



## 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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MOSAIC VIRUS - PETROBIA LATENS - BARLEY INTERACTIONS**

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## 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.

1/9/95  
Date

Thomas W. Carroll  
Chairperson, Graduate Committee

Approved for the Major Department

1/9/95  
Date

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Head, Major Department

Approved for the College of Graduate Studies

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## TABLE OF CONTENTS

|                                                              | Page |
|--------------------------------------------------------------|------|
| APPROVAL .....                                               | ii   |
| STATEMENT OF PERMISSION TO USE .....                         | iii  |
| ACKNOWLEDGEMENTS .....                                       | iv   |
| TABLE OF CONTENTS .....                                      | v    |
| LIST OF TABLES .....                                         | viii |
| LIST OF FIGURES .....                                        | ix   |
| ABSTRACT .....                                               | x    |
| INTRODUCTION .....                                           | 1    |
| MATERIALS AND METHODS .....                                  | 5    |
| General Procedures .....                                     | 5    |
| Mite colony establishment .....                              | 5    |
| Mite colony cage construction .....                          | 5    |
| Mite transfers .....                                         | 7    |
| Growth of barley seedlings for experiments .....             | 7    |
| The plastic tube method of mite confinement .....            | 8    |
| The ziplock bag method of containment .....                  | 9    |
| The plastic exclusion method .....                           | 12   |
| Production of BaYSMV-infected, mite-free barley plants ..... | 12   |
| Greenhouse conditions .....                                  | 13   |
| Growth chambers .....                                        | 14   |
| ELISA procedures .....                                       | 14   |
| MATERIALS AND METHODS .....                                  | 15   |
| Specific Experiments .....                                   | 15   |

## TABLE OF CONTENTS - Continued

|                                                                                                                                                 | Page |
|-------------------------------------------------------------------------------------------------------------------------------------------------|------|
| I. Association of severe BaYSM outbreaks and large mite populations with warm and dry conditions . . . . .                                      | 15   |
| The influence of ambient air temperature and host plant water status on BaYSM incidence in barley . . . . .                                     | 15   |
| Red egg submersion experiments . . . . .                                                                                                        | 21   |
| The influence of damp substrates on mite egg laying . . . . .                                                                                   | 21   |
| Mite counts on healthy vs. BaYSMV-infected barley plants . . . . .                                                                              | 22   |
| II. The efficiency of the brown wheat mite as vector for BaYSMV . . . . .                                                                       | 24   |
| Red egg infestations . . . . .                                                                                                                  | 24   |
| Inoculation of BaYSMV by viruliferous preadults . . . . .                                                                                       | 25   |
| BaYSMV acquisition by nonviruliferous preadults . . . . .                                                                                       | 27   |
| Nonviruliferous mite counts at 21°C and 30°C . . . . .                                                                                          | 27   |
| RESULTS . . . . .                                                                                                                               | 29   |
| I. Associations of severe BaYSM outbreaks and large mite populations with warm and dry conditions . . . . .                                     | 29   |
| The influence of ambient air temperature and host plant water status on BaYSM incidence in the barley cultivars Alpine and Harrington . . . . . | 29   |
| Red egg submersion experiments . . . . .                                                                                                        | 35   |
| The influence of damp substrates on mite egg laying . . . . .                                                                                   | 35   |
| Mite counts on healthy vs. BaYSMV-infected barley plants . . . . .                                                                              | 36   |
| II. The efficiency of the brown wheat mite as a vector for BaYSMV . . . . .                                                                     | 42   |
| Red egg infestations . . . . .                                                                                                                  | 42   |
| Inoculation by adult viruliferous mites . . . . .                                                                                               | 42   |
| Inoculation of BaYSMV by viruliferous preadults . . . . .                                                                                       | 43   |
| Acquisition of BaYSMV by nonviruliferous adults and preadults . . . . .                                                                         | 43   |
| Nonviruliferous mite counts at 21°C and 30°C . . . . .                                                                                          | 43   |
| DISCUSSION . . . . .                                                                                                                            | 45   |
| I. Association of severe BaYSM outbreaks and large mite populations with warm and dry conditions . . . . .                                      | 45   |

# TABLE OF CONTENTS - Continued

|                                                                                                     | Page |
|-----------------------------------------------------------------------------------------------------|------|
| The influence of air temperature and host plant water status on BaYSM incidence in barley . . . . . | 45   |
| The influence of soil moisture on the brown wheat mite vector . . . . .                             | 51   |
| Mite counts on healthy vs. BaYSMV-infected barley plants . . . . .                                  | 54   |
| II. The efficiency of the brown wheat mite as a vector for BaYSMV . . . . .                         | 63   |
| Efficiency of acquisition and inoculation of BaYSMV by the brown wheat mite . . . . .               | 63   |
| Nonviruliferous mite population expansion at 21°C and 30°C . . . . .                                | 63   |
| CONCLUSIONS . . . . .                                                                               | 66   |
| REFERENCES CITED . . . . .                                                                          | 70   |
| APPENDIX . . . . .                                                                                  | 77   |

## LIST OF TABLES

| Table                                                                                                                                                                                                                                                                    | Page |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 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 . . . . .                                              | 17   |
| 2. 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 BaYSM incidence in the barley cultivars Alpine and Harrington . . . . .                 | 30   |
| 3. 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 BaYSM incidence in the barley cultivars Alpine and Harrington . . . . . | 31   |
| 4. Red egg counts on periodically dampened vs. dry perlite granules . . . . .                                                                                                                                                                                            | 36   |
| 5. Mite counts on healthy vs. infected barley plants over a 21 day counting period beginning approximately 14 days after infestation of each plant with four adult mites . . . . .                                                                                       | 37   |
| 6. Summary of highest one day mite counts for all 10 infected group plants combined compared to 10 healthy group plants combined for each replicate . . . . .                                                                                                            | 40   |
| 7. Summary of average of highest one day mite count for each infected group plant compared to each healthy group plant for each replicate . . . . .                                                                                                                      | 41   |
| 8. Nonviruliferous mite counts on barley plants at 21°C and 30°C . . . . .                                                                                                                                                                                               | 44   |

**LIST OF FIGURES**

| Figure                                                                                      | Page |
|---------------------------------------------------------------------------------------------|------|
| 1. Mite colony cage . . . . .                                                               | 11   |
| 2. Plastic tube method of mite confinement . . . . .                                        | 11   |
| 3. Ziplock bag method of containment . . . . .                                              | 11   |
| 4. The influence of temperature and host plant water status on BAYSM<br>incidence . . . . . | 32   |

## 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.

## INTRODUCTION

Barley yellow streak mosaic virus (BaYSMV) is a recently discovered plant virus transmitted in nature only by the brown wheat mite, Petrobia latens Muller (Acari: Tetranychidae) (35). The preferred agricultural host for the virus is barley (Hordeum vulgare L.) , however wheat (Triticum aestivum 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 Nicotiana benthamiana 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.

