold for efficient BaYSMV-induced disease expression in barley appeared to exist between 21°C and 26°C. Temperatures at the lower end of that range supported only a very low incidence of disease while temperatures toward the upper end of the range supported disease at about a tenfold greater incidence. Disease incidence at 30°C was not greater than at 26°C, however. The influence of the drought-stressing of barley host plants on disease incidence was neutral, positive, or negative, depending upon ambient temperature. Periodic soil moisture restricted mite egg-laying activities. Dry conditions may, therefore, be more influential in favoring the biology of the vector rather than of disease expression itself. Mite counts tended to be higher on BaYSMV-infected barley plants than on healthy plants.

The mite appeared to be an efficient vector of BaYSMV. Indirect evidence for transovarial passage of BaYSMV within its mite vector was obtained, confirming earlier work. 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. This differential effect of temperature on virus-induced disease in the host plant and on the mite vector may permit the purging of BaYSMV from mite populations expanding at relatively low temperatures in the field.

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Plant Pathology

MONTANA STATE UNIVERSITY
Bozeman, Montana

January 1995

APPROVAL

of a thesis submitted by

Eric Daniel Smidansky

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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STATEMENT OF PERMISSION TO USE

ACKNOWLEDGEMENTS

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ABSTRACT

Barley yellow streak mosaic virus (BaYSMV), the cause of disease in barley, is transmitted in nature only by the brown wheat mite (Petrobia latens Muller). Many aspects of the ecology of the BaYSMV-brown wheat mite-barley host plant system have never been studied. Therefore, greenhouse and growth chamber experiments were conducted to test specific hypotheses that may help explain field reports associating severe BaYSMV-induced disease outbreaks and large mite populations with warm and dry conditions. In addition, experiments were undertaken to define the efficiency of the mite as a vector for BaYSMV. The presence of BaYSMV antigen in experimental plants and mites was confirmed by ELISA.

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INTRODUCTION

Barley yellow streak mosaic virus (BaYSMV) is a recently discovered plant virus transmitted in nature only by the brown wheat mite, <u>Petrobia</u> <u>latens</u> Muller (Acari: Tetranychidae) (35). The preferred agricultural host for the virus is barley (<u>Hordeum</u> <u>vulgare</u> L.) , however wheat (<u>Triticum aestivum</u> L.) has also been infected by BaYSMV (34). In addition, several wild annual grass species have been identified as hosts of the virus (34). BaYSMV cannot be transmitted to barley, its usual host, via mechanical inoculation, but it can be mechanically transmitted to <u>Nicotiana</u> <u>benthamiana</u> Domin, an exotic tobacco species susceptible to many viruses (37). BaYSMV has been partially characterized and has no apparent affinities with any other known plant viruses (29). Virus particles are very large, averaging about 64 nm in diameter and ranging in length from 127 nm to 4000 nm and they appear to be enveloped (36). Preliminary chemical analysis of BaYSMV-enriched preparations indicated the presence of proteins of 32 and 100 kDA and high molecular weight RNAs of 11-13 kb (37). Virus particles are most often seen within cavities of endoplasmic reticulum in cells of infected barley plants (36).

The brown wheat mite vector, a spider mite in the family Tetranychidae, has a cosmopolitan distribution, having been reported not only from North America but also from parts of Europe, northern Africa, Asia, and Australia (17). The mite goes

through a complex life cycle involving egg, larva, protochrysalis, protonymph, deutochrysalis, deutonymph, teleiochrysalis, and adult (7,8,10). It is not a web spinner and uses surface soil layers for egg laying and for quiescent stages of its life cycle (17). Two types of eggs are laid (7,10,24). During the portion of the year climatically favorable for both mite activity and host plant growth, nondiapausal red eggs are laid which readily hatch, generally within 6-10 days depending upon temperature (7). In anticipation of the end of the growing season and the onset of adverse climatic conditions, diapausal white eggs, the life stage utilized to survive until favorable conditions return, are predominantly laid (7,17). In a closely related mite species, the switch from laying nondiapausal to diapausal eggs was found to be mediated by multiple cues including nutritional availability, temperature, and photoperiod (20) but this switch has not been studied in the brown wheat mite. In extremely hot regions, active mite stages are present during cooler parts of the year and the mite diapauses as white eggs during the hot summer (8). In more temperate regions with cold winters, such as Montana, the mite is active from spring through about mid-summer and diapauses, again as white eggs, over the autumn and winter (8). The brown wheat mite appears to have a very broad host range, including both monocots (gramineous and nongramineous) and dicots (17). The details of the feeding style of the brown wheat mite, very important in understanding how the mite vectors BaYSMV, have never been reported. However, based upon work on other tetranychid mites, it is presumed that the brown wheat mite first punctures epidermal and mesophyll cells with a pair of sharp stylets and then sucks up the cell contents with