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

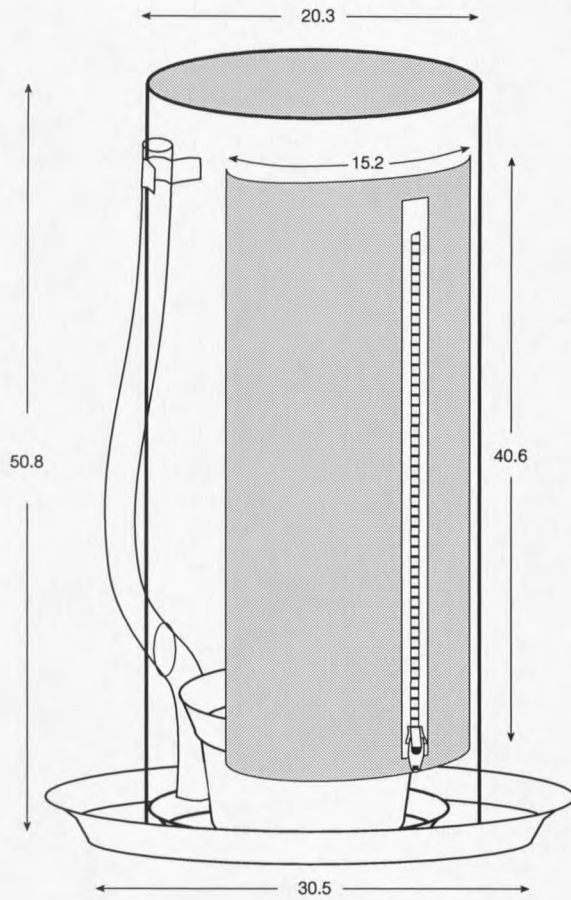


**Figure 1.** Mite colony cage.

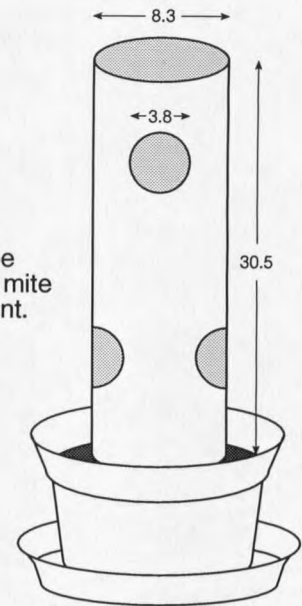


**Figure 2.**  
Plastic tube  
method of mite  
confinement.

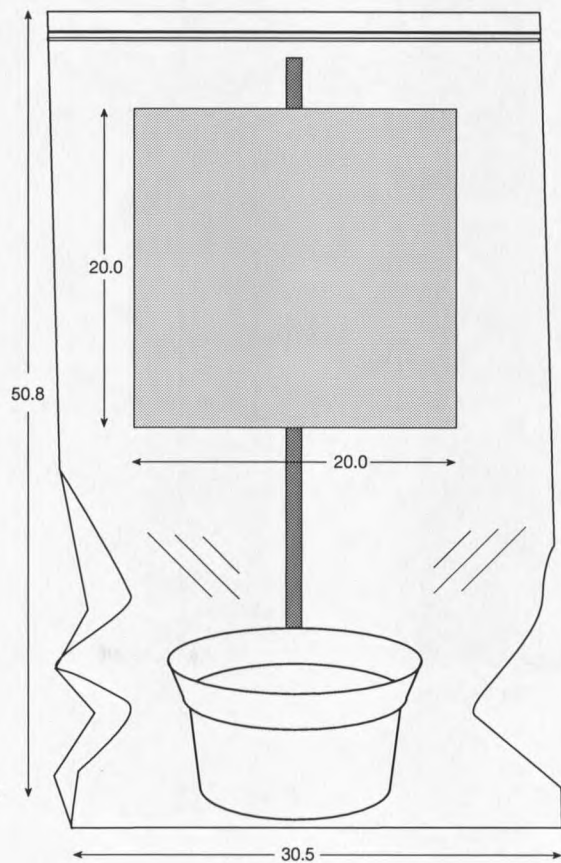


**Figure 3.** Ziplock  
bag method of  
containment.

### 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.

| <u>Exp<sup>a</sup></u> | <u>Date plants<br/>infested<sup>b</sup></u> | <u>Temp<sup>c</sup></u> | <u>Cultivar<sup>d</sup></u> | <u>Number of<br/>watered<br/>plants<sup>e</sup></u> | <u>Number of<br/>droughted<br/>plants<sup>e</sup></u> |
|------------------------|---------------------------------------------|-------------------------|-----------------------------|-----------------------------------------------------|-------------------------------------------------------|
| 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 fluorescent

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  $450 \mu\text{E} \cdot \text{s}^{-1} \text{m}^{-2}$  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

21°C. Three replicates were done at each temperature. For each replicate, ten Alpine barley seedlings were each infested with four adult mites from the nonviruliferous colony. Containment was achieved using the plastic tube method. Contained infested plants were placed in a Conviron growth chamber set at the desired temperature. Plants were bottom-watered with 100 ml of water nine, 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 previously for counting mites on healthy vs. BaYSMV-infected plants. To count mites, plants with their containment tubes in place were brought from the Conviron growth chambers into the greenhouse room. Because some mites tended to drop off the plants during the transfer from the growth chambers to the greenhouse, it was necessary to wait two hours after the transfer before counting to allow more than enough time for mites to move back onto plant surfaces. After counting, plants with containment tubes in place were replaced in the appropriate growth chamber.

## RESULTS

### I. Association of severe disease 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

Data collected from growth chamber experiments intended to provide insight into the influence of temperature (21°C, 26°C, and 30°C) and host plant water status (W=watered;D=droughted) on BaYSM incidence in the barley cultivars Alpine (A) and Harrington (H) are shown in table 2.

An analysis of variance showed no statistically significant differences in incidence of disease between the two cultivars for any temperature-host plant water status combination tested. In addition, the proportion of Alpine plants becoming symptomatic under all six treatments combined ( $115/260 = 0.442$ ) was nearly the same as the proportion of Harrington plants becoming symptomatic under all six treatments combined ( $113/269 = 0.420$ ). Significant differences in disease frequency at different temperatures ( $p < 0.0001$ ) and a significant temperature x water interaction ( $p = 0.0089$ ) were indicated, however. With these analysis of variance findings in mind, table 3 summarizes both fitted mean proportions and raw data mean proportions of diseased plants, combining results for the two cultivars for each temperature-host plant water status combination.

Table 2. 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 BaYSM incidence in the barley cultivars Alpine and Harrington.

| <u>Temp<sup>a</sup></u> | <u>Cult<sup>b</sup></u> | <u>Water status<sup>c</sup></u> | <u>No. of symptomatic plants/<br/>no. of total plants tested</u> | <u>% Symptomatic</u> |
|-------------------------|-------------------------|---------------------------------|------------------------------------------------------------------|----------------------|
| 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                 |

a) Temperature in degrees centigrade.

b) Cultivar (A=Alpine; H=Harrington).

c) Host plant water status (W=watered; D=droughted; see Materials and Methods for differential care of the two groups).

Table 3. 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 BaYSM incidence in the barley cultivars Alpine and Harrington<sup>h</sup>.

| <u>Temperature<sup>i</sup></u> | <u>Water status<sup>j</sup></u> | <u>Raw data mean proportions<sup>k</sup></u> | <u>Fitted mean proportions<sup>l</sup></u> |
|--------------------------------|---------------------------------|----------------------------------------------|--------------------------------------------|
| 21                             | W                               | 0.1290                                       | 0.0610 a <sup>z</sup>                      |
| 21                             | D                               | 0.1330                                       | 0.0685 a                                   |
| 26                             | W                               | 0.6118                                       | 0.6014 b                                   |
| 26                             | D                               | 0.7765                                       | 0.7656 b                                   |
| 30                             | W                               | 0.6136                                       | 0.5922 b                                   |
| 30                             | D                               | 0.3636                                       | 0.3422 c                                   |

h) Data for the barley cultivars 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.

i) Temperature in degrees centigrade.

j) Host plant water status (W=watered; D=droughted).

k) Proportions of plants becoming symptomatic based on actual experimental data.

l) 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  $p=0.05$ .

Figure 4. The Influence of Temperature and Host Plant Water Status on BaYSM Incidence

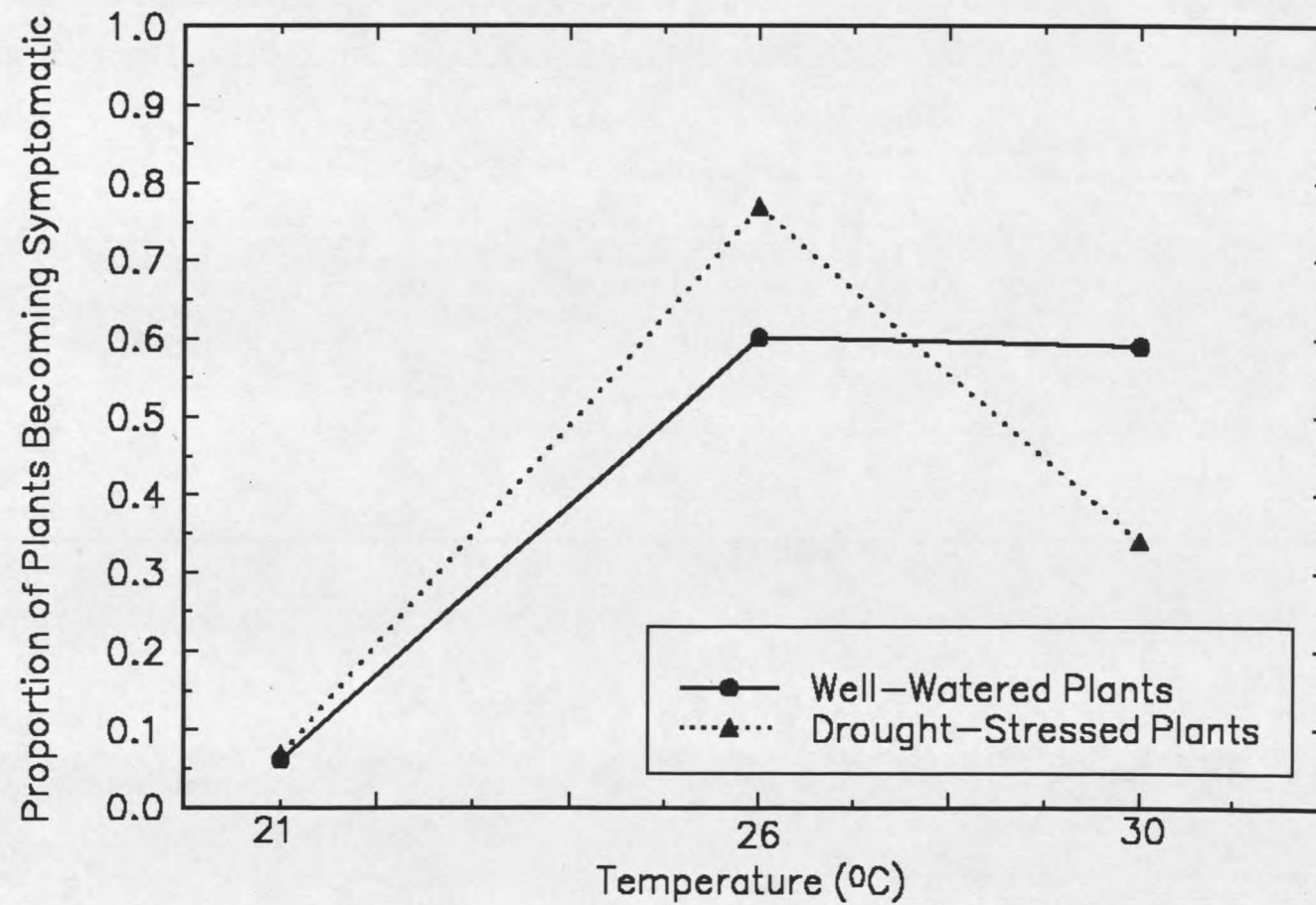


Figure 4 graphically illustrates the relationships between temperature, host plant water status, and BaYSM incidence.

Disease incidence at 21°C, whether plants were watered or droughted, was significantly less than disease incidence of either watered or droughted plants at either 26°C or 30°C. Disease incidence for watered plants at 21°C and droughted plants at 21°C was nearly the same. Disease incidence for watered plants at 26°C and for watered plants at 30°C was nearly the same. However, disease incidence was significantly less for droughted plants at 30°C than for droughted plants at 26°C ( $p=0.0014$ ). Disease incidence for droughted plants at 30°C was significantly less than for watered plants at 30°C ( $p=0.0076$ ). Preliminary greenhouse observations suggested that disease incidence may be somewhat higher in droughted plants than in watered plants at 26°C. Data from these growth chamber studies further suggests this possibility ( $p=0.0573$ ). However, further experimentation is required to more clearly define the influence of host plant water status on disease incidence at 26°C.

Of 93 ELISA tests done on plants which remained nonsymptomatic over the 25 day observational period even though initially infested with adult mites from the viruliferous colony, 84 generated ELISA signals indistinguishable from those of negative healthy barley controls (refer to appendix for a summary of all ELISA results). The remaining nine generated higher signals. Of these nine, five became visibly symptomatic within 24-48 hours after the ELISA test. The other four were not observed after the ELISA test. Symptomatic leaves from 21 classically symptomatic plants were tested with ELISA and, in every case, maximum ELISA signals near to

or slightly above 1.00 were generated (vs. average signals of 0.07 generated from healthy barley negative controls). Six nonsymptomatic first leaves from otherwise classically symptomatic plants were tested and, in each case, high ELISA signals, averaging 0.80 for the six samples, were generated. ELISA results from the experiment involving the plant having a nonsymptomatic first leaf (the only leaf present at the time of mite infestation), a nonsymptomatic second leaf and apical half of the third leaf (neither region accessible to mites at the time of infestation), and a classically symptomatic basal half of the third leaf (also not accessible at the time of mite infestation) further define the relationship between symptom presence/absence and BaYSMV presence/absence. The initially fed-on nonsymptomatic first leaf generated an elevated ELISA signal of 0.58. The nonsymptomatic second leaf and apical half of the third leaf generated ELISA signals of 0.04 and 0.03 respectively, comparable to the healthy barley negative control. The symptomatic basal half of the third leaf generated an ELISA signal of 1.04, comparable to the symptomatic barley positive control. The inoculation process itself, accomplished by mite feeding activity, did not result in detectable levels of BaYSMV in the leaves fed on in any of the three experiments that examined this. Detectable levels of BaYSMV, as evidenced by ELISA signals of 3.4X to 6X over healthy barley negative controls, were found in four of the nine batches of three to four mites from the viruliferous colony that were tested. Interestingly, mites originally from the viruliferous colony in the five batches that did not generate elevated ELISA signals had all been permitted to feed on healthy barley for one to about 20 days prior to the ELISA test. ELISAs done on dilutions of

the standard symptomatic barley tissue preparation (0.1 g tissue/1.0 ml general extraction buffer) indicated that dilutions down to 1/1000 consistently generated elevated (average of 0.34, which is about 5X over healthy negative controls, for four different tests) ELISA signals. In not a single instance did any of the negative controls (usually healthy barley tissue but also barley tissue from plants heavily fed on over extended periods of time by nonviruliferous mites and also batches of three or four nonviruliferous mites) generate elevated ELISA signals.

#### Red egg submersion experiments

Red eggs that had been laid on white Sunshine Mix Perlite granules were submerged in tap water for 7, 9, 11, 13, or 15 days after which time they were removed to dry, mesh-enclosed plastic capsules for observation of hatching. Many eggs hatched after seven days of submersion. Even after 13 days of complete submersion in water, at least some red eggs hatched within 24 hours of drying. The proportion of red eggs surviving submersion was not studied.

#### The influence of damp substrates on mite egg-laying

Red egg counts from five replicates of an experiment designed to assess the influence of periodically dampened vs. dry egg-laying substrates on the number of red eggs laid by mites are shown in table 4.

Table 4. Red egg counts on periodically dampened vs. dry perlite granules<sup>a</sup>.

| <u>Rep<sup>b</sup></u> | <u>No. plants with dampened granules</u> | <u>Red egg<sup>c</sup> count</u> | <u>No. plants with dry granules</u> | <u>Red egg<sup>d</sup> count</u> |
|------------------------|------------------------------------------|----------------------------------|-------------------------------------|----------------------------------|
| 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                              |

a) All replicates done at 21°C.

b) Replicate number.

c) Red egg counts are for all plants with dampened granules combined within a given replicate.

d) Red egg counts are for all plants with dry granules combined within a given replicate.

The five experiment total of red eggs laid on periodically dampened white Sunshine Mix granules was 143 (group I) and the total was 633 on dry granules (group II). The difference in egg numbers laid between the two groups is statistically significant at  $p = 0.0231$ .

#### Mite counts on healthy vs. BaYSMV-infected barley plants

Mite counts for each of the eight replicates are summarized in table 5. In each replicate, mites were counted daily for the 21 day counting period; the daily counts have been summed for week one, week two, week three, and totaled.