the help of a powerful pharyngeal pump (1,17,21). Viruses, by definition, can only replicate in cells having functional protein synthesis machinery (29). Therefore, arthropods that feed in this manner are not considered to have widespread potential as vectors of plant viruses because the cells fed on tend to be either significantly damaged or destroyed (43), although there are well-known exceptions to this generalization such as the thrips/tomato spotted wilt virus system (12). Recent findings that indicate that punctured plant cells have efficient, rapid sealing mechanisms may be of significance in understanding how this rather destructive feeding style may nevertheless permit the vectoring of some plant viruses (39). In contrast to the presumed feeding style of the brown wheat mite, many homopterous insects have evolved the unique ability to tap moving streams of phloem sap in phloem sieve tube members without incapacitating the cells they feed on (33). Insects with this feeding style comprise the majority of efficient arthropod vectors of plant viruses (29).

Field observations have associated severe outbreaks of barley yellow streak mosaic (BaYSM), the barley disease caused by BaYSMV infection, and large mite populations with warm temperatures and dry conditions (34). This association has been especially apparent in poorly-irrigated or unirrigated fields, particularly where barley had been planted over several successive years (recrop barley) (34). BaYSM was prevalent in northcentral Montana between 1982 and 1988, a period characterized by generally warm and dry growing season months (6). Since 1988, growing season months have been, on average, slightly wetter and cooler (6) and, perhaps related to

this, BaYSM has not been nearly as prevalent. Another feature of BaYSM is its very

limited known geographic distribution in spite of the extremely widespread

distribution of both the brown wheat mite vector and the barley host plant (34,41).

With these considerations in mind, the specific objectives of this research were to (1)

develop and test hypotheses that could help explain field observations associating

severe BaYSM outbreaks and large mite populations with warm and dry conditions

and (2) conduct experiments designed to help define the efficiency of the brown wheat

mite as a vector for BaYSMV.

MATERIALS AND METHODS

General Procedures

Mite Colony Establishment

A brown wheat mite that failed to bring about BaYSM when confined on a

barley seedling was obtained from the Valier colony of mites (originating from

Valier, Montana) maintained by S. Brumfield. Subsequent infestations of barley

seedlings over time by many generations of progeny from this mite (reproduction in

the brown wheat mite is generally believed to be parthenogenetic) (14,17, but see 26)

also failed to produce BaYSM in the barley host. This line of mites, stemming from

one individual, served as the foundation for a colony of nonviruliferous mites.

Subsequently, a group of mites from the newly established nonviruliferous colony was

confined on a BaYSMV-infected but mite-free barley plant (produced as described

below). The mites and/or their progeny that successfully acquired BaYSMV from the

infected host plant similarly served as the foundation for a colony of viruliferous

mites. Therefore, mites comprising both the nonviruliferous and viruliferous colonies

used in all experiments described in this thesis were derived from one initial

nonviruliferous mite selected from the Valier colony.

Mite Colony Cage Construction

Mite colonies were maintained over time on Alpine barley (Hordeum vulgare

L. cv. Alpine, CI 9578) contained within cages designed and constructed to (1) allow

convenient mite transfers into and out of cages, (2) allow rapid and contamination-free watering of host plants, (3) minimize the chances of unwanted mite movement into or out of cages, and (4) permit thorough cleaning of cages between uses. Cages with these specific qualities were necessary to permit the completion of experiments required to fulfill the research objectives. Colony cages were constructed starting with 20.3 cm diameter, 50.8 cm tall cast acrylic tubes having 0.3 cm thick walls (Interstate Plastics, Boise, ID) (see Figure 1). Two 15.2 cm by 40.6 cm rectangular openings were cut opposite from one another and equidistant from the top and bottom into the walls of each acrylic tube. Mesh (Stencron imitation silk polyester, Nazdar/KC Western Supply, Garden Grove, CA) windows were cut to fit the two 15.2 cm x 40.6 cm openings and also one circular end of each tube. A 35.6 cm straight metal zipper was sewn into one 15.2 cm x 40.6 cm mesh window. The three mesh windows were glued in place with thermoplastic glue and a gluegun. A 1.9 cm diameter hole was cut into one side of the acrylic tube 10.2 cm from the bottom and into this hole was inserted a 45.7 cm section of 1.9 cm diameter plastic tubing. The plastic tubing was glued in place so that 10.2 cm extended inside the cage pointing down and 35.6 cm extended outside the cage pointing up. Once constructed in this fashion, a cage was placed over a potted barley seedling resting in a plastic watering dish and setting on top of a 30.5 cm diameter plastic dinner plate. The bottom of the cage was secured to the plastic plate by gluing in 4 places and then sealing the joint circumferentially with spaghetti-shaped weather stripping putty. The portion of the plastic plate outside the cage and a 2.5 cm width of cage extending up from the plate were smeared with