Table 5. Mite counts on healthy vs. infected barley plants over a 21 day counting period beginning approximately 14 days after infestation of each plant with four adult mites<sup>a</sup>.

| Rep <sup>b</sup> | Gp <sup>c</sup> | Wk 1<br>counts <sup>d</sup> | Wk1<br>I/H <sup>e</sup> | Wk 2<br>counts <sup>d</sup> | Wk 2<br>I/H <sup>e</sup> | Wk 3<br>counts <sup>d,f</sup> | Wk 3<br>I/H <sup>e</sup> | Total<br>counts <sup>d</sup> | Total<br>I/H <sup>e</sup> |
|------------------|-----------------|-----------------------------|-------------------------|-----------------------------|--------------------------|-------------------------------|--------------------------|------------------------------|---------------------------|
| 1                | I               | 2992                        | 1.67                    | 2995                        | 1.67                     | 1579                          | 1.59                     | 7566                         | 1.65                      |
|                  | H               | 1792                        |                         | 1795                        |                          | 990                           |                          | 4577                         |                           |
| 2                | I               | 396                         | 0.62                    | 1345                        | 1.13                     | 1347                          | 1.87                     | 3088                         | 1.21                      |
|                  | H               | 639                         |                         | 1188                        |                          | 720                           |                          | 2547                         |                           |
| 3                | I               | 1176                        | 1.09                    | 1511                        | 1.29                     | 255                           | 1.14                     | 2942                         | 1.19                      |
|                  | H               | 1077                        |                         | 1174                        |                          | 224                           |                          | 2475                         |                           |
| 4                | I               | 1587                        | 1.94                    | 688                         | 3.13                     | 321                           | 6.42                     | 2596                         | 2.39                      |
|                  | H               | 816                         |                         | 220                         |                          | 50                            |                          | 1086                         |                           |
| 5                | I               | 1211                        | 1.35                    | 1583                        | 1.92                     | 1090                          | 1.47                     | 3884                         | 1.57                      |
|                  | H               | 900                         |                         | 826                         |                          | 743                           |                          | 2469                         |                           |
| 6                | I               | 1049                        | 0.89                    | 1393                        | 1.52                     | 1043                          | 1.90                     | 3485                         | 1.32                      |
|                  | H               | 1174                        |                         | 915                         |                          | 550                           |                          | 2639                         |                           |
| 7                | I               | 613                         | .074                    | 1837                        | 1.61                     | 894                           | 0.72                     | 3344                         | 1.04                      |
|                  | H               | 824                         |                         | 1140                        |                          | 1248                          |                          | 3212                         |                           |
| 8                | I               | 258                         | 0.88                    | 1097                        | 1.71                     | 713                           | 1.12                     | 2068                         | 1.32                      |
|                  | H               | 293                         |                         | 640                         |                          | 639                           |                          | 1572                         |                           |
| Tot <sup>g</sup> | I               | 9282                        | 1.24                    | 12449                       | 1.58                     | 7242                          | 1.40                     | 28973                        | 1.41                      |
|                  | H               | 7515                        |                         | 7898                        |                          | 5164                          |                          | 20577                        |                           |

a) Counts are for all ten plants in a group (replicate four had only nine plants in each of the two groups).

b) Replicate number.

c) Group; 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).

d) Counts are for all seven days combined within the week indicated.

e) Quotient of number of mites counted on infected group plants/number of mites counted on healthy group plants for the period indicated.

f) Only three days of counting during week three for replicate three.

g) Composite data from all eight replicates combined.

For the eight replicates combined, a total of 28,973 mites were counted on the infected group plants (infested with mites from the viruliferous colony) and 20,577 mites were counted on the healthy group plants (infested with mites from the nonviruliferous colony), giving a composite I/H ratio of  $28,973/20,577 = 1.41$ . Approximately 75% of the 79 total infected group plants were symptomatic 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 seven days after mite infestation at the earliest to approximately 30 days after infestation at the latest. Of 79 total healthy group plants, five became symptomatic by the end of the 21 day counting period, indicating the inadvertent transfer of a viruliferous mite, probably early in the counting period. The I/H ratio varied in individual replicates from 1.04 (replicate seven, in which three of the inadvertent contaminations leading to symptomatic healthy group plants occurred) to 2.39 (replicate four). The I/H ratio was greater than one for each of the eight replicates, indicating a statistically significant difference in mite counts between the infected group plants and healthy group plants at  $p < 0.002$ . In four of the replicates (two, six, seven, and eight), mite counts for the healthy group plants were greater than mite counts for the infected group plants over the first week of counting. In the other four replicates (one, three, four, and five), mite counts for the infected group plants were greater than mite counts for the healthy group plants over the first week of counting. However, for the second week of counting, mite counts were greater for the infected group plants than for the healthy group plants in all eight replicates. This was also the

case in seven of eight replicates for the third week of counting. The only exception was replicate seven (again, in which three of the inadvertent contaminations leading to symptomatic healthy group plants occurred) where more healthy group mites were counted than infected group mites in the third week of counting. Of the three counting weeks, the eight replicate average I/H ratio was lowest (1.24) for the first week, highest (1.58) for the second week, and then dropped to an intermediate value of 1.41 for the third counting week.

Tables 6 and 7 summarize data that compare mite counts on infected group plants vs. healthy group plants in two additional ways.

Table 6. Summary of highest one day mite counts for all 10 infected group plants combined compared to 10 healthy group plants combined for each replicate.

| <u>Rep<sup>a</sup></u> | Maximum one day<br>mite count on<br>all 10 infected<br>group plants combined | Maximum one day<br>mite count on<br>all 10 healthy<br>group plants combined | <u>MaxI/<br/>MaxH</u> |
|------------------------|------------------------------------------------------------------------------|-----------------------------------------------------------------------------|-----------------------|
|                        | <u>(MaxI)</u>                                                                | <u>(MaxH)</u>                                                               |                       |
| 1                      | 689                                                                          | 423                                                                         | 1.63                  |
| 2                      | 272                                                                          | 220                                                                         | 1.24                  |
| 3                      | 309                                                                          | 246                                                                         | 1.26                  |
| 4                      | 300                                                                          | 154                                                                         | 1.95                  |
| 5                      | 268                                                                          | 186                                                                         | 1.44                  |
| 6                      | 230                                                                          | 196                                                                         | 1.17                  |
| 7                      | 320                                                                          | 286                                                                         | 1.19                  |
| 8                      | 245                                                                          | 138                                                                         | 1.76                  |
| Average ratio          |                                                                              |                                                                             | 1.46                  |

a) Replicate number.

For each replicate, the number of mites counted on all infected group plants combined reached a maximum on a certain day. Likewise, the number of mites counted on all healthy group plants combined reached a maximum on a certain day. The particular days on which mite counts for the two groups peaked were the same in some replicates and different in others. In each of the eight replicates, however, the highest single day mite count for all infected group plants combined exceeded the highest single day mite count for all healthy group plants combined, as shown in table 6. This difference is statistically significant at  $p < 0.002$ .

Table 7. Summary of average of highest one day mite count for each infected group plant compared to each healthy group plant for each replicate.

| <u>Rep<sup>a</sup></u> | Average highest one<br>day mite count per<br>infected group plant<br><u>(ImaxAve)</u> | Average highest one<br>day mite count per<br>healthy group plant<br><u>(HmaxAve)</u> | <u>ImaxAve/<br/>HmaxAve</u> |
|------------------------|---------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|-----------------------------|
| 1                      | 83.3                                                                                  | 51.8                                                                                 | 1.61                        |
| 2                      | 40.7                                                                                  | 36.3                                                                                 | 1.12                        |
| 3                      | 39.2                                                                                  | 30.5                                                                                 | 1.29                        |
| 4                      | 41.4                                                                                  | 25.1                                                                                 | 1.65                        |
| 5                      | 40.2                                                                                  | 30.4                                                                                 | 1.32                        |
| 6                      | 38.0                                                                                  | 26.3                                                                                 | 1.44                        |
| 7                      | 41.3                                                                                  | 36.4                                                                                 | 1.13                        |
| 8                      | 27.1                                                                                  | 18.0                                                                                 | 1.51                        |
| Average ratios         |                                                                                       |                                                                                      | 1.38                        |

a) Replicate number.

Mite counts on individual plants within each replicate reached a maximum on a certain day within the 21 day counting period and the days on which these individual plant maxima were reached varied greatly from one individual plant to another.

However, in each of the eight replicates, the average maximum mite count per infected group plant exceeded the average maximum mite count per healthy group plant, as shown in table 7, again yielding a statistically significant difference at  $p < 0.002$ .

Out of 164 total counting days in all eight replicates combined, the number of mites counted on infected group plants was greater than the number of mites counted on healthy group plants on 129 of the days (79%). Out of 56 week one counting days, the number of mites counted on infected group plants was greater than the number of mites counted on healthy group plants on 33 days (59%). Out of 56 week two

counting days, the number of mites counted on infected group plants was greater than the number of mites counted on healthy group plants on 53 days (95%). Out of 52 week three counting days, the number of mites counted on infected group plants was greater than the number of mites counted on healthy group plants on 43 days (83%).

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

### Red egg infestations

Of 24 plants, each infested with four to 14 red eggs from the viruliferous colony, four became symptomatic. In these four plants, ELISA tests confirmed the presence of BaYSMV.

### Inoculation by adult viruliferous mites- data from various experiments

Approximately 75% of barley seedlings became BaYSMV-infected under conditions nearly ideal 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. Under all 12 treatment combinations used in the previously described growth chamber experiments combined (two barley cultivars at three temperatures with both well-watered and drought-stressed plants at each temperature), 43% (227/528) of one-leaf barley seedlings infested with four adult mites from the viruliferous colony became BaYSMV-infected. In initial greenhouse trials, some infestations with only one adult viruliferous mite resulted in symptomatic barley seedlings, sometimes with inoculation access periods as brief as 20 to 120 minutes.

### Inoculation of BaYSMV by viruliferous preadults

Of 12 plants, each infested with five to 15 preadults from the viruliferous colony, one became symptomatic. The presence of BaYSMV was confirmed in this plant by ELISA. These experiments did not attempt to differentiate larvae from nymphs.

### Acquisition of BaYSMV by nonviruliferous adults and preadults

Preliminary greenhouse experiments strongly suggested that nonviruliferous adult mites can acquire and inoculate BaYSMV. However, in those experiments, containment was achieved with the plastic tube method and the presence of BaYSMV in symptomatic plants was not confirmed by ELISA.

In the one experiment that tested the ability specifically of six-legged larvae to acquire and inoculate BaYSMV, acquisition occurred and the assay plant became symptomatic. Of the five experiments that tested the ability of preadults in general (without distinguishing between larvae and nymphs) to acquire and transmit BaYSMV, successful acquisition occurred in three, as evidenced by development of symptoms in assay plants. The presence of BaYSMV in the four assay plants that became symptomatic was confirmed by ELISA.

### Nonviruliferous mite counts at 21°C and 30°C

Data from replicates of the experiment designed to provide insight into the influence of differing temperatures (21°C vs. 30°C) on mite population expansion are summarized in Table 8.

Table 8. Nonviruliferous mite counts on barley plants at 21°C and 30°C<sup>a</sup>

| <u>Temp<sup>b</sup></u> | <u>Rep<sup>c</sup></u> | <u>Ten plant mite counts at<sup>d</sup></u> |                |                |                |                |
|-------------------------|------------------------|---------------------------------------------|----------------|----------------|----------------|----------------|
|                         |                        | <u>11 days</u>                              | <u>15 days</u> | <u>21 days</u> | <u>25 days</u> | <u>30 days</u> |
| 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              |

a) Each plant was initially infested with four adult nonviruliferous mites.

b) Temperature in degrees centigrade.

c) Replicate number; 21°C and 30°C replicates bearing the same number were conducted simultaneously. During the time interval in which replicate three was conducted, mite population expansion in general throughout the colonies and in individual experiments taking place at that time was low. This appeared to be due, at least in part, to a high proportion of mites laying diapausal white eggs.

d) Within each replicate, mite counts are for the day indicated after infestation for all ten plants combined.

## DISCUSSION

### 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

In light of the substantial differences in genetic background and growth habit between Alpine and Harrington barleys, it was surprising to find that BaYSM incidences in the two cultivars did not differ significantly for any of the six temperature-host plant water status combinations studied. The finding that disease incidence in Alpine and Harrington was so similar across a range of experimental conditions may render other studies involving the use of Alpine barley as a host for BaYSMV and its mite vector more meaningful and, perhaps, more applicable in general.

In agreement with the outcome of preliminary greenhouse experiments, data from these growth chamber studies strongly suggest that the incidence of BaYSM is heavily influenced by temperature between 21°C and 26°C. All comparisons between 21° C-grown plants, whether watered or droughted, and 26°C-grown plants, whether watered or droughted, yielded highly significant differences having P-values of <0.001. There was virtually no difference in incidence of disease in watered and

droughted 21°C-grown plants. Data are suggestive that disease incidence is somewhat higher in droughted 26°C-grown plants than in watered 26°C grown plants ( $p=0.0573$ ), as was also suggested by preliminary greenhouse studies. It should be noted that this P-value was calculated from a two-sided test (testing the hypothesis that the proportions of watered and droughted plants becoming symptomatic at 26°C are not equal). In actuality, the hypothesis to be tested, based on preliminary greenhouse observations, was one-sided (the proportion of droughted 26°C-grown plants becoming symptomatic is higher than the proportion of watered 26°C-grown plants becoming symptomatic). Therefore, the P-value for this one-sided test is half the reported value. Nevertheless, to more clearly define the influence of host plant water status on disease incidence at 26°C, further experimentation is required. Based primarily on field observations associating severe BaYSM outbreaks and high mite vector populations with warm and dry weather conditions, it was hypothesized that disease frequency would be even higher at 30°C, particularly within the droughted group of plants, than at 26°C. It should be noted, however, that preliminary greenhouse studies were not conducted at temperatures this high. This hypothesized outcome was not observed in the growth chamber experiments. Disease incidence in watered 30°C-grown plants was indistinguishable from disease incidence in watered 26°C-grown plants. Unexpectedly, disease incidence in droughted 30°C-grown plants was significantly lower than in watered 26°C-grown plants ( $p=0.0262$ ), droughted 26°C-grown plants ( $p=0.0014$ ), and watered 30°C-grown plants ( $p=0.0076$ ).

In summary, comparing fitted mean proportions of plants becoming diseased

(see table 3), disease incidence in watered plants was very low at 21°C and was approximately ten-fold higher at both 26°C and 30°C with no detectable difference in disease incidence at the two higher temperatures. Disease incidence in droughted plants was also very low at 21°C (being indistinguishable from disease incidence in watered plants at 21°C) and again increased approximately ten-fold at 26°C.

However, in contrast to the situation in watered plants, disease incidence dropped in droughted 30°C-grown plants to about half the level of that in droughted 26°C-grown plants. One may speculate on the reasons behind these findings. At 21°C, host plant water status may fail to influence the disease development process because virus-induced disease may be so severely temperature-limited. At 26°C, droughted plants may be at a slight disadvantage in comparison to watered plants in terms of disease likelihood, perhaps because of slightly impaired plant defenses as plants marshal their resources to cope with moderate drought stress and/or because BaYSMV is able to more efficiently reach new growing regions within slowly growing drought-stressed plants (25). Maximum disease incidence may be reached at approximately 26°C and beyond that temperature, disease incidence may level off and remain on a plateau until conditions are reached that begin to diminish disease expression, either because of effects on the virus itself or on the response of the host plant. Watered 26°C-grown plants and watered 30°C-grown plants appear to exist on this maximum disease plateau. However, the combination of drought stress and a temperature of 30°C appears to prematurely push plants off the disease maximum plateau. A variety of highly stressful conditions are known to cause a global downregulation of protein

synthesis within both prokaryotic and eukaryotic cells (2,9,16,28,38). Since all viruses are, by definition, absolutely dependent on the use of host cell protein synthesis machinery, a stress-induced downregulation of host cell protein synthesis capacity may inhibit virus replication and thus disease incidence. While a temperature of 30°C may not be sufficiently stressful to trigger a downregulation of host cell protein synthesis under conditions of adequate water, the combination of high temperature and inadequate water may be sufficiently stressful for such downregulation to occur. Therefore, under highly stressful conditions, virus-induced disease expression in susceptible plants may serve as a barometer of the status of host cell protein synthesis capacity. Highly stress-tolerant cultivars that are susceptible to a virus may actually express disease symptoms at higher incidence under more stressful environmental conditions than comparably susceptible but less stress-tolerant cultivars.

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 (29). Therefore, an important question that needed to be addressed was whether or not BaYSMV was detectable in plants that remained nonsymptomatic over the 25 day observational period even though initially infested with mites from the viruliferous colony. More broadly, the question revolves around the relationship between disease symptom presence/absence and BaYSMV presence/absence. A variety of ELISA experiments were conducted to define this relationship. Evidence strongly suggests that detectable levels of BaYSMV are generally absent when symptoms are absent. However, two specific exceptions to

this generalization were identified. BaYSMV levels were high in nonsymptomatic leaves of plants in which symptoms first began to appear within 24-48 hours after the ELISA test. ELISA can thus detect elevated BaYSMV levels in nonsymptomatic plants where symptom appearance is imminent. In addition, BaYSMV levels were consistently high in nonsymptomatic first leaves (the only leaves present at the time of mite infestation) of otherwise classically symptomatic plants. The ELISA results from different regions of the plant having a nonsymptomatic first leaf, second leaf, and apical half of the third leaf but a symptomatic basal half of the third leaf indicate that different leaves on the same plant and even different sections of one leaf appear to adhere to the symptoms absent/BaYSMV absent generalization, again with the exception of the two specific situations described above. Based on the outcome of three experiments, the inoculation process itself, resulting from mite feeding activity, does not appear to place ELISA-detectable levels of BaYSMV within barley leaves. Nevertheless, the ELISA test used throughout these experiments appears to be quite sensitive. It was able to detect BaYSMV in some batches of three to four adult mites from the viruliferous colony crushed on healthy barley leaf tissue and then ground in 2 ml of general extraction buffer. The ratio of virus-containing mite tissue/ volume of buffer was extremely small in these ELISA tests. The finding that ELISA was consistently able to detect BaYSMV in 1/1000 dilutions of the standard symptomatic plant tissue preparation further defines the sensitivity of the test for detecting the presence of BaYSMV.