vaseline to serve as an additional barrier to mite movement. The 10.2 cm section of plastic tubing inside the cage was directed into the plastic watering dish in which the pot rested. The portion of the plastic tubing outside the cage was glued in a vertical position to the outside of the cage. The opening of the plastic tubing outside the cage was plugged with a piece of putty. To water the plant serving as a host for the caged mite colony, the putty plug was removed and a funnel was inserted into the end of the plastic tubing. Mite colony cages were kept in a greenhouse of the plant growth center (conditions described below).

Mite Transfers

To transfer adult mites from one plant to another, a dampened artist's brush, 0.3 cm in diameter but with most bristles cut off, was used. Mites were harmlessly carried on the dampened bristles, to which they readily adhered, and were placed on a small square of butcher's paper placed at the base of the plant to be infested. Preadult mites were transferred by knocking them off a donor plant onto a white paper disk placed at the base of the plant. The disk carrying the mites was then placed up against a recipient plant.

Growth of Barley Seedlings for Experiments

To produce barley seedlings for experiments and for colony maintenance, seeds were planted in Sunshine Mix (Fisons Horticulture, Inc., Vancouver, B.C., Canada) in either 14 cm pots (plants used in experiments) or 15.2 cm pots (plants used for colony maintenance). All pots were placed in individual plastic watering dishes. To start seedlings, pots filled to within 2.5 cm of the top with Sunshine Mix

were first soaked from the top with 300 ml of water. Water draining out the bottom of the pots into the plastic dishes was eventually reabsorbed. Two barley seeds per pot were then planted in the Sunshine Mix at a depth of about 1.3 cm (when both seeds germinated, one was removed). After seed planting, an additional 100 ml of water (for most experiments) or 300 ml of water (for "watered" plants in the growth chamber experiments that examined the influence of temperature and host plant water status on disease incidence- see below) was then added, again from the top. The fertilizer/water mixture for all work reported in this thesis was 0.6 grams of plant food to 3.77 liters of tap water (Peters Professional Water Soluble Fertilizer, General Purpose 20-20-20, Grace-Sierra Horticultural Products, Milpitas, CA). All plants were started in a start-up growth chamber (Percival, model PGW-108, Boone, Iowa) at 24°C with lights on between 6 a.m. and 6 p.m.. Barley seedlings were infested with mites in experiments described below when in the late one-leaf/early two-leaf stage. Except where noted, all barley plants used in experiments were the Alpine winter barley cultivar (seeds obtained from T.W. Carroll and S. Brumfield, Montana State University Department of Plant Pathology, from a 1991 seed increase).

The Plastic Tube Method of Mite Confinement

For many experiments, mites were confined on test barley plants using 8.3 cm x 30.5 cm cast acrylic tubes with 0.3 cm wall thickness (Interstate Plastics, Boise, ID) and having four circular 3.8 cm diameter mesh windows and a mesh top (see Figure 2). After starting barley seedlings as described above, a 1.3 cm deep layer of dry Sunshine Mix was spread on the soil surface around the base of the plant to maximize

the dryness of soil conditions (which mites were thought to prefer). A plastic containment tube was then placed over the seedling. Finally, A 2.5 cm thick layer of damp Sunshine Mix was packed outside the base of the plastic tube, slightly overfilling the pot. This is the plastic tube method. Plants on which mites were confined using the plastic tube method were bottom-watered by dispensing a measured amount of water into the plastic dish in which each individual pot rested. The plastic tube method of containment was less suitable than the ziplock bag method (see below) when absolute avoidance of unwanted brown wheat mite contaminants was essential.

The Ziplock Bag Method of Containment

For certain experiments, mites were confined on or excluded from test plants by sealing a potted barley seedling in a ziplock bag cage (30.5 cm x 50.8 cm, 4 mil, Consolidated Plastics Company, Twinsburg, OH) (see Figure 3). This type of cage was constructed by cutting two 20.0 cm x 20.0 cm squares of plastic from opposite sides of the bag, two inches below the ziplock seal and centered side to side. Two slightly larger squares of mesh were then taped into place covering these cutout areas. The ziplock cages were supported in an upright position by taping a 40.6 cm long piece of 0.6 cm thick bamboo to the outside of each bag. This is the ziplock bag method. Disposable ziplock cages were used in those experiments where it was absolutely essential to avoid contamination by even a single brown wheat mite or mite egg but where frequent access to the host plant was not required. To water a plant confined in a disposable ziplock cage, the ziplock-caged plant was taken out of the greenhouse into the adjoining hallway, the ziplock seal was partially opened, and

water was added in such a way that it was absorbed through the holes at the bottom of the pot in which the plant was growing. A sprinkling can never in contact with mite-colonized plants was used for watering.

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Figures 1, 2 and 3. Cages used for mite confinement.
(All dimensions are in centimeters)

Figure 2.
Plastic tube method of mite confinement.

Figure 1. Mite colony cage.
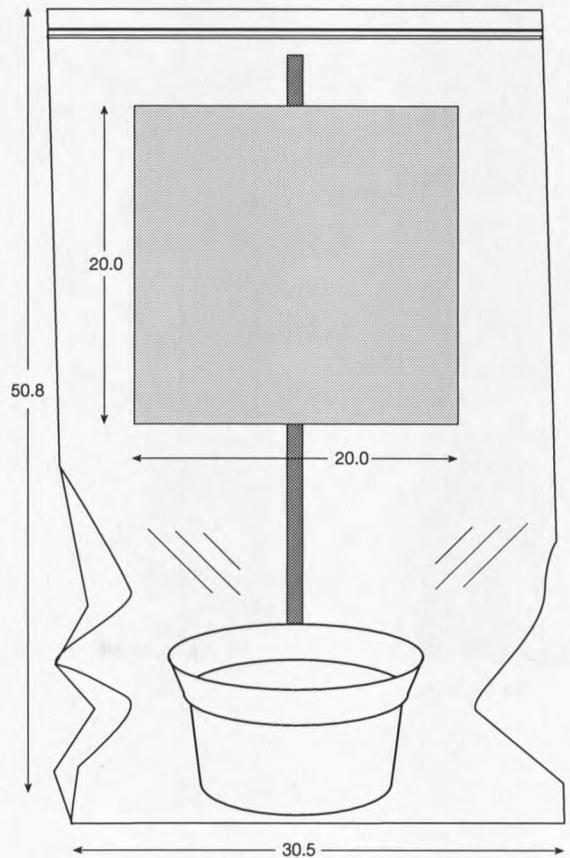
Figure 3. Ziplock bag method of containment.

The Plastic Exclusion Method

For certain purposes, such as the production of virus-infected but mite-free plants (see below), it was necessary to prevent mites used to infest a plant from gaining access to the soil at the base of the plant. To accomplish this, a 22.9 cm x 22.9 cm white plastic square was cut from a plastic garbage can liner. A small hole was made in the center of the plastic square and the square was then lowered down over the leaf of a one-leaf barley seedling until the plastic square rested at the base of the stem of the barley seedling on the surface of the Sunshine Mix. The opening in the plastic square around the base of the barley seedling was then sealed with two 2.5 cm pieces of weather stripping putty. Finally, a 0.5 cm layer of dry Sunshine Mix was placed over the plastic square around the base of the plant. This is the plastic exclusion method. When using the plastic exclusion method, mites were first allowed the opportunity to feed on plants for a specified period of time (to either inoculate or acquire BaYSMV, depending on the experiment). After this period of access to the plant, because mites were confined above the plastic square, it was possible to completely remove not only the initially transferred 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).

Production of BaYSMV-infected, Mite-free Barley Plants

To produce virus-infected but mite-free barley plants, used in acquisition studies (see below), the plastic exclusion method was utilized as previously described. The plastic tube method was then used for containment around the barley seedling and

on top of the plastic square. Four to eight adult mites from the viruliferous colony were transferred to the contained barley seedling. The mites were allowed access to feed on the seedling for two to three days. After that period of time, the containment tube was removed, all visible mites were removed from the plant, all Sunshine Mix on top of the plastic square was removed and discarded, and the putty seal and plastic square were then carefully removed from the base of the barley plant. The barley plant was then fumigated overnight using PT1200 (Resmethrin, Whitmire Corp., St. Louis, MO). After fumigation and ventilation, the inoculated but mite-free barley plant was contained using the ziplock bag method during the period of symptom development.