It appears, then, that an important temperature threshold for BaYSM expression exists somewhere between 21°C and 26°C. Toward the lower end of this range, little disease occurs but toward the upper end, disease incidence becomes substantial. Temperatures beyond 26°C do not appear to enhance disease frequency. However, the influence of barley host plant drought stress on disease incidence may be either neutral, positive, or negative, depending upon ambient temperature. The temperature range over which the drought-stressing of host plants enhances BaYSM frequency seems rather narrow and the influence, when it does occur, does not appear strong. These findings do not support the notion that host plant drought stress is an important factor in promoting BaYSMV-induced disease, at least under the experimental conditions utilized and over the time interval examined. It is possible that extended periods of drought stress in the field significantly beyond the 25 day period utilized in these experiments do significantly enhance the prevalence and severity of BaYSMV-induced disease in barley (although the outcome of experiments combining a 30°C temperature with inadequate water suggest that extremely stressful conditions may have the opposite effect on disease likelihood). However, the interval studied in these growth chamber experiments, though not extremely lengthy, may nevertheless be informative because it begins very early in host plant development (the one-leaf stage) when new growing regions of the plant are readily accessible to a virus replicating and moving within the plant.

The influence of soil moisture on the brown wheat mite vector

Irrigation practices have been found to influence population densities of spider mites, closely related to the brown wheat mite, in the field (5). Varying rainfall amounts were reported to differentially influence brown wheat mite numbers in field surveys (7) and this mite has long been considered primarily a dryland pest (10). The brown wheat mite is unusually dependent on the use of surface soil layers, compared to most arthropod vectors of plant viruses, for completion of its life cycle (17). Eggs are laid on suitable substrates within surface layers of soil and inactive chrysalids between each of the four active mite stages also lie within surface soil layers. Active mite stages appear to frequently move back and forth between surface soil layers and host plants, perhaps to maximize time at preferred temperatures, to minimize the dessicating influences of sun and wind, and possibly to avoid predators. The lack of evidence that dry conditions (which field reports have associated with severe BaYSM outbreaks and large mite populations) strongly promote the expression of BaYSM in barley suggests that the main influence of dryness may be on promotion of the mite vector. The dependence of the brown wheat mite on use of surface soil layers for completion of its life cycle suggested the possibility that the frequent presence of moisture within that surface soil layers (such as from natural rainfall and/or from regular irrigation) may be a major cause of mite mortality or at least a major hindrance to important mite activities. Several experiments were conducted to gain insight into these possibilities. When nondiapausal red eggs were fully submerged in water, even soapy water, for seven days, many still hatched within 24-48 hours after

drying out, yielding live larvae. In addition, some proportion of red eggs fully submerged in water for up to 13 days still hatched after drying out. Laboratory observations by S. Brumfield (unpublished data) indicated that adult mites can remain alive when placed in liquids for at least several days. Based upon these findings, it appears that mite eggs and adults have a surprising ability to survive not only soil dampness but even full submersion in liquids. With this in mind, the possibility that soil moisture could negatively influence a critical aspect of mite behavior, egg-laying, rather than mite survival per se was examined using the experimental design involving the placement of white Sunshine Mix perlite granules, either periodically dampened or left dry, on a layer of fine white silica sand around the bases of mite-infested barley seedlings. Previous observations indicated that the white granules in Sunshine Mix are favored sites for mite egg-laying, apparently because some granule surface porosities are precisely the right size to receive individual mite eggs. It has also been observed that mites lay diapausal white eggs in similar areas of surface porosity on natural substrates such as rocks in barley fields. It thus appears that mites may tend to preferentially locate tiny lacunae of just the right size for their egg-laying so that eggs are protected in or at least retained by tiny individual caverns. The surface porosity of white Sunshine Mix granules provides just such locations. However, the fine silica sand, consisting of very minute smooth-surfaced particles, surrounding the infested plants and underlying the white granules offers no such preferred egg-laying sites. Thus, given the choice between white Sunshine Mix granules and fine sand, mites strongly prefer the white granules for their egg-laying. White granules are also

convenient from an experimental standpoint because red eggs are easy to see and count (under a dissecting microscope) when present on white granules.

The outcome of the five replicates of this experiment strongly suggests that mites lay far fewer eggs on periodically dampened substrates than on dry substrates (143 red eggs vs. 633 red eggs respectively for all five replicates combined over a six day egg-laying period in each replicate). An influence of this magnitude could be a major factor underlying the difference in mite numbers and BaYSM severity between nonirrigated or poorly-irrigated barley and well-irrigated barley. Thus, periodic irrigation or rainfall may not directly lead to high mite mortality but, rather, may greatly interfere with mite egg-laying activity, thus diminishing the rate of mite population expansion. The brown wheat mite appears to strongly prefer dry substrates for optimum egg-laying and the longer potential egg-laying substrates are damp, the more egg-laying activity may be restricted. If relatively few eggs can be laid to begin with because of the presence of moisture on preferred egg-laying substrates within soil, mite population development will be greatly restricted regardless of the ability of various mite life cycle stages to survive the presence of water. The brown wheat mite may have evolved in very arid regions and may have little tolerance for even thin films of moisture on egg-laying substrates within the soil. The conditions created with this experimental design were intended to temporarily dampen preferred egg-laying substrates daily without hindering mite movement onto and off of nearby host plants. The ten white granules near the base of each host plant were spread over a circular area approximately 2.5 cm in diameter. Applying 3 ml of water once a day to that

surface area provides about 6 ml of water per unit area per day. After dampening, excess water quickly drained off the white granules and out of the porous sand layer, leaving behind, for some period of time, thin films of water over the surface porosities of the white granules. When red eggs were counted on white granules six days after mite infestation, mites were occasionally found walking over and between the white granules, both on dry granules and on the periodically dampened granules. The finding that periodic dampening of preferred egg-laying substrates strongly interferes with mite egg-laying activities implies that any edaphic and climatic characteristics which favor the maintenance of dry surface soil layers where preferred mite egg-laying substrates are located may tend to favor the fulfillment of maximum mite egg-laying potential, mite population expansion, and thus mite BaYSMV vectoring activity. 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 seems significant that this list of characteristics very much describes the conditions extant in the early to midseason of the barley growing regions of northcentral Montana where BaYSMV in barley was first discovered and where disease outbreaks have tended to be most severe.

#### Mite counts on healthy vs. BaYSMV-infected barley plants

Observations made over time of the mite colonies maintained in the greenhouse led to the impression that greater numbers of adult mites were generally visible within the viruliferous colony than within the nonviruliferous colony. Eight replicates of an experiment were conducted to compare mite counts on BaYSMV-

infected barley plants with mite counts on healthy barley plants. Data from the eight replicates suggest that mite counts are usually higher on BaYSMV-infected plants than on healthy plants under the experimental conditions utilized. The data further suggest that peak mite counts tend to be higher on virus-infected plants than on healthy plants.

The interval chosen for mite counting, starting approximately 14 days after mite infestation and then continuing, in general, for 21 consecutive days was selected for several reasons. First, mites used in the infestations undoubtedly varied in their inherent reproductive capacities at the time of infestation. Therefore, even though comparable in size, mites from the nonviruliferous colony may have been older or younger on average or more or less fecund on average than mites from the viruliferous colony at the time of infestation for any one replicate. Mite counts obtained over the first week of counting seem to support this possibility. In four of the replicates, mite counts were higher on healthy group plants than on infected group plants while in the other four replicates the reverse situation occurred. Waiting 14 days after infestation before beginning to count was intended to diminish the influence of the initial reproductive capacities of the infesting mites (which was not the parameter being examined with this experimental design) on mite counts obtained over the counting period. In theory the earlier after infestation counting begins, the more the counts in the early part of the counting period should reflect the reproductive ability of the infesting mites, an outcome to be minimized as much as possible. In addition, because the phenomenon to be studied is the influence of virus-infected plants vs. healthy plants on countable mites, waiting 14 days before the beginning of

counting allowed a significant proportion of plants infested with viruliferous mites to become BaYSMV-infected and, thus, to potentially impact mites feeding on those plants. It should be kept in mind, however, that symptom appearance during the counting period is not necessarily a prerequisite for a virus-inoculated plant to influence the physiology of mites feeding on that plant. Early changes within a virus-inoculated plant prior to symptom development may be important in host plant-mite interactions.