Greenhouse Conditions

All greenhouse and growth chamber experiments described in this thesis were conducted in the Plant Growth Center at Montana State University-Bozeman, Bozeman, MT. Greenhouse experiments were conducted in range GH2B. The desired temperature in the greenhouse for most experiments was 26.7°C; to attain this, the temperature setting in the greenhouse was varied from 24.4°C to 26.7°C, depending upon outside weather conditions. To minimize unwanted temperature increases resulting from sunlight, the overhead curtain was drawn at all times. In addition, the side windows of the greenhouse were whitewashed over the half of the room where the experimental material was located. Temperatures were monitored in the greenhouse using a drum thermograph (cat. no. 594, Belfort Instr. Co., Baltimore, MD). In spite of efforts to maintain constant temperatures, fluctuations from 20°C to

greater than 31°C occurred occasionally over time. Overhead lights were set to come

on at 6 p.m. and to go off at 11 a.m. All cages and pots were kept on 30.5 cm x 50.8

cm plastic trays set on rolling metal utility table tops. A barrier of vaseline about 2.5

cm wide was applied to the periphery of each plastic tray as a further hindrance to

unwanted mite movement.

Growth Chambers

Growth chamber experiments were conducted in three Conviron growth

chambers (Two model E15, one model PGR15, Conviron, Winnipeg, Manitoba,

Canada). Temperature and light settings were as described below for each

experiment.

ELISA Procedures

Direct double antibody sandwich-enzyme-linked immunosorbent assays

(ELISAs) were performed on material in a number of the experiments to determine

the presence or absence of BaYSMV antigen. ELISAs were performed using purified

rabbit polyclonal anti-BaYSMV antibodies provided courtesy of Dr. J. Skaf.

Antibodies were the IgG fraction of anti-BaYSMV serum cross-absorbed with a

concentrated host protein preparation of Nicotiana benthamiana. Procedures for

ELISA were as described by Skaf (41) with the following exceptions. Immulon I

polystyrene plates (Dynatech Corp., Chantilly, VA) were used to provide a solid

phase substrate for the binding of coating antibodies. Plant tissue was prepared by

grinding in a general extraction buffer pH 7.4 (described in 31) at a ratio of 0.1 g

tissue/1.0 ml buffer. Mites to be tested were prepared by crushing them on 0.2 g of

healthy barley leaf tissue which was then ground in 2.0 ml of general extraction buffer. Between all steps, five washings with PBS-T pH 7.4 (31) were done using a Miniwash (Dynatech Corp., Alexandria, VA). Four microtiter wells were used for each sample tested. Wells were read at a wavelength of 410 nm using a Minireader II spectrophotometer (Dynatech Corp., Alexandria, VA) at 15 minutes and at 30 minutes after adding the substrate (Sigma 104 phosphatase substrate, Sigma Chemical Co., St. Louis, MO) for alkaline phosphatase.

Specific Experiments

I. Association of severe BaYSM outbreaks and large mite populations with warm and dry conditions

The influence of air temperature and host plant water status on BaYSM incidence in barley

Preliminary experiments conducted in the greenhouse, where ambient air temperature can be varied but not with precision or constancy, suggested that BaYSM incidence in Alpine barley increased dramatically as the temperature was increased from approximately 20°C to 27°C and also that disease incidence was somewhat higher in droughted plants than in well-watered plants, although this latter effect was not as strong and was more difficult to interpret. In addition, field observations (34) associated severe disease with warm and dry conditions. Based on these considerations, two specific hypotheses were developed; (1) as temperature increases, disease incidence increases and (2) disease incidence in droughted plants is greater than in well-watered plants. To test these hypotheses, growth chamber experiments

were undertaken which involved twelve treatment combinations (two barley cultivars and three temperature levels with both well-watered and droughted plants at each temperature). The two barley cultivars chosen were Alpine and Harrington (Hordeum vulgare L. cv. Harrington). Alpine is an older cultivar of six row facultative winter barley not currently of any commercial importance (45). However, Alpine barley has been used as the greenhouse host plant for BaYSMV because it is highly susceptible to the virus, it is very acceptable as a host for the mite vector of BaYSMV, and because it does not generally enter the reproductive phase (which would cause it to outgrow the mite containment cages) when grown under nonvernalizing conditions. In contrast, Harrington is a much more recently released two row spring barley cultivar (13) that is currently of widespread commercial importance, both for malting and nonmalting purposes, in Montana and surrounding states and provinces. The substantial differences between these two cultivars in genetic background and growth habit suggested a third hypothesis to be tested: (3) BaYSM incidences in Alpine barley and Harrington barley will differ under some conditions. Table 1 summarizes the sequence and conditions of growth chamber experiments completed.

Table 1. Summary of experimental conditions in growth chamber experiments designed to examine the influence of temperature and host plant water status on BaYSM incidence in the barley cultivars Alpine and Harrington.

Exp[a]	Date plants infested[b]	Temp[c]	Cultivar[d]	Number of watered plants[e]	Number of droughted plants[e]
1	May 1	26	A	20	20
2	May 21	21	H	18	14
3	May 26	26	H	21	21
4	June 12	21	A	22	22
			H	9	10
5	July 9	26	A	22	22
6	June 23	21	H	22	22
7	July 9	30	H	22	22
8	July 13	26	H	22	22
9	July 18	21	A	22	22
10	Aug 1	30	A	22	22
11	Aug 9	30	H	22	22
12	Aug 22	30	A	22	22

a) Experiment number.
b) Date of mite infestations (all dates are 1994).
c) Temperature in degrees centigrade.
d) Cultivar (A=Alpine; H=Harrington).
e) Differential care of watered vs. droughted plants is described in Materials and Methods.

Each of the twelve treatment combinations was imposed upon approximately 44 mite-infested barley seedlings (the exact number of seedlings varied slightly in the first several experiments because of lack of germination of some seeds). Harrington seeds were provided courtesy of Dr. T. Blake (Montana State University Department of Plant, Soil and Environmental Sciences). Each potted seedling was infested with four adult mites from the viruliferous colony. Mite confinement was accomplished with the plastic tube method. After infestation, contained plants were placed in one of the Conviron growth chambers set at the desired temperature. Banks of flourescent

and incandescent lights (to provide a mixture of proper wavelengths) were stepped on

at 5, 6, and 7 a.m. and stepped off at 9, 10, and 11 p.m. in all experiments. Three

banks of lights produce approximately 450 $\mu E \cdot s^{-1} m^{-2}$ of light energy at six inches

below the lights. Plants were maintained in the growth chamber for 25 days after

infestation. The difference in watering schedules for "watered" vs. "droughted" plants

was a matter of judgement and was designed to achieve specific biological objectives

in terms of differential rates of growth for the two groups of plants. The biological

goal for the "droughted" plants was to maintain new leaf development at a very slow

pace (but not to stall it altogether) and to allow plants to reach a mild temporary

wilting point shortly before the next increment of water was to be added. The

biological goal for the "watered" plants was to maintain the rate of new leaf

development as close to the maximum as possible but without watering (always

accomplished from the bottom) so much that surface soil layers became damp (which

could hinder mite activities and population development). To achieve these biological

goals, fine tuning of the watering schedules based on temperature being studied and

also current growth stage of plants (plants with increasingly more leaves transpire

increasingly greater volumes of water) was necessary. Thus, a rigid, predetermined,

and totally uniform watering schedule would not have been appropriate to achieve the

desired biological effects over time. Seeds of plants to be used in "droughted" groups

were given 400 ml of water at the time of planting. Seeds of plants to be used in

"watered" groups were given 600 ml of water at the time of planting. After

infestation, plants in "droughted" groups were bottom watered with 150-225 ml in 75

ml increments at intervals over the 25 day observational period. After infestation,

plants in the "watered" groups were bottom watered with 600-900 ml in 75 ml

increments at much more frequent intervals over the 25 day observational period.

Daily observations of symptom appearance and plant growth stages were made during

the 25 day experimental period.

Differences in proportions of plants becoming symptomatic in the twelve

treatment condition combinations were analyzed using a general linear model to

accommodate a non-balanced blocking variable and the slight imbalance associated

with use of three trials for 21°C-grown Harrington plants and only two trials for all

the others. The blocking variable defined three blocks across the time duration of the

experiment in order to account for the varying infectivity of mites. Output included an

analysis of variance table in which all main effects and interactions for the three

factors (cultivar, temperature, and host plant water status) were tested. Fitted means

were supplied for which the proportion of symptomatic plants was adjusted to a mean

level of mite infectivity across the experimental duration. Statistical analysis was

accomplished with the help of the MSUSTAT statistical software package (27).

A variety of ELISA experiments were conducted to define the relationship

between the presence or absence of disease symptoms and the presence or absence of

BaYSMV. Specific hypotheses to be tested were (1) BaYSMV is not detectable in

nonsymptomatic barley leaf tissue and (2) BaYSMV is always present within

symptomatic tissue. Leaf tissue from 93 plants that remained nonsymptomatic over the