Once beginning to count at approximately 14 days after mite infestation, another factor limited the length of the subsequent counting period. Not long beyond the end of the 21 day counting period (which is 34 days after infestation of one-leaf barley seedlings), the size of host plants began to impinge upon the space available within containment tubes. Such impingement probably leads to changes in within-tube microclimatic conditions which can become significant and unwanted influences on mite development and behavior (15). The counting period was thus constrained by the volume available within the containment tubes but within that constraint, an attempt was made to choose the most biologically relevant counting interval based on the hypothesis to be tested.

A number of aspects of the data summarized in table 5 appear meaningful. As mentioned, the fact that in four of the replicates, mite counts on infected plants were higher than mite counts on healthy plants over the first week of counting while in the other four replicates the reverse situation occurred seems to indicate that, at times, mites chosen from the viruliferous colony were inherently more fecund while at other

times mites from the nonviruliferous colony were more fecund. These differences in initial reproductive capacity seem to be reflected not only in the week one counts, but also in the observation that mite counts on infected plants were greater than mite counts on healthy plants on only 59% (33/56) of week one counting days and, finally, in the fact that of the three counting weeks, the infected plant mite count/healthy plant mite count ratio was lowest for week one (1.24). Over the week two counting period, evidence of the initial differences in mite reproductive capacity seems to be largely lost. In all eight replicates, even those in which healthy plant mite counts were greater than infected plant mite counts over week one, infected plant mite counts were greater than healthy plant mite counts during week two. Of the three counting weeks, the infected plant mite count/healthy plant mite count ratio was greatest for week two (1.58). In addition, infected plant mite counts were greater than healthy plant mite counts on 95% (53/56) of week two counting days. Week two may, on average, represent the period when mite counts maximally reflect changes in host plant physiology resulting from virus infection. Over the week three counting period, the dominance of infected plant mite counts over healthy plant mite counts began to diminish somewhat on average. Infected plant mite counts were greater than healthy plant mite counts in seven of the eight replicates (replicate seven is the exception), the infected plant mite count/healthy plant mite count ratio dropped to 1.40, and infected plant mite counts were greater than healthy plant mite counts on 83% (43/52) of week three counting days. In summary, it appears that the first week of counting still tended to reflect initial differences in reproductivity capacity of the infesting mites,

week two appeared to reflect maximal influences of host plant physiology on mite counts, and week three began to reflect a diminution of those effects.

Data summarized in tables 6 and 7 suggest that peak mite counts on BaYSMV-infected barley plants tend to be higher on average than peak mite counts on healthy plants. It is interesting that the infected plant mite count/healthy plant mite count ratios derived from the three different methods of comparison summarized in tables 5, 6, and 7 are all close to 1.4/1.

Findings from the eight replicates have intentionally been described in terms of "mite counts" rather than "mite numbers" or "mite populations". In spite of the fact that attempts were made to examine the influence of BaYSMV-infected host plants vs. healthy host plants on the brown wheat mite in a biologically meaningful fashion, and in spite of the fact that the experiment was repeated eight times, the results must be viewed with caution. An additional difficulty in completing this group of replicates was that temperatures in the greenhouse fluctuated substantially and unpredictably (from about 20°C to greater than 31°C) at times over the course of the six-month experimental period. Much further experimentation would be required to conclude definitively that BaYSMV-infected barley plants do or do not have some form of net positive effect on brown wheat mites feeding on those plants. Even more work would be required to identify the specific nature of the effect(s). Kennedy (19) was the first to describe influences of virus-infected host plants on vectoring arthropods. The barley yellow dwarf virus (BYDV)-aphid vector systems have been most heavily investigated. A summary by Power and Gray (32) of those investigations points out

the differing influences of BYDV-infected host plants on vectoring aphids in different virus-vector combinations studied by different workers. Similarly, Fritzsche and Thiele (11) reported opposite influences of virus-infected host plants on reproduction of a nonvectoring spider mite (Tetranychus urticae Koch) in two different host plant/virus-mite combinations. Jones (18) has very logically described, using vector diagrams, the possible outcomes of the effects of pathogen-infected host plants on arthropods feeding on those plants. In seven out of ten possible outcomes described by Jones, a net positive effect of pathogen-infected host plants on feeding arthropods was not predicted to occur. The data collected in these eight replicates represent only a 21 day "snapshot", conducted under artificial conditions, of a phenomenon that occurs over 90 or more days in the field and in which migration, predation, variable weather conditions, and many other factors potentially influence the overall process. It is also possible that the influence of virus-infected host plants on phytophagous arthropods (including vectors) varies with the stage of disease development in the plant (3,4). At times, positive effects on feeding arthropods may occur, perhaps early in disease development when host plant physiology is changing significantly but before structural host plant defenses are in place or before significant tissue necrosis has occurred. Perhaps later in disease development, potential benefits may be overwhelmed by host plant changes of a structural defensive nature or because of tissue death that actually render the plant less supportive of feeding arthropods. In addition, the nature of the influence of pathogen-induced host plant disease on feeding arthropods may depend on the feeding style of the arthropod (3). Such feeding style-

specific effects are analogous to those suggested by Larsson (23) involving the influence of drought-stressed host plants on feeding arthropods.

If mite counts do tend to be higher on BaYSMV-infected plants than on healthy plants, as suggested by these data (under the experimental conditions imposed), there are many possible explanations, along with what may be the most likely explanation—a combination of reasons. Mite populations may not be greater on infected plants. Rather, individual mites may feed longer or more frequently on virus-infected plants, perhaps because of phagostimulants produced in diseased plants (42), or because nutrition is more difficult to obtain per unit feeding time on diseased plants thus necessitating longer or more frequent feedings, or because somewhat drier, diseased plant tissue creates problems in maintaining water balance in feeding mites, again necessitating longer or more frequent feedings. Alternatively, mites may have longer lifespans on diseased plants but their reproductive output may not be increased or more time may be required to go through each active stage of the life cycle, perhaps because of diminished nutrition on virus-infected plants. With any of these possibilities, mite populations on infected plants may not expand more rapidly and become larger than on healthy plants but the number of countable mites on infected plants at any one time may be greater on average thus creating the appearance that populations are expanding more rapidly.

It is also possible that systemic virus disease inhibits certain plant defenses that would normally be deployed in response to mite feeding. Thus, it may be that mite numbers and/or mite lifespans are not increased on plants sustaining a systemic virus

infection but rather, they are decreased less because of virus disease-impaired anti-mite defenses. It should be noted that this notion is counter to the findings of Krischik (22), which seem to indicate that plants utilize certain common core defenses in the face of various types of attack, including both virus infection and arthropod feeding.

An additional possibility is that BaYSMV-infected plants may provide enhanced nutrition, perhaps especially in the form of essential amino acids, at least during a certain phase of disease development, 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 expanded egg-laying lifetime, or some combination of these, culminating in accelerated expansion of mite populations. White proposed that a variety of plant stresses, including disease, lead to transiently increased nitrogen levels which can benefit invertebrate herbivores feeding on those plants (44). Some studies have suggested that BYDV-infected plants may provide enhanced nutrition for some phloem-sap feeding aphid vector species (32). However, because epidermal and mesophyll whole-cell contents (which the brown wheat mite is thought to obtain during feeding) contain approximately ten-fold higher levels of nitrogen than does phloem sap (33), it would seem that an arthropod with the feeding style of the brown wheat mite would have much less to gain from virus infection-induced improvements in host plant nutrition than would a specialist phloem sap feeder.

Finally, it is also possible that the influence of BaYSMV infection leading somehow to greater mite counts on infected plants is 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.

If BaYSMV-infected host plants do tend to have a net positive effect on the rate of expansion of brown wheat mite populations at the individual plant level, this effect could have an even more consequential effect at the community level in an agricultural field. Under field circumstances, more rapidly expanding mite populations on virus-infected plants could lead to earlier and heavier migration of mites onto neighboring plants leading to more rapid expansion of the mite population spatially, earlier inoculation of adjacent healthy barley plants with BaYSMV leading to a greater negative impact on plant productivity and yield, to a higher proportion of plants becoming BaYSMV-infected in the field, and, because of a larger and spatially more widespread mite population by the end of the growing season, a heavier and more widespread diapausal white egg load available to impact plants the following season. Thus, a net positive effect on mite population expansion at the individual plant level may have an even greater impact on disease dynamics at the community level during one growing season and may have carryover effects from season to season. The phenomenon of greater brown wheat mite counts on BaYSMV-infected barley plants than on healthy barley plants may, in part, explain field observations associating more severe disease and greater mite numbers with warm and dry growing conditions.

The possibility of greater mite numbers on BaYSMV-infected host plants than on healthy host plants potentially has both agricultural and evolutionary implications. Whether greater numbers of mites on plants at any given time are the result of more

time spent feeding by mites or the result of a larger population of mites, greater mite numbers on plants should mean more direct feeding damage by mites (40) and, more importantly, more virus inoculation events. Thus, increased mite numbers on plants, regardless of cause(s) could be consequential agriculturally. From an evolutionary standpoint, disease-induced changes in host plant physiology, resulting from vector inoculation of a virus, that lead to enhanced fitness of the vectoring species (more rapid reproduction and/or greater longevity of individuals) may be a factor underlying the initiation and stabilization of some virus-vector relationships.

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

### Efficiency of acquisition and inoculation of BaYSMV by the brown wheat mite

Data from experiments designed to provide insight into the ability of the brown wheat mite to acquire and inoculate BaYSMV suggest that the mite is an efficient vector for this virus. Acquisition of BaYSMV by nonviruliferous individuals 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-laying, as evidenced by the production of diseased plants following infestation with red eggs. Adult viruliferous mites efficiently inoculate barley plants with BaYSMV and preadults also appear to be able to inoculate the virus.

### Nonviruliferous mite population expansion at 21°C and 30°C

Data from replicates of the experiment designed to gain insight into the influence of two different temperatures on mite population expansion suggest an

aspect of mite biology that may tend to decrease the likelihood that the brown wheat mite will vector BaYSMV in nature. It was initially hypothesized, based on the reported association of greater mite populations in the field with warm and dry conditions, that mite populations would grow extremely rapidly at 30°C but suboptimally at 21°C. Contrary to this expectation, mite populations increased very little at 30°C over the 30 day observational period while at 21°C, mite counts reached extremely high numbers by the thirtieth day after initial infestation of host plants. Vigorous population expansion at 21°C is consistent with data reported by Cox and Lieberman (7) regarding time required by the mite to complete different stages of the life cycle at 68°F (20°C). As previously discussed, BaYSM occurs at a very low incidence in barley at an ambient temperature of 21°C. Brumfield (unpublished data) found that when initially 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 because of lack of ongoing access to BaYSMV within the plant cells fed on, resulting eventually in a nonviruliferous colony. At temperatures too low to support virus-induced disease, barley becomes, in effect, a virus nonhost. Initially 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. In summary, lower temperatures that will only support virus-induced disease in barley at very low incidences seem well able to sustain vigorous mite population expansion and this differential effect of temperature on the virus-induced disease in barley and on

mite population growth may diminish the likelihood that brown wheat mite populations will retain BaYSMV over time. It should also be noted that the host range of the brown wheat mite is thought to be much broader than the host range of BaYSMV and this differential host range of virus and vector may similarly tend to purge BaYSMV from mite populations in nature. This possibility may account, in part, for the observed importance of the growing of barley in the same location over several successive seasons in the development of severe BaYSM outbreaks.

## CONCLUSIONS

Data from experiments described in this thesis provide insight into possible factors underlying the field reports which have associated severe BaYSM outbreaks and large brown wheat mite populations with warm and dry conditions. A critical temperature threshold for efficient BaYSM expression in barley appears to occur between 21°C and 26°C. Temperatures at the lower end of that range support only a very low incidence of disease while temperatures toward the upper part of the range support disease at perhaps a tenfold greater frequency. Disease incidence was not greater at 30°C than at 26°C, however. Therefore, sufficient warmth within the microenvironment where BaYSMV has been inoculated by its vector into barley plants appears critical for significant disease to occur. Conversely, warmer temperatures do not appear to be necessary for the generation of large mite populations based on the finding that mite populations expand very well at 21°C. The most important impact of dry conditions may be on the brown wheat mite vector rather than on the expression of BaYSM itself. The presence of periodic soil moisture appears to significantly constrain mite egg-laying activities. Because of this constraint, mite population expansion may be much more rapid under unirrigated or poorly-irrigated circumstances than under conditions of heavy irrigation. In contrast, data suggest that the direct influence of dry conditions on the incidence of BaYSM in barley may be

neutral, positive, or even negative, depending upon ambient temperature. Therefore, considering the range of temperatures likely to occur in the field over time, the drought-stressing of barley host plants may not play a critical role in severe disease outbreaks. Finally, the finding that mite counts frequently tend to be higher on BaYSMV-infected barley plants than on healthy barley plants for reasons other than the influence of soil moisture on mite egg-laying activities may also underlie field observations linking greater disease severity and high mite numbers with warm and dry conditions.

On balance, experimental evidence suggests that the brown wheat mite is potentially a very capable vector of BaYSMV. Viruliferous adults efficiently transmit the virus to barley and preadults can also inoculate the virus. Nonviruliferous preadults readily acquire BaYSMV from infected barley plants and preliminary studies have suggested that adults can also acquire the virus, although more rigorously controlled experiments need to be conducted to confirm this. Experiments which have provided indirect but strong evidence for the occurrence of transovarial passage of the virus within its mite vector, confirming earlier work (35), highlight an additional aspect of the BaYSMV-brown wheat mite relationship that enhances the potential efficiency of the mite as vector. However, not all data support the notion that the brown wheat mite is likely to vector BaYSMV in the field. The finding that brown wheat mite populations can expand substantially at temperatures too low to support all but very low incidences of BaYSMV in barley suggests the possibility that BaYSMV may tend to become purged from mite populations when relatively cool mite-supportive but virus disease-restrictive temperatures are prolonged.

BaYSMV was undetectable by ELISA in nonsymptomatic plants initially infested with mites from the viruliferous colony with the exception of two specific situations. High levels of BaYSMV, as indicated by strong ELISA signals, were detected in nonsymptomatic leaves of plants that began to develop symptoms within 24-48 hours after the ELISA test. In addition, nonsymptomatic first leaves of otherwise symptomatic plants consistently contained high levels of BaYSMV. The ELISA test used in these experiments proved to be both reliable and sensitive and should be useful in the future for the diagnosis of disease caused by BaYSMV and in experiments designed to better understand the biology of the BaYSMV-brown wheat mite-barley system.

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**APPENDIX**

## APPENDIX - SUMMARY OF ELISA RESULTS

| Sample <sup>a</sup><br>type                                                                                                    | Number of examples<br>tested | Absorbance at 410nm <sup>c</sup> |             |
|--------------------------------------------------------------------------------------------------------------------------------|------------------------------|----------------------------------|-------------|
|                                                                                                                                |                              | Mean                             | Range       |
| Healthy barley                                                                                                                 | 29                           | 0.07                             | 0.03 - 0.11 |
| Symptomatic barley                                                                                                             | 29                           | 0.99                             | 0.74 - 1.11 |
| Dilutions of symptomatic barley <sup>b</sup>                                                                                   |                              |                                  |             |
| 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 |
| 1/1000 dilution                                                                                                                | 4                            | 0.34                             | 0.27 - 0.40 |
| 1/2000 dilution                                                                                                                | 2                            | 0.19                             | 0.16 - 0.22 |
| 1/4000 dilution                                                                                                                | 2                            | 0.13                             | 0.08 - 0.17 |
| 1/5000 dilution                                                                                                                | 3                            | 0.13                             | 0.12 - 0.14 |
| 1/10000 dilution                                                                                                               | 1                            | 0.05                             | ...         |
| Healthy <u>Nicotiana benthamiana</u>                                                                                           | 5                            | 0.09                             | 0.08 - 0.11 |
| Symptomatic <u>N. benthamiana</u>                                                                                              | 5                            | 0.94                             | 0.70 - 1.10 |
| Nonsymptomatic plants from cultivar-temperature-host plant water status growth chamber experiments testing negative for 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 |
| Barley leaf inoculated by 20 adult mites from the viruliferous colony                                                          | 3                            | 0.10                             | 0.08 - 0.15 |
| Batches of 3-4 mites from the viruliferous colony                                                                              | 9                            | 0.19                             | 0.05 - 0.33 |
| Batches of 3-4 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                             | ...         |

a) Unless otherwise noted, plants tested are barley.

b) Dilutions done in general extraction buffer - see Materials and Methods.

c) Readings taken at 30 minutes.