25 day observational period even though initially infested with mites from the

viruliferous colony were tested for the presence of BaYSMV antigen with ELISA. In the vast majority of plants that did become symptomatic, the first leaf (the only leaf present at the time of mite infestation) failed to develop symptoms. ELISA tests were done on a number of nonsymptomatic first leaves of otherwise symptomatic plants as well as on classically symptomatic leaves from those plants. To even more precisely define the correspondence between symptom presence/absence and BaYSMV presence/absence, an ELISA experiment was done on a barley plant having a nonsymptomatic first leaf (the only leaf present at the time of mite infestation), a nonsymptomatic second leaf (not yet accessible at the time of mite infestation), and a third leaf in which the apical half was nonsymptomatic but the basal half was symptomatic (also not yet accessible at the time of mite infestation). ELISA tests were done using tissue from each of these four regions. To test whether the inoculation process itself, resulting from mite feeding activity, places within barley leaf tissue detectable quantities of BaYSMV, 20 adult mites from the viruliferous colony were confined for inoculation access periods of six to 48 hours on one-leaf barley seedlings after which time ELISA tests were done on the barley leaves that had been fed on. To test the ability of the ELISA procedure to detect small quantities of BaYSMV when the virus is, in fact, present, groups of three or four adult mites (an extremely small quantity of tissue) from the viruliferous colony were crushed on a segment of healthy barley leaf tissue which was then prepared for ELISA in the standard way (0.2 g plant tissue ground in 2 ml of general extraction buffer). To better define the sensitivity of the ELISA test, dilution series were done of standard symptomatic leaf tissue

preparations. Dilutions (done in general extraction buffer) down to 1/10,000 were tested with ELISA. Healthy barley leaf tissue, barley leaf tissue heavily fed on over an extended period of time by nonviruliferous mites, and batches of three or four adult mites from the nonviruliferous colony served as negative controls at various times during the ELISA experiments.

Red egg submersion experiments

Red egg-containing white Sunshine Mix granules were located using a dissecting microscope. The egg-containing white granules were placed in plastic containers of tapwater for specific periods of time, after which the egg-containing granules were removed from the water and placed in 1.9 cm diameter x 1.3 cm tall plastic tubes sealed at both ends with mesh and gluegun glue. Evidence of egg hatching was assessed using a dissecting microscope over a period of ten days after removal from water. The hypothesis to be tested was that red eggs are rapidly rendered nonviable when submerged in water.

The influence of damp substrates on mite egg laying

Five replicates of an experiment designed to determine the influence of damp vs. dry egg deposition substrates on egg laying activity were done. The specific hypothesis to be tested was that mites lay fewer eggs when their preferred egg-laying substrates are periodically dampened than when their egg-laying substrates remain dry. For each barley plant in each replicate, a 1.3 cm layer of 50 mil white silica sand (0.15 mm effective size in filtration, Unimin Corp., Emmett, Idaho) was placed on top of the Sunshine Mix around the base of the potted seedling. Ten white perlite

granules (a chemically inert, siliceous, volcanic rock), 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 barley seedling. For half of the plants in each replicate (group I), the white granules and underlying sand were initially dampened with 13 ml of tap water. For the other half of the plants (group II), no dampening of the white granules was done. Each plant was then infested with six adult mites (viruliferous in some replicates and nonviruliferous in others) and containment was achieved using the plastic tube method minus the layer of damp Sunshine Mix as an outer packing. Infested seedlings were maintained at 21°C in a growth chamber. The white granules and underlying sand of group I plants were dampened daily with 3 ml of tap water. No dampening was done of granules of group II plants. After six days, the white granules were removed from each contained plant and the number of red eggs on the white granules was counted using a dissecting microscope. Beyond initial watering to start seedlings, plants were not watered during the six day experimental period. Numbers of red eggs laid on dry white granules were compared statistically with numbers of red eggs laid on dampened white granules using a two-sample t-test accomplished by the tpaired procedure of the MSUSTAT statistical software package (27).

Mite counts on healthy vs. BaYSMV-infected barley plants

Eight replicates of an experiment designed to compare mite counts on healthy Alpine barley plants with mite counts on BaYSMV-infected barley plants were done. The specific hypothesis to be tested was that more mites can be counted over time on

BaYSMV-infected plants than on healthy plants. For each replicate, ten potted barley seedlings were each infested with four adult mites from the nonviruliferous colony and ten potted barley seedlings were each infested with four adult mites from the viruliferous colony. Containment was achieved with the plastic tube method. Infested plants were maintained in the greenhouse. Within a given replicate, 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. After infestation with mites, an incubation period of approximately 14 days was utilized during which mites were not counted. Beginning at about day 14 after 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 by tapping the plant 30 times with a 25.4 cm segment of coathanger and also by tapping the containment tube eight times by hand. Each white paper disk was cut from the edge to the center and, in addition, a small hole was cut out in the center of each disk. By cutting the disk in this fashion, it was possible to slide the disk around the base of each plant. After counting mites on an individual plant, the paper disk was slid back away from the base of the stem and set against the plant (allowing mites to either move into soil at the base of the plant or climb back onto the plant) and the containment tube was replaced down over the plant onto the Sunshine Mix surface. Prior to the first day of counting, it was necessary to remove the now-dry layer of Sunshine Mix used as packing outside the containment tube so that the surface of the Sunshine Mix in the pot was flat. This allowed the

white paper disk to lie flat during counting procedures. In addition, all particles of

Sunshine Mix were carefully brushed from the inner and outer surfaces of the

containment tube so that small particles of Sunshine Mix would not fall onto the paper

disk during the tapping procedure, thus obscuring the mites. The ten plants infested

with mites from the nonviruliferous colony were counted consecutively as were the

ten plants infested with mites from the viruliferous colony. The group counted first

was alternated each day. A separate tapping rod was used for each of the two groups

of plants. The two groups of plants were kept on separate vaseline barrier-containing

plastic trays. To minimize possible effects of slightly differing light and temperature

conditions in different locations in the greenhouse, each of the eight replicates was

conducted in a slightly different location in the greenhouse and the spatial

arrangement of trays on the benchtop containing plants infested with nonviruliferous

and viruliferous mites was varied. To take into account possible seasonal effects on

mite counts, the eight replicates were conducted over approximately a six month

period (May-October). Statistical comparisons of mite counts on BaYSMV-infected

and healthy plants were done using the nonparametric sign test (30).

II. The efficiency of the brown wheat mite as a vector for BaYSMV

Red egg infestations

Robertson (35) previously demonstrated that barley plants infested with

diapausal white mite eggs can lead to BaYSMV-infected plants. Preliminary

greenhouse experiments, which relied on the plastic tube method of containment and

in which the presence of BaYSMV was not verified by ELISA, suggested that

infestation of barley seedlings with nondiapausal red eggs can also lead to BaYSMV-infected plants. Rigorously controlled infestations of barley plants with nondiapausal red eggs were therefore undertaken to indirectly test the hypothesis that transovarial passage of BaYSMV occurs within its mite vector. Red eggs laid by mites on white Sunshine Mix granules near the base of the stem of barley plants colonized by viruliferous mites were located using a dissecting microscope. Four to 14 red eggs on white granules were then transferred to the base of the stem of each of 24 potted healthy barley seedlings. Containment of the infested barley seedlings was achieved using the ziplock bag method. Caged infested barley seedlings were maintained in the greenhouse. The presence of BaYSMV in assay plants that became symptomatic was confirmed by ELISA.

Inoculation of BaYSMV by viruliferous preadults

Preliminary greenhouse experiments, relying exclusively on the plastic tube method of containment and in which the presence of BaYSMV was not confirmed by ELISA, suggested that viruliferous preadults can infect barley plants with BaYSMV. Rigorously controlled infestations of barley seedlings with viruliferous preadults were therefore done to test the hypothesis that preadult mites can inoculate barley plants with BaYSMV, leading to infected plants. Preadults were tapped from barley plants colonized by viruliferous mites onto 6.4 cm diameter white paper disks. A disk containing five to 15 preadult mites was placed against each of 12 potted healthy barley seedlings. Containment was achieved using the plastic exclusion method combined with the containment tube method. After a 24-48 hour 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 overnight. After fumigation and ventilation, the soil surface around the seedling was smeared with a layer of vaseline out for a radius of 2.5 cm circumferentially from the base of the seedling and then the entire soil surface was wetted. Containment was achieved around the seedling using the ziplock bag method and plants were maintained in the greenhouse for observation of symptom development. Presence of BaYSMV in assay plants that became symptomatic was confirmed by ELISA.

BaYSMV acquisition by nonviruliferous preadults

Carefully controlled experiments were done to test the hypothesis that preadult mites can acquire BaYSMV and become viruliferous by feeding on BaYSMV-infected barley plants. In one experiment, eight six-legged larvae were obtained, with the aid of a dissecting microscope, from the Sunshine Mix at the base of a plant colonized by nonviruliferous mites. In five experiments, nonviruliferous preadults were tapped from plants colonized by nonviruliferous mites onto 6.4 cm diameter white paper disks, 18 to 30 mites per disk. In each of the six experiments, the mites were then confined on virus-infected but mite-free barley plants (produced as described above) for acquisition access periods of 24-48 hours using the plastic exclusion method in combination with the plastic tube method (using steam-cleaned tubes). After this time, mites were 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 ziplock bag method. Contained infested seedlings were maintained and observed in the greenhouse. The presence of BaYSMV in assay plants becoming symptomatic was confirmed by ELISA.

Nonviruliferous mite counts at 21°C and 30°C

To study the impact of ambient air temperature on mite population expansion, mites were counted on five occasions over a period of 30 days after infestation on groups of barley plants maintained at either 21°C or 30°C. The hypothesis to be tested was that mite populations would grow extremely rapidly at 30°C but slowly at